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Clinical consequences of cytochrome P450 2C9 polymorphisms

The gene coding for the cytochrome P450 (CYP) enzyme 2C9 (CYP2C9) carries numerous inherited polymorphisms. Those coding for R144C (*2) and I359L (*3) amino acid substitutions have both significant functional effects and appreciable high population frequencies, and their in vivo consequences have been studied in humans with regard to drug metabolism. This review summarizes present knowledge about the pharmacokinetics, drug responses, and outcomes of clinical studies in individuals with different CYP2C9 genotypes. Tentative estimates of how CYP2C9 genotyping might be applied to dose adjustments in clinical therapy were based on dose-related pharmacokinetic parameters such as clearance or trough drug concentrations. Mean clearances in homozygous carriers of the *3 allele were below 25% of that of the wild type for S-warfarin, tolbutamide, glipizide, celecoxib, and fluvastatin. In the more frequent heterozygous carriers (genotype *1/*3), the clearances were between 40% and 75%. In these cases in which individual dosages are derived from clinical drug effects, such as for the oral anticoagulants, the pharmacogenetics-based dose adjustments showed a good correlation with the genotype-specific empirically derived doses. In addition to its role in pharmacokinetics, CYP2C9 contributes to the metabolism of fatty acids, prostanoids, and steroid hormones, and it may catalyze potentially toxic bioactivation reactions. However, our current understanding of the role of CYP2C9 in biotransformation of endogenous signaling molecules and in drug toxicity is relatively meager. (Clin Pharmacol Ther 2005;77:1-16.)

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The structure and function of the cytochrome P450 (CYP) enzyme 2C9 and its genetic variants have been extensively studied by means of enzymology, clinical pharmacokinetic studies, observational clinical studies measuring drug responses and side effects, and, more recently, protein crystallography.1,2 More than 100 currently used drugs have been identified as substrates of CYP2C9 by biochemical analysis, corresponding to about
10% to 20% of commonly prescribed drugs. However, the clinical consequences of CYP2C9 polymorphisms have been investigated for only some of these drugs. More than 50 single nucleotide polymorphisms (SNPs) have been described in the regulatory and coding regions of the CYP2C9 gene, but only 2 coding variants, termed CYP2C9*2 and CYP2C9*3, with functional consequence are common, having allele frequencies of around 11% (*2) and 7% (*3) in white subjects (Tables I and II). They have significantly lower frequencies, however, in African and Asian populations. Some CYP2C9 alleles were detected in African-derived populations only, such as CYP2C9*5, CYP2C9*6, CYP2C9*8, and CYP2C9*11 (allele frequencies in Africans of 1.8%, <1%, 8%, and 2.7%, respectively). A single African subject with epilepsy was identified as carrying a homozygous deletion of the CYP2C9 gene (CYP2C9*6), indicating that absence of CYP2C9 activity is compatible with life. The allele frequency of this variant was 0.6% in the African population studied, and this allele was not found in Asian or white populations. It remains to be elucidated whether some of the numerous noncoding CYP2C9 polymorphisms can contribute to genotypic prediction of the CYP2C9 metabolic phenotype.

CYP2C9*2 codes for an R144C substitution, whereas CYP2C9*3 reflects an I359L change in the amino acid sequence. The 2 substitutions have never been found on the same chromosome; thus 3 alleles result from these 2 SNPs, CYP2C9*1 coding for R144, I359, and the wild-type allele, CYP2C9*2 coding for C144 and L359, and CYP2C9*3 coding for R144 and Leu359. According to most in vitro data, substrate affinity is not affected substantially by the *2 variant, but the maximum rate of metabolism (Vmax) is reduced to around 50% of that for CYP2C9*1, resulting in a lower intrinsic clearance (Vmax/Km [Michaelis-Menten constant]). Although the amino acid substitution coded by the CYP2C9*3 allele is a conservative amino acid substitution, which should not have major effects on the protein function, catalytic activity of the *3-encoded protein is significantly reduced for most CYP2C9 substrates because of both an increase in Km and a reduction in Vmax.

### Table I. Genotype frequencies of CYP2C9 polymorphisms in Asian, African, and white subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Activity</th>
<th>Population frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>African</td>
</tr>
<tr>
<td>CYP2C9*1/*1</td>
<td>Normal</td>
<td>87.0</td>
</tr>
<tr>
<td>CYP2C9*1/*2</td>
<td>Minor reduction</td>
<td>8.7</td>
</tr>
<tr>
<td>CYP2C9*2/*2</td>
<td>Moderately reduced</td>
<td>0</td>
</tr>
<tr>
<td>CYP2C9*1/*3</td>
<td>Moderately reduced</td>
<td>4.3</td>
</tr>
<tr>
<td>CYP2C9*2/*3</td>
<td>Moderately reduced</td>
<td>0</td>
</tr>
<tr>
<td>CYP2C9*3/*3</td>
<td>Very low</td>
<td>0</td>
</tr>
</tbody>
</table>

Frequency data are derived from references 2, 5, 8, 26, and 73-76.

### Table II. Allele frequencies of CYP2C9 polymorphisms in Asian, African, and white subjects

<table>
<thead>
<tr>
<th>Allele and genotype</th>
<th>Protein variation</th>
<th>African subjects</th>
<th>Asian subjects</th>
<th>White subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C9*2</td>
<td>R144C</td>
<td>4</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>CYP2C9*3</td>
<td>I358L</td>
<td>2</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>CYP2C9*4</td>
<td>I264M</td>
<td>0</td>
<td>？</td>
<td>0</td>
</tr>
<tr>
<td>CYP2C9*5</td>
<td>D360E</td>
<td>1.8</td>
<td>？</td>
<td>0</td>
</tr>
<tr>
<td>CYP2C9*6</td>
<td>Null allele</td>
<td>0.6</td>
<td>？</td>
<td>0</td>
</tr>
<tr>
<td>CYP2C9*11</td>
<td>R335W</td>
<td>2.7</td>
<td>？</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Frequency data are derived from references 2, 5, 8, 26, and 73-76. The question mark indicates that the allele frequency in the respective ethnic group has not yet been studied.
PHARMACOKINETIC DIFFERENCES ASSOCIATED WITH CYP2C9*2 AND *3

As summarized in Table III, we identified 17 drugs for which an effect of the CYP2C9 polymorphisms *2 and *3 has been shown in pharmacokinetic studies. Although homozygous carriers of *2/*2 and *3/*3 show the most pronounced effects, because of the greater number of affected persons, the estimates of pharmacokinetic effects for most drugs are more precise for the heterozygous *1/*2 and *1/*3 carriers. A surrogate parameter for possible clinical effects of the variants may be area under the curve or total clearance, and Fig 1 illustrates how much this parameter differs between carriers of 0, 1, and 2 CYP2C9*2 alleles. In this figure, the drugs are given in the same order as for CYP2C9*3, illustrating that there is only a weak correlation between the CYP2C9*2 and *3 effects. As illustrated, in carriers of the CYP2C9*2/*2 genotype, the total clearance values of S-acenocoumarol and S-warfarin were 21% and 32%, respectively, of the value in CYP2C9*1/*1 carriers, whereas for phenytoin, tolbutamide, ibuprofen, nateglinide, fluvastatin, and phenprocoumon, this clearance was between 68% and 90% (Fig 1). Interestingly, even in heterozygous carriers, losartan clearance was only 58%, but, unfortunately, no clinical pharmacokinetic data for carriers of the homozygous genotype *2/*2 have been published.

For a few drugs such as diclofenac and celecoxib, mean clearances tended to be higher in carriers of the CYP2C9*2 allele than in carriers of CYP2C9*1/*1 (Fig 1). These group differences, however, were not statistically significant and may simply reflect random variation associated with the small sample size of most of the pharmacokinetic studies (Table III). However, because CYP2C9*2 is tightly linked to allele *3 in the related enzyme CYP2C8 and also to CYP2C9 promoter variants, there might be a true effect elevating enzyme activity as a result of linkage with high-activity variants in CYP2C8 or other genes within the CYP2C9 gene locus, which includes the homologous CYP2C8, CYP2C9, CYP2C18, and CYP2C19.
In the case of the CYP2C9*3 allele, larger group differences in pharmacokinetic parameters are observed. For most substrates, even heterozygous carriers of CYP2C9*3, who constitute between 3% and 15% of most populations studied to date, had approximately 50% of the mean clearance compared with that of the wild type for drugs such as S-warfarin, tolbutamide, celecoxib, fluvastatin, phenytoin, glimepiride, candesartan, and tenoxicam. Interestingly, large differences resulting from the CYP2C9*2 allele do not always run in parallel with CYP2C9*3 effects (compare Fig 1 with Fig 2). Whereas both CYP2C9 alleles, *2 and *3, had a significant effect on S-warfarin and acenocoumarol clearance, for drugs such as tolbutamide

<table>
<thead>
<tr>
<th>Table III. Summary of pharmacokinetic consequences of CYP2C9 polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total clearance (L/h)</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Oral anticoagulants</td>
</tr>
<tr>
<td>S-Acenocoumarol</td>
</tr>
<tr>
<td>19.8 (16.3-23.3)</td>
</tr>
<tr>
<td>0.53 (0.2-0.8)§</td>
</tr>
<tr>
<td>0.23 (0-0.4)§</td>
</tr>
<tr>
<td>S-Phenprocoumon</td>
</tr>
<tr>
<td>0.057 (0.045-0.069)</td>
</tr>
<tr>
<td>0.047 (0.037-0.057)</td>
</tr>
<tr>
<td>0.044 (0.032-0.056)</td>
</tr>
<tr>
<td>S-warfarin</td>
</tr>
<tr>
<td>39.6 (34.4-44.8)∥</td>
</tr>
<tr>
<td>22.8 (17.5-28.1)∥</td>
</tr>
<tr>
<td>12.8 (9.2-16.4)∥</td>
</tr>
<tr>
<td>Antidiabetics</td>
</tr>
<tr>
<td>Glimepiride</td>
</tr>
<tr>
<td>4.3 (3.2-5.4)</td>
</tr>
<tr>
<td>4 (3.0-5.0)</td>
</tr>
<tr>
<td>Glyburide</td>
</tr>
<tr>
<td>3.5 (2.8-4.2)</td>
</tr>
<tr>
<td>4.3 (2.7-5.9)</td>
</tr>
<tr>
<td>2.9 (1.8-4.0)</td>
</tr>
<tr>
<td>Tolbutamide</td>
</tr>
<tr>
<td>0.9 (0.8-1.0)</td>
</tr>
<tr>
<td>1 (0.7-1.3)</td>
</tr>
<tr>
<td>0.7 (0.5-0.9)</td>
</tr>
<tr>
<td>0.78 (0.75-0.8)</td>
</tr>
<tr>
<td>0.9 (0.8-1.0)</td>
</tr>
<tr>
<td>0.6 (0.5-0.7)</td>
</tr>
<tr>
<td>0.9 (0.9-0.9)</td>
</tr>
<tr>
<td>0.8 (0.7-0.9)</td>
</tr>
<tr>
<td>0.6</td>
</tr>
<tr>
<td>Nateglinide</td>
</tr>
<tr>
<td>9.1 (7.7-10.5)</td>
</tr>
<tr>
<td>8.4 (6.9-9.9)</td>
</tr>
<tr>
<td>8.1 (4.8-11.4)</td>
</tr>
<tr>
<td>Angiotensin antagonists</td>
</tr>
<tr>
<td>Candesartan</td>
</tr>
<tr>
<td>109 (not given)</td>
</tr>
<tr>
<td>73 (34-112)</td>
</tr>
<tr>
<td>64 (45-83)</td>
</tr>
<tr>
<td>Losartan</td>
</tr>
<tr>
<td>125 (50-200)</td>
</tr>
<tr>
<td>73 (34-112)</td>
</tr>
<tr>
<td>57 (7-107)</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>Celecoxib</td>
</tr>
<tr>
<td>30 (12-48)</td>
</tr>
<tr>
<td>42 (17-67)</td>
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<tr>
<td>48 (24-72)</td>
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<td>38 (32-44)</td>
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<td>43 (37-49)</td>
</tr>
<tr>
<td>32 (21-43)</td>
</tr>
<tr>
<td>45 (38-52)</td>
</tr>
<tr>
<td>45</td>
</tr>
<tr>
<td>Diclofenac</td>
</tr>
<tr>
<td>53 (44-62)</td>
</tr>
<tr>
<td>58 (51-65)</td>
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<td>31 (30-32)</td>
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<td>20 (16-24)</td>
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<td>29 (23-35)</td>
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<td>30 (19-41)</td>
</tr>
<tr>
<td>46 (33-59)</td>
</tr>
<tr>
<td>29 (17-41)</td>
</tr>
<tr>
<td>53</td>
</tr>
<tr>
<td>22 (19-25)</td>
</tr>
<tr>
<td>Flurbiprofen</td>
</tr>
<tr>
<td>1.7 (1.3-2.1)</td>
</tr>
<tr>
<td>1.3 (1.0-1.6)</td>
</tr>
<tr>
<td>3.3 (2.6-4.0)</td>
</tr>
<tr>
<td>3.2 (2.5-3.9)</td>
</tr>
<tr>
<td>3.1 (2.3-3.9)</td>
</tr>
<tr>
<td>5.2 (4.1-6.2)</td>
</tr>
<tr>
<td>4.8 (2.0-7.6)</td>
</tr>
<tr>
<td>2.0 (0.4-5.4)</td>
</tr>
<tr>
<td>Tenoxicam</td>
</tr>
<tr>
<td>0.11 (0.09-0.13)</td>
</tr>
<tr>
<td>0.08 (0.076-0.084)</td>
</tr>
<tr>
<td>Others</td>
</tr>
<tr>
<td>35,5R-Fluvastatin</td>
</tr>
<tr>
<td>88 (42-134)</td>
</tr>
<tr>
<td>95 (79-111)</td>
</tr>
<tr>
<td>70 (41-99)</td>
</tr>
<tr>
<td>Phenytoin</td>
</tr>
<tr>
<td>4.2 (4-4.5)‡</td>
</tr>
<tr>
<td>5.5 (5-6)‡</td>
</tr>
<tr>
<td>6.6 (2-12)‡</td>
</tr>
<tr>
<td>15.8 (2.36)‡</td>
</tr>
<tr>
<td>16.1 (9-32)‡</td>
</tr>
<tr>
<td>14 (11-18)‡</td>
</tr>
<tr>
<td>1.7 (1.4-2.0)</td>
</tr>
<tr>
<td>0.8 (0.6-1.0)</td>
</tr>
<tr>
<td>0.3</td>
</tr>
<tr>
<td>Torsemide (INN, torasemide)</td>
</tr>
<tr>
<td>3.5 (2.8-4.2)</td>
</tr>
<tr>
<td>3.7 (3.0-4.4)</td>
</tr>
<tr>
<td>2.3</td>
</tr>
</tbody>
</table>

If not otherwise marked, data are given as mean and 95% confidence interval. §Sample size in CYP2C9 diplotype groups *1/*1, *1/*2, *2/*2, *1/*3, *2/*3, and *3/*3. †Therapeutically adjusted doses were recorded and then genotyping was performed, which revealed significant dependence of the therapeutically adjusted dose on CYP2C9 genotype. If doses were adjusted according to therapeutic drug monitoring alone, this might be considered redundant; however, we assume that, generally, the effects and adverse effects guided clinical dose adjustment. §Steady-state trough concentrations are given in nanograms per milliliter. The unbound clearance of warfarin is given in this article, which is thus about 100-fold smaller than the total clearance as given in the study by Herman et al.78
and celecoxib, clearance was decreased more in carriers of CYP2C9*3 than in carriers of CYP2C9*2. This might indicate differences in substrate specificity among the 3 enzyme variants encoded by CYP2C9*1, *2, and *3, but, as mentioned, linkage with CYP2C8 might be another explanation.

### NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

At least 16 different registered nonsteroidal anti-inflammatory drugs (NSAIDs) are currently known to be at least partially metabolized via CYP2C9. These include aceclofenac, acetylsalicylic acid, apazone

---

#### Total clearance (L/h)

<table>
<thead>
<tr>
<th></th>
<th>*1/*3</th>
<th>*2/*3</th>
<th>*3/*3</th>
<th>Sample size†</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.9 (7.5-14.3)</td>
<td>3/0/0/0/3/0</td>
<td>0.4 (0-1.1)§</td>
<td>0.053 (0.032-0.074)</td>
<td>170/45/0/32/9/0</td>
<td>50</td>
</tr>
<tr>
<td>0.11 (0.09-0.13)</td>
<td>118/32/2/27/6/3</td>
<td>0.06 (0.05-0.07)</td>
<td>0.045 (0.033-0.057)</td>
<td>7/4/3/4/5/3</td>
<td>77</td>
</tr>
<tr>
<td>20.7 (15.3-26.1)</td>
<td>54/15/2/16/4/2</td>
<td>2 (0-5.3)§</td>
<td>0.038 (0.02-0.056)</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>1.6</td>
<td>1.6</td>
<td>2.8</td>
<td>2.5 (2-2.6)</td>
<td>4/4/3/3/4/3</td>
<td>80</td>
</tr>
<tr>
<td>0.5 (0.4-0.6)</td>
<td>0.038 (0.02-0.056)</td>
<td>0.045 (0.033-0.057)</td>
<td>7/4/3/4/5/3</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>0.6 (0.5-0.7)</td>
<td>6/4/3/4/3</td>
<td>0.5 (0-0.5)</td>
<td>12/00/6/0/0</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>0.5 (0.5-0.5)</td>
<td>5/5/0/0/5/0</td>
<td>0.053 (0.032-0.074)</td>
<td>7/4/3/4/5/3</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>6.9 (5.7-8.1)</td>
<td>15/7/13/0/0</td>
<td>5.8 (4.1-7.5)</td>
<td>3.9 (3.6-4.2)</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>52.6</td>
<td>6/0/0/1/0/0</td>
<td>110 (35-185)</td>
<td>5/5/0/0/5/0</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>87 (18-156)</td>
<td>54 (22-86)</td>
<td>39</td>
<td>6/3/3/4/5/1</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>25 (15-35)</td>
<td>43 (37-49)</td>
<td>15</td>
<td>10/6/2/0/4/1</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>70 (53-87)</td>
<td>76</td>
<td>12/20/0/2/1</td>
<td>86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22 (15-29)</td>
<td>23 (18-28)</td>
<td>10/6/2/0/4/1</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31 (14-48)</td>
<td>63</td>
<td>6/3/1/4/5/1</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 (22-38)</td>
<td>6/0/0/0/6/0</td>
<td>87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (0-9-1-1)</td>
<td>11/4/0/5/0/0</td>
<td>88</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (1-3-2.7)</td>
<td>2.4 (1.8-3.0)</td>
<td>1.5 (1.0-2.0)</td>
<td>4/4/3/3/4/3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>3.6 (1-4.5-4)</td>
<td>1.2 (0.1-1.9)</td>
<td>0.9 (0.4-1.8)</td>
<td>69/34/4/11/7/5</td>
<td>89</td>
<td></td>
</tr>
<tr>
<td>0.06 (0.058-0.062)</td>
<td>11/4/0/5/0/0</td>
<td>76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.7 (5-6)</td>
<td>5.9</td>
<td>68/13/3/0/16/1</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13.8 (5-18)</td>
<td>20.4 (13-28)</td>
<td>37/9/3/9/2/0</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7 (0-1.1-3)</td>
<td>0.2</td>
<td>18/7/14/1/0</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2 (1-2.5)</td>
<td>1.9 (1-7-2.1)</td>
<td>1.2 (1-1.6-1.24)</td>
<td>12/9/1/9/3/2</td>
<td>91a</td>
<td></td>
</tr>
</tbody>
</table>
(INN, azapropazone), celecoxib, diclofenac, flurbiprofen, ibuprofen, indomethacin (INN, indometacin), lornoxicam, mefenamic acid, meloxicam, naproxen, phe-nylbutazone, piroxicam, suprofen, and tenoxicam. Thus far, the relative contribution of CYP2C9 to pharmacokinetic differences has been studied in only a few cases (Table III). The pharmacokinetics of diclofenac was found to be independent of CYP2C9 polymorphisms in several clinical studies, and, correspondingly, diclofenac-induced hepatitis did not appear to be related to CYP2C9 polymorphism (Table IV). However, there were significant intergenotypic differences in the pharmacokinetics of celecoxib, flurbiprofen, ibuprofen, and tenoxicam (Table III), and these were reflected in the duration of cyclooxygenase (COX) 1 and COX-2 inhibition. Gastritis, peptic ulcer, and minor or major upper intestinal bleeding, analgesic nephropathy, and fluid retention are typical dose-related adverse events of NSAIDs. Recently, the frequency of CYP2C9 polymorphisms was reported to be higher in patients with acute gastric bleeding complications after NSAID use (Table IV). In addition, allergic skin reaction and other idiosyncratic reactions also have to be considered as adverse effects of NSAIDs, but there is no current information regarding involvement of CYP2C9 variants.

**ANTIDIABETIC DRUGS**

As illustrated in Figs 1 and 2, the consequences of CYP2C9 on the pharmacokinetics of the oral antidiabetic drugs glyburide (INN, glibenclamide), glimepiride, glipizide, and tolbutamide were similar, in that oral clearance in carriers of the CYP2C9*1/*3 genotype was approximately 50% of that in CYP2C9*1/*1 homozygotes and the clearance in *3/*3 homozygotes was only 20% of that in wild-type individuals (Table III and Fig 2). With the newer antidiabetic drug nateglinide, the genotype-associated differences were smaller (Table III), and, to our knowledge, there are no published clinical studies on possible pharmacokinetic differences in the peroxisome proliferator–activated receptor γ agonists troglitazone, pioglitazone, and rosiglitazone. The latter drugs may be substrates of CYP2C9 on the basis of in vitro data, but they are also metabolized by the closely related and genetically linked CYP2C8 enzyme. The clinical studies with oral antidiabetic drugs were generally performed in healthy volunteers, and CYP2C9 polymorphism had little or no effect on glucose regulation. However, in healthy volunteers studied after a single dose, extensive counterregulatory responses may exist, and it is reasonable to postulate that, as a result of the prolonged action of oral antidiabetics, slow-metabolizer patients may be at higher risk for hypoglycemia. Indeed, a recent analysis of 20 patients hospitalized for severe hypoglycemia found 2 carriers of the CYP2C9 genotypes CYP2C9*2/*3 and *3/*3, which corresponded to an incidence of 10%, in contrast to an expected rate of less than 2%. Although these data are preliminary, they should stimulate further clinical studies in patients.

**ANGIOTENSIN I RECEPTOR ANTAGONISTS**

Losartan is metabolized via CYP2C9 to the active and long-acting metabolite E-3174, and less stable blood pressure reduction in carriers of the slow-metabolizer CYP2C9 genotypes might be anticipated, particularly in the *3/*3 genotype. Whether this is clinically relevant has not yet been shown, but the administration of phenytoin, a CYP2C9 substrate, reduced the formation of the active E-3174 metabolite by 63%. This might be due to induced metabolism of E-3174 by other CYPs, because phenytoin is a strong inductor of, for example, CYP3A enzymes, or it might be due to competitive substrate inhibition of losartan and phenytoin at CYP2C9. For irbesartan, Hallberg et al found an enhanced blood pressure reduction in carriers of the *1/*2 and *1/*3 genotypes compared with carriers of the *1/*1 genotype. For candesartan, 1 carrier of the slow-metabolizing *1/*3 genotype had an excessive blood pressure reduction, but a comprehensive pharmacokinetic-pharmacodynamic evaluation of the effect of CYP2C9 polymorphisms in patients receiving candesartan and irbesartan still remains to be performed.

**VITAMIN K ANTAGONISTS**

The 3 vitamin K antagonists acenocoumarol, phenprocoumon, and warfarin are currently used as oral anticoagulants. The S-enantiomers of all 3 drugs are substrates of CYP2C9. For warfarin, as well as phenprocoumon, the S-enantiomers are predominantly responsible for the anticoagulant effect. In contrast, the pharmacologic activities of S- and R-acenocoumarol are comparable, and as a result of slower elimination of the S-enantiomer, R-acenocoumarol is largely responsible for the overall anticoagulant response. Warfarin is mainly prescribed in North America and Asia, whereas acenocoumarol and phenprocoumon are more commonly used in Europe. As illustrated in Figs 2 and 3, both CYP2C9 alleles, CYP2C9*2 and *3, had
### Table IV. Clinical outcomes in patients according to CYP2C9 genotype

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Frequency of adverse events in genotypes at risk (%)</th>
<th>Frequency of adverse events in genotypes not at risk (%)</th>
<th>Significance</th>
<th>Genotype at risk</th>
<th>Sample size: Genotype at risk/remaining genotypes (wild type)</th>
<th>Effect of polymorphisms on effects and clinical end points</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oral anticoagulants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>311/685 Major bleeding complication after 460 days of treatment</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>S-Acenocoumarol</td>
<td>6.8</td>
<td>4.5</td>
<td>Hazard ratio of 1.6</td>
<td>All variant CYP2C9 genotypes</td>
<td>41/181 INR &gt; 4.5</td>
<td>55</td>
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<td></td>
<td>44</td>
<td>12</td>
<td>(P &lt; .001)</td>
<td>(CYP2C9^*3) carriers</td>
<td>46/146 INR &gt; 6.0</td>
<td>57</td>
<td></td>
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<tr>
<td></td>
<td>22</td>
<td>6</td>
<td>(P = .004)</td>
<td>(CYP2C9^*3) carriers</td>
<td>31/51 INR &gt; 4</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>47</td>
<td>NS</td>
<td>All variant CYP2C9 genotypes</td>
<td>70/134 INR &gt; 6</td>
<td>54</td>
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<tr>
<td>S-Phenprocoumon</td>
<td>16</td>
<td>16</td>
<td>NS</td>
<td>All variant CYP2C9 genotypes</td>
<td>15/164 Bleeding complications</td>
<td>60</td>
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<tr>
<td></td>
<td>40</td>
<td>18</td>
<td>(P = .048)</td>
<td>(CYP2C9^*3) carriers</td>
<td>60/187 INR &gt; 6</td>
<td>60</td>
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<td></td>
<td>26.2</td>
<td>9.1</td>
<td>(P = .001)</td>
<td>Any *2 allele carriers</td>
<td>37/187 INR &gt; 6</td>
<td>60</td>
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<tr>
<td></td>
<td>21.6</td>
<td>9.1</td>
<td>(P = .041)</td>
<td>Any *3 allele carriers</td>
<td>31/42 At least 1 asymptomatic INR &gt; 6.0 per 100 patient-years</td>
<td>92</td>
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<td>S-warfarin</td>
<td>14.7</td>
<td>4.5</td>
<td>NS</td>
<td>All variant CYP2C9 genotypes</td>
<td>58/127 Bleeding event</td>
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<td>24</td>
<td>11</td>
<td>(P = .02)</td>
<td>All variant CYP2C9 genotypes</td>
<td>54/75 INR &gt; 3</td>
<td>93</td>
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<tr>
<td></td>
<td>65</td>
<td>33</td>
<td>(P = .01)</td>
<td>All variant CYP2C9 genotypes</td>
<td>169/392 INR &gt; 8</td>
<td>41</td>
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<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>NS</td>
<td>All variant CYP2C9 genotypes</td>
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<td>Angiotensin antagonists</td>
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<td>Candesartan</td>
<td>12</td>
<td>Not given</td>
<td>NS</td>
<td>(CYP2C9^*1/*3)</td>
<td>Change in diastolic blood pressure after 12 weeks</td>
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<td>Irbesartan</td>
<td>14</td>
<td>8</td>
<td>(P = .04)</td>
<td>(CYP2C9^*1/*2)</td>
<td>Extensive blood pressure reduction in the interaction with comorbidity and comedication</td>
<td>30</td>
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<td>NSAIDs</td>
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<td></td>
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<tr>
<td>Diclofenac</td>
<td>16</td>
<td>22</td>
<td>NS</td>
<td>All variant CYP2C9 genotypes</td>
<td>44/77 Diclofenac-induced liver toxicity</td>
<td>18</td>
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<tr>
<td>Other NSAIDs</td>
<td>51</td>
<td>37</td>
<td>(P = .02)</td>
<td>All variant CYP2C9 genotypes</td>
<td>101/117 Acute gastrointestinal bleeding</td>
<td>20</td>
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<tr>
<td>Other Phenytoin</td>
<td>33</td>
<td>64</td>
<td>NS</td>
<td>(CYP2C9^*3) carriers</td>
<td>3/25 Gingival overgrowth</td>
<td>94</td>
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</table>

INR, International normalized ratio; NS, not significant; NSAID, nonsteroidal anti-inflammatory drug.
a substantial effect on the intrinsic clearance of $S$-warfarin. 31-34

Most clinical studies with warfarin that have been performed in patients have assessed differences in mean daily dose or international normalized ratio (INR) in relation to CYP2C9 genotypes. If differences in pharmacokinetic parameters of warfarin resulting from the CYP2C9 genotype are compared with differences in empirically obtained doses in patients, the differences in clearance lead to a larger reduction in calculated dose than that obtained from INR (Fig 3). In Fig 3 the weighted means of the results of several studies performed in patients that have assessed the daily doses of warfarin adjusted according to INR are depicted and compared with the pharmacokinetic data from Scordo et al. 33

As illustrated with the example of warfarin, the pharmacogenetics-based reduced doses in carriers of *1/*2 and *2/*2 would be 80% and 32% of the standard dose, respectively, according to pharmacokinetic studies and 87% and 82%, respectively, according to clinical INR-based dose adjustment (which was blinded with regard to CYP2C9 genotype). The corresponding doses in carriers of *1/*3, *2/*3, and *3/*3 would be 56%, 23%, and 9%, respectively, according to pharmacokinetics and 68%, 57%, and 33%, respectively, according to the clinical effects. Thus the concept of deriving genotype-based dose adjustments from pharmacokinetic studies is confirmed by the empiric clinical data. However, the empirically obtained doses in the slow metabolizer genotypes were significantly higher than the dose adjustments that one might recommend on the basis of the pharmacokinetic differences between the CYP2C9 genotypes (Fig 3). This discrepancy may be explained by the fact that the $R$-enantiomer, which is affected less or not at all by CYP2C9 polymorphisms, contributes to some extent to the anticoagulant activity. 48

Patients with reduced clearance of oral anticoagulants are more susceptible to adverse bleeding events. Especially during initiation of warfarin treatment, the dose an individual requires for an optimal response is unknown. A higher rate of major bleeding complications during initiation of warfarin therapy and longer hospitalization times have been re-
ported in patients who were CYP2C9*2 or *3 carriers.\textsuperscript{36,46} Furthermore, patients carrying 1 or 2 variant CYP2C9 alleles had a higher incidence of supratherapeutic INR values (>4), required a longer time to achieve a stable dose, and had a higher rate of serious or life-threatening bleeding events (Table IV). In contrast to the exaggerated problems of finding the right dose in carriers of slow CYP2C9 metabolic genotypes at initiation of anticoagulant therapy, no difference in the likelihood of severe overanticoagulation or antithrombotic instability was detected during long-term treatment with warfarin.\textsuperscript{41}

In Asian populations, larger differences in the oral clearance of S-warfarin have been reported in CYP2C9*1/*3 heterozygotes compared with studies performed in white subjects (63% reduced oral clearance in Asian CYP2C9*1/*3 carriers versus no difference in white subjects and in 1 Asian CYP2C9*3/*3 individual, with only 10% of the clearance in wild-type carriers). Furthermore, Japanese patients with the homozygous wild-type genotype had a higher oral clearance of S-warfarin compared with that in white subjects even after normalization for body weight.\textsuperscript{31,32,37} A number of SNPs in the −2.1-kb 5′-flanking region have been described and found to be in linkage disequilibrium with common functional SNPs in the coding region,\textsuperscript{49,50} and differences in haplotypes between different ethnic groups might contribute to these differences in CYP2C9 activity in Asians versus white subjects. Therefore it might be more appropriate to perform haplotype analyses rather than single SNP genotyping. However, at present, there is no explanation for the ethnic differences, and further study is required. Such studies will need to include polymorphisms in other genes mediating the anticoagulant effect, as well as environmental determinants such as nutrition and other lifestyle factors, age, body weight, and comediations, all of which might contribute to ethnic variability in the pharmacokinetic and anticoagulant response of warfarin.

For acenocoumarol, there are pharmacokinetic differences associated with the CYP2C9*3 allele and differences in empirically obtained daily doses. Heterozygous carriers of CYP2C9*3 had only 40% of the clearance measured in CYP2C9*1/*1 carriers, whereas differences in INR values between these 2 groups resulted in a mean daily dose that was 70% that of wild-type patients in *1/*3 carriers.\textsuperscript{51-55} A higher risk
of bleeding complications was described in carriers of CYP2C9 variants, and a higher percentage had overt-anticoagulation with INR values greater than 6, particularly CYP2C9*3 carriers. However, CYP2C9 polymorphisms play a smaller role in acenocoumarol than in warfarin therapy, because the S-enantiomer is only the minor compound and R-acenocoumarol is metabolized by several CYPs, including CYP1A2, CYP2C19, and CYP3A4.

Only very small differences attributable to CYP2C9 genotype were detected in the pharmacokinetics of phenprocoumon, and one study found no effect of CYP2C9 polymorphisms on the clinical dose requirements. Two other studies, however, found an approximately 2- to 3-fold increased bleeding risk or risk of severe overanticoagulation in carriers of CYP2C9*2 and *3 alleles.

CYP2C9 polymorphisms, together with variants at the pharmacodynamic sites, such as coagulation factor polymorphisms and the polymorphisms in vitamin K epoxide reductase (VKORC1), might explain variability in the overall pharmacodynamic responses to oral anticoagulants. However, the question of whether genotyping really improves anticoagulant drug therapy by preventing overanticoagulation and minimizing the risk for bleeding complications has not yet been answered. Sufficiently powerful randomized controlled clinical trials in patients will have to be performed to evaluate the clinical benefit of using an individual's CYP2C9 genotype alone or in combination with the VKORC1 genotype for treatment optimization based on pharmacokinetic dose adjustments and empiric clinical data. Treatment with oral anticoagulants that antagonize vitamin K is one area where the concept of pharmacogenetics-based dose adjustments could become clinical reality.

DISCUSSION

Differences in pharmacokinetic parameters such as oral clearance could be overcome by adjustment of the drug dose. In Figs 2 and 3 the differences in the oral clearance of drugs that have been studied in humans with regard to CYP2C9 polymorphisms are depicted. However, the potential clinical benefit of dose adjustment according to genotype differences in pharmacokinetics is not evident. The differences in the drug effects between the genotype groups are almost always smaller than the pharmacokinetic differences, and minor dose adjustments are neither necessary nor clinically helpful. For some drugs, plasma concentrations correlate poorly with clinical efficacy, and empiric dose-finding rather than drug concentrations is used to avoid adverse drug effects.

Thus the list of drugs for which CYP2C9 genotype-specific dose reductions may be clinically useful has been restricted to those with a clear relationship between genotype and effect. At the moment, this is probably the case only for warfarin. For phenytoin, the routine use of therapeutic drug monitoring would preclude the need for genetic information, except possibly as confirmation of a very low dose requirement. Genetically predicted dose adjustments for phenytoin would lead to 37% of the dose in homozygous carriers of CYP2C9*2 or *3 compared with wild-type carriers. However, doses obtained on the basis of blood concentration measurements of phenytoin did not differ in carriers of the CYP2C9*2 allele and were only moderately reduced in CYP2C9*3 carriers (77% in homozygous carriers).

For NSAIDs, the incidence and severity of fluid and sodium retention and other renal adverse events may be related to CYP2C9 genotype. However, such a correlation has not been demonstrated in clinical studies. A relationship between CYP2C9 genotype and upper gastrointestinal bleeding was shown previously and might suggest that such drugs should be avoided in carriers of the CYP2C9 slow-metabolic genotypes, but this suggestion requires further prospective evaluation before one can judge its clinical utility. Also, with regard to therapy with oral antidiabetics, further prospective studies are needed, and, at the moment, routine CYP2C9 genotyping is not evidence-based.

