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Evaluation of inhibitory drug interactions during drug development: Genetic polymorphisms must be considered

Lois S. Lee, PharmD, Anne N. Nafziger, MD, MHS, and Joseph S. Bertino, Jr, PharmD  Cooperstown, NY

The evaluation of drug interactions during the drug development process is an important requirement that provides information for the product label and medical references. References such as Micromedex (Thomson Micromedex, Greenwood Village, Colo) or ePocrates (Epocrates, San Mateo, Calif) are readily available in the clinical setting and are often used to identify and quantify the extent of potential drug interactions when 2 or more drugs are administered to a patient. When an interaction is sufficiently large, dose adjustment is recommended to minimize the risk of therapeutic failure or drug toxicity.

However, data on drug interactions are often limited or incomplete. For example, the metabolism of aripiprazole, a substrate of the cytochrome P450 (CYP) 2D6 enzyme, is inhibited by quinidine, a potent CYP2D6 inhibitor, causing increases in the area under the plasma concentration–time curve (AUC) of aripiprazole and its active metabolite by 112% and 35%, respectively.1,2 On the basis of this unpublished study, the manufacturer recommends that the aripiprazole dose should be reduced by 50% when quinidine or other potent CYP2D6 inhibitors are initiated.2 However, some crucial details of the study that are required to evaluate this recommendation, such as the number of subjects, the range or mean values of the magnitude of inhibition by quinidine, and, most important, the subjects’ CYP2D6 metabolizer status, are not provided.

CYP enzymes such as CYP2C19 and CYP2D6 show genetic polymorphisms (defined as the presence of variant genes in at least 1% of a population3,4) that may result in significant variability of drug metabolism5-7, leading to significant interindividual variability of the extent of drug interactions8-12 but often only the mean pharmacokinetic values for the interaction are reported and taken into account when making recommendations. The variable magnitude of a drug interaction between individuals can be at least partially explained by genetic polymorphisms. Knowledge of an individual’s metabolizer status of the polymorphic CYP enzyme that is responsible for metabolizing the affected drug (substrate) can offer vital information when evaluating drug interac-
Many drug interaction studies performed during drug development are done in healthy volunteers, where genotype can appropriately categorize enzyme activity. The following examples of inhibitory drug interactions via CYP2C19 and via CYP2D6 illustrate the variability in the extent of inhibition based on genotype and suggest that more complete information must be provided to appropriately judge the potential for drug interactions.

### CYP2C19

A study that evaluated the effect of omeprazole, a CYP2C19 inhibitor, on the pharmacokinetics of moclobemide in genotyped extensive metabolizers (EMs) and poor metabolizers (PMs) showed that omeprazole significantly inhibited (up to 2-fold) moclobemide metabolism in the EMs but not in the PMs.13 In the study 8 homozygous EMs (CYP2C19*1/*1) and 8 PMs (CYP2C19*2/*3) were assessed. After 8 days of administration of 40 mg of omeprazole, the mean AUC of moclobemide more than doubled in the EMs (Table I). In addition, the metabolite to parent AUC ratio of Ro 12-8095 (the major metabolite of moclobemide produced by CYP2C19) to moclobemide in the EMs was significantly reduced. In the PMs, the AUC ratio of Ro 12-8095 to moclobemide was not significantly changed. The inhibition of moclobemide metabolism by omeprazole in the EMs caused the plasma concentration–time graphs of moclobemide and its 2 metabolites to resemble those of the PMs on day 8. In a subsequent study in the same subjects, the pharmacokinetic parameters of omeprazole were determined with and without single 300-mg moclobemide doses to evaluate whether moclobemide inhibits CYP2C19 enzyme activity.14 In the EMs an approximately 2-fold increase in the AUC and in the maximum plasma concentration (Cmax) of omeprazole was observed when moclobemide was given simultaneously. Coadministration of moclobemide increased the mean AUC of omeprazole and AUC ratio of omeprazole to 5-hydroxyomeprazole in the PMs to an insignificant extent.

### Differences in inhibitory effects between genotyped EMs and PMs

A study by Yasui-Furukori et al.15 The effect of fluvoxamine, an inhibitor of CYP2C19, on omeprazole metabolism was compared in 12 EMs (CYP2C19*1/*1 [n = 6], CYP2C19*1/*2 [n = 3], and CYP2C19*1/*3 [n = 3]) and 6 PMs (CYP2C19*2/*2 [n = 4] and CYP2C19*2/*3 [n = 2]). In the EMs twice-daily administration of 25 mg fluvoxamine significantly increased the Cmax and AUC of omeprazole when compared with the placebo phase. In addition, the AUC ratio of 5-hydroxyomeprazole to omeprazole decreased.

### Table I. Differences in inhibitory effect of pharmacokinetic parameters between CYP2C19 EMs and PMs

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Pharmacokinetic parameters of substrate</th>
<th>EMs</th>
<th>S</th>
<th>S + I</th>
<th>PMs</th>
<th>S</th>
<th>S + I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole13</td>
<td>AUC of moclobemide (µg · h/mL)</td>
<td>13.0 ± 5.8</td>
<td>28.7 ± 8.5*</td>
<td>46.0 ± 9.5</td>
<td>36.8 ± 8.8*</td>
<td>121.9 ± 48.7</td>
<td>55.2 ± 25.2*</td>
</tr>
<tr>
<td></td>
<td>AUC ratio of Ro 12-8095/moclobemide (%)</td>
<td>121.9 ± 48.7</td>
<td>55.2 ± 25.2*</td>
<td>25.3 ± 5.1</td>
<td>28.7 ± 8.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moclobemide14</td>
<td>AUC of omeprazole (ng · h/mL)</td>
<td>1821.4</td>
<td>3774.3*</td>
<td>10348.3</td>
<td>12112.4</td>
<td>2.5</td>
<td>5.3*</td>
</tr>
<tr>
<td></td>
<td>AUC ratio of omeprazole/5-hydroxyomeprazole</td>
<td>2.5</td>
<td>5.3*</td>
<td>25.7</td>
<td>38.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine15</td>
<td>AUC of omeprazole (ng · h/mL)</td>
<td>1483 (HM)</td>
<td>8340.4*</td>
<td>15044</td>
<td>17348</td>
<td>4411 (HET)</td>
<td>10507*</td>
</tr>
<tr>
<td></td>
<td>AUC ratio of 5-hydroxyomeprazole/omeprazole</td>
<td>0.39 (HM)</td>
<td>0.07*</td>
<td>0.047</td>
<td>0.018</td>
<td>0.25 (HET)</td>
<td>0.11*</td>
</tr>
<tr>
<td>Fluvoxamine16</td>
<td>AUC of lansoprazole (ng · h/mL)</td>
<td>6373 (HM)</td>
<td>24390*</td>
<td>26666</td>
<td>27774</td>
<td>8906 (HET)</td>
<td>22237*</td>
</tr>
<tr>
<td></td>
<td>AUC of 5-hydroxylansoprazole/lansoprazole</td>
<td>0.118 (HM)</td>
<td>0.028*</td>
<td>0.015</td>
<td>0.014</td>
<td>0.107 (HET)</td>
<td>0.034*</td>
</tr>
</tbody>
</table>

EMs, Extensive metabolizers; PMs, poor metabolizers; S, substrate; I, inhibitor; HM, homozygous; HET, heterozygous; AUC, area under plasma concentration–time curve; Ro 12-8095, metabolite of moclobemide produced by CYP2C19; 5-hydroxyomeprazole, metabolite of omeprazole produced by CYP2C19; 5-hydroxylansoprazole, metabolite of lansoprazole produced by CYP2C19.

*P < 0.05, compared with receiving substrate only or with placebo.
Genetic polymorphisms and drug interactions

CYP2D6

Although CYP2D6 expression constitutes only 2% to 5% of the total CYP content, approximately 20% of all marketed drugs are metabolized by CYP2D6.18,19 CYP2D6 has the greatest number of genetic variants identified to date. In addition to the wild-type allele (CYP2D6*1A), more than 70 variant alleles have been identified.20 To summarize the allele activity patterns, CYP2D6*1, *2, *33, and *35 have normal enzyme activity whereas CYP2D6*3, *4, *6, *7, and *8 have null enzyme activity. CYP2D6*5 represents deletion of the enzyme, and CYP2D6*9, *10, *17, and *41 code for decreased enzyme activity.20

Several studies have consistently shown a larger magnitude of inhibition in EMs of CYP2D6 (Table II). A placebo-controlled crossover study evaluated the inhibitory effect of diphenhydramine on the metabolism of metoprolol, a CYP2D6 substrate, in 10 EMs (CYP2D6*1A/*1A [n = 8] and CYP2D6*1A/*5 [n = 2]) and 6 PMs (CYP2D6*4/*4 [n = 5] and CYP2D6*4/*4 deletion [n = 1]).21 Placebo or 50 mg diphenhydramine (a CYP2D6 inhibitor) was given 3 times a day for 5 days, and a dose of 100 mg metoprolol was given on day 3. With coadministration of diphenhydramine in the EMs, the Cmax and AUC extrapolated to infinity of metoprolol increased by approximately 16% and 61%, respectively. Diphenhydramine decreased the conver-

Table II. Differences in inhibitory effect of pharmacokinetic measures between CYP2D6 EMs and PMs21-26

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Pharmacokinetic parameters of substrate</th>
<th>EMs</th>
<th>S</th>
<th>S + I</th>
<th>PMs</th>
<th>S</th>
<th>S + I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenhydramine21</td>
<td>AUC of metoprolol (µg · h/mL)</td>
<td>940 ± 403</td>
<td>1514 ± 382*</td>
<td>3956 ± 935</td>
<td>4354 ± 992</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinidine22</td>
<td>AUC of (S)-venlafaxine (µmol · h/L)</td>
<td>0.41</td>
<td>1.58*</td>
<td>0.80</td>
<td>0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diphenhydramine23</td>
<td>AUC of venlafaxine (µmol · h/L)</td>
<td>0.17</td>
<td>2.07*</td>
<td>1.47</td>
<td>1.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine24</td>
<td>AUC of risperidone (ng · h/mL)</td>
<td>45 ± 31</td>
<td>16 ± 11*</td>
<td>3 ± 1</td>
<td>4 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thoridazine25</td>
<td>Debrisoquine metabolic ratio</td>
<td>&lt;12.6</td>
<td>Increased (2-fold)*</td>
<td>&gt;12.6</td>
<td>Unchanged or insignificantly changed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terbinafine26</td>
<td>Dextromethorphan/dextromethan</td>
<td>0.005</td>
<td>0.282</td>
<td>1.534</td>
<td>0.803</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < .05, compared with receiving substrate only or with placebo.

CYP2C19 enzyme. However, in the PMs the pharmacokinetic parameters of omeprazole metabolism did not change significantly from the control phase. The degree of inhibition caused by fluvoxamine was significantly greater in the homozygous EMs (CYP2C19*1/*1) than in the heterozygous EMs (CYP2C19*1/*2 and CYP2C19*1/*3). This is consistent with the finding that the magnitude of inhibition of CYP2C19-metabolized drugs depends on the individual’s baseline CYP2C19 activity as identified by genotype because heterozygous EMs have reduced enzyme activity compared with homozygous EMs. These inhibitory effects of fluvoxamine in relation to CYP2C19 genotypes were confirmed with the use of lansoprazole, another CYP2C19 substrate, in healthy adults with similar genotypes.16

As summarized in Table I, a greater extent of inhibitory drug interactions exists among EMs compared with PMs of the CYP2C19 enzyme. In contrast, changes in the pharmacokinetic parameters of a CYP2C19 substrate among CYP2C19 PMs are expected to be insignificant because of the PMs’ decreased baseline enzyme activity. This may be further interpreted in the clinical setting to indicate that dose adjustment may be necessary only in CYP2C19 EMs who are taking a CYP2C19 substrate when a CYP2C19 inhibitor is initiated but not in CYP2C19 PMs.
sion of metoprolol to \( \alpha \)-hydroxymetoprolol (the CYP2D6-produced metabolite) by 3-fold in the EMs but did not cause significant changes in metoprolol disposition in the PMs.

A study conducted by Eap et al.\(^2\) showed the differences in drug interaction potential with use of venlafaxine, a CYP2D6 substrate. Quinidine, a potent CYP2D6 inhibitor, was administered to 7 EMs (CYP2D6*1/*1 [n = 4] and CYP2D6*1/*4 [n = 3]) and 5 PMs (CYP2D6*3/*4 [n = 1] and CYP2D6*4/*4 [n = 4]) to assess the stereoselectivity of venlafaxine metabolism in EMs and PMs. Quinidine increased the AUC of (S)-venlafaxine by 4-fold and (R)-venlafaxine by 12-fold in the EMs. The partial metabolic clearance to \( O \)-demethylated metabolites of (S)- and (R)-venlafaxine via CYP2D6 decreased by 7-fold and 113-fold, respectively, in the EMs. In contrast, no significant changes were observed in the pharmacokinetic parameters when coadministration of quinidine in the PMs.

Another study evaluated the inhibitory effect of diphenhydramine on the pharmacokinetics of venlafaxine in 9 EMs (CYP2D6*1/*1 [n = 6] and CYP2D6*1/*4 [n = 3]) and 6 PMs (CYP2D6*4/*4 [n = 4], CYP2D6*3/*4 [n = 1], and CYP2D6*7/*7 [n = 1])\(^2\). Coadministration of diphenhydramine resulted in a more than 2-fold increase in the AUC of venlafaxine in the EMs but did not alter the AUC in the PMs. The partial metabolic clearance to \( O \)-demethylated metabolites was decreased approximately 3-fold by diphenhydramine in the EMs, but no significant changes occurred in the PMs. These results further imply that concurrent administration of venlafaxine with a CYP2D6 inhibitor to CYP2D6 EMs may require a dose reduction of venlafaxine, whereas a dose reduction in CYP2D6 PMs could result in therapeutic failure.

Other studies evaluating drug interactions caused by CYP2D6 inhibition have consistently shown a greater degree of interactions in CYP2D6 EMs compared with CYP2D6 PMs.\(^2\) They are summarized in Table II. These findings further support the concept that the variant CYP alleles comprising an individual’s CYP enzyme activity should be incorporated into the decision-making process when evaluating the occurrence and extent of drug interactions.

**APPLICATION OF GENETIC POLYMORPHISM IN DRUG DEVELOPMENT AND CLINICAL IMPLICATIONS**

The studies discussed\(^2\) indicate that (1) a greater magnitude of inhibitory drug interactions occurs in EMs than in PMs and (2) the extent of inhibition can be predicted based on genotypically identified baseline activity of the responsible CYP enzyme. Although it is logical that the extent of drug interactions should be greater in EMs,\(^2\) only a limited number of drug interaction studies are currently performed that distinguish genotyped EMs and PMs during the drug development process. When metabolizer status is not determined, the role of genetic polymorphisms is ignored, and the potential for, and range of, variation in drug interactions cannot be evaluated for a relationship to genotype. For example, a drug interaction study that primarily includes PMs can result in insignificant inhibitory effects, and it will be concluded that there is no drug interaction. However, very different results can be expected if only EMs are enrolled in the study. In addition, because a different extent of drug interactions exists between EMs and PMs,\(^2\) a study with an anonymous number or combination of EMs and PMs will likely show a large range in the magnitude of a drug interaction. This is frequently seen in drug interaction studies.\(^8\) For these reasons, drug interaction information on the product label or common medical references can be misleading if presented as a mean or a range without identification of the subjects’ metabolizer status.

A greater degree of drug interaction may lead to a greater likelihood of toxicity or therapeutic failure. The above-mentioned findings can be further interpreted as EMs being at greater risk for clinically significant drug interactions when an inhibitory drug of the same CYP enzyme is introduced. Although changes in pharmacokinetics cannot always be translated into expected changes in pharmacodynamics, altered pharmacodynamics caused by increased drug exposure have been shown with some commonly used drugs. Hamelin et al.\(^\) found that the coadministration of diphenhydramine and metoprolol to EMs not only significantly altered pharmacokinetics but also prolonged negative chronotropic and inotropic effects. Such changes were not seen in the PMs. When diphenhydramine impairs the metabolism of a drug dosed on a long-term basis with a narrow therapeutic range taken by an EM, there is a considerable chance of reaching toxic drug exposures with a subsequent occurrence of adverse events.

Antipsychotics and antidepressants are often prescribed concurrently to treat comorbid symptoms. Antidepressants such as paroxetine and fluoxetine are potent inhibitors of CYP2D6, the enzyme responsible for metabolizing many typical and atypical antipsychotic medications (many of which have a narrow therapeutic range).\(^2\) Studies have reported that a significant rise in the concentrations of the antipsychotic drug, resulting from initiation of paroxetine or fluoxetine, can lead to an increased fre-
quency or worsening of extrapyramidal symptoms.\textsuperscript{24,31-33} Such adverse drug interactions are more likely to be seen in EMs because the extent of inhibition is more significant in EMs than PMs.

There are additional points to consider when drug interactions are evaluated. Although we have discussed the extent of drug interactions in EMs versus PMs, it is worthwhile to note that because of the variability of enzyme activity among EMs (eg, homozygous versus heterozygous EMs)\textsuperscript{34} the extent of inhibitory drug interactions may also vary among EMs.\textsuperscript{15,16,35} A heterozygous EM with low enzyme activity may have an insignificant change in drug exposure after the addition of an inhibitory agent. Therefore data from homozygous EMs should be applied with caution to heterozygous EMs. This concept suggests that individuals with duplicated or amplified genes of the CYP2D6 enzyme, also known as ultrarapid metabolizers,\textsuperscript{36} would have the greatest extent of inhibitory effect as a result of elevated baseline enzyme activity and an increased baseline drug dose. Second, the data we present are from examples of drug interactions with use of the substrates and the inhibitors of the same CYP enzyme. Using a substrate that is metabolized through more than 1 CYP enzyme pathway in EMs and PMs of 1 CYP enzyme and adding an inhibitor of another CYP enzyme that is also responsible for metabolizing the substrate may result in contradictory data. This is seen in the inhibitory effect of clarithromycin (CYP3A inhibitor) on omeprazole metabolism in CYP2C19 EMs and PMs.\textsuperscript{37} Third, we have assessed only data for CYP2C19 and CYP2D6 enzymes. Recent data suggest that the presence of \textit{CYP3A5*1} is associated with increased baseline intestinal activity of CYP3A enzyme and a larger degree of inhibition.\textsuperscript{38} However, more data are needed for this enzyme.

For more accurate evaluation of drug interactions, genotyping of drug-metabolizing enzymes that exhibit clinically significant genetic polymorphisms should become an imperative part of drug development and the extent of inhibitory drug interactions should be reported according to metabolizer types. Although it is difficult to untangle genetic factors from nongenetic factors when evaluating and predicting drug interactions, the influence of the interindividual variability from genetic factors can be minimized by genotyping. This makes both the drug development process and the clinical use of drugs more challenging. However, we believe it is essential for providing more accurate information and for clinicians to better assess potential drug interactions and recommend appropriate dose adjustments when necessary. Clinicians should be cautious when using commonly available drug information resources to determine drug dosage adjustments with interacting drugs. These adjustments are often based on mean data without regard to the large ranges of the interactions, and this variability may be a result of subjects’ unidentified metabolizer status. The premise that some data, even though they are not good data, are better than no data is erroneous and can potentially lead to therapeutic failure or toxicity for patients.

Drs Lee and Nafziger have no conflict of interest to disclose. Dr Bertino is a consultant for Ortho-McNeil Pharmaceutical (Raritan, NJ) and Merck & Co (Whitehouse Station, NJ) and has contracts with Ortho-McNeil Pharmaceutical, Merck & Co, and ViroPharma (Exton, Pa).

References

12. Hansten PD. Clinical and pharmacoeconomic significance of metabolic drug interactions. In: Levy RH,
Personal digital assistant–based drug reference software as tools to improve rational prescribing: Benchmark criteria and performance

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CURRENT NEED FOR TOOLS TO IMPROVE RATIONAL PRESCRIBING

The need for rational prescribing, that is, the selection of the most appropriate therapeutic regimen for a specific patient, is greater than ever. For example, a recent study identified physicians’ poor prescribing performance as the source for over 70% of clinically significant medication errors in a tertiary-care setting.1 This may not be surprising given that physicians are expected to incorporate vast amounts of critical information on an ever-increasing number of prescription medications, over-the-counter medications, vitamins, and herbal supplements into prescribing in real time. New data on genetically determined differences in drug action and drug metabolism become available on a daily basis. The problem is further confounded by an aging population with multiple medical problems taking multiple drugs, vitamins, and herbal preparations. Limitations imposed by formulary requirements of health insurance plans and hospital systems further complicate the matter. At the same time, public awareness of prescribing errors as a significant source of preventable patient injuries has risen.2 In fact, prescription errors and adverse drug events are a leading cause for malpractice litigation.3

Physicians have always relied on tools such as drug handbooks, pocket cards, and electronic drug databases for rapid access to accurate prescribing information, yet these tools are often limited in scope, are difficult to access, or lack the most current information. The recent advent of electronic drug databases on personal digital assistants (PDAs) holds the promise of giving physicians a new prescribing tool that almost instantaneously provides the most up-to-date and clinically relevant prescribing information and can be directly linked to patient-specific data and laboratory results.4 Increasing physician usage of such electronic prescribing tools has been advocated as a means to reduce the number of prescribing errors.5,6 Although not yet tested, such an approach seems rational given that lack of pharmacotherapy knowledge has been identified as the primary reason for the poor prescribing performance of physicians resulting in medication errors.1 This may explain why reliance by physicians on programs such as ePocrates (Epocrates, San Mateo, Calif) has grown exponentially over the last decade, especially among medical students and physicians in training. For example, a survey in 2002 among medical residents found that the majority of respondents used their PDAs daily to access drug reference databases.7 At the same time, the number of drug reference software products has risen steeply over the past several years. Although a number of studies have evaluated different PDA-based drug reference software products,8-13 these studies have primarily examined their utility from the perspective of a librarian or a pharmacist.
The purpose of this commentary is to suggest a set of benchmark criteria for the utility of PDA-based drug reference software as tools for rational prescribing and to provide an initial assessment on how currently available drug reference software performed when assessed by a novel rating scale based on such benchmark criteria.

**BENCHMARK CRITERIA FOR PDA DRUG REFERENCE SOFTWARE AS TOOLS FOR RATIONAL PRESCRIBING**

To assemble a list of criteria by which the utility of PDA drug reference software as tools of rational prescribing is assessed, we conducted an informal survey among the fellows and faculty of the Division of Clinical Pharmacology at Georgetown University Medical Center, Washington, DC. All participants had experience in teaching rational prescribing as part of a required therapeutics course for fourth-year medical students. As such, the benchmark criteria primarily reflect the opinion of physicians with training in clinical pharmacology and subspecialty training in internal medicine, oncology, nephrology, pediatrics, and drug development sciences. The following quality criteria were identified.

**Up-to-date information**

One of the main advantages of electronic databases over printed material is the ability to be updated on a periodic basis. Thus the update frequency of an electronic database represents a significant quality criterion for its clinical utility as a prescribing tool.

**Evidence-based dosing information for labeled and unlabeled indications**

In addition to the standard information on indications, dosage, frequency, route of administration, dosage forms, contraindications, and precautions, rational prescribing tools should contain the following in the drug monograph: (1) dosage recommendations for special populations (e.g. pediatric patients, geriatric patients, pregnant women, and patients with renal impairment), (2) estimates of the level of evidence that supports the use of a drug for a particular indication (e.g. randomized, placebo-controlled trial), and (3) estimates of the anticipated size of drug effect (e.g. number needed to treat). Such data should be provided for both labeled and unlabeled indications. This information would allow the prescriber to better estimate the risk-benefit ratio of a given drug for an individual patient. It would also help the prescriber to choose between alternative treatment options.

**Structured information on side effects of drugs**

To maximize the utility to the prescriber, possible side effects should not merely be listed, but information should be structured to give the frequency and severity of each side effect. In addition, recommendations on how to manage side effects and drug toxicity (i.e., overdose) should be provided.

**Structured information on drug interactions**

Any good electronic prescribing tool should contain comprehensive information on drug-drug, drug-food, and drug-herbal interactions. The information should be structured to provide the likelihood and severity of the interactions, explain the underlying mechanism in brief, and give recommendations for clinical management of the interaction. In addition, the electronic prescribing tool should be capable of screening a list of drugs, herbs, and food items for possible drug interactions.

**Herbals and nutritional supplements**

Given the high prevalence of use of herbals and nutritional supplements among patients, any good drug database should contain basic information (e.g., claimed benefits, dosage, contraindications, and side effects) on herbals and nutritional supplements.

**Special features**

Good drug reference software should contain a number of special features that would further help the prescriber to select the most appropriate drug and dosage for a specific patient. In particular, features such as medical calculators, treatment guidelines based on national consensus documents, pharmacokinetic information, free-text search capability, drug cost information, drug picture, compatibility of intravenous solutions, and cross-referencing of drug information to the Internet and the medical literature would significantly enhance the utility of a prescribing tool.

The above features should ideally be combined in a single user interface that provides quickly accessible, searchable, and cross-referenced prescribing information. At the same time, the information should be comprehensive in scope.

**DEVELOPMENT OF A RATING SCALE FOR PDA-BASED DRUG REFERENCE SOFTWARE AS RATIONAL PRESCRIBING TOOLS**

The benchmark criteria identified above were used to develop a 40-point rating scale to compare different software products (Table I). Each criterion was weighted depending on how important we thought it...
was for a rational prescribing tool. For example, information on indication and dosage was considered most important and was weighted with 10 points. Information on side effects, drug interactions, and special features was weighted with 8 points each. The availability of an herbal and nutritional supplement database was considered the least important feature and was weighted with only 2 points.

For the main quality criteria of prescribing software products identified in the previous section (eg, the presence of rational prescribing features such as evidence-based dosage recommendations, structured information on drug interactions and side effects, and searchable drug database), 4 to 8 points were given, depending on whether the prescribing features were present (see Table I for details). As a result, rational prescribing features represented a total of 22 points, or 55% of the maximally possible score.

To also obtain a comprehensiveness score for indication, side effect, and drug interaction data, rather than comparing the results against a gold standard, software products were assigned points based on their quintile rank: products ranking in the top quintile received 4 points, and products ranking in the bottom quintile received no points. Together with the herbal category, a total of 14 points (35% of total score) were possible for comprehensiveness. Update frequency was tested by assessing whether drugs with different approval dates were contained in the database and was rated on a scale from 0 to 4.

A battery of drugs, foods, and nutritional supplements was then used to assess the performance of the different software products (see Table I). The drug indication and side effect data were evaluated with 3 test drugs that have numerous labeled and unlabeled indications, have an extensive side effect profile, and represent 3 different drug categories: generic (carbamazepine), over the counter (acetaminophen), and brand name (sildenafil). To evaluate whether the drug reference software can provide the most rational evidence-based dosage recommendation, gentamicin was used as a test drug. Once-a-day or extended interval dosing of aminoglycosides such as gentamicin has clearly been established as at least as efficacious and possibly less side effect–prone for adults \(^{15,16}\) and children \(^{17}\) compared with traditional multiple daily dosing regimens. Once-a-day aminoglycoside dosing could be considered the standard of care for adult patients, given that such regimens have been adopted by more than 75% of US hospitals surveyed in 2000.\(^{18}\)

The drug interaction data were evaluated by use of 3 sets of drug-herbal, drug-drug, and drug-food combinations. The first set—cyclosporine (INN, ciclosporin) and St John’s wort—was chosen to represent a well-documented, potentially life-threatening drug-herbal interaction.\(^{19,20}\) The second set—carbamazepine, sildenafil, acetaminophen, ethanol, and grapefruit juice—was chosen to test whether the software can detect drug-food interactions in a list of multiple drugs and food. The final set—erythromycin and ketoconazole—was chosen to test whether the software could predict a potentially life-threatening drug interaction (cardiac arrhythmia) based on established pharmacologic principles. This drug interaction is plausible because it is well established that ketoconazole inhibits cytochrome P450 (CYP) 3A4,\(^{21}\) the P450 enzyme that metabolizes erythromycin.\(^{22}\) Although neither pharmacokinetic interaction data nor increased incidences of QT prolongation or cardiac arrhythmia have been reported for the ketoconazole-erythromycin combination, increased erythromycin blood concentrations resulting from ketoconazole coadministration are clearly a risk factor for QT prolongation, torsades de pointes cardiac arrhythmias, and sudden death.\(^{23}\)

**SURVEY OF AVAILABLE PDA-BASED ELECTRONIC DRUG REFERENCE SOFTWARE**

An Internet search conducted in August 2003 identified 11 PDA-based electronic databases that fulfilled at least 5 of the above quality criteria: 5 stand-alone products and 6 software suites. Software suites were defined as individual databases that can be combined into the same user interface and are cross-linked. Products that covered only specific areas (eg, drug interactions, herbal databases) or were merely electronic reproductions of handbooks published annually were not included in the analysis. All products surveyed could be frequently updated via download from the company’s Web site. In all cases the updates were performed automatically during the hot-sync process of the handheld device. Prices ranged from $27 to $155 for an annual license. For a number of products (Pepid, CP OnHand, ePocrates, and mobileMDX), the use was restricted to the 1-year term. For the remainder, the products were still usable after 1 year, but no further updates were provided. All products were available for either the Palm or Pocket PC operating system. The size of the drug database varied considerably among products, ranging from 900 to over 7000 drug names. Table II summarizes the basic features of the 11 products; it was updated.
### Table I. Rating scale to evaluate PDA-based drug reference software

<table>
<thead>
<tr>
<th>PDA rating scale (range of possible scores)</th>
<th>CP OnHand</th>
<th>ePharmacopoeia</th>
<th>ePocrates</th>
<th>mobileMDX</th>
<th>mobilePDR</th>
<th>Pepid PDC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Update frequency</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Which of the following drugs are in the</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>database (yes = 1, no = 0)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aciphex (rabeprazole), NDA 9/30/02</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gleevec (imatinib), NDA 4/18/03</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aggrastat (tirotiban), NDA 2/28/03</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Levitra (vardenafil), NDA 8/19/03</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Update frequency score (0-4)</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<td>3</td>
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<tr>
<td><strong>Indications and dosing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Which of the following features are present (yes = 1, no = 0)?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1. Labeled and unlabeled indications</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>2. Dosing in special populations</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>3. Level of evidence for efficacy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>4. Drug effect size</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>5. Gentamicin ODD listed</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td>6. Gentamicin ODD as primary regimen</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>Indications and dosing features (0-6)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
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<tr>
<td><strong>How many indications are present for each of the following drugs?</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1. Carbamazepine</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>7</td>
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<td>2. Acetaminophen</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>3</td>
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<td>3. Sildenafil</td>
<td>4</td>
<td>1</td>
<td>1</td>
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<td>Raw score</td>
<td>25</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>12</td>
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<td>Indications comprehensiveness (0-4)</td>
<td>4</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Combined score for indications (0-10)</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td><strong>Side effects and toxicity</strong></td>
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<tr>
<td>Which of the following side effect features are present (yes = 1, no = 0)?</td>
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<td>1. Side effects listed</td>
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<tr>
<td>2. Frequency listed</td>
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<td>3. Severity rated</td>
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<td>4. Management recommendations</td>
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<td>5. Side effect features (0-4)</td>
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<tr>
<td>Combined score for side effects (0-8)</td>
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<td>4</td>
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</table>

Data reflect software product versions that were updated from the respective company Web sites on August 19, 2003, and evaluated during the month of September 2003. Software products evaluated: CP OnHand (Gold Standard Multimedia, Tampa, Fla), ePharmacopoeia (Tarascon Publishing, Lompoc, Calif), ePocrates (Epocrates), mobileMDX (Thomson Healthcare, Montvale, NJ), mobilePDR (Thomson Healthcare), Pepid Portable Drug Compendium (PDC; Pepid LLC, Skokie, Ill), A2zDrugs (Skyscape, Marlborough, Mass), Clin-eRX (Skyscape), DrDrugs (Skyscape), Lexi-Drugs (Lexi-Comp, Hudson, Ohio), and PDRDrugs (Skyscape). NDA, New Drug Application; PDA, personal digital assistant; ODD, once-daily dosing. *Combined with iFacts drug interaction database. †Combined with Lexi-Interact and Lexi-Natural Products databases.
<table>
<thead>
<tr>
<th>Software suites</th>
<th>A2zDrugs*</th>
<th>Clin-eRX*</th>
<th>DrDrugs*</th>
<th>Lexi-Drugs†</th>
<th>PDRDrugs*</th>
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<td>15</td>
<td>8</td>
<td>27</td>
<td>76</td>
<td>16</td>
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<td>9</td>
<td>1</td>
<td>5</td>
<td>10</td>
<td>9</td>
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<td>122</td>
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<td>15</td>
<td>39</td>
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<td>146</td>
<td>14</td>
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<td>125</td>
<td>145</td>
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</tr>
<tr>
<td>6</td>
<td>1</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>
to reflect product versions available for purchase in December 2004.