CLINICAL UTILITY OF CYP2C9 POLYMORPHISMS

Study design considerations. In our exploration of the value of CYP2C9 genotyping as a therapeutically valuable diagnostic test in clinical practice, the different levels of evidence may be summarized as follows. First, there is no doubt that genotyping for CYP2C9*2, CYP2C9*3, and possibly other alleles predicts slow biotransformation of numerous drugs. This is important information that could be useful with regard to therapeutic decisions and has to be taken into consideration together with information about drug interactions occurring as a result of enzyme and transporter inhibition and induction. However, the major question about the relevance of this pharmacokinetic variability for clinical end points remains to be unequivocally answered. Different types of studies confer different levels of
Clinical consequences of \textit{CYP2C9} polymorphisms

Evidence. At the lowest level, case reports of patients with severe adverse events or greatly enhanced effects and carrying \textit{CYP2C9} variant genotypes offer important clues; however, such reports do not prove that a clinical meaningful relationship exists in the general patient population. Case-control studies are more informative. However, a major problem is selection bias; in the worst situation the most severe cases of adverse events or exaggerated effects resulting from CYP2C9 poor metabolism may escape detection because patients may have died or may have been switched to alternative therapies before inclusion in the study. This problem is solved by a cohort study design, in which patients are prospectively included in the study and all patients are followed up with regard to surrogate parameters and clinical end points. However, cohort studies also may yield erroneous conclusions, as was recently learned from studies about postmenopausal estrogen replacement therapy. The value of pre-prescription CYP2C9 genotyping will have to be proved in prospective randomized clinical trials in which one group of patients is treated with consideration of genotype information and compared with another group in which physicians do not have genotype information and conventional empiric therapy is used. The design of such a study should be similar to that supporting the introduction of an alternative diagnostic test.\textsuperscript{64} Another major problem is that the really functionally important slow-metabolic genotype (\textit{CYP2C9*3/*3}) has a prevalence of only 0.5% or even lower in most populations. Thus genotyping large numbers of patients to identify the small number with a markedly increased risk of adverse effects will not be cost-effective unless the toxicity is severe and not detected by standard clinical monitoring.

The cost-benefit aspect of pre-prescription genotyping is currently not evident for CYP2C9. In other fields of drug therapy, preliminary estimations of possible cost-benefit relationships have been published, and in a study on CYP2D6, the genotypes causing ultrarapid or poor metabolism were found to result in up to $4000 to $6000 more in health care costs per year.\textsuperscript{65} With regard to CYP2C9 substrates, similar analyses would be valuable for the oral anticoagulants, oral antidiabetic drugs, and NSAIDs.

\textbf{Role of \textit{CYP2C9} in idiosyncratic drug reactions and in biotransformation of physiologic substances.} Numerous substrates of \textit{CYP2C9} are known to cause severe toxic and immunologic adverse drug reactions. Hepatotoxicity is a well-known adverse effect of the CYP2C9 substrates diclofenac, leflunomide, troglitazone, and valproic acid. Recently, a case of severe leflunomide-induced hepatotoxicity was described in a carrier of the rare \textit{CYP2C9*3/*3} genotype.\textsuperscript{66} However, the relationship between leflunomide hepatotoxicity and \textit{CYP2C9} genotype has not been systematically studied, and the single case report does not prove any causal relationship between toxicity and \textit{CYP2C9}. Severe hemotoxicity and adverse skin drug reactions are clinically relevant problems in treatment with sulfamethoxazole and other sulfonamides, phenytoin, and NSAIDs including the newer COX-2–selective agents. All of these drugs are substrates of \textit{CYP2C9}, and many are also inhibitors of the enzyme. Prominent inhibitors of \textit{CYP2C9} include chloramphenicol and sulfonamides such as sulfaphenazole, sulfamethazine (INN, sulfadimidine), and sulfamethoxazole.\textsuperscript{13}

It is not yet known whether variations in \textit{CYP2C9} activity contribute to adverse drug reactions. One possible mechanism might involve generation of reactive metabolites from the drugs, and there may be a difference depending on \textit{CYP2C9} genotype. Alternatively, inhibition of \textit{CYP2C9} activity might play an important role, because \textit{CYP2C9} may be involved in the biosynthesis of mediators of inflammation and angiogenesis, blood pressure, and regulation of renal functions.\textsuperscript{67} Among several enzymes tested, \textit{CYP2C9} had the highest activity in epoxidation of linoleic acid to 9,10-epoxy-12-octadecenoate and 12,13-epoxy-9-octadecenoate.\textsuperscript{68} These so-called leukotoxins play an important role in many inflammatory reactions, acute respiratory distress syndrome, and in reperfusion injury after myocardial infarction.\textsuperscript{69} In addition, such epoxyeicosatrienoic acids induce angiogenesis.\textsuperscript{70} However, the relative importance of \textit{CYP2C9} with regard to these reactions in humans is not clear, and other enzymes such as \textit{CYP2C8} and \textit{CYP2J2} may be even more important. Data have been derived from inhibition experiments with chloramphenicol or sulfamethoxazole, but there are no data studying the role of the specific \textit{CYP2C9} genotypes.

\textit{CYP2C9} is also capable of metabolizing arachidonic acid to 14\textit{R,15S}–epoxyeicosatrienoic acid (EET) and 11,12-EET. Whereas these reactions can also be catalyzed by \textit{CYP2C8}, 8,9-EET appears to be a \textit{CYP2C9}-specific metabolite.\textsuperscript{68} Thus apparently dual functions of \textit{CYP2C9} exist in the cardiovascular system and in the kidneys. On the one hand, the enzyme metabolizes cardiovascular and anti-inflammatory drugs such as NSAIDs and angiotensin receptor antagonists and, on the other hand, \textit{CYP2C9} is involved in biotransformation of vasoactive leukotriene and arachidonic acid derivatives. Thus the impact of the \textit{CYP2C9*2} and \textit{*3} polymorphisms may
extend beyond a role in drug metabolism and may influence physiologic processes. In addition, the impact of CYP2C9 polymorphisms on idiosyncratic toxic or immune-mediated adverse drug reactions and on the metabolism of physiologic signaling molecules is incompletely understood but is an exciting area for future studies.

CONCLUSIONS

On the basis of pharmacokinetic data and clinical data on drug effects, CYP2C9 genotype–based dose recommendations can be derived for warfarin, acenocoumarol, and phenytoin. As in most other areas of pharmacogenetics, the concept of therapy based on genotype has not been evaluated prospectively in a randomized controlled clinical trial in which one arm was dosed according to genotype and the other treated as usual. In the current era of evidence-based medicine and with the increasing consideration of pharmacoeconomics in medicine, it is unlikely that CYP2C9 genotyping will become routine clinical practice unless its value is demonstrated by such rigorous evaluations. This view is shared by other investigators.71,72 Such studies may, however, be justified because many of the CYP2C9-metabolized drugs such as hypoglycemic agents, anti-inflammatories, and, in particular, anticoagulants have a narrow therapeutic index.

None of the authors has any conflict of interest.

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PHARMACOKINETICS AND
DRUG DISPOSITION

Different effects of three transporting
inhibitors, verapamil, cimetidine, and
probenecid, on fexofenadine
pharmacokinetics

**Objective:** Fexofenadine is a substrate of P-glycoprotein and organic anion transporting polypeptides. The aim of this study was to compare the inhibitory effects of different transporting inhibitors on fexofenadine pharmacokinetics.

**Methods:** Twelve male volunteers took a single oral 120-mg dose of fexofenadine. Thereafter three 6-day courses of either 240 mg verapamil, an inhibitor of P-glycoprotein, 800 mg cimetidine, an inhibitor of organic cation transporters, or 2000 mg probenecid, an inhibitor of organic anion transporting polypeptides, were administered on a daily basis in a randomized fashion with the same dose of fexofenadine on day 6. Plasma and urine concentrations of fexofenadine were monitored up to 48 hours after dosing.

**Results:** Verapamil treatment significantly increased the peak plasma concentration by 2.9-fold (95% confidence interval [CI], 2.4- to 4.0-fold) and the area under the plasma concentration–time curve from time 0 to infinity [AUC(0-∞)] of fexofenadine by 2.5-fold (95% CI, 2.0- to 3.3-fold). No changes in any plasma pharmacokinetic parameters of fexofenadine were found during cimetidine treatment. AUC(0-∞) was slightly but significantly increased during probenecid treatment by 1.5-fold (95% CI, 1.1- to 2.4-fold). Renal clearance of fexofenadine was significantly decreased during cimetidine treatment to 61% (95% CI, 50%-98%) and during probenecid treatment to 27% (95% CI, 20%-58%) but not during verapamil treatment.

**Conclusion:** This study suggests that verapamil increases fexofenadine exposure probably because of an increase in bioavailability through P-glycoprotein inhibition and that probenecid slightly increases the area under the plasma concentration–time curve of fexofenadine as a result of a pronounced reduction in renal clearance. However, it may be difficult to explain these interactions by simple inhibitory mechanisms on target transporters. (Clin Pharmacol Ther 2005;77:17-23.)

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Recently, it has become increasingly evident that drug transporters have a pivotal role in pharmacokinetics of numerous drugs with therapeutic implications.1-6 Numerous studies have revealed that targeted expression of drug uptake and efflux transport to specific cell membrane domains allows for the efficient directional movement of many drugs in clinical use.1-6 Transport by adenosine triphosphate–dependent efflux pumps, such as P-glycoprotein and multidrug resistance–related proteins, influences the intestinal absorption2,8
and renal or hepatic elimination and central nervous system concentrations of many drugs. Members of the organic anion transporting polypeptides (OATPs) and organic cation transporter (OCT) families of drug uptake transporters have been found to be capable of transporting a large array of structurally divergent drugs. OATPs are expressed in the liver, kidney, brain, and intestine and OCTs are expressed in the liver and kidney, suggesting that they may play a critical role in drug interaction, as well as in drug absorption, elimination, and tissue penetration.

Fexofenadine, an active metabolite of terfenadine, is a selective histamine receptor antagonist and is clinically effective in the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria for first-line therapeutic agents like loratadine and cetirizine. In clinical trials fexofenadine did not prolong the QT interval or decrease heart rate, unlike terfenadine, astemizole, and ebastine. Fexofenadine is regarded as a substrate of P-glycoprotein and OATP-A and OATP-B on the basis of several in vitro studies.

Drug-drug and drug-food interaction reports have shown that rifampin (INN, rifampicin), St John’s wort, fruit juice, and verapamil influence alternation of fexofenadine disposition. Because fexofenadine is not metabolized, these interactions are explained by modulation of P-glycoprotein and OATPs. However, there are no published data suggesting an in vivo contribution of these transporters to the disposition of fexofenadine.

Verapamil, a short-term inhibitor of mainly P-glycoprotein, has been used to increase the therapeutic effectiveness of cytotoxic anticancer drugs in cancer chemotherapy. More recently, P-glycoprotein reversal agents including verapamil have been demonstrated to alter the pharmacokinetic properties of coadministered agents in therapeutic areas other than oncology. Meanwhile, to identify the renal secretion pathway for a particular drug, in vivo pharmacokinetic drug interaction studies are usually conducted with inhibitors such as cimetidine and probenecid. Because cimetidine is known to compete for active tubular secretion primarily with basic drugs, this drug is regarded as an efficient inhibitor of OCT1 and OCT2. In addition, cimetidine has recently been identified as a potent inhibitor of the OATP-C–mediated transport of organic anions. In contrast, probenecid has been known not only as a direct inhibitor of glucuronide conjugation but also as a potent inhibitor of OATPs, because probenecid competitively inhibited the secretion of many weak organic acids.

The aim of this study was to compare the inhibitory effects of different transporting inhibitors, verapamil, cimetidine, and probenecid, on fexofenadine disposition. Renal clearance was also examined to clarify the effect of these transporters on excretion of fexofenadine in the kidney. The results suggest that there is an in vivo contribution of these transporters to the disposition of fexofenadine.

METHODS

Subjects. Twelve healthy Japanese male volunteers were enrolled in this study. The mean (±SD) age was 25.2 ± 5.6 years (range, 20-40 years), and the mean body weight was 60.9 ± 6.2 kg (range, 54-80 kg). The Ethics Committee of Hirosaki University School of Medicine, Hirosaki, Japan, approved the study protocol, and written informed consent had been obtained from each participant before any examinations.

Study design. The volunteers took a single oral 120-mg dose of fexofenadine (Allegra; Aventis Pharma Co, Tokyo, Japan) at 9 AM with 240 mL of tap water as a control phase. Thereafter, a randomized crossover study design in 3 treatment phases was conducted at intervals of 2 weeks. Verapamil (two 40-mg tablets) 3 times daily (240 mg/d) (Vasolan; Eisai Pharmaceutical Co, Ltd, Tokyo, Japan), cimetidine (two 200-mg tablets) twice daily (800 mg/d) (Tagamet; Sumitomo Pharmaceutical Co, Ltd, Osaka, Japan), or probenecid (four 250-mg tablets) twice daily (2000 mg/d) (Benecid; Kaken Pharmaceutical Co, Ltd, Tokyo, Japan) with 240 mL of tap water was given for 6 days. Four volunteers within each group were allocated to 1 of 3 different drug sequences as follows: verapamil-cimetidine-probenecid, probenecid-verapamil-cimetidine, or cimetidine-probenecid-verapamil. On day 6, they took a single oral 120-mg dose of fexofenadine with 240 mL of tap water (9 AM) 1 hour after the last 80-mg dose of verapamil, 400-mg dose of cimetidine, or 1000-mg dose of probenecid after overnight fasting (9 AM) with 240 mL of tap water. Treatment medication was not taken after oral administration of fexofenadine. Compliance was confirmed by pill count. No other medications were taken during the study periods. No meal was allowed until 4 hours after dosing (1 AM). No volunteers ingested any fruit juice until at least 8 hours after dosing. The use of alcohol, tea, coffee, and cola was forbidden during the test days.

Sample collections. Blood samplings (10 mL each) for determination of fexofenadine were taken into heparinized tubes just before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after the administration of fexofenadine. Urine samples were collected from 0 to 4 hours, 4 to 12 hours, 12 to 24 hours, and 24 to 48 hours after dosing. Plasma was separated immediately and kept at −30°C until analysis. A part of the urine sample
(30 mL) was stored at −30°C until analysis. The remaining urine samples were discarded after the urine volume was recorded.

Assay. Plasma and urine concentrations of fexofenadine were quantitated by use of an HPLC method developed in our laboratory. In brief, 10 μL of 10-μg/mL diphenhydramine (internal standard) diluted with 1 mL of acetate buffer (0.2 mol/L, pH 4.0) was added to samples (1 mL plasma or 0.1 mL urine). Sample purification was performed by solid-phase extraction on C18 minicolumns (BondElut C18, 3 mL, 500 mg packing; Varian, Palo Alto, Calif). The cartridges were preconditioned with 2 mL of methanol, 2 mL of water, and 1.5 mL of acetate buffer (0.2 mol/L, pH 4.0). After sample load, the cartridges were washed with water (2 mL), methanol-water (50:50 [vol/vol]) (2 mL), and methanol (1 mL). After the cartridges were dried, fexofenadine and internal standard were eluted with 50-mmol/L triethylamine in methanol (1 mL). The eluates were dried with airflow, and the residue dissolved in 10 to 300 μL of eluent A was injected into an HPLC system (Shimadzu Class-VP; Shimadzu Co, Kyoto, Japan). The mobile phases were as follows: 0.05-mol/L monobasic potassium phosphate buffer/acetonitrile/methanol (60:35:10 [vol/vol/vol]) (A) and 0.05-mol/L monobasic potassium phosphate buffer/acetonitrile (40:60 [vol/vol]) (B). Chromatographic separation was achieved on an octadecylsilane-80A column (internal diameter, 150 × 4.6 mm; particle size, 5 μm) by use of a linear gradient from A to B in 10 minutes. The peak was detected with a fluorescence detector set at an excitation wavelength of 220 nm and an emission wavelength of 290 nm, and the total time for a chromatographic separation was approximately 17 minutes. The validated quantitation ranges of this method were 1.0 to 500 ng/mL, with coefficients of variation of 0.6% to 6%. Mean recoveries were 72.8% to 76.7%, with coefficients of variation of 0.6% to 9.1%. Mean recoveries were 72.8% to 76.7%, with coefficients of variation of 0.6% to 9.1%. Free fraction was separated from plasma by use of an ultrafiltration technique (Ultracent; Tosou Co, Tokyo, Japan).

Data analyses of pharmacokinetics. The peak concentration (Cmax) and concentration peak time (tmax) were obtained directly from the original data. The terminal elimination rate constant (kE) was determined by log-linear regression of the final data points (n = 4). The apparent elimination half-life of the log-linear phase (t1/2) was calculated as follows: t1/2 = 0.693/ke. The area under the plasma concentration–time curve (AUC) from 0 to 48 hours [AUC(0-48)] was calculated with use of the linear-linear trapezoidal rule. The AUC from time 0 to infinity [AUC(0-∞)] and elimination half-life were determined by a noncompartmental model with WinNonlin software (Pharsight Corporation, Cary, NC). Residual area was about 0.5% to 6%, and the Console Calculator was used. The amount of drug excreted in urine (Ae) was calculated by volume of urine volume and urine concentration of fexofenadine. Renal clearance was obtained from Ae(0-48)/Free AUC(0-48).

Statistical analyses. Repeated-measures ANOVA was used for comparisons of pharmacokinetic parameters between the control phase and the 3 treatment phases. The comparison of tmax was performed by use of the Friedman test. The amount of drug excreted in each sample was compared between the control phase and the 3 treatment phases by use of 2-way ANOVA. A P value of .05 or less was regarded as significant. Geometric mean ratios to corresponding values in the control phase with 95% confidence intervals (CIs) were used for detection of significant differences as post hoc analyses. When the 95% CI did not cross 1.0, the result was regarded as significant. SPSS 12.0 for Windows (SPSS Japan Inc, Tokyo, Japan) was used for these statistical analyses.

RESULTS

Although mild side effects were experienced (slight chest pain in 2 cases during verapamil treatment, tendency of diarrhea in 1 case during cimetidine treatment, mild stomach disturbance in 3 cases during probenecid treatment), no clinically significant adverse events were reported throughout the study.

Plasma kinetics. There were significant differences among the 4 treatment groups in pharmacokinetic parameters such as Cmax (F = 13.785; df = 3,9; P = .001), AUC(0-48) (F = 10.857; df = 3,9; P = .002), and AUC(0-∞) (F = 10.677; df = 3,9; P = .003) (Fig 1). However, no differences were found in tmax (F =
1.202; $df = 3.9; P = .364$) or elimination half-life (F = 3.667; $df = 3.9; P = .056$). Post hoc analyses showed that verapamil treatment significantly increased the $C_{\text{max}}$ of fexofenadine by 2.9-fold (95% CI, 2.4- to 4.0-fold), the $AUC(0-48)$ of fexofenadine by 2.5-fold (95% CI, 2.0- to 3.4-fold), and the $AUC(0-\infty)$ of fexofenadine by 2.5-fold (95% CI, 2.0- to 3.3-fold). In contrast, elimination $t_{1/2}$ during verapamil treatment was 24% shorter than during the control phase. No changes in plasma pharmacokinetic parameters were found during cimetidine treatment. Probenecid treatment significantly increased the $AUC(0-48)$ of fexofenadine by 1.5-fold (95% CI, 1.1- to 2.5-fold) and the $AUC(0-\infty)$ of fexofenadine by 1.5-fold (95% CI, 1.1- to 2.4-fold). All other pharmacokinetic parameters of fexofenadine did not differ during probenecid treatment.

**Urinary excretion.** There was a significant difference in the amount of drug excretion among the 4 phases (Fig 2). Post hoc analysis showed that verapamil significantly increased the cumulative urinary excretion at 4 hours ($P < .001$), 12 hours ($P < .001$), 24 hours ($P < .001$), and 48 hours ($P < .001$) and the total amount of drug excreted between 0 and 48 hours after administration, whereas the amounts of drug excreted between 0 and 48 hours after administration were significantly decreased during cimetidine treatment by 0.68-fold (95% CI, 0.52- to 0.98-fold) and during probenecid treatment by 0.43-fold (95% CI, 0.27- to 0.97-fold). Renal clearance of fexofenadine was significantly decreased during cimetidine treatment to 61% (95% CI, 50%-98%) and during probenecid treatment to 27% (95% CI, 20%-58%) but not during verapamil treatment (Table I).

### DISCUSSION

The results of this study showed a significant increase in plasma concentration of fexofenadine ($C_{\text{max}}$ and AUC) during verapamil treatment, without prolonging the elimination half-life of fexofenadine, which is in accordance with previous in vivo and in vitro reports. These findings suggest that verapamil increased the bioavailability of fexofenadine but did not inhibit the elimination of fexofenadine, and this might be partially explained by an inhibitory effect of verapamil on P-glycoprotein. Moreover, although the amount of fexofenadine excretion was significantly increased during verapamil treatment, renal clearance was unchanged by verapamil. When the plasma and urine findings are taken together, verapamil increased only the bioavailability of fexofenadine but not the excretion of fexofenadine, suggesting that this interaction occurs in the small intestine or in the liver but not in the kidney. A recent jejunal single-pass perfusion study suggested that verapamil treatment did not alter the permeability of fexofenadine. Therefore the interaction between verapamil and fexofenadine might be attributed to the decreased first-pass liver extraction of fexofenadine. Likewise, ketoconazole did not have an impact on the intestinal permeability of fexofenadine.

Verapamil is a nonspecific inhibitor of several membrane transport proteins including P-glycoprotein and OATP-A, whereas several in vitro studies have shown that P-glycoprotein and OATPs have an important role in penetration of the membrane by fexofenadine. Therefore it is most likely that verapamil alters fexofenadine pharmacokinetics through inhibition of P-glycoprotein and OATPs. In comparison with control values, in this study the relative percent of fexofenadine AUC during verapamil coadministration was greater than during probenecid coadministration. Because verapamil has an inhibitory effect on both P-glycoprotein and OATPs, the magnitude of the verapamil-mediated increase in plasma kinetics of fexofenadine might be greater than that of the probenecid-mediated increase.

The renal clearance of fexofenadine was decreased to 27% during probenecid treatment in this study. This finding suggests that fexofenadine excretion in the kidney was inhibited by probenecid probably through OATP inhibition. This is the first report suggesting a specific in vivo contribution of OATPs to fexofenadine excretion. In contrast, only a small, although statistically significant, difference in the plasma kinetics of fexofenadine was found during probenecid treatment. We have 2 plausible explanations for this discrepancy. First, even a pronounced decrease in renal clearance might not have a major impact on fexofenadine dispo-
endobiotics and xenobiotics. MRP2 generally pertathione, glucuronide, and sulfate conjugates of many adenosine triphosphate–binding cassette transporter forms excretory or protective roles, and it is expressed on the apical domain of hepatocytes, enterocytes of the proximal small intestine, and proximal renal tubular cells, as well as in the brain and placenta. Thus the possibility that several cellular processes other than membrane proteins are involved in this interaction cannot be excluded entirely on the basis of the current data.

It is possible that fexofenadine is a substrate of both OCTs and OATPs because the chemical structure of fexofenadine contains not only a carboxyl group with a negative logarithm of the acid ionization constant (pK_a) equal to 4.25 but also a piperidino group with pK_a equal to 9.53 (Aventis Pharma Co, Tokyo, Japan, personal communication, November 2000), although an in vitro study has suggested that the rat organic cation transporter rOCT1 did not mediate fexofenadine celluar uptake. In this study, however, a relatively small but statistically significant change in urine kinetics was found during coadministration of cimetidine, which is a potent inhibitor of OCT. In contrast, there was no change in any plasma kinetics of fexofenadine during cimetidine treatment. Therefore it is less likely that fexofenadine disposition is predominantly dependent on changes in OCT activity, suggesting that OCT does not have a major in vivo contribution to fexofenadine disposition.

Verapamil, cimetidine, and probenecid were given before rather than after fexofenadine administration in this study. Because the mean cumulative urinary excretion rate of fexofenadine reached 50% at 4 hours and 89% at 12 hours in comparison with the rate at 48 hours after administration during the control period, urinary excretion was almost complete by 12 hours after fexofenadine administration. Even if these drugs had been continued until the last sampling time point (48 hours), it is unlikely that there would have been significantly different because there was only a relatively small amount (about 10%-15%) of fexofenadine excretion in the urine during the control period. Second, it is possible that OATP inhibition by probenecid in the small intestine or liver leads to the decreased bioavailability of fexofenadine. A recent study showed that several fruit juices decreased the plasma concentration of fexofenadine whereas they did not alter renal clearance. Thus the authors of this study concluded that fruit juices decreased oral availability through OATP inhibition in the small intestine.

Probenecid is also an inhibitor of multidrug resistance protein (MRP)–mediated cell efflux. MRP2 is an adenosine triphosphate–binding cassette transporter accepting a diverse range of substrates, including glutathione, glucuronide, and sulfate conjugates of many endobiotics and xenobiotics. MRP2 generally performs excretory or protective roles, and it is expressed on the apical domain of hepatocytes, enterocytes of the proximal small intestine, and proximal renal tubular cells, as well as in the brain and placenta. Thus the significant interaction between fexofenadine and probenecid in this study might be ascribable to MRP inhibition in the liver, small intestine, or kidney, although there is no information indicating the involvement of MRP-mediated cell efflux in fexofenadine disposition.

### Table I. Effects of verapamil, cimetidine, and probenecid treatments on pharmacokinetic parameters of fexofenadine after single oral 120-mg dose of fexofenadine in 12 healthy volunteers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Verapamil</th>
<th>Cimetidine</th>
<th>Probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (ng/mL)</td>
<td>611 ± 206</td>
<td>1807 ± 692</td>
<td>609 ± 318</td>
<td>767 ± 490</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>2.92 (2.37-3.95)</td>
<td>0.93 (0.69-1.49)</td>
<td>1.14 (0.85-1.74)</td>
</tr>
<tr>
<td>t_max (h)</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Median (range)</td>
<td>0.5-4.0</td>
<td>1.0-4.0</td>
<td>1.0-4.0</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>AUC(0-48) (ng · h/mL)</td>
<td>3569 ± 1222</td>
<td>9092 ± 3586</td>
<td>4028 ± 1903</td>
<td>6097 ± 3973</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>2.52 (2.02-3.20)</td>
<td>1.09 (0.79-1.77)</td>
<td>1.54 (1.13-2.46)</td>
</tr>
<tr>
<td>AUC(0-∞) (ng · h/mL)</td>
<td>3637 ± 1199</td>
<td>9136 ± 3573</td>
<td>4124 ± 2019</td>
<td>6150 ± 3972</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>2.48 (2.00-3.32)</td>
<td>1.08 (0.77-1.78)</td>
<td>1.53 (1.13-2.40)</td>
</tr>
<tr>
<td>Elimination half-life (h)</td>
<td>11.0 ± 5.1</td>
<td>7.9 ± 2.4</td>
<td>9.7 ± 3.1</td>
<td>8.5 ± 1.6</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>0.76 (0.66-0.90)</td>
<td>0.92 (0.80-1.25)</td>
<td>0.84 (0.70-1.13)</td>
</tr>
<tr>
<td>Amount excreted (mg)</td>
<td>14.1 ± 4.9</td>
<td>35.5 ± 18.9</td>
<td>10.0 ± 5.1</td>
<td>9.2 ± 11.2</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>2.40 (1.99-3.20)</td>
<td>0.68 (0.52-0.98)</td>
<td>0.43 (0.27-0.97)</td>
</tr>
<tr>
<td>CLrenal (mL/min)</td>
<td>230 ± 78</td>
<td>224 ± 93</td>
<td>152 ± 70</td>
<td>74 ± 52</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1.00</td>
<td>0.95 (0.77-1.29)</td>
<td>0.61 (0.50-0.98)</td>
<td>0.27 (0.20-0.58)</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD for pharmacokinetic parameters (except for t_max) and geometric mean and 95% confidence interval for ratio to control. C_max: Peak concentration; t_max, time to peak concentration in plasma; AUC(0-48), area under plasma concentration–time curve from 0 to 48 hours; AUC(0-∞), area under plasma concentration–time curve from time 0 to infinity; CLrenal, renal clearance.
greater differences in plasma and urine kinetics during cimetidine or probenecid treatment.

There was a marked interindividual variation in the pharmacokinetics of fexofenadine in this study (coefficient of variation, 37%-50%; maximum difference, 5.9-fold). This could be explained by large interindividual variation in transporter activity such as P-glycoprotein and OATPs. The MDR1 genotypes T3435C and G2677T/A have a different expression of P-glycoprotein in the small intestine, although a previous study failed to find any significant difference in fexofenadine disposition between MDR1 genotypes. Recently, it has been suggested that polymorphisms in OATP-C, such as T521C (Val174Ala), are associated with altered pharmacokinetics of pravastatin. Meanwhile, there is still no evidence of functional polymorphisms in OATP-A, of which fexofenadine is a substrate. Further studies are, therefore, required to explain interindividual variations seen in the pharmacokinetics of fexofenadine, including the contribution of transporters other than OATPs and P-glycoprotein.

A limitation of our study is the 4-way sequence used in the protocol. The control phase was always the first in sequence. Although sufficient washout periods (2 weeks) were used in this study, bias as a result of a sequence effects cannot be excluded.

In conclusion, this study suggests that verapamil increases fexofenadine exposure probably because of an increase in bioavailability through P-glycoprotein inhibition and that probenecid also increases the AUC of fexofenadine, to some extent, as a result of a pronounced reduction in renal clearance. Changes in the regulation of transporters such as P-glycoprotein and OATPs, although not simple, may lead to significant alternation of fexofenadine pharmacokinetics.

We thank Miss Sayaka Nagasama (Hirosaki University School of Medicine) for her expertise in measuring the plasma and urine fexofenadine levels.

The authors and their institutes have no conflicts of interest.

References
P-glycoprotein and surfactants: Effect on intestinal talinolol absorption

Background and Objective: Surfactants used in pharmaceutical formulations can modulate drug absorption by multiple mechanisms including inhibition of intestinal P-glycoprotein (P-gp). Our objective was to analyze the effect of 2 surfactants with different affinity for P-gp in vitro on the intestinal absorption and bioavailability of the P-gp substrate talinolol in humans.

Methods: In vitro, the influence of surfactants on talinolol permeability was studied in Caco-2 cells. In vivo, an open-label 3-way crossover study with 9 healthy male volunteers was performed. Subjects were intubated with a 1-lumen nasogastrointestinal tube. The study solution, containing either talinolol (50 mg), talinolol and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (0.04%), or talinolol and Poloxamer 188 (0.8%), was administered through the tube.

Results: TPGS, but not Poloxamer 188, inhibited the P-gp–mediated talinolol transport in Caco-2 cells. In healthy volunteers TPGS increased the area under the plasma concentration–time curve with extrapolation to infinity (AUC₀₋∞) of talinolol by 39% (90% confidence interval, 1.10-1.75) and the maximum plasma concentration (Cₘₐₓ) by 100% (90% confidence interval, 1.39-2.88). Poloxamer 188 did not significantly alter the AUC₀₋∞ or Cₘₐₓ of talinolol.

Conclusions: This in vivo intraduodenal perfusion study showed that low concentrations of TPGS, close to the concentrations that showed P-gp inhibition in vitro, significantly increased the bioavailability of talinolol. The study design excluded modulation of solubility by TPGS and unspecific surfactant-related effects. The latter was supported by the absence of modulation of the talinolol pharmacokinetics by Poloxamer 188, which does not modulate P-gp. Therefore we consider intestinal P-gp inhibition by TPGS as the major underlying mechanism for the increase in talinolol bioavailability. (Clin Pharmacol Ther 2005;77:24-32.)

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Low oral bioavailability of drugs can be caused by low drug solubility, low intrinsic membrane permeability, drug instability, high first pass, active transport back into the intestinal lumen, or combinations thereof. A relevant mechanism of drug excretion from the enterocyte back into the gut lumen is represented by efflux pumps such as P-glycoprotein (P-gp). P-gp is present at the apical membrane of enterocytes along the whole intestine, and substrates of P-gp often show low oral bioavailability. Many drugs are substrates of both P-gp and the cytochrome P450 (CYP) isof orm CYP3A4. Both mechanisms work synergistically as a defense mechanism to limit the absorption and bioavailability of xenobiotics. Clinical studies indicate that P-gp may be involved in intestinal efflux of drugs such as talinolol, digoxin, and cyclosporine (INN, ciclosporin) and that the process is inducible by drugs such as rifampin and St John’s wort. Interindividual variation of intestinal expression levels of P-gp could account for the high variability in substrate bioavailability as shown for cyclosporine. To date, 29 single nucleotide polymorphisms (SNPs) have been reported in the MDR1 gene (multidrug resistance gene encoding for P-gp). A synonymous SNP in exon 26 (C3435T) was the first variant to be associated with altered protein expression. It could be shown that individuals ho-
been widely investigated. Various mechanisms play-a role in pharmacokinetics. The use of surface-active agents to increase intestinal absorption of drugs has been widely investigated. Various mechanisms playing a role in their absorption-enhancing effects have been identified, such as increasing the solubility of hydrophobic macromolecules in the aqueous boundary layer, increasing membrane fluidity or damaging of the intestinal epithelium, disrupting of tight junctions, interacting with metabolic enzymes, and inhibiting excretory processes including P-gp-mediated efflux.

Selective inhibition of the intestinal secretory transporter P-gp could be a strategy to improve absorption of substrates with low permeability and to reduce variability in pharmacokinetics. The use of surface-active agents to increase intestinal absorption of drugs has been widely investigated. Various mechanisms playing a role in their absorption-enhancing effects have been identified, such as increasing the solubility of hydrophobic macromolecules in the aqueous boundary layer, increasing membrane fluidity or damaging of the intestinal epithelium, disrupting of tight junctions, interacting with metabolic enzymes, and inhibiting excretory processes including P-gp-mediated efflux.

In vitro assays have been used to distinguish between surfactants that do or do not inhibit the P-gp efflux transporter. Some of these surfactants have been tested in animal experiments, and evidence has been provided that they may also inhibit P-gp in vivo. However, to date, a limited number of clinical studies have explored the relevance of P-gp inhibition by surfactants for improving oral bioavailability of P-gp substrates in humans. The surfactants investigated were d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS), Cremophor EL, and Cremophor RH40. The P-gp substrates used in the above-mentioned studies were cyclosporine, saquinavir, amprenavir, and digoxin. Although these studies provided useful pharmacokinetic information, questions about the underlying mechanism of absorption enhancement remained open. Most P-gp substrates used had low water solubility, were administered in high doses, or were CYP3A4 substrates (except for digoxin). In addition, the surfactants were often given at very high doses. Therefore the increased plasma drug levels after addition of a surfactant to the oral formulation could result from improved particle wetting, faster dissolution rate, increased drug solubility, inhibition of efflux transporters, or inhibition of CYP3A4 metabolism of the drug or a combination of these.

The focus of this clinical study was on P-gp-surfactant interactions in humans. Therefore the study design was such that it allowed elimination of most non-P-gp-related factors on drug absorption. Talinolol, a β1- antagonist with medium bioavailability (55%), minimal affinity for CYP3A4, low metabolism, and medium solubility (1.23 mg/mL), was selected as the P-gp substrate. A buffered solution containing 50 mg of talinolol was administered directly into the duodenum via a nasogastric intestinal tube to avoid wetting, dissolution, and solubility problems. Furthermore, to eliminate non-P-gp surfactant-related effects, 2 surfactants were investigated, one that inhibited P-gp in vitro and another that did not (Poloxamer 188). Both surfactants were administered at concentrations twice their critical micelle concentration values (0.04% TPGS and 0.8% Poloxamer 188). By use of this design, talinolol pharmacokinetics was measured in subjects genotyped for the MDR1 C3435T polymorphism.

METHODS
Materials
Talinolol was kindly provided by Arzneimittelwerk Dresden (Dresden, Germany). Synperonic F68 (Poloxamer 188) was kindly provided by BASF (Ludwigshafen, Germany) and TPGS by the Peboc Division of Eastman (Llangefni, England). The perfusion buffer contained 35-mmol/L mannitol, 5-mmol/L potassium chloride, 5-mmol/L sodium phosphate monobasic monohydrate, 2.5-mmol/L sodium phosphate dibasic dihydrate, 10-mmol/L glucose, and 119-mmol/L sodium chloride (290 mOsm/L). All materials were of pharmaceutical grade for the clinical study and of the best quality for the in vitro study.

In vitro Caco-2 permeability studies
Caco-2 permeation experiments were performed as described previously. In brief, Caco-2 cells (passage No. 104-112) were grown in 96-well polycarbonate filter plates for 7 to 10 days. After washing, 140-μL samples of 300-μmol/L talinolol in transport buffer, with or without surfactant, were added to the apical or basolateral side of the cells. Vinblastine and rhodamine 123 were used as P-gp substrate controls in the experiments, and the basolateral-to-apical/apical-to-basolateral ratios were 10 and 20 to 30 for vinblastine and rhodamine 123, respectively, which indicate P-gp activity in the passage number used. Talinolol was quantified in these experiments by measuring the ultraviolet (UV) absorption at 240 nm by use of a SpectraMax Plus 96-well microtiter plate UV reader (Molecular Devices Corp, Sunnyvale, Calif) as described previously. After incubation for 90 minutes at 37°C at pH 7.4, talinolol appearing in the receiver compartment was detected by direct UV analysis, and the permeability coefficient was calculated as described by Alsenz and Haenel. Experiments were performed in triplicate, and results are expressed as mean ± SD.
In vivo clinical trial

Subjects. Nine healthy male nonsmoking volunteers participated in the study after having given informed consent. None was taking any other medication or vitamin preparations for 30 days before the start of the study until study end. Before study inclusion, the individuals were ascertained to be healthy by medical history, clinical examination, laboratory screening results, including hematologic and biochemical blood tests, urinalysis, and a 12-lead electrocardiogram. All were white, the mean age was 30 years (range, 24-38 years), and the mean body weight was 73 kg (range, 55-94 kg). The SNP in exon 26 (C3435T) of the MDR1 gene was determined for all subjects according to the method described in the following text.

Study design. The study protocol was approved by the Ethics Committee of Basel, Switzerland; the study was conducted at the University Hospital Basel, Basel, Switzerland.

A placebo-controlled, randomized, 3-way crossover study design was used with at least 7 days’ washout between the treatment periods. The study medication was formulated as an aqueous buffer solution (pH 6.0) containing either talinolol (50 mg), talinolol (50 mg) and TPGS (0.04%), or talinolol (50 mg) and Poloxamer 188 (0.8%). The solution was freshly prepared by a pharmacist on the day before administration. After an overnight fast of at least 12 hours, subjects were intubated with a 1-lumen nasogastrointestinal tube (Flocare-Nutrisoft feeding tube, 125 cm; Pfrimmer Nutricia, Meyrin, Switzerland). The tube was placed under fluoroscopic control with the tip in the duodenum. The study medication was perfused for 25 minutes into the duodenum by use of a perfusion pump (Flocare Micromax 200i; Nutricia Norge AS, Oslo, Norway) at a rate of 5 mL/min. For 4 hours after drug administration, subjects remained in the supine position. Thereafter the tube was removed, and subjects received a liquid meal (Ensure Plus, 2520 kJ; Ross Products Division, Abbott Laboratories, Columbus, Ohio); 8 hours after drug administration, a standardized snack was given. Alcohol and caffeine-, xanthine-, or grapefruit-containing beverages or meals were not allowed 24 hours before study drug administration until the last blood sample was drawn. Blood samples (7.5 mL), taken through an intravenous catheter, were collected into ethylenediaminetetraacetic acid–containing tubes immediately before and at 10, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, and 48 hours after dosing. Blood samples were centrifuged at 3000 rpm for 10 minutes and were immediately put on dry ice. Study samples were stored at −70°C until analysis. Peripheral blood pressure and heart rate were measured after 5 and 20 minutes and 1, 3, 4, 5, 6, 8, 12, 24, 36, and 48 hours following drug administration.

Allelic discrimination of MDR1 C3435T by use of 5′-nuclease assay. Genomic deoxyribonucleic acid (DNA) was isolated from ethylenediaminetetraacetic acid–blood with the QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The MDR1 polymorphism C3435T was tested by allele-specific quantitative polymerase chain reaction (5′-nuclease assay). A total of 25 ng complementary DNA was used as a template for realtime quantitative polymerase chain reaction analysis, which was performed with the TaqMan assay by use of a GeneAmp 5700 Sequence Detector (Applied Biosystems, Rotkreuz, Switzerland), a combined thermocycler and fluorescence detector. Two allele-specific probes, one labeled at the 5′ end with the fluorescent dye FAM or with VIC (Applied Biosystems), which is able to discriminate between the 2 alleles, were used. The fluorescent dye at the 5′ end of the probe, FAM, or VIC serves as the reporter, and its emission is quenched by the second fluorescent dye at the 3′ end of the probe (6-carboxy-tetramethyl-rhodamine). During elongation, the 5′ to 3′ exonuclease activity of the Taq DNA polymerase cleaves the probe, thus releasing the reporter from the quencher. Primers and the probe were designed according to Fellay et al.38 The DNA (2.5 μL) was amplified in a 25-μL volume containing TaqMan PE Mastermix (Applied Biosystems), 22 pmol of each primer, and 200 pmol of probe. Cycling conditions were as follows: initial denaturation and activation of AmpliTaq Gold DNA polymerase (Applied Biosystems) for 10 minutes at 95°C, followed by denaturation for 40 cycles for 15 seconds at 95°C and combined annealing and primer extension for 1 minute at 60°C.

Determination of talinolol concentrations. A specific liquid chromatography–tandem mass spectrometry assay, validated according to Food and Drug Administration guidelines, was used for determination of talinolol concentrations in human plasma. This assay used online solid phase extraction (Prospekt II; Spark Holland BV, Emmen, The Netherlands) reversed-phase HPLC combined with atmospheric pressure chemical ionization–tandem mass spectrometry for detection (Q TRAP; AB/MDS Sciex, Concord, Ontario, Canada) in the selected reaction-monitoring mode. The method involved precipitation of plasma proteins with perchloric acid and injection of a 500-μL aliquot of the supernatant onto a Prospekt C8 end-capped 10 × 2-mm cartridge for compound retention. By use of the backflush mode, the analytes were transferred onto an ana-
lytic column (XTerra C18, 4.6 × 50 mm; Waters Corporation, Milford, Mass) for chromatographic separation and mass spectrometry detection in positive mode. Propranolol was used as internal standard. The mean precision and accuracy, calculated from the quality control samples (3, 20, and 150 ng/mL), were found to be 7.47% and 103%, respectively. The data were assessed from quality control samples during analysis of study samples. The lower limit of quantitation was 2.5 ng/mL by use of a 0.25-mL plasma aliquot for the range 2.5 to 200 ng/mL.

**Pharmacokinetic evaluation.** Noncompartmental analysis with WinNonlin 4.1 (Pharsight Corporation, Mountain View, Calif) was performed to determine pharmacokinetic parameters of talinolol. Maximum plasma concentration \(C_{\text{max}}\) and time of maximum plasma concentration \(t_{\text{max}}\) were obtained directly from the raw data. The area under the plasma concentration–time curve \(AUC\) was calculated by the trapezoidal rule, with extrapolation to infinity \(AUC_{0-\infty}\). \(AUC_{0-6}\) is the AUC from 0 to 6 hours. The elimination rate constant \(k_e\) was determined by linear regression analysis with the last 3 to 5 points on the plot of the log-linear plasma concentration–time curve. The apparent terminal half-life \(t_{1/2}\) was calculated by the following equation: \(t_{1/2} = \ln 2/k_e\).

### Statistical analysis

The results are expressed as arithmetic mean values \((±SEM)\) in the figures and as arithmetic mean values \((±SD)\) and geometric mean ratios (with 90% confidence intervals [CIs]) in the text and tables. Descriptive analysis of all parameters was performed. A 3-way ANOVA by use of treatment, subject, and period effects was performed through use of log-transformed values of \(AUC\) and \(C_{\text{max}}\) ratios, assuming common variance among the 3 treatments. The 90% CIs for the geometric mean ratios were calculated from CIs of the corresponding contrasts of the log-transformed data by exponentiation. Relative bioavailabilities were estimated by use of the exposure ratio of surfactants containing treatments relative to talinolol administered without surfactants. Comparisons for \(t_{\text{max}}\) and \(t_{1/2}\) were performed by ANOVA with Tukey post hoc multiple comparisons. Interindividual variability was determined by use of coefficients of variation (CVs). All comparisons were performed as 2-tailed analyses. The level of significance was \(P < .05\). All statistic analyses were performed with SPSS for Windows (version 11.0; SPSS Inc, Chicago, Ill).

<table>
<thead>
<tr>
<th>Table I. Talinolol permeability across Caco-2 cell monolayers in presence of TPGS or Poloxamer 188</th>
<th>Permeability coefficient (cm/s) ((×10^{-6}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant (%)</td>
<td>Apical to basolateral</td>
</tr>
<tr>
<td>Buffer</td>
<td>0</td>
</tr>
<tr>
<td>Talinolol plus TPGS</td>
<td>0.005</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>0.1</td>
</tr>
<tr>
<td>TPGS</td>
<td>0.2</td>
</tr>
<tr>
<td>Poloxamer 188</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Permeability assays were performed in triplicate at pH 7.4 with 300-μmol/L talinolol solutions. Talinolol alone or in the presence of varying concentrations of surfactant was added to either the apical or basolateral chamber of Caco-2 cells, and its appearance in the receiver compartment was determined. Values represent mean permeability coefficient values \((±SD)\).

**TPGS, 1,1,1,3,3,3-Hexamethyl-2,4-pentanediol succinate.**

**RESULTS**

**In vitro Caco-2 cell experiments**

For TPGS and Poloxamer 188, the affinity for P-gp was analyzed in a previous study. Their interaction with talinolol transport in vitro has never been investigated. Therefore the effect of TPGS and Poloxamer 188 on talinolol transport through Caco-2 cell monolayers was studied before the conduct of the clinical trial. Results from the in vitro permeability experiments in Caco-2 cell monolayers are shown in Table I. A 10-times higher transport in the basolateral-to-apical direction compared with the apical-to-basolateral direction is seen for talinolol, which indicates efflux by a luminal active transporter such as P-gp. TPGS, but not Poloxamer 188, increases the permeability of talinolol in the apical-to-basolateral (absorptive) direction (maximum, 6.7 times) and decreases its transport in the basolateral-to-apical direction (maximum, 2.5 times). At the highest Poloxamer 188 concentration tested (0.4%), a reduced talinolol permeability was seen in the apical-to-basolateral direction.

**Tolerability**

Nine subjects completed the study and received all 3 treatments without adverse drug events. Two subjects were excluded from the investigation because of non-adherence to the study protocol; these dropouts were replaced.
Pharmacokinetics

The plasma concentration–time profiles of talinolol, administered with and without surfactants (TPGS or Poloxamer 188), are shown in Fig 1. The AUC of talinolol was analyzed on the 3 different occasions, and the AUC of talinolol in the presence of TPGS or Poloxamer 188 was compared with the AUC of talinolol given alone. The exposure of talinolol when administered with TPGS was statistically (P < .05 for AUC\text{0-6}, AUC\text{0-6}, and C_{\text{max}}) higher compared with talinolol administered without surfactant (reference solution). The exposure of talinolol when administered with Poloxamer 188 was not statistically (P > .5 for AUC\text{0-6}, AUC\text{0-6}, and C_{\text{max}}) different from the exposure achieved with the reference solution. No significant changes in t_{\text{max}} and t_{\text{0.5}} were noticed for the TPGS and Poloxamer 188 treatment as compared with the reference. Pharmacokinetic data of talinolol after single intraduodenal administration of 50 mg talinolol with or without surfactants are summarized in Table II. The TPGS effect is distinct during the first 6 hours after talinolol administration, as shown by an approximately 2-fold increase in geometric mean AUC\text{0-6} in the TPGS group compared with talinolol administration alone (P = .001). On the other hand, AUC\text{0-6} was virtually unchanged in the Poloxamer 188 group. The presence of TPGS in the talinolol formulation increased the AUC\text{0-6} of talinolol by 39% (90% CI, 1.10–1.75) compared with the reference formulation; C_{\text{max}} was increased by 100% (90% CI, 1.39–2.88). For the TPGS treatment, the 90% CIs for the geometric mean ratios for AUC\text{0-6} and C_{\text{max}} did not include the 100% reference.

All talinolol concentrations at 36 hours were above the limit of quantification for each subject. The inter-subject variability in AUC was high for all treatments, with a tendency toward lower CVs for the surfactant-containing formulations. The CVs were 58%, 28%, and 36% for the reference, TPGS, and Poloxamer 188 treatment, respectively. Because of this high intersubject variability, arithmetic and geometric means differ.

The individual talinolol AUC\text{0-6} values after single intraduodenal administration of 50 mg talinolol with or without surfactant are depicted in Fig 2 and are linked to genotype information. Among the 9 subjects, 2 were homozygous TT (considered to have low P-gp expression), 4 were heterozygous CT, and 3 were homozygous wild-type CC. No significant effect of the MDR1 genotype (C3435T) on talinolol pharmacokinetics was observed.
concentration (IC50) values for P-gp inhibition in was 20 to 200 times higher than the 50% inhibitory trials, the estimated intestinal concentration of surfactant intental design of the study. In several previous clinical inhibition of metabolism) can be ruled out by the exper-

imental permeabilization, and a direct P-gp–related effect of a surfactant on the flux can already sufficient to increase its permeability. Therefore effects on drug concentration and permeability coefficient of the drug. Surfactants may af-

fect both drug solubility and permeability. It was shown lumenal side of the surface of the enterocytes and the flux is driven by 2 factors, the drug concentration at the of relatively poorly absorbed P-gp substrates. In princi-

ple, the amount of orally absorbed drug depends on the flux of the drug across the intestinal membrane. This flux is driven by 2 factors, the drug concentration at the luminal side of the surface of the enterocytes and the permeability coefficient of the drug. Surfactants may a-

ffect both drug solubility and permeability. It was shown for amprenavir that high TPGS concentrations improved solubilization whereas much lower concentrations were already sufficient to increase its permeability. Therefore a direct P-gp–related effect of a surfactant on the flux can only be proven if an influence on drug concentration and on non–P-gp–related permeability (such as a reduction in the unstirred water layer, membrane permeabilization, and inhibition of metabolism) can be ruled out by the experimental design of the study. In several previous clinical trials, the estimated intestinal concentration of surfactant was 20 to 200 times higher than the 50% inhibitory concentration (IC50) values for P-gp inhibition in vitro. Furthermore, most P-gp substrates used, except for digoxin, are metabolized by CYP3A4, which is present in the intestine. Therefore the influence of surfac-

tants on the presystemic metabolism of P-gp substrates could not always be excluded.

In our study the uncertainty with regard to solubility enhancement was ruled out because perfectly soluble solutions were perfused directly into the duodenum. No precipitation of components from the perfusion solution was visible on mixing (1:1) of perfusion solutions with clear human intestinal juice in vitro. In addition, before the study was started, all 3 perfusion solutions were tested for adsorption to the tubes by measuring the UV absorption of talinolol at entry and exit of the 125-cm nasogastrointestinal tubes. UV absorption of the talinolol solutions at exit were similar (3%) to the

Table II. Pharmacokinetic data of talinolol after single intraduodenal administration of 50 mg talinolol with or without surfactants in 9 healthy individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Talinolol</th>
<th>Talinolol plus TPGS</th>
<th>Talinolol plus Poloxamer 188</th>
<th>Talinolol plus TPGS/talinolol ratio and 90% CI*</th>
<th>Talinolol plus Poloxamer 188/talinolol ratio and 90% CI*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-6 (ng · h/mL)</td>
<td>942 ± 487</td>
<td>1130 ± 318†</td>
<td>773 ± 239‡</td>
<td>1.39 (1.10-1.75)</td>
<td>0.91 (0.73-1.15)</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>222 ± 169</td>
<td>327 ± 120§</td>
<td>183 ± 110‡</td>
<td>2.11 (1.54-2.90)</td>
<td>0.99 (0.73-1.35)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>64.5 ± 53.2</td>
<td>88.0 ± 36 0†</td>
<td>58.1 ± 35.9‡</td>
<td>2.00 (1.39-2.88)</td>
<td>1.13 (0.79-1.61)</td>
</tr>
<tr>
<td>AUC0-6/CI*</td>
<td>3.6‡</td>
<td>1.0‡</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CI*</td>
<td>1.3‡</td>
<td>1.3‡</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Three formulations were administered as follows: talinolol alone, talinolol with 0.04% TPGS, and talinolol with 0.8% Poloxamer 188. Values are arithmetic mean values (±SD) unless otherwise indicated.

CI, Confidence interval; AUC0-6, area under plasma concentration–time curve with extrapolation to infinity; AUC0-6/CI* area under plasma concentration–time curve from 0 to 6 hours; Cmax, maximum plasma concentration; Tmax, time of maximum plasma concentration; t0.5, apparent terminal half-life; NA, not applicable.

*Geometric mean ratios and 90% CI from ANOVA.

†Significantly different from control treatment (talinolol) at P < .05.

§Not significantly different from control treatment (talinolol).

§§Significantly different from control treatment (talinolol) at P = .001.

Pharmacodynamics

In this low-dose talinolol study, no relevant changes in heart rate or blood pressure were observed after the surfactant-containing treatments when compared with the reference treatment.

DISCUSSION

To date, a limited number of clinical studies have reported that inhibition of intestinal P-gp by certain surfactants can be used for improving the oral bioavailability of relatively poorly absorbed P-gp substrates. In principle, the amount of orally absorbed drug depends on the flux of the drug across the intestinal membrane. This flux is driven by 2 factors, the drug concentration at the luminal side of the surface of the enterocytes and the permeability coefficient of the drug. Surfactants may a-

ffect both drug solubility and permeability. It was shown for amprenavir that high TPGS concentrations improved solubilization whereas much lower concentrations were already sufficient to increase its permeability. Therefore a direct P-gp–related effect of a surfactant on the flux can only be proven if an influence on drug concentration and on non–P-gp–related permeability (such as a reduction in the unstirred water layer, membrane permeabilization, and inhibition of metabolism) can be ruled out by the experimental design of the study. In several previous clinical trials, the estimated intestinal concentration of surfactant was 20 to 200 times higher than the 50% inhibitory concentration (IC50) values for P-gp inhibition in vitro. Furthermore, most P-gp substrates used, except for digoxin, are metabolized by CYP3A4, which is present in the intestine. Therefore the influence of surfac-
MDR1 gene have been identified recently, which could be a result of an inhibition of P-gp in the small intestine. The absence of significant changes in the talinolol pharmacokinetics on coadministration of the control surfactant (Poloxamer 188) further supports the mechanism of selective P-gp inhibition.

Intersubject variability was high, with CVs for AUC and $C_{\text{max}}$ that were higher than 50% for the reference treatment. A CV of 38% for AUC was reported in another talinolol intestinal perfusion study. The high variability in the current study can be explained in part by the small sample size. Of 9 subjects, 3 showed an AUC$_{0-\infty}$ that was lower for the TPGS treatment compared with the reference treatment (Fig 2); on the other hand, only 1 of 9 subjects showed this pattern for AUC$_{0-6}$. This effect is probably related to the sampling scheme; the later phase in the pharmacokinetic profile is more prone to variability of single-concentration measurements, which has an impact on the calculation of AUC$_{0-\infty}$. The variability in exposure tended to be lower for the surfactant-containing treatments, especially the treatment containing TPGS. Reducing variability in drug bioavailability is a known feature of surfactant-containing formulations. Besides the intersubject variation in intestinal transit time, different concentrations of intestinal P-gp are considered as a possible cause for high intersubject variability in drug pharmacokinetics. Indeed, CVs for the mean duodenal P-gp content assessed with Western blot range between 60% and 135%. In this regard, inhibition of P-gp–mediated efflux would explain the reduced variability. It was shown that the human MDR1 gene is highly polymorphic, but contradictory results on the correlation between MDR1 genotype, duodenal P-gp expression, and disposition of P-gp substrates have been reported. In our study no correlation between the C3435T genotype and the talinolol exposure could be drawn, which is in accordance with findings from other studies. However, given that different SNPs of the MDR1 gene have been identified recently, which could also affect the MDR1 phenotype, a haplotype analysis should be performed in future studies.

The results of this study indicate that not all excipients are inert but that they can have the potential for MDR reversal. These pharmaceutical excipients could be used in drug formulations to increase drug absorption and to reduce intersubject variability. In general, only low amounts of surfactants are absorbed and thus no systemic effects are expected. At extreme acidic or alkaline pH conditions, small amounts of TPGS could be hydrolyzed to polyethylene glycol and vitamin E; the latter can be absorbed but is not able to inhibit P-gp. In vitro screening assays such as overexpressing cells and Caco-2 cells represent a valuable tool to select the appropriate surfactant for rational optimization of drug formulations.

Regarding our results, the interpretation of the clinical relevance of a P-gp–mediated TPGS drug interaction is not straightforward. Although changes in systemic exposure were statistically significant ($P < .05$), they are probably not clinically relevant. Estimation of clinical significance of the interaction should be done on the basis of what is known about the dose-response relationship and safety margin of the drug that will be coadministered with the surfactant. In our study the inhibition of intestinal P-gp and hence 39% increase in exposure did not influence talinolol pharmacodynamics or safety.

In conclusion, this in vivo intraduodenal perfusion study showed for the first time that low concentrations of TPGS, close to in vitro IC$_{50}$ values for P-gp inhibition, increased the bioavailability of talinolol significantly. A modulation of talinolol metabolism or solubility could be excluded, as well as unspecific surfactant-related effects. The latter was supported by the absence of modulation of the talinolol pharmacokinetics by Poloxamer 188, which does not modulate P-gp. The clinical data are consistent with results from in vitro studies. Therefore we consider intestinal P-gp inhibition by TPGS as the major underlying mechanism for the increase in talinolol bioavailability.

We declare that none of the authors has any conflict of interest.

References


Hepatic technetium Tc 99m–labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer

Background and Objective: The adenosine triphosphate–binding cassette transporter ABCB1 (P-glycoprotein) mediates terminal excretion of many chemotherapeutic agents, and variable ABCB1 activity may be an important contributor to interpatient variability in the clearance of chemotherapeutic agents. Our objective was to determine the elimination constant \(k_{H}\) for hepatic elimination of technetium Tc 99m–labeled sestamibi (99mTc-MIBI) in patients with cancer and to compare this putative indicator of ABCB1 phenotype with clinical features and common ABCB1 genetic variants.

Methods: 99mTc-MIBI \(k_H\) was determined from the time-dependent elimination profile of 99mTc-MIBI over a 90-minute hepatic scanning period in 66 patients with cancer. Single nucleotide polymorphisms (SNPs) in ABCB1 exons 12 (C1236T), 21 (G2677T/A), and 26 (C3435T) were documented by polymerase chain reaction–restriction fragment length polymorphism analysis.

Results: There was a 12-fold variation in 99mTc-MIBI \(k_H\) across the cohort, which was not correlated with sex, age, conventional liver function test results, previous chemotherapy treatment, or history of liver metastasis. Mean 99mTc-MIBI \(k_H\) was significantly reduced in patients with SNPs in exons 21 and 26 such that mean 99mTc-MIBI \(k_H\) was 1.90 times (95% confidence interval, 1.14-2.66; \(P = .02\)) and 2.21 times (95% confidence interval, 1.47-2.97; \(P < .01\)) higher in subjects homozygous for the wild-type alleles than in those homozygous for these SNPs, respectively.

Conclusion: Hepatic elimination of 99mTc-MIBI is a potential in vivo probe of hepatic ABCB1 activity that is significantly associated with the presence of common SNPs in ABCB1. 99mTc-MIBI hepatic scanning may provide a useful pretreatment indicator of ABCB1-mediated drug clearance in cancer patients. (Clin Pharmacol Ther 2005;77:33-42.)

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There is a wide interindividual variation in drug clearance for most agents as a result of genetic and environmental factors that affect elimination mechanisms. Dose calculation of cytotoxic chemotherapy is a particular challenge because the therapeutic range of these drugs is narrow and drug clearance varies by up to 10-fold despite dose individualization by use of body surface area.1 The importance of dose individualization

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is illustrated by studies that show better outcomes for cancer patients who have posttreatment dose adjustments based on therapeutic drug monitoring or on toxicity. However, with these methods, the optimum dose for each patient is often not reached until after the majority of the treatment course has been given. It is clear that robust pretreatment clearance indicators would be an advantage to ensure optimal dose effect from the first treatment.

The clearance of anticancer drugs is a complex multistep process involving a number of metabolism and elimination mechanisms. An important component of the process is the multidrug resistance 1 (MDR1) (P-glycoprotein, ABCB1) protein. ABCB1 is an adenosine triphosphate–binding cassette transporter, located on the canalicular membrane of hepatocytes, proximal renal tubular cells, and other sites such as the small intestine, that mediates the terminal elimination of important classes of chemotherapeutic agents such as anthracyclines, taxanes, and vinca alkaloids. Considerable variation in drug pharmacokinetics has been observed in different subjects by use of ABCB1 substrates such as fexofenadine and digoxin, suggesting that there is considerable interindividual variation in ABCB1 activity. This is supported by the finding that ABCB1 expression in peripheral mononuclear cells and the small intestine was highly variable between subjects. In addition, many common drugs are ABCB1 inhibitors or inducers that can alter ABCB1 activity and potentially affect drug clearance when given concomitantly. Therefore, variation in ABCB1 activity may be one of the important determinants of the observed variation in chemotherapy drug clearance, and indicators of ABCB1 activity may be useful in predicting the chemotherapy clearance capability of individual patients. However, to date, there is no established method of measuring ABCB1 activity clinically.

Technetium Tc 99m–labeled sestamibi (hexakis-2-methoxyisobutyl isonitrile) (99mTc-MIBI) is a radioactive compound commonly used in cardiac perfusion imaging. Its cationic charge allows accumulation in the cytosolic and mitochondrial membrane potentials. Thus it is retained in organs with high metabolic rates such as the heart, kidney, lung, and liver. 99mTc-MIBI undergoes minimal metabolism in the guinea pig and has been shown to be a substrate of ABCB1 in both in vitro and in vivo studies. In addition, hepatobiliary clearance of 99mTc-MIBI is markedly reduced in the presence of ABCB1 inhibitors in humans and in vivo elimination of 99mTc-MIBI from solid tumors is associated with tumor expression of ABCB1. Therefore, it is likely that ABCB1 is the principal mediator of 99mTc-MIBI elimination, and hepatic 99mTc-MIBI scanning may provide an indicator of ABCB1 activity useful for prediction of drug clearance.

Altered ABCB1 activity and expression have been associated with genetic variants of ABCB1, suggesting a contribution of genotype to constitutional differences in drug clearance. For example, Hoffmeyer et al described the association of a single nucleotide polymorphism (SNP) in exon 26 (C3435T) of ABCB1 with reduced clearance of an ABCB1 substrate, digoxin, and reduced ABCB1 expression in the duodenum, and subsequent studies have supported the presence of functional polymorphisms in ABCB1.

The aim of this study was to evaluate the clinical utility of measuring 99mTc-MIBI hepatic elimination in cancer patients receiving chemotherapy as an indicator of ABCB1 activity and to determine the relationship between this measure and common genetic variants of ABCB1.

**METHODS**

**Patients.** This study included patients from a previously described cohort. In brief, patients belonged to 1 of 2 treatment groups, as follows: Group 1 comprised patients with a variety of malignancies to be treated with the vinca alkaloid vinorelbine and group 2 comprised patients with chronic myeloid leukemia or gastrointestinal stromal tumor to be treated with the oral tyrosine kinase inhibitor imatinib mesylate. All patients had an expected survival greater than 1 month, Eastern Cooperative Oncology Group performance score of 0 to 2, and adequate bone marrow, renal, and hepatic function. Patients with known primary liver disease were excluded, although those with hepatic metastases were allowed.

Patients were encouraged not to take medications that are known ABCB1 inducers or inhibitors unless these medications were regarded as essential. Two patients were taking a known ABCB1 inhibitor (verapamil), and 1 patient was taking a known inducer (dexamethasone). Analysis was performed separately with these patients excluded as described here.

Patients were categorized as Caucasian or non-Caucasian, and the country of birth was documented for patients not born in Australia. Body surface area was determined by use of the Du Bois and Du Bois formula, as follows: Body surface area = 0.007184 × Height\(^{0.725}\) (in centimeters) × Weight\(^{0.425}\) (in kilograms). Body mass index was determined as follows: Body mass index = Weight (in kilograms)/Height\(^2\) (in meters).
The study protocol was approved by the Western Sydney Area Health Service Human Research and Ethics Committee, and informed consent was obtained from all patients.

**99mTc-MIBI hepatic scanning.** 99mTc-MIBI hepatic scanning was performed within 7 days before administration of the first cycle of chemotherapy. 2-Methoxyisobutyl isonitrile (sestamibi) labeled with technetium Tc 99m was prepared by use of a commercial kit (Cardiolite; DuPont Ltd, Sydney, Australia). Radiochemical purity was determined with 99mTc-MIBI content not less than 90%.

Patients fasted for 4 hours before receiving a bolus intravenous injection of 99mTc-MIBI at around 200 MBq. The precise activity of 99mTc-MIBI administered was calculated by subtracting the residual 99mTc-MIBI activity of the syringe from the activity before injection. Immediately after the administration of 99mTc-MIBI, patients remained in a supine position for 90 minutes while radioactivity in the abdominal region was measured by a gamma camera (Basicam; Siemens Corporation, New York, NY) as counts per minute at 1-minute intervals up to 90 minutes. The gamma camera has a high-resolution collimator with a 15% window around the 99mTc-MIBI emission of 140 keV. Change in hepatic radioactivity over time was determined by outlining an area of liver parenchyma on the abdominal images, with the biliary tree, gallbladder, and kidney regions avoided. The total radioactivity of the area was determined by a custom design program on the ICON workstation (Siemens Corporation) and automatically adjusted for the natural decay of the radioisotope.

**Pharmacokinetic modeling of 99mTc-MIBI hepatic elimination.** A compartmental model was developed to represent the region from which the radioactivity was measured. In brief, the liver vasculature was assumed to be a constant fraction of the body vascular space. The image, therefore, represented the summation of the liver parenchymal and vascular spaces. The major segments of the biliary tree were excluded from the area of imaging, and biliary radioactivity was, therefore, excluded from the calculations. Because uptake of 99mTc-MIBI into the liver is extremely rapid and concentration, efflux from the liver back into the vasculature was assumed to be negligible. The summation of the equations describing the kinetics of radioactivity in the vascular and parenchymal areas resulted in a biexponential equation of the following form: Radioactivity at time \( t = A e^{-kDt} - B e^{-kHt} \), where A and B are constants, \( kD \) is the distribution constant, and \( kH \) is the elimination constant. This equation was fitted to the radioactivity data from all patients with weighting of 1/y by use of the solver function of Microsoft Excel (Office XP; Microsoft Corp, Redmond, Wash) (Fig 1, A). The elimination constant (\( kH \)) was used as the measure of 99mTc-MIBI hepatic elimination rate.

The elimination of 99mTc-MIBI was extremely rapid in 2 patients, which led to the appearance of a third, slow phase of reduction in radioactivity. Hence, a 3-compartment model was required for adequate curve fitting for these 2 patients (Fig 1, B). This late phase is most likely a result of the redistribution of the radioisotope from peripheral tissue into the circulation, as noted in other studies of sestamibi clearance.14 In these cases the following equation was used: Radioactivity at time \( t = A e^{-kDt} - B e^{-kHt} - C e^{-kRt} \), where A, B and C are constants; \( kD \) is the distribution constant; \( kH \) is the elimination constant; and \( kR \) is the redistribution constant.

To test for interobserver variability in measuring radioactivity of the liver parenchyma, determination of \( kH \) was repeated in a number of cases in which the area of liver parenchyma was defined in duplicate by 2 independent observers. Results were highly correlated (Pearson correlation coefficient \( r^2 = .85, n = 53, P < .01 \). Mean \( kH \) was used in this subset of patients.

**ABCB1 genotyping.** Genomic deoxyribonucleic acid (DNA) was extracted from peripheral mononuclear cells collected from patients’ whole blood by use of a Qiagen DNA extraction kit (Qiagen, Valencia, Calif). Polymerase chain reaction and restriction fragment length polymorphism assays, previously described by Cascorbi et al,25 were used, with slight modifications, to genotype DNA for the exon 12 C1236T, exon 21 G2677T/A, and exon 26 C3435T polymorphisms of ABCB1. Identical primers were used to amplify the fragment of exon 21 to detect 2677A and 2677T. To detect 2677A, exon 21 polymerase chain reaction products were incubated with KpnI instead of BsrII, and DpnII was used instead of Sau3AI to detect 3435T in exon 26. Details of primer sequences, restriction endonucleases, and fragment lengths are listed in Table I. Haplotype frequencies were estimated by use of the HAPIPF implementation of the EM algorithm in Stata v7.0 (Stata Corporation, College Station, Tex).

**Statistical analysis.** Two-tailed tests with a significance level of 5% were used in all statistical analyses with the statistical software package SPSS for Windows v11 (SPSS, Chicago, Ill). 99mTc-MIBI elimination was correlated with ABCB1 genotypes by use of the nonparametric Jonckheere-Terpstra test.
A general linear model was used to correlate log $^{99m}$Tc-MIBI elimination with other variables, in which categoric variables including sex, cancer type, radiologic evidence of liver metastasis, and prior use of chemotherapy were considered as factors and continuous variables including age, body surface area, body mass index, international normalized ratio of prothrombin time, serum aminotransferase levels, and albumin levels were treated as covariates. Variables from univariate analyses with a $P$ value $<.1$ were included in multiple regression analysis to select independent predictors of log $^{99m}$Tc-MIBI elimination. Allelic frequencies of $ABCB1$ were tested for deviation from Hardy-Weinberg equilibrium by use of the chi square test.

RESULTS

Patient characteristics. Sixty-seven patients (45 in group 1 and 22 in group 2) participated in this study. Of the patients, 58 (87%) were Caucasian and 9 (13%) were of non-Caucasian origin (2 Chinese, 1 Vietnamese, 1 Fijian, 1 Samoan, 1 Filipino, 1 Sri Lankan, 1 South American, and 1 Lebanese). Other patient characteristics are summarized in Table II.

$^{99m}$Tc-MIBI hepatic elimination. One patient in group 1 was unable to lie flat to complete $^{99m}$Tc-MIBI hepatic scanning and was excluded from the analysis. For the overall cohort of 66 patients, $^{99m}$Tc-MIBI radioactivity reached a peak at a mean time of 11.5 minutes, followed by a decrease to a mean of 46% of the peak at the end of the 90-minute study (Fig 1, A and B). The profile of radioactivity was deemed to represent an initial net accumulation of the agent from the vascular space followed by excretion from the liver parenchyma.