**PERFORMANCE OF PDA-BASED DRUG REFERENCE SOFTWARE AS RATIONAL PRESCRIBING TOOLS**

There was a large range in the performance of the different drug reference software products when assessed by the 0- to 40-point rating scale. The lowest-scoring product, mobilePDR, had only 30% (12 points) of the quality criteria and scope considered important for a rational prescribing tool, whereas the highest-scoring product, Lexi-Drugs, achieved 73% (29 points). Table I describes the point scores of the different software products in detail for each prescribing category.

### Table I—Cont’d

<table>
<thead>
<tr>
<th>Drug interactions</th>
<th>CP</th>
<th>ePharmacopoeia</th>
<th>ePocrates</th>
<th>mobileMDX</th>
<th>mobilePDR</th>
<th>Pepid</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDA rating scale (range of possible scores)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug interactions</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Severity rated</td>
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<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
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<tr>
<td>Mechanism described</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dosing adjustments recommended</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Drug interaction features (0-4)</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

**How many drug-drug, drug-food, and drug-herbal interactions are listed?**

| 1. St John’s wort and cyclosporine | 1 | 0 | 1 | 0 | 0 | 0 |
| 2. Carbamazepine, acetaminophen, sildenafil, ethanol, and grapefruit juice | 6 | 3 | 4 | 1 | 1 | 3 |
| 3. Ketoconazole and erythromycin | 0 | 1 | 0 | 0 | 0 | 3 |
| Total raw score | 7 | 4 | 5 | 1 | 1 | 6 |
| Interaction comprehensiveness (0-4) | 3 | 2 | 2 | 0 | 0 | 3 |
| Combined score for drug interactions | 7 | 6 | 5 | 4 | 2 | 7 |

**Herbals**

| How many names of herbals, supplements, or alternative medicines are present? | 25 | 50 | 477 | 256 | 0 | 200 |
| Scoring (0 = 0, 1-150 = 1, and >150 = 2) | 25 | 50 | 477 | 256 | 0 | 200 |
| Total score for herbals and supplements (0-2) | 1 | 1 | 2 | 2 | 0 | 2 |

**Special features**

| Which of the following special features are present (yes = 1, no = 0)? | 0 | 1 | 1 | 0 | 0 | 1 |
| 1. Medical calculators | 0 | 1 | 1 | 0 | 0 | 1 |
| 2. Tables and therapeutic algorithms | 0 | 1 | 1 | 0 | 1 | 1 |
| 3. Search capability | 0 | 0 | 0 | 0 | 0 | 0 |
| 4. Pharmacokinetic data | 0 | 0 | 1 | 0 | 0 | 1 |
| 5. Drug cost | 0 | 1 | 1 | 0 | 0 | 1 |
| 6. Intravenous compatibility | 0 | 0 | 0 | 0 | 0 | 1 |
| 7. Drug picture available | 0 | 0 | 0 | 0 | 0 | 0 |
| 8. Data hyperlinked to Internet resources | 0 | 0 | 0 | 0 | 0 | 0 |
| Total special features score (0-8) | 0 | 3 | 4 | 0 | 1 | 5 |
| Total score (0-40) | 24 | 21 | 22 | 18 | 12 | 25 |
| Percentage score (0%-100%) | 60% | 53% | 55% | 45% | 30% | 63% |
| Rank among drug reference software | 3 | 5 | 4 | 9 | 11 | 2 |
All products contained data on drugs approved by the Food and Drug Administration within the last year period, suggesting that the delivery of up-to-date information is achieved, which is surely 1 of the strongest aspects of PDA-based drug reference software. CP On-Hand was the only product that provided information on drugs that were in the late phase of clinical development and had not yet been approved and thus scored highest in this category.

Information structure and rational prescribing features

As outlined above, providing structured information on indications, side effects, and drug interactions, as well as special prescribing features, was considered the
most important aspect for a rational prescribing tool. Accordingly, points in this category reflected more than 50% of the overall score (22/40). As illustrated in Fig 1, even the highest-scoring product, Pepid PDC, had only 70% of the features considered essential for a good prescribing tool. Many products had less than 50%, with mobilePDR and CP OnHand scoring lowest in this category. The overall average of 50% ± 11% suggests that there is significant room for improvement for the different software products. There was also significant variability in how different products performed in each category. This is discussed below.

**Indications and dosage.** With the exception of mobilePDR, all products contain information on drug use for labeled and unlabeled indications and on dosing in special populations. However, because none of the software products provided information on the level of evidence that supports the use of a drug for a particular indication or on the anticipated size of drug effect, one would have to rate the performance of all products as

<table>
<thead>
<tr>
<th>Name (URL)</th>
<th>Price ($)*</th>
<th>Company</th>
<th>Size (MB)†</th>
<th>No. of drug names</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stand-alone drug reference software</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP OnHand (<a href="http://www.cponhand.gsm.com">www.cponhand.gsm.com</a>)</td>
<td>99</td>
<td>Gold Standard Multimedia</td>
<td>16.4</td>
<td>6700</td>
<td>IV Alert costs additional $49 annually</td>
</tr>
<tr>
<td>ePharmacopoeia (<a href="http://www.tarascon.com">www.tarascon.com</a>)</td>
<td>27</td>
<td>Tarascon Publishing</td>
<td>4.0</td>
<td>&gt;4000</td>
<td>Drug interaction tool based on Medical Letter database</td>
</tr>
<tr>
<td>ePocrates Rx Pro (<a href="http://www.epocrates.com">www.epocrates.com</a>)</td>
<td>60</td>
<td>Epocrates</td>
<td>3.8</td>
<td>3400</td>
<td>Regular ePocrates Rx available free to physicians and medical students</td>
</tr>
<tr>
<td>mobileMDX (<a href="http://www.micromedex.com">www.micromedex.com</a>)</td>
<td>75</td>
<td>Thomson Healthcare</td>
<td>8.3</td>
<td>3200</td>
<td>Available free with institutional subscription</td>
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<tr>
<td>mobilePDR (<a href="http://www.mobilepdr.com">www.mobilepdr.com</a>)</td>
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<td>Thomson Healthcare</td>
<td>3.9</td>
<td>No information</td>
<td>Available free to physicians and medical students</td>
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<tr>
<td>Pepid PDC (<a href="http://www.pepid.com">www.pepid.com</a>)</td>
<td>70</td>
<td>Pepid LLC</td>
<td>5.7</td>
<td>5000</td>
<td>Pepid Portable Drug Compendium (PDC) is included at no charge with any clinical suite</td>
</tr>
</tbody>
</table>

Software suites§

<table>
<thead>
<tr>
<th>Name (URL)</th>
<th>Price ($)‡ (50 + 70)</th>
<th>Company</th>
<th>Size (MB)§</th>
<th>No. of database names</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2zDrugs and iFacts (<a href="http://www.skyscape.com">www.skyscape.com</a>)</td>
<td>108</td>
<td>Facts and Comparisons</td>
<td>4.3 and 3.3</td>
<td>&gt;5000</td>
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</tr>
<tr>
<td>PDRDrugs and iFacts (<a href="http://www.skyscape.com">www.skyscape.com</a>)</td>
<td>120</td>
<td>Thomson Healthcare</td>
<td>2.1 and 3.3</td>
<td>&gt;1500</td>
<td>Not currently available</td>
</tr>
<tr>
<td>Clin eRx and iFacts (<a href="http://www.skyscape.com">www.skyscape.com</a>)</td>
<td>90</td>
<td>McGraw-Hill</td>
<td>0.8 and 3.3</td>
<td>&gt;1000</td>
<td></td>
</tr>
<tr>
<td>DrDrugs and iFacts (<a href="http://www.skyscape.com">www.skyscape.com</a>)</td>
<td>108</td>
<td>F.A. Davis</td>
<td>2.4 and 3.3</td>
<td>&gt;4000</td>
<td></td>
</tr>
<tr>
<td>Lexi-Drugs, Lexi-Interact, and Lexi-Natural Products (<a href="http://www.lexi.com">www.lexi.com</a>)</td>
<td>155</td>
<td>Lexi-Comp</td>
<td>5.3, 2.8, and 0.3</td>
<td>7000</td>
<td>Each additional database is $40</td>
</tr>
</tbody>
</table>

*With the exception of PDR Drugs, data reflect product versions updated from the respective company Web sites on December 21, 2004.
*Price either for annual usage license or for product purchase with free updates for a 1-year period.
†Size of software when installed on Tungsten C with Palm OS.
‡Discounted price when purchased together as software suite.
§Suites provide a single user interface that links separate drug or natural product databases (or both) with drug interaction software (iFacts from Skyscape and Lexi-Interact from Lexi-Comp).
mediocre in this category. The only exception was CP OnHand, which summarized data from published studies that support the use of a particular drug for unla-beled indications but did not provide any specific references.

**Evidence-based dosage recommendations.** None of the software products recommended once-a-day gentamicin dosing as the primary dosing regimen. Of the 11 programs, 9 mentioned it as an alternative dosing regimen. A2zDrugs and mobilePDR did not list it at all.

**Side effects and toxicity.** Products differed widely in the category of side effects and toxicity. Although all software products listed side effects, only 2 (Lexi-Drugs and mobileMDX) provided structured information on the frequency, severity, and clinical management of side effects and drug overdoses. Interestingly, both the largest database (CP OnHand) and the smallest database (Clin-eRX) scored lowest in this category.

**Drug interactions.** The drug interaction category was a significant strength of drug reference software because all programs except ePocrates and mobilePDR had all of the drug interaction features that we would expect from a good prescribing tool. However, this was also the only category where all of the databases contained significant errors (see below for details).

**Special features.** The special features category represents a significant weakness of the currently available drug reference software products. None of the products had a free-text search capability or drug pictures. None was able to hyperlink drug data to Internet resources (eg, PubMed). Only 1 product, Pepid PDC, provided information on the compatibility of various intravenous solutions (eg, total parenteral nutrition, 5% dextrose injection, 0.9% sodium chloride injection, and 8.5% amino acid injection). CP OnHand and mobileMDX had no special prescribing features whatsoever. Even the overall highest-scoring product, Lexi-Drugs, did not have medical calculators or treatment algorithms. Pepid PDC and ePocrates scored highest in this category.

**Comprehensiveness of information**

The comprehensiveness of drug information was assessed for indications, side effects, drug interactions, and herbals. Fig 2 compares the scores of the different products. The size of the database clearly played a role in determining the score in this category. For example, the largest databases (CP OnHand, 16 MB) scored high, and the smallest product (Clin-eRX, 0.8 MB) scored low. However, factors other than database size were also important, because the highest-scoring product, Lexi-Drugs, and the lowest-scoring product, mobilePDR, were of similar size (5.3 MB and 3.9 MB, respectively).
A surprising finding was that even in our limited evaluation using several test drugs, every drug reference software product contained errors in its ability to detect clinically significant drug interactions. Three products (mobilePDR, mobileMDX, and ePharmacopoeia) were not able to detect potentially life-threatening herbal-drug interactions because their respective drug interaction tools could not screen for herbal medications. One product (Pepid PDC) did detect the St John’s wort–cyclosporine interaction but misclassified the interaction as minor, not clinically significant. None of the products could correctly identify the potential risk of a ketoconazole-erythromycin combination for QT prolongation and cardiac arrhythmias. The 3 products that detected the interaction classified it as minor, of questionable clinical significance.

No significant errors were detected in the drug monograph information on labeled indications, dosage, and side effects. Accuracy of herbal information was not assessed. CP OnHand contained a number of programming errors, such as duplication of monograph pages or incorrect linkage of drug and side effects (eg, wrong side effects listed for acetaminophen [INN, paracetamol]). The latter was corrected in a version obtained in December 2004.

Re-evaluation of drug interaction data

Given the significant errors identified in all of the drug interaction databases, we decided to re-evaluate the drug interaction component of the different products in December 2004, approximately 15 months after the initial purchase. The results were somewhat disappointing: only 4 (CP OnHand, ePharmacopoeia, ePocrates, and Pepid PDC) of 11 products were able to correctly identify potentially serious consequences of the ketoconazole-erythromycin interaction. This was the case even after a study published in October 2004 in the *New England Journal of Medicine* strongly advised against the concomitant use of erythromycin with strong CYP3A4 inhibitors such as ketoconazole. Furthermore, mobilePDR, mobileMDX, and ePharmacopoeia were still unable to screen for drug-herbal interactions. The new version of Pepid PDC correctly identified the cyclosporine–St John’s wort interaction.

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**Comparison with previously published evaluations of PDA-based drug reference software**

On the basis of the data presented here (Table 1), Lexi-Drugs and Pepid PDC presently provide the best combination of features (Fig 1) and comprehensiveness (Fig 2) as rational prescribing tools for physicians. In contrast, mobilePDR had significant deficiencies across all categories and cannot be recommended as a first-line mobile prescribing reference. Programs such as CP OnHand and mobileMDX have a wealth of drug information and may have significant potential in the future if made more user friendly for physicians. From our
analysis, it also appears that currently available PDA-based drug reference products come in 2 flavors: programs that evolved out of pharmacy reference databases (eg, mobileMDX and CP OnHand) and programs that evolved out of drug references for medical students and physicians (eg, ePharmacopoeia, ePocrates, and Pepid PDC). As a result, programs such as CP OnHand excel in the comprehensiveness and quality of drug information (Fig 2) but fall short in providing prescribing features important to physicians (Fig 1). The opposite appears to be the case for programs such as ePharmacopoeia. Interestingly, ePocrates, which is currently the PDA drug reference that is most widely used among physicians, was average in both categories (Figs 1 and 2).

Using a validated set of drug questions critical to pharmacists, Clauson et al recently compared scope, completeness, and ease of use of PDA-based drug reference software. With the exception of Pepid PDC, the same drug reference products were included in their study. Overall, their results were consistent with our study in that Lexi-Drugs ranked highest and mobilePDR ranked lowest. Although they did not assess it formally, these authors also concluded that the only significant errors were found in the drug interaction database. These results confirm an earlier survey that also ranked Lexi-Drugs highest. It should be noted that none of these studies considered the clinical utility of drug reference software for physicians, which may explain why Pepid PDC was not included in any of the previous studies. Pepid PDC, which ranked highest in terms of rational prescribing features in our analysis, was developed as part of a suite of medical decision-making products for physicians.

Barrons recently compared the accuracy, comprehensiveness, and ease of use of PDA-based drug interaction software. Again, with the exception of Pepid PDC, all of the products covered in our study were included in his review. Similar to our results, iFacts and Lexi-Interact scored highest overall and mobileMDR scored lowest. CP OnHand had the highest score for sensitivity of detected drug-drug interactions but scored lowest in usability. Drug-food or drug-herbal interactions were not assessed in this study. The author noted that both iFacts and Lexi-Drugs were more accurate than several drug interaction databases used routinely by pharmacies. The lack of a rating of the severity of the drug interaction was cited as one of the deficiencies in several database products. This appears to have been corrected in the more current versions of the drug interaction products examined here (Table I).

Medical students and physicians in training (interns, residents, and fellows), who usually do not have routine access to office-based drug reference software, currently are the primary consumer of PDA-based drug reference software. Thus such software products should strive to educate their users by providing evidence-based drug information. For example, information on the mechanism of action, size of drug effect, level of evidence, and literature references for specific indications, side effects, or drug interactions would be valuable. Unfortunately, among the currently available software products, such an approach has been taken only for drug interactions and only by a few products (Lexi-Interact, iFacts, and ePharmacopoeia). This is unfortunate, because the availability of inexpensive memory expansion cards makes the size of the drug database much less of a concern. Furthermore, intelligent and user-friendly structuring and linking of drug information also remain an issue. For example, it is surprising that none of the databases provided a free-text search tool that would allow the user to retrieve information in the database specific to his or her questions.

It should be recognized that the evaluation of up-to-dateness, comprehensiveness, and accuracy was only a snapshot using a small number of test drugs and drug combinations and, therefore, may not reflect the overall database. Furthermore, product accuracy and ease of use were not reflected in the overall score. However, our results were largely consistent with the results of studies that examined individual components of the databases in greater detail. We would encourage the reader to also consult these studies before selecting an individual product. Given that software companies continually update their content and features, reviews such as ours should be conducted regularly. At the same time, it is hoped that the companies will respond to the criticisms raised here and address them in the next versions of the software products. It should be remembered that, though likely, it remains to be determined whether use of better PDA-based drug reference products will improve rational prescribing and reduce medication errors.

To summarize, the spectrum of currently available PDA-based drug reference software products provides physicians with a range of reasonable prescribing tools from which to choose. Their major strengths are the provision of up-to-date information on indications, dosage, and detection, rating and management of drug interactions, and side effects. Their major weaknesses as rational prescribing tools are as follows: (1) lack of information on the scientific evidence and size of drug
effect for specific indications, (2) lack of free-text search capability, (3) lack of data linkage to Internet resources, and (4) inaccuracy of drug interaction information. Hopefully, these weaknesses will be addressed in future versions of the drug reference products. Meanwhile, given the large range of performance of the different products across different prescribing categories, it may be advisable for physicians to use not just 1 but 2 or 3 different products as prescribing tools.

We thank Yvonne M. Hernandez for her helpful suggestions and proofreading of the manuscript. All of the authors have no conflict of interest related to the content of this manuscript. None of the software companies mentioned in this article provided any financial support for this study.

References
St John’s wort–associated drug interactions: Short-term inhibition and long-term induction?

Hong-Guang Xie, MD, PhD, and Richard B. Kim, MD
Nashville, Tenn

In this issue of the Journal, Rengelshausen et al report an interesting observation that short-term administration of St John’s wort (SJW) resulted in a marked increase in voriconazole exposure (22% higher than control during the initial 10 hours) whereas long-term exposure to SJW was associated with a significant reduction in voriconazole’s bioavailability (59% lower than control on day 15) as a result of increased oral clearance. The mechanisms underlying the ability of SJW to both inhibit and induce metabolism of a drug are of interest.

SJW, an extract made from the flowers of the perennial plant Hypericum perforatum L (Fig 1), is widely used as an over-the-counter herbal antidepressant and accounts for a significant portion of the $4 billion a year spent on alternative medicines in the United States. Recent in vitro and focused clinical studies have shown that clinically important drug interactions associated with the use of SJW can be explained by altered drug metabolism or drug transport. Importantly, a large number of clinically relevant SJW-drug interactions, including numerous case reports and clinical studies, have been reported (Table I). These types of studies and reports of interactions have led to the Food and Drug Administration to issue a warning to the medical community about the use of SJW.

SJW is a natural product of more than 1 constituent. Commercially available SJW preparations vary greatly in their major constituents, contents, formulations, and recommended daily dose. Hypericin (a presumed active ingredient) and hyperforin (considered to be the constituent with antidepressant properties), pseudohypericin, and the flavonoids quercetin and its methylated form isorhamnetin have been noted to be the main components detectable in human plasma after single and multiple oral dosing. What is now widely appreciated is that SJW can alter the pharmacokinetic profiles of many coadministered drugs. In an in vitro induction study, when the LS180 intestinal carcinoma cell line was exposed for 3 days to SJW and hypericin, multidrug resistance 1 (MDR1) expression was
Table I. Examples of St John’s wort–associated drug interactions

<table>
<thead>
<tr>
<th>Affected drugs</th>
<th>Responsible mechanisms</th>
<th>Clinical relevance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunosuppressants</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Increased daily dose requirements or transplant rejection</td>
<td>3-8</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Increased daily dose requirements or transplant rejection</td>
<td>3-8</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Daily dose adjustments or organ/tissue rejection or nephrotoxicity</td>
<td>9-11</td>
</tr>
<tr>
<td>HIV drugs</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Treatment failure or drug resistance</td>
<td>12</td>
</tr>
<tr>
<td>Indinavir</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Treatment failure or drug resistance</td>
<td>13-15</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Treatment failure or drug resistance</td>
<td>16</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>Induction of CYP3A4 and MDR1</td>
<td>Treatment failure or drug resistance</td>
<td>16</td>
</tr>
<tr>
<td>CNS drugs</td>
<td>Induction of CYP3A4 or MDR1</td>
<td>Increased dose requirements</td>
<td>17</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>Induction of CYP3A4 or MDR1</td>
<td>Increased dose requirements</td>
<td>17</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>19</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>19</td>
</tr>
<tr>
<td>Hypnotics</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>19</td>
</tr>
<tr>
<td>Alprazolam</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Quazepam</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Buspirone</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Others</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Midazolam</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Methadone</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Methadone</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Chlorzoxazone</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Anesthetic drugs*</td>
<td>Induction of intestinal CYP3A4</td>
<td>Reduced plasma drug levels</td>
<td>20</td>
</tr>
<tr>
<td>Cancer drugs</td>
<td>Irinotecan (prodrug)</td>
<td>Reduced plasma levels of active metabolite (SN-38)</td>
<td>30, 31</td>
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<tr>
<td>Imatinib</td>
<td>Induction of intestinal CYP3A4</td>
<td>Decreased drug bioavailability</td>
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<tr>
<td>Oral contraceptives</td>
<td>Induction of CYP3A4</td>
<td>Breakthrough bleeding or contraceptive failure</td>
<td>24, 34-36</td>
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<tr>
<td>Anticoagulants</td>
<td>Warfarin</td>
<td>Increased apparent clearance</td>
<td>25, 37</td>
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<tr>
<td>Warfarin</td>
<td>Induction of CYP2C9</td>
<td>Increased apparent clearance</td>
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<tr>
<td>Phenprocoumon</td>
<td>Induction of CYP2C9</td>
<td>Decreased drug bioavailability</td>
<td>25, 38</td>
</tr>
<tr>
<td>Other drugs</td>
<td>Digoxin</td>
<td>Induced MDR1 efflux activity</td>
<td>Decreased drug bioavailability</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Induced MDR1 efflux activity</td>
<td>Decreased drug bioavailability</td>
<td>25, 26, 42</td>
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<tr>
<td>Omeprazole</td>
<td>Induction of CYP2C19 and 3A4</td>
<td>Decreased drug bioavailability</td>
<td>43</td>
</tr>
<tr>
<td>Verapamil</td>
<td>Induction of intestinal CYP3A4</td>
<td>Decreased drug bioavailability</td>
<td>44</td>
</tr>
<tr>
<td>Simvastatin (prodrug)</td>
<td>Induction of intestinal CYP3A4</td>
<td>Increased bioactivation</td>
<td>25, 45</td>
</tr>
<tr>
<td>Theophylline†</td>
<td>Induction of CYP1A2 (only in female subjects)</td>
<td>Reduced plasma drug levels</td>
<td>25, 46-48</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>Induction of CYP2C8</td>
<td>Reduced plasma drug levels</td>
<td>49</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>Induction of CYP2C19, CYP3A4, and CYP2C9</td>
<td>Increased daily dose requirements or possible treatment failure</td>
<td>1</td>
</tr>
</tbody>
</table>

HIV, Human immunodeficiency virus; CNS, central nervous system.
*Including fentanyl, propofol, and sevoflurane.
†Because CYP1A2 appears to be induced by SJW only in female subjects and not in male subjects, plasma theophylline concentrations were not affected by SJW in male subjects.
strongly induced by SJW (4-fold at 300 µg/mL) and by hypericin (7-fold at 3 µM), resulting in reduced accumulation of rhodamine 123, an MDR1 substrate. In porcine brain capillary endothelial cells, quercetin and hyperforin were observed to be potent modulators of MDR1 function when assessed by use of calcein-AM as the fluorescent marker of MDR1 function.55 In clinical studies, coadministration of high-dose hyperforin-rich extract (LI160) resulted in a markedly reduced value of the area under the plasma concentration–time curve (AUC) of digoxin (a well-known MDR1 substrate).41 Furthermore, the magnitude of cytochrome P450 (CYP) 3A4 induction, assessed by use of midazolam as the probe drug, revealed that the extent of the inductive effect associated with SJW appeared to be linked to hyperforin content.56 These findings are also consistent with a study showing significantly reduced AUC values for cyclosporine (INN, ciclosporin), a dual substrate of both MDR1 and CYP3A4, when the use of SJW with high hyperforin content was compared with a low-hyperforin SJW preparation.8 Such findings suggest that hypericin, hyperforin, and quercetin are the major constituents that mediate the apparent reduction in intestinal absorption and bioavailability of affected drugs via induction of intestinal MDR1 and intestinal and hepatic CYP3A4. Of the primary constituents in SJW, hyperforin57 but not hypericin appears to be the key activator of the xenobiotic nuclear receptor PXR (pregnane X receptor). Indeed, in vitro reporter assays have shown that hyperforin can directly activate PXR, thereby leading to the transcriptional activation of drug metabolism genes, such as CYP3A458,59 and CYP2C9.60 These findings have been validated by use of human hepatocytes for CYP3A461,62 and CYP2C9.62 Prolonged exposure of quercetin has also been shown to significantly increase MDR1 and CYP3A4 messenger ribonucleic acid levels in Caco-2 cells.15 Because PXR can also regulate the transcriptional activation of CYP2C19,63 long-term SJW use may also lead to induction of CYP2C19 in vivo.

In addition to the widely recognized ability of SJW to induce drug-metabolizing enzymes and transporters, a number of investigations have shown that SJW appears to also possess inhibitory properties. An in vitro study found that hyperforin was a competitive inhibitor of CYP3A4 and CYP2C9 (inhibition constant, 0.5 and 1.8 µM, respectively) and a potent noncompetitive inhibitor of CYP2D6 (inhibition constant, 1.5 µM), and hypericin had a similar inhibitory effect.64 In a subsequent in vitro study, hyperforin and quercetin were shown to significantly inhibit complementary deoxyribonucleic acid–expressed recombinant human P450 isoforms (CYP2C19, CYP3A4, CYP1A2, CYP2C9, and CYP2D6). Similarly, quercetin and hypericin were able to inhibit MDR1-mediated efflux of ritonavir in either Caco-2 cells or MDR1–Madin-Darby canine kidney cells and also substantially inhibit CYP3A-mediated cortisol metabolism.15 Short-term administration of hyperforin (at 5 and 10 µM) inhibited CYP3A4 activity,62 but short-term administration of SJW did not affect CYP3A4 activity in vivo when assessed by use of midazolam as the probe drug.22 However, short-term exposure to a single dose of SJW significantly decreased the oral clearance of fexofenadine (an MDR1 substrate) and increased its maximum plasma concentration,42 indicating an inhibition of intestinal MDR1 activity. Similarly, hyperforin and hypericin inhibited MDR1-mediated efflux of the fluorescent markers daunorubicin and calcein-AM in vitro.66 Quercetin inhibited MDR1-mediated digoxin transport in Caco-2 cells67 and CYP2C9-mediated flurbiprofen hydroxylation in vitro (50% inhibitory concentration, 25.8 µmol/L).68 However, in contrast to short-term exposure, long-term SJW administration resulted in significantly increased oral clearance of MDR1 substrates, such as digoxin,25,39-41 fexofenadine,25,26,42 and cyclosporine.5-8,25 Similarly, concomitant SJW ingestion was associated with a significant increase in the oral and, to a lesser extent, systemic clearance of midazolam,7,22,24-26 indicating that induction of intestinal CYP3A4 and, likely, MDR1 is responsible for drug interactions associated with SJW therapy.

In addition to the combined up-regulation of expression and activity of intestinal MDR17,8,23,25,26,40 and intestinal and hepatic CYP3A4,19,20,22,23,25,44,45,48,69 which are consistent with activation of PXR by SJW constituents, SJW was shown to significantly induce CYP2C19-dependent 4’-hydroxylation of 5-methylenetetrahydrofolate and 5-hydroxylation of omeprazole,43 CYP2C9-catalyzed 7-hydroxylation of S-warfarin,43 CYP2E1-mediated 6-hydroxylation of chlormezazone,23 and CYP2C8-mediated metabolism of rosiglitazone (a peroxisome proliferator–activated receptor γ agonist and CYP2C9 substrate).49 CYP2D6,19,22,48,62,70,71 CYP1A2,22,43,48,62 N-acetyltransferase 2,48 and xanthine oxidase48 were unaffected by SJW.

In this issue of the Journal, Rengelshausen et al compare the effects of short-term and long-term SJW intake on the metabolism of the antifungal agent voriconazole, a substrate for CYP2C19, CYP3A4, and CYP2C9.72 Repeated comedication with SJW resulted in a significant reduction in voriconazole exposure, which is consistent with SJW’s known properties as a potent inducer of such enzymes, whereas short-term
dosing with SJW was associated with increased exposure to voriconazole. Interestingly, the extent of induction was significantly lower among subjects who carry CYP2C19 variant alleles. However, from the data provided, it is not clear whether induction of MDR1 had any role in the disposition profiles for voriconazole during short-term and long-term administration of SJW.

In summary, SJW appears to exert a biphasic effect on MDR1 and certain CYP enzymes, where the initial doses often lead to an apparent inhibition of such proteins, followed by significant induction during long-term use, particularly for genes regulated by PXR. Indeed, most of the reported clinical interactions associated with concomitant use of SJW appear to be due to the inductive effect of SJW, resulting in a loss of drug efficacy or treatment failures from concomitantly administered drugs (Table I). Accordingly, for drugs whose primary route of elimination is through CYP3A4, CYP2C19, CYP2C9, CYP2C8, CYP1A2 (in female subjects), or MDR1, concurrent use of SJW would be predicted to lower the plasma level and efficacy of such drugs. In addition, it is clear that herbal preparations such as SJW have multiple constituents that can also inhibit CYP enzymes and transporter proteins and that a constituent that mediates the inductive response may not be the constituent that mediates the inhibitory effect. These types of interactions point to the need for more systematic assessments of herbal remedies for their potential to interact with drug-metabolizing enzymes and transporters so that clinically relevant interactions can be predicted or avoided before their widespread use.