There was a difference of approximately 6-fold in $^{99m}$Tc-MIBI $k_H$ across 95% of the patient population (mean $^{99m}$Tc-MIBI $k_H$, 0.013 min$^{-1}$ [SD, 0.009 min$^{-1}$]) and 12-fold across the entire cohort. The frequency distribution of $^{99m}$Tc-MIBI $k_H$ is shown in Fig 2. Log $^{99m}$Tc-MIBI $k_H$ was not associated with age ($P = .63, n = 66$), sex ($P = .52, n = 66$), cancer type ($P = .65, n = 66$), history of chemotherapy treatment ($P = .74, n = 65$), dose of $^{99m}$Tc-MIBI administered ($P = .73, n = 60$), presence of liver metastasis ($P = .60, n = 63$), serum albumin level ($P = .15, n = 66$), or serum hepatic transaminase levels (ALT, $P = .82, n = 66$; AST, $P = .32, n = 65$). Prothrombin time (international normalized ratio), which was available in 36 patients (55%) from group 1 (excluding 3 patients taking warfarin), did not correlate with log $^{99m}$Tc-MIBI $k_H$ ($P = .43, n = 36$). Log $^{99m}$Tc-MIBI $k_H$ was not associated with body mass index ($P = .55, n = 66$). Body surface
area correlated inversely with log \(^{99m}\text{Tc-MIBI kH}\) \(r^2 = 0.13, P < .01, n = 66\).

**ABCB1 genotyping.** The frequencies of \(ABCB1\) alleles in exons 12, 21, and 26 are summarized in Table III. Polymorphisms involving thymine substitution were present in all 3 exonal loci of \(ABCB1\) and were found in both alleles (TT genotype) of 35% of patients in exon 12, 28% in exon 21, and 30% in exon 26. There were no significant deviations from Hardy-Weinberg equilibrium of the allelic frequencies of any of the loci tested (Table III). The genotypes of the 3 loci were in strong linkage disequilibrium: the overall DA = 0.93 for exon 12–exon 21 haplotypes and 0.60 for exon 21–exon 26 haplotypes, respectively. Two 3-locus (exon 12-21-26) haplotypes were estimated to account for most (82%) of the chromosomes, as follows: T-T-T, which was present at 48% (compared with 18% expected), and C-G-C, which was present at 34% (com-

Table I. Primer sequences for detecting common genetic variants in exons 12, 21, and 26 of \(ABCB1\)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer sequence</th>
<th>Restriction enzyme</th>
<th>Resultant fragment length (base pairs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 12 C1236T</td>
<td>5'-TATCCTGTGTCTGTAATTGCC</td>
<td>HaeIII</td>
<td>Wild type: 63, 35, 272</td>
</tr>
<tr>
<td></td>
<td>5'-CCTGACTCAACACAAATG</td>
<td></td>
<td>SNP: 98, 272</td>
</tr>
<tr>
<td>Exon 21 G2677A</td>
<td>5'-TGCAAGGCTATAGTTTCCAGG</td>
<td>KpnI</td>
<td>Wild type: 224</td>
</tr>
<tr>
<td></td>
<td>5'-TTAGATTGACTCACCTCCCG</td>
<td></td>
<td>SNP: 202, 22</td>
</tr>
<tr>
<td>Exon 21 G2677T</td>
<td>5'-TGCAAGGCTATAGTTTCCAGG</td>
<td>BanI</td>
<td>Wild type: 198, 26</td>
</tr>
<tr>
<td></td>
<td>5'-TTAGATTGACTCACCTCCCG</td>
<td></td>
<td>SNP: 224</td>
</tr>
<tr>
<td>Exon 26 C3435T</td>
<td>5'-TGTTTTCAAGCTGCTTGATGG</td>
<td>DpnII</td>
<td>Wild type: 158, 39</td>
</tr>
<tr>
<td></td>
<td>5'-AAGGCATGTATGTTGCGCTC</td>
<td></td>
<td>SNP: 197</td>
</tr>
</tbody>
</table>

Primer sequences were taken from Cascorbi et al.\(^{25}\). Fragment lengths were verified from \(ABCB1\) sequence (bacterial artificial chromosome [BAC] clones of genomic sequence from exon 8-28 of \(ABCB1\) gene) (GenBank accession No. AC005068). Variations from the described method are the use of identical primers to detect both the 2677A and 2677T SNPs and digestion with \(KpnI\) to detect 2677A and \(Sau3AI\) to detect 3435T. Fragment lengths for wild-type and SNP sequences are shown. SNP, Single-nucleotide polymorphism.

Table II. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>45</td>
<td>22</td>
</tr>
<tr>
<td>Age (y) (mean, range)</td>
<td>62 (42-81)</td>
<td>55 (37-81)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Female</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>ECOG performance score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Body surface area (m(^2))</td>
<td>1.84 ± 0.23</td>
<td>1.86 ± 0.26</td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>27.3 ± 6.5</td>
<td>27.5 ± 6.5</td>
</tr>
<tr>
<td>(mean ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer types</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>Breast</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>Bladder</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>GIST</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>CML</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Radiologic evidence of liver metastasis*</td>
<td>14 (n = 42)</td>
<td>8 (n = 22)</td>
</tr>
<tr>
<td>Prior chemotherapy*</td>
<td>27 (n = 44)</td>
<td>1 (n = 22)</td>
</tr>
</tbody>
</table>

ECOG, Eastern Cooperative Oncology Group; GIST, gastrointestinal stromal tumor; CML, chronic myeloid leukemia.

*Numbers in parentheses are the numbers of patients available for analysis.

Fig 2. Frequency distribution of elimination constant (k\(H\)). The rate of \(^{99m}\text{Tc-MIBI hepatic scanning and ABCB1 genotyping}\) 37
pared with 8% expected). The next most frequent haplotypes had estimated frequencies of 6% or less.

\[ \text{99mTc-MIBI hepatic elimination rate and ABCB1 genetic variants.} \]

There was an ordered increase in mean 99mTc-MIBI \( k_H \) in subjects with TT, CT, and CC genotypes in exon 26 (Jonckheere-Terpstra test, \( P < .01 \)) (Fig 3, A) and subjects with TT, GT/GA, and GG genotypes in exon 21 (Jonckheere-Terpstra test, \( P < .01 \)) (Fig 3, B). A similar trend was observed in subjects with TT, CT, and CC genotypes in exon 12, but results did not reach statistical significance (Jonckheere-Terpstra test, \( P = .08 \) ) (Fig 3, C). Mean 99mTc-MIBI \( k_H \) was 2.21 times higher in CC subjects than in TT subjects in exon 26 (95% confidence interval, 1.47-2.97; \( P < .01 \)) and 1.90 times higher in GG subjects than in TT subjects in exon 21 (95% confidence interval, 1.14-2.66; \( P = .02 \)). By use of a general linear model, 19% of the overall variability of log 99mTc-MIBI \( k_H \) was accounted for by the \( ABCB1 \) genotype in exon 26 whereas 14% and 10% were accounted for by the \( ABCB1 \) genotype in exons 21 and 12, respectively. \( ABCB1 \) genotype in exon 26 was the strongest predictor \( (P < .01) \) of log 99mTc-MIBI \( k_H \) when all variables with \( P < .1 \) from univariate analysis (including \( ABCB1 \) polymorphisms in the 3 exonal loci and body surface area) were included in multiple regression analysis. Partitioning of subjects into equal groups of high and low 99mTc-MIBI eliminators showed that the C-G-C haplotype (exon 12-21-26) was more frequent in high 99mTc-MIBI eliminators (38%) than in low 99mTc-MIBI eliminators (24%) whereas the T-T-T haplotype was more common in low 99mTc-MIBI eliminators (59%) than in high 99mTc-MIBI eliminators (37%), as estimated by the EM algorithm.

The 2 patients with extremely rapid 99mTc-MIBI elimination requiring 3-compartment modeling to determine \( k_H \) had the CC genotype in exon 26. One of these patients was taking an \( ABCB1 \) inducer (8 mg dexamethasone daily). After these 2 patients were excluded, significant changes in mean 99mTc-MIBI \( k_H \) remained with different \( ABCB1 \) genotypes in exon 26 \( (P = .02) \), exon 21 \( (P = .06) \), and exon 12 \( (P = .07) \).

Two patients receiving ongoing treatment with a known \( ABCB1 \) inhibitor (240 mg verapamil daily) had 99mTc-MIBI \( k_H \) values within the lowest quartile range and were of CT \( (k_H = 0.008 \text{ min}^{-1}) \) and TT \( (k_H = 0.007 \text{ min}^{-1}) \) genotypes in exon 26, respectively. After these 2 patients and the above-mentioned patient receiving dexamethasone were excluded, \( ABCB1 \) genotype in exon 26 and exon 21 remained significantly correlated with 99mTc-MIBI \( k_H \). In addition, the result became significant with \( ABCB1 \) genotype in exon 12 (Jonckheere-Terpstra test, \( P = .03 \)).

**DISCUSSION**

We have investigated the use of pre-chemotherapy treatment 99mTc-MIBI hepatic scanning as an in vivo probe of \( ABCB1 \) activity in a cohort of cancer patients and demonstrated an association between 99mTc-MIBI hepatic elimination rate and \( ABCB1 \) genetic variants.

Standard pharmacokinetic modeling was used to describe the hepatic accumulation and elimination of 99mTc-MIBI detected by scanning. An elimination constant \( (99mTc-MIBI k_H) \), determined from the shape of the time-dependent elimination profile, was used as the indicator of elimination rate phenotype for comparison between individuals and in the genotype-phenotype analysis. The rationale for this was that \( k_H \) most di-

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**Table III. Frequency of \( ABCB1 \) variants**

<table>
<thead>
<tr>
<th>( ABCB1 ) locus</th>
<th>Genotype</th>
<th>Actual frequency (%)</th>
<th>Predicted frequency (%) (Hardy-Weinberg equilibrium)</th>
<th>( \chi^2 ) (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 12</td>
<td>CC</td>
<td>17</td>
<td>17</td>
<td>0.021 (.885)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>48</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>35</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Exon 21</td>
<td>GG</td>
<td>17</td>
<td>20</td>
<td>3.125 (.373)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>52</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>28</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Exon 26</td>
<td>CC</td>
<td>17</td>
<td>19</td>
<td>0.597 (.440)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>53</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>30</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

T1236C in exon 12, G2677TA in exon 21, and C3435T polymorphisms in exon 26 of \( ABCB1 \) were detected by polymerase chain reaction–restriction fragment length polymorphism. Actual allelic frequencies were compared with predicted frequencies if alleles were in Hardy-Weinberg equilibrium by chi square test.
directly reflects intrinsic elimination pump activity and is not affected by factors that could confound regional radioactivity measurements of exposure (area under the curve) such as variations in dose, vascular volume, distance of target from camera, specific activity of the preparation, collimation, or tissue thickness. Conversely, hepatic $^{99m}$Tc-MIBI clearance may be the appropriate measure to reflect ABCB1-mediated drug clearance. This requires that hepatic volume is taken into account according to conventional calculation of clearance being the product of elimination rate and volume of distribution. Hepatic volume can be estimated from body surface area, and a hepatic $^{99m}$Tc-MIBI clearance measure could, therefore, be determined for the purpose of predicting the drug clearance capability of individual patients.

In this patient cohort the variation in $^{99m}$Tc-MIBI $k_H$ was approximately 6-fold among 95% of the patient cohort and there was more than a 12-fold variation overall. $^{99m}$Tc-MIBI $k_H$ did not correlate with age, sex, or conventional liver function test results. In addition, $^{99m}$Tc-MIBI $k_H$ did not appear to be influenced by tumor uptake of $^{99m}$Tc-MIBI, because it was not influenced by the presence of liver metastasis. There was an unexpected inverse relationship between $^{99m}$Tc-MIBI $k_H$ and body surface area, with low body surface area being associated with slightly increased $^{99m}$Tc-MIBI $k_H$. This may indicate that the elimination rate is faster in subjects with a smaller hepatic volume. However, the correlation coefficient indicated that only approximately 13% of the variability of $^{99m}$Tc-MIBI $k_H$ could be accounted for by this factor.

The 3 most common variant alleles in the $ABCB1$ gene were examined. The C3435T in exon 26 is the best-studied SNP that has an allele frequency of more than 50% in the Caucasian population. In this study 83% of patients carried this SNP and 30% were homozygotes. It is a synonymous SNP and does not cause any alterations in the amino acid sequence of the transporter protein. Our results, together with other published data, suggest that this SNP is linked to G2677T in exon 21, which causes an amino acid change from

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Fig. 3. Correlation between $^{99m}$Tc-MIBI $k_H$ and $ABCB1$ genotype in exons 26 (A), 21 (B), and 12 (C) on the basis of the Jonckheere-Terpstra test. $^{99m}$Tc-MIBI hepatic scanning was performed before the administration of chemotherapy. Boxes represent the interquartile range, asterisks represent outliers, lines across boxes represent median, and bars represent upper and lower adjacent values. #1, $P < .01$; #2, $P = .02$; #3, $P = .08$. 
alanine to serine in the second transmembrane domain of ABCB1, and another synonymous SNP, C1236T in exon 12.

In this study, mean pretreatment $^{99m}$Tc-MIBI $k_{H}$ was significantly higher in subjects with the CC genotype compared with those with the TT genotype in exon 26, as well as in GG subjects compared with TT subjects in exon 21. Heterozygote subjects showed an intermediate result. Exon 26 genotype was the most powerful predictor of $^{99m}$Tc-MIBI $k_{H}$ among the genetic variants in 3 exonal loci of ABCB1. The absence of deviation from Hardy-Weinberg equilibrium in these ABCB1 genotypes suggests that this association is unlikely to be a result of population admixture.

Previous studies examining the relationship between the ABCB1 C3435T SNP in exon 26 and phenotype have returned variable results. Our finding is consistent with a report showing that Caucasian subjects who had the CC genotype in exon 26 had a 2-fold higher ABCB1 expression in the small intestine than those who had the TT genotype and heterozygous subjects had an intermediate expression by Western blot analyses. It has also been suggested that ABCB1 activity is higher in CC subjects than in TT subjects in studies taking ABCB1 substrates such as digoxin and antiepileptic compounds. However, there is also evidence suggesting higher ABCB1 activity associated with the TT genotype in exon 26. Intestinal ABCB1 expression was found to be higher in TT genotype carriers in a small study performed in Japanese subjects. The serum level of the antihistamine ABCB1 substrate fexofenadine was lower in TT subjects after a single oral dose in a cohort including predominantly Caucasian and African American subjects, and lower steady-state serum levels of anti–human immunodeficiency virus drugs have been associated with the TT genotype. Furthermore, Goh et al found no impact of the C3435T SNP in exon 26 on excretion of docetaxel, an ABCB1 substrate, in an Asian population.

There are also conflicting results on the functional impact of the G2677T SNP in exon 21. Kim et al showed that this SNP was associated with an increase in ABCB1 activity, whereas other studies have shown no effects when different ABCB1 substrates and patients of different ethnicity were involved.

It is possible that studies to date have perceived an indirect relationship between the SNPs examined and ABCB1 activity that is the result of linkage disequilibrium between these SNPs and unrecognized causal genetic variants. Causal variants may be within non-coding regions of ABCB1, or within neighboring genes, and may reside on different haplotypes in different populations. This would be consistent with in vitro studies which found that C3435T and G2677T/A did not alter ABCB1 activity and may explain the contradiction in intestinal ABCB1 expression between Caucasian and Japanese studies, because different haplotype patterns may exist in different ethnic groups. Consistent with this hypothesis are results from another Japanese study showing an association between C3435T and low intestinal expression of cytochrome P450 (CYP) 3A4, which, like ABCB1, is located at chromosome 7q21. Another possible explanation is that ABCB1 elimination phenotype is influenced by combinations of genetic variants at multiple loci. Clearly, further studies are needed to resolve these possibilities.

Because drug elimination is complex, any single measure of drug clearance is unlikely to be completely predictive. Most ABCB1 substrates are subject to other drug clearance mechanisms such as CYP3A-mediated metabolism and cellular uptake by organic anion transporters, variation in these may also have an impact on the apparent ABCB1 genotype-phenotype relationship.

High-throughput genotyping technology, which allows rapid screening of multiple drug clearance genes at a relatively low cost, is a candidate method for more comprehensive assessment of the overall drug clearance capability of an individual. However, as with ABCB1, precise genotype-phenotype relationships are yet to be determined for many drug clearance genes. Furthermore, as illustrated by this study, in which only up to 19% of the overall variability in $^{99m}$Tc-MIBI $k_{H}$ was attributable to the ABCB1 variants, genotype alone may be a weak indicator of phenotype.

In vivo functional studies using drug probes to estimate ABCB1 activity are more cumbersome to perform but have the advantage of reflecting the combined impact of genotype and nongenetic factors, such as drug interactions, on function. $^{99m}$Tc-MIBI hepatic scanning avoids the multiple venous samplings required for formal clearance studies of ABCB1 substrates such as digoxin or fexofenadine and can be repeated safely because of the low radiation exposure. Furthermore, because most cytotoxic agents are given intravenously, measurement of hepatic ABCB1 activity by $^{99m}$Tc-MIBI hepatic scanning has relevance over clearance of orally administered agents. One drawback is that $^{99m}$Tc-MIBI hepatic scanning requires continuous scanning for 90 minutes, which can be troublesome for some cancer patients. Limited sampling techniques with shorter scanning time are under investigation.
In conclusion, \( ^{99}\text{Tc-MIBI} \) hepatic scanning is a potential in vivo probe of hepatic ABCB1 activity that may provide a useful a priori approach to estimating clearance of cytotoxic agents that are ABCB1 substrates. Further studies that examine the relationship between \( ^{99}\text{Tc-MIBI} \) clearance and pharmacokinetics of chemotherapeutic agents are underway.

We thank Karen Byth for her assistance in statistical analysis and Nicholas Wilcken for helpful comments on the manuscript. All authors have declared no conflicts of interest in relation to this study.

References


Relationship of systemic exposure to unbound docetaxel and neutropenia

**Objective:** Our objective was to evaluate the association between exposure to unbound docetaxel and neutropenia in patients with cancer and to identify factors influencing unbound docetaxel clearance.

**Methods:** Docetaxel was administered once every 3 weeks at a dose of 75 mg/m² to 49 patients with normal liver function (n = 40, group 1) or mild elevations in liver function test results (n = 9, group 2) or at a dose of 50 mg/m² to patients with moderate elevations in liver function test results (n = 6, group 3). Pharmacokinetic studies and toxicity assessments were performed during the first cycle of therapy. Total docetaxel concentrations were determined by HPLC and tandem mass spectrometry, and unbound docetaxel fraction was determined by equilibrium dialysis.

**Results:** In patients with normal liver function, unbound docetaxel disposition was characterized by mean (±SD) maximum plasma concentration (C_{max}), area under the curve (AUC), and clearance values of 233 ± 101 ng/mL, 32 ± 143 ng/mL · h, and 565 ± 329 L/h, respectively. Unbound clearance varied 8.5-fold; polysorbate 80 exhibited mean (±SD) C_{max}, AUC, and clearance values of 451 ± 221 µg/mL, 528 ± 217 µg/mL · h, and 8.18 ± 3.66 L/h, respectively; and clearance varied 6.7-fold. Unbound docetaxel clearance was reduced in patients with moderate liver impairment (groups 1 and 2 versus group 3, P = .020). From multiple linear regression analysis, only polysorbate 80 AUC and liver impairment were significantly associated with unbound docetaxel clearance. Both unbound docetaxel AUC and total AUC were correlated with the percentage decrements in absolute neutrophil count (P = .002 and P = .029, respectively), as well as the worst grade of neutropenia (P = .013 and P = .220, respectively), where higher exposure was associated with worse hematologic toxicity.

**Conclusions:** Exposure to unbound docetaxel is closely related to drug-induced hematologic toxicity and should be considered in future pharmacologic investigations. (Clin Pharmacol Ther 2005;77:43-53.)

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Docetaxel is a semisynthetic taxane derived from an extract of the needles of the European yew tree (*Taxus baccata*) and acts by disrupting the microtubule network.¹ The drug has significant antitumor activity against numerous tumors and is approved for treatment of locally advanced or metastatic breast, non–small cell lung, and androgen-independent prostate cancers at doses ranging from 60 to 100 mg/m² administered as a 1-hour infusion every 3 weeks. Neutropenia is the dose-limiting toxicity of docetaxel administered on schedules of once every 3 weeks. In patients with normal liver function receiving docetaxel at 75 mg/m², grade 3/4 and febrile neutropenia occur in 65% and 6.3% of patients, respectively.² Other side effects include alopecia, asthenia, dermatologic reactions, fluid retention, hypersensitivity reactions, and stomatitis.

Docetaxel’s pharmacokinetic profile is characterized by substantial interpatient variability in total clearance (>10-fold), which has important clinical consequences. In a population pharmacokinetic-pharmacodynamic study of more than 600 patients receiving docetaxel monotherapy at doses ranging from 75 to 100 mg/m² administered every 3 weeks, a reduction in total do-
cetaxel clearance by 50% was associated with increases in the odds of development of grade 4 neutropenia and febrile neutropenia by 430% and 300%, respectively. Even a 25% decrease in docetaxel clearance is associated with a 150% increase in the odds of development of febrile neutropenia. In addition, docetaxel exposure has also been related to treatment efficacy. The area under the curve (AUC) was a significant predictor of time to tumor progression in non–small cell lung cancer, and data from a clinical trial simulation indicated that underdosing (eg, lower docetaxel AUC because of increased clearance) was associated with a worse time to progression and time to death.

Elimination routes of docetaxel are mediated principally by the hepatic cytochrome P450 (CYP) 3A isoforms CYP3A4 and CYP3A5. The major docetaxel metabolites and less than 10% of the parent drug are excreted into the feces, whereas total urinary excretion is less than 10%. The metabolites demonstrate substantially reduced cytotoxic activity as compared with the parent drug, making biotransformation by CYP3A a major route of inactivation. Liver impairment with concurrent elevations in serum transaminase concentrations in the presence of a normal total bilirubin level is associated with reduced total clearance by 25%, and an elevated total bilirubin level greater than or equal to 1.5 times the upper limit of institutional normal is associated with reduced total docetaxel clearance by greater than 50%.

Docetaxel is extensively bound to albumin and α₁-acid glycoprotein (AAG), with the latter being the main determinant of variability in docetaxel serum binding. In cancer patients, AAG concentrations vary approximately 5-fold between patients, and these variations may contribute to differences in protein binding and systemic drug clearance. From population pharmacokinetic modeling analyses, AAG has been identified as a significant predictor of total docetaxel clearance, with high AAG levels being associated with reduced docetaxel clearance. Recently, it has been shown that the plasma binding of docetaxel is further influenced by the presence of its formulation vehicle, polysorbate 80, such that the unbound fraction of docetaxel increases during the docetaxel infusion. This time-dependent change in unbound fraction resulted, on average, in a 12% higher unbound fraction on the basis of the AUC ratio of unbound to total drug, as compared with that obtained from pretreatment plasma concentrations.

The clinical significance of an increasing unbound fraction of docetaxel during the infusion is unknown. The purpose of this study was to investigate the association between unbound docetaxel exposure and the principal toxicity, neutropenia. In addition, an analysis was undertaken to identify factors influencing unbound docetaxel clearance.

METHODS

Patient eligibility. Patients were eligible when they had histologically or cytologically confirmed solid tumors, for which docetaxel was a viable treatment option. Other criteria for patient enrollment were as follows: (1) age of 18 years or older; (2) performance score of less than 3 according to the Eastern Cooperative Oncology Group criteria; (3) adequate bone marrow function as defined by pretherapy values of hemoglobin of 8.0 g/dL or greater, absolute neutrophil count (ANC) of 1500/µL or greater, and platelet count of 100,000/µL or greater; (4) creatinine level less than or equal to 2.0 times the institutional upper limit of normal (ULN); (5) total bilirubin level less than 1.5 × ULN; (6) any elevations in AST/ALT (if alkaline phosphatase concentration was <2.5 × ULN) or any elevation in alkaline phosphatase concentration (if AST/ALT was <1.5 × ULN); patients with ALT or AST greater than or equal to 1.5 × ULN with concomitant alkaline phosphate greater than or equal to 2.5 × ULN, considered mild liver impairment, were eligible for treatment and received a reduced dose of docetaxel; (7) peripheral neuropathy of grade 1 or lower and no symptomatic brain metastasis; (8) no previous treatment with docetaxel; and (9) no concomitant use of phenytoin, carbamazepine, barbiturates, rifampin (INN, rifampicin), phenobarbital, St John’s wort, and ketoconazole. All concomitant drugs and the use of herbal medicines were recorded. The clinical protocols were approved by the local institutional review boards (Rotterdam, The Netherlands, Baltimore, Md, and Washington, DC), and all patients provided written informed consent before enrollment. Before treatment, a complete registration form was received by the coordinating center (Baltimore, Md) and a study number was assigned.

Retrospectively, patients were grouped according to baseline liver function to determine the association between elevations in liver transaminase concentrations or alkaline phosphatase and unbound docetaxel clearance. Patients in liver function group 1 had no elevations in AST/ALT or alkaline phosphatase concentrations as described for liver function groups 2 and 3. Patients in liver function group 2 had concurrent elevations in transaminase and alkaline phosphatase concentrations as follows: AST/ALT greater than 1.0 × ULN concurrent with alkaline phosphatase greater than or equal to 2.5 × ULN, AST/ALT greater than or equal to 1.5 × ULN concurrent with alkaline phosphatase concentration.
greater than 1.0 × ULN, or isolated elevations of AST/ALT or alkaline phosphatase greater than or equal to 5.0 × ULN. Patients in liver function group 3 had concurrent elevations in transaminase concentrations as previously described:3 AST/ALT greater than or equal to 1.5 × ULN concurrent with alkaline phosphatase greater than or equal to 2.5 × ULN.

Drug treatment. The clinical docetaxel preparation (Taxotere; Aventis Pharmaceuticals, Bridgewater, NJ) containing 20 or 80 mg of the drug formulated in 0.5 mL and 2.0 mL of polysorbate 80, respectively, was diluted with a solution of 13% ethanol in water to a concentration of 10 mg docetaxel per milliliter. This solution was diluted further in a 250-mL infusion bag containing 20 or 80 mg of the drug formulated in 0.5 mL and 2.0 mL of polysorbate 80, respectively, was diluted with a solution of 13% ethanol in water to a concentration of 10 mg docetaxel per milliliter. This solution was diluted further in a 250-mL infusion bag to produce a final concentration of 10 mg docetaxel per milliliter. After 1 cycle of therapy, treatment continued at the discretion of the treating physician until tumor progression, development of unacceptable toxicity, or patient withdrawal.

Patient evaluation. The extent of prior cytotoxic treatment was assessed with regard to the following 2 factors: (1) the number of prior treatment regimens and (2) whether patients were considered to be heavily pretreated (ie, if they received ≥2 cycles of mitomycin C, ≥4 cycles of carboplatin, or ≥6 cycles of cisplatin or an alkylating agent). Pretreatment evaluations included assessment of performance score, height, weight, toxicity assessment, a complete blood cell count with differential cell count, and the following serum chemistry analyses: creatinine, alkaline phosphatase, AST, ALT, total bilirubin, and albumin. AAG concentrations were determined in serum obtained from each patient on the first day, immediately before the administration of docetaxel.

Toxicity assessment and a complete blood cell count with differential cell count were performed weekly for a total of 3 weeks (1 cycle). Toxicity assessments were performed according to the National Cancer Institute Common Toxicity Criteria, version 2.0.12a Management of toxicity was done at the discretion of the treating physician per institutional guidelines.

Pharmacokinetic sampling and assay. Blood samples were collected for docetaxel and polysorbate 80 pharmacokinetic studies during the first cycle of treatment at the following time points: before the infusion, at 30 minutes into the infusion, at 59 minutes into the infusion (immediately before the end of the infusion), and after the infusion at 10 and 30 minutes and 1, 3, 7, 24, and 48 hours and on day 8. Samples were collected in a 10-mL heparinized tube and placed on ice until further processing within 30 minutes of collection. Plasma was isolated by centrifugation at 4°C. at 1000g for 10 minutes, and frozen at or below −20°C until the time of analysis.

Total docetaxel concentrations and polysorbate 80 concentrations were quantitated in plasma over the range of 0.50 to 100 nmol/L and 1 to 100 μg/mL, respectively, by use of a validated liquid chromatographic method with tandem mass spectrometric detection, as previously described.13 The bias and precision of quality control samples were less than 15%. At the assay lower limit of quantitation, bias and precision were less than 20%, as per the guidelines provided in the Food and Drug Administration Guidance for Industry Bioanalytic Method Validation.14

The unbound fraction of docetaxel in plasma (fU) was determined by use of a validated method based on equilibrium dialysis as previously described.15 In brief, equilibrium dialysis was conducted on a rotator at 37°C in a humidified atmosphere of 5% carbon dioxide by use of 96-well microdialysis plates (Harvard Apparatus, Holliston, Mass). The dialysis compartments in each well were separated by a regenerated cellulose membrane with a 5-kd molecular weight cutoff (Harvard Apparatus). Experiments were carried out with 250-μL aliquots of plasma containing a tracer amount of \textsuperscript{[G-3H]}docetaxel (Moravek Biochemicals, Brea, Calif) against an equal volume of 0.01-mol/L phosphate buffer (pH 7.4). After a 4-hour reaction time, measurement of total radioactivity in both compartments was measured by liquid scintillation counting for 1 minute. The tritium label is relatively stable, and the volume shift during dialysis is negligible; hence the results were used directly without application of a correction factor. The concentration of unbound docetaxel (C\textsubscript{u}) was calculated from the concentration of total...
Docetaxel in plasma (\(C_u\)) as follows: \(C_u = C_p \times f_u\). The within-run and between-run variability (reproducibility) and bias (accuracy) of the method were less than 15% on the basis of repeat analyses of quadruplicate samples with differing docetaxel \(f_u\) values (depending on the spiked polysorbate 80 concentration) on 6 consecutive days. The mean relative SD was less than 10%, ensuring high discriminatory power in the detection of changes in the \(f_u\) in patient samples with different AAG and polysorbate 80 concentrations.

With the pharmacokinetic sampling scheme used, total docetaxel concentrations are best described by a 3-compartment model.\(^{16}\) Because the unbound docetaxel concentrations increased at a different rate during the infusion because of the apparent binding disequilibrium, standard noncompartmental methods were used for calculation of pharmacokinetic parameters, including those for total docetaxel and polysorbate 80. To determine the contribution of increasing unbound fraction to overall unbound docetaxel exposure, unbound docetaxel concentrations were calculated for estimation of pharmacokinetic parameters twice, as follows: (1) Unbound fraction measured at each sampling time point was multiplied by the corresponding total concentration (\(C_u = C_p \times f_u\)) and (2) unbound fraction measured before treatment was calculated from the total concentration at each time point after the start of the docetaxel infusion (\(C_u = C_p \times \text{Pretreatment } f_u\)). Values for observed unbound maximum plasma concentration (\(C_{\text{max}}\)) and predicted unbound \(C_{\text{max}}\) based on the pretreatment \(f_u\) (\(C_{\text{max, pretreatment } f_u}\)) and values for observed unbound AUC and predicted AUC based on the pretreatment \(f_u\) (\(AUC_{\text{pretreatment } f_u}\)) were then compared. Noncompartmental analysis was performed by use of the software program WinNonlin, version 3.0 (Pharsight Corporation, Mountain View, Calif).

**Statistical analysis.** Docetaxel and polysorbate 80 pharmacokinetic parameters were summarized as the mean (SD), and range. For continuous variables, nonparametric tests were used to compare mean values between different groups. When 3 or more groups were compared, a trend test was used.\(^{17}\) Categoric variables were compared by use of the 2-tailed Fisher exact test for 2-by-2 tables. Multiple linear regression models were used to assess the influence of polysorbate 80 exposure (\(C_{\text{max}}, \text{AUC}\), AAG, age, and liver function group (2 or 3) (predictor variables) on unbound docetaxel clearance (outcome variable). Predictor variables were evaluated for colinearity; if several variables were correlated, only 1 was maintained in the model. Regression coefficients, SEs of the coefficients, and the associated \(P\) values were determined from the multiple linear regression modeling. Stepwise backward elimination was performed to systematically exclude the least significant factors until the \(P\) value was <.05. Multiple linear regression modeling was performed by use of the software program Stata, version 8.2 (Stata Corporation, College Station, Tex). Because this study was mainly exploratory in intent, a Bonferroni adjustment was not used to evaluate the significance of the multiple comparisons, and hence the a priori level of significance was set at \(P < .05\).

**RESULTS**

Pharmacokinetic studies were performed in 55 cancer patients receiving docetaxel therapy. Forty-eight patients received 75 mg/m\(^2\) docetaxel and 7 patients received 50 mg/m\(^2\). One patient receiving 50 mg/m\(^2\) erroneously received a lower docetaxel dose; the intended dose was 75 mg/m\(^2\). Patient characteristics as a function of liver function group are summarized in Table I. None of the patients received any growth factors. Pharmacokinetic parameters for total docetaxel and the \(f_u\) have been published previously in 9 and 5 patients, respectively.\(^{13,15}\)

**Docetaxel pharmacokinetics.** Concentration-time curves for unbound and total docetaxel from a representative patient are shown in Fig 1, A and B; this patient, representing liver function group 1, had average values for unbound and total docetaxel pharmacokinetic parameters and change in \(f_u\) during the docetaxel infusion. Unbound docetaxel pharmacokinetic parameters are summarized in Table II. In 40 patients receiving 75 mg/m\(^2\) docetaxel with normal liver function tests (liver function group 1), unbound docetaxel disposition was characterized by mean (±SD) \(C_{\text{max}},\text{AUC, and clearance values of 233 ± 101 ng/mL, 321 ± 143 ng/mL · h, and 565 ± 329 L/h, respectively, and unbound clearance varied 8.5-fold. Patients in liver function group 2 did not have reduced unbound clearance as compared with patients with normal liver function parameters (Table II and Fig 2, A); unbound clearance varied 13.0-fold, which was more variable than that in liver function group 1, most likely as a result of the inclusion of 1 patient with an outlier value for unbound clearance (2770 L/h). A trend for reduced unbound docetaxel clearance in patients with moderate elevations in liver function tests (liver function group 3) was observed (\(P = .020\)).

In 40 patients with normal liver function parameters, the mean (±SD) pretreatment \(f_u\) expressed as a percentage was 4.72% ± 1.79% and protein binding as determined by the ratio of unbound AUC (AUC\(_{\text{unbound}}\)) to total AUC (AUC\(_{\text{total}}\)) expressed as a percentage was
5.66% ± 1.40% (Table II); overall, protein binding as assessed from the ratio of AUC\textsubscript{unbound} to AUC\textsubscript{total} varied over a 2.5-fold range, and the mean values were not significantly different in the 3 liver function groups (Table II), suggesting that protein binding was independent of liver function. Although statistically associated, a strong linear correlation was not observed between pretreatment f\textsubscript{u} and AUC\textsubscript{unbound}/AUC\textsubscript{total} ratio ($R^2 = 0.4593$, $P < .0001$), demonstrating that accurate assessment of unbound docetaxel exposure cannot be determined reliably from the pretreatment f\textsubscript{u} only. The lack of complete concordance between pretreatment f\textsubscript{u} and AUC\textsubscript{unbound}/AUC\textsubscript{total} ratio is most likely a result of increasing unbound fraction after the start of the infusion. Compared with pretreatment values, the unbound fraction was significantly increased at 30 minutes during the infusion and at the end of the infusion ($P = .0012$), with a decline in f\textsubscript{u} after the end of the infusion and a return to values close to baseline f\textsubscript{u} at 3 to 7 hours after infusion (data not shown), as described previously for docetaxel in another cohort of cancer patients. The maximum increase in f\textsubscript{u} occurred at the end of the infusion, where the mean (±SD) f\textsubscript{u} value was 5.68% ± 1.56% (range, 2.78%-9.56%) (N = 55); this represents an average increase in the f\textsubscript{u} of 24% compared with the pretreatment f\textsubscript{u} (mean ± SD, 4.60% ± 1.63%; range, 1.19%-8.63%; N = 55). To determine the contribution of time-dependent changes in f\textsubscript{u} on the exposure to

<table>
<thead>
<tr>
<th>Table I. Patient demographics</th>
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<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Liver function group</strong></td>
</tr>
<tr>
<td><strong>Group 1</strong> (n = 40)</td>
</tr>
<tr>
<td><strong>Group 2</strong> (n = 9)</td>
</tr>
<tr>
<td><strong>Group 3</strong> (n = 6)</td>
</tr>
<tr>
<td>Docetaxel dose (mg/m\textsuperscript{2})</td>
</tr>
<tr>
<td>Age (y) (median, range)</td>
</tr>
<tr>
<td>Body surface area (m\textsuperscript{2}) (median, range)</td>
</tr>
<tr>
<td>Sex (No.)</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>AAG (mg/dL) (median, range)</td>
</tr>
<tr>
<td>Liver function tests (×ULN) (median, range)</td>
</tr>
<tr>
<td>AST</td>
</tr>
<tr>
<td>ALT</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>Total bilirubin</td>
</tr>
<tr>
<td>ECOG performance status* (No.)</td>
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<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Primary tumor type (No.)</td>
</tr>
<tr>
<td>Breast</td>
</tr>
<tr>
<td>Head and neck</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Melanoma</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Angiosarcoma</td>
</tr>
<tr>
<td>Unknown</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Prior treatment (No.)</td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>1-2 Cytotoxic regimens</td>
</tr>
<tr>
<td>≥3 Cytotoxic regimens</td>
</tr>
<tr>
<td>Light</td>
</tr>
<tr>
<td>Heavy</td>
</tr>
</tbody>
</table>

AAG, α\textsubscript{1}-acid glycoprotein; ULN, institutional upper limit of normal; ECOG, Eastern Cooperative Oncology Group.