We thank Mr Mark Udsetuen of Shooting Star Native Seeds, Spring Grove, Minn, who gave us the permission to reproduce the photograph of the SJW flower.

The authors have no conflict of interest to disclose.

References
Opposite effects of short-term and long-term St John’s wort intake on voriconazole pharmacokinetics

Objectives: Constituents of St John’s wort (SJW) in vivo induce the cytochrome P450 (CYP) isozymes 3A4, 2C9, and 2C19 but in vitro were shown to inhibit them. This study investigates both short- and long-term effects of SJW on the antifungal voriconazole, which is metabolized by these enzymes.

Methods: In a controlled, open-label study, single oral doses of 400 mg voriconazole were administered to 16 healthy men stratified for CYP2C19 genotype before and on day 1 and day 15 of concomitant SJW intake (300 mg LI 160 3 times daily). Plasma and urine concentrations of voriconazole were determined by liquid chromatography with mass-spectrometric detection.

Results: During the initial 10 hours of the first day of SJW administration, the area under the voriconazole plasma concentration–time curve was increased by 22% compared with control (15.5 ± 6.84 h · μg/mL versus 12.7 ± 4.16 h · μg/mL, P = .02). After 15 days of SJW intake, the area under the plasma concentration–time curve from hour 0 to infinity was reduced by 59% compared with control (9.63 ± 6.03 h · μg/mL versus 23.5 ± 15.6 h · μg/mL, P = .0004), with a corresponding increase in oral voriconazole clearance (CL/F) from 390 ± 192 to 952 ± 524 mL/min (P = .0004). The baseline CL/F of voriconazole and the absolute increase in CL/F were smaller in carriers of 1 or 2 deficient CYP2C19*2 alleles compared with wild-type individuals (P < .03).

Conclusions: Coadministration of SJW leads to a short-term but clinically irrelevant increase followed by a prolonged extensive reduction in voriconazole exposure. SJW might put CYP2C19 wild-type individuals at highest risk for potential voriconazole treatment failure. (Clin Pharmacol Ther 2005;78:25-33.)

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Extracts of St John’s wort (Hypericum perforatum L) (SJW) are widely used as over-the-counter remedies for the treatment of mild to moderate depressive disorders.1 Coadministration of SJW are widely used as over-the-counter remedies for the treatment of mild to moderate depressive disorders.1 At variance with the in vivo effects, SJW failed to reduce the pharmacokinetics of ciclosporine, tacrolimus, warfarin, amlodipine, and imatinib.11 Most of these drugs are metabolized by cytochrome P450 (CYP) 3A4 or transported by P-glycoprotein (or both). As the basis for these interactions, SJW was shown to induce the expression of intestinal and hepatic CYP3A4, as well as intestinal P-glycoprotein, in humans.14
The extracts of SJW contain different groups of compounds such as hypericin, hyperforin, and flavonoids. These compounds vary widely in their content between different preparations. In clinical studies the content of hyperforin determined the extent of the interaction with digoxin and cyclosporine. These findings are in line with in vitro data showing a relevant increase in messenger ribonucleic acid and protein of CYP3A4 and CYP2C9 in cultured human hepatocytes after hyperforin treatment for 48 hours. Hyperforin mediates the induction of CYP3A4 expression by high-affinity binding and subsequent activation of the pregnane X receptor (PXR). In addition to the long-term inducing effect, hyperforin was shown to inhibit the activity of CYP isozymes 1A2, 2C9, 2C19, 2D6, and 3A4 in vitro when added to the culture medium for a short term. Therefore the primary aim of this study was to investigate the short- and long-term effects of SJW on the pharmacokinetics of voriconazole.

The new triazole antifungal voriconazole is used for the treatment of severe fungal infections. In immunocompromised patients with invasive aspergillosis, initial treatment with voriconazole led to improved survival rates compared with initial therapy with amphotericin B. The extensive metabolism of voriconazole is primarily mediated by CYP2C19 and CYP3A4, as well as by CYP2C9 to a lesser extent. Genetic polymorphisms of CYP2C19 influence the plasma concentrations of voriconazole, which are approximately 3 times higher in poor metabolizers compared with homozygous extensive metabolizers, with intermediate concentrations in heterozygous extensive metabolizers. Poor metabolizers for CYP2C19 (3%-5% in white subjects) carry 2 defective alleles, of which the CYP2C19*2 allele accounts for about 80% of mutated alleles. With the use of mephenytoin as a test substance, SJW was shown to induce the activity of CYP2C19 in extensive metabolizers but not in poor metabolizers. Therefore the secondary aim of this study was to investigate, in an exploratory way, the influence of the CYP2C19 genotypes on the interaction between SJW and voriconazole.

**METHODS**

The study was approved by the Ethics Committee of the Medical Faculty of the University of Heidelberg, Heidelberg, Germany, and was conducted at the Department of Internal Medicine VI, Clinical Pharmacology and Pharmacoepidemiology in accordance with the Declaration of Helsinki, as amended in Somerset West 1996, and the specific legal requirements in Germany.

**Study population**

Seventeen healthy, nonsmoking men participated in the study after they had been fully informed about the study and given written informed consent. None was receiving any other systemic drug treatment during the study, and systemic drug treatment had to be discontinued for at least 10 elimination half-lives of the respective compound. The participants were ascertained to be healthy by medical history, physical examination, laboratory screening including hematologic and biochemical blood tests, and a 12-lead electrocardiogram. The mean age, body weight, and body mass index (BMI) were not significantly different between the CYP2C19 wild-type group and the combined CYP2C19*1/*2 and *2/*2 group (P > .15).

**Study design and procedures**

According to a controlled, open-label, fixed-dose schedule design, each participant received a single oral dose of 400 mg voriconazole (2 VFEND 200-mg film tablets; Pfizer, Sandwich, Kent, United Kingdom) on study days 1, 3, and 17. In addition, each participant received 300 mg SJW extract LI 160 (Jarsin, 300 mg; Lichtwer Pharma, Berlin, Germany) orally 3 times daily (approximately every 8 hours) for 15 days from
study day 3 to 17 (Fig 1). The intake of SJW was monitored by an electronic device (MEMS V TrackCap; Aardex, Zug, Switzerland). On study days 3 and 17, SJW was ingested 60 minutes before voriconazole administration to reduce the probability of a galenic interaction. After an overnight fast from 12 hours before until 4 hours after voriconazole administration, each participant received a standard hospital lunch and dinner served 4 and 9 hours after voriconazole dosing. Alcoholic and caffeinated beverages were not allowed from 24 hours before study day 1 until study day 4 and from study day 16 until study day 18. During study days 1 to 18, beverages containing grapefruit juice had to be avoided. On study days 1, 3, and 17, venous blood samples (7.5 mL each) were collected through an intravenous catheter into heparinized tubes immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24 hours after the administration of voriconazole. The blood samples were immediately centrifuged (3000 g for 10 minutes at 4°C), and separated plasma samples were stored at −20°C until analysis. After completely voiding the bladder immediately before voriconazole intake, the participants collected urine from 0 to 24 hours after voriconazole administration. After measurement of the urine volume, a 10-mL aliquot of each fraction was stored at −20°C until analysis.

**Determination of voriconazole concentrations in plasma and urine**

Voriconazole concentrations in plasma and urine were determined after solid-phase extraction on the basis of a previously described HPLC assay and were determined after solid-phase extraction on the plasma and urine. Determination of voriconazole concentrations in study day 3 to 17 (Fig 1). The intake of SJW was monitored by an electronic device (MEMS V TrackCap; Aardex, Zug, Switzerland). On study days 3 and 17, SJW was ingested 60 minutes before voriconazole administration to reduce the probability of a galenic interaction. After an overnight fast from 12 hours before until 4 hours after voriconazole administration, each participant received a standard hospital lunch and dinner served 4 and 9 hours after voriconazole dosing. Alcoholic and caffeinated beverages were not allowed from 24 hours before study day 1 until study day 4 and from study day 16 until study day 18. During study days 1 to 18, beverages containing grapefruit juice had to be avoided. On study days 1, 3, and 17, venous blood samples (7.5 mL each) were collected through an intravenous catheter into heparinized tubes immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24 hours after the administration of voriconazole. The blood samples were immediately centrifuged (3000 g for 10 minutes at 4°C), and separated plasma samples were stored at −20°C until analysis. After completely voiding the bladder immediately before voriconazole intake, the participants collected urine from 0 to 24 hours after voriconazole administration. After measurement of the urine volume, a 10-mL aliquot of each fraction was stored at −20°C until analysis.

In brief, plasma and urine samples (500 μL of standards, controls, and unknown samples) were spiked with internal standard (UK-115,794) and 0.2-mol/L borate buffer (pH 9.0, 700 μL) was added. Buffered and vortexed samples were loaded onto conditioned C18 SPE columns (BondElut, 100 mg; Varian, Darmstadt, Germany) and washed with borate buffer and methanol/water (50:50) before being eluted with 1 mL of methanol/acetic acid mixture (99:1). The extracts were dried under streams of nitrogen, and the dried residues were reconstituted with mobile liquid chromatography phase, centrifuged, and analyzed by liquid chromatography–mass spectrometry. For mass selective detection, the liquid chromatography system consisted of a Luna C18 column (3 μm, 50 × 2.0 mm at 40°C) and an isocratic mobile phase with the use of 0.02-mol/L ammonia acetate including 0.1% acetic acid and acetonitrile (65:35 [vol/vol]) at a flow rate of 0.35 mL/min. The injection volume was 10 μL. An atmospheric pressure chemical ionization source (4.5 kV, 400°C) was used for ionization, and the mass spectrometer worked in the selected ion monitoring mode at mass-to-charge ratio (m/z) 350 (voriconazole) and m/z 348 (internal standard). The limit of quantification was 0.05 μg/mL, and the calibration ranged between 0.05 μg/mL and 10.0 μg/mL. Correlation coefficients were always r² > 0.99. The analytic methods were validated according to Food and Drug Administration validation guidelines and fulfilled the respective quality assurance requirements for accuracy and precision. For plasma, the precision of determination (coefficient of variation) was 9.5%, 7.0%, 9.2%, and 7.9% and the accuracy was 105.3%, 100.9%, 96.1%, and 96.8% at quality-control concentrations of 0.16, 0.38, 3.15, and 6.38 μg/mL, respectively. For urine, the precision of determination (coefficient of variation) was 4.8%, 6.1%, 2.7%, and 3.9% and the accuracy was 110.3%, 114.3%, 92.0%, and 93.2% at concentrations of 0.17, 0.43, 3.01, and 6.14 μg/mL, respectively.

**Pharmacokinetic analysis**

Noncompartmental analysis by use of WinNonlin 4.0 software (Pharsight, Mountain View, Calif) was performed to determine pharmacokinetic parameters of voriconazole. The peak plasma concentration (Cmax) and the time to reach Cmax (tmax) were obtained directly from the raw data. The terminal plasma elimination half-life (t½) was calculated as follows: % = ln2/λ z, where λ z represents the slope of the terminal part of the plasma concentration–time curve obtained by linear regression after semilogarithmic transformation. The area under the plasma concentration–time curve (AUC) from hour 0 to infinity (AUC 0-24) as time limits were calculated as follows: CL/F = D/(λ z · AUC 0-24), where D is dose and F is oral bioavailability. The oral systemic clearance was calculated as follows: CL/F = D/AUC 0-24. The renal clearance during the time interval from 0 to 24 hours was calculated as follows: CL renal 0-24 = Aeq 0-24/AUC 0-24, where Aeq 0-24 is the amount of voriconazole excreted into urine during the time interval from 0 to 24 hours.
Determination of CYP2C19 genotypes

The presence of the CYP2C19*2 or *3 allele in the genomic deoxyribonucleic acid derived from leukocytes of the participants was determined by use of the hybridization probes format (LightCycler CYP2C19 Mutation Detection Kit with specific primers) on a LightCycler (both obtained from Roche Applied Science, Mannheim, Germany). The presence of the wild-type allele CYP2C19*1 was inferred from the absence of the *2 and *3 alleles.

Statistical analysis

Data are expressed as mean values ± SD or mean values ± SEM (Fig 2). Differences in pharmacokinetic parameters of voriconazole between concomitant SJW treatment and control were assessed with the nonparametric Wilcoxon signed rank test for paired data. Differences in age, body weight, and body mass index and in the pharmacokinetic parameters of voriconazole between the CYP2C19*1/*1 group and the combined CYP2C19*1/*2 and *2/*2 group were assessed with the Wilcoxon rank sum (Mann-Whitney U) test for unpaired data. Assuming an SD of 30% for the difference in voriconazole AUC with and without SJW based on data for the interaction between SJW and omeprazole, a sample size of 6 in each group was estimated to detect a 40% change in voriconazole AUC with a significance level of 5% and a statistical power of 90%. P < .05 was considered statistically significant.

RESULTS

Voriconazole was generally well tolerated by all participants, without serious adverse events. Transient enhanced light perception during the first hour after voriconazole intake was reported by most participants. This visual disturbance has been recognized as the most frequent adverse reaction to voriconazole and is related to transient electric changes in the retina but without detection of ocular lesions. One participant (CYP2C19*1/*1 genotype) discontinued the study after the first part because of elevated liver enzyme values and sonographic signs of hepatic steatosis possibly related to previous alcohol consumption.

The plasma concentration–time curves of voriconazole before and during SJW (LI 160) administration are shown in Fig 2. On the first day of SJW intake (D1 SJW), the AUC of voriconazole was increased by 22% compared with control, whereas after 15 days of SJW administration (D15 SJW), the AUC of voriconazole was reduced by 43% compared with control (Table I). AUC0-10 values were taken for primary comparison because on D15 SJW, plasma concentrations of voriconazole were below the limit of quantification 24 hours after drug administration in 11 of 16 participants. The changes in C_{max} of voriconazole were similar to those in the AUC0-10. In contrast, AUC_{0-\infty} was not significantly changed on D1 SJW but was extensively reduced by 59% on D15 SJW. Correspondingly, CL/F of voriconazole was not affected on D1 SJW but was increased by 144% on D15 SJW. AUC_{0-\infty} and CL/F varied widely between the participants (Fig 3). Despite an unchanged CL/F, the t_{1/2} of voriconazole was already reduced on D1 SJW, with further reduction on D15 SJW. CL_{\text{renal} 0-24} of voriconazole was only small compared with CL/F but yielded a significant reduction on D1 SJW (Table I).

When analyzed according to the different CYP2C19 genotypes, AUC_{0-10} and AUC_{0-\infty} of voriconazole were not significantly changed on D1 SJW but were extensively reduced on D15 SJW in both the CYP2C19 wild-type group and the CYP2C19*1/*2 group (Table II). A similar trend occurred in the 2 participants with the CYP2C19*2/*2 genotype. Corresponding results were obtained for CL/F in the 3 genetic groups (Fig 4). In the control phase, the CL/F of voriconazole was higher in the CYP2C19 wild-type group than in the combined CYP2C19*1/*2 and *2/*2 group (493 ± 123 mL/min versus 287 ± 199 mL/min, P = .03). On D15 SJW, the absolute increase in the CL/F of voriconazole
Table I. Pharmacokinetic parameters of single oral dose of 400 mg voriconazole before (control) and on first day and last day of coadministration of 300 mg St John’s wort (LI 160) 3 times daily for all participants (N = 16)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>D1 SJW</th>
<th>P value*</th>
<th>D15 SJW</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC₀-10 (h · µg/mL)</td>
<td>12.7 ± 4.16</td>
<td>15.5 ± 6.84</td>
<td>.02</td>
<td>7.26 ± 3.43</td>
<td>.0004</td>
</tr>
<tr>
<td>AUC₀-∞ (h · µg/mL)</td>
<td>23.5 ± 15.6</td>
<td>25.2 ± 16.2</td>
<td>.12</td>
<td>9.63 ± 6.03</td>
<td>.0004</td>
</tr>
<tr>
<td>Cmax (µg/mL)</td>
<td>2.56 ± 0.56</td>
<td>3.13 ± 0.91</td>
<td>.02</td>
<td>1.87 ± 0.75</td>
<td>.001</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>1.84 ± 0.94</td>
<td>1.75 ± 0.91</td>
<td>.97</td>
<td>1.25 ± 0.63</td>
<td>.01</td>
</tr>
<tr>
<td>Cmax (h)</td>
<td>8.18 ± 4.73</td>
<td>6.37 ± 2.45</td>
<td>.004</td>
<td>4.95 ± 1.34</td>
<td>.0005</td>
</tr>
<tr>
<td>Vz/F (L)</td>
<td>227 ± 80.5</td>
<td>180 ± 79.5</td>
<td>.03</td>
<td>377 ± 199</td>
<td>.0004</td>
</tr>
<tr>
<td>CL/F (mL/min)</td>
<td>390 ± 192</td>
<td>371 ± 215</td>
<td>.18</td>
<td>952 ± 524</td>
<td>.0004</td>
</tr>
<tr>
<td>CLrenal 0-24 (mL/min)</td>
<td>1.60 ± 0.92</td>
<td>1.31 ± 0.64</td>
<td>.04</td>
<td>2.20 ± 1.31†</td>
<td>.11†</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.
D1 SJW, First day of St John’s wort administration; D15 SJW, last day of St John’s wort administration; AUC₀-10, area under plasma concentration–time curve from 0 to 10 hours; AUC₀-∞, area under plasma concentration–time curve from hour 0 to infinity; Cmax, peak plasma concentration; tmax, time to peak plasma concentration; t½, terminal plasma elimination half-life; Vz/F, apparent volume of distribution; CL/F, oral systemic clearance; CLrenal 0-24, renal clearance during time interval from 0 to 24 hours.
*P values are given for the differences with respect to control.
†Values were calculated for 15 participants because of a urine collection error in 1 participant.

compared with control was also higher in the CYP2C19 wild-type group than in the combined CYP2C19*1/*2 and *2/*2 group (675 ± 232 mL/min versus 448 ± 479 mL/min, P = .02), but the relative increase in the CL/F of voriconazole in relation to the control phase was not different between the CYP2C19 wild-type group and the combined CYP2C19*1/*2 and *2/*2 group (148% ± 66.8% versus 150% ± 67.3%, P = .67). Corresponding results were obtained for the AUC₀-∞ of voriconazole in the control phase (14.3 ± 3.48 h · µg/mL versus 32.7 ± 17.7 h · µg/mL, P = .03) and the relative reduction in the AUC₀-∞ on D15 SJW in relation to control (−57.1% ± 11.5% versus −57.9% ± 9.1%, P = .67) when the 2 genetic groups were compared.

DISCUSSION

The SJW preparation LI 160 has been used in most clinical studies investigating drug interactions with SJW. As a hyperforin-rich methanolic extract of SJW, LI 160 induces intestinal and hepatic CYP3A4 and intestinal P-glycoprotein. Consequently, the plasma AUC of orally administered substrates of CYP3A4 or P-glycoprotein is reduced by a mean range of 20% to 60%. Because voriconazole is metabolized mainly by CYP2C19 and CYP3A4, an interaction with SJW was expected. Knowing the extent of this interaction is essential, given the high prevalence of SJW exposure in hospitalized patients.

In this study the reduction of the voriconazole AUC₀-∞ by 59% after 15 days of LI 160 coadministration is similar to the AUC reduction of oral cyclosporine and tacrolimus and about twice as high as the AUC reduction of digoxin after 2 weeks of daily treatment with 600 mg and 900 mg LI 160, respectively. The t½ of digoxin remained unchanged after SJW treatment. Because digoxin is hardly metabolized but is a substrate of P-glycoprotein, the unchanged t½ reflects a predominant influence on the intestinal absorption of digoxin limited by P-glycoprotein. In this study the concomitant reduction of voriconazole’s Cmax (27%) and t½ (39%) after 15 days of SJW intake suggests that both pre-systemic processes (intestinal absorption or intestinal first-pass metabolism) and systemic elimination of voriconazole are altered by prolonged intake of SJW. In previous studies on the interaction between SJW and the CYP3A4 substrate midazolam, the increase in the CL/F of midazolam was more than 3 times higher after oral administration than after intravenous administration, indicating that induction of intestinal CYP3A4 mainly contributes to the interaction.

Intestinal transport of voriconazole had not been described yet, but the related azole antifungal intraconazole was identified to be transported by P-glycoprotein. Thus induction of both absorption-limiting drug transport proteins and intestinal metabolism might contribute to the reduction in voriconazole exposure after prolonged administration of SJW.

Rifampin (INN, rifampicin) is a well-known and potent inducer of CYP-mediated drug metabolism and P-glycoprotein–mediated drug transport. Most drugs interacting with rifampin are also known to interact with SJW in a similar way. For drugs such as tacrolimus, verapamil, imatinib, and digoxin, the extent of reduction in exposure was higher after rifampin than after SJW administration. Coadministration of rifampin led to a 96% reduction in the AUC of voriconazole (German product labeling for voriconazole, Pfizer, Ger-
many, August 2004), which is also higher than the 59% reduction observed in our study. Apart from dose and factors related to tissue distribution, an inhibitory effect of SJW on CYP3A4 as observed in vitro\textsuperscript{21,22} was suggested as a possible explanation for this difference.\textsuperscript{12}

The effect of short-term coadministration of SJW on the pharmacokinetics of digoxin, caffeine, tolbutamide, dextromethorphan, and midazolam was investigated in 2 previous studies, without detection of significant changes.\textsuperscript{4,31} In a further study a single dose of SJW led to a 45% increase in the $C_{\text{max}}$ of fexofenadine, which is a substrate of P-glycoprotein.\textsuperscript{34} In our study a 22% increase in the AUC$_{0-10}$ of voriconazole occurred on the first day of SJW administration. This increase was reflected also in C$_{\text{max}}$ but not in AUC$_{0-\infty}$ or CL/F. These results suggest that the short-term effect of SJW is limited to the absorption phase of voriconazole. Several mechanisms for this effect can be discussed. First, acceleration of the dissolution of voriconazole tablets by constituents of SJW appears unlikely because the $t_{\text{max}}$ of voriconazole remains unchanged. Second, apical intestinal export pumps such as P-glycoprotein might be inhibited either by hyperforin\textsuperscript{34,35} or by the pharmaceutic aid Macrogol 6000, similar to P-glycoprotein inhibition by Cremophor RH40.\textsuperscript{36} This inhibition might lead to enhanced absorption of voriconazole during the intestinal presence of the SJW preparation. Third, enhanced absorption of voriconazole might be related to a short-term inhibition of the intestinal voriconazole metabolism. The 50% inhibitory concentration ($IC_{50}$) values required for inhibition of CYP3A4 by hyperforin in vitro (2-4 \textmu mol/L)\textsuperscript{21,22} are much higher than the 50% effective concentration.

Table II. Mean exposure of voriconazole after single oral dose of 400 mg before and on first day and last day of coadministration of 300 mg St John’s wort (LI 160) 3 times daily differentiated for CYP2C19 genotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>D1 SJW</th>
<th>P value*</th>
<th>D15 SJW</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2C19 wild type (n = 8)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-10}$ (h · \textmu g/mL)</td>
<td>10.2 ± 2.19</td>
<td>11.6 ± 2.85</td>
<td>.21</td>
<td>5.08 ± 0.65</td>
<td>.01</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (h · \textmu g/mL)</td>
<td>14.3 ± 3.48</td>
<td>15.4 ± 4.12</td>
<td>.26</td>
<td>5.84 ± 0.84</td>
<td>.01</td>
</tr>
<tr>
<td>CYP2C19*1/*2 (n = 6)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-10}$ (h · \textmu g/mL)</td>
<td>14.3 ± 4.49</td>
<td>17.7 ± 7.87</td>
<td>.12</td>
<td>8.84 ± 4.21</td>
<td>.03</td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (h · \textmu g/mL)</td>
<td>31.2 ± 20.7</td>
<td>32.4 ± 20.5</td>
<td>.60</td>
<td>12.5 ± 7.61</td>
<td>.03</td>
</tr>
<tr>
<td>CYP2C19*2/*2 (n = 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-10}$ (h · \textmu g/mL)</td>
<td>18.1</td>
<td>24.8</td>
<td></td>
<td>11.2</td>
<td></td>
</tr>
<tr>
<td>AUC$_{0-\infty}$ (h · \textmu g/mL)</td>
<td>37.1</td>
<td>42.8</td>
<td></td>
<td>16.2</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. *P values are given for the differences with respect to control.
(EC$_{50}$) value for PXR activation (23 nmol/L).

This supports the hypothesis that relevant inhibition can only be achieved locally in the gut whereas the lower systemic concentrations are sufficient for PXR activation and subsequent induction of metabolism and transport.

The basis for the reduction in the voriconazole $t_{1/2}$ after short-term administration of SJW remains unclear.

First, oral voriconazole pharmacokinetics does not appear to have flip-flop characteristics because $t_{1/2}$ after oral intake is rather shorter than after intravenous administration. Thus an unmasking of the “real” oral $t_{1/2}$ by SJW is unlikely. Second, a short-term increase in systemic voriconazole clearance by induction of metabolism is also unlikely because cell cultures were exposed to hyperforin for 48 hours to yield significant CYP induction. Finally, an alteration of voriconazole metabolism by the preceding administration of voriconazole on study day 1 appears unlikely because autoinduction of voriconazole metabolism was only found in rats and dogs but not observed in humans.

Third, the 20% reduction in the V$\text{$_{d}$/F}$ of voriconazole on the first day of SJW administration observed in our study may be caused by an increase in oral bioavailability or by a reduction in the distribution of voriconazole. However, the absolute bioavailability of voriconazole is about 90%, indicating that the observed change cannot be explained by a change in oral bioavailability alone. Thus a short-term reduction of the systemic voriconazole distribution by SJW, for example, by alteration of transport processes, may lead to the observed reduction in $t_{1/2}$ on the first day of SJW administration.

Voriconazole is metabolized mainly by CYP2C19 and CYP3A4. Therefore genetic polymorphisms of CYP2C19 are expected to influence the interaction with SJW. In the exploratory analysis of our study, the pattern of interaction was similar in the 3 genetic groups (CYP2C19 wild type, CYP2C19*1/*2, and CYP2C19*2/*2) with a trend toward an increase in the AUC$_{0-10}$ of voriconazole on the first day of SJW intake and an extensive reduction in the AUC$_{0-\infty}$ after prolonged SJW intake. Because genetic screening included only the alleles *2 and *3, which account for more than 85% of defective CYP2C19 alleles in white subjects, the occurrence of other defective alleles in the study population and thus misclassification of individuals cannot be ruled out.

For statistical comparison, data from individuals with CYP2C19*1/*2 and *2/*2 genotype were pooled. In line with a previous report, the presence of the deficient CYP2C19*2 allele resulted in a higher baseline AUC$_{0-\infty}$ and lower CL/F of voriconazole compared with the CYP2C19 wild-type group in our study. In addition, the absolute increase in the CL/F after prolonged intake of SJW was smaller in the presence of the CYP2C19*2 allele compared with the wild-type group. Therefore the induction of voriconazole metabolism might not be limited to the induction of CYP3A4, which would be expected to result in equal absolute increases in CL/F in the 2 genetic groups. In addition, CYP2C19 also appears to be induced, leading to a smaller increase in CL/F in the presence of the CYP2C19*2 allele (Fig 4). This hypothesis is supported by recent data on the induction of CYP2C19-mediated
metabolism of mephenytoin by SJW, where an increase in CYP2C19 activity occurred in the extensive metabolizer group but not in the poor metabolizer group.\textsuperscript{28} PXR was found to up-regulate the transcription of CYP2C19\textsuperscript{39}; thus it is reasonable that SJW might induce the metabolism of voriconazole, depending on both CYP3A4 and CYP2C19. Because the recommended voriconazole dosage is independent of the CYP2C19 genotype (German product labeling for voriconazole, Pfizer, Germany, August 2004), combination therapy with potent inducers such as SJW might result in the lowest antifungal exposure in CYP2C19 wild-type patients because of the highest CL/F of voriconazole achieved in this group.

In conclusion, coadministration of the SJW preparation LI 160 results in a short-term small and clinically irrelevant increase in voriconazole exposure, which is followed by an extensive reduction after prolonged administration of SJW. This halving of voriconazole exposure is probably of clinical relevance because plasma concentrations of voriconazole might fall below the levels needed for antifungal activity, although a clear correlation between plasma concentrations and efficacy of voriconazole was not yet been described.\textsuperscript{40} In contrast to SJW, voriconazole is mainly used in the inpatient setting, but the recent finding of a prevalence of about 7% undeclared exposure to SJW in hospitalized patients\textsuperscript{30} underlines the importance of considering this interaction in the clinical setting.

We thank Mrs Brigitte Tubach for study support and Mrs Andrea Deschlmayr, Mrs Magdalena Longo, and Mrs Jutta Kocher for their excellent technical assistance.

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References


29. Pennick GJ, Clark M, Sutton DA, Rinaldi MG. Development and validation of a high-performance liquid chro-


Higher exposure to mycophenolic acid with sirolimus than with cyclosporine cotreatment

**Introduction:** Therapeutic drug monitoring of mycophenolate mofetil is recommended because of the interindividual variability in the exposition to its active moiety, mycophenolic acid. However, most of the pharmacokinetic studies involved patients cotreated with cyclosporine (INN, ciclosporin).

**Methods:** We analyzed the pharmacokinetics of mycophenolic acid in 13 renal graft recipients treated with sirolimus in an anticalcineurin-free regimen and compared it with that of 17 patients cotreated with cyclosporine. The area under the concentration versus time curve over a 12-hour period (AUC0-12) of mycophenolic acid was estimated at 2 weeks, 1 month, 2 months, and 3 months after transplantation.

**Results:** At the first 3 time points, patients cotreated with sirolimus had significantly higher mycophenolic acid AUC0-12 values compared with patients cotreated with cyclosporine, as follows: 81 mg · h/L (SD, 39 mg · h/L) versus 43 mg · h/L (SD, 11 mg · h/L) ($P < .001$), 72 mg · h/L (SD, 17 mg · h/L) versus 48 mg · h/L (SD, 13 mg · h/L) ($P < .001$), and 70 mg · h/L (SD, 25 mg · h/L) versus 47 mg · h/L (SD, 17 mg · h/L) ($P < .01$) at week 2, month 1, and month 2, respectively. At all time points, patients cotreated with sirolimus had significantly higher dose-normalized mycophenolic acid AUC0-12 values. At months 1 and 2, white blood cell counts were lower in the sirolimus group than in the cyclosporine group, as follows: 4.8 × 10^3/mL (SD, 1.1 × 10^3/mL) versus 6.5 × 10^3/mL (SD, 2.2 × 10^3/mL) ($P < .01$) at month 1 and 4.6 × 10^3/mL (SD, 1.1 × 10^3/mL) versus 5.9 × 10^3/mL (SD, 2.0 × 10^3/mL) ($P < .05$) at month 2.