*Baseline performance status was not established in 1 patient in liver function group 1.
unbound docetaxel, $C_{\text{max}}$ and AUC values were calculated by use of the pretreatment $f_u$ and the observed $f_u$ at each time point (Table II). On average, unbound $C_{\text{max}}$ and AUC values were 28% and 19% higher, respectively, when the observed $f_u$ was used versus the pretreatment unbound fraction, and this effect was consistently observed in all 3 liver function groups (Table II).

Total docetaxel pharmacokinetic parameters (Table II) are similar to those reported previously after administration of 75 mg/m$^2$ docetaxel as a 1-hour infusion.$^{16}$

**Polysorbate 80 pharmacokinetics.** A concentration-time curve for polysorbate 80 from a representative patient is shown in Fig 1, C, and polysorbate 80 pharmacokinetic parameters are summarized in Table II. As described previously,$^{13,18}$ polysorbate 80 concentra-

---

**Table II.** Pharmacokinetic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (75 mg/m$^2$)*</th>
<th>Group 2 (75 mg/m$^2$)</th>
<th>Group 3 (50 mg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 40)</td>
<td>(n = 9)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>Unbound docetaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>233 ± 101 (57.4-489)</td>
<td>213 ± 92.4 (43.8-329)</td>
<td>190 ± 40.3 (137-228)</td>
</tr>
<tr>
<td>AUC (ng/mL·h)</td>
<td>321 ± 143 (96.4-584)</td>
<td>296 ± 176 (50.9-654)</td>
<td>361 ± 132 (217-533)</td>
</tr>
<tr>
<td>Clearance (L/h)</td>
<td>565 ± 329 (207-1763)</td>
<td>715 ± 790 (199-2770)</td>
<td>269 ± 94.9 (161-414)</td>
</tr>
<tr>
<td>Pretreatment $f_u$ (%)</td>
<td>4.72 ± 1.79 (1.19-8.63)</td>
<td>4.42 ± 1.32 (2.71-6.58)</td>
<td>4.00 ± 0.56 (3.34-4.75)</td>
</tr>
<tr>
<td>$C_{\text{max}}$/AUC$_{\text{total}}$ (%)</td>
<td>5.66 ± 1.40 (3.41-8.59)</td>
<td>4.92 ± 0.98 (3.24-6.33)</td>
<td>4.93 ± 0.84 (3.68-5.80)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ pretreatment $f_u$ (ng/mL)</td>
<td>181 ± 92.1 (42.4-390)</td>
<td>164.8 ± 71.3 (40.8-247)</td>
<td>151 ± 55.3 (83.5-241)</td>
</tr>
<tr>
<td>AUC$_{\text{pretreatment}}$ $f_u$ (ng/mL·h)</td>
<td>268 ± 147 (59.1-667)</td>
<td>233 ± 140 (42.5-496)</td>
<td>323 ± 141 (178-565)</td>
</tr>
<tr>
<td>Total docetaxel</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>3.89 ± 1.49 (0.88-6.44)</td>
<td>4.14 ± 1.60 (1.51 ± 6.52)</td>
<td>3.56 ± 1.27 (1.82-5.35)</td>
</tr>
<tr>
<td>AUC (µg/mL·h)</td>
<td>5.75 ± 2.51 (2.30-12.1)</td>
<td>5.95 ± 3.55 (1.57-13.4)</td>
<td>7.54 ± 3.06 (3.75-12.6)</td>
</tr>
<tr>
<td>Clearance (L/h)</td>
<td>30.0 ± 14.2 (12.4-74.0)</td>
<td>30.7 ± 23.8 (9.69-89.8)</td>
<td>13.4 ± 6.02 (7.17-24.0)</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>451 ± 221 (210-1199)</td>
<td>379 ± 163 (28.9-593)</td>
<td>344 ± 88.5 (244-458)</td>
</tr>
<tr>
<td>AUC (µg/mL·h)</td>
<td>528 ± 217 (244-1212)</td>
<td>466 ± 190 (56.2-725)</td>
<td>484 ± 173 (208-708)</td>
</tr>
<tr>
<td>Clearance (L/h)</td>
<td>8.18 ± 3.66 (3.00-20.2)</td>
<td>13.1 ± 19.6 (4.30-65.2)</td>
<td>5.62 ± 2.50 (3.11-9.95)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD and range. $C_{\text{max}}$, Maximum plasma concentration; AUC, area under curve; $f_u$, unbound fraction of docetaxel in plasma; AUC$_{\text{unbound}}$, unbound area under curve; AUC$_{\text{total}}$, total area under curve; $C_{\text{max}}$ pretreatment $f_u$, predicted unbound maximum plasma concentration based on pretreatment unbound fraction; AUC$_{\text{pretreatment}}$ $f_u$, predicted area under curve based on pretreatment unbound fraction.

*One patient erroneously received 50 mg/m$^2$, and $C_{\text{max}}$ and AUC values for this patient were not included in the summary statistics.
tions were undetectable after 4 hours (3 hours after infusion) in all but a few patients. In patients receiving 75 mg/m² docetaxel in liver function group 1, polysorbate 80 exhibited mean (±SD) $C_{\text{max}}$, AUC, and clearance values of 451 ± 221 µg/mL, 528 ± 217 µg/mL · h, and 8.18 ± 3.66 L/h, respectively; in this population, polysorbate 80 clearance varied 6.7-fold. The clearance of polysorbate 80, similar to that of unbound docetaxel, was not reduced in patients in liver function group 2, but a trend for reduced clearance was observed in patients with moderate elevations in liver function tests (liver function group 3), although this did not reach a level of statistical significance ($P = .092$).

**Predictors of unbound docetaxel clearance.** AAG concentration was significantly correlated with the pretreatment unbound fraction ($R^2 = 0.3863$, $P < .0001$) (Fig 3, A). However, no association was noted between AAG concentration and unbound docetaxel clearance (Fig 3, B). This was expected because the unbound clearance is classically used to examine factors other than protein binding that influence clearance (eg, hepatic blood flow, enzyme activity) and because the use of unbound clearance eliminates the confounding effects of binding to proteins or other macromolecules in the systemic circulation. This lack of association is also supported by the notion that the mean unbound clearance of docetaxel in normal patients (liver function group) was 565 ± 329 L/h, which is approximately 2-fold greater than cardiac output. Considering what is known regarding predictors of total docetaxel clearance, specifically AAG concentration, age, and liver function, a multiple linear regression analysis was performed to determine whether these variables in addition to polysorbate 80 exposure were associated with un-
Table III. Multiple regression model for unbound clearance of docetaxel*

<table>
<thead>
<tr>
<th></th>
<th>Coefficient</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>5.94</td>
<td>0.0998</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>1/Polyorbate 80 AUC</td>
<td>112</td>
<td>29.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Liver function (group 3)</td>
<td>-0.663</td>
<td>0.209</td>
<td>.003</td>
</tr>
</tbody>
</table>

For the overall model, $R^2 = 0.3224$ and $P < .0001$.

*Because unbound clearance has a skewed distribution, log-transformed clearance was used as the primary end point in this analysis. Unbound clearance is lower, with a negative coefficient value.

bound docetaxel clearance. Although polysorbate $C_{\text{max}}$ and AUC were associated with unbound docetaxel clearance, polysorbate 80 AUC was a better predictor. In addition, because $C_{\text{max}}$ and AUC were highly correlated, only polysorbate AUC was included in the multivariate model. However, the reciprocal of polysorbate 80 AUC yielded better prediction than untransformed AUC, and thus 1/Polyorbate AUC was included in the model. Furthermore, because unbound clearance had a skewed distribution, unbound clearance was log-transformed. After stepwise backward deletion, only 1/Polyorbate AUC and liver function group 3 were retained in the final model ($R^2 = 0.3224$, $P < .00001$) (Table III), which explained 32% of the total variability. Fig 2, B, illustrates the relationship between polysorbate 80 AUC and unbound docetaxel clearance, where higher AUC values are associated with lower unbound clearance values; polysorbate 80 alone accounted for 19% of the variability in unbound docetaxel clearance.

Relationship between unbound docetaxel exposure and neutropenia. Because of missing blood cell counts in 3 patients, only 52 of 55 patients were evaluable for hematologic toxicity. The incidence of grade 3 and 4 neutropenia and febrile neutropenia and percentage decrements in ANC is summarized in Table IV. Overall, 26 of 52 patients had grade 4 neutropenia and 4 of 26 patients with grade 4 neutropenia had febrile neutropenia.

The association between unbound docetaxel AUC and neutropenia was assessed, as was the relationship between total docetaxel AUC and neutropenia, for the purpose of comparison. Patients in liver function group 3 received a reduced dose of docetaxel to achieve total docetaxel AUC values similar to those in liver function groups 1 and 2. 3,7 AUC values for both unbound docetaxel and total docetaxel were similar in group 3 and groups 1 and 2 combined ($P = .395$); therefore AUC values for both unbound docetaxel and total docetaxel at the different dose levels were combined to assess the relationship between docetaxel exposure and neutropenia. Both unbound and total AUC correlated significantly with neutropenia. The association appeared stronger with both percentage decrements in ANC ($P = .002$ for unbound versus $P = .029$ for total) (Fig 4) and worst grade of neutropenia ($P = .013$ for unbound versus $P = .220$ for total) (Fig 5) for unbound docetaxel AUC. However, the study, which was exploratory in nature, was not powered for a direct comparison between these 2 measures of exposure. The percentage decrements in ANC were greater in those patients with unbound AUC values in the upper quartile (mean decrement, 95.4%) compared with those with AUC values in the interquartile range (mean decrement, 76.9%) ($P = .020$) (Fig 4, A). Likewise, patients in whom grade 4 neutropenia developed had significantly higher unbound AUC values (mean, 374 ng/mL · h) than patients with grade 0 to 3 neutropenia (mean, 275 ng/mL · h) ($P = .013$) (Fig 5, A). In addition, all patients in whom neutropenic fever developed had unbound AUC values above the overall mean value, whereas only 3 of 4 patients had total AUC values above the overall mean value.

DISCUSSION

Previous studies examining relationships between the pharmacokinetics of the anticancer drug docetaxel and treatment outcome have consistently focused on measurement of total docetaxel concentrations in plasma, disregarding effects of variable levels of systemic binding on the fraction-unbound drug. This study demonstrates that the hematologic toxicity induced by docetaxel is significantly correlated with the systemic exposure to unbound drug.

Variability in systemic drug binding has frequently been demonstrated in humans. However, the clinical significance of this variability to drug disposition and pharmacodynamics depends largely on intrinsic pharmacokinetic characteristics of the drug. For most anticancer drugs, the interindividual variation in plasma protein binding is quite small in patients with normal liver function and drug-metabolizing capability. Therefore vascular binding is usually not an important consideration in therapeutic drug monitoring and in the evaluation of pharmacokinetic-pharmacodynamic relationships. However, in rare instances, the total concentration is not reflective of the unbound drug level. For some anticancer agents, this situation arises if the drug demonstrates protein concentration–dependent binding (eg, imatinib and 7-hydroxystaurosporine) or when irreversible or near-covalent binding occurs after therapeutic doses of an anticancer drug (eg, platinum-
containing agents). Indeed, cisplatin, carboplatin, and oxaliplatin are currently the only agents for which unbound concentrations are routinely measured and for which the relationship between unbound drug and therapeutic effects has been demonstrated.24

The significant relationship between exposure to total docetaxel and the percentage decrease in neutrophil count at nadir, as described previously in a study involving more than 600 patients receiving docetaxel in similar regimens,7 was not observed in our investigation. It is likely that this apparent inconsistency is a direct consequence of the differences in sample size, which might become an important consideration when the pharmacodynamic evaluation is based on a sparse set of hematologic toxicity data (ie, with blood cells measured once a week). Experimental evidence has

Table IV. Hematologic toxicity

<table>
<thead>
<tr>
<th>Liver function group</th>
<th>No. of patients</th>
<th>Neutropenia (No. of patients)</th>
<th>Decrease in ANC (%) (median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grade 3</td>
<td>Grade 4</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>6 (15%)</td>
<td>23 (58%)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3 (43%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0 (0%)</td>
<td>2 (40%)</td>
</tr>
</tbody>
</table>

ANC, Absolute neutrophil count.
also suggested that information on the entire time course of changes in blood cell counts might be a more physiologically relevant end point than the nadir count.\textsuperscript{12} To further resolve this issue with respect to docetaxel, a mechanism-based population analysis for docetaxel-mediated neutropenia is currently being planned with the entire time course of neutrophils taken into account in a larger population of patients.

The current data on docetaxel describe a previously unrecognized type of time dependence for the fraction-unbound drug, which is consistent with in vitro evidence indicating a concentration-dependent influence of its formulation vehicle, polysorbate 80, on the \( f_u \).\textsuperscript{12} In our study, although the \( f_u \) in pretreatment samples was correlated with individual levels of AAG, only exposure to polysorbate 80 (and not AAG) was significantly associated with the clearance of unbound docetaxel in both univariate and multivariate analyses.

The mechanistic basis for a possible influence of polysorbate 80 on docetaxel \( f_u \) during the infusion is as yet unknown, although, on the basis of the available data from our study, it cannot be excluded that this correlation is the result of the fixed ratio of the doses of the 2 agents; patients with a high volume of distribution (eg, lean body mass) and high hepatic blood flow and function would be expected to have high volumes of distribution and clearance, respectively, of both agents. Likewise, the converse would be expected such that the AUCs of both compounds would very likely be interrelated but not necessarily through a causative association. Nevertheless, on the basis of theoretic considerations, it is possible that polysorbate 80 or its metabolites (eg, oleic acid) interfere with the binding of docetaxel to albumin and AAG\textsuperscript{1} and lead to a temporary increase in the fraction-unbound drug. However, other possible mechanisms, including inhibition of CYP3A-mediated\textsuperscript{26,27} or P-glycoprotein–mediated elimination\textsuperscript{28} of docetaxel by polysorbate 80 early after drug administration, cannot be excluded. These and several other possible mechanisms are currently under further investigation.

Collectively, the findings of this study demonstrate that the exposure to unbound docetaxel is more closely related than total drug to drug-induced hematologic toxicity. Given that an increase in systemic exposure to unbound drug was associated with more severe neutropenia, these findings suggest that determination of unbound docetaxel concentrations is indicated for future pharmacologic investigations. Currently, a population analysis for docetaxel-mediated neutropenia is being planned with the entire time course of blood cell counts taken into account through the use of a semiphysiologic mechanism-based pharmacodynamic model.

We thank Drs Ming Zhao (Baltimore, Md), Milin R. Acharya (Bethesda, Md), and Luca Paoluzzi (Bethesda, Md) for their contribution to this work and the participating investigators at The Sidney Kimmel Comprehensive Cancer Center (Dr Michael Carducci) and Franklin Square Hospital Center (Dr William McGuire), Baltimore, Md, and Howard University Cancer Center (Dr Fitzroy Dawkins), Washington, DC.

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References


Marked effect of liver and kidney function on the pharmacokinetics of selegiline

**Objectives:** The pharmacokinetics of selegiline was investigated in an open study with 4 parallel groups of 10 subjects in each. Patients with liver disease, those receiving a drug that induced hepatic enzyme activity, and those with impaired kidney function were compared with control subjects.

**Methods:** A single oral 20-mg dose of selegiline was administered after an overnight fast, and blood samples were collected over a period of 48 hours. Concentrations of serum selegiline and its main metabolites were determined and pharmacokinetic parameters calculated.

**Results:** The pharmacokinetic parameters of selegiline differed considerably between the patient groups and the control subjects. The area under the concentration-time curve of serum selegiline was, on average, 18-fold higher ($P < .05$) in patients with impaired liver function, 23-fold lower ($P < .001$) in patients with drug-induced liver function, and 6-fold higher ($P < .05$) in patients with impaired kidney function as compared with the control subjects. There was a large interindividual variation in every group. The changes in selegiline metabolite kinetics supported the changes in the kinetics of the parent compound.

**Conclusion:** The elimination rate of selegiline was substantially increased in patients with drug-induced liver function and decreased in patients with impaired liver or kidney function when compared with control subjects. These results suggest that selegiline dosage adjustments may be required in patients with altered liver and kidney function. (Clin Pharmacol Ther 2005;77:54-62.)

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Selegiline (l-deprenyl) is a selective irreversible inhibitor of monoamine oxidase type B. It inhibits the breakdown of dopamine in the human brain and is thus useful in the treatment of Parkinson’s disease both as monotherapy and together with levodopa.1,2 It has been shown to prolong and potentiate the duration of action of levodopa. At the neuronal level, selegiline is also a dopamine reuptake inhibitor. Interest has also been directed toward the drug as a neuroprotective or neuronal rescue agent.3 Selegiline is usually used at a dose of 5 to 10 mg daily, and the maximum dose used in the treatment is 30 mg daily.

Selegiline is rapidly and completely absorbed from the gastrointestinal tract; however, the oral bioavailability is only about 10% as a result of extensive hepatic first-pass metabolism.4,5 Selegiline is metabolized primarily into desmethylselegiline and l-methamphetamine and further into l-amphetamine.6,7 In vitro studies suggest that several hepatic cytochrome P450 (CYP) enzymes are involved in the formation of metabolites (Fig 1). At least CYP2B6, CYP2C19, and CYP1A2 are active catalysts of primary oxidative routes of selegiline metabolism.8,10 On this basis, it is expected that liver impairment or activation may influence the pharmacokinetics of selegiline. Because of negligible urinary excretion of unchanged selegiline,7 renal impairment is not expected to greatly affect the kinetics of selegiline. Most patients receiving selegiline therapy (eg, patients with Parkinson’s disease) are elderly subjects in whom age, liver disease, or concomitant drug therapy may influence the pharmacokinetics of selegiline and require dosage adjustments. Thus the aim of this study was to investigate the effect of altered (impaired and drug-induced) liver and im-
paired kidney function after a single oral selegiline dose.

METHODS

Subjects. This study comprised 40 volunteers (21 women and 19 men) who were classified into 4 groups (10 each) according to their liver and kidney function (Table I). Subjects in group 1 (control group) included normal healthy subjects who had no evidence of liver or kidney disease. These subjects were ascertained to be in good health by medical history, clinical examination, and standard hematologic and blood chemistry tests before entering the study. Patients in group 2 (liver disease group) had a diagnosis of liver dysfunction that had been confirmed histologically, and their liver function test results were abnormal and clearly distinguished from those of the control subjects. There was no evidence of kidney disease. Group 3 (drug-induced liver group) included patients undergoing therapy with anticonvulsants known to activate the drug-metabolizing enzymes. They had normal liver and kidney function test results. Group 4 (kidney disease group) included patients with stable long-term renal impairment with elevated serum creatinine values. Their liver function test results were in the normal range. Patients in group 4 were older on average than those in the other groups.

Before inclusion in the study and after informed consent was obtained, the medical history was obtained from each participating subject and a clinical examination was performed. Data recorded during the clinical examination included age, height, weight, information on previous diseases, and both previous and concomitant medication (Table II). Electrocardiographic recording was also performed. The subjects underwent a laboratory screening including liver and kidney function tests and glucose balance. The study was conducted according to the principles of the Declaration of Helsinki of the World Medical Assembly. The study protocol was reviewed by the Ethics Committee of Deaconess Institute of Oulu, Oulu, Finland. Before entering the study, each subject received an information text, in which the purpose, possible risks, and duration of the study were explained and the procedures described. After the subject had sufficient time to acquaint himself or herself with this information, the investigator asked for his or her written consent. Written informed consent was always obtained before actual screening procedures were started.

Study procedure. The subjects arrived at the clinical research facility 12 hours before drug administration and remained there until 24 hours after administration. They fasted from the evening (at least 12 hours) before and until 3 hours after the drug administration. Smoking was not permitted during the fasting period. Intake of food containing large amounts of tyramine (aged cheese, yeast products) within 1 week before and 1 week after the drug administration was not allowed. Alcohol intake was prohibited for 2 days before and 48 hours after the drug administration. A light standardized meal was served 3 hours after drug ingestion. No other food was allowed for 7 hours after dosing.

Subjects were given a single dose of two 10-mg tablets of selegiline hydrochloride (Eldepryl; Orion Pharma, Turku, Finland) with 150 mL of tap water. Venous blood samples (10 mL) were collected just before the drug administration and at the following times thereafter: 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 7, 10, 14, 24, 28, and 48 hours (17 samples). Blood samples were centrifuged, and the separated sera were stored at −20°C until the time of assays.

Bioanalytic methods. The concentrations of selegiline and its major metabolites (desmethylselegiline, l-methamphetamine, and l-amphetamine) in serum were determined by a validated gas chromatographic method with nitrogen-selective detection.12 The lower limit of quantitation was 0.05 ng/mL for selegiline, 0.2 ng/mL for desmethylselegiline, and 0.5 mg/mL for both l-methamphetamine and l-amphetamine. The interassay coefficients of variation at a medium serum level of each compound were 9.8% for selegiline (at 1.8 ng/mL), 8.2% for desmethylselegiline (at 10 ng/mL),
11.2% for l-methamphetamine (at 16 ng/mL), and 14.2% for l-amphetamine (at 8 ng/mL).

**Pharmacokinetic analysis.** Noncompartmental pharmacokinetic parameters were calculated by standard methods. Peak concentration (C_{max}) was taken as the maximum observed concentration in serum, and time to peak concentration (t_{max}) was taken as the sampling time at which C_{max} was observed. Area under the serum concentration–time curve (AUC) was calculated by use of the linear trapezoidal rule to the last nonzero concentration and extrapolated to infinity by use of the terminal elimination rate constant (λ_z), which was cal-

### Table I. Demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Control</th>
<th>Impaired liver function</th>
<th>Drug-induced liver function</th>
<th>Impaired kidney function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Women</td>
<td>7</td>
<td>3</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Mean ± SD</td>
<td>56.4 ± 6.8</td>
<td>57.9 ± 8.0</td>
<td>53.5 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>48-68</td>
<td>47-69</td>
<td>45-71</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td></td>
<td>63.5 ± 10.4</td>
<td>87.8 ± 16.8</td>
<td>68.9 ± 14.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td></td>
<td>163.3 ± 9.4</td>
<td>174.2 ± 10.1</td>
<td>163.7 ± 9.4</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td>23.7 ± 3.0</td>
<td>28.7 ± 3.4</td>
<td>25.5 ± 3.8</td>
</tr>
<tr>
<td>Serum chemistry studies*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td></td>
<td>23.9 ± 9.3</td>
<td>46.4 ± 29.7</td>
<td>24.6 ± 7.4</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td></td>
<td>23.1 ± 7.7</td>
<td>64.1 ± 53.3</td>
<td>30.5 ± 12.1</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td></td>
<td>127 ± 30</td>
<td>253 ± 164</td>
<td>176 ± 66</td>
</tr>
<tr>
<td>γ-Glutamyltransferase (U/L)</td>
<td></td>
<td>25.2 ± 7.8</td>
<td>254 ± 422</td>
<td>57.4 ± 35.5</td>
</tr>
<tr>
<td>PIIINP (μg/L)</td>
<td></td>
<td>3.48 ± 0.68</td>
<td>5.47 ± 2.30</td>
<td>3.06 ± 0.78</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td></td>
<td>5.19 ± 1.20</td>
<td>5.67 ± 1.94</td>
<td>5.38 ± 1.56</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td></td>
<td>78.0 ± 15.1</td>
<td>79.6 ± 11.9</td>
<td>79.6 ± 11.8</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD (n = 10 in each group).

PIIINP, Type III procollagen amino terminal peptide.

*The range of institutional normal values for the serum chemistry parameters are as follows: AST and ALT, 10 to 35 U/L; alkaline phosphatase, 50 to 200 U/L; γ-glutamyltransferase, 5 to 50 U/L; PIIINP, 1.7 to 4.2 μg/L; urea, 1.7 to 8.3 mmol/L; and creatinine, 55 to 115 μmol/L.

### Table II. Medications taken by study subjects in different groups

<table>
<thead>
<tr>
<th>Subject group</th>
<th>No medication</th>
<th>Potentially inducing drugs*</th>
<th>Other drugs*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>None</td>
<td>Diltiazem, enalapril, and sotalol</td>
</tr>
<tr>
<td>Impaired liver function</td>
<td>1</td>
<td>None</td>
<td>Furosemide (n = 4), sotalol (n = 3), prednisolone (n = 3), digoxin (n = 2), glipizide (n = 2), insulin (n = 2), glyburide (INN, glibenclamide), betaxolol, ibuprofen, and guaram</td>
</tr>
<tr>
<td>Drug-induced liver function</td>
<td>0</td>
<td>Phenobarbital (n = 7), phenytoin, carbamazepine, and amobarbital</td>
<td>Sotalol (n = 4), metoprolol, propranolol, verapamil, digoxin, furosemide, glyceryl nitrate, lisinopril, and nefedipine</td>
</tr>
<tr>
<td>Impaired kidney function</td>
<td>1</td>
<td>None</td>
<td>Furosemide (n = 4), aspirin (n = 4), glycercyl nitrate (n = 3), lisinopril (n = 3), metformin (n = 2), digoxin (n = 2), isosorbide-5-mononitrate (n = 2), diltiazem, insulin, diprydamole, prednisolone, propranolol, metoprolol, enalapril, glyburide, captopril, and warfarin</td>
</tr>
</tbody>
</table>

*The number of study subjects taking each medication is given in parentheses (no number indicates that only 1 subject received the drug).
concentration-time profiles of serum selegiline are changes related to the study drug. Tests (data not shown) revealed no clinically relevant abnormalities reported during the study. The poststudy laboratory samples from 40 subjects completed the study. A single dose of 20 mg selegiline was well tolerated; no adverse events were reported. The individual concentrations could be determined in only a few serum samples and the calculation of kinetic parameters had to be based on a few measured values. In other groups, individual concentration-time curves were highly variable, but patient groups were still definitely different with respect to selegiline elimination. Some concentration-time curves of a few individuals were in the same range as those in control patients, but in each patient group there were a number of individuals with highly prolonged selegiline elimination and extremely high serum AUC values. Attempts to correlate the severity of liver or kidney disease with selegiline pharmacokinetic parameters were made, but no significant correlations were observed (data not shown). This was perhaps expected, because subject groups were relatively small (ie, 10 subjects), and consequently, the power of calculations is very weak.

**RESULTS**

**Clinical observations.** The results of prestudy laboratory tests are shown in Table I. No signs of clinically relevant abnormality were apparent in the control subjects or in the patients with drug-induced liver function. The patients with impaired liver and kidney function had expected abnormalities (Table I). All 40 study subjects completed the study. A single dose of 20 mg selegiline was well tolerated; no adverse events were reported during the study. The poststudy laboratory tests (data not shown) revealed no clinically relevant changes related to the study drug.

**Selegiline pharmacokinetics.** The individual concentration-time profiles of serum selegiline are shown in Fig 2, and the pharmacokinetic parameters are listed in Table III. After the ingestion of two 10-mg selegiline tablets, the peak serum concentration of selegiline in control subjects was achieved in 0.5 hour, with an average peak serum concentration of 3.1 ng/mL (range, 0.4-5.8 ng/mL). The half-life of unchanged selegiline could not be reliably estimated.

Large, statistically highly significant changes (P < .001) in serum selegiline concentrations were observed in patients with altered liver function as compared with the control group. The peak serum concentrations and AUC values were, respectively, 7- and 18-fold higher (P < .05) on average in patients with impaired liver function and 15- and 23-fold lower (P < .001) on average in patients with drug-induced liver function when compared with subjects with normal liver function. In patients with impaired kidney function, peak serum concentrations and the AUC values of selegiline were approximately 4- and 6-fold higher (P < .05), respectively, than in control subjects.

It has to be emphasized that the changes described here are average values. Interindividual variability in selegiline kinetics was extremely large (Fig 2). In patients with drug-induced liver function, selegiline concentrations could be determined in only a few serum samples and the calculation of kinetic parameters had to be based on a few measured values. In other groups, individual concentration-time curves were highly variable, but patient groups were still definitely different with respect to selegiline elimination. Some concentration-time curves of a few individuals were in the same range as those in control patients, but in each patient group there were a number of individuals with highly prolonged selegiline elimination and extremely high serum AUC values. Attempts to correlate the severity of liver or kidney disease with selegiline pharmacokinetic parameters were made, but no significant correlations were observed (data not shown). This was perhaps expected, because subject groups were relatively small (ie, 10 subjects), and consequently, the power of calculations is very weak.

**Metabolite pharmacokinetics.** The mean profiles of selegiline and its 3 metabolites are given in Fig 3. Table III contains the mean pharmacokinetic parameters with summary statistics. Desmethylselegiline parameters were comparable between control subjects and patients with impaired liver function but 2- to 3-fold lower (P < .01) in patients with drug-induced liver function. No significant changes in desmethylselegiline kinetics were observed in patients with impaired kidney function.

Pharmacokinetic parameters of l-methamphetamine were comparable between subjects in the control group and patients with drug-induced liver function, but the peak serum concentrations were 2-fold lower (P < .01) and half-life values were 2-fold higher (P < .01) in patients with impaired liver function when compared with control subjects. The AUC and half-life values of l-methamphetamine were almost 2-fold higher (P < .01) in patients with impaired kidney function.

Pharmacokinetic parameters of l-amphetamine differed between the groups studied in each group of subjects. The AUC values and peak concentrations were significantly lower (P < .01) and the half-lives longer (P < .05) in patients with impaired liver function compared with the control subjects, and the peak concentrations were higher (P < .05) and the half-lives shorter (P < .01) in patients with drug-induced liver function compared with the control subjects. In patients with kidney disease, a lower peak concentration (P < .05) and a prolongation of half-life (P < .001) were observed.

**DISCUSSION**

The elimination rate of selegiline was substantially increased in subjects with drug-induced liver function and decreased in subjects with impaired liver function.
Fig 2. Individual serum selegiline concentration–time curves after oral administration of 20 mg selegiline in control subjects and in patients with impaired liver function, drug-induced liver function, and impaired kidney function (n = 10 in each group).
consideration in the interpretation of in vivo results. 9,10 CYP enzymes suggest that CYP2B6 and CYP2C19 are partly, by the drug-induced induction of some CYP enzymes. In cultured human hepatocytes and in precision-cut human liver slices, CYP2B6 is strongly inducible by phenobarbital, by rifampin (INN, rifampicin), and presumably by other antiepileptic drugs, whereas the induction of CYP2C19 seems less prominent. 14-16 Although the role of CYP2B6 in vivo remains to be confirmed, the induction of CYP2B6 by antiepileptic drugs remains the most plausible explanation for the drastic enhancement of selegiline elimination. In our study desmethylselegiline concentrations were actually lower in patients with drug-induced liver function, but this fact could be reconciled with a strong induction of CYP2C19 (or other inducible CYPs) if further metabolism of desmethylselegiline is also catalyzed by these enzymes. l-Methamphetamine concentrations were similar in control subjects and drug-induced patients, but this finding could be explained by induction affecting both metabolic steps similarly. The role of CYP2C19 in inducible selegiline metabolism remains to be studied. At least in healthy volunteers, the CYP2C19 poor metabolizer phenotype seems not to affect the pharmacokinetics of selegiline. 17 In addition, Selegiline elimination was strongly augmented in patients taking antiepileptic and other inducing drugs. This increased elimination can be explained, at least partly, by the drug-induced induction of some CYP enzymes. In cultured human hepatocytes and in precision-cut human liver slices, CYP2B6 is strongly inducible by phenobarbital, by rifampin (INN, rifampicin), and presumably by other antiepileptic drugs, whereas the induction of CYP2C19 seems less prominent. 14-16 Although the role of CYP2B6 in vivo remains to be confirmed, the induction of CYP2B6 by antiepileptic drugs remains the most plausible explanation for the drastic enhancement of selegiline elimination. In our study desmethylselegiline concentrations were actually lower in patients with drug-induced liver function, but this fact could be reconciled with a strong induction of CYP2C19 (or other inducible CYPs) if further metabolism of desmethylselegiline is also catalyzed by these enzymes. l-Methamphetamine concentrations were similar in control subjects and drug-induced patients, but this finding could be explained by induction affecting both metabolic steps similarly. The role of CYP2C19 in inducible selegiline metabolism remains to be studied. At least in healthy volunteers, the CYP2C19 poor metabolizer phenotype seems not to affect the pharmacokinetics of selegiline. 17 In addition,
the role of inducible CYP3A4 in the 18-fold increase in selegiline clearance in patients with drug-induced liver function remains to be studied. However, in young volunteers, the CYP3A4 selective inhibitor itraconazole did not affect selegiline elimination.18 Thus both this in vivo study and microsomal and recombinant in vitro studies (see previous text) suggest that the role of CYP3A4 is negligible in selegiline metabolism.

Selegiline elimination was considerably retarded in patients with impaired liver function. This finding is in good agreement with a large number of studies in which activities of various CYP enzymes were shown to be decreased, although variably and depending on the type of liver derangement, in patients with compromised liver function.19 The levels of major metabolites were also decreased, suggesting lowered enzyme activities in the liver. However, decreases in metabolite concentrations were somewhat blunted, perhaps because the further metabolism of primary metabolites is also equally decreased.

Very high selegiline concentrations occurring in some patients with liver or kidney disturbances may lead to clinical consequences; incidences of more common side effects such as insomnia, dyskinesias, dizziness, nausea, and hypotension may increase. The loss of monoamine oxidase type B selectivity at high concentrations may predispose patients to the elevation of blood pressure in connection with indirectly acting sympathomimetics, although this risk is generally regarded as being only theoretic. Interactions with selective serotonin reuptake inhibitors or tricyclic antidepressants may become more pronounced. Furthermore, because main metabolites are variably active, they might also add to the side effect profile and intensity, if their concentrations are elevated.

It is of interest that selegiline elimination, which is practically completely dependent on metabolism, was markedly reduced in patients with long-term kidney disease, although their liver function test results were within normal limits. However, there is some indication that compromised kidney function leads to wider effects on the pharmacokinetic processes, including liver drug metabolism.20 Recent studies have demonstrated that hepatic CYP enzymes are also down-regulated, although to a variable extent depending on a specific enzyme, in kidney diseases. For example, 3 recent studies in patients with severe or end-stage renal disease suggested that CYP2E1, CYP2C9, and CYP3A4,
measured by chlorzoxazone elimination, S-/R-warfarin ratio, and erythromycin breath test, respectively, were reduced. Consequently, it seems quite possible, albeit not proven, that kidney disease impairs the hepatic metabolism of selegiline, even in the absence of clinical signs of overt liver involvement.