**Conclusion:** These data show that exposure to mycophenolic acid is higher in patients cotreated with sirolimus than in those cotreated with cyclosporine. (Clin Pharmacol Ther 2005;78:34-42.)

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Mycophenolate mofetil (MMF) is routinely used in combination with other immunosuppressive agents for the prevention of acute rejection after organ transplantation. On the basis of clinical dose-ranging studies, the recommended MMF dose is 1 g twice a day.1 Nevertheless, therapeutic drug monitoring of its active metabolite, mycophenolic acid (MPA), is now recommended,2 because substantial interindividual variability in MPA pharmacokinetics has been described.3,4 Relationships between the area under the concentration versus time curve (AUC) of MPA and the risk of side effects, particularly neutropenia and gastrointestinal disorders,5 on the one hand, and the risk of rejection,3,4 on the other hand, have been reported. Target ranges of MPA AUC over a 12-hour period (AUC0-12) of 30 to 60 mg · h/L when measured with an HPLC technique6 or of 35 to 70 mg · h/L when measured with an enzyme-multiplied immunoassay technique (EMIT)7 have been proposed in renal graft recipients cotreated with cyclosporine (INN, ciclosporin). Such recommendations are not yet available for combinations with other immunosuppressive drugs, particularly tacrolimus or sirolimus.

The pharmacokinetics of MPA is influenced by medications because higher MPA concentrations are observed in transplant recipients receiving MMF alone.
or combined with tacrolimus than in patients receiving MMF together with cyclosporine. The higher MPA concentrations observed in patients cotreated with tacrolimus were initially attributed to an effect of tacrolimus on MPA glucuronidation. However, it is now believed that the difference in MPA exposure according to comedication is explained by an inhibition of MPA enterohepatic circulation by cyclosporine as demonstrated in a rat model. Of note, most studies exploring MPA pharmacokinetics were performed in patients treated with MMF together with cyclosporine. In this population MPA trough concentrations correlate poorly with MPA AUC\textsubscript{0-12} and drug monitoring should, therefore, be based on MPA AUC\textsubscript{0-12}.

Tacrolimus and sirolimus are both macrolides and share the same intracellular transporter (FK506 binding protein [FKBP]), but their mechanisms of action are very different. Indeed, tacrolimus inhibits calcineurin, a key enzyme of T-cell activation, whereas sirolimus is an inhibitor of mTOR (mammalian target of rapamycin), a key enzyme of the transduction signal of interleukin 2 in activated T cells. Of note, both drugs are metabolized by cytochrome P450 (CYP) 3A4.

Sirolimus, contrary to calcineurin inhibitors, does not appear to induce nephrotoxicity and is used in the prevention of acute rejection after kidney transplantation. A multicenter prospective study has shown that sirolimus is associated with a low rejection rate and that, overall, the renal function is better in patients treated with this drug than in those treated with cyclosporine. Although, as in this study, sirolimus is usually used in association with MMF and steroids, the pharmacokinetic parameters of MPA have not been described in detail during this drug combination. We, therefore, performed a prospective multicenter study to compare MPA pharmacokinetics in renal graft recipients cotreated with sirolimus with that of patients cotreated with cyclosporine.

**METHODS**

**Patients.** A total of 36 white recipients of a first cadaveric kidney transplant from 4 transplant centers (Amiens, Clermont-Ferrand, Limoges, and Tours, France) were included in this pharmacokinetic study. They were taking part in a large prospective, randomized, multicenter French clinical trial enrolling 150 renal graft recipients. The aim of this study was to compare the safety and efficacy of sirolimus and cyclosporine during the first year after transplantation. The pharmacokinetic study was approved by the local ethics committee, and written consent was obtained from all patients. No power calculation was performed before the study. A total of 18 patients was recruited in both groups. One patient in the cyclosporine group and 3 patients in the sirolimus group were excluded because not all 4 pharmacokinetic studies were performed. Moreover, 2 patients in the sirolimus group dropped out of the study because their immunosuppressive regimen was changed during the first month after transplantation—1 because of cellular rejection and 1 because of the occurrence of a lymphocele. Thirteen patients cotreated with sirolimus and 17 patients cotreated with cyclosporine were, therefore, analyzed.

The patients received an induction therapy with antithymocyte globulin (Thymoglobulin; Imix-Sangstat, France) for 5 days together with MMF (CellCept; Roche, Neuilly-sur-Seine, France) and prednisolone. All patients were treated by a trimethoprim-sulfamethoxazole combination. MMF was given at an initial dose of 2 g/d, which was reduced only in the case of suspicion of MMF-related side effects (ie, neutropenia or abdominal pain) but not on the basis of MPA plasma concentrations.

Thirteen patients received sirolimus (Rapamune; Wyeth Europa) at a dose of 15 mg on day 1 and 10 mg on days 2 and 3 and then a dose adjusted to achieve trough blood concentrations between 10 and 20 ng/mL (liquid chromatography–mass spectrometry technique). These were the recommended target levels when the study was performed (2002-2003). Seventeen patients received cyclosporine (Neoral; Novartis Pharmaceuticals, Basel, Switzerland) starting on day 1 at a dose of 8 mg · kg\textsuperscript{-1} · d\textsuperscript{-1}, with adjustment on the following days to achieve trough blood concentrations between 200 and 300 ng/mL during month 1 and between 150 and 250 ng/mL during months 2 and 3 after transplantation (EMIT technique; Dade Behring, Deerfield, Ill). The morning dosing of cyclosporine or sirolimus was concomitant with that of MMF, with the drugs being given before breakfast.

**MPA pharmacokinetics.** MPA plasma concentrations were determined at week 2 and months 1, 2, and 3 after transplantation. A total of 10 blood samples were drawn before (C\textsubscript{0}) and at 20 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 3 hours, 4 hours, 6 hours, and 9 hours after MMF intake. Blood was collected in tubes containing edetic acid, and plasma was rapidly separated by centrifugation. The plasma concentrations of MPA were measured by use of the EMIT Mycophenolic Acid Test (Dade Behring).

The following MPA pharmacokinetic parameters were calculated: AUC\textsubscript{0-12} by use of the linear trapezoidal method and with the assumption that the concentration at 12 hours was equal to C\textsubscript{0}, AUC\textsubscript{0-12} normalized to a 1-g dose every 12 hours (observed MPA...
AUC$_{0-12}/$dose), maximal concentration ($C_{\text{max}}$), and time to reach $C_{\text{max}}$ ($t_{\text{max}}$).

**Statistical methods.** The quantitative data obtained in the 2 groups of patients were compared by use of a nonparametric Mann-Whitney $U$ test. The sex ratios in the 2 groups were compared by use of a chi-square test. Repeated-measures ANOVA was used to analyze the evolution of parameters with time. The relationships between 2 quantitative parameters were studied by linear regression. All of these statistics were considered to be significant at $P < .05$. Results are given as mean and SD unless otherwise stated.

**RESULTS**

As shown in Table I, baseline characteristics, renal function, and daily doses of MMF and prednisolone were not different between the 2 groups. Delayed graft function, defined by the need for at least 1 dialysis session during the first week after transplantation, occurred similarly in the 2 groups (3 of 13 patients in the sirolimus group and 4 of 17 patients in the cyclosporine group). No graft loss occurred in either of the study groups. Trough blood concentrations (mean $\pm$ SD) of sirolimus and cyclosporine were within the target ranges, as follows: 16.6 $\pm$ 7.3 ng/mL, 15.7 $\pm$ 6.6 ng/mL, 16.0 $\pm$ 4.5 ng/mL, and 14.0 $\pm$ 3.6 ng/mL for sirolimus and 255 $\pm$ 72 ng/mL, 226 $\pm$ 48 ng/mL, 197 $\pm$ 45 ng/mL, and 194 $\pm$ 36 ng/mL for cyclosporine at week 2 and months 1, 2, and 3 after transplantation, respectively. The number of patients who had a reduction of the MMF dose at 3 months was slightly higher in the group cotreated with sirolimus (6/13) than in the

**Table I.** Baseline characteristics, renal function, and doses of immunosuppressive agents in renal graft recipients

<table>
<thead>
<tr>
<th></th>
<th>Sirolimus group</th>
<th>Cyclosporine group</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>13</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>39 $\pm$ 12</td>
<td>45 $\pm$ 10</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>7/6</td>
<td>13/4</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.5 $\pm$ 12.0</td>
<td>70.2 $\pm$ 13.1</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>168 $\pm$ 9</td>
<td>169 $\pm$ 9</td>
<td>NS</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month 1</td>
<td>37.3 $\pm$ 4.6</td>
<td>39.8 $\pm$ 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Month 3</td>
<td>37.8 $\pm$ 4.2</td>
<td>40.5 $\pm$ 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine ($\mu$mol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>186 $\pm$ 85</td>
<td>145 $\pm$ 53</td>
<td>$P = .04$</td>
</tr>
<tr>
<td>Month 1</td>
<td>113 $\pm$ 37</td>
<td>131 $\pm$ 42</td>
<td>NS</td>
</tr>
<tr>
<td>Month 2</td>
<td>112 $\pm$ 36</td>
<td>131 $\pm$ 41</td>
<td>NS</td>
</tr>
<tr>
<td>Month 3</td>
<td>114 $\pm$ 34</td>
<td>124 $\pm$ 40</td>
<td>NS</td>
</tr>
<tr>
<td>Mycophenolate mofetil dose (g/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>1.90 $\pm$ 0.4</td>
<td>1.94 $\pm$ 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Month 1</td>
<td>1.80 $\pm$ 0.4</td>
<td>1.90 $\pm$ 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Month 2</td>
<td>1.66 $\pm$ 0.4</td>
<td>1.86 $\pm$ 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Prednisolone dose (mg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>31.2 $\pm$ 6.7</td>
<td>31.8 $\pm$ 6.5</td>
<td>NS</td>
</tr>
<tr>
<td>Month 1</td>
<td>19.2 $\pm$ 4.1</td>
<td>20.1 $\pm$ 5.9</td>
<td>NS</td>
</tr>
<tr>
<td>Month 2</td>
<td>10.7 $\pm$ 1.5</td>
<td>13.6 $\pm$ 4.6</td>
<td>NS</td>
</tr>
<tr>
<td>Month 3</td>
<td>9.0 $\pm$ 1.3</td>
<td>9.2 $\pm$ 1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sirolimus dose (mg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>9.1 $\pm$ 2.1</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Month 1</td>
<td>8.7 $\pm$ 2.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Month 2</td>
<td>7.3 $\pm$ 2.0</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Month 3</td>
<td>5.8 $\pm$ 1.7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cyclosporine dose (mg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 2</td>
<td>—</td>
<td>422 $\pm$ 70</td>
<td></td>
</tr>
<tr>
<td>Month 1</td>
<td>—</td>
<td>366 $\pm$ 98</td>
<td></td>
</tr>
<tr>
<td>Month 2</td>
<td>—</td>
<td>320 $\pm$ 80</td>
<td></td>
</tr>
<tr>
<td>Month 3</td>
<td>—</td>
<td>304 $\pm$ 76</td>
<td></td>
</tr>
</tbody>
</table>

Patients were treated with mycophenolate mofetil and prednisolone associated with either sirolimus or cyclosporine. NS, Not significant.
group cotreated with cyclosporine (3/17). All but 1 of these MMF dose reductions was performed because of leukopenia. In 1 patient the dose was reduced because of persistent abdominal pain.

MPA trough concentrations (C₀) were significantly higher in patients cotreated with sirolimus than in those cotreated with cyclosporine at the 4 time points (Table II). Of note, 2 patients treated with sirolimus showed MPA trough concentrations higher than 10 ng/mL at week 2. MPA maximum concentrations (Cₘₐₓ) did not differ significantly between patients taking cyclosporine and those taking sirolimus. There was a slight but nonsignificant increase in Cₘₐₓ with time from week 2 to month 3 in patients cotreated with cyclosporine (mean ± SD, 11.9 ± 4.9 mg/L to 14.9 ± 7.8 mg/L, P = .3), whereas it remained stable in patients cotreated with sirolimus (15.2 ± 5.0 mg/L to 14.9 ± 5.7 mg/L, P = .8). The time to reach Cₘₐₓ (tₘₐₓ) did not differ significantly between patients cotreated with sirolimus and those cotreated with cyclosporine, even though a trend toward shorter MPA tₘₐₓ was observed after 1 month with sirolimus as compared with cyclosporine (Table II).

![Graphs](null)

**Fig 1.** Mycophenolic acid (MPA) concentrations measured at week 2 and months 1, 2, and 3 after transplantation in patients cotreated with either sirolimus (solid circles) or cyclosporine (open circles). Results are displayed as means and SDs.

At the initial MMF dose of 1 g twice daily, which was maintained in all patients at least until week 2, MPA AUC₀₋₁₂ (mean ± SD) was significantly higher in those cotreated with sirolimus compared with those cotreated with cyclosporine, as follows: 81 ± 39 mg · h/L versus 43 ± 11 mg · h/L (P < .001) (Fig 1). At this time point, 4 patients cotreated with cyclosporine had an MPA AUC₀₋₁₂ lower than 35 mg · h/L whereas all patients cotreated with sirolimus had an MPA AUC₀₋₁₂ greater than 35 mg · h/L. At the same time point, none of the patients cotreated with cyclosporine had an MPA AUC₀₋₁₂ greater than 70 mg · h/L whereas 5 patients cotreated with sirolimus had an MPA AUC₀₋₁₂ greater than 70 mg · h/L (Fig 2). In the sirolimus group the dose of MMF had been reduced in 2, 3, and 6 patients at month 1, month 2, and month 3, respectively. The corresponding numbers were 1, 1, and 3 patients in the cyclosporine group. MPA AUC (mean ± SD) was still higher in sirolimus-cotreated patients at months 1 and 2, as follows: 72 ± 17 mg · h/L versus 48 ± 13 mg · h/L (P < .001) and 70 ± 25 mg · h/L versus 47 ± 17 mg · h/L (P < .01), respectively. A trend toward higher MPA AUC (mean ± SD) in sirolimus-cotreated patients than in cyclosporine-cotreated patients was still observed at month 3 (58 ± 27 mg · h/L versus 49 ± 20 mg · h/L, P = .06) despite...
a lower dose of MMF in the former group of patients (1.66 g/d versus 1.86 g/d, \( P = \) not significant).

Dose-normalized MPA AUC\(_{0-12}\) values were significantly higher in patients taking sirolimus than in those taking cyclosporine at all 4 time points (Table II). In the former patients, dose-normalized MPA AUC\(_{0-12}\) (mean \( \pm \) SD) remained unchanged over time (81 \( \pm \) 36 mg \( \cdot \) h/L \( \cdot \) g and 71 \( \pm \) 26 mg \( \cdot \) h/L \( \cdot \) g at week 2 and month 3, respectively; \( P = .8)\), whereas in the latter patients, it tended to increase with posttransplantation time, although not significantly, from 43 \( \pm \) 11 mg \( \cdot \) h/L \( \cdot \) g at week 2 to 56 \( \pm \) 31 mg \( \cdot \) h/L \( \cdot \) g at month 3 (\( P = .9)\).

When all time periods were analyzed together, a trend toward a better correlation was observed between \( C_0 \) and AUC\(_{0-12}\) of MPA in patients cotreated with sirolimus than in those cotreated with cyclosporine but the difference was not significant (\( r^2 = 0.631 \) versus \( r^2 = 0.462, P = .12\)) (Fig 3). Of note, all but 2 sirolimus-cotreated patients with an MPA \( C_0 \) greater than 2 ng/mL had an MPA AUC\(_{0-12}\) greater than 40 mg \( \cdot \) h/L and only 3 patients with an MPA \( C_0 \) lower than 5 ng/mL had an MPA AUC\(_{0-12}\) greater than 80 mg \( \cdot \) h/L.

At baseline, the white blood cell count (mean \( \pm \) SD) was not statistically different between patients cotreated with sirolimus and those treated with cyclosporine (7.8 \( \pm \) 1.7 \( \times \) 10\(^3\)/mL versus 6.6 \( \pm \) 1.7 \( \times \) 10\(^3\)/mL, \( P = \) not significant). At months 1 and 2, the white blood cell count (mean \( \pm \) SD) was significantly lower in patients cotreated with sirolimus than in those cotreated with cyclosporine, as follows: 4.8 \( \pm \) 1.1 \( \times \) 10\(^3\)/mL versus 6.5 \( \pm \) 2.2 \( \times \) 10\(^3\)/mL (\( P < .01\)) at month 1 and 4.6 \( \pm \) 1.1 \( \times \) 10\(^3\)/mL versus 5.9 \( \pm \) 2.0 \( \times \) 10\(^3\)/mL (\( P < .05\)) at month 2. At month 3, the white blood cell counts (mean \( \pm \) SD) were no longer different, as follows: 5.7 \( \pm \) 2.4 \( \times \) 10\(^3\)/mL versus 5.8 \( \pm \) 2.3 \( \times \) 10\(^3\)/mL for sirolimus and cyclosporine, respectively. Of note, there was no correlation between white blood cell count and MPA dose or MPA AUC. At 3 months after transplantation, hemoglobin tended to be lower in patients treated with sirolimus (11.0 \( \pm \) 1.5 g/mL) than in patients treated with cyclosporine (12.0 \( \pm \) 1.9 g/mL) but the difference was not statistically different. None of the patients in this study had high proteinuria (defined as >2 g/d).

**DISCUSSION**

MMF is an immunosuppressive drug that is widely used in the prevention of acute cellular rejection after solid organ transplantation. Initially, a fixed dose of 1 g twice daily was recommended in adult transplant recipients. More recently, guidelines regarding the analytic technique, the pharmacokinetic parameters to monitor, the target concentrations, and the indications for MPA monitoring were published. In this study we observed a significantly higher exposure to MPA in renal graft recipients cotreated with sirolimus than in patients cotreated with cyclosporine. Many patients cotreated with sirolimus had MPA AUC\(_{0-12}\) values above the target concentration range recommended in solid organ recipients cotreated with cyclosporine. High MPA AUCs have been associated with an increased risk of side effects, mostly leukopenia. One might speculate that some of the side effects observed in patients treated with sirolimus, particularly in the immediate posttransplant period, such as leukopenia, might be partly related to an overdosing of MPA.

We used the EMIT assay, which has been proposed for therapeutic drug monitoring of patients taking MMF. It quantifies not only MPA but also one of its metabolites, MPA–acyl glucuronide. The HPLC technique allows a more specific measurement of MPA. However, MPA–acyl glucuronide inhibits inosine

**Table II. Pharmacokinetic parameters of mycophenolic acid at 4 posttransplantation time points in patients cotreated with either sirolimus (n = 13) or cyclosporine (n = 17)**

<table>
<thead>
<tr>
<th></th>
<th>Week 2</th>
<th>Month 1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sirolimus</td>
<td>Cyclosporine</td>
<td>( P )</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td>group</td>
<td>value</td>
</tr>
<tr>
<td>( C_0 ) (mg/L)</td>
<td>5.2 (3.0)</td>
<td>1.3 (0.5)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>( C_{max} ) (mg/L)</td>
<td>15.2 (5.0)</td>
<td>11.9 (4.9)</td>
<td>NS</td>
</tr>
<tr>
<td>( t_{max} ) (min)</td>
<td>77 (59)</td>
<td>69 (61)</td>
<td>NS</td>
</tr>
<tr>
<td>AUC(_{0-12}) (mg ( \cdot ) h/L)</td>
<td>81 (36)</td>
<td>43 (11)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Dose-normalized AUC(_{0-12}) (mg ( \cdot ) h/L ( \cdot ) g)</td>
<td>81 (36)</td>
<td>43 (11)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Results are given as means and SDs.

\( C_0 \), Trough concentration; \( C_{max} \), maximal concentration; \( t_{max} \), time to maximal concentration; AUC\(_{0-12}\), measured area under curve.

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monophosphate dehydrogenase, as does MPA, and induces the release of cytokines from human mononuclear leukocytes. The EMIT test, therefore, has the advantage of measuring the total active concentration of the drug. In addition, the relationships between MPA concentrations and its effects were demonstrated in renal graft recipients by use of the EMIT technique, which can, therefore, be used for therapeutic drug monitoring of MPA. However, the meaning of MPA–acyl glucuronide in relation to clinical efficacy or side effects is still unclear.

Most studies describing MPA pharmacokinetics were performed in organ recipients cotreated with calcineurin inhibitors, mainly cyclosporine. This drug may inhibit the biliary secretion of MPA’s major metabolite, MPA glucuronide, thus leading to a decrease in the enterohepatic circulation of MPA. This interaction is thought to be the consequence of the inhibition of an adenosine triphosphate–binding cassette transporter, P-glycoprotein (product of the MDR1 gene), or multidrug resistance–associated protein 2 (mrp-2, product of MDR2). Lower MPA concentrations are indeed observed in patients cotreated with cyclosporine as compared with graft recipients treated with MMF alone. Pharmacokinetic studies in renal graft recipients cotreated with cyclosporine have also shown an increase in MPA AUC0-12 with time. Of note, this increase can be detected by measuring MPA AUC0-12 or Cmax, whereas MPA C0 values do not vary. We observed the same phenomenon in the cyclosporine-treated patients in our study, with an increase in MPA AUC0-12 with time associated with a persistently low C0. The increase in MPA AUC0-12 might be a result of the decrease in cyclosporine dose and, more precisely, in cyclosporine blood concentrations with time, as reported previously. Other factors, such as steroid dose or renal function, which have been shown to influence MPA pharmacokinetics, are probably less involved, because our patients who were cotreated with sirolimus had the same dose of steroids and comparable renal function.

The pharmacokinetic profiles of MPA in patients cotreated with sirolimus may be closer to the profiles observed in patients cotreated with tacrolimus, given that higher MPA concentrations and higher C0 values have been reported in association with tacrolimus than in association with cyclosporine. Indeed, the MMF dose of 0.5 g twice daily was reported to lead to MPA concentrations within the therapeutic range in renal graft recipients cotreated with tacrolimus.

For practical reasons, the determination of MPA AUC is difficult because multiple blood samples collected at precise time points are needed. Because a poor correlation between MPA C0 and MPA AUC was previously shown in cyclosporine-treated patients, limited sampling strategies were developed to estimate MPA AUC0-12 with the use of a few blood samples collected during the absorption phase. The current study shows that MPA C0 tends to be better correlated with MPA AUC in renal graft recipients cotreated with sirolimus than in patients cotreated with cyclosporine, although the difference was not significant. However, more studies are needed before C0 is considered as a reliable surrogate of MPA AUC in sirolimus-treated patients.

This study was not designed to explore the relationship between pharmacokinetic parameters and side effects possibly related to MPA blood concentrations. Nevertheless, we observed a significantly lower white blood cell count in the group of patients cotreated with sirolimus at months 1 and 2 after transplantation. Higher MPA AUC0-12 values in these patients may
Fig 2. Mycophenolic acid (MPA) area under concentration versus time curve over 12-hour period (AUC\textsubscript{0-12}) at week 2 and months 1, 2, and 3 after transplantation in kidney graft recipients treated with either sirolimus (SRL) or cyclosporine (CsA). Dotted lines represent the recommended therapeutic range for MPA AUC\textsubscript{0-12} measured by the enzyme-multiplied immunoassay technique (EMIT).

Fig 3. Correlation between MPA trough concentrations (C\textsubscript{0}) and MPA AUC\textsubscript{0-12} in renal graft recipients treated with either cyclosporine (open circles) or sirolimus (solid circles). Regression lines are shown as a dotted line for cyclosporine and a solid line for sirolimus.
favor leukopenia, but we could not demonstrate a linear relationship between MPA AUC and total white blood cell count. One can speculate that some of the side effects previously attributed to sirolimus might, in fact, be related to a higher exposure of these patients to MPA as compared with those cotreated with cyclosporine.

Overall, this is the first prospective study analyzing full pharmacokinetics of MPA in renal graft recipients cotreated with sirolimus in a calcineurin inhibitor–free protocol within the first 3 months after transplantation. Higher MPA trough concentrations were previously reported in sirolimus-treated patients compared with patients cotreated with cyclosporine. However, trough concentrations are not a good index of exposure to MPA. Our study confirms the higher concentrations of MPA in sirolimus-cotreated patients. Thus therapeutic drug monitoring of MPA should be performed in renal graft recipients cotreated with sirolimus because of the risk of overexposure to this immunosuppressive agent.

None of the authors has a conflict of interest.

References


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You will receive an e-mail to confirm that you have been added to the mailing list. Note that table of contents e-mails will be sent out when a new issue is posted to the Web site.
Effect of verapamil on pharmacokinetics and pharmacodynamics of risperidone: In vivo evidence of involvement of P-glycoprotein in risperidone disposition

Objective: A recent in vitro study has shown that risperidone is a substrate of P-glycoprotein. The aim of this study was to confirm the effects of verapamil, a P-glycoprotein inhibitor, on the pharmacokinetics of risperidone.

Methods: Two 6-day courses of either 240 mg verapamil daily, an inhibitor of P-glycoprotein, or placebo were administered in a randomized crossover fashion with at least a 4-week washout period. Twelve male volunteers took a single oral 1-mg dose of risperidone on day 6 of both courses. Plasma concentrations of risperidone, 9-hydroxyrisperidone, and prolactin were monitored up to 24 hours after dosing.

Results: Compared with placebo, verapamil treatment significantly increased the peak plasma concentration of risperidone by 1.8-fold and the area under the plasma concentration–time curve (AUC) from 0 to 24 hours of risperidone by 2.0-fold but did not alter the elimination half-life. The AUC from 0 to 24 hours of 9-hydroxyrisperidone, but not other pharmacokinetic parameters, was significantly increased during verapamil treatment. However, the AUC from 0 to 4 hours and the AUC from 0 to 8 hours of prolactin concentrations were not increased by verapamil treatment despite the pharmacokinetic alterations.

Conclusion: This study demonstrated that the bioavailability of risperidone was increased by verapamil, suggesting in vivo involvement of P-glycoprotein in the pharmacokinetics of risperidone. (Clin Pharmacol Ther 2005;78:43-51.)

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Recently, it has become increasingly evident that drug transporters have a pivotal role in the 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, influences the intestinal absorption,7,8 renal9,10 or hepatic elimination,11 and central nervous system concentrations6 of many drugs.

Risperidone is one of the representative atypical antipsychotic drugs and has potent antagonistic properties for serotonin 5-HT2 and dopamine D2 receptors.12,13 This drug is characterized by its effectiveness against both positive and negative symptoms in the treatment of schizophrenia.14 Furthermore, it produces fewer side effects, including extrapyramidal side effects, than conventional antipsychotic drugs.15 A recent in vitro study has examined the activity of P-glycoprotein toward 4 atypical and 2 conventional antipsychotics and a proven substrate, verapamil, by their P-glycoprotein adenosine triphosphatase activity, a putative measure of P-glycoprotein affinity.16 The rank order of the ratio of maximum velocity to Michaelis-Menten constant was as follows: verapamil (2.6) > quetiapine fumarate (INN, quetiapine) (1.7) > risperidone (1.4) > olanza-
pine (0.8) > chlorpromazine (0.7) > haloperidol (0.3). The atypical antipsychotics quetiapine fumarate and risperidone were relatively good P-glycoprotein substrates, although their affinities were not as high as that of verapamil. These results suggest that P-glycoprotein is likely to influence absorption in the small intestine or excretion in the liver or kidney of all atypical antipsychotics to various degrees. However, there are no in vivo data indicating that quetiapine fumarate or risperidone as a substrate of P-glycoprotein is of clinical relevance, although some in vivo data have shown a lack of impact of the multidrug resistance 1 (MDR1) genotype on steady-state plasma concentrations of risperidone and 9-hydroxyrisperidone.\(^{17}\)

Verapamil, a short-term inhibitor of mainly P-glycoprotein, has been used to increase the therapeutic effectiveness of cytotoxic anticancer drugs in cancer chemotherapy.\(^{18}\) 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.\(^{19}\)

The aim of this study was to confirm the effects of verapamil, a transporting inhibitor, on the disposition of risperidone and its active metabolite, 9-hydroxyrisperidone. Prolactin response to risperidone was also examined to clarify the effect of P-glycoprotein activity modulated by verapamil on the pharmacodynamics of risperidone.

**METHODS**

**Subjects**

Twelve healthy Japanese male volunteers were enrolled in this study. Their mean age (±SD) was 24.0 ± 2.0 years (range, 20–28 years), and their mean body weight was 64.8 ± 6.2 kg (range, 53–86 kg). The Ethics Committee of Hirosaki University School of Medicine, Hirosaki, Japan, approved the study protocol, and written informed consent was obtained from each participant before any examinations.

**Study design**

A randomized crossover study design was conducted at intervals of 4 weeks. Two 40-mg tablets of verapamil (Vasolan; Eisai Pharmaceutical, Tokyo, Japan) 3 times daily (at 8 AM, 1 PM, and 6 PM) or matched placebo with 240 mL of tap water was given for 6 days. The volunteers took a single oral 1-mg dose of risperidone at 9 AM on day 6 with 240 mL of tap water. Compliance with taking the test drug was confirmed by pill count. No other medications were taken during the study periods. No meal was allowed until 4 hours after dosing (at 1 AM). The use of alcohol, tea, coffee, and cola was forbidden during the test days.

**Sample collections**

Blood samples (5 mL each) for determination of risperidone and 9-hydroxyrisperidone and prolactin concentrations were taken into heparinized tubes just before and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours after the administration of risperidone. Plasma was separated immediately and kept at −30°C until analysis. At the same time of blood sampling, blood pressure, heart rate, and Udvalg for Kliniske Undersøgelser (UKU) side effects rating scales\(^{20}\) were monitored. The UKU consisted of 18 items, ie, psychic (concentration difficulties, asthenia, sleepiness, failed memory, and depression), extrapyramidal (dystonia, rigidity, hypokinesia, hyperkinesia, tremor, akinesia, and increased salivation), and autonomic (accommodation disturbances, reduced salivation, constipation, micturition disturbance, orthostatic dizziness, and palpitation) side effects, and was classified from 0 to 3 for each item.

**Assay**

Plasma concentrations of risperidone and 9-hydroxyrisperidone were measured via the liquid chromatography–tandem mass spectrometry method described by Yasui-Furukori et al.\(^{17}\) In brief, the extraction procedure was as follows: to 200 \(\mu\)L of plasma sample was added 200 \(\mu\)L of 0.1-mol/L phosphate buffer (pH 7), 50 \(\mu\)L of internal standard solution (R068808; Jansen Research Foundation, Beerse, Belgium), and 100 \(\mu\)L of methanol. Thereafter, 400 \(\mu\)L of 0.1-mol/L Borax (Sigma Coatings, Hasselt, Belgium) was added. The mixture was whirled in a vortex blender and poured over an Extrelut NT 1 (Merck, Boston, Mass) column, which was eluted with 7 mL of ethyl acetate. The eluate was evaporated under a nitrogen stream at 65°C and was redissolved in 100 \(\mu\)L of 0.1-mol/L ammonium acetate (50/50, pH 9.0), and 5 \(\mu\)L was injected onto the liquid chromatography–mass spectrometry–mass spectrometry system. The system consisted of API 3000 (Applied Biosystems, Foster City, Calif) and a column (Hypersil BDS C18, 100 × 4.6, 3 \(\mu\)m [Chemco Scientific, Brussels, Belgium]). The mobile phase was gradient ammonium acetate (0.01 mol/L, pH 9.0)–acetonitrile. Among the fragment ions of the compounds, the mass-to-charge ratio \((m/z)\) 207.0 for risperidone, \(m/z\) 191.0 for 9-hydroxyrisperidone, and \(m/z\) 201.0 for the internal standard were selected for ion monitoring. The lower limit of detection was 0.1 ng/mL for risperidone and 9-hydroxyrisperidone, and the values of...
the intra-assay and interassay coefficient of variation were less than 5% at all of the concentrations (0.1-100 ng/mL) of calibration curves for both compounds.