The interpretation of changes in selegiline metabolites in various clinical conditions studied here was sometimes possible but often conjectural (see previous text). In this respect, one has to keep in mind that concentration profiles of selegiline metabolites are dependent on the rate of formation and further metabolism (i.e., the balance between input and output), as well as the rate of urinary excretion. More research is needed to further elucidate the metabolism, especially enzyme kinetics, of the primary selegiline metabolites desmethylselegiline and l-methamphetamine.

In conclusion, with consideration of the marked impact of impaired liver and kidney function and hepatic enzyme–inducing drugs on selegiline pharmacokinetics and metabolism, it is advisable to modify selegiline dosing accordingly. Because some individuals in all patient groups had selegiline elimination rates that were close to “normal,” standard modifications cannot be instituted routinely and very careful monitoring of patient response will be required.

Drs Sotaniemi, Pelkonen, and Rautio have no conflict of interest to disclose. Mr Anttila was an employee of Orion Pharma, the manufacturer of selegiline, until 2001.

References


Effect of tadalafil on cytochrome P450 3A4–mediated clearance: Studies in vitro and in vivo

Objectives: Tadalafil was examined in vitro and in vivo for its ability to affect human cytochrome P450 (CYP) 3A–mediated metabolism.

Methods: Reversible and mechanism-based inhibition of CYP3A by tadalafil was examined in human liver microsomes. The ability of tadalafil to influence CYP3A activity was also examined in primary cultures of human hepatocytes. The effect of tadalafil on the pharmacokinetics of CYP3A probe substrates was evaluated in human volunteers before and after coadministration with either a single dose or multiple doses of tadalafil (10 or 20 mg).

Results: Negligible competitive inhibition of CYP3A was observed in vitro. Mechanism-based inhibition of CYP3A was detected, albeit with a low potency. In human hepatocytes, exposure to 1 μmol/L or greater of tadalafil resulted in increased CYP3A protein expression; however, as with a combined effect of induction and inhibition, a corresponding increase in CYP3A activity did not occur. The clinical pharmacokinetics of midazolam and lovastatin, probe substrates of CYP3A, were unaffected by up to 14 days of tadalafil administration (90% confidence intervals for the ratio of least squares means for the pharmacokinetic parameters of tadalafil were contained within the no-effect boundaries of 0.7 to 1.43).

Conclusions: In vitro results suggested that tadalafil would have little effect on the pharmacokinetics of drugs metabolized by CYP3A. Clinical studies demonstrated that the pharmacokinetics of 2 different CYP3A substrates, midazolam and lovastatin, were virtually unchanged after tadalafil coadministration. Thus therapeutic concentrations of tadalafil do not produce clinically significant changes in the clearance of drugs metabolized by CYP3A. (Clin Pharmacol Ther 2005;77:63-75.)

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Indianapolis, Ind

Tadalafil (Cialis) is a potent reversible phosphodiesterase-5 (PDE5) inhibitor used for the treatment of erectile dysfunction. The recommended starting dose of tadalafil is generally 10 or 20 mg taken before anticipated sexual activity. The pharmacokinetic profile of tadalafil has been determined from dose-normalized data pooled across 13 single-dose studies of 10 or 20 mg tadalafil in healthy subjects. Peak concentrations of tadalafil are achieved over a range of 0.5 to 6 hours (median time to reach peak concentration, 2 hours). The half-life of tadalafil is 17.5 hours, with an apparent oral clearance (Cl/F) of 2.48 L/h. Within 5 days of once-daily dosing, steady-state plasma concentrations are attained and exposure is approximately 1.6-fold greater than after a single dose.

Physiologically, in response to sexual stimulation, nitric oxide is released into the smooth muscle of the corpus cavernosum of the penis, resulting in elevation of cyclic guanosine monophosphate (cGMP) levels and relaxation of the smooth muscle to produce an erection. Because PDE5 inactivates cGMP, inhibition of PDE5 by tadalafil increases the intracellular levels of cGMP in the corpus cavernosum, facilitating the erectile response. Patients who are treated for erectile dysfunction are likely to take additional medications for pre-existing conditions such as cardiovascular disease.
and diabetes mellitus. Therefore the potential for tadalafil to cause metabolism-based drug-drug interactions was assessed in vitro and in vivo.

The human cytochromes P450 (CYPs) are the major family of enzymes involved in the oxidative metabolism of drugs. In this family of enzymes, CYP3A is the dominant CYP in terms of both expression levels in the liver and the number of drugs metabolized. As a result of the broad substrate specificity exhibited by CYP3A and the polypharmacy often used by patients, alterations in CYP3A activity by induction or inhibition can result in metabolically based drug-drug interactions. To evaluate the potential for tadalafil to cause drug-drug interactions, in vitro studies were performed examining the ability of tadalafil to alter metabolism mediated by CYP3A (and CYP1A2) in primary cultures of human hepatocytes or reversibly inhibit metabolism mediated by CYP3A (and CYP2D6, CYP2C9, CYP1A2, and CYP2C19) in human liver microsomes. In addition, tadalafil was examined for its ability to cause mechanism-based inhibition of CYP3A-mediated reactions in human liver microsomes. Mechanism-based inhibition occurs when the drug is converted to a metabolite that binds irreversibly to the enzyme active site, permanently inactivating the enzyme. The inactivated enzyme must be replaced by newly synthesized CYP to regain activity; thus recovery is slowed after mechanism-based inactivation as compared with reversible inhibition. Tadalafil was examined as a mechanism-based inhibitor because of the presence of a methylenedioxyphenyl functional group in its structure.

The in vitro results led to the conduct of clinical studies evaluating the effect of 2 dose levels of tadalafil on the pharmacokinetics of coadministered drugs that are metabolized by CYP3A. These in vivo studies were designed to examine the impact of a single dose (to investigate competitive inhibition) and multiple doses (to investigate mechanism-based inhibition and/or induction) of tadalafil on the pharmacokinetics of 2 well-recognized, sensitive probe substrates of CYP3A, midazolam and lovastatin. In the clinical studies reported, 2 separate probes were tested to provide conclusive evidence concerning the effect of tadalafil on CYP3A-mediated clearance. This was thought to be especially valuable because the in vitro signals, which triggered the initial midazolam study, did not correlate with a clinical effect.

METHODS
Materials
Tadalafil and [13C2H3]-tadalafil were obtained from Lilly ICOS LLC (Indianapolis, Ind, and Bothell, Wash). Midazolam for in vitro studies was obtained from Hoffmann-La Roche (Nutley, NJ). Bufuralol, 1'-hydroxy (OH)-bufuralol, 1'-OH-midazolam, S-mephenytoin, and 4'-OH-mephenytoin were obtained from Ultrafine Ltd (Manchester, United Kingdom). Midazolam for the clinical bioanalytic assay was obtained from Radiant International (Austin, Tex). For in vitro bioanalytic studies, 1'-OH-[13C5]-midazolam was biologically derived from [13C5]-midazolam synthesized at Eli Lilly and Company, and for clinical bioanalytic assays, [13C3]-midazolam was purchased from Bridge Organics (Vicksburg, Mich). Diclofenac, phenacetin, NADPH, flunitrazepam, rifampin (INN, rifampicin), 3-methylcholanthrene, metoprolol, and salicylamide were purchased from Sigma Chemical Co (St Louis, Mo). 4'-OH-diclofenac was obtained from BD Gentest (Woburn, Mass). Acetaminophen was obtained from Kodak (Rochester, NY). Trolox and 4'-OH-phenytoin were obtained from Aldrich Chemical Co (Milwaukee, Wis). Resorufin and 7-ethoxyresorufin were obtained from Molecular Probes (Eugene, Ore). Lovastatin and simvastatin for bioanalytic assays were obtained from US Pharmacopeia (Rockville, Md). Hepatocyte Maintenance Medium (HMM) was purchased from BioWhittaker (Walkersville, Md). Mouse monoclonal antibody to CYP3A was a gift from P. Beaune at Universite de Liege (Liege, Belgium). Horseradish peroxidase–conjugated secondary antibodies were purchased from Bio-Rad (Hercules, Calif). ECL + detection reagents were purchased from Amersham (Arlington Heights, Ill).

Human liver samples designated HLB, HLH, HLM, and HLP were obtained from the liver transplant units at the Medical College of Wisconsin (Milwaukee, Wis) and Indiana University School of Medicine (Indianapolis, Ind) under protocols approved by the appropriate committees for the conduct of human research. Microsomes were prepared by differential centrifugation and stored at −70°C. A mixture of equal protein concentrations of microsomes from HLB, HLH, HLM, and HLP was prepared and used in the in vitro microsomal studies. Microsomes prepared from a baculovirus-infected insect cell system containing complementary deoxyribonucleic acid–expressed CYP3A4, CYP reductase, and cytochrome b5 (Supersomes) were obtained from BD Gentest (Woburn, Mass). Primary cultures of human hepatocytes were obtained from S. Strom at the University of Pittsburgh (Pittsburgh, Pa).

In vitro reversible inhibition
Microsomal incubations were performed in duplicate with form-selective CYP substrates and 1-mmol/L
NADPH under linear rate conditions (CYP1A2 [acetaminophen (INN, paracetamol) formation], 0.5 mg/mL, protein and 30-minute incubation; CYP2C9 [4′-OH-diclofenac formation], 0.25 mg/mL, protein and 15-minute incubation; CYP2C19 [4′-OH-mephenytoin formation], 0.5 mg/mL, protein and 30-minute incubation; CYP2D6 [1′-OH-bufuralol formation], 0.1 mg/mL, protein and 30-minute incubation; and CYP3A [1′-OH-midazolam formation], 0.5 mg/mL, protein and 1-minute incubation), with or without tadalafil. The samples were analyzed for the formation of the form-selective metabolite, and where warranted, an apparent K_inact value (dissociation constant for the enzyme inhibitor complex) was generated by fit of the appropriate inhibition model to the data. Concentrations of substrate and tadalafil for the various reactions were as follows: midazolam (CYP3A4), 5, 10, 25, 50, or 100 μmol/L, and tadalafil, 1, 10, 25, or 50 μmol/L; bufuralol (CYP2D6), 5 μmol/L, and tadalafil, 0.5, 1, 10, 25, 50, or 100 μmol/L; diclofenac (CYP2C9), 2.5, 5, 10, 25, or 50 μmol/L; phenacetin (CYP1A2), 12.5, 25, 50, 75, or 100 μmol/L; phenoxybenzamine (CYP2C19), 5, 10, 25, 50, or 100 μmol/L; and tadalafil, 35, 50, 65, or 80 μmol/L. The potential for significant drug-drug interaction was evaluated by calculation of a ratio of inhibitor concentration (I) over K_inact, where a ratio lower than 0.1 suggests low risk for drug-drug interactions, 0.1 to 1 suggests medium risk, and greater than 1 suggests high risk.

In vitro mechanism-based inhibition (CYP3A)

CYP3A4 Supersomes (20 pmol CYP/mL) were preincubated in 100-mmol/L sodium phosphate buffer, pH 7.4, containing 1-mmol/L ethylenediaminetetraacetic acid, and tadalafil (0, 1, 2.5, 5, 10, or 20 μmol/L), erythromycin (0, 5, 10, 25, or 50 μmol/L), or diltiazem (0, 0.1, 0.5, 1, or 5 μmol/L) for 3 minutes at 37°C in duplicate. The mechanism-based inhibition reaction was initiated with the addition of NADPH (1 mmol/L). After incubations at various times, an aliquot of the mixture was withdrawn and diluted 20-fold into a prewarmed (37°C) CYP3A4 activity assay incubation system containing 1-mmol/L NADPH and midazolam (100 μmol/L). This activity assay mixture was allowed to incubate a further 2 minutes (linear rate conditions), and the supernatant was analyzed for 1′-OH-midazolam levels.

To obtain the mechanism-based inhibition kinetic parameters of k_inact (the formation rate constant of the inactive complex with the enzyme) and K_i (the dissociation constant for the inactivator), equation 2 was fit to the observed rate of 1′-OH-midazolam formation by the samples after different times of incubation with tadalafil, erythromycin, or diltiazem by use of WinNonlin Professional software (Pharsight Corporation, Mountain View, Calif) as follows:

\[
\text{Percent inhibition} = 100 \times \left(1 - e^{-\lambda t}\right)
\]

where \(\lambda\), the pseudo first-order rate constant for enzyme inactivation, was defined by the following:

\[
\lambda = \frac{\text{k}_{\text{inact}} - 1}{\text{K}_{\text{f}} + 1}
\]

The mechanism-based inhibitory potency of the tested compounds was evaluated by calculating inactivation clearance (Cl_{inact}) (ratio of k_{inact}/K_i).

In vitro human hepatocyte incubations

Hepatocyte monolayers in 6-well culture plates (approximately 1 × 10^6 cells/well) were incubated in triplicate with tadalafil (0.1, 1, 3, or 10 μmol/L), vehicle control (0.1% dimethylsulfoxide), or known inducers (1 μg/mL 3-methylcholanthrene or 10 μmol/L rifampin) in HMM for 48 hours. For the short-term experiment, cultures were treated for 0, 5, 15, 30, or 60 minutes with 0.1, 1, and 10 μmol/L tadalafil. After incubation with tadalafil, the medium was removed and cells were rinsed with HMM and incubated with midazolam (10 μmol/L) or 7-ethoxyresorufin (2 μmol/L) in HMM (containing 3 mmol/L salicylamide) for 30 minutes. Samples of the medium were analyzed by validated assays for the formation of the products 1′-OH-midazolam or resorufin.

To determine which treatment groups were statistically different from controls, a variety of statistical evaluations were performed by use of JMP software (SAS Institute, Cary, NC) as described previously. After 48 hours of exposure to tadalafil, CYP3A4 immunoreactive protein content in the hepatocyte cultures was determined by Western blot analysis. Proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10%) followed by transfer to nitrocellulose paper. The blot was probed with a mouse monoclonal antibody to CYP3A4 followed by a goat antimouse horseradish peroxidase–conjugated antibody. The blots were developed with ECL reagents according to the manufacturer’s instructions, visualized by use of a Storm 860 imager (Molecular Dynamics, Sunnyvale, Calif) and quantified by use of ImageQuant version 3.3 (Molecular Dynamics).

Clinical investigations

Open-label outpatient studies were conducted to examine the effect of coadministration of 10 or 20 mg
tadalafil on the pharmacokinetics of 2 well-established probes of CYP3A activity, midazolam and lovastatin. Studies were conducted and patient written informed consent was obtained in conformity with the ethical principles of the Declaration of Helsinki (adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, and revised at the World Medical Assembly, Tokyo, Japan, 1975; Venice, Italy, 1983; and Hong Kong, 1989) and the applicable European laws. Approval of the midazolam protocol and consent form was obtained by the Medieval Independent Ethics Committee, and the study was conducted by Medeval Ltd, Manchester, United Kingdom. Approval of the lovastatin protocol and consent form was obtained by the Independent Ethical Committee of the Phase I Clinical Trials Unit Ltd, Plymouth, United Kingdom, and the study was conducted by the Phase I Clinical Trials Unit Ltd. All subjects were overtly healthy as determined by medical history and physical examination. Alcohol use was not permitted for 48 hours preceding the days of pharmacokinetic blood sampling. At other times during both studies, alcohol use was permitted and smoking was permitted in the lovastatin interaction study, because neither of these habits affects CYP3A activity.28

Study subjects and experimental protocol for midazolam-tadalafil interaction study. For the midazolam-tadalafil interaction study, 12 male subjects (10 white, 1 biracial [black and white], and 1 black Caribbean subject) entered the study. All subjects were nonsmokers or former smokers who had stopped smoking more than 6 months before screening. Eleven subjects reported alcohol consumption ranging from 2 to 26 U (1 U of alcohol defined as 0.5 pt [284 mL] of beer or lager, 1 glass of wine, or 25 mL of spirits) per week before the study. Although alcohol use was discouraged, alcohol consumption of no more than 2 U/d was allowed during the study.

Tadalafil (10-mg tablets) was given once daily for 14 consecutive days (days 15 to 28). Midazolam (Dormicum; Hoffmann-La Roche) was administered as a single 15-mg tablet on 5 separate occasions (days 1, 8, 15, 28, and 42). Subjects were required to abstain from food and fluids, with the exception of water, starting at midnight before each dose. Water was not permitted from the beginning of dosing until 2 hours after tadalafil dosing. Food was consumed at least 2 hours after either midazolam or tadalafil dosing. Xanthine-containing drinks were restricted to 2 cups per day from 48 hours before the first dose of tadalafil until discharge, and consumption of grapefruit-containing products was not allowed from 48 hours before the first dose of midazolam until discharge.

Plasma concentrations of midazolam were measured on days 1 and 8 (to obtain baseline concentrations from midazolam administered alone on both days), 15 (first dose of tadalafil), 28 (last dose of tadalafil), and 42 (after a 2-week washout period after completion of tadalafil dosing). Samples for midazolam were collected before dosing and at 0.25, 0.50, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 9, 12, 16, and 24 hours after dosing. Plasma concentrations of tadalafil were measured before dosing and 3 hours after dosing on days 15, 21, 28, and 42.

Midazolam concentrations were measured by use of a validated HPLC assay with tandem mass spectrometric detection (HPLC-MS/MS), with a lower limit of quantitation of 0.5 ng/mL. After addition of [13C5]-midazolam internal standard to the sample, the analytes were extracted with methyl tert-butyl ether and reconstituted in methanol for direct injection by the autosampler. Chromatography of the extracts was performed by use of a BetaBasic (100 × 2 mm, 5 μmol/L) C18 column (Keystone Scientific, Bellfonte, Pa) at 45°C and a mobile phase consisting of 2.5-mmol/L ammonium acetate buffer in 50:50 (vol/vol) methanol/water (mobile phase A) and methanol (mobile phase B). The gradient profile was as follows (in minutes per percent mobile phase B): 0/0, 2/40, 2.2/90, 2.7/90, and 3/0, with a column flow rate of 0.4 mL/min and a total run time of 6 minutes. The extracts were analyzed on a Finnegan TSQ-7000 equipped with a Finnegan APCI Interface (Finnegan Corporation, San Jose, Calif). Tandem mass spectrometry (positive ion mode) was used to monitor the transitions mass-to-charge ratio (m/z) 326.1 → 291.1 for midazolam and m/z 329.1 → 294.1 for internal standard. Standard curves and quality control samples were analyzed with the study samples, with the overall accuracy (percent relative error) of the method being lower than 17% and overall precision (percent coefficient of variation) being lower than 7%.

Tadalafil concentrations were measured by use of a validated HPLC-MS/MS assay with a lower limit of quantitation of 0.5 ng/mL. After addition of [13C2H3]-tadalafil internal standard to the sample, the analytes were extracted by use of 3M Empore 3-mL/7-mm C2 disk extraction cartridges (3M Company, St Paul, Minn). Elution was achieved with 150 μL of methanol/water (90:10 [vol/vol]). Extracts were diluted 1:2 in water and underwent chromatography by use of a Luna (100 × 4.6 mm, 5 μm) phenyl-hexyl column (Phenomenex, Torrance, Calif), ambient column temperature, and a mobile phase consisting of methanol/water (90:10 [vol/vol]). The isotropic flow rate was 1.0 mL/min, with a total run time of 3 minutes. The extracts were analyzed on a PE Sciex API III Plus mass spec-
trometer equipped with a Sciex APCI Interface (Applied Biosystems/MDS Sciex, Foster City, Calif). Tandem mass spectrometry (positive ion mode) was used to monitor the transitions m/z 390 → 268 for tadalafil and m/z 394 → 272 for the internal standard. Standard curves and quality control samples were analyzed with the study samples, with the overall accuracy (percent relative error) of the method being lower than 10% and overall precision (percent coefficient of variation) being lower than 11%.

**Study subjects and experimental protocol for lovastatin-tadalafil interaction study.** For the lovastatin-tadalafil interaction study, 16 white subjects (10 women and 6 men) entered the study. Eight subjects smoked tobacco (ranging from 2 to 8 cigarettes per day) and 14 consumed alcohol (ranging from 1 to 20 U/ wk) before screening. Female subjects of childbearing potential (not surgically sterilized between menarche and menopause) had a negative pregnancy test result at the time of enrollment and were using a reliable method of contraception (no subjects were taking oral contraceptives).

Tadalafil (two 10-mg tablets) was given once daily for 14 consecutive days (study days 8 to 21). Lovastatin (Mevacor; Merck Frosst Canada Ltd, Montreal, Quebec, Canada) was administered as a single 40-mg dose on 4 occasions (study days 1, 9, 21, and 35). Subjects were required to eat a standard breakfast 1 hour before tadalafil and/or lovastatin administration on each pharmacokinetic sampling day to maximize the absorption of lovastatin. Water was not permitted for up to 2 hours after lovastatin dosing. Restrictions regarding xanthine-containing drinks, alcohol, and grapefruit products were similar to those for the midazolam study.

Plasma concentrations of lovastatin were measured on days 1 (baseline concentrations from lovastatin administered alone), 9 (second dose of tadalafil), 21 (last dose of tadalafil), and 35 (after a 2-week washout period after completion of tadalafil dosing). Samples for lovastatin were collected before dosing and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9, 12, 16, and 24 hours after dosing. Plasma concentrations of tadalafil were measured before dosing and at 0.5, 1, 2, 3, 4, 6, 12, 16, and 24 hours after dosing on days 9 and 21.

Tadalafil concentrations were measured by use of a validated HPLC-MS/MS assay as outlined above. Lovastatin concentrations were measured by use of a validated HPLC-MS/MS assay, with a lower limit of quantitation of 0.25 ng/mL. After addition of simvastatin internal standard to the sample, the analytes were extracted by use of Isolute C18 solid-phase extraction cartridges (100 mg sorbent; Isolute International Sorbent Technology, Ystrad Mynach, United Kingdom). The cartridges were conditioned with methanol and 10-mmol/L ammonium acetate (pH 4.5) and washed with HPLC-grade water and 10:90 water/acetonitrile (vol/vol), and the analytes were eluted with acetonitrile. After evaporation, extracts were reconstituted in HPLC mobile phase for direct injection by the autosampler. Extracts underwent chromatography by use of a Phenomenex Luna C18(2) (50 × 2.1 mm, 5 μmol/L) column and a mobile phase consisting of 50% 4-mmol/L ammonium acetate buffer (pH 4.5)/acetonitrile (20:80 [vol/vol]) and 50% acetonitrile with a column flow rate of 0.3 mL/min and a total run time of 3.5 minutes. The extracts were analyzed on a PE Sciex API III Plus equipped with a Turbo Ion Spray interface (PE Sciex, Concord, Ontario, Canada). Tandem mass spectrometry (positive ion mode) was used to monitor the transitions m/z 405 → 285 for lovastatin and m/z 419 → 285 for internal standard. Standard curves and quality control samples were analyzed with the study samples, with the overall accuracy (percent relative error) of the method being lower than 14% and overall precision (percent coefficient of variation) being lower than 8%.

**Pharmacokinetic and statistical analyses**

Pharmacokinetic parameters were calculated by non-compartmental methods by use of WinNonlin Professional software. Statistical evaluation was conducted by use of a mixed effects model by ANOVA techniques on the log-transformed data. The geometric least squares (LS) mean ratios and 90% confidence intervals (confidence intervals were predefined in the clinical protocols) for comparisons were calculated. An equivalence approach was used to compare area under the curve (AUC) and peak concentration (Cmax) values for midazolam or lovastatin with and without coadministration of tadalafil. No clinically significant interactions were declared if the 90% confidence intervals for the ratio of geometric LS means of these parameters were contained within the equivalence limits of 0.70 to 1.43. The sample size of the studies was chosen to have a greater than 90% chance to rule out a clinically significant effect by use of these confidence intervals.

**RESULTS**

**In vitro reversible inhibition**

Tadalafil was examined for its ability to reversibly inhibit CYP form–selective catalytic activities. The Ki values for the inhibition by tadalafil of CYP3A4-, CYP2C9-, CYP2C19-, and CYP1A2-mediated metabolism were 41 ± 5 μmol/L (noncompetitive), 66 ± 6
μmol/L (competitive), 73 ± 8 μmol/L (noncompetitive), and 14 ± 1 μmol/L (noncompetitive), respectively. By use of these $K_i$ values, given an inhibitor concentration (I) of 2.02 μmol/L, which is the highest individual plasma tadalafil concentration observed for once-daily dosing of 20 mg, $I/K_i$ ratios of 0.05, 0.03, 0.03, and 0.14 were obtained for CYP3A, CYP2C9, CYP2C19, and CYP1A2, respectively. Little inhibition (≤15%) of bufuralol 1′-hydroxylation by CYP2D6 occurred at a Michaelis-Menten constant concentration of bufuralol (5 μmol/L) with up to 100 μmol/L tadalafil.

**In vitro mechanism-based inhibition**

In these studies tadalafil (Fig 1), erythromycin (data not shown), or diltiazem (data not shown) inhibited 1′-OH-midazolam formation in a time- and concentration-dependent manner. Values for $k_{inact}$ and $K_I$ of 0.21 ± 0.04 min$^{-1}$ and 12 ± 4 μmol/L, respectively, were determined by fit$^{19}$ of equation 1 to the data for tadalafil inhibition and used to calculate a $Cl_{inact}$ of 17 min$^{-1}$ · mmol/L$^{-1}$. An erythromycin $k_{inact}$ value of 0.30 ± 0.02 min$^{-1}$ and $K_I$ value of 5.1 ± 1.5 μmol/L and a diltiazem $k_{inact}$ value of 0.17 ± 0.02 min$^{-1}$ and $K_I$ value of 0.52 ± 0.11 μmol/L were also determined. Calculated values of $Cl_{inact}$ for erythromycin and diltiazem were 59 min$^{-1}$ · mmol/L$^{-1}$ and 327 min$^{-1}$ · mmol/L$^{-1}$, respectively, which were 3- to 19-fold greater than the $Cl_{inact}$ value for tadalafil.

**Hepatocyte incubations**

Tadalafil (0.1 to 10 μmol/L) was examined for its ability to induce or inhibit CYP1A2 (7-ethoxyresorufin deethylation) and CYP3A (midazolam 1′-hydroxylation) after incubation for 48 hours in cultures of primary human hepatocytes. These activities were compared with vehicle-treated cultures and in cultures exposed to known inducers of CYP1A2 (3-methylcholanthrene) or CYP3A (rifampin). In hepatocyte cultures from 2 different donors, the positive control 3-methylcholanthrene induced CYP1A2 activity by 47- and 118-fold. In 1 culture a slight increase (1.7-fold) in CYP1A2 activity was observed after exposure to 10 μmol/L tadalafil, and in the second culture, no significant induction was observed (data not shown). Tadalafil was also examined for its ability to alter CYP3A activity (Fig 2). A significant induction of 1′-OH-midazolam formation (6.6- and 13.3-fold increase in activity) was observed with 10 μmol/L rifampin (positive control). In contrast, exposure to 0.1 μmol/L tadalafil did not affect either CYP3A activity or immunoreactive protein levels in these samples. Induction of CYP3A protein levels clearly occurred with exposure of the hepatocytes to 1 μmol/L tadalafil or greater. Increased CYP3A activity was observed with 1 μmol/L tadalafil, but this induction response was decreased after exposure to 10 μmol/L tadalafil (Fig 2).
These results suggest both inductive and inhibitory effects on CYP3A.

For an evaluation of mechanism-based inhibition of CYP3A activity in hepatocyte cultures, an additional experiment was performed to investigate the effect of short-term exposure (0 to 60 minutes) to tadalafil. At 0.1 and 1 μmol/L tadalafil, a slight inhibitory effect (15% to 31%) on CYP3A-mediated midazolam 1'-hydroxylase activity was observed.

**Table I.** Geometric mean (percent coefficient of variation) and statistical comparison of midazolam pharmacokinetic parameters after single oral dose (15 mg midazolam) on days 1, 8, 15, 28, and 42 and daily oral dose of tadalafil from days 15 to 28 in 10 healthy subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1: Midazolam alone</th>
<th>Day 8: Midazolam alone</th>
<th>Day 15: Midazolam with day 1 tadalafil</th>
<th>Day 28: Midazolam with day 14 tadalafil</th>
<th>Day 42: Midazolam alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μg · h/L)</td>
<td>206 (37.8)</td>
<td>222 (50.2)</td>
<td>235 (51.7)</td>
<td>185 (48.0)</td>
<td>218 (54.0)</td>
</tr>
<tr>
<td>Ratio and 90% CI of geometric LS means</td>
<td>1.08 (0.89-1.30)</td>
<td>1.10 (0.96-1.25)</td>
<td>0.87 (0.76-0.99)</td>
<td>1.02 (0.90-1.16)</td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (μg/L)</td>
<td>110 (46.3)</td>
<td>115 (39.5)</td>
<td>114 (43.4)</td>
<td>117 (28.9)</td>
<td>127 (45.0)</td>
</tr>
<tr>
<td>Ratio and 90% CI of geometric LS means</td>
<td>1.04 (0.75-1.43)</td>
<td>1.02 (0.85-1.23)</td>
<td>1.05 (0.87-1.26)</td>
<td>1.13 (0.94-1.36)</td>
<td></td>
</tr>
<tr>
<td>Cl/F (L · h&lt;sup&gt;-1&lt;/sup&gt; · kg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.357 (35.9)</td>
<td>0.334 (53.7)</td>
<td>0.319 (54.0)</td>
<td>0.404 (48.7)</td>
<td>0.342 (55.0)</td>
</tr>
<tr>
<td>Ratio and 90% CI of geometric LS means</td>
<td>0.93 (0.77-1.12)</td>
<td>0.91 (0.80-1.04)</td>
<td>1.15 (1.02-1.31)</td>
<td>0.98 (0.86-1.12)</td>
<td></td>
</tr>
</tbody>
</table>

AUC, Area under curve; CI, confidence interval; LS, least squares; C<sub>max</sub>, peak concentration; Cl/F, apparent oral clearance.

*Ratio and 90% CI for day of treatment versus baseline (mean of days 1 and 8).
hydroxylase activity was seen. With 10 μmol/L tadalafil, statistically significant time-dependent inhibition of 1'-OH-midazolam formation was observed, with inhibition ranging from 51% to 82% at 5 and 60 minutes of exposure, respectively. Ten subjects completed the study, whereas 2 discontinued because of adverse events. These 2 subjects received 3 of the 5 planned doses of midazolam (days 1, 8, and 15) and 11 of the 14 planned doses of tadalafl (days 15 to 25). Adverse events were similar to those reported previously and included headache and myalgia.

Summary pharmacokinetic parameters for midazolam are shown in Table I. Summary statistics were representative of individual subject data (data not shown). Data are shown for the 10 subjects completing the study, although midazolam summary pharmacokinetic parameters were similar on days that data were available for all 12 subjects (data not shown). The distributions of midazolam AUC and C\textsubscript{max} values suggested a negligible effect of tadalafl relative to the control treatments on days 1, 8, and 42 (Fig 3). A statistical comparison of the primary pharmacokinetic parameters of midazolam was conducted for baseline day 8 and compared with baseline day 1 to assess the variability in midazolam pharmacokinetics (Table I). The 90% confidence intervals for the ratio of geometric LS means for the pharmacokinetic parameters of midazolam were evaluated on days 15 (first dose of tadalafl), 28 (last dose of tadalafl), and 42 (after a 2-week washout period after completion of tadalafl dosing) versus mean baseline values (mean of days 1 and 8) to determine whether they were contained within the equivalence limits of 0.70 to 1.43 (Table I). Comparison of these geometric means showed no statistically significant differences relative to baseline for midazolam AUC, C\textsubscript{max}, or Cl/F on tadalafl treatment days 1 or 14 (study days 15 or 29) or after a 2-week washout period after the last dose of tadalafl.

Plasma concentrations of tadalafl were measured to verify that subjects were exposed to the study drug. Tadalafl concentrations were measured before dosing and at 3 hours after dosing on days 15 (first dose of tadalafl), 21 (day 7 of tadalafl dosing), 28 (last dose of tadalafl), and 42 (after a 2-week washout period after completion of tadalafl dosing) (Fig 4). From the 3-hour data, it was determined that exposures after single and multiple tadalafl doses were within the ranges expected for a 10-mg tadalafl dose.lovastatin-tadalafil interaction study. All 16 subjects completed the lovastatin-tadalafil interaction study. Their ages, body weights, and heights were between 22 and 47 years (mean, 38 ± 7.2 years), 57.8 and 99.1 kg (mean, 73.7 ± 12.91 kg), and 156 and 185 cm (mean, 170 ± 9.8 cm), respectively. Previously reported adverse events with tadalafl were also reported in this study as outlined here.
The AUC was calculated for 9 to 10 subjects on each lovastatin dosing day. The distribution of AUC and Cmax values for lovastatin is displayed in Fig 5, with considerable overlap of the interquartile ranges being noted between study days. As in the midazolam study, summary statistics were representative of individual subject data (data not shown).

A statistical comparison of the primary pharmacokinetic parameters of lovastatin on days 9 (second dose of tadalafil), 21 (last day of tadalafil treatment), and 35 (after a 2-week washout period after completion of tadalafil dosing) versus baseline values (day 1) was conducted (Table II). For both tadalafil treatment groups (days 9 and 21), as well as at 2 weeks after tadalafil treatment (day 35), lovastatin AUC and Cmax were considered to be equivalent to day 1 values, because the 90% confidence intervals for the geometric LS mean ratios were contained within the limits of 0.70 to 1.43.

Exposure to tadalafil was confirmed on days 9 (second dose of tadalafil) and 21 (last day of tadalafil treatment), with concentrations determined over 10 time points up to 24 hours after dosing (Fig 6). The results exhibited tadalafil exposure consistent with 20-mg dosing, with the expected slight accumulation of tadalafil occurring on multiple dosing.

DISCUSSION

The first in vitro experiments indicated that coadministration of tadalafil had a low potential to cause any clinically significant, reversible inhibition of the metabolism of coadministered drugs cleared by CYP3A or the other CYPs tested. However, methylenedioxypyrenyl functional groups have been implicated in mechanism-based inhibition of CYPs, and tadalafil contains this group. With mechanism-based inhibition, the substrate is metabolized by a CYP to an intermediate that binds either irreversibly or essentially reversibly to the catalytic site of the enzyme. Because CYP3A is the major CYP involved in drug metabolism and tadalafil is metabolized by CYP3A, tadalafil was evaluated in vitro for its ability to inactivate CYP3A metabolism. The inactivation parameters obtained with tadalafil were compared with those generated for known mechanism-based inhibitors that have exhibited moderate levels of in vivo inhibition, erythromycin and diltiazem. As a measure of inhibitory potency, Cl_inact values were calculated and exhibited the following rank order: diltiazem (327 min⁻¹ · mmol/L⁻¹) > erythromycin (59 min⁻¹ · mmol/L⁻¹) > tadalafil (17.1 min⁻¹ · mmol/L⁻¹). These results indicate that tadalafil is a substantially less efficient
mechanism-based inhibitor in vitro than either of the 2 positive controls.

Tadalafil was also evaluated for its ability to induce CYP3A and CYP1A2 activity in primary cultures of human hepatocytes. On the basis of the results of these studies, it does not appear that tadalafil would have a marked effect on in vivo CYP1A2 activity. Hepatocyte preparations exposed to 1 μmol/L tadalafil over a 2-day period demonstrated induction of CYP3A protein and activity. However, at higher doses of tadalafil, although CYP3A protein levels were induced, a reduction of CYP3A activity relative to protein levels was observed. These results suggest that tadalafil caused both inhibition and induction of CYP3A. Although there was a biphasic effect on activity dependent on concentration, measurement of CYP3A protein demonstrated that induction of CYP3A protein occurred with hepatocytes from all donors at nearly all tadalafil concentrations tested. A short-term exposure study of tadalafil with hepatocytes (0 to 60 minutes) confirmed that there was a time-dependent loss of CYP3A activity, further suggesting that mechanism-based inhibition occurs. This pattern of inhibition and induction by tadalafil suggests that mechanism-based inhibition of CYP3A is occurring in concert with CYP3A induction in hepatocytes. The net effect of these processes in vitro appears to be related to tadalafil concentration and results in essentially little change in CYP3A activity.

The clinical significance of the in vitro results depends on the ability of tadalafil to bind to the appropriate receptor for induction and reach the enzyme for inhibition. In vivo results may range from either inhibition or induction prevailing or no net effect. Interestingly, it has been reported that other methylenedioxyphenyl-containing compounds can not only cause mechanism-based inhibition but also induce enzymatic activity. Therefore clinical studies with recognized CYP3A probe substrates were conducted to definitively evaluate possible inhibition or induction of CYP3A activity in vivo by tadalafil. Evaluation after 1 or 2 or multiple tadalafil doses would distinguish the potential effect of time of onset on the inhibition or induction of CYP3A by tadalafil. The progressive nature of mechanism-based inhibition is exemplified by erythromycin treatment, where erythromycin dosing for 1 day had a negligible effect on systemic clearance of alfentanil but treatment for 7 days decreased alfentanil clearance by 26%. In another study accumulation of the CYP3A substrate terfenadine was observed after 7 days of co-administration with erythromycin. Because CYP3A probe substrates typically exhibit high between- and within-subject variability in pharmacokinetics, the equivalence limits for the 90% confidence intervals of the geometric LS means were set at 0.73 and 1.43. These equivalence limits were selected on the basis of the knowledge that the 0.8 to 1.25 limits are unnecessarily rigorous for highly variable probes, which would lead to a false-positive claim of a statistically significant difference even for the data obtained on the control days (days 1 and 8, before tadalafil dosing). Midazolam pharmacokinetics after the first and last dose during a 2-week daily-dose regimen of 10 mg tadalafil, as well as 2 weeks after completion of tadalafil dosing, was determined to be equivalent to the pharmacokinetics determined before

Fig 5. Lovastatin pharmacokinetic parameters after administration of 40 mg lovastatin at baseline (day 1), after second dose (day 9) and last dose (day 21) of daily dosing with 20 mg tadalafil, and after 2-week washout period (day 35). Boxes indicate 25th and 75th percentiles, and whisker bars indicate 10th and 90th percentiles. Solid horizontal bars within boxes indicate mean data. Circles represent values falling outside 10th and 90th percentiles.
tadalafil administration. Thus these results indicate that there was no evidence that single or multiple doses of 10 mg tadalafil resulted in a change in CYP3A activity in vivo. These findings are in stark contrast with trials evaluating the interaction of midazolam with known potent CYP3A4 inhibitors, such as itraconazole, or known CYP3A4 inducers, such as rifampin. In such studies coadministration of itraconazole increased midazolam exposure by approximately 800% and coadministration of rifampin decreased midazolam exposure by 98%. An additional clinical study was conducted to confirm the lack of an effect on CYP3A4 activity when a higher dose of tadalafil (20 mg) was administered. Because no change was detected after 1 day of tadalafil exposure in the midazolam study, the pharmacokinetics of lovastatin was evaluated after the second tadalafil dose. Furthermore, lovastatin pharmacokinetics was evaluated after the second 20-mg tadalafil dose, rather than the first, to best detect a mechanism-based inhibitory effect because this may occur rapidly but may be subsequently disguised by offsetting induction. The
90% confidence intervals for ratios (lovastatin with tadalafil:lovastatin alone) of LS mean AUC and C\text{max} values were completely contained in the prespecified no-effect boundary. Therefore it was concluded that 20 mg tadalafil administered daily had no significant effect on the pharmacokinetics of the CYP3A probe substrate lovastatin. These results are in contrast to the observed inhibition of CYP3A4 by itraconazole, which increased lovastatin AUC and C\text{max} values by approximately 15-fold.\textsuperscript{40}

The findings with midazolam and lovastatin provide strong evidence that short-term and long-term exposure to therapeutic concentrations of tadalafil do not alter the clearance of other drugs metabolized by CYP3A4. As further support of this conclusion, it is known that mechanism-based inhibitors often alter their own metabolic clearance in vivo. Thus exposure to a drug that may be a mechanism-based inhibitor would be predicted to be dose- and time-dependent. However, for tadalafil, over a dose range of 2.5 to 20 mg, exposure increased proportionally with dose, and single-dose pharmacokinetics (half-life, 17.5 hours) is predictive of observed plasma concentrations during multiple dosing.\textsuperscript{5,41}

In conclusion, in vitro results suggest that tadalafil would not reversibly inhibit the metabolism of coadministered substrates of the major human CYPs but may have the potential to be a weak mechanism-based inhibitor and an inducer of CYP3A. Definitive clinical assessments demonstrated that, if induction and inhibition occurred, they were offset, because the pharmacokinetics of midazolam and lovastatin, CYP3A probe substrates, was virtually unchanged after coadministration with 10 and 20 mg tadalafil. Thus therapeutic concentrations of tadalafil do not produce clinically significantly changes in the clearance of drugs metabolized by CYP3A.

We gratefully acknowledge Jessica L. Fayer, Shelly N. Binkley, Kenneth Ruterbories, and Steve C. Kasper for their performance of the reversible interaction studies for CYP1A2, 2D6, 2C9, 2C19, and 3A; David Petullo for the performance of the statistical analysis for the hepatocyte work; and Karen Lee for preparation of Figs 4 and 5. In addition, we also thank David J. Humphries, Kirk D. Knotts, Kenneth Ruterbories, Bradley Ackerman, and Frank J. Belas for development of the tadalafil assay and Mary McIntosh, Amanda Marland, and Ernest Wong for tadalafil concentration measurements.

All authors are employed by Eli Lilly and Company and own stock and/or stock options in the company.

References


PHARMACODYNAMICS AND DRUG ACTION

Population pharmacokinetics of CCI-779: Correlations to safety and pharmacogenomic responses in patients with advanced renal cancer

Objective: Our objective was to estimate the pharmacokinetic parameters of CCI-779 and its metabolite, sirolimus, and evaluate associations of exposure parameters with safety and clinical activity. Exposure parameters were also correlated with pharmacogenomic responses in peripheral blood mononuclear cells (PBMCs).

Methods: In this randomized, double-blind, multicenter trial, once-weekly intravenous doses of 25, 75, or 250 mg CCI-779 were administered to patients with advanced renal cancer. Whole blood for CCI-779 and sirolimus concentrations was drawn. Population pharmacokinetic analyses yielded Bayesian-predicted exposure metrics that were correlated with severity and duration of adverse events and survival. PBMC samples taken before and after treatment were examined for pharmacogenomic responses. Ribonucleic acid samples were converted to labeled probes and hybridized to oligonucleotide arrays containing more than 12,600 human sequences.

Results: The final population pharmacokinetic models of CCI-779 and sirolimus included 235 and 305 observations, respectively, from 50 patients. For CCI-779, dose, single versus multiple dose, and body surface area were significant pharmacokinetic covariates. For sirolimus, dose and hematocrit were significant covariates. Age, sex, or race did not influence drug disposition. CCI-779 area under the curve correlated with adverse event severity for thrombocytopenia ($P = .007$), pruritus ($P = .011$), and hyperlipemia ($P = .040$). Exposure (CCI-779 cumulative area under the curve) correlated with a specific subset of gene transcripts in PBMCs following 16 weeks after therapy ($P < .001$, Spearman correlation).

Conclusions: Concentrations of CCI-779 and sirolimus were adequately described with a population model incorporating factors for dose, attenuated exposure of multiple doses, body surface area, and hematocrit. Correlations with adverse event severity and duration profiles were provided to aid in the detection of treatment-emergent effects. Pharmacogenomic profiling of PBMCs identified altered ribonucleic acid transcript expression levels that correlate with exposure. These transcripts represent potential biomarkers of CCI-779 exposure in peripheral blood. (Clin Pharmacol Ther 2005;77:76-89.)

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CCI-779 is a unique anticancer agent with demonstrated significant inhibitory effects on tumor growth in a number of in vitro and in vivo models.1-6 Mechanistically, both CCI-779 and its major metabolite, siroli-
mus, have exhibited equipotent activity to inhibit kinase activity of mammalian target of rapamycin (mTOR), a key mediator of phosphoinositide 3-kinase signal transduction. Inhibition of mTOR leads to arrest of cells in the G1 phase of the cell cycle, which may in turn delay time to tumor progression or recurrence.

CCI-779 has been evaluated in a phase 2 study in patients with advanced renal cell carcinoma. When CCI-779 (25, 75, or 250 mg) was administered intravenously weekly, it produced an objective response rate of 7% and a minor response rate of 26%. It was generally well tolerated, with hyperglycemia (17%), hypophosphatemia (13%), anemia (9%), and hypertriglyceridemia (6%) being the most frequently occurring grade 3 or 4 adverse events. Neither toxicity nor efficacy was significantly influenced by CCI-779 dose level.

The pharmacokinetics of CCI-779 in these patients was studied. Exposure in whole blood after intravenous administration generally increases less than proportionally with dose. Steady-state volume of distribution ($V_{ss}$) is large, increases with dose, and ranges from 230 L (after a 25-mg dose) to as high as 900 L (after a 250-mg dose). Distribution of CCI-779 into red blood cells appears to be preferential at lower doses and saturable at higher doses. Metabolism of CCI-779 occurs mainly via oxidative hydrolysis to form sirolimus, with a mean area under the curve (AUC) ratio (sirolimus to CCI-779) ranging from 2.8 to 5.3. Both CCI-779 and sirolimus are extensively metabolized via cytochrome P450 (CYP) 3A enzymes to form various demethylated and hydroxylated isomeric products that are predominantly excreted in the feces. Clearance from whole blood also increases with increasing dose, as observed with $V_{ss}$, with mean values ranging from 20 L/h after a 25-mg dose to 100 L/h after a 250-mg dose (coefficient of variation, approximately 16%-27%). The terminal half-life of CCI-779 is approximately 13 hours for CCI-779 and 50 hours for sirolimus.

A primary objective of this study was to evaluate the relationship of pharmacokinetic (PK) exposure to clinical safety responses in patients with advanced, refractory renal cell carcinoma who were administered weekly doses of CCI-779 (25, 75, and 250 mg). Formal clinical safety and efficacy results for this study population were previously reported by Atkins et al. The most frequently occurring grade 3 or 4 adverse events observed included hyperglycemia (17%), hypophosphatemia (13%), and anemia (9%). Neither toxicity nor efficacy was significantly influenced by CCI-779 dose level.

A secondary goal in this study was to evaluate any potential pharmacogenomic effects of CCI-779, as measured in the peripheral blood in renal cancer patients. The use of microarrays in clinical settings has received increased attention in the clinical and regulatory communities based on early reports that expression profiles of tumor tissues may identify transcriptional patterns associated with disease, disease severity, and even clinical responsiveness. Access to fresh tumor tissue, however, is often impractical in clinical settings, including in patients with advanced renal cell carcinoma entering a phase II trial. Because of its accessibility, peripheral blood represents an attractive alternative (surrogate) tissue for identification of markers of drug exposure. The possibility of discovering biomarkers by expression profiling of surrogate tissues had been anticipated and a recent report identified disease-associated transcripts in peripheral blood mononuclear cells (PBMCs) collected from renal cancer patients before initiation of therapy in the present clinical trial. Therefore an additional objective in this PK study was to identify transcripts in PBMCs that,
after initiation of CCI-779 therapy, exhibit temporal profiles correlated with PK measures of CCI-779 exposure in vivo.

**METHODS**

**Study design**

This was a randomized, double-blind, multicenter, outpatient phase II study of CCI-779 administered by 30-minute intravenous infusion (via an automatic dispensing pump) in patients with advanced renal cell carcinoma. Eligible patients were randomly assigned to treatment in a 1:1:1 ratio to receive 25, 75, or 250 mg of CCI-779 weekly until evidence of disease progression was demonstrated.

Full PK profiling was planned in a subset of patients, with remaining patients to provide sparse sampling. Time points for whole-blood concentrations of CCI-779 and sirolimus in fully sampled patients were scheduled at 0 hours (predose) and at 0.5, 1, 2, 6, 24, 74, 96, and 168 hours after the start of the 30-minute infusion during weeks 1 and 4 of treatment. For patients undergoing limited PK blood sampling, whole blood was drawn for CCI-779 and sirolimus at 0 hours (predose), at 0.5 hour, and at the discretion of the investigator and patient, on 1 other day after dosing during week 4.

Consent for the pharmacogenomic portion of the clinical study was requested separately after the project was approved by the local institutional review boards of participating clinical sites. A total of 50 of the original 111 patients enrolled in the study provided consent for pharmacogenomic analysis. In total, 45 evaluable baseline profiles were obtained; 33 evaluable week-8 profiles and 23 evaluable week-16 profiles were obtained for the purposes of pharmacogenomic analysis. Blood samples (8 mL) for pharmacogenomic characterization were drawn into cell purification tubes (CPT; Becton Dickinson, Rutherford, NJ) before therapy and after approximately 8 and 16 weeks of treatment. All blood samples were shipped overnight to the Wyeth Department of Molecular Medicine, Andover, Mass, and PBMCs were isolated from the whole blood samples according to the manufacturer’s direction.

**Analytic method**

The bioanalytic method for CCI-779 was performed by use of whole blood in a liquid chromatography–tandem mass spectrometry procedure with deuterated internal standard. Plasma was not used because of limitations in analyte stability. The method was validated through the quantitation range of 0.25 to 100 ng/mL by use of 1 mL of ethylenediaminetetraacetic acid–treated whole blood and, during validation, exhibited interday and intraday variabilities, expressed as coefficient of variation of 5% or lower and biases of 9.4% or lower. The bioanalytic method for sirolimus also used a liquid chromatography–tandem mass spectrometry procedure that was validated through the quantitation range of 0.1 to 100 ng/mL by use of 1 mL of blood. For cases in which concentrations exceeded the validated range, blood samples were reassayed by use of a dilution appropriate to the calibration range. Collectively, the interday and intraday variabilities of sirolimus in quality control samples measured during validation were 12.7% or lower (coefficient of variation), and biases were 11.3% or lower.

**Population PK analyses**

To characterize the PK profiles of all patients, fully and sparsely sampled patient data were analyzed collectively by use of a population PK method and the NONMEM application (version 1.0, revision 5.0, on Windows NT 4.0 computer [Microsoft Corporation, Redmond, Wash] with Pentium processor [Intel Corporation, Santa Clara, Calif]). From earlier study, we reported that sirolimus was the major metabolite resulting from CCI-779 treatment in humans. Because sirolimus is equipotent to CCI-779 and is formed to a significant degree, preliminary efforts to analyze CCI-779 and sirolimus with a common model were attempted. However, the modeling proved unsuccessful in part because of complexities of competition for binding in red blood cells, the multicompartmental nature of disposition, and uncertainty regarding the fraction of drug metabolized. PK data were, therefore, modeled separately and segregated by analyte into 2 separate data sets with dose, time and duration of administration, and demographic information. Construction of the NONMEM data set was performed by use of SAS (version 8.1) on a Sun Microsystems mainframe computer with Sun OS 5.8 (Sun Microsystems, Inc, Santa Clara, Calif). For CCI-779, a 3-compartment model with zero-order infusion was found to most adequately describe the data. For sirolimus, a 2-compartment model with first-order input was appropriate. In both cases, the model-derived value for AUC for a given patient and analyte was obtained from the quotient of CCI-779 dose/clearance (CL), in which CL was obtained from Bayesian estimation and the POSTHOC option of NONMEM. AUC$_{\text{sum}}$ for each patient was calculated as the sum of CCI-779 and sirolimus AUCs.
During model building, an exponential error model was applied to the kinetic model. A proportional model best described the intraindividual residual error. To assess goodness of fit, the criteria described by Pai et al\textsuperscript{20} were considered and included (1) decrease of the objective function of more than 3.84 (\(P < .05\)) during model building and more than 7.88 (\(P < .005\)) during model reduction (\(P\) values assume a normal chi square distribution), (2) minimization of the SEs with respect to the parameter estimates, (3) random scatter of points around a horizontal line of identity at 0 in plots of weighted residual versus predicted concentrations, (4) minimization of interindividual variances and an improvement in their precision, and (5) a reduction in the magnitude of residual variability.

**PK associations with safety**

*Correlation to adverse event severity.* Tests for association of drug exposure (CCI-779 AUC, AUC\textsubscript{sum}, and observed concentration of CCI-779 at end of infusion) with adverse event severity were performed using a nonparametric correlation analysis (Spearman rank correlation).

**Fig 1.** Observed and predicted patient concentrations of CCI-779 and sirolimus after multiple intravenous doses of CCI-779. Observed concentrations (circles) and predicted concentrations (lines) for CCI-779 (A, C, and E) and sirolimus (B, D, and F) are indicated for 25-mg (A and B), 75-mg (C and D), and 250-mg (E and F) doses of CCI-779.
sion \([C_{\text{cum}}]\) with safety end points were performed to evaluate whether the severity of a given adverse event (AE) was significantly associated with single-dose or cumulative-dose drug exposure. For the cumulative-dose drug exposure, CCI-779 cumulative AUC and cumulative AUC\(_{\text{cum}}\) were determined. Each patient’s specific dosage history while taking the trial medication was determined and used to derive respective AUCs for each patient for the duration of time from the start of treatment to the time of the highest-severity AE. This test included all of the data, regardless of AE severity, and was examined graphically and analyzed statistically by use of the asymptotic Mantel-Haenszel test for ordinal association.\(^{21}\) A severity score of 1 indicating the lowest severity and 3 indicating the highest severity was used; a score of 0 indicated that the patient did not have the AE. PK parameters were grouped into low, medium, and high categories with an equal number of patients in each category. Given the general utilitarian value of this method as a screening technique, a \(P\) value < .05, without adjustment for multiple comparisons, was considered indicative of a potentially clinically relevant association.

**Correlation with AE duration.** Tests for association of drug exposure (AUC\(_{\text{cum}}\) and cumulative AUC\(_{\text{cum}}\)) with safety end points were performed to evaluate whether the duration of a given AE was significantly associated with single-dose or cumulative-dose drug exposure. Estimation of cumulative exposure was determined for each patient as described. Duration of an AE was determined by calculating the time interval for which a given patient had a given AE. Only patients who had an AE were included in this test. The correlations between AE duration and the continuous PK variables were calculated by use of a Spearman rank correlation test.\(^{19}\) A \(P\) value < .05 was considered indicative of a potentially clinically relevant association.

**Pharmacogenomics analytic method**

For expression profiling analyses, total ribonucleic acid (RNA) was isolated from PBMC pellets by use of the RNeasy mini kit (Qiagen, Valencia, Calif), and the labeled probe for oligonucleotide arrays was prepared by use of a modification of the procedure described by Lockhart et al.\(^{22}\) Labeled probes were hybridized to oligonucleotide arrays comprising more than 12,600 human sequences (HgU95A; Affymetrix, Santa Clara, Calif) according to the manufacturer. Expression levels, expressed as “average difference” and absent or present call determinations, were computed from raw fluorescent intensity values by use of GENECHIP 3.2 software (Affymetrix). “Present” calls were calculated by GENECHIP 3.2 software by estimating if a transcript was detected in a sample on the basis of the strength of the gene-specific hybridization signal compared with background. The “average difference” values for each transcript were normalized to “frequency” values by use of the scaled frequency normalization method,\(^{23}\) in which the average differences for 11 control complementary RNAs with known abundance spiked into each hybridization solution were used to generate a global calibration curve. This calibration was then used to convert average difference values for all transcripts to frequency estimates, stated in units of parts per million and ranging from 1:300,000 (approximately 3 ppm) to 1:1000 (1000 ppm).

**PK associations to gene expression in PBMCs**

Expression profiling analysis of the 45 baseline PBMC samples, 33 samples at week 8, and 23 samples at week 16 revealed that of the 12,626 genes on the HgU95A chip 5469 genes met the initial criteria for further analysis (at least 1 sample with a “present” call across the data set and at least 1 sample with a frequency \(\geq 10\) ppm). The filter that removes transcripts with a frequency of 10 ppm or less in all samples is designed to remove low-abundance transcripts that demonstrate variable expression in technical replicates.

After data reduction, a Spearman rank correlation test was used to correlate the individually derived exposure metrics of CCI-779 cumulative AUC and cumulative AUC\(_{\text{cum}}\) observed at 8 or 16 weeks with either (1) static expression levels of PBMC transcripts at 8 or 16 weeks or (2) changes in expression from pretreatment levels to 8 or 16 weeks in patient PBMCs. Changes from pretreatment levels were calculated on the basis of log-transformed expression levels.

**RESULTS**

**Population PK analysis**

Mean demographic factors of patients providing samples for population analysis are shown in Table I. The typical demographic profile of patients was that of a 58-year-old white (90%) male (66%) weighing 83 kg and having a hematocrit level of 38.1%.

**CCI-779.** Blood samples from 90 patients were obtained from both fully and sparsely sampled patients for consideration in the population analyses. In this analysis 235 measurable observations from 50 patients were ultimately used to determine the final population model. A number of patients had concentrations below the limit of quantitation, especially when sampling for a given patient was limited to the later time course, and

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**Table I.**

<table>
<thead>
<tr>
<th>Demographic Factor</th>
<th>Mean</th>
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<td>Age (years)</td>
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<tr>
<td>Gender</td>
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<td>Weight (kg)</td>
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<tr>
<td>Hematocrit (%)</td>
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**Note:**

- Gender: 90% male, 66%
- Weight: 83 kg
- Hematocrit: 38.1%
were, therefore, dropped from analysis. Other typical causes for censoring PK observations included a high (>4) weighted residual, an aberrant concentration observation that adversely affected model fitting for the population, or questionable sample identification. A 3-compartment model was used with factors for nonlinear dose effect, multiple-dose decrease in exposure, and interpatient variability. Observed steady-state concentrations of CCI-779 appeared lower than expected from a linear prediction of accumulation. This phenomenon was empirically modeled in this study with the DNUM variable (effect after single or multiple doses) for CCI-779 clearance (Table II). Covariate analysis revealed that body surface area (BSA) is a significant factor affecting CCI-779 clearance (data not shown). Differences in age, sex, and race did not influence parent drug disposition (data not shown).

Final model typical values were used to generate the concentration versus time profiles shown in Fig 1. The final model was internally validated by the bootstrap approach. Results of this analysis indicate that most of the final model estimates lie within the 5th and 95th

| Table II. Final model typical values of pharmacokinetic parameters for CCI-779 |
|---------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Parameter estimate              | 0.139  | 37.6   | 6.48   | 271    | 0.585  | 323    | 0.551  | 1.203  |
| Precision of estimate (%)       | 29.9   | 14.8   | 14.4   | 11.5   | 24.0   | 18.5   | 6.5    | 35.0   |
| Interpatient variability (%)    | 23.7   | —      | —      | 44.1   | —      | 129    | —      | —      |

| Table III. Results of validation of final population pharmacokinetic model for CCI-779 |
|---------------------------------|--------|----------------|-----------|--------|
| Parameter                       | Unit   | Symbol          | 5th Percentile | 95th Percentile | Median | % Difference between median bootstrap and final model estimate |
| CL                              | L/h    | 0.76            | 2.56       | 1.45   | 1.39   | 4.3 |
| V1                              | L      | 27.7            | 47.2       | 38.3   | 37.6   | 1.9 |
| Q2                              | L/h    | 4.77            | 9.13       | 6.46   | 6.48   | 0.3 |
| V2                              | L      | 224             | 337        | 275    | 271    | 1.5 |
| Q3                              | L/h    | 0.164           | 0.430      | 0.267  | 0.258  | 3.5 |
| V3                              | L      | 139             | 1311       | 339    | 323    | 5.0 |
| Dose effect on CL               | —      | 0.480           | 0.629      | 0.553  | 0.551  | 0.4 |
| Interperiod variability on CL   | —      | 0.049           | 0.218      | 0.110  | 0.103  | 6.8 |
| BSA on CL                       | —      | 0.405           | 2.07       | 1.21   | 1.28   | 5.5 |
| Intersubject variability on CL  | —      | 0.028           | 0.412      | 0.215  | 0.056  | 283.9 |
| Intersubject variability on V2  | —      | 0.222           | 0.641      | 0.414  | 0.194  | 113.4 |
| Intersubject variability on V3  | —      | 0.247           | 1.56       | 1.08   | 1.67   | 35.3 |
| Proportional error              | —      | 0.335           | 0.436      | 0.383  | 0.157  | 143.9 |

BSA, Body surface area.

*Derived from 1000 successful bootstrap sample runs.
percentile confidence intervals of the bootstrap values (Table III). Exceptions to conformity with the confidence intervals were observed for intersubject variability on V2 and V3, as well as residual error. Bias between the final model and median bootstrap values appeared low (<7%) for all of the structural PK parameters. Somewhat higher differences for the pharmacostatistical parameters for intersubject and residual variabilities were seen. This is thought to occur when a data set is limited in size for a given model or when the model exhibits a substantial degree of parameterization relative to the data set.

**Sirolimus.** Blood samples from 90 patients were obtained from both fully and sparsely sampled patients for consideration in the population analyses. Of this number, 305 observations from 50 patients were ultimately included in the final analyses for sirolimus.

For sirolimus, a 2-compartment model with apparent first-order formation into the central compartment was used. Factors for nonlinear dose effect on apparent clearance and intersubject variability were incorporated. An analysis to identify demographic factors of variability indicated that hematocrit is a significant covariate of sirolimus volume of distribution. Final results are shown in Table IV and Fig 1.

The final model for sirolimus was also validated through bootstrapping. Results of this analysis are shown in Table V and indicate that most of the final model estimates lie within the 5th and 95th percentiles of confidence intervals of the bootstrap values. In addition, bias between the final model and median bootstrap sample data appears moderate (<38%) for all of the structural PK parameters between the final and median bootstrap values. Higher differences for the structural and pharmacostatistical parameters for inter-

### Table IV. Final model typical values of pharmacokinetic parameters for sirolimus

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<th>Parameter</th>
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<td>Dose effect on V3</td>
<td>—</td>
<td>(0_7)</td>
<td>0.055</td>
<td>0.414</td>
<td>0.231</td>
<td>0.302</td>
<td>23.5</td>
</tr>
<tr>
<td>Hematocrit effect on V3</td>
<td>—</td>
<td>(0_8)</td>
<td>0.314</td>
<td>1.41</td>
<td>0.768</td>
<td>0.719</td>
<td>6.8</td>
</tr>
<tr>
<td>Intersubject variability on CL</td>
<td>—</td>
<td>(\sigma_1)</td>
<td>0.382</td>
<td>0.930</td>
<td>0.545</td>
<td>0.406</td>
<td>34.2</td>
</tr>
<tr>
<td>Intersubject variability on V2</td>
<td>—</td>
<td>(\sigma_2)</td>
<td>0.592</td>
<td>4.843</td>
<td>1.418</td>
<td>2.72</td>
<td>47.9</td>
</tr>
<tr>
<td>Intersubject variability on V3</td>
<td>—</td>
<td>(\sigma_3)</td>
<td>0.000</td>
<td>0.363</td>
<td>0.179</td>
<td>0.052</td>
<td>244.2</td>
</tr>
<tr>
<td>Intersubject variability on (k_a)</td>
<td>—</td>
<td>(\sigma_4)</td>
<td>0.175</td>
<td>0.424</td>
<td>0.316</td>
<td>0.120</td>
<td>163.3</td>
</tr>
<tr>
<td>Proportional error</td>
<td>—</td>
<td>(\epsilon^2)</td>
<td>0.166</td>
<td>0.278</td>
<td>0.212</td>
<td>0.0543</td>
<td>290.4</td>
</tr>
</tbody>
</table>

*Derived from 1000 successful bootstrap sample runs.
subject and residual variabilities were observed; these may be a result of limitations in the data set for the given model or the inherently greater variability associated with metabolite data.

**Correlation of exposures to adverse events**

Pharmacodynamic analysis results by use of $AUC_{sum}^\dagger$ (discrete predictor), cumulative $AUC_{sum}^\dagger$ (cumulative predictor variable), and end-of-infusion concentration of CCI-779 ($C_{\text{ei}}^\dagger$) are shown in Table VI with representative figures (Figs 2 and 3) as derived from 49 differing AEs recorded for those patients included in the population analysis.

Clinically interesting associations between $AUC_{sum}^\dagger$ and AE severity were observed for thrombocytopenia ($P = .007$), pruritus ($P = .011$), and hyperlipemia ($P = .040$) (Table VI and Fig 2, A). Increased $AUC_{sum}^\dagger$ values were associated with increased duration of thrombocytopenia ($P = .015$) and dry mouth ($P = .036$) (Fig 3, A).

Analysis of cumulative exposures indicated potential associations between cumulative $AUC_{sum}^\dagger$ and AE severity for acne ($P = .003$), infection ($P = .003$), mucositis ($P = .004$), nail discoloration ($P = .005$), pruritus ($P = .011$), maculopapular rash ($P = .012$), and cough ($P = .05$ (Table VI and Fig 2, B). Similarly, correlations between cumulative $AUC_{sum}^\dagger$ and AE duration indicate that increased exposure was associated with increased duration of rash ($P < .001$), anorexia ($P = .001$), hyperglycemia ($P = .019$), diarrhea ($P = .03$), and maculopapular rash ($P = .046$) (Fig 3, B).

Thrombocytopenia, which was a clinically significant and frequent AE, was not associated with cumulative drug exposure ($AUC_{sum}^\dagger$) ($P = .834$), presumably because thrombocytopenia frequently led to a delay or reduction of dose.

Correlations between $C_{\text{ei}}^\dagger$ and AE severity revealed associations for myalgia ($P = .013$) and fever ($P = .022$) (Table VI). Similarly, correlations be-
between Ceoi and AE duration indicated possible associations with abdominal pain \( (P = .01) \) and anorexia \( (P = .015) \). A negative correlation was observed with fever \( (P = .04) \).

Correlation of exposures with PBMC gene expression levels

Pairwise correlations were calculated to assess the association between individually derived exposure metrics and gene expression levels measured by HgU95A Affymetrix microarrays during the course of therapy. Correlations were run for 2 PK parameters (CCI-779 cumulative AUC and cumulative AUC\(_{\text{sum}}\)) and for 4 measures of RNA expression level (log\(_2\)-transformed scaled frequency at 8 weeks, log\(_2\)-transformed scaled frequency at 16 weeks, the difference between log\(_2\)-transformed scaled frequency at 8 weeks and baseline, and the difference between log\(_2\)-transformed scaled frequency at 16 weeks and baseline).

The correlation analyses were based on Spearman rank correlations, which are not sensitive to potential nonnormal distribution properties of the PK param-
The P value for the hypothesis that the correlation was equal to 0 was calculated for each pairwise correlation. For each comparison between PK parameters and gene expression, the number of tests that were nominally significant of the 5469 tests performed was calculated for 3 type I (ie, false-positive) error levels. To appropriately adjust for the fact that 5469 non-independent tests were performed, a permutation-based approach was used to evaluate how often the observed number of significant tests would be found under the null hypothesis of no correlation. The only set of correlations for which there appeared to be substantially more statistically significant transcripts than would naturally be expected by chance alone was that between CCI-779 cumulative AUC versus change in gene expression at 16 weeks compared with pretreatment levels. The results of permutation tests run for CCI-779 cumulative AUC versus expression change at 8 weeks and at 16 weeks indicate that there was reasonably strong evidence for an association between CCI-779 cumulative AUC and the changes in gene expression in 19 transcripts (P < .001) at 16 weeks compared with pretreatment levels (data not shown). Table VII presents the results of these correlations at 8 weeks and 16 weeks for each of these 19 transcripts, and representative plots for 4 transcripts with the strongest association between exposure and expression are shown in Fig 4.
All expression data are available to the public in the Gene Expression Omnibus.26

DISCUSSION

In this phase 2 study, the PK profile of CCI-779 was characterized through use of a mixed sampling design in which 1 subset of 16 patients was extensively sampled during weeks 1 and 4 and the remaining patients were sparsely sampled during week 4 only. Certain results from patients sampled extensively were provided by Atkins et al8 but were incorporated in this analysis to support the pharmacostatistical structure for the more comprehensive population model.

To describe CCI-779 pharmacokinetics, a 3-compartment model with zero-order infusion was used. One feature of CCI-779 pharmacokinetics characterized in this study was the polyexponential and nonlinear nature of disposition when measured from whole

Table VII. Transcripts with changes in expression levels at 16 weeks correlated with CCI-779 cumulative AUC*

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Gene description</th>
<th>GO biologic</th>
<th>Average %CV in healthy individuals</th>
<th>Correlation with CCI-779 cumulative AUC 8 wk</th>
<th>P value 8 wk</th>
<th>Correlation with CCI-779 cumulative AUC 16 wk</th>
<th>P value 16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>U44839</td>
<td>Ubiquitin-specific protease 11</td>
<td>Deubiquitylation mRNA processing</td>
<td>18</td>
<td>0.16</td>
<td>.3998</td>
<td>0.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>AI762438</td>
<td>U2 snRNP auxiliary factor (65 kd)</td>
<td></td>
<td>21</td>
<td>0.23</td>
<td>.2209</td>
<td>0.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>U90917</td>
<td>Forkhead box M1</td>
<td>Transcription or oxidative stress</td>
<td>30</td>
<td>0.24</td>
<td>.206</td>
<td>0.75</td>
<td>0.0001</td>
</tr>
<tr>
<td>AF038661</td>
<td>UDP-Gal:betaGlcNAc beta-1,4-galactosyltransferase, polypeptide 3</td>
<td>Sugar metabolism</td>
<td>15</td>
<td>0.21</td>
<td>.2589</td>
<td>0.73</td>
<td>0.0002</td>
</tr>
<tr>
<td>AL046394</td>
<td>Clone DKFZp434M217 5' mRNA sequence</td>
<td></td>
<td>20</td>
<td>0.31</td>
<td>.0999</td>
<td>0.72</td>
<td>0.0002</td>
</tr>
<tr>
<td>AI540925</td>
<td>Cytochrome c oxidase subunit VIa polypeptide 1</td>
<td>Energy pathways</td>
<td>10</td>
<td>−0.5</td>
<td>.005</td>
<td>−0.73</td>
<td>0.0002</td>
</tr>
<tr>
<td>H98552</td>
<td>cDNA DKFZp586f0523</td>
<td>Energy pathways</td>
<td>30</td>
<td>0.39</td>
<td>.0308</td>
<td>0.71</td>
<td>0.0003</td>
</tr>
<tr>
<td>U48734</td>
<td>Actinin, alpha 4</td>
<td>Cell motility, invasive growth</td>
<td>22</td>
<td>0.33</td>
<td>.0721</td>
<td>0.71</td>
<td>0.0003</td>
</tr>
<tr>
<td>AI147237</td>
<td>RP immunoglobulin heavy-chain FW2-JH region gene</td>
<td>Immunoglobulin</td>
<td>27</td>
<td>0.32</td>
<td>.0877</td>
<td>0.70</td>
<td>0.0004</td>
</tr>
<tr>
<td>M19309</td>
<td>Troponin T1, skeletal, slow</td>
<td>Muscle contraction regulation</td>
<td>80</td>
<td>0.21</td>
<td>.2684</td>
<td>0.70</td>
<td>0.0004</td>
</tr>
<tr>
<td>J05257</td>
<td>Dipeptidase 1 (renal)</td>
<td>Enzyme metabolism</td>
<td>39</td>
<td>0.39</td>
<td>.0348</td>
<td>0.69</td>
<td>0.0005</td>
</tr>
<tr>
<td>AL022318</td>
<td>Clone 150C2 on chromosome 22q13.1-13.2</td>
<td>Unknown</td>
<td>22</td>
<td>0.21</td>
<td>.2617</td>
<td>0.69</td>
<td>0.0005</td>
</tr>
<tr>
<td>U92315</td>
<td>Sulotransferase family 2B, member 1</td>
<td>Steroid metabolism</td>
<td>34</td>
<td>0.3</td>
<td>.1066</td>
<td>0.69</td>
<td>0.0005</td>
</tr>
<tr>
<td>AF070548</td>
<td>Solute carrier family 25, member 11</td>
<td>Small molecule transport</td>
<td>29</td>
<td>0.32</td>
<td>.0881</td>
<td>0.69</td>
<td>0.0006</td>
</tr>
<tr>
<td>AB020664</td>
<td>KIAA0857 protein</td>
<td>Unknown</td>
<td>45</td>
<td>0.01</td>
<td>.9627</td>
<td>0.69</td>
<td>0.0006</td>
</tr>
<tr>
<td>M14565</td>
<td>Cytochrome P450, subfamily XIA</td>
<td>Steroid biosynthesis</td>
<td>22</td>
<td>0.32</td>
<td>.0838</td>
<td>0.69</td>
<td>0.0006</td>
</tr>
<tr>
<td>U17566</td>
<td>Solute carrier family 19 member 1</td>
<td>Folate transport</td>
<td>28</td>
<td>0.29</td>
<td>.122</td>
<td>0.68</td>
<td>0.0007</td>
</tr>
<tr>
<td>AF074382</td>
<td>I kappa B kinase gamma</td>
<td>Apoptosis</td>
<td>28</td>
<td>0.3</td>
<td>.1013</td>
<td>0.68</td>
<td>0.0007</td>
</tr>
<tr>
<td>M79463</td>
<td>Promyelocytic leukemia</td>
<td>Oncogenesis</td>
<td>22</td>
<td>0.38</td>
<td>.0378</td>
<td>0.68</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

GO biologic, Gene Ontology Biological Category; CV, coefficient of variation; mRNA, messenger ribonucleic acid; UDP, uridine diphosphate; cDNA, complementary deoxyribonucleic acid; snRNP, soluble neutrophil 1.