Plasma concentrations of prolactin were quantitated via enzyme immunoassay (IMX Prolactin Dainapack; Dainabot, Tokyo, Japan). The lower limit of detection was 0.6 ng/mL, and the values of the interassay coefficient of variation were 3.7%, 3.5%, and 3.5% at the concentrations of 8, 20, and 40 ng/mL for prolactin, respectively.

**Cytochrome P450 2D6 genotypes**

For the determination of cytochrome P450 (CYP) 2D6 genotype, deoxyribonucleic acid was isolated from peripheral leukocytes by a guanidium isothiocyanate method. The CYP2D6*1, CYP2D6*3, and CYP2D6*4 alleles were identified by allele-specific polymerase chain reaction (PCR) analysis according to Heim and Meyer. A long-PCR analysis was used to detect the CYP2D6*5 allele. The CYP2D6*10 allele was identified as the C188T mutation by use of 2-step PCR analysis as described by Johansson et al. The CYP2D6*14 allele was identified as the G1846T/A mutation by use of 2-step PCR analysis as described by Kubota et al. CYP2D6*2 does not result in decreased CYP2D6 activity. Therefore CYP2D6*2 was regarded as the wild-type (wt) allele, together with CYP2D6*1.

**Date analyses of pharmacokinetics**

The peak concentration (C max) and concentration peak time (t max) were obtained directly from the original data. The terminal elimination rate constant (k e) was determined by log-linear regression of the final data points (4). The apparent elimination half-life of the log-linear phase (t1/2) was calculated as follows: 0.693/ k e. The area under the plasma drug concentration–time curve (AUC) from 0 to 24 hours [AUC(0-24)] was calculated with use of the linear-linear trapezoidal rule. AUC from time 0 to infinity [AUC(0-∞)] or total AUC was calculated by AUC(0-24) + C last/ k e, where C last was the plasma drug concentration at the last detectable time point.

**Statistical analysis**

Data are shown as mean ± SD in tables and mean ± SE in figures. Paired t test was used for the comparison of the plasma drug concentrations and scores of clinical assessments between 2 phases (ie, placebo and verapamil). The comparison of t max was performed by use of the Wilcoxon signed-sample test. One-way ANOVA was used to compare CYP2D6 genotype effect on this interaction. A P value of .05 or less was regarded as significant. Geometric mean ratios to corresponding values in the placebo phase with 95% confidence intervals (CIs) were used for identification of significant differences. When the 95% CI did not cross 1.0, the result was also regarded as significant. SPSS 12.0J for Windows (SPSS Japan, Tokyo, Japan) was used for these statistical analyses.

**RESULTS**

The subjects had the following CYP2D6 genotypes: wt/wt in 3 subjects, *10/wt in 5, *10/*10 in 1, and *5/*10 in 3. No subjects regarded as poor metabolizers were included. The subjects were divided into 3 groups according to the number of mutated alleles: there were no mutated alleles in 2 subjects, 1 mutated allele in 6, and 2 mutated alleles in 4.

**Pharmacokinetics**

Plasma drug concentration–time curves during both placebo and verapamil treatments are shown in Fig 1, and their pharmacokinetic parameters are summarized in Table I. The C max of risperidone during verapamil treatment was higher than the corresponding value during the placebo phase by 1.76-fold (95% CI, 1.36- to 2.49-fold). The AUC(0-24) of risperidone during verapamil treatment was higher than during placebo by 1.97-fold (95% CI, 1.37- to 3.01-fold). The total AUC of risperidone during verapamil treatment was higher than during placebo by 1.82-fold (95% CI, 1.24- to 2.82-fold). No change was found in t max (0.88-fold [95% CI, 0.60- to 1.52-fold]) or elimination t1/2 (0.92-fold [95% CI, 0.78- to 1.19-fold]) of risperidone.

The geometric mean ratio of C max of 9-hydroxy-risperidone was 1.14 (95% CI, 0.99- to 1.36-fold). The geometric mean ratios of AUC(0-24) and total AUC of 9-hydroxyrisperidone were 1.46-fold (95% CI, 1.00- to 2.24-fold) and 1.50-fold (95% CI, 0.76- to 2.89-fold), respectively. No differences in t max (1.07-fold [95% CI, 0.78- to 1.68-fold]) or elimination t1/2 (1.15-fold [95% CI, 0.83- to 1.74-fold]) were found.

The C max values of active moiety (risperidone plus 9-hydroxyrisperidone) (1.34-fold [95% CI, 1.19- to 1.55-fold]) were increased during verapamil treatment. The AUC(0-24) (1.39-fold [95% CI, 1.28- to 1.52-fold]) and total AUC (1.38-fold [95% CI, 1.24- to 1.57-fold]) of active moiety during verapamil treatment were significantly higher than the corresponding value before the coadministration.

The AUC ratio of risperidone to 9-hydroxyrisperidone was not altered (1.21-fold [95% CI, 0.78- to 2.31-fold]). The mean magnitude (±SD) of the risperidone-verapamil interaction was 3.2 ± 2.5-fold in subjects with no mutated allele, 1.7 ± 0.3-fold in subjects with 1 mutated allele,
and 2.0 ± 1.3-fold in subjects with 2 mutated alleles (df = 2.9; F = 1.72; P = not significant).

**Pharmacodynamics**

UKU score–time curves and plasma concentration–time curves of prolactin and during both treatments are shown in Figs 2 and 3, and the pharmacokinetic parameters of prolactin are summarized in Table II. Although there were mild side effects (chest pain) in 1 subject, no clinically significant adverse events were reported during days 1 to 5. Mild to moderate psychic side effects (eg, concentration difficulty, latency, and sleepiness)
were observed from 2 to 12 hours after the risperidone administration in almost all subjects. There was no change in the peak UKU score (4.7 ± 2.3 versus 3.2 ± 1.6, \( P = \) not significant; 0.67-fold [95% CI, 0.52- to 1.15-fold]). The area under the UKU score–time curve during placebo was significantly higher than that during verapamil (29.7 ± 21.3 versus 14.4 ± 10.8, \( P < .05 \)). However, the geometric mean ratio of the area under the UKU score–time curve was 0.53 (95% CI, −0.29- to 3.05-fold).

The geometric mean ratio of \( C_{\text{max}} \) of prolactin between placebo and verapamil treatments was 1.08 (95% CI, 0.96- to 1.27-fold). Those of AUC(0-4), AUC(0-8), AUC(0-24), and incremental AUC of prolactin were 1.15-fold (95% CI, 1.01- to 1.26-fold), 1.12-fold (95% CI, 1.02- to 1.25-fold), 1.24-fold (95% CI, 1.12- to 1.39-fold), and 1.20-fold (95% CI, 0.73- to 2.23-fold), respectively.

**DISCUSSION**

The results of this study showed a significant increase in the plasma concentration of risperidone (\( C_{\text{max}} \) and AUC) during verapamil treatment. These findings imply that verapamil increases the bioavailability of risperidone or decreases the total clearance of risperidone. However, because verapamil did not alter the elimination \( t_{1/2} \) of risperidone in this study, it appears that only the bioavailability of risperidone was increased by verapamil, which might be attributed to increased absorption of risperidone in the small intestine or inhibition of extraction to bile in the liver. Our recent study showed that verapamil increased the bioavailability of fexofenadine,\(^{25} \) probably through P-glycoprotein inhibition. Because a recent jejunal single-pass perfusion study suggested that verapamil treatment did not alter the permeability of fexofenadine,\(^{26} \) it is more likely that the interaction between verapamil and risperidone occurs in the first-pass liver extraction process.

Our previous report showed a lack of effects of major polymorphisms of the \( MDR1 \) gene on steady-state plasma drug concentrations in 85 schizophrenic patients receiving 3 mg risperidone twice daily.\(^{17} \) When \( MDR1 \) variants alter the activity of P-glycoprotein in the small intestine, which limits oral bioavailability, the peak concentration of risperidone may differ between \( MDR1 \) genotypes. However, plasma concentrations of risperidone were monitored at 12 hours after dosing in the present study. Therefore any difference in the peak plasma concentration of risperidone caused by \( MDR1 \) variants was offset by large interindividual variability in risperidone metabolism during the elimination phase.

Verapamil is regarded as an inhibitor of CYP3A, as well as P-glycoprotein, on the basis of several in vitro and in vivo investigations.\(^{27-29} \) Fang et al\(^{30} \) demonstrated that CYP3A is a major enzyme catalyzing 9-hydroxylation of risperidone. On the basis of these facts, therefore, it is possible that significant interaction between these drugs.
CYP3A was 6.65 and 25.2 hydroxylation of risperidone catalyzed by CYP2D6 was generally relevant concentrations of risperidone. In fact, P-glycoprotein exists in the renal proximal tubule.34/P-gp may be mediated by other transporters. In substrate of P-glycoprotein or that renal excretion of the either that 9-hydroxyrisperidone is not such a specific treatment as much as risperidone. This finding suggests 9-hydroxyrisperidone were not influenced by verapamil be larger. However, the pharmacokinetic parameters of difference in 9-hydroxyrisperidone concentration would P-glycoprotein activity, and we assumed that the differ-ence in 9-hydroxyrisperidone depends on 9-hydroxyrisperidone cannot be excluded. Consequently, it is possible that the plasma concentration of 9-hydroxyrisperidone depends on P-glycoprotein activity, and we assumed that the differ-ence in 9-hydroxyrisperidone concentration would be larger. However, the pharmacokinetic parameters of 9-hydroxyrisperidone were not influenced by verapamil treatment as much as risperidone. This finding suggests either that 9-hydroxyrisperidone is not such a specific substrate of P-glycoprotein or that renal excretion of the compound may be mediated by other transporters. In addition, the formation of 9-hydroxyrisperidone is mainly catalyzed by CYP2D6, but plasma concentrations of 9-hydroxyrisperidone are not dependent CYP2D6 activity.35-38 Further study is required to clarify the disposition of 9-hydroxyrisperidone. Likewise, verapamil was associated with the active moeity concentration. This is a reasonable finding in light of the alteration of plasma concentrations of ris-peridone. Apart from steady state, the active moeity concentration is influenced by both plasma concentra-tions of risperidone and 9-hydroxyrisperidone because the plasma concentration of 9-hydroxyrisperidone was similar to that of risperidone after a single oral dose of risperidone.39 From this pharmacokinetic point of view, we concluded that P-glycoprotein modulation has potential clinical implications based on changes in peak plasma concentrations. The major physiologic role of P-glycoprotein is to serve as a barrier to entry and as an efflux mechanism for xenobiotics and cellular metabolites.40 Not only may P-glycoprotein limit intestinal drug absorption to constrain oral drug bioavailability, but the rate of P-glycoprotein efflux transport can also mediate brain penetration of lipophilic drugs.41,42 This is based on several kinetic studies showing large differences in brain concentration between the knockout animal [mrd1a (−/−) and mrd1a/1b (−/−) mice] and normal animal [mrd1a (+/+ and mrd1a/1b (−/+ mice).43 Therefore interindividual variability of P-glycoprotein function in the brain contributes to this variability of clinical response to neuropsychiatric agents. A few studies have suggested that inhibition of P-glycoprotein in the central nervous system affects pharmacodynamic alteration. Loperamide produced no respiratory depression when administered alone, but respiratory depression occurred when loperamide (16 mg) was given with quinidine at a dose of 600 mg.44 These changes were not explained by increased plasma loperamide concentrations. This study demonstrates the potentially important drug interactions that occur by inhibition of P-glycoprotein.
Several positron emission tomography studies suggested that risperidone disposition in plasma is not associated with that in the brain. Because the inhibition of P-glycoprotein activity during verapamil treatment probably leads to elevation of risperidone concentrations in the brain, we assumed that the prolactin concentration or prolactin concentration normalized by plasma concentration should be increased during verapamil treatment. In addition, several animal studies showing that the brain concentration of P-glycoprotein substrates increased after verapamil treatment support our hypothesis, although these studies used experimental doses of verapamil. Contrary to our expectation, the prolactin concentration was not affected during verapamil treatment. We do not have a clear explanation for this finding. A previous study demonstrated that verapamil infusion decreased the prolactin concentration in patients with hyperprolactinemia induced by sulpiride, which is a dopamine D2 antagonist, but increased the prolactin concentration in patients with hyperprolactinemia induced by prolactinoma. Further studies with respect to the pharmacodynamic effect of verapamil are required. The area under the UKU score–time curve during placebo was significantly higher than that during verapamil treatment, although the active moiety concentration was increased during verapamil treatment in this study. The possibility that this finding is ascribable to more sequence effects in the placebo-verapamil group than that in the verapamil-placebo group cannot be excluded entirely. In conclusion, this study showed that verapamil increased risperidone exposure, probably because of an increase in bioavailability through P-glycoprotein inhibition. Change in the regulation of transporters such as P-glycoprotein, though not simple, may lead to significant alteration of risperidone pharmacokinetics.

We thank Dr Ronald De Vries (Pharmacokinetics, Janssen Research Foundation, Beerse, Belgium) for her expertise in measuring the risperidone and 9-hydroxyrisperidone levels.

None of the authors or their institutes has conflicts of interest.

References


27. Zhao XJ, Ishizaki T. A further interaction study of quinine with clinically important drugs by human liver microsomes: determinations of inhibition constant (Ki) and type of inhibition. Eur J Drug Metab Pharmacokinet 1999;24:272-8.


Oral therapy with dipyridamole limits ischemia-reperfusion injury in humans

Background: Adenosine receptor stimulation induces several effects that could limit ischemia-reperfusion injury. We hypothesize that treatment with the nucleoside uptake inhibitor dipyridamole increases endogenous adenosine and limits ischemia-reperfusion injury in humans.

Methods: Ischemia-reperfusion injury was studied in forearm skeletal muscle by technetium Tc 99m–labeled annexin A5 scintigraphy. Ischemia-reperfusion injury was induced by unilateral forearm ischemic exercise. Immediately on reperfusion, annexin A5 labeled with technetium Tc 99m was administered intravenously, and ischemia-reperfusion injury was expressed as the percentage difference in radioactivity between the experimental arm and the control arm 1 and 4 hours after reperfusion. Targeting was quantified in the region of the thenar muscle and forearm flexor muscles. This approach was used in 9 healthy male volunteers after a 1-week treatment with dipyridamole (200 mg, slow release, twice daily) and in 23 control subjects.

Results: Dipyridamole treatment significantly reduced annexin A5 targeting in skeletal muscle compared with the control group (thenar region, 13% ± 7% versus 22% ± 15% at 1 hour after reperfusion and 9% ± 6% versus 27% ± 13% at 4 hours for dipyridamole and control groups, respectively [P = .01]; flexor region, 4% ± 8% versus 7% ± 6% at 1 hour after reperfusion and 1% ± 4% versus 10% ± 9% at 4 hours for dipyridamole and control groups, respectively [P = .01]).

Conclusions: One week of oral treatment with the nucleoside uptake inhibitor dipyridamole (200 mg, slow release, twice daily) significantly limits ischemia-reperfusion injury in humans in vivo, as assessed by technetium Tc 99m–labeled annexin A5 scintigraphy of forearm skeletal muscle. (Clin Pharmacol Ther 2005;78:52-9.)

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Despite state-of-the-art reperfusion therapy, the 30-day mortality rate of acute myocardial infarction is still approximately 7%. In addition, as a result of improved survival after acute myocardial infarction, the incidence and prevalence of chronic heart failure, which is often caused by ischemic death of cardiomyocytes, increase rapidly. Therefore the development of new strategies to reduce morbidity and mortality rates once infarction has occurred could provide substantial clinical benefit.

The most potent endogenous protective mechanism against ischemia-reperfusion injury of the myocardium, other than early reperfusion, is ischemic preconditioning. Initially described by Murry et al, this phenomenon describes the powerful infarct size-limiting effect of brief sublethal periods of ischemia and reperfusion preceding a sustained period of ischemia. The endogenous purine nucleoside adenosine, which is released during the preconditioning ischemia, has been shown to play a pivotal role in the protection afforded by preconditioning. Endogenous adenosine also has several additional effects other than inducing preconditioning, which ultimately protect against ischemia-reperfusion damage to the heart.
Methods

Subjects. After approval of the protocol by the Institutional Review Board of the Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands, 32 healthy male volunteers signed written informed consent statements before participation in the study. They had no history of cardiovascular disease or asthma and did not use any medication. In all participants a physical examination, electrocardiography, and laboratory investigation were performed to exclude cardiovascular and pulmonary disease, hypertension, and diabetes mellitus. Because caffeine is a potent adenosine receptor antagonist, all volunteers were asked to abstain from caffeine-containing beverages for at least 24 hours before each visit on days 1, 6, and 7 of the study.

Because of these beneficial effects, pharmacologic elevation of the concentration of endogenous adenosine is an attractive target to attenuate ischemia-reperfusion injury. The nucleoside uptake inhibitor dipyridamole effectively increases the plasma concentration of adenosine and may, therefore, serve this goal. Because myocardial ischemic events are seldom planned or accurately predicted, it is desirable to offer a sustained protection against ischemia and reperfusion in patients who are at risk for myocardial ischemia. In this study we, therefore, aimed to determine whether oral treatment with dipyridamole limits ischemia-reperfusion injury in humans in vivo. For this purpose, we used the recently developed and well-validated technique of technetium Tc 99m–labeled annexin A5 scintigraphy to detect ischemia-reperfusion injury in forearm skeletal muscle in healthy male volunteers.

This model is based on the specific and high-affinity binding of annexin A5 to phosphatidylserine residues. Loss of membrane asymmetry occurs shortly after an ischemic insult and results in phosphatidylserine exposure on the outer membrane leaflet, either as an early sign of apoptosis or as a reversible event, preceding commitment to apoptotic cell death. By labeling recombinant annexin A5, it is possible to visualize this loss of membrane asymmetry in vivo. Using the protocol described in this study, we previously showed that ischemic exercise of forearm muscle increases targeting of annexin A5 and that this can be prevented by ischemic preconditioning, as well as by the infusion of adenosine into the brachial artery.

Experimental protocol. All participants in the active treatment group (n = 9) were treated orally for 1 week with 200 mg slow-release dipyridamole twice daily in an open-label design (Pershant Retard, 200 mg; Boehringer-Ingelheim, Alkmaar, The Netherlands). On days 1, 6, and 7 of the study, they visited our department in the morning for hemodynamic monitoring, venous blood sampling, and supervised administration of the study medication. Heart rate and blood pressure were measured with an automated blood pressure device (Dinamap; Critikon, Tampa, Fla) on the dominant arm, with 4 measurements performed at 3-minute intervals, starting after 5 minutes of supine rest. On day 1, blood pressure and heart rate were measured before administration of the first dose and at 2 hours thereafter. On day 6, hemodynamic measurements were performed and blood was drawn for determination of the plasma dipyridamole concentration before administration of the morning dose. On day 7, heart rate and blood pressure were measured again, before and at 2 hours after administration of the morning dose of dipyridamole. Venous blood samples were taken on these 2 occasions for determination of the plasma concentration of dipyridamole and caffeine. On day 7, immediately after the final hemodynamic measurement (approximately 145 minutes after administration of the final dose of dipyridamole), the ischemic-exercise experiment was performed as previously described (see later). This active treatment group was compared with a control group of 23 healthy male volunteers who only performed the ischemic-exercise experiment without pretreatment. A part of this control group (n = 10) has been reported on previously in a recent study from our group.

Ischemic-exercise protocol. In brief, all volunteers were studied in a sitting position after cannulation of an antecubital vein of the dominant forearm. Maximal voluntary contraction was determined in the nondominant arm with a handgrip dynamometer (Baseline Hydraulic Hand Dynamometer; Fabrication Enterprise, Irvington, NY). Subsequently, the circulation of the nondominant arm was occluded for 10 minutes by inflation of an upper arm cuff to 200 mm Hg. Simultaneously, the subjects performed rhythmic isometric hand gripping at 50% of maximal voluntary contraction for 5 seconds every 10-second period until exhaustion. The total duration of ischemia was 10 minutes. Immediately on reperfusion, 0.1 mg of hydrazinonicotinamide-derivatized recombinant human annexin A5 (NAS2020; Theseus Imaging, Boston, Mass), radiolabeled with 450 MBq technetium Tc 99m was administered intravenously into the dominant arm. Both forearms and hands were imaged at 1 and 4 hours after...
injection by use of a gamma camera (Siemens Orbiter; Siemens, Hoffman Estates, Ill) (equipped with low-energy high-resolution collimators) connected to a Hermes Gold image processing system (Nuclear Diagnostics, Stockholm, Sweden) as previously described.\(^{8}\)

Radiolabeled annexin A5 was freshly prepared before each experiment as previously described.\(^{8}\)

**Ex vivo determination of nucleoside uptake inhibition.** In the last 3 participants of the active treatment group, blood was drawn in ethylenediaminetetraacetic acid–containing Vacutainer blood collection tubes (BD Vacutainer Systems, Plymouth, United Kingdom) on day 1, before administration of the first dipyridamole dose, and on day 7, immediately before the start of the experiment, approximately 145 minutes after administration of the final dose for ex vivo determination of transport inhibition of adenosine and uridine. Immediately after the venous sample was taken, the blood was centrifuged, and the erythrocytes were washed twice in normal saline solution and resuspended in MOPS-[3-(N-morpholino)propanesulfonic acid] buffer to obtain a 20% solution.

For uridine transport measurements, a 50-µL uridine solution was added to 100 µL of 10% erythrocytes in MOPS buffer to obtain final concentrations of 10, 30, 100, 200, 400, and 1000 µmol/L. After 3 seconds, uridine uptake was completely blocked by 100 µL of 25-µmol/L dipyridamole, and the erythrocytes were isolated by immediate centrifugation through a dibutylphthalate layer. After removal and washing of the upper layer, the erythrocytes were lysed with Triton X-100 (formerly registered trademark of Rohm and Haas, Philadelphia, Pa; now registered trademark of Union Caribe, Houston, Tex) (Sigma-Aldrich BV, Zwijndrecht, The Netherlands) and treated with perchloric acid for protein precipitation. After centrifugation, the uridine concentration in the supernatant was determined by HPLC.

For adenosine uptake determinations, adenosine (in a final concentration of 3 µmol/L) was added to 100 µL of 1% erythrocytes in Tris–sodium chloride buffer. After 0, 3, 6, 10, and 15 minutes, adenosine uptake and deamination were completely blocked by high-dose dipyridamole (10 µmol/L) and erythro-9-(2-hydroxynon-3-yl)-adenine (8 µmol/L), respectively. Subsequently, the cells were separated from the supernatant by centrifugation through a dibutylphthalate layer, and the adenosine concentration in the supernatant was determined as described later.

**Analytic procedures.** Plasma caffeine concentrations were determined by use of reversed-phase HPLC with ultraviolet detection set at 273 nm according to Schreiber-Deturmeny and Bruguerolle.\(^{11}\) Plasma dipyridamole concentrations were determined in deproteinized plasma by use of HPLC with fluorescence detection set at 286 nm/470 nm as previously described by Wolfram and Björnsson.\(^{12}\)

In consideration of the ex vivo nucleoside uptake experiments, uridine concentration was determined by HPLC with ultraviolet detection set at 254 nm by use of a Polaris C18 column (Varian BV, Middelburg, The Netherlands). For the mobile phase, 10-mmol/L tetrabutylammonium hydrogen sulfate in 0.1% acetic acid was used. Uridine uptake was expressed as nanomoles per minute per milligram protein in the membranous fraction, determined according to the well-documented assay of Lowry et al.\(^{12a}\) The adenosine concentration was determined with reversed-phase HPLC with ultraviolet detection set at 260 nm. Adenosine was separated by a linear gradient of 2% acetonitrile (in 10-mmol/L tetrabutylammonium hydrogen sulfate, 20-mmol/L ammonium dihydrogen phosphate, pH 6.0) to 35% acetonitrile in 15 minutes at 1 mL/min.

**Statistical analysis.** All data are presented as mean ± SD unless otherwise stated. Because not all baseline characteristics showed a Gaussian distribution, the Mann-Whitney U test was used to compare groups. Effects of dipyridamole on hemodynamic parameters were tested with a paired-sample 2-sided t test.

All of the digitized gamma camera images were analyzed offline by the same investigator (W.J.G.O.) by use of Hermes Gold software. Two predefined regions of interest were drawn in each forearm representing the flexor muscles and thenar muscles, respectively. Annexin A5 targeting was expressed as the percentage difference between the experimental (nondominant) arm and the control arm (“targeting”) as previously described.\(^{8}\) The differences between the groups and the interaction between group and time were analyzed with ANOVA for repeated measures (SPSS for Windows, release 10.0.7; SPSS, Chicago, Ill). In this model we corrected for workload, which was defined as the product of maximal voluntary force and duration of ischemic exercise (ANOVA for repeated measures with workload as covariate).

All ex vivo measurements were performed in duplicate and averaged for each subject. The ex vivo uridine uptake was plotted for each subject according to Michaelis-Menten kinetics. The adenosine uptake was plotted according to 1-phase exponential decay, and half-lives were calculated for each subject. The calculated maximum velocity (V\(_{\text{max}}\)) and half-lives were compared by use of paired t tests.
Table I. Baseline characteristics of study groups

<table>
<thead>
<tr>
<th></th>
<th>Control group*</th>
<th>Dipyridamole group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>23</td>
<td>9</td>
</tr>
<tr>
<td>Age (y)</td>
<td>23 ± 3</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181 ± 7</td>
<td>182 ± 7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75 ± 9</td>
<td>75 ± 11</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>23 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>131 ± 7</td>
<td>130 ± 8</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)†</td>
<td>74 ± 9</td>
<td>76 ± 6</td>
</tr>
<tr>
<td>Heart rate (beats/min)†</td>
<td>69 ± 10</td>
<td>77 ± 10‡</td>
</tr>
<tr>
<td>Random glucose (mmol/L)</td>
<td>4.9 ± 0.7</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>Workload (kg · s)</td>
<td>3528 ± 9958</td>
<td>9958 ± 1727</td>
</tr>
</tbody>
</table>

*Part of this control group has been reported on previously by our group.8
†Blood pressure was measured by auscultation while subjects were in the supine position by use of sphygmomanometry, and heart rate was measured by pulse frequency counting.
‡P = .05, versus control group.

RESULTS

Subjects. The demographic characteristics of both groups are summarized in Table I. Baseline heart rate was higher in the dipyridamole-treated group (P = .05). Other baseline parameters were not significantly different between groups (P > .1). The plasma caffeine concentration in the dipyridamole group was lower than 0.07 mg/L in 8 subjects but was 1.19 mg/L in 1 subject, indicating that the instructions regarding caffeine intake were not adequately followed by the latter subject. However, exclusion of this subject did not change the overall results. Trough plasma dipyridamole concentrations in the morning on days 6 and 7 were 0.79 ± 0.41 mg/L and 0.70 ± 0.46 mg/L, respectively, indicating steady state. The peak plasma concentration on day 7, immediately before the experiment, averaged 1.33 ± 0.33 mg/L. Both peak and trough levels are comparable to the plasma concentrations of dipyridamole mentioned in the large European Stroke Prevention Study 2 (ESPS-2).13

Hemodynamic parameters are depicted in Fig 1. The first dose of dipyridamole did not change systolic blood pressure but did significantly decrease diastolic blood pressure from 65 ± 3 mm Hg to 60 ± 6 mm Hg (P = .005) and increased heart rate from 66 ± 10 beats/min to 74 ± 8 beats/min (P = .003). This hemodynamic effect is consistent with earlier reports on hemodynamic changes in response to intravenously administered dipyridamole.14 On day 7, ingestion of the dipyridamole dose no longer induced any immediate hemodynamic effects. Moreover, heart rate and blood pressure immediately before the main experiment on day 7 did not differ from baseline values obtained on day 1, although the diastolic blood pressure tended to be lower on day 7 (62 ± 5 mm Hg versus 65 ± 3 mm Hg, P = .06).

Annexin A5 scintigraphy. Annexin targeting at 1 and 4 hours after reperfusion is illustrated in Fig 2. In the thenar region of the hand, the targeting of annexin A5 was significantly reduced in the dipyridamole group compared with the control group (P = .01 for between-group effect and P = .01 for interaction between group and time). Annexin A5 targeting was also reduced by dipyridamole in the flexor region (P = .04 for between-group effect and P = .01 for the interaction between group and time).

Ex vivo determination of nucleoside uptake inhibition. Uridine uptake into the erythrocytes via the dipyridamole-sensitive nucleoside transporter was significantly inhibited on day 7 compared with day 1 (before the first dose of dipyridamole): Vmax decreased from 328 ± 28 nmol · min⁻¹ · mg⁻¹ protein to 169 ± 28 nmol · min⁻¹ · mg⁻¹ protein (n = 3, P = .02) (Fig 3, A). In addition, the uptake of adenosine was significantly inhibited on day 7 compared with day 1 (half-life increased from 8.0 ± 0.7 minutes to 9.9 ± 0.8 minutes) (n = 3, P = .004) (Fig 3, B).

DISCUSSION

The main finding of this study is that a 1-week treatment with the nucleoside uptake inhibitor dipyrid-
amole, with a dose of 200 mg twice daily, effectively inhibits cellular adenosine uptake and potently limits ischemia-reperfusion injury of forearm skeletal muscle in humans in vivo. Extrapolating this finding, dipyridamole may well confer additional clinical benefit in patients who are at risk for myocardial ischemia.

**Fig 2.** Annexin targeting in thenar region of hand (A) and in flexor region of arm (B) at 1 and 4 hours after reperfusion (mean ± SE). Open bars represent dipyridamole group, and filled bars represent control group. Annexin targeting was reduced in the dipyridamole-treated group, in both flexor and thenar regions (P = .01 for interaction between group and time, ANOVA for repeated measures).