*For the 19 transcripts, accession numbers, a short description of the gene encoding the transcript, the functional annotation, the CV observed in healthy individuals, and the actual magnitude of the correlation with CCI-779 cumulative AUC and P value from the Spearman correlation test at both 8 and 16 weeks are presented.
blood. This behavior is thought to occur from specific drug binding to FK506 binding protein, an FKBP-class immunophilin in red blood cell membranes. Given this complexity, CCI-779 and sirolimus kinetics could not be characterized simultaneously with a common model; therefore sirolimus disposition was separately described by use of a 2-compartment model with first-order input. An exponential function that accounts for less-than-proportional exposure with dose and a factor for repeated doses were found to significantly minimize variability of the model for CCI-779 and were included. The final model for CCI-779 also incorporated a covariate for BSA on clearance. For a given dose, clearance of CCI-779 increases approximately 45% when administered to patients with BSA values that range from 1.5 to 2 m². Although substantial, previous data had shown that the effect of BSA on total concentrations (CCI-779 plus sirolimus) in blood is negligible and was confirmed in the current study by simulation. As expected with a drug that preferentially binds to red blood cells, the hematocrit exerted an important effect on sirolimus concentrations, with decreasing hematocrit level causing an overall decrease in composite drug concentrations.

To evaluate possible pharmacodynamic relationships to safety, discrete (Ceoi and AUCsum) and composite (cumulative AUCsum) Bayesian predictor variables were collated or derived for individual patients. The intent of the PK-AE correlations was to screen for potential relationships between drug exposure and AE severity or duration as an aid in identifying treatment-emergent effects. Testing was not corrected for multiple comparisons, increasing the potential for type I error and decreasing the probability of type II error. Therefore, by not making adjustments for multiple comparisons, the possibility of detecting a health risk when none exists is increased, allowing for conservative screening of potential exposure-response relationships. It is envisioned that this approach may generate hypotheses regarding the temporal relationship between CCI-779 exposure and toxicity, which can be tested further.

It was reported by Atkins et al8 that activity was observed for all CCI-779 doses administered; however, no relationship with patient survival or tumor shrinkage could be identified. PK parameters as predictor variables similarly failed to show a significant relationship (data not shown). Given the complexities between sig-
nal transduction interruption and measurable clinical effects, it followed that investigation to identify relevant biologic correlates of exposure appeared justified.

The search for transcriptional biomarkers correlated with drug exposure in surrogate tissues (eg, PBMCs) is a relatively new application of clinical pharmacogenomics in the field of oncology, which has to date largely focused on studies examining the expression profiles of primary tumors. The accessibility of surrogate tissues and the ability to perform rapid and noninvasive sampling for the analysis of drug effects will undoubtedly drive the search for expression profiles in these tissues in clinical trials in the future.

The pharmacogenomic objective of this study was to identify transcripts in PBMCs that appeared to covary with independently derived exposure metrics for patients in the study. By correlating exposure to expression, this analysis identified 19 transcripts with alterations in expression from pretreatment levels that were significantly correlated ($P < .001$) with individual values for CCI-779 cumulative AUC measured in the patients receiving CCI-779. Although the transcripts significantly correlated with CCI-779 exposure at 16 weeks were not significantly altered ($P < .001$) after only 8 weeks of therapy, the directions of the correlations (positive or negative) of the individual transcripts with CCI-779 exposure were conserved at 8 and 16 weeks for every transcript (Table VII).

In the absence of a control arm, there was no opportunity to understand any placebo effects on RNA expression profiles of disease or other factors in patients with advanced renal cell carcinoma. As an approximation, we analyzed expression profiles from PBMCs harvested at 8-week intervals ($n = 3$ time points) from 10 disease-free individuals to determine transcripts in peripheral blood that appear to vary naturally over a similar time course as measured in this study. A coefficient of variation was calculated by use of a 1-way ANOVA to estimate within-individual variation for each transcript. Only 1 of 19 transcripts, troponin, was found to possess a high (>80%) average coefficient of variation in PBMCs from normal individuals measured over time. The remaining 18 transcripts did not vary significantly (average coefficients of variation ranging between 10% and 45%) in the PBMCs of disease-free individuals measured at different time points, suggesting that the variation in the 18 transcripts in CCI-779–treated renal cancer patients may be explained by the presence of drug.

In the future, it will be important to determine whether these transcripts are specific markers of CCI-779 exposure in peripheral blood. A phase III clinical trial of CCI-779 in advanced renal cell carcinoma is comparing clinical outcomes in patients receiving interferon $\alpha$ alone, CCI-779 plus interferon $\alpha$, or CCI-779 alone. This ongoing study will enable comparison of longitudinal expression profiles among 3 treatments and provide an opportunity to confirm the transcriptional changes that appear to be specific to CCI-779 exposure in vivo observed in this study.

We thank Nicole Hinton for data management, Edward Faith for clinical programming, and Ron Yannuzzi for coordinating bioanalytic analysis. We also thank Susan Leinbach for assistance in manuscript preparation.

During this study, Joseph P. Boni, Cathie Leister, Natalie Twine, Jennifer Stover, Andrew Dorner, Fred Immermann, and Michael E. Burczynski were employees of Wyeth Research and held stock options. Gregor Bender was an employee of Wyeth Research, and Virginia Fitzpatrick was an independent contractor.

References


Neurocognitive changes in patients with hepatitis C receiving interferon alfa-2b and ribavirin

**Background:** During antiviral therapy of chronic hepatitis C, patients frequently report impairment of concentration or memory. Therefore we prospectively investigated neurocognitive performance in patients receiving interferon alfa and ribavirin.

**Methods:** Repeated computer-based testing of neurocognitive function was performed in 70 patients with chronic hepatitis C receiving interferon alfa-2b (pegylated or conventional) and ribavirin. In addition, depression scores were obtained (Hospital Anxiety and Depression Scale).

**Results:** Reaction times were significantly increased during treatment (mean reaction time increase after 3 months of therapy: alertness, 46.76 ms [95% confidence interval (CI), 26.86-66.66 ms], \( P < .001 \); divided attention, 47.04 ms [95% CI, 26.44-67.64 ms], \( P < .001 \); vigilance, 60.78 ms [95% CI, 29.24-92.32 ms], \( P < .001 \); and working memory, 38.53 ms [95% CI, 1.22-75.83], \( P = .34 \)). Accuracy measures (number of false reactions) were affected for the working-memory task exclusively. Cognitive performance returned to pretreatment values after the end of therapy. Cognitive impairment was not significantly correlated with the degree of concomitant depression (0.04 < \( r \) [absolute value] < 0.10, \( P > .390 \)).

**Conclusions:** Interferon-based combination therapy of chronic hepatitis C causes significant but reversible impairment of neurocognitive performance. Consequences for the requirements of an active life in patients with chronic hepatitis C receiving antiviral therapy need to be assessed. (Clin Pharmacol Ther 2005;77:90-100.)

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Chronic hepatitis C is one of the most frequent infectious diseases worldwide and a major cause of chronic liver disease. At diagnosis, approximately 20% of patients with chronic hepatitis C already have liver cirrhosis. Therapy for hepatitis C is still unsatisfactory, although at present a sustained loss of hepatitis C virus (HCV) will be reached in about 50% of patients treated with a combination of peginterferon alfa and ribavirin for up to 1 year.

Psychiatric side effects of interferon alfa are well known and may necessitate dose reduction or even premature discontinuation of therapy. In addition, patients undergoing interferon treatment frequently report concentration or memory impairment that in some cases interferes considerably with their capacity to manage the requirements of everyday life. This raises the possibility of a deterioration of cognitive functions during antiviral therapy of chronic hepatitis C.

Such side effects of interferons are suggested by very limited data from patients with cancer, amyotrophic lateral sclerosis, or multiple sclerosis. However, the results available so far are inconsistent, with reports even suggesting that interferon may not have any effect on neurocognitive parameters at all. Hence, an evaluation of potential interferon-induced neurocognitive side effects in a homogeneous study sample with a sufficient sample size has become necessary.

Although chronic hepatitis C is the most frequent indication for interferon therapy, no systematic data are available so far on possible impairment of cognitive...
function in these patients. Therefore we prospectively investigated timing, intensity, and specificity of neurocognitive changes induced by therapy with interferon alfa-2b (either pegylated or conventional) and ribavirin in patients with chronic hepatitis C.

**METHODS**

**Patients**

The participants were 83 consecutive patients in whom chronic hepatitis C was diagnosed at our institution or who were referred for antiviral therapy of known chronic hepatitis C. All patients had documented antibodies to HCV and circulating HCV ribonucleic acid as measured by reverse-transcription polymerase chain reaction (COBAS AMPLICOR HCV MONITOR Test; Roche Diagnostics, Basel, Switzerland). Patients were recruited from August 1998 to August 2001. The last patient finished the study in August 2002. Patients were not included if they were aged less than 18 years or more than 65 years or had coinfections (hepatitis B virus or human immunodeficiency virus), severe internal diseases (eg, cancer, ischemic heart disease, and autoimmune disease), psychiatric illness (severe depression or psychosis and so on), active intravenous drug use or alcohol abuse (within the last 6 months before possible study entry), obvious intellectual impairment, or insufficient knowledge of the German language. If liver cirrhosis was already present, it had to be well compensated (Child stage A).

All patients gave written consent to participate in the study before enrollment. The study was approved by the Ethics Committee for Medical Research of Würzburg University, Würzburg, Germany, in accordance with the Declaration of Helsinki.

**Study design and procedures**

This was a prospective, longitudinal, single-center study. Of 83 patients, 13 (15.7%) could not be included in the final evaluation and statistical analysis; 9 patients terminated therapy prematurely within the first 3 months because of intolerable side effects, and 4 patients withdrew their consent to participate in the study. Of 83 patients, 70 completed the study.

According to the changing recommendations in Germany during the study period, patients were treated with interferon alfa-2b and ribavirin from August 1998 until August 2000 (38/70 patients [54.3%]) or with peginterferon alfa-2b and ribavirin from September 2000 until August 2002 (32/70 patients [45.7%]). In the case of virologic response, 5 MIU of interferon alfa-2b 3 times weekly or 80 to 150 μg of peginterferon alfa-2b (1.5 μg/kg) once weekly was given for 12 months (genotype 1) or 6 months (genotypes 2, 3, and 4). All patients received oral ribavirin (800-1200 mg daily).

In all eligible patients, psychometric scores (depression) and computer-based test results for cognitive functions were obtained before therapy (t1) and after 4 weeks (t2), 3 to 4 months (t3), and 6 to 8 months (t4) of treatment, as well as 4 to 6 weeks (t5) after termination of therapy.

Blood samples were obtained during the patients’ medical visits at time points t1 to t5 for measurement of blood count and levels of transaminase, anti-HCV antibodies, and HCV ribonucleic acid. Genotype identification and liver biopsy (staging and grading: inflammation, fibrosis, or cirrhosis) were performed before therapy. The mode of infection was documented.

**Psychometric instruments**

*Hospital Anxiety and Depression Scale.* Depression and anxiety were assessed by the well-validated Hospital Anxiety and Depression Scale (HADS, German version, as published by Herrmann et al17). HADS is a 14-item questionnaire with dimensions of anxiety and depression. All items exclusively refer to the emotional state and do not reflect somatic symptoms.17

*Computer-based testing of neurocognitive functions.* Neurocognitive performance was assessed by a set of computer-assisted psychologic tests (Test for Attentional Performance [TAP], version 1.02c, as published by Zimmermann and Fimm18,19).

The core of the procedures comprises reaction-time tasks of low complexity allowing the evaluation of very specific deficiencies. The tasks consist of simple and easily distinguishable stimuli to which the patients react by a simple motor response. Out of a total of 12 subtests, the 4 most relevant computerized tasks were selected to monitor cognitive function during the treatment period in patients with chronic hepatitis C. During each session, the patients performed the tests in the following sequence:

1. **Alertness (testing time, 10 minutes):** This examination includes a simple and a cued reaction time task (visual test stimulus with and without an additional acoustic cue). The simple reaction time has been shown to be a valid measure of general slowness, whereas the difference between simple and cued reaction time is a measure of phasic alertness. The visual stimulus consists of a white cross on a black background presented approximately every 3 seconds. (A total of 80 stimuli are presented in this subtask.)
2. Divided attention (testing time, 5 minutes): Situations that require divided attention (attention to various aspects) are the rule, not the exception. This performance can be investigated by dual tasks. In this examination this is realized by independent visual and acoustic tasks. The visual task consists of crosses that appear in a random configuration in a $4 \times 4$ matrix. The subject has to detect whether the crosses form the corners of a square. The acoustic task comprises a regular sequence of high and low beeps. The subject has to detect an irregularity in the sequence.

3. Vigilance (testing time, 20 minutes): This is a bimodal task (combined visual and acoustic) that assesses sustained attention or vigilance over a time period of 15 minutes. Out of a series of monotonously presented acoustic and visual stimuli (beeps and letters alternately), the patient must press a button if the sequence “high beep followed by E” or “low beep followed by N” occurs.

4. Working memory (testing time, 15 minutes): The test measures the control of a continuous flow of information through short-term memory. Numbers are presented on the screen that must be compared with previously exposed numbers. The repetition of a number within a short interval has to be acknowledged by pressing a key. The frequency of the numbers appearing on the screen is about 1 per second. The key has to be pressed when the presented number equals the last number except 1.

**Statistical analysis**

Data were registered and analyzed by use of the Statistical Package for Social Sciences (SPSS for Windows, German version 10.0.7\(^{19}\)). All tests of significance were 2-tailed. \(P\) values \(< .05\) were considered statistically significant. Because of the explorative character of the study, we did not consider \(\alpha\) adjustment in multiple comparisons.

**Descriptive analysis.** Results describing quantitative measures are expressed as median or mean \(\pm\) SD or SE. Qualitative variables are presented as counts and percentages.

**Tests of significance.** Comparison of variables representing categoric data was performed with the chi square test. Mean differences of continuous variables between patient subgroups were examined by either \(t\) tests for independent samples or ANOVA if more than 2 subgroups were included. Group means of dependent samples (eg, time course of continuous variables) were compared by means of repeated-measures ANOVA (general linear model procedure, repeated-measures design). Corresponding contrasts were analyzed by paired \(t\) tests. Pearson correlation was used when appropriate (assessment of associations between quantitative variables).

**RESULTS**

**Study population**

Table I shows the characteristics of the 70 patients who were included in the final evaluation. There were no significant differences in sociodemographic or biomedical parameters between both subgroups (treatment with conventional or pegylated interferon) with the one exception that patients treated with pegylated interferon alfa-2b were slightly but significantly older than patients receiving conventional interferon alfa-2b (\(P = .048\)). Sex, acquisition mode, virus genotype, and liver histologic characteristics did not differ significantly.

A total of 11 of 70 patients (15.7\%) received antidepressant medication (selective serotonin reuptake inhibitor [SSRI]) (20 mg paroxetine daily) during the evaluation period, as follows: Two patients were receiving (prophylactic) SSRI medication before the onset of interferon treatment. Another 9 patients received concomitant SSRI therapy subsequent to substance-induced major depression (without suicidal ideation) for the remainder of the antiviral treatment. Subgroup analysis revealed that the factor SSRI treatment had no significant effect on the neurocognitive parameters evaluated in our study (data not shown).

**Tests of cognitive function**

Both reaction times and accuracy measures did not differ significantly between patients treated with conventional or pegylated interferon alfa. Consequently, the results are presented for the study group as a whole.

Fig 1 shows the reaction times for the TAP alertness, divided attention (visual and acoustic subtasks), and vigilance tasks at the different measuring points. During therapy with interferon and ribavirin, reaction times increased for all tasks, with significance failing only for the visual subtask of divided attention (\(P = .060\)) (reported \(P\) values refer to repeated-measures ANOVA analysis). The increase in reaction time reached a maximum at either t3 or t4 (3 or 6 months of therapy, respectively). At t4, only 66 of 70 patients were receiving interferon therapy because 4 patients had to stop antiviral treatment prematurely (Fig 1). On average, reactions were delayed by 46.8 ms (alertness) and 65.8 ms (divided attention, acoustic subtask). Observed effect sizes ranged from 0.65 (vigilance) to 0.98 (divided attention, acoustic subtask). Mean relative reaction time increases were between 5.4\% (working-
memory task) and 17.7% (alertness subtask). After termination of therapy (t5), reaction times returned to the pretreatment values.

Regardless of the increase in reaction times, the “correctness” of the reactions (omissions and false alarms) remained unchanged during interferon-ribavirin therapy as compared with the pretreatment and posttreatment values. Overall, 54 of 70 patients (77.1%) had deterioration in at least 1 of the TAP alertness, divided attention, or vigilance subtasks at t3 (after 3 months of therapy). Patients who experienced worsening could not be characterized by pretherapeutic variables (age, sex, type of interferon, baseline neurocognitive performance, acquisition mode, liver histologic characteristics, or education level) by use of logistic regression analysis.

The TAP working-memory task was available for only 59 of the 70 hepatitis C patients. (As indicated in Fig 2, at t4, 56 of 59 patients were receiving therapy because of premature termination of antiviral medication.) Reasons for the missing data comprise in particular the high task complexity and the sequence of the tests; the memory subtest was the last test presented to the study patients. In our view, however, the evaluation of this test in only some of the patients is justified because the working-memory task covers one important aspect of cognitive performance frequently reported as impaired by patients during therapy with interferon alfa plus ribavirin.

Mean reaction times (Fig 2, A) increased significantly over time (P = .034) (reported P values refer to repeated-measures ANOVA analysis) with a maximum at t4 (effect size, 0.36). In addition, there was a significant impairment of cognitive performance concerning both the number of correct reactions (Fig 2, B; decline, P < .001) and the number of omissions (increase, P < .001; data not shown). The frequency of false alarms, however, did not change during the treatment (P = .578).

To control these results for potential biasing effects, we compared the 11 patients who were not able to complete the working-memory tasks in our study with the patients without missing data. Subgroup analysis revealed that there was no significant difference between these subgroups with respect to sex, acquisition mode, HADS scores, or neurocognitive performance (both before and during antiviral treatment with interferon alfa-2b). However, the 11 patients without complete working-memory data were slightly but significantly older than the remainder of the study sample (47.7 versus 41.3 years, P = 0.033).

### Table I. Pretherapeutic patient characteristics and HADS depression scores

<table>
<thead>
<tr>
<th>Sociodemographic or biomedical factor</th>
<th>All patients (N = 70)</th>
<th>Peginterferon plus ribavirin (n = 32)</th>
<th>Interferon alfa-2b plus ribavirin (n = 38)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) (mean ± SD, range)</td>
<td>42.3 ± 9.3 (22-65)</td>
<td>44.7 ± 7.9 (25-63)</td>
<td>40.3 ± 10.0 (22-65)</td>
<td>.048</td>
</tr>
<tr>
<td>Women</td>
<td>30 (42.9%)</td>
<td>15 (46.9%)</td>
<td>15 (39.5%)</td>
<td>.533</td>
</tr>
<tr>
<td>Men</td>
<td>40 (57.1%)</td>
<td>17 (53.1%)</td>
<td>23 (60.5%)</td>
<td></td>
</tr>
<tr>
<td>Acquisition mode</td>
<td></td>
<td></td>
<td></td>
<td>.277</td>
</tr>
<tr>
<td>Unknown</td>
<td>21 (30.0%)</td>
<td>12 (37.5%)</td>
<td>9 (23.7%)</td>
<td></td>
</tr>
<tr>
<td>IVDU</td>
<td>36 (51.4%)</td>
<td>15 (46.9%)</td>
<td>21 (55.3%)</td>
<td></td>
</tr>
<tr>
<td>After transfusion</td>
<td>13 (18.6%)</td>
<td>5 (15.6%)</td>
<td>8 (21.0%)</td>
<td></td>
</tr>
<tr>
<td>Virus genotype</td>
<td></td>
<td></td>
<td></td>
<td>.354</td>
</tr>
<tr>
<td>Genotype 1</td>
<td>36 (51.4%)</td>
<td>17 (53.1%)</td>
<td>19 (50.0%)</td>
<td></td>
</tr>
<tr>
<td>Genotype 2</td>
<td>10 (14.3%)</td>
<td>5 (15.6%)</td>
<td>5 (13.2%)</td>
<td></td>
</tr>
<tr>
<td>Genotype 3</td>
<td>22 (31.4%)</td>
<td>8 (25.0%)</td>
<td>14 (36.8%)</td>
<td></td>
</tr>
<tr>
<td>Genotype 4</td>
<td>2 (2.9%)</td>
<td>2 (6.3%)</td>
<td>0 (0.0%)</td>
<td>.184</td>
</tr>
<tr>
<td>Liver biopsy* or liver damage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis only</td>
<td>36 (52.2%)</td>
<td>13 (40.6%)</td>
<td>23 (62.2%)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>18 (26.1%)</td>
<td>11 (34.4%)</td>
<td>7 (18.9%)</td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>15 (21.7%)</td>
<td>8 (25.0%)</td>
<td>7 (18.9%)</td>
<td></td>
</tr>
<tr>
<td>Nonresponder</td>
<td>38 (54.3%)</td>
<td>16 (50.0%)</td>
<td>22 (57.9%)</td>
<td>.509</td>
</tr>
<tr>
<td>Responder</td>
<td>32 (45.7%)</td>
<td>16 (50.0%)</td>
<td>16 (42.1%)</td>
<td></td>
</tr>
<tr>
<td>HADS depression score before therapy</td>
<td>4.56 ± 3.42</td>
<td>4.50 ± 3.55</td>
<td>4.61 ± 3.37</td>
<td>.899</td>
</tr>
</tbody>
</table>

HADS, Hospital Anxiety and Depression Scale; IVDU, intravenous drug use.

*In 1 patient treated with conventional interferon alfa-2b, liver biopsy was declined by the patient.
Depression

As expected, depression occurred during the treatment period. HADS depression scores rose significantly to a maximum at t4 (P < .001). The interferon-induced increase of HADS depression scores in the study sample is displayed in Fig 1 (with corresponding data and stratified numbers given in Table I). The therapy mode (conventional versus pegylated interferon) had no significant effect on either extent (main effect therapy mode, P = .752) or time course (interaction time × therapy mode, P = .068) of depression.

Given that patients with depressive disorders frequently complain about symptoms such as difficulty in concentrating, slowed thinking, or indecisiveness, we tested whether the cognitive impairment observed in our study patients could be explained by depression. Pearson correlation coefficients revealed that there was neither a statistically significant (P < .500) nor a clinically relevant association (r [absolute value] <0.1) between changes in HADS depression scores and therapy-induced decreases observed in TAP subtasks (changes from t1 to t3).

Anxiety

Confirming the results of previous studies, HADS anxiety scores increased significantly to maximum values at t4 (P < .001). Interferon-induced anxiety was reversible after the end of interferon treatment. Neurocognitive performance was not significantly correlated with the extent of therapy-induced anxiety (data not shown).

Anemia

To assess a potential effect of ribavirin-induced hemolysis and anemia, the changes in hemoglobin levels between t1 and t3 were correlated with the respective changes in reaction times. Hemoglobin values decreased significantly during antiviral therapy. Pearson correlation coefficients (r = 0.27 for vigilance, P = .024) showed that cognitive impairment during combination therapy for chronic hepatitis C was weakly but significantly linked to the drop in hemoglobin values.

Severity of liver disease

There was no significant correlation between the degree of liver disease (histologic staging and grading) and the attentional performance in the TAP tasks (data not shown). Liver function or Child stage did not deteriorate in any of our patients during the study period.

Further factors possibly influencing neurocognitive symptoms

Age and education level are well-known factors that may have an impact on neuropsychologic tasks; therefore we correlated these variables with measures of interferon-induced cognitive changes. However, we were not able to find any statistically or clinically relevant associations of the extent of neurocognitive decline with age or education level (data not shown; correlation coefficients were all below 0.3).

The increase in reaction time in the single subtasks did not show any significant correlation with pretreatment cognitive performance (r [absolute value] <0.01). However, there was a correlation between absolute performances before and during interferon treatment (eg, for alertness subtask, r = 0.6).

Some studies have suggested a direct association between hepatitis C infection (presence of HCV) and cognitive impairment. Therefore we compared the subgroups with (n = 32 [45.7%]) and without (n = 38 [54.3%]) virologic response with respect to their post-treatment cognitive performance. Neither reaction times nor measures of accuracy differed significantly between both subgroups (.10 < P < .80, t tests for independent samples).

Likewise, in our patients (both total study sample and responders), no significant improvement was found between pretreatment and posttreatment levels of neurocognitive performance (time points t1 and t5; P > .05, paired t tests).

Fig. 1. Time course of mean Test for Attentional Performance (TAP) reaction times (in milliseconds) (±SEM) in 70 patients before, during, and after interferon alfa therapy for alertness subtask (A); divided attention, visual subtask (B); divided attention, acoustic subtask (C); and vigilance subtask (D) (measure of variation, SEM) and time course of mean Hospital Anxiety and Depression Scale (HADS) depression scores (±SEM) (E) in 70 hepatitis C patients before, during, and after interferon alfa therapy. n.s., Not significant; t1, before therapy; t2, after 4 weeks of treatment; t3, after 3 to 4 months of treatment; t4, after 6 to 8 months of treatment; t5, 4 to 6 weeks after termination of therapy.
A

Working Memory - Mean Reaction Times

Reaction Time [ms]

p=0.034

Evaluation Time Points

t1 (n=59)  t2 (n=59)  t3 (n=59)  t4 (n=56)  t5 (n=59)

B

Working Memory - Number of Correct Reactions

Number of Correct Reactions [c]

p<0.001

Evaluation Time Points

t1 (n=59)  t2 (n=59)  t3 (n=59)  t4 (n=56)  t5 (n=59)
DISCUSSION

Data from small studies suggest that, among the central nervous side effects of interferon, an impairment of neurocognitive performance may also occur. However, these observations have been challenged by some reports rejecting the hypothesis of cytokine-induced neurocognitive deficits. Systematic data on the extent and spectrum of deficiencies induced by this drug, especially as part of the combination therapy of chronic hepatitis C, are not available so far. Neurocognitive impairment has only been systematically studied in untreated hepatitis C patients.

Therefore this study was performed prospectively in a homogeneous group of hepatitis C patients in a longitudinal design. Because there may be a possible discrepancy between patients’ complaints and actual neurocognitive performance, as may occur in depression (a major side effect of interferon therapy), a standardized and objective computer-based neurocognitive test battery was used. Furthermore, we designed the study to control changes in neurocognitive performance for potentially confounding variables such as interferon-induced depressive symptoms and ribavirin-induced hemolysis, which had not been done in previous studies.

In contrast to hepatic encephalopathy, for example, no specific psychometric tests to measure interferon-induced neurocognitive symptoms have been validated so far.

Standardized data for the TAP subtasks, as well as information concerning learning effects in healthy control subjects, are available. Performance in the TAP subtasks used in our study has been shown not to be influenced by significant learning effects when administered repeatedly. Therefore a control group without antiviral therapy was considered unnecessary in our study design.

The results of our study demonstrate a marked and significant decline of important aspects of cognitive performance. This is basically consistent with the results of Poutiainen et al in patients with amyotrophic lateral sclerosis undergoing high-dose interferon alfa therapy or Capuron et al (interferon alfa/interleukin [IL] 2 in cancer patients) as far as cytokine-induced cognitive effects in general are concerned. However, our specific findings for the combination therapy with interferon alfa and ribavirin in patients with chronic hepatitis C comprise additional and more detailed aspects of quality and time course of cognitive impairment.

With regard to the alertness, divided-attention, and vigilance tasks, the respective reaction times are markedly and significantly increased during therapy with peginterferon alfa. On the other hand, accuracy measures such as the number of correct answers or omissions are not significantly changed in these subtests. Therefore cognitive functions were slowed down but did not become less accurate during antiviral therapy of chronic hepatitis C.

However, this is not the case for the working-memory task: Both reaction time and accuracy are impaired during therapy with peginterferon alfa and ribavirin. This finding is in contrast to the results of Capuron et al, who did not observe any impairment in measures of memory performance accuracy. However, their study focused exclusively on short-term cognitive effects of cytokine immunotherapy (IL-2, interferon alfa) during a treatment period of only 1 month in cancer patients. In our study a significant impairment of both reaction time latency and accuracy in the working-memory task did not occur before 3 months after initiation of antiviral therapy (t3).

In general, by means of TAP testing, we were able to monitor transient and reversible signs of cognitive deficits. In all of the 4 tested domains of cognitive performance, both reaction time and accuracy levels reached pretreatment values after termination of interferon therapy. These results are not consistent with the findings of Meyers et al, who suggested persistent neurotoxicity in some cases even after termination of interferon alfa therapy in cancer patients.

In addition, we found no evidence that pegylated interferon alfa, because of the higher dose applied, causes significantly more or stronger neurocognitive deficits than conventional interferon alfa. Both therapies were similar in quality and intensity of the cognitive changes they produced in patients with chronic hepatitis C.

As shown here, interferon alfa adversely affects cognition, attention, and memory. Several pathophysiological mechanisms of immune-to-brain communication can be discussed in this context.

Fig. 2. Mean reaction times (A) and number of correct reactions (B) for TAP working-memory task before, during, and after therapy with interferon alfa and ribavirin (measure of variation, SEM).
It has been demonstrated by several groups that cytokines present in the circulation outside the blood-brain barrier may exert effects on the central nervous system either directly or indirectly. In particular, activation of IL-1 by interferon alfa and the interaction of IL-1 with specific neurohormones and neurotransmitters in the brain might play an important role in this context. IL-1α crosses the blood-brain barrier by a saturable transport system. With regard to the localization of cytokine pathways within the brain, IL-1, especially IL-1α, has been found in several brain regions, including the hippocampus. Nevertheless, the mapping of the various cytokine pathways and their receptors and the precise pathophysiologic mechanisms of the observed cognitive impairment in the brain remain unclear.

Our findings suggest that the observed cognitive impairment is not substantially linked to depression or decline of hemoglobin values. Both factors are potential candidates in models of attentional and memory-related deficits during interferon-based antiviral therapy. However, in the case of depression, our study clearly indicates that the observed deterioration of cognitive or attentional performance is not significantly related to the occurrence or aggravation of affective or mood disorders.

Regarding the decline in hemoglobin values, we found a weak association with measures of neurocognitive impairment. Although the correlation was statistically significant ($P < .03$), it cannot, to a substantial degree, account for the variation of attentional and memory-related deficits ($r < 0.30$). Therefore ribavirin-induced hemolysis may be an aggravating factor with respect to attentional performance but does not represent the major cause for it. This finding is in accordance with results of other studies reporting on cognitive or neuropsychologic impairment in patients receiving interferon monotherapy.

In addition, reduced performance was not correlated with HCV positivity per se. After treatment, no difference was found between responders and nonresponders to therapy. Similar results have been reported by Cordoba et al. who found no direct effect of HCV on neurocognitive function. We are, however, aware that this evaluation of possible effects of mere HCV presence on neurocognitive function is a preliminary one, because this was not a primary aim of our investigation. Studies with the main focus on this issue must be designed with a longitudinal analysis, as well as higher sample sizes, to detect possible differences based on smaller effect sizes.

In conclusion, the decline of neurocognitive functions induced by interferon-based antiviral therapy is marked and significant and can be measured by means of the TAP test battery. The observed cognitive impairment is transient (ie, fully reversible after termination of therapy). According to our findings, accuracy was exclusively affected in the working-memory task whereas a deterioration of reaction time was a characteristic of all tests.

The magnitude of reaction time increases found in our study (mean values between 5.4% in the working-memory task and 17.7% in the alertness subtask) may be put in perspective by a comparison with side effects of other drugs in therapeutic doses. At least the latter result (TAP alertness subtask) lies within the dimension of what has been found for antihistamines. Theunissen et al reported a 15.4% increase in reaction time subsequent to mequitazine administration. The effects of this drug are similar to those of other second-generation antihistamines in that it causes mild driving impairment.

With regard to benzodiazepines, Kelly et al found a significant increase in reaction time (audiologic reaction times, 10 dB) of 12% (500 ms at baseline versus 560 ms after application) in 15 subjects receiving midazolam (0.04 mg/kg; clinical, sedating dose).

To summarize, the interferon-induced prolongation of reaction times observed in our study is within the range of prolonged reaction times observed for other drugs known to impair cognitive performance. We are, however, aware of the fact that the comparisons described have to be interpreted with caution because the applied tests were different in the respective studies and the percentage of reaction time changes can only be a clue for clinical significance. Finally, only a subgroup of the patients in our study was particularly affected by interferon-induced reaction time prolongation.

The implication of our findings with regard to the patients’ performance in their daily activities or work is not clear. However, it cannot be excluded that an increase in reaction time as observed in our study may affect performance, at least in certain specialized professions. Our data raise some important questions with regard to interferon-induced neurocognitive deterioration; implications and consequences for clinical practice, however, can only be derived from specific tests (eg, driving performance tests with regard to the issue of driving safety). In addition, the effect of interferon on reaction times varied considerably between single individuals. Whereas in the alertness (simple reaction time) subtask reaction time increased by a mean of about 47 ms, in 12 of 70 patients a prolongation of
reaction time between 100 and 380 ms was found. A car with a speed of 120 km/h will cover a distance of 1.6 m in 47 ms but 12.6 m in 380 ms. In the divided-attention test (acoustic subtask), 19 of 70 patients even had an increase in reaction time by more than 100 ms. Because complex tasks are the rule rather than the exception in real life, the latter test may better simulate reality.

Given the considerable number of patients with chronic hepatitis C treated during an active phase of their lives for up to 1 year, further systematic evaluations of interferon-induced neurocognitive side effects with respect to their significance, especially with regard to sophisticated tasks and quality of life, are necessary.

Dr. Kraus is a member of the Scientific Advisory Board of Essex Pharma (Munich, Germany), a subsidiary of Schering-Plough (Kenilworth, NJ) and has served on speakers bureaus for Schering-Plough and Essex Pharma. The other authors have no conflicts of interest. The sponsor (Essex Pharma, Munich, Germany) was not involved in any way in the design, interpretation, analysis, or writing of the study.

References