**Fig 3.** Ex vivo uridine (A) and adenosine (B) uptake of isolated erythrocytes (mean ± SE). Uridine uptake is depicted, expressed as nanomoles per minute per milligram protein (A). Maximum velocity (V_max) was lower after dipyridamole treatment (P = .02). B, Concentration of adenosine in the supernatant 3, 6, 10, and 15 minutes after the addition of adenosine to the erythrocyte suspensions. Erythrocytes were obtained on day 1, before administration of first dose of dipyridamole (open squares, n = 3), and on day 7, 2 hours after administration of last dose of dipyridamole (filled squares, n = 3). Half-life was increased after dipyridamole treatment (P = .004).
Originally introduced as an antianginal drug in 1959, dipyridamole has since been used especially as an antithrombotic drug, because of its inhibitory effect on platelet aggregation. Only in the last decade has dipyridamole been used in experimental settings to modulate ischemia-reperfusion injury. Results in animal studies have been equivocal. Although short-term pretreatment with dipyridamole protected against myocardial stunning in the rabbit heart and potentiated the infarct size–limiting effects of ischemic preconditioning in rabbit and canine hearts, short-term pretreatment with dipyridamole without preconditioning did not reduce infarct size in rabbit and canine hearts. In humans, several experimental studies suggested clinical beneficial effects of dipyridamole on ischemia-reperfusion injury, but the methods used in these studies all suffer from methodologic shortcomings. In patients with coronary artery disease it was shown that short-term intravenous administration of dipyridamole, as well as 4-day oral treatment with dipyridamole, increased tolerance to dipyridamole stress echocardiography. However, pretreatment with dipyridamole might well have influenced sensitivity to dipyridamole, thus affecting ischemic load and not resistance to ischemia. In patients with stable coronary artery disease, short-term intravenous pretreatment with dipyridamole, but not long-term oral treatment with dipyridamole, increased tolerance to exercise testing. Increased collateral circulation could have confounded these findings. Finally, increased collateral circulation might also have affected the finding that intracoronary administration of dipyridamole before percutaneous transluminal coronary angioplasty in patients with symptoms of coronary artery disease increased tolerance to balloon inflation.

With ischemic or pharmacologic preconditioning, the time frame of protection is limited. Because sustained pharmacologic protection is preferred in patients at risk for myocardial ischemia, several studies have explored whether the short-term protection afforded by pharmacologic preconditioning with exogenous or endogenous adenosine persists over time. Although the rabbit heart showed desensitization to the protective effect of a selective adenosine A1 receptor agonist, when administered as a 72-hour continuous infusion, protection was maintained after repeated intravenous bolus infusions at 48-hour intervals for 10 days. Moreover, long-term oral administration of dipyridamole for 2 to 6 weeks in guinea pigs provided sustained protection to stunning and creatine kinase release. In the current study we showed that oral therapy with dipyridamole for 1 week also provides sustained protection to ischemia-reperfusion in humans in vivo.

Although this study was an experimental study with surrogate end points, on the basis of our results, one would also expect to see benefits from dipyridamole therapy in clinical trials. Protection by dipyridamole in patients at risk for myocardial ischemia would lead to attenuation of infarct size and, subsequently, less progression to heart failure and a reduction in mortality rate. However, the vast majority of end point studies are characterized by disappointing results. There are several possible explanations for this discrepancy, although these are speculative. Increased tolerance to ischemia will not decrease the incidence of vascular events but rather will limit infarct size and, subsequently, the incidence of heart failure and death once infarction has occurred. However, most studies used the incidence of vascular events as the primary end point, and when vascular death was reported, studies were often underpowered for this end point. Moreover, when vascular death was indeed one of the end points, most studies were concentrated on patients with cerebrovascular disease and not coronary artery disease. Because timely reperfusion still remains indispensable for protection to occur and reperfusion in cerebral ischemic events occurs less often than in cardiac events, the protection afforded in these patients might be expected to be less than in patients who are prone to coronary events.

The observed effects of dipyridamole are most likely mediated by an increase in the endogenous adenosine concentration. Although dipyridamole has several other effects besides nucleoside uptake inhibition, previous reports showed that the protective effect of dipyridamole was completely prevented by the adenosine receptor antagonist theophylline, as were the hemodynamic effects of dipyridamole in humans. Only a few studies have directly shown that oral treatment with dipyridamole indeed increased the endogenous adenosine concentration, because measurement of adenosine is severely complicated by its extremely short half-life. In the current study we convincingly showed that dipyridamole, in the dose given, effectively inhibited uptake of both uridine and adenosine in erythrocytes. Because erythrocytes lack uridine kinases and phosphorylases, this nucleoside can be very useful to reliably obtain the transport characteristics of the equilibrative nucleoside transporter. On the contrary, adenosine is rapidly deaminated and rephosphorylated after uptake into the cell. In the normal situation, intracellular deamination of adenosine, rather than its transport into the cell, is the rate-limiting step in the overall catabolism of adenosine. Consequently, at least 90% occupancy of the transporter by a nucleoside
uptake inhibitor is required to inhibit adenosine breakdown ex vivo, which explains why, in our study, the disappearance of uridine is more potently inhibited than adenosine.\textsuperscript{34}

In this study the healthy volunteers were treated with extended-release preparations of dipyridamole in a dose of 200 mg twice daily, which is the recommended dose in The Netherlands for the secondary prevention of cerebrovascular events. The safety of this dose has been evaluated extensively in the ESPS-2 study.\textsuperscript{13} Short-term administration of dipyridamole increases heart rate and decreases diastolic blood pressure,\textsuperscript{14} as also observed after the first dose of dipyridamole in our study. A long-term increase in heart rate, however, is undesirable in patients at risk for cardiac ischemia. In our study the described hemodynamic effects of dipyridamole waned after 1 week. Moreover, on day 7 of the study, dipyridamole no longer induced any short-term effects on heart rate and blood pressure. This adaptation may have been induced by adenosine receptor down-regulation. However, any receptor down-regulation did not abolish the protection of dipyridamole against ischemia-reperfusion.

Several limitations of this study need to be addressed. First, the study was open-labeled and without randomization. However, both groups were similar with regard to baseline characteristics, except for an increased baseline heart rate in the dipyridamole group, and the experimental and analytic procedures were highly standardized, leaving little room for significant confounding. Second, ischemia-reperfusion injury was assessed in the forearm skeletal muscle and not directly in the myocardium. Although extrapolation of these findings to the heart must be done with great caution, the mechanisms of ischemic preconditioning in skeletal muscle and myocardium do share many common pathways.\textsuperscript{35-37} Moreover, with the use of this model, it is possible to circumvent potential confounding by collateral circulation. Finally, we studied young healthy volunteers, whereas older patients who are at increased risk for ischemia might be less amenable to pharmacologic preconditioning.\textsuperscript{38}

In conclusion, this study showed that oral treatment with the nucleoside uptake inhibitor dipyridamole, in a dose of 200 mg twice daily, potently protects against ischemia-reperfusion injury in forearm skeletal muscle of healthy volunteers, as detected with technetium Tc 99m–labeled annexin A5 scintigraphy.

We thank Dr N. Steinmetz and Theseus Imaging (Boston, Mass) for kindly donating the NAS 2020 kits for preparation of \textsuperscript{99m}Tc HYNIC-rh-annexin A5. We thank Dr S. Levison-Keating, Department of Hematology, Radboud University Nijmegen Medical Center, for correction of the English.

None of the authors has any competing financial interest with regard to this manuscript.

References

Dipyridamole limits ischemia-reperfusion injury

High-dose statins and skeletal muscle metabolism in humans: A randomized, controlled trial

**Background:** Myopathy, probably caused by 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibition in skeletal muscle, rarely occurs in patients taking statins. This study was designed to assess the effect of high-dose statin treatment on cholesterol and ubiquinone metabolism and mitochondrial function in human skeletal muscle.

**Methods:** Forty-eight patients with hypercholesterolemia (33 men and 15 women) were randomly assigned to receive 80 mg/d of simvastatin (n = 16), 40 mg/d of atorvastatin (n = 16), or placebo (n = 16) for 8 weeks. Plasma samples and muscle biopsy specimens were obtained at baseline and at the end of the follow-up.

**Results:** The ratio of plasma lathosterol to cholesterol, a marker of endogenous cholesterol synthesis, decreased significantly by 66% in both statin groups. Muscle campesterol concentrations increased from 21.1 ± 7.1 nmol/g to 41.2 ± 27.0 nmol/g in the simvastatin group and from 22.6 ± 8.6 nmol/g to 40.0 ± 18.7 nmol/g in the atorvastatin group (P < 0.005, repeated-measurements ANOVA). The muscle ubiquinone concentration was reduced significantly from 39.7 ± 13.6 nmol/g to 26.4 ± 7.9 nmol/g (P = 0.031, repeated-measurements ANOVA) in the simvastatin group, but no reduction was observed in the atorvastatin or placebo group. Respiratory chain enzyme activities were assessed in 6 patients taking simvastatin with markedly reduced muscle ubiquinone and in matched subjects selected from the atorvastatin (n = 6) and placebo (n = 6) groups. Respiratory chain enzyme and citrate synthase activities were reduced in the patients taking simvastatin.

**Conclusions:** High-dose statin treatment leads to changes in the skeletal muscle sterol metabolism. Furthermore, aggressive statin treatment may affect mitochondrial volume. (Clin Pharmacol Ther 2005;78:60-8.)

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CK or rhabdomyolysis, which occur rarely.\textsuperscript{11,12} The incidence of myopathy and rhabdomyolysis increases when statins are used together with other compounds, particularly those that inhibit their metabolism.\textsuperscript{11,13} The new aggressive goals of low-density lipoprotein (LDL) cholesterol reduction may increase the risk of side effects even when drugs affecting statin metabolism are not used concomitantly.\textsuperscript{14}

It is not known whether statins affect cholesterol metabolism in the extrahepatic tissues. We have previously shown that simvastatin at low doses (20 mg/d) does not decrease skeletal muscle ubiquinone in humans.\textsuperscript{15,16} Thus we designed this study to test whether high doses of simvastatin and atorvastatin are able to function in nonhepatic tissues. In this article we report the effects of high-dose statin treatment on skeletal muscle cholesterol and ubiquinone metabolism and respiratory chain enzyme activity. In addition to cholesterol, we analyzed lathosterol, a cholesterol precursor, and plant sterol (campesterol and sitosterol) concentrations. Lathosterol and the lathosterol-to-cholesterol ratio were used as markers for the endogenous cholesterol synthesis rate.\textsuperscript{17-19} Plasma plant sterol concentrations and their ratios to cholesterol were used to assess intestinal sterol absorption rates.\textsuperscript{19}

METHODS

Patients

Forty-eight subjects aged between 31 and 69 years (33 men and 15 women) were recruited from the University Hospital of Tampere and Primary Health Care Centres of neighboring municipalities of Tampere, Finland. The mean serum total cholesterol concentration was 5.9 ± 0.9 mmol/L, and the mean serum triglyceride concentration was below 4.5 mmol/L. These patients had never been treated with statins before. They were instructed to adhere to their normal diet during the study. Patients with familial hypercholesterolemia and those with a serum total cholesterol concentration greater than 7.0 mmol/L in the initial screening were excluded, as were women of childbearing potential. Other exclusion criteria were as follows: use of concurrent lipid-altering medication or antioxidant vitamins, renal or hepatic dysfunction, and use of medication known to affect the metabolism of atorvastatin or simvastatin. The study protocol was accepted by the Ethics Committee of the University Hospital of Tampere, and written informed consent was obtained from all participants.

Design

This was a randomized, double-blind, placebo-controlled trial with 3 treatment groups: placebo, 40 mg/d atorvastatin, and 80 mg/d simvastatin (Fig 1). A block-of-18 randomization scheme generated by the Pharmacy of University Hospital of Tampere was used. For every 18 subjects enrolled, 6 were assigned to the placebo group, 6 to the atorvastatin group, and 6 to the simvastatin group. Placebo was simvastatin-matched, and to also ensure blinding of atorvastatin, all study drugs were supplied in sealed, identical, numbered containers. All investigators and subjects were blinded until all analyses were done. The duration of the follow-up was 8 weeks. Muscle biopsy specimens were obtained at baseline and at the end of the treatment period. With the patient under local anesthesia, biopsy specimens were taken from the lateral portion of the quadriceps femoris muscle at about the midpoint be-
between the greater trochanter and the knee joint with a biopsy needle (Tru-Cut; Baxter, McGaw Park, Ill). The muscle specimens were frozen within 1 to 2 seconds in liquid nitrogen and stored at −80°C until analyzed. Blood samples for lipid, CK, and hepatic transaminase assessments were collected at baseline and at 1, 2, 4, and 8 weeks during the treatment. Serum samples were stored at −80°C and analyzed in random order blinded to treatment at the end of the study.

SERUM LIPIDS AND LIPOPROTEINS

Plasma triglyceride and total and high-density lipoprotein (HDL) cholesterol concentrations were analyzed colorimetrically by use of a Cobas Integra 700 automatic analyzer with reagents and calibrators as recommended by the manufacturer (Roche Diagnostics, Basel, Switzerland). The LDL concentration was calculated by use of the Friedewald formula.20 The interassay coefficients of variation were 1.4% for the assessment of total cholesterol, 1.0% for the assessment of triglycerides, and 3.7% for the assessment of HDL cholesterol.

Muscle and plasma sterols

Muscle specimens were thawed, rinsed 3 times in 500 μL of 0.9% sodium chloride solution, and centrifuged at 10,800 U/min for 2 minutes to remove residual blood. The tissues were then lyophilized and dried in a SpeedVac (Savant Instruments, Holbrook, NY). One microgram of [26,26,26,27,27,27-2H6] cholesterol (10 μL from a stock solution of d6-cholesterol in cyclohexane, 0.1 mg/mL) and 1 μg of epicoprostanol (Sigma) (10 μL from a stock solution of epicoprostanol in cyclohexane, 100 μg/mL) were added as internal standards. The residue was dissolved in 60 μL of n-decane. Fifty microliters of the solution was transferred into a microvial for GC-MS analysis of cholesterol precursors, metabolites, and plant sterols. Ten microliters was diluted with 90 μL of n-decane for analysis of cholesterol by gas-liquid chromatography–flame ionization detection (GC-FID). Solvolysis, extraction, and derivatization were performed as described.21,22

The concentrations of lathosterol, campesterol, and sitosterol were calculated from standard curves by use of epicoprostanol as internal standard. The identity of all sterols was proved by comparison with the full scan mass spectra of authentic compounds. Additional qualifiers (characteristic fragment ions) were used for structural identification.

Serum and muscle ubiquinone

The ubiquinone assays were done as described previously by a specific HPLC method.23 The lowest level of detection for plasma samples was 0.1 mg/L, and the coefficients of variation were 5% and 8% for within- and day-to-day variations, respectively, for all measured plasma levels. The muscle biopsy specimens were homogenized (Ultra-Turrax; Janke and Kunkel, Staufen, Germany) and centrifuged, and an aliquot of the supernatant underwent chromatography. The homogenization time (3 × 20 seconds) was selected to obtain the maximal recovery of ubiquinone from the muscle tissue. Coenzyme Q10 exists in oxidized (ubiquinone) and in reduced (ubiquinol) form in circulation and within human tissue. This redox equilibrium may be altered while processing the samples; therefore all serum and muscle tissue samples were oxidized before analysis, and the results are expressed as ubiquinone.

Respiratory chain enzyme activities

Sample preparation was performed as described previously.24 In brief, the muscle specimens (5-15 mg) were kept at −80°C until studied. Twenty volumes of buffer (10-mmol/L Tris–hydrochloric acid, 0.25-mol/L sucrose, 2-mmol/L ethylenediaminetetraacetic acid, and 50 U/mL heparin [pH 7.4]), with a minimum final volume of 200 μL, were added to 1 volume of muscle
sample for homogenization in a glass/glass pestle. The homogenate was then centrifuged at 5600g for 1 minute. The supernatant was kept on ice. Before the spectrophotometric measurements, the supernatant was sonicated with a "point-sonification" device for 2 seconds.

For the analysis of complex II, complex II + III, complex IV, and citrate synthase and for the measurement of the protein content, a minimum volume of 200 μL of homogenate was needed. Protein concentrations were in the range of 1.2 to 4.9 mg/mL.

Spectrophotometric assays were used to measure complex II (succinate coenzyme Q reductase, malonate-sensitive), complex II + III (succinate cytochrome c reductase), complex III (ubiquinol–cytochrome c reductase), complex IV (cytochrome c oxidase), and citrate synthase, as described previously. Evaluation of complex I was not possible because of the small size of the samples.

Statistical analyses

The data were analyzed with SPSS software (SPSS, Chicago, Ill). The normality of the distribution and the homogeneity of the variance were evaluated by the Kolmogorov-Smirnov test before further analyses. Overall differences in the responses of reported parameters over time between the treatment groups were compared with ANOVA for repeated measurements (RANOVA). Within the study groups, the paired t test was used to test the significance of differences over time (baseline versus after 8 weeks of treatment). Continuous and normally distributed parameters (ie, age and body mass index) were compared between study groups by the t test for independent samples. In addition, for some variables of interest, Pearson correlation coefficients were calculated. Data are presented as mean ± SD unless otherwise stated. Percentage differences between baseline and end point are given as the absolute differences of the mean values. A P value of less than .05 was considered statistically significant. The power of the study was designed to be greater than .90 with the current number of subjects, with a probability for type I error (α) of .05 to detect a difference in plasma cholesterol metabolite and ubiquinone levels between the control group and the simvastatin or atorvastatin group.

RESULTS

Forty-four subjects were included in the final analysis. Three subjects withdrew from the study because of personal reasons. One dropout patient in the simvastatin group reported unspecific symptoms. No other adverse events, including muscle pain, were reported during the study. However, a 3-fold increase in hepatic transaminase levels was recorded in 1 patient receiving simvastatin. CK and hepatic transaminase levels remained within reference limits in all other patients during the follow-up.

After 8 weeks, simvastatin (80 mg/d) and atorvastatin (40 mg/d) treatment resulted in a significant lowering of LDL cholesterol by about 50% and of total cholesterol by 40%. A similar reduction of about 30% was also recorded in both treatment groups regarding plasma triglyceride concentrations. HDL cholesterol levels increased slightly in both groups (Table I).

A significant decrease of 76% and 78% (P < .001 between groups over time, RANOVA) was measured in plasma lathosterol concentrations in the simvastatin and atorvastatin treatment groups, respectively. The lathosterol-to-cholesterol ratio, an indicator of cholesterol synthesis, decreased significantly by about 66% (P < .001 between groups over time, RANOVA) during both statin treatments. In addition, atorvastatin treatment caused an elevation in plasma campesterol (31%; P = .056 between groups over time, RANOVA) and sitosterol (41%; P = .019 between groups over time, RANOVA) concentrations; in the simvastatin group, circulating plant sterol levels remained unchanged (Table I). However, the campesterol-to-cholesterol ratio, an indicator of cholesterol absorption, increased significantly in both statin-treated groups. An increase of 107% (P < .001) was recorded in the atorvastatin group, and an increase of 52% (P = .001) was observed in the simvastatin-treated subjects.

At baseline, a significant association was observed between plasma ubiquinone and age (r = −0.32, P = .035) and between plasma ubiquinone and serum total cholesterol concentration (r = 0.49, P = .003). Both simvastatin and atorvastatin resulted in a significant decrease of 36% and 39% (P < .001 between groups over time, RANOVA) in the plasma levels of ubiquinone, respectively (Fig 2). However, the difference between groups was not significant when adjusted for the change in serum total cholesterol concentration.

In muscle an increase of 38% was observed for cholesterol concentrations in the simvastatin group (P = .055 between groups over time, RANOVA). Muscle cholesterol levels remained unchanged in the atorvastatin and placebo groups. On the other hand, muscle lathosterol levels decreased by 13% (P = .308) and 25% (P = .004) in the simvastatin and atorvastatin groups, respectively. The ratio of lathosterol to cholesterol in muscle decreased by 35% (P = .002) during simvastatin treatment and by 28% (P = .001) during
Table I. Plasma lipid and sterol concentrations at baseline and end of follow-up

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>Lathosterol (μmol/L)</th>
<th>Campesterol (μmol/L)</th>
<th>Sitosterol (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Placebo group</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>5.90 ± 0.91</td>
<td>1.41 ± 0.43</td>
<td>1.79 ± 0.89</td>
<td>3.68 ± 0.84</td>
<td>7.37 ± 3.20</td>
<td>12.81 ± 9.01</td>
<td>5.84 ± 3.48</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>6.10 ± 1.03</td>
<td>1.40 ± 0.41</td>
<td>1.76 ± 1.07</td>
<td>3.90 ± 0.87</td>
<td>7.59 ± 3.42</td>
<td>12.73 ± 8.47</td>
<td>5.82 ± 3.18</td>
</tr>
<tr>
<td><strong>Simvastatin group</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>5.75 ± 1.00</td>
<td>1.26 ± 0.36</td>
<td>1.86 ± 0.87</td>
<td>3.65 ± 0.85</td>
<td>6.93 ± 2.57</td>
<td>12.40 ± 6.39</td>
<td>5.95 ± 3.12</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>3.68 ± 0.68‡</td>
<td>1.27 ± 0.30</td>
<td>1.33 ± 0.53*</td>
<td>1.68 ± 0.68‡</td>
<td>1.64 ± 0.98‡</td>
<td>12.36 ± 5.67</td>
<td>5.78 ± 2.70</td>
</tr>
<tr>
<td><strong>Atorvastatin group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>5.80 ± 0.85</td>
<td>1.27 ± 0.37</td>
<td>1.94 ± 0.94</td>
<td>3.65 ± 0.64</td>
<td>7.15 ± 2.53</td>
<td>12.16 ± 5.70</td>
<td>5.56 ± 2.43</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>3.77 ± 0.73‡</td>
<td>1.32 ± 0.36</td>
<td>1.25 ± 0.58‡</td>
<td>1.88 ± 0.46‡</td>
<td>1.59 ± 1.11‡</td>
<td>15.93 ± 7.32†</td>
<td>7.84 ± 3.76†</td>
</tr>
</tbody>
</table>

\(P < .001\) for baseline versus treatment comparison within group (paired t test), \(P < .05\), for baseline versus treatment comparison within group (paired t test). The association was not significant with other complex enzyme activities.

atorvastatin treatment. These changes were not significant in the RANOVA. Treatment with simvastatin and atorvastatin resulted in a significant enrichment in skeletal muscle of campesterol (95% and 78%, respectively) and sitosterol (55% and 43%, respectively) \((P = .004\) between groups over time for both changes, RANOVA) \((Table II)\).

Mean muscle ubiquinone concentrations in men \((47 ± 15 \text{ nmol/g})\) were significantly higher \((P = .005)\) than in women \((34 ± 12 \text{ nmol/g})\). At baseline, the concentration range for muscle ubiquinone was 12 to 73 nmol/g in all subjects. No association was observed between serum and muscle ubiquinone concentrations. At the end of the follow-up, the mean muscle ubiquinone levels were unchanged in the placebo and atorvastatin groups compared with the baseline values. However, simvastatin resulted in a significant reduction of muscle ubiquinone concentrations by 30% \((P = .031\) between groups over time, RANOVA adjusted for gender). However, skeletal muscle ubiquinone levels in simvastatin-treated subjects remained within the low normal range as defined at baseline \((Fig 2)\). Exceptionally low muscle ubiquinone concentrations were not observed.

Respiratory chain enzyme activities were measured in 6 subjects taking simvastatin with markedly reduced muscle ubiquinone concentrations \(\text{from} \sim 24\% \text{ to} \sim 74\%)\) during statin treatment. Assays were also performed in 12 age- and gender-matched subjects selected from the placebo \((n = 6)\) and atorvastatin \((n = 6)\) groups. Complex II, complex II + III, complex III, and complex IV activity, as well as citrate synthase activity, were reduced in patients taking simvastatin \((Table III)\). However, the ratio between complex activities and citrate synthase activity remained unchanged during statin treatment. Baseline respiratory chain enzyme activities tended to be lower in these individuals selected based on a highly significant change in muscle ubiquinone level during simvastatin treatment than in the matched subjects in the other groups. The muscle ubiquinone level was significantly associated with complex III activity \((r = 0.55, P = .019)\) at baseline. The association was not significant with other complex activities. However, there was no association between plasma ubiquinone level and respiratory chain enzyme activities.

**DISCUSSION**

The results of this study prove that high clinically approved doses of simvastatin and atorvastatin have an effect on skeletal muscle in humans. We observed increased levels of plant sterols in both treatment groups. Decreased levels of lathosterol, a cholesterol precursor used in this study as an indicator of cholesterol synthesis, were recorded in the atorvastatin group. In addition, 80 mg/d simvastatin increased total cholesterol concentrations and decreased ubiquinone concentrations in muscle tissues. Furthermore, decreased respiratory chain enzyme and citrate synthase activity was recorded in 6 patients with a substantial reduction in muscle ubiquinone level during simvastatin treatment.
In theory, inhibition of HMG-CoA reductase in muscle cells could lead to reduced synthesis of cholesterol in the cells and a compensatory up-regulation of lipoprotein receptors and increased receptor-mediated uptake of cholesterol and plant sterols, which are also transported within serum lipoproteins. In addition, an increased uptake of lathosterol by the same mechanism cannot be ruled out. This could explain the increased muscle cholesterol levels in simvastatin-treated subjects. Because campesterol and sitosterol are entirely of dietary origin, we suggest that their concentrations were increased in muscle at least partially as a result of enhanced uptake via lipoprotein receptors. Miettinen and Gylling\textsuperscript{31} have previously proposed that a reduction in circulating plasma cholesterol levels in humans could cause an up-regulation of cholesterol absorption from the intestine. In line with those earlier results, a significantly increased sterol absorption rate (assessed as plasma campesterol/cholesterol ratio) was observed in this study during both statin treatments. Thus it is possible that the lipoprotein load of the plant sterols increased accordingly, which could have further augmented tissue accumulation of campesterol and sitosterol.

Intriguingly, Phillips et al\textsuperscript{32} have previously reported that muscle biopsy specimens obtained from patients with muscle symptoms that developed during statin therapy revealed extensive lipid-filled vacuoles and lipid droplet accumulation. Taken together, our results and those of Phillips et al indicate that levels of sterols and lipids are increased in muscles during statin treatment. Physiologic concentrations of plant sterols, especially sitosterol, have been shown in experimental studies to decrease cellular growth, de novo synthesis of cholesterol, and synthesis of deoxyribonucleic acid, as well as to stimulate apoptosis.\textsuperscript{33-37} Thus increased levels of cellular plant sterols may increase toxic effects of statins. This could be an additional mechanism for statin-induced muscle damage, similar to observed changes in erythrocyte membrane fluidity and rigidity in phytosterolemic patients.\textsuperscript{38} Interestingly, it has previously been reported that free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway.\textsuperscript{39,40}

Simvastatin treatment significantly reduced mean skeletal muscle ubiquinone levels. There was no reduction in the concentration of muscle ubiquinone in the atorvastatin group, despite a similar reduction in serum cholesterol and ubiquinone levels. Thus a reduction in plasma ubiquinone seems to reflect mainly decreased concentrations of LDL particles, which transport ubiquinone in circulation.\textsuperscript{23} These results indicate that serum and muscle ubiquinone levels are differently regulated and that serum ubiquinone levels may not be used as a marker for tissue ubiquinone levels. Furthermore, plasma ubiquinone concentration did not correlate with the respiratory chain enzyme activities. Thus it is not a useful biomarker when evaluating the effects of statins on tissue energy metabolism.

Importantly, respiratory chain enzyme activities decreased substantially in patients selected for analyses based on a significant reduction in muscle ubiquinone during statin treatment. The mitochondrial function assays revealed reductions in all measured mitochondrial
in mitochondrial number or volume could also explain, none and mitochondrial enzyme activity. The reduction actually explains the decrease in both muscle ubiquinone and citrate synthase activity. In other words, mitochondrial effects were related to treatment and not analyzing the whole study population for mitochondrial function was the limited capacity of performing these studies. Thus these results on mitochondrial function are limited to 12 patients (6 receiving 80 mg/d simvastatin and 6 receiving 40 mg/d atorvastatin), and therefore these results should be considered as preliminary findings. Interestingly, mitochondrial effects were related to treatment with 80 mg/d simvastatin. Whether these observed differences in muscle effects relate to the physicochemical

**Table II.** Skeletal muscle sterol concentrations at baseline and end of follow-up

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol (µmol/g)</th>
<th>Lathosterol (nmol/g)</th>
<th>Campesterol (nmol/g)</th>
<th>Sitosterol (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo group</td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>12.33 ± 3.47</td>
<td>20.17 ± 6.10</td>
<td>25.22 ± 21.73</td>
<td>17.35 ± 10.14</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>12.80 ± 2.90</td>
<td>19.48 ± 5.60</td>
<td>24.31 ± 12.09</td>
<td>13.60 ± 4.13</td>
</tr>
<tr>
<td>Simvastatin group</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>11.59 ± 1.98</td>
<td>16.50 ± 4.27</td>
<td>21.09 ± 7.10</td>
<td>15.02 ± 2.78</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>16.06 ± 5.72*</td>
<td>14.40 ± 5.27</td>
<td>41.22 ± 26.98†</td>
<td>23.23 ± 12.06*</td>
</tr>
<tr>
<td>Atorvastatin group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>11.52 ± 2.68</td>
<td>16.09 ± 4.37</td>
<td>22.58 ± 8.58</td>
<td>15.82 ± 6.11</td>
</tr>
<tr>
<td>End of follow-up</td>
<td>12.47 ± 2.97</td>
<td>12.04 ± 3.91†</td>
<td>40.03 ± 18.68†</td>
<td>22.67 ± 11.25*</td>
</tr>
</tbody>
</table>

*P < .05, for baseline versus treatment comparison within group (paired t test).
†P < .01, for baseline versus treatment comparison within group (paired t test).

Results are given as mean ± SD. P values are given for group-time interaction (repeated-measurements ANOVA).

**Table III.** Respiratory chain enzyme activity in muscle of 6 patients taking statins with reduction of 45% to 74% in skeletal muscle ubiquinone level during intervention and of 6 matched control subjects receiving placebo

<table>
<thead>
<tr>
<th></th>
<th>Simvastatin patients (n = 6)</th>
<th>Atorvastatin patients (n = 6)</th>
<th>Control subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmol · min⁻¹ · mg protein⁻¹)</td>
<td>(nmol · min⁻¹ · mg protein⁻¹)</td>
<td>(nmol · min⁻¹ · mg protein⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>End of follow-up</td>
<td>Baseline</td>
</tr>
<tr>
<td>Complex II</td>
<td>32.5 ± 15.2</td>
<td>17.7 ± 11.3*</td>
<td>47.3 ± 26.3</td>
</tr>
<tr>
<td>Complex II + III</td>
<td>30.2 ± 13.1</td>
<td>18.7 ± 13.0*</td>
<td>37.0 ± 16.9</td>
</tr>
<tr>
<td>Complex III</td>
<td>78.5 ± 40.0</td>
<td>40.3 ± 18.9*</td>
<td>76.0 ± 19.4</td>
</tr>
<tr>
<td>Complex IV</td>
<td>143.3 ± 41.3</td>
<td>91.3 ± 45.1*</td>
<td>201.7 ± 138.7</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>177.7 ± 54.7</td>
<td>97.8 ± 47.9*</td>
<td>221.0 ± 93.3</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>4/2</td>
<td>4/2</td>
<td>4/2</td>
</tr>
<tr>
<td>Age (y)</td>
<td>56.7 ± 9.8</td>
<td>54.9 ± 7.3</td>
<td>57.4 ± 7.8</td>
</tr>
</tbody>
</table>

Results are given as mean ± SD.
*P < .05, for baseline versus treatment comparison within group (paired t test).

for example, statin-induced muscle weakness without CK elevations and could initiate the development of myopathy symptoms.

The rationale of selecting these patients with lowered muscle ubiquinone levels and not analyzing the whole study population for mitochondrial function was the limited capacity of performing these studies. Thus these results on mitochondrial function are limited to 12 patients (6 receiving 80 mg/d simvastatin and 6 receiving 40 mg/d atorvastatin), and therefore these results should be considered as preliminary findings. Interestingly, mitochondrial effects were related to treatment with 80 mg/d simvastatin. Whether these observed differences in muscle effects relate to the physicochemical
properties of simvastatin and atorvastatin or whether, for instance, the baseline mitochondrial content or activity is significantly affecting the effect of statins on muscle mitochondria must await confirmation in future studies.

In conclusion, this study proves that high-dose treatment with atorvastatin or simvastatin has significant effects on skeletal muscle sterol metabolism even in patients with no concomitant drug treatment affecting pharmacokinetics of statins. It is of importance to determine next the possible link between statins and mitochondrial biogenesis.

We are indebted to the personnel of the outpatient clinic and laboratory of the University Hospital of Tampere and Department of Clinical Pharmacology, University of Helsinki, for their valuable help during the study. We also thank all of the volunteers who participated in this study.

Dr von Bergmann has offered consultancy advice to Merck Sharpe & Dohme and Schering-Plough (in Germany, Essex Pharma). He has also given lectures at symposia organized by these companies. The other authors have no conflicts of interest to declare.

References

7. Olsson AG. Statin therapy and reductions in low-density lipoprotein cholesterol: initial clinical data on the potent new statin rosuvastatin. Am J Cardiol 2001;87:33B-6B.


Pharmacokinetic-pharmacodynamic comparison of a novel multiligand somatostatin analog, SOM230, with octreotide in patients with acromegaly

Objective: Acromegaly is a serious hormonal disorder resulting from a pituitary adenoma causing excess growth hormone (GH) production. Somatostatin analogs such as octreotide have been the medical treatment of choice. SOM230, a novel somatostatin analog, was compared with octreotide with respect to pharmacokinetic (PK) profiles and inhibition of GH secretion in acromegalic patients.

Methods: In a double-blind, 3-period, crossover, proof-of-concept study, 12 patients with active acromegaly were randomized to single subcutaneous doses of SOM230 (100 and 250 μg) and octreotide (100 μg). Concentrations of SOM230, octreotide, and GH were determined at designated times after dosing and at baseline. The PK properties of SOM230 and octreotide and the relationship of PK with GH were investigated by a nonlinear mixed-effects modeling analysis.

Results: The apparent clearance for SOM230 is approximately half of that for octreotide (8.0 L/h versus 15.8 L/h). The elimination half-life for SOM230 is about 5 times longer than that for octreotide (11.8 hours versus 2.3 hours). The relationship between GH levels and plasma concentrations of SOM230 and octreotide is well described by a direct inhibitory model. The test drug concentration level at which half of the maximum drug effect is observed (EC50) is 46 and 553 pg/mL for octreotide and SOM230, respectively, with large interpatient variability (coefficients of variation, 164% and 65%, respectively), mainly attributable to the heterogeneous responses among patients.

Conclusions: SOM230 demonstrates a lower clearance and longer half-life than octreotide, which compensates for the lower potency in GH inhibition. As a result of the lower interpatient variability for EC50, SOM230 is expected to have a more uniform clinical GH inhibition than octreotide for acromegalic patients at a clinically effective dosing regimen. (Clin Pharmacol Ther 2005;78:69-80.)

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Tokyo, Japan, East Hanover, NJ, Basel, Switzerland, and Rotterdam, The Netherlands

Acromegaly is a rare, serious disease characterized by chronic hypersecretion of growth hormone (GH), also called hypsomatotropism, which in the majority of patients is caused by a pituitary adenoma of the somatotrophs. Clinical features of acromegaly comprise changes in practically all body organs, both structural and functional, such as soft-tissue hypertrophy, exaggerated growth of limbs, and coarsened facial features. Increased cardiovascular risk, respiratory abnormalities, and malignancies in acromegalic patients are considered as the first and most relevant cause of mortality, whereas bone complications are considered next as the most important cause of morbidity. The clinical manifestations may be a result of the peripheral actions of excessive GH and elevated insulin-like growth factor 1 levels or the central pressure effects of the tumor mass (or both).

For patients with acromegaly, the drug treatment of choice is somatostatin analogs such as octreotide (or
octreotide acetate with brand name Sandostatin (Biochemie GmbH, Schaftenau, Austria) and lanreotide acetate (INN, lanreotide), which exert inhibitory effects on the release of pituitary and gastroenteropancreatic hormones including GH by preferentially binding to \( \text{hsst} \, 2 \), one of the 5 somatostatin receptor subtypes (\( \text{hsst} \, 1-5 \)).\(^2,3\) Using pituitary GH–secreting adenomas from acromegalic patients who underwent transsphenoidal surgery, Saveanu et al\(^4\) quantified both \( \text{hsst} \, 2 \) and 5 messenger ribonucleic acid (mRNA) expression and showed that the octreotide-sensitive adenomas equally expressed both \( \text{hsst} \, 2 \) and \( \text{hsst} \, 5 \) mRNA, whereas in the adenomas that were poorly responsive to octreotide, the loss of \( \text{hsst} \, 2 \) mRNA contrasted with a 30-fold higher expression of \( \text{hsst} \, 5 \) versus \( \text{hsst} \, 2 \) mRNA. This finding suggests a better suppressive effect of GH from somatostatin analogs acting on both \( \text{hsst} \, 2 \) and \( \text{hsst} \, 5 \) subtypes.

SOM230 is a cyclohexapeptide, currently in phase II development by Novartis Pharma (Basel, Switzerland) as a successor compound to octreotide. This novel multiligand compound exhibits a unique binding profile with high affinity to 4 of the 5 human somatostatin receptors. In vitro assays show that SOM230 binds with a 30- to 40-fold higher affinity than octreotide to the \( \text{hsst} \, 1 \) and \( \text{hsst} \, 5 \) receptor subtypes and a 5-fold higher affinity to \( \text{hsst} \, 3 \), although its affinity to \( \text{hsst} \, 2 \) is somewhat lower than that of octreotide (0.4-fold).\(^5\) The clinical relevance of high-affinity binding of SOM230 to \( \text{hsst} \, 1 \), \( \text{hsst} \, 3 \), and \( \text{hsst} \, 5 \) has yet to be demonstrated, but it is hypothesized that through a greater binding to these subtype receptors SOM230 may show improved efficacy in the present octreotide indications and broaden the scope of indications for somatostatin analogs. In an in vitro study Hofland et al\(^6\) compared the effects of SOM230 and octreotide on hormone release by primary cultures of human pituitary adenoma cells. Their results showed that the inhibition by SOM230 of tumoral pituitary hormone release was probably mediated via both \( \text{hsst} \, 2 \) and \( \text{hsst} \, 5 \), again suggesting a greater potential of controlling GH levels by SOM230 than by octreotide.

In preclinical studies SOM230 was shown to have potent and long-lasting inhibitory effects on the growth factor/insulin-like growth factor 1 axis across species.\(^7\) The safety of SOM230 has been investigated in healthy volunteers after single and multiple subcutaneous doses.\(^8-11\) SOM230 is well tolerated at single doses up to 1500 \( \mu \)g or daily infusion doses up to 2050 \( \mu \)g/d for 7 days. The most frequently reported treatment-related side effects were predominantly gastrointestinal symptoms, such as loose stools and nausea at high doses. Dutreix et al\(^12\) studied the pharmacokinetics of SOM230 in healthy male volunteers. It was shown that SOM230 was rapidly absorbed after subcutaneous doses, generally reaching peak concentrations within an hour. SOM230 demonstrated a linear dose-exposure (area under the plasma concentration–time curve and maximum plasma concentration) relationship between 50 and 600 \( \mu \)g at single daily doses and at steady state. The effective half-life of SOM230 was approximately 10 hours. Detailed pharmacokinetic (PK) properties of SOM230 in acromegalic patients have not appeared in the literature so far.

In this proof-of-concept study, data from 12 acromegalic patients were collected for the purpose of investigating the PK and GH relationship. van der Hoek et al\(^13\) previously analyzed part of the GH data from this study and concluded that SOM230 is an effective GH-lowering drug in acromegalic patients, with the potential to increase the number of patients controlled during long-term medical treatment. In this analysis, the pharmacokinetics of SOM230 and its GH inhibition activity were studied at 100- and 250-\( \mu \)g doses and compared with 100 \( \mu \)g octreotide. The relationship between the serum GH concentration level as a pharmacodynamic (PD) response and the plasma concentration level of SOM230 and octreotide as a PK variable was investigated by a modeling approach. Factors of age, gender, and body weight were evaluated in the kinetic analysis. The chronobiologic rhythm of GH secretion was also considered in the PK-PD model.

**METHODS**

**Study design.** This was a single-center, double-blind, 3-period crossover study designed to compare the efficacy of single subcutaneous doses of SOM230 (100 and 250 \( \mu \)g) and a standard, subcutaneous dose of octreotide (100 \( \mu \)g). Twelve patients with active acromegaly were randomized into the 6 possible sequences, with 2 patients in each sequence. On study day 0 (the day before treatment), all 12 patients had GH levels assessed. On study days 1, 8, and 15, each patient received a subcutaneous injection of one of the following 3 treatments: 100 \( \mu \)g of SOM230, 250 \( \mu \)g of SOM230, or 100 \( \mu \)g of octreotide. Study days 0, 1, 8, and 15 are hereafter designated the control day and periods 1, 2, and 3, respectively.

The study drugs in each treatment period were administered between 7 and 9 AM. Blood samples for determining serum GH concentration levels were taken 30 minutes and 1 minute before and hourly for 24 hours after injection of the study drug during treatment periods 1 through 3 and at the same time points on the...
control day. An additional blood sample for measuring the GH level was collected at 48 hours after each administration of the study drugs.

A subset of the blood samples for GH analysis was used for PK analysis. Plasma concentration levels of octreotide and SOM230 were measured for all patients in the 3 periods from the GH samples collected before dosing and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 hours after dosing. The lower limit of quantification was 50 and 30 pg/mL for octreotide and SOM230, respectively. Serum GH concentrations were analyzed by a chemiluminometric immunoassay method (Nichols Advantage HGH system; Quest Diagnostics, San Juan Capistrano, Calif). The lower limit of quantification was 0.1 ng/mL.

**Analysis method.** The PD response in this modeling analysis was the GH concentration level, and the PK represented the SOM230 or octreotide plasma concentration measurements. Early work has shown that the time delay of GH response to SOM230 concentration is minimal, as has been documented similarly for octreotide by Grass et al and Comets et al. Consistent with the earlier findings, the GH level showed a negligible time delay or no time delay when plotted against SOM230 or octreotide plasma concentration after a subcutaneous injection. Hence a direct relationship between the concentration level of SOM230 and octreotide and the corresponding GH level was investigated by modeling with different inhibitory sigmoid E_max models.

For a large number of GH measurements, there were no corresponding PK data. Hence a 2-step modeling method was considered in order to maximally use all available measurements. First, a population PK model describing the time courses of SOM230 and octreotide concentration was established. The individual patient’s predicted concentrations of SOM230 or octreotide were then used as input in the PK-PD model. Using the predicted PK concentration as an independent explanatory variable in the PK-PD model was later shown to be a reasonable approach, because the PK predictions were accurate, with small variability.

The models were developed with NONMEM (non-linear mixed-effects modeling) software, version V. The first-order conditional method with interaction was used in the model-building process, where both multiplicative and additive errors were imposed.

It is known that GH level is correlated with both age and gender. Therefore demographic covariates are studied in the models to assess whether they account for any variability of pharmacokinetics and pharmacodynamics in acromegalic patients. Whether a covariate x has any influence on the PK or PD parameters was tested in the framework of hierarchic model pairs, that is, one is a submodel of the other when some constraints of the parameters are made. The following functional form of the covariate was used as a multiplier in the model structure: \( \frac{x}{x_m} \) for a continuous variable x with a median value of \( x_m \), and \( \theta^x \) for a dichotomous variable x taking a value of 1 (for yes) or 0 (for no), where \( \theta \) is a parameter to be estimated. In addition, multiplicative random effects were studied on most PK and PD parameters. If \( \theta \) represents any of the parameters, then a quantity of the form \( \theta \cdot \exp(\eta_j) \), instead of \( \theta \) alone, was used in the functional form of the model, where \( \eta_j \) is a subject-specific random effect and assumed to be normally distributed with a mean of 0. In doing so, the value of the parameter \( \theta \) can be viewed as representative of the patient population and is, therefore, called a typical value.

The final model was developed from progressively more elaborate models, namely, when 2 models were related as hierarchic, the difference of the minimum objective function (MOF) values was taken as guidance for favoring inclusion of a covariate. A reduction of MOF of more than 6.63 was regarded to be significant at the .01 level, approximated by the chi-square distribution with 1 df. The final model was obtained when, in general, eliminating any covariate would lead to an MOF value increase of greater than 6.63. The usual diagnostic techniques such as examination of the residual plot and correlation plots of predicted versus observed concentrations were applied in model selection when appropriate. Other factors such as the size of the estimated random effects, random errors, and standard errors of the parameter estimates were also considered.

**PK model.** The time courses of SOM230 and octreotide concentrations were assumed to follow compartmental models with first-order disposition processes. Because the sample collection at early time points after dosing does not allow complete characterization of the subcutaneous drug absorption phase, instantaneous (ie, bolus-like) drug input into the central compartment was incorporated into the PK models. The following parameters were assumed in the models: the elimination clearance (CL) from the central compartment, the volume of distribution for the central compartment (V1), the volume of distribution for the peripheral compartment (V2), and the intercompartmental clearance (Q). In this analysis the parameters CL, V1, V2, and Q were all apparent terms (ie, already divided by the bioavailability term).

The actual model fitting was done after logarithmic transformation. Thus the model takes the form of \( \log(Y) = \log(F) + \epsilon \), which is equivalent to \( Y =
Table I. Summary statistics of age and weight by gender

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th></th>
<th></th>
<th>Weight (kg)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Mean</td>
<td>SD</td>
<td>Minimum</td>
<td>Median</td>
<td>Maximum</td>
</tr>
<tr>
<td>Male</td>
<td>6</td>
<td>42.5</td>
<td>13.2</td>
<td>33</td>
<td>36.5</td>
<td>67</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>50.8</td>
<td>15.6</td>
<td>36</td>
<td>47.5</td>
<td>79</td>
</tr>
<tr>
<td>All</td>
<td>12</td>
<td>46.7</td>
<td>14.4</td>
<td>33</td>
<td>41.5</td>
<td>79</td>
</tr>
</tbody>
</table>

F·exp(ε), where Y is the observed concentration of either SOM230 or octreotide and F is the fitted value. The random error term ε represents model mispecification, error in the analytic measurement (eg, resulting from assay quantification limit), and residual random noise and is assumed to be normally distributed with a mean of 0 and an unknown variance to be estimated.

PK-PD model. A direct inhibitory PK-PD model was considered—that is, GH was studied as directly related to the study drug concentrations, which in turn were functions of time, as follows:

\[
G(t) = A + B \cdot \cos(2 \cdot \pi(t - T_1)/24) + C \cdot \cos(2 \cdot \pi(t - T_2)/12)
\]

where \( G(t) \) represents the predicted GH level, \( A \) is the predicted GH level when there was no treatment (see subsection below), \( C(t) \) is the concentration level of SOM230 or octreotide, \( E_{\text{max}} \) is the maximum drug effect (ie, the maximum GH change from baseline that can be attributed to the presence of the test drug), \( EC_{50} \) is the test drug concentration level at which half of \( E_{\text{max}} \) is observed, and \( \gamma \) is the Hill constant.

All 3 parameters (\( E_{\text{max}}, EC_{50}, \) and Hill constant) were allowed to be different between SOM230 and octreotide. Also assigned to the model were independent random errors, composed of 2 parts—an additive part (\( \varepsilon_2 \)) and a multiplicative part \([G(t) \cdot \varepsilon_1] \), the latter assumed to be proportional to the predicted GH level. The errors \( \varepsilon_1 \) and \( \varepsilon_2 \) were assumed to be normally distributed with means of 0 and unknown variances to be estimated. Thus the model took the form \( Z(t) = G(t) \cdot (1 + \varepsilon_1) + \varepsilon_2 \), where \( Z \) was the observed GH level and \( G \) the fitted value at time \( t \). Similarly, as in the case of the population PK model, the random error terms represented quantities such as model misspecification, analytic error, and residual noise. A diagonal covariance structure of the random effects for \( E_{\text{max}} \) and \( EC_{50} \), expressing the independent interpatient variation, and multiplicative and additive residual errors were imposed on all models during the PK-PD model-building process.

Circadian and circatidal rhythms. It has been documented that GH presents chronobiologic rhythms that include circadian rhythm, pulsatile variation, and other types. Under the current experimental conditions, certain rhythmic patterns were observed in all patients, which were particularly apparent on the control days. To consider such rhythmic patterns, the GH levels were studied by fitting models that consisted of periodic functions. The identified model with the estimated parameter values then served as guidance for a component of the above-described PK-PD model.

When there was no test drug, \( \alpha(t) \) was assumed to be a function of 2 combined cosine terms, as follows:

\[
\alpha(t) = A + B \cdot \cos(2 \cdot \pi(t - T_1)/24) + C \cdot \cos(2 \cdot \pi(t - T_2)/12)
\]

where \( A \) represents the baseline or average GH level; \( B \) is the amplitude for the component representing the GH circadian rhythm; \( C \) is the amplitude of the component representing the semicircadian, or circatidal, rhythmic pattern; and \( T_1 \) and \( T_2 \) are the corresponding times at which maxima of the circadian and circatidal rhythms occur, respectively. As described previously, multiplicative interpatient random effects were imposed on the baseline and amplitude parameters (\( A, B, \) and \( C \)), and demographic covariates were studied for their influence on the baseline parameter \( A \). For the time parameters \( T_1 \) and \( T_2 \), only additive interpatient random effects were added. Both multiplicative and additive errors were again assumed in the model for the GH rhythmic patterns.

RESULTS

Summary statistics of demographic information (age and body weight by gender) are listed in Table I. Because of the small number of patients in the nonwhite ethnic groups (1 black and 1 other), the relationship of PK and race was not investigated. Age and body weight were moderately correlated, with a correlation coefficient of −0.457.

SOM230 PK model. A 2-compartment model appeared to be adequate for describing the pharmacokinetics of SOM230. A multiplicative random effect was imposed on the covariate model for \( CL, V_1, \) and \( Q, \) respectively, but not for \( V_2, \) because no model im-
Improvement was obtained when such a random effect was imposed. The 2 parameters CL and V₁ were assumed to have dependent random effects in the final model. Either age or weight, but not both, was found to be a significant covariate relating to CL. However, only age was selected in the final model. Also found to be important was IOV, the random effect of occasion, which represents the differences in a patient between the 2 periods of SOM230 administration and is called the interoccasion random effect. In the final model the parameter structure of CL, V₁, Q, and V₂ was identified as follows:

\[
CL = \theta_{CL} \cdot \text{age}/42^{\beta_{AGE}} \exp(\eta_{CL}) \exp(\eta_{IOV})
\]

\[
V_1 = \theta_{V_1} \cdot \exp(\eta_{V_1})
\]

\[
Q = \theta_Q \cdot \exp(\eta_Q)
\]

\[
V_2 = \theta_{V_2}
\]

where \(\theta\) represents typical values of the PK parameters, \(\beta_{AGE}\) is a fixed effect quantifying the influence of age on CL, and \(\eta\) is random effects. The PK parameter estimates from NONMEM output for the final model are provided in Table II. The SD for the multiplicative error was small, at about 15% for the final model.

The model predicted that for a patient with a median age of 42 years the apparent CL is 7.96 L/h, and for a patient aged 53 years the apparent CL is 6.35 L/h, a decrease of about 20%. Fig 1 shows the predicted time course from the final model of SOM230 concentration in relation to the measured concentration values in linear and in semilogarithmic scales. The prediction curve is based on a hypothetic patient with a median age of 42 years. In Fig 1 SOM230 concentrations were normalized by the dose administered (in micrograms).

Table II. SOM230 and octreotide: Fixed effects and variances of random effects of final model

<table>
<thead>
<tr>
<th></th>
<th>(\theta_{CL}) (L/h)</th>
<th>(\theta_{V_1}) (L)</th>
<th>var((\eta_{CL}))</th>
<th>var((\eta_{V_1}))</th>
<th>cov((\eta_{CL}, \eta_{V_1}))</th>
<th>(\beta_{AGE}) (L/h)</th>
<th>(\theta_Q) (L/h)</th>
<th>var((\eta_{IOV}))</th>
<th>var((\eta_Q))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octreotide</td>
<td>15.8 (6.8%)</td>
<td>53.3 (9.7%)</td>
<td>0.057 (132%)</td>
<td>0.109 (161%)</td>
<td>0.065 (174%)</td>
<td>-0.648 (18.7%)</td>
<td>0.057 (132%)</td>
<td>0.238 (42%)</td>
<td>0.065 (174%)</td>
</tr>
<tr>
<td>SOM230</td>
<td>7.96 (7.0%)</td>
<td>59.2 (14.3%)</td>
<td>0.0456 (50%)</td>
<td>0.238 (42%)</td>
<td>0.0734 (59%)</td>
<td>-0.971 (20.6%)</td>
<td>6.56 (17.5%)</td>
<td>45.2 (51.3%)</td>
<td>0.037 (51.3%)</td>
</tr>
</tbody>
</table>

Percentage standard errors are shown in parentheses. \(\theta\) represents typical values of the pharmacokinetic parameters shown in their subscripts; thus the typical value of the apparent clearance (CL) for SOM230 was estimated to be 7.96 L/h with its standard error equal to 7%.

Var, Variance of \(\eta\); \(\eta_{IOV}\), intersubject random effects; cov, covariance of \(\eta\); \(\beta_{AGE}\), fixed effect quantifying influence of age on CL; \(\eta_{IOV}\), random effect for interoccasion variability.

Fig 1. Time course of dose-normalized observed concentration values and population-predicted concentration curve for SOM230 (left, linear scale; right, logarithmic scale).

Octreotide PK model. A 1-compartment model was adequate to characterize the octreotide pharmacokinetics. A multiplicative random effect was assumed for CL and V₁, respectively, and the 2 random effects were assumed to be correlated. Age was the only significant covariate relating to CL in the final selected model,
where the structure models of CL and $V_1$ were identified as follows:

$$CL = \theta_{CL}(\text{age}/42)^{\beta_{AGE}} \exp(\eta_{CL})$$

$$V_1 = \theta_{V_1} \cdot \exp(\eta_{V_1})$$

where $\theta$ represents typical values of the PK parameters, $\beta_{AGE}$ is a fixed effect quantifying the influence of age on CL, and $\eta$ is random effects. The PK parameter estimates for the final model from NONMEM output are shown in Table II.

The model predicted that for a patient aged 42 years the apparent CL is 15.8 L/h, and for a patient aged 53 years the apparent CL is 13.6 L/h, a decrease of about 14%. The SD for the multiplicative error was small, at about 10% from the final model. Fig 2 shows the predicted time course from the final model of octreotide concentration in relation to the measured concentration values in regular and in semilogarithmic scales. The prediction curve is based on a hypothetic patient with a median age of 42 years. In the figure octreotide concentrations were normalized by the dose administered (in micrograms).

Population PK-PD model. For GH data collected during all treatment periods at 48 hours after the administration of the study drug, the corresponding predicted concentrations for either SOM230 or octreotide were essentially zero. These data points were excluded from the PK-PD modeling analysis to avoid numeric difficulties in the fitting process of the sigmoid Emax model.

GH levels on the control day were first examined for possible rhythmic changes. A final model describes the overall GH on the control day as following a pattern that is a combination of circadian and circatidal rhythms. From the model, the maxima of the circadian and circatidal rhythms occur in the early morning hours and in the afternoon.

Age and weight did not have any significant influence on the overall GH levels. For GH levels of all periods, patient 12 (a female patient with the minimum weight of 53.3 kg in the study) had GH levels during the study that were considerably higher than those of other patients. Moreover, the GH levels of this patient were not suppressed when octreotide was given in period 3. Thus the $E_{\text{max}}$ type of model could not be applied to the patient. She was excluded from the PK-PD analysis.

The modeling process showed that SOM230 and octreotide inhibit GH levels to a similar extent. Therefore $E_{\text{max}}$ was taken to be the same for both treatments. However, $EC_{50}$ differed between the treatments. Models of $E_{\text{max}}$ types in which no sigmoidicity was incorporated (ie, Hill constant = 1) were clearly inferior, based on, for example, the MOF values. The final PK-PD model combines the study drug–related changes as a direct sigmoid $E_{\text{max}}$ model with the rhythmic patterns as similarly seen on the control day. No demographic covariates (age, weight, and gender) were significantly correlated with either $EC_{50}$ or $E_{\text{max}}$.

The results of fitting the final model to the GH and PK concentration data in NONMEM are summarized in Table III. The values of the parameter estimates from the final model were consistent with those obtained
from other, less elaborate models with successful runs. However, the covariance step for the final model failed, and as a result, the standard errors of the parameter estimates were not available. It is shown in Table III that, on average, the maximum inhibition level of GH secretion by SOM230 and octreotide is about 10.4 ng/mL, 78% of the total average GH level of the patients in this study. The typical EC$_{50}$ is 46 pg/mL for octreotide and 553 pg/mL for SOM230 with large interpatient variability (coefficients of variation, 164% and 65%, respectively).

Fig 3 shows the population model-predicted SOM230 and octreotide concentration versus GH in logarithmic scale during the study drug periods as a curve, where the component of rhythmic variation was not included. Also drawn on the figure are the model-predicted individual SOM230 or octreotide versus observed GH concentrations. Fig 4 shows the observed GH levels over a 24-hour period after the study drug treatment with the predicted time course of GH levels, obtained by composing the PK and PK-PD modeling functions of time, with rhythmic variations. SOM230 at 250 μg showed comparable potency but longer-lasting activity in comparison with 100 μg octreotide.

Fig 5 shows the individual model-predicted time course over the observed GH levels for the first 3 listed patients, where the 4 columns of plots were for the control day, 100 μg SOM230, 250 μg SOM230, and 100 μg octreotide, respectively.

Model validation. SOM230 and octreotide PK models appear to fit the concentration data well. Large variability of GH was seen, because of factors such as the pulsatile secretion pattern often in the time range of minutes. Diagnostic plots for the final population PK models for SOM230 and octreotide and the PK-PD model were examined. Constancy of multiplicative errors was not violated. Other than the large variability that could not be adequately explained, the final PK-PD model also appeared to be unbiased in data fitting when all data were included.

DISCUSSION

The GH data from this study have previously been analyzed, and the short-term effects, defined by the area under the effect curve of GH from 2 to 8 hours after dosing, were compared between octreotide and SOM230. It was found that doses of 100 and 250 μg SOM230 were not significantly different from 100 μg octreotide in suppressing GH levels ($P > .1$), although there were some preferential differences in GH inhibition between SOM230 and octreotide in the 12 patients. In this analysis the PK of SOM230 and octreotide and their inhibition of GH secretion were studied by a nonlinear mixed-effects modeling method. The PK analysis results showed that the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent. For a typical patient who is aged 42 years, the apparent clearance for SOM230 and for octreotide is age-dependent.
distribution for octreotide is 53.3 L. The terminal elimination half-life for SOM230 is 11.8 hours, about 5 times longer than that of 2.3 hours for octreotide. A relatively low clearance and long half-life for SOM230, as compared with octreotide, suggest that SOM230 can probably be administered less frequently (e.g., by twice-daily dosing instead of 3 times daily, as recommended for octreotide subcutaneous injection).

In this analysis the PK models of SOM230 and octreotide were used to predict concentration levels at any time points where GH levels were measured. Subsequently, all predictions were used as input to the PK-PD model. This approach made use of the GH measurements at time points where SOM230 and octreotide concentration values were not available and, therefore, minimized the loss of data information. Although using only the predicted values, instead of the observed values, may seem to ignore an additional source of error, the prediction plots and the diagnostic plots showed that the models were adequate in fitting.
the concentration data, therefore supporting the use of this approach.

The PK of both SOM230 and octreotide was linear and showed low variability. The GH level, as the PD response variable of this study, showed large variations. In addition to the PK variability, other factors contributing to GH variations include demographic influences (not detected in this study) and the episodic or pulsatile release of GH, the magnitude and timing of which are affected by arousal, sleep states, nutritional state, level of stress, and so on. Under the experimental condition of this study in acromegalic patients, the variability resulting from the pulsatile nature of GH release cannot be accounted for because of the limited sampling strategy. Receptor expression differences for different hsst subtypes may have also contributed to the GH variability. Among those patients who responded to the study drug, there were patients whose GH levels were not adequately controlled by either of the 2 study drugs (eg, patients 2, 3, and 12 taking octreotide and patient 8 taking SOM230). (See van der Hoek et al13) Data from such patients added further to the variability and had an inadvertent effect on the precision of the model estimates.

Despite the above-mentioned factors, observations made on acromegalic patients in this study were consistent with the general understanding of GH secretion in endocrinology.19 Circadian and circatidal rhythmic patterns were observed in patients as shown in Fig 5, representing types of GH patterns beyond the pulsatile secretion mode. The primary peak representing the maximum GH release was reached between midnight and 2:30 AM. A secondary peak occurred between 1 and 4 PM. The amplitudes of such rhythms were small and apparently overwhelmed by the interpatient differences and other

Fig 5. GH during all periods for the first 3 listed patients: Time profiles of observed concentration and individual predicted curves. The first column of the plots is for the control day, the second column is for 100 µg SOM230, the third column is for 250 µg SOM230, and the fourth column is for 100 µg octreotide.
types of variations described. Therefore the additive error of the model for rhythmic patterns appeared to be relatively large. However, the size of the additive error was reduced when study drugs were given to regulate the GH levels. This is also clear from visual inspection of the data. The analysis here provides first-hand experience regarding rhythmic patterns of GH and needs to be further confirmed.

A direct inhibitory model was found to be adequate to describe the GH and PK concentration relationship in patients with acromegaly, and incorporating circadian and circatidal rhythms into the equation improved the model fit. Although it is believed that age and gender affect the GH levels in general, neither was found to be influential on GH in this analysis. One possible reason for this is the small size of this study. Results regarding the effects of demographic covariates should, therefore, be understood in such a context. It is perhaps of interest to note that identifying such a PK-GH relationship in healthy volunteers has not always been possible, because of the low basal GH concentration in healthy subjects and the low sampling frequency in such clinical studies. The discrepancy in identifying a PK-GH relationship between acromegalic patients and healthy subjects is perhaps also attributed to the different neurohormonal mechanisms governing the GH dynamics.

The potency estimate of EC\textsubscript{50} (or EC\textsubscript{90}) represents the plasma concentration level needed for 50% (or 90%) of E\textsubscript{max} to be inhibited. A large interpatient variability for this parameter for the same drug treatment (SOM230 or octreotide) and the large difference in the EC\textsubscript{50} estimates between drug treatments and their random effects (Table III) suggest that there is a great difference among patients with regard to GH responses at the same plasma concentration level, possibly as a result of interactions with different subsets of \textit{hsst} receptors.

Highly variable post hoc ratios between treatments were seen from the model-derived individual parameters of EC\textsubscript{50}, again suggesting interactions with different subsets of \textit{hsst} receptors. Therefore different subsets of \textit{hsst} receptors, responsible for different GH response and for the large interpatient variability, cannot be excluded. A different Hill constant value between SOM230 and octreotide may imply different in vivo binding profiles between the 2 compounds in acromegalic patients. The estimates of EC\textsubscript{50} are, furthermore, consistent with the in vitro findings in the literature. In their study comparing the effects of SOM230 and octreotide on hormone release by secreting pituitary adenoma cultures, Hofland et al\textsuperscript{16} showed that at the same concentration levels of 10 nmol/L, the potency of SOM230 on GH release inhibition (50% inhibitory concentration [IC\textsubscript{50}], 0.5 nmol/L) was relatively low, as compared with octreotide (IC\textsubscript{50}, 0.02 nmol/L). However, in one culture completely resistant to octreotide, SOM230 significantly inhibited GH release in a dose-dependent manner, with an IC\textsubscript{50} value in the low nanomolar range. Their in vitro findings for IC\textsubscript{50} showed similar relationships between the 2 compounds, as did the values of EC\textsubscript{50} estimated here from the clinical data.

The typical EC\textsubscript{50} is 46 pg/mL for octreotide and 553 pg/mL for SOM230, with large interpatient variability (Table III), and the EC\textsubscript{90} values of SOM230 and octreotide (equal to EC\textsubscript{50} \cdot 9^{1/\gamma}, where \gamma is the Hill constant) are 204.3 pg/mL and 1659 pg/mL, respectively. However, for appropriate comparison of clinical pharmacologic activity between SOM230 and octreotide, other factors including clearance, dosing regimen, and the sigmoidicity of the PK-PD relationship have to be considered, because the relationship between GH inhibition and plasma concentration is nonlinear. Octreotide appears to be highly potent, resulting in an adequate duration of GH inhibitory activity despite its short half-life (2.3 hours). At a single dose of 100 \mu g, the octreotide plasma concentration decreases to the level of EC\textsubscript{50} after 12 hours. The apparent clearance of SOM230 is about half as much as that of octreotide (Table II), and the half-life of SOM230 is about 5 times longer. Therefore more accumulation and less concentration fluctuation would be expected of SOM230 than octreotide at steady state if the same dosing regimen were used. As predicted on the basis of the PK and PK-PD models (Fig 4), 250 \mu g SOM230 had pharmacologic activity comparable to that of 100 \mu g octreotide. Similar to this conclusion, the analysis results reported previously for the study (van der Hoek et al\textsuperscript{13}) showed that 250 \mu g SOM230 had a short-term inhibitory effect in the same range as that of 100 \mu g octreotide. Therefore, when expressed by dose, the potency difference between 250 \mu g SOM230 and 100 \mu g octreotide is 250/100 or 2.5-fold. The lower potency for SOM230 may not be a concern because SOM230 is well tolerated and higher doses can be administered.

SOM230 was shown in this proof-of-concept study via modeling analysis to have superior PK properties with small variability. SOM230 has a lower clearance and longer half-life than octreotide, which compensates for the lower potency in GH inhibition. SOM230 was effective in directly inhibiting GH
levels at selected doses. The modeling analysis results are important for designing the phase II and III clinical studies in which the efficacy, safety, plasma exposure, and dosing regimen will be further investigated in a larger patient population. As a result of the lower interpatient potency (EC$_{50}$) variability, SOM230 is expected to have a more uniform clinical GH inhibition than octreotide for acromegalic patients with a clinically effective dosing regimen. The clinical results together with the in vitro findings on somatostatin receptor subtypes suggest that SOM230 and octreotide may have a complementary effect in inhibiting GH levels. Further mechanism studies are needed to better understand the importance of the diversified binding profile of SOM230 to different hss receptor subtypes in inhibiting GH secretion in acromegalic patients.

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References
19. Parker K, Schimmer BP. Pituitary hormones and their


Drug interaction between mycophenolate mofetil and rifampin: Possible induction of uridine diphosphate–glucuronosyltransferase

The tuberculostatic compound rifampin (INN, rifampicin) induces the expression of a number of drug metabolism–related genes involved in multidrug resistance (P-glycoprotein and multidrug resistance proteins 1 and 2), cytochromes (cytochrome P450 [CYP] 3A4), uridine diphosphate–glucuronosyltransferases, monoamine oxidases, and glutathione S-transferases. Drugs that depend on these enzymes for their metabolism are prone to drug interactions when coadministered with rifampin. A novel, clinically relevant drug interaction is described between rifampin and mycophenolate mofetil (MMF), a cornerstone immunosuppressive molecule used in solid organ transplantation. Long-term rifampin therapy caused a more than twofold reduction in dose-corrected mycophenolic acid (MPA) exposure (dose-interval area under the concentration curve from 0 to 12 hours \([AUC_{0-12}]\) when administered simultaneously in a heart-lung transplant recipient, whereas subsequent withdrawal of rifampin resulted in reversal of these changes after 2 weeks of washout (dose-corrected \(AUC_{0-12}\) after rifampin withdrawal, 19.7 mg·h·L\(^{-1}\)·g\(^{-1}\) versus 6.13 mg·h·L\(^{-1}\)·g\(^{-1}\) before rifampin withdrawal [221% change]; dose-uncorrected \(AUC_{0-12}\) after rifampin withdrawal, 29.6 mg·h/L [daily MMF dose, 3 g] versus 18.4 mg·h/L [daily MMF dose, 6 g] during rifampin administration [60.8% change]). Failure to recognize this drug interaction could potentially lead to MPA underexposure and loss of clinical efficacy. The effect of rifampin on MPA metabolism can, at least in part, be explained by simultaneous induction of renal, hepatic, and gastrointestinal uridine diphosphate–glucuronosyltransferases and organic anion transporters with subsequent functional inhibition of enterohepatic recirculation of MPA. (Clin Pharmacol Ther 2005;78:81-8.)

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Rifampin (INN, rifampicin) is a well-known inducer of multidrug resistance transport proteins (P-glycoprotein [P-gp], multidrug resistance protein 1 and 2 [MRP1 and MRP2, respectively], and lung resistance–related protein [LRP]) and of cytochrome P450 (CYP) 3A4. Clinically relevant interactions between rifampin and calcineurin inhibitors (tacrolimus and cyclosporine [INN, ciclosporin]) have been extensively documented and have led to guidelines for prompt dose adjustments and strict drug monitoring in allograft recipients when both drugs are combined. Mycophenolate mofetil (MMF) is an immunosuppressive drug that is mainly metabolized by uridine diphosphate–glucuronosyltransferases (UGTs) to the inactive 4-hydroxyphenyl-β-glucuronide (MPAG) metabolite and, to a lesser extent, to the active acyl-glucuronide (AcMPAG). Mycophenolic acid (MPA) and MPAG are subject to enterohepatic (re)circulation (EHC), which can account for up to 40% of the total dose-interval MPA area under the concentration curve (AUC) from 0 to 12 hours.
The biliary excretion of MPA/MPAG and subsequent distal (re)absorption involves several transport mechanisms including organic anion–transporting polypeptides, multidrug resistance–related proteins, and UGTs. Because UGTs play an important role in the metabolism and disposition of MPA, induction of UGT activity could lead to significant MPA underexposure and loss of clinical efficacy, resulting in acute or chronic graft rejection. Conversely, inhibition of UGT activity would increase the risk for MMF-related side effects (anemia, leukopenia, diarrhea). Calcineurin inhibitors (tacrolimus and cyclosporine) coadministered with MMF have a differential effect on MPA exposure, probably through opposing effects on hepatic excretion of MPA/MPAG and enterohepatic (re)circulation. As a result, dose-corrected posing effects on hepatic excretion of MPA/MPAG and differential effect on MPA exposure, probably through organic anion–transporting polypeptides, multidrug resistance–related proteins, and UGTs. Consequently, MMF dose requirements are on average 50% higher in cyclosporin-treated renal recipients. Consequently, MMF dose requirements are on average 50% higher in cyclosporin-treated renal recipients.

In this report a novel clinically relevant drug interaction between MPA and rifampin is documented for the first time in a solid organ recipient and explanatory mechanisms for this interaction are discussed.

METHODS

Case

A 51-year-old male patient received a combined heart–double-lung allograft in June 2004 for chronic respiratory failure as a result of histiocytosis X and secondary therapy-resistant pulmonary hypertension. The immediate postoperative course was uneventful except for a hemorrhagic pleural effusion requiring surgical reintervention on day 2 after grafting. Initial immunosuppressive drug therapy consisted of 0.2 mg·kg⁻¹·d⁻¹ oral tacrolimus (Prograf; Fujisawa, Munich, Germany), targeted at therapeutic trough concentrations between 8 and 10 ng/mL, 1 g MMF (CellCept; Roche, Basel, Switzerland) twice daily (target trough plasma concentration of 2.5 μg/mL), and 20 mg methylprednisolone (Medrol; Upjohn, Kalamazoo, Mich). Induction therapy with antithymocyte globulin (ATG-Fresenius; Hoechst Marion Roussel, Frankfurt, Germany) was administered in a daily dose of 5 mg/kg for 5 days. Concomitant medication consisted of the following: nystatin oral suspension (Nystatine; Pharma Logistics, Huizingen, Belgium), 150 mg ranitidine daily (Zantac; GlaxoSmithKline, Brentford, Middlesex, United Kingdom), amphotericin B, 5 mg aerosols (Fungizone; Bristol-Meyers Squibb, Braine-L’alleud, Belgium), 230 mg ganciclovir daily (Cymevène; Roche), 400/80 mg sulfamethoxazole-trimethoprim (Bactrim; Roche) twice weekly, calcium carbonate with cholecalciferol (INN, colecalciferol) (Cacit Vitamine D3 500/440; Procter & Gamble/Pharma Logistics, Strombeek-Bever, Belgium), and 20 mg enoxaparin subcutaneously daily (Clexane; Aktuapharma, Heverlee, Belgium) as thrombosis prophylaxis until discharge from the hospital. Routine histologic examination of the left explanted lung unexpectedly revealed a necrotizing granulomatous lesion in the upper lobe that was clearly Ziehl stain–positive. Despite the fact that repetitive bronchoalveolar specimens, obtained during consecutive routine bronchoscopic examinations, did not grow mycobacterium species, triple antimycobacterial therapy was initiated. On day 13 after transplantation, 600 mg rifampin (Rifadine; Aventis, Brussels, Belgium) daily was started together with 300 mg isoniazid (Nicotibine; Aventis), 1 g/d pyrazinamide (Tebrazid; Continental Pharma, Brussels, Belgium), and 250 mg pyridoxine weekly. This treatment was well tolerated. A few weeks later, a febrile episode was successfully treated with meropenem (Meronem; AstraZeneca, Basel, Switzerland) for 10 days; no infectious agent was identified.

After commencing tuberculostatic therapy, daily oral dose requirements for tacrolimus started to increase progressively from 7 mg twice per day on postoperative day 14 to as high as 22 mg twice daily on postoperative day 74, continuously aiming at target trough concentrations between 8 and 10 ng/mL. In an attempt to moderate this drug interaction, the dose of rifampin was tapered to 450 mg daily on postoperative day 47, but high tacrolimus dose requirements persisted and the highest trough blood concentration obtained was 11 ng/mL on postoperative day 74 (total daily dose of 44 mg). Surprisingly, dose requirements for MMF increased simultaneously from 2 g/d to a total of 6 g daily, without being able to achieve a preset target trough plasma concentration of 2.5 μg/mL. The highest MPA plasma trough concentration achieved with 2 g of MMF was 0.84 mg/L (postoperative day 44), whereas increasing the dose to 6 g of MMF led to a maximum MPA trough concentration of 3.16 mg/L (on postoperative day 81). Because of these continually high dose requirements of both tacrolimus and MMF and the associated risk for the development of serious drug-induced side effects and toxicity, it was decided to discontinue rifampin and continue with dual antimycobacterial therapy.

Pharmacokinetic studies. To assess the effects of rifampin on MPA exposure, a 12-hour dose-interval concentration-time curve of MPA and tacrolimus was obtained, before withdrawal of rifampin and again after
a 13-day washout period. Measuring the evolution of tacrolimus exposure simultaneously with MPA served as a control for the enzyme-inducing effect of rifampin. On day 92, after an overnight fast, MPA and tacrolimus blood samples were collected from a peripheral venous catheter, just before both drugs were taken ($C_0$) and after 30, 60, and 90 minutes and 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12 hours. Thirteen days after rifampin therapy was discontinued, the same measurements were repeated. After rifampin was stopped, the treating physicians reduced the tacrolimus and MMF dose in the following days, according to predose trough target concentrations, to avoid acute drug intoxication after withdrawal of rifampin. Blood tacrolimus concentrations were determined by a microparticulate enzyme immunoassay (Tacrolimus II, MEIA/IMx analyzer; Abbott Laboratories, Abbott Park, Ill). Plasma concentrations of MPA were determined by HPLC–mass spectrometry. Interference of rifampin with the HPLC determination of MPA concentrations is unlikely considering the differences in molecular weight and after comparison of the MPA chromatograms before and after withdrawal of rifampin. Renal function, albumin and hematocrit concentrations, liver function, and concomitant medication had not changed between pharmacokinetic assessments. The patient gave his informed consent, and the study was approved by the ethics committee of the Faculty of Medicine of the University of Leuven, Leuven, Belgium.

Pharmacokinetic modeling was performed by use of WinNonlin 3.2 Pro software (Pharsight, Mountain View, Calif). The following model-independent pharmacokinetic parameters for tacrolimus and MPA were calculated and dose-corrected when appropriate: dose-interval MPA AUC0-12 still increased from 18.4 mg · h/L to 29.6 mg · h/L (+60.8%) (Table I). The corresponding dose-corrected AUC0-12 increased by 221% after discontinuation of rifampin therapy, whereas apparent MPA total body clearance diminished from 163 L/h to 50.6 L/h (−68.9%). Interestingly, the terminal portion of the MPA concentration-time curve under rifampin therapy did not show a second concentra-

tion peak, indicating a low degree of enterohepatic recycling of MPA, whereas after withdrawal of rifampin, a prominent secondary peak occurred between 9 and 12 hours after drug dosing (Fig 1). Without a secondary peak, the corresponding 12-hour trough plasma MPA concentration was 0.14 mg/L, whereas after rifampin discontinuation (and a marked secondary concentration peak), it was 1.13 mg/L. The predose trough concentration showed similar differences (0.18 mg/L versus 1.63 mg/L). To estimate the relative contribution of EHC to the total dose-interval AUC, the percentage ratio of the partial MPA AUC6-12 (estimate of EHC) and total 12-hour AUC0-12 was calculated. With rifampin therapy, the EHC contributed only 8.5% of total MPA exposure whereas after rifampin was stopped, EHC was responsible for an estimated 28% of the MPA AUC0-12, corresponding to a relative increase of 230%.

**Tacrolimus pharmacokinetics**

The dose-corrected tacrolimus AUC0-12 increased by 121% after withdrawal of rifampin (Table I), whereas trough concentration–targeted dose requirements were simultaneously reduced from 0.39 mg/kg body weight to 0.16 mg/kg (−59%) (Fig 2). The daily dose of tacrolimus was tapered from 44 mg to 18 mg (59% dose reduction) to obtain comparable drug exposure, as reflected by the relatively stable tacrolimus AUC0-12 before and after rifampin withdrawal (217 ng · h/mL versus 197 ng · h/mL). Total body clearance of tacrolimus fell from 101.2 L/h to 45.6 L/h (−54.8%) after rifampin therapy was stopped.

**DISCUSSION**

In this report a strong and clinically relevant drug interaction between MPA and rifampin is documented for the first time. Discontinuation of long-term rifampin therapy caused a dramatic increase in dose-corrected MPA exposure ($AUC_{0-12}$, $C_0$, and $C_{max}$) or, alternatively, reduced weight-corrected drug dose requirements by more than 50%. The concomitant increase in the oral bioavailability of tacrolimus occurring after rifampin withdrawal confirms that drug metabolism was indeed induced prominently by the dose administered, and these effects disappeared approximately 2 weeks after discontinuation of the drug. Rifampin is a pleiotropic inducer of P-gp, MRPs, and other cytochromes, UGTs, monoamine oxidase B, and certain glutathione S-transferases. The upregulation of CYP3A4 and P-gp expression in the gut and liver mediated by rifampin and the effect on calcineurin-inhibitor metabolism have been extensively
documented.11–13 Rifampin induces gene expression of CYP3A and P-gp by activating the nuclear pregnane X receptor in hepatocytes and enterocytes.14,15 The result is increased hepatic and intestinal clearance of tacrolimus with drastically reduced blood concentrations and a loss of efficacy.4,5 The pregnane X receptor is also involved in the regulation of the rodent organic anion–transporting polypeptide and the human MRP2 (ABCC2) hepatic transporter, respectively located at the sinusoidal and canalicular membrane of hepatocytes and responsible for hepatic uptake of organic anion transporter substrates from the blood and their excretion into the bile.16 The exact mechanism through which rifampin induces MPA metabolism is currently not known, but induction of UGTs in the liver, kidney, and intestinal mucosa is a candidate pathway.17 UGT expression and activity are regulated at least in part through the pregnane X receptor18–20 and thus inducible by rifampin. UGT1A9 is the key UGT responsible for glucuronidation of MPA to its inactive hydroxy-β-glucuronide (MPAG) and is predominantly active in the kidney, liver, and intestine.18–20 UGT2B7, predominantly active in the liver, is responsible for generation of the active acyl-glucuronide metabolite (AcMPAG)21 of MPA, which has been related to clinical side effects of MMF.24 UGT1A7 and UGT1A8 are predominantly located in the gastrointestinal tract and have also been implicated in the metabolism and first-pass effect of MPA.22 This could explain why withdrawal of rifampin and decreased UGT activity caused a strong increase in dose-corrected MPA exposure. The obvious lack of a secondary concentration peak under rifampin therapy, indicating a loss of enterohepatic recirculation, adds to the complexity of the rifampin effect on MPA metabolism. Induction of primarily UGT1A9-mediated conversion of MPA into MPAG in combination with the

Table I. Model-independent pharmacokinetic parameters of mycophenolic acid and tacrolimus before and 13 days after withdrawal of rifampin in heart-lung recipient

<table>
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<tr>
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<th>Before rifampin withdrawal</th>
<th>After rifampin withdrawal</th>
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<tr>
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<td>Dose-uncorrected parameters</td>
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<td>Dose (g)</td>
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<td>AUC₀-₁₂ (mg · h · L⁻¹ · g⁻¹)</td>
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<td>Dose (mg)</td>
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<tr>
<td>AUC₀-₁₂ (ng · h/mL)</td>
<td>217.2</td>
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<tr>
<td>Cₘ₅₆₇ (ng/mL)</td>
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<td>tₘ₅₆₇ (h)</td>
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<td>CL (L/h)</td>
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<td>Dose-corrected parameters</td>
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<td>Weight-corrected dose (mg/kg)</td>
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<td>0.89</td>
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<tr>
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<td>21.89</td>
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<td>Cₘ₅₆₇ (ng · mL⁻¹ · mg⁻¹)</td>
<td>1.5</td>
<td>2.67</td>
<td>+78</td>
</tr>
</tbody>
</table>

C₀, Predose trough blood/plasma concentration; AUC₀-₁₂, area under concentration curve from 0 to 12 hours; AUC₀-₁₂, area under concentration curve from 0 to 12 hours; Cₘ₅₆₇, maximum blood/plasma concentration; tₘ₅₆₇, time to maximum blood/plasma concentration; CL, total steady-state body clearance.
activation of CYP3A4 and P-gp, as one can assume from the concomitant changes in tacrolimus exposure, would lead to an increase in enterohepatic recirculation rather than a decrease, especially if a possible additive inductive effect of rifampin on MRP2-mediated transport of MPAG into bile is taken into account. One possible explanation for the absence of a secondary MPA concentration peak is the simultaneous induction of intestinal UGT1A9 (and possibly UGT1A7 and UGT1A8) activity by rifampin, which could lead to increased levels of MPAG in the (portal and systemic) blood compartment and gut lumen, thereby counteracting the effect of deglucuronidation of MPAG to MPA in the distal gut by the microbial flora. This could ultimately lead to lower effective MPA reabsorption from the distal small bowel and hence less enterohepatic recirculation of the latter. Under normal (not induced) circumstances, UGT1A9 is responsible for approximately 40% of intestinal MPAG production. It is, therefore, conceivable that rifampin therapy aug-

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**Fig 1.** Dose-uncorrected (A) and dose-corrected (B) mycophenolic acid (MPA) dose-interval concentration-time curve before (daily dose, 6 g) (solid squares) and 13 days after (daily dose, 3 g) (open squares) withdrawal of rifampin in heart-lung recipient.
ments not only renal and liver but also intestinal UGT1A9 (and UGT1A7, UGT1A8) activity, leading to even higher plasma (and subsequently intestinal) MPAG concentrations and less MPA absorption from the gut in its deglucuronated form, thereby leading to lower “secondary peak” MPA concentrations. In addition, rifampin itself could modify the capacity of the intestinal flora for deglucuronidation of MPAG, thereby reducing enterohepatic recirculation even further.25

Another reason for reduced dose-corrected MPA exposure under rifampin therapy could be a strong induction of MRP2-mediated excretion of MPAG in the urine and hence lower MPA plasma concentrations. Simultaneous measurement of plasma and urine MPAG concentrations could have been helpful in unraveling these proposed mechanisms but was unfortunately not performed. Whether hepatic UGT2B7 induction would similarly lead to increased concentrations of AcMPAG21 was not studied in this patient, but typical side effects attributed to the presence of this metabolite (anemia and diarrhea) were absent.24

Although small amounts of MPA are metabolized by CYP3A4/5 to 6-O-desmethyl MPA,26 it is unlikely that rifampin-induced CYP3A4 activity could have produced the current changes in MPA exposure. Similarly,
Drug interaction between mycophenolate mofetil and rifampin


LETTERS TO THE EDITOR

Leflunomide and peripheral neuropathy: A potential interaction between uracil/tegafur and leflunomide

To the Editor:

In response to the recent report in the Journal of a possible association between leflunomide and peripheral neuropathy, we report on a patient in whom peripheral neuropathy developed during combination treatment with UFT (tegafur/uracil) and leflunomide. This may represent a potential drug interaction between UFT and leflunomide. Because leflunomide is being evaluated in combination with cytotoxic drugs for the treatment of malignancies, we consider this an important observation.

Our patient, a 75-year-old man with a history of rheumatoid arthritis, had been diagnosed with rectal carcinoma and had undergone anterior resection in July 2001. In October 2002 he was examined in our outpatient clinic and progress-

Fig 1. Biosynthesis of pyrimidines and mechanism of action of 5-fluorouracil (5-FU) and leflunomide. 5-FU is mainly converted with phosphoribosyl pyrophosphate (PRPP) as a cofactor to fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP), thereby causing ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) damage. Uracil inhibits the rate-limiting enzyme in 5-FU catabolism, dihydropyrimidine dehydrogenase (DPD), in both tumor and normal cells. Leflunomide inhibits orotate biosynthesis at an early stage and may promote conversion of 5-FU to fluorouracil monophosphate (FUMP) by increasing PRPP levels. In addition, it may be speculated that leflunomide has inhibiting action on DPD additive to uracil, thereby increasing intracellular 5-FU levels. DHFU, Dihydrofluorouracil; OMP, orotate monophosphate; FUDP, fluorouridine diphosphate; FdUDP, fluorodeoxyuridine diphosphate; dTMP, deoxymethylidine 5-monophosphate; dTDP, deoxymethylidine 5-diphosphate; dTTP, deoxymethylidine 5-triphosphate.
ing disease was diagnosed. We initiated oral treatment with UFT (600 mg daily in 3 divided doses, combined with 30 mg calcium folinate 3 times daily, days 1-28; days 29-35, pause), which was followed by a partial response and not associated with adverse effects until an episode of minor duodenal bleeding occurred in December 2002. In this situation we decided to pause chemotherapy. Three months later, the patient had increasing pain from the rheumatoid arthritis, and treatment with leflunomide (N-[4-(trifluoromethyl)-phenyl]-5-methylisoxazole-4-carboxamide) (Arava; Aventis Pharma, Frankfurt, Germany) was initiated with a starting dose of 100 mg/d for 3 consecutive days followed by a maintenance dose of 20 mg/d. This measure was followed by a good response of polyarthritis, and the patient reported good quality of life. Therefore the locally progressing tumor was again treated with UFT (dosage as described earlier). After completion of 2 cycles, the patient had increasing numbness of both lower extremities in a stocking pattern, which was suggestive of polyneuropathy (PNP). Nerve conduction studies confirmed axonal sensorimotor PNP. In addition, the patient had severe diarrhea and hand-foot syndrome. The latter 2 adverse events were self-limited and manageable in an outpatient setting.

Because (1) there were no further risk factors for the development of PNP, (2) there was no exposure to any other neurotoxic drugs, and (3) the patient had previously tolerated UFT alone well, we believe this to be a case of PNP and gastrointestinal toxicity that was induced by a potential drug interaction between UFT and leflunomide. This is of special interest because leflunomide is currently being investigated—as an inhibitor of platelet-derived growth factor receptor signaling—in ongoing phase I and II studies in combination with cytotoxic agents for the treatment of various cancers.

Both 5-fluorouracil (5-FU) and leflunomide have been reported to cause neurotoxicity on their own, but we infer a potential drug interaction as the cause of this unexpected toxicity: Leflunomide’s active metabolite A77 1726 inhibits the key enzyme in pyrimidine synthesis, dihydroorotate dehydrogenase. In UFT, uracil is used to increase the bioavailability of the 5-FU prodrug tegafur by inhibiting dihydroxyrimidine dehydrogenase, which is the rate-limiting enzyme in 5-FU catabolism. Thus leflunomide may increase 5-FU toxicity either by upstream inhibition of pyrimidine synthesis, thereby increasing conversion of 5-FU to fluorouracil monophosphate (FUMP), or by additional blockade of dihydroxyrimidine dehydrogenase (Fig 1).

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The authors have no conflicts of interest directly relevant to the content of this letter.

References

Pharmacokinetics of glimepiride and cytochrome P450 2C9 genetic polymorphisms

To the Editor:

Recently, Niemi et al have reported in the Journal that cytochrome P450 (CYP) 2C9*1/*3 individuals showed significant alterations in glimepiride pharmacokinetics compared with *1/*1 individuals. In individuals heterozygous for the CYP2C9*3 allele (n = 3), the median total area under the plasma concentration–time curve (AUC) of glimepiride was 267% of the values in subjects with the CYP2C9*1/*1 genotype. However, only 3 individuals heterozygous for the CYP2C9*3 allele were involved in this experiment. The concentrations of hydroxy metabolite (M1) and carboxy metabolite (M2) were not measured, and the parameters related to M1 and M2 were not mentioned. Glimepiride is metabolized mostly in the liver to the active M1 metabolite by CYP2C9, which shows genetic polymorphism, with further dehydrogenation to the inactive M2 (carboxy) metabolite. Thus we assessed the pharmacokinetics of oral administration of glimepiride in relation to CYP2C9 genetic polymorphism in healthy Chinese subjects.

The protocol was designed according to Good Clinical Practice principles and approved by the Ethics Committee of the Chinese People Liberation Army General Hospital, Beijing, China. All volunteers were determined to be healthy and signed informed consent forms before participation. CYP2C9 genotype was determined by oligonucleotide microarray as described earlier. Nineteen subjects (9 expressing CYP2C9*1/*1, 9 expressing CYP2C9*1/*3, and 1 expressing
CYP2C9*3/*3 were invited to participate in the phenotype phase of the study. Blood and urine samples were collected after 4 mg glimepiride was administered orally. Plasma glimepiride and urine M1 and M2 concentrations were determined by HPLC.

CYP2C9 genotype significantly affected the pharmacokinetics of glimepiride. The concentration-time profiles of glimepiride for each of the genotyped groups are illustrated in Fig 1, and the pharmacokinetic data are presented in Table I. The AUC from time 0 to infinity (AUC0-\infty) was significantly greater in the CYP2C9*1/*3 subjects than in *1 homozygotes (\(P<0.05\)), with the *1/*3 and *3/*3 individuals demonstrating 1.3- and 1.4-fold increases in mean glimepiride AUC0-\infty, respectively. In subjects with the CYP2C9*1/*3 allele, the half-life (t\(\text{half}\)) of glimepiride was 163\% of the values in subjects expressing CYP2C9*1/*1 (\(P<0.05\)). Glimepiride oral clearance (CL\(\text{oral}\)) was significantly reduced in CYP2C9*1/*3 individuals to 75\% of that in *1 homozygotes (\(P<0.05\)), suggesting that significant differences in glimepiride elimination exist among individuals expressing different genotypes. Glimepiride formation clearance (CL\(\text{form}\)) to its M1 and M2 metabolites was also significantly reduced in CYP2C9*1/*3 individuals (65\%, \(P<0.05\)), as compared with subjects expressing *1/*1. However, no significant differences were found in the AUC from 0 to 48 hours (AUC0-48), amount of M1 and M2 secreted in urine, and blood glucose variables of glimepiride between the subjects with different genotypes. In clinical diabetes therapeutics, glimepiride is always administered at a dosage of 2 to 4 mg/d for several months, whereas the volunteers in our study were given a single administration of 4 mg glimepiride orally. The relationship of CYP2C9 genetic polymorphisms to pharmacokinetics and pharmacodynamics of glimepiride may be more clear after multiple administrations or 8 mg glimepiride. The results will be more useful to clinical therapeutics after multiple administrations. These preliminary observations suggest that the CYP2C9 genotype should be evaluated with regard to drug interactions or individualized therapy.

**Fig 1.** Mean (\(\pm\)SD) plasma concentrations of glimepiride in healthy Chinese volunteers with different CYP2C9 genotypes after single oral 4-mg dose of glimepiride.

**Table I.** Measures of glimepiride pharmacokinetics and metabolism by CYP2C9 genotype

<table>
<thead>
<tr>
<th>CYP2C9*1/*1</th>
<th>CYP2C9*1/*3</th>
<th>CYP2C9*3/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(\text{half}) (h)</td>
<td>11.4 ± 4.3</td>
<td>18.6 ± 6.1†</td>
</tr>
<tr>
<td>AUC0-48 ((\mu\text{g} \cdot \text{L}^{-1} \cdot \text{h}))</td>
<td>1355.4 ± 314.0</td>
<td>1707.5 ± 500.2</td>
</tr>
<tr>
<td>AUC0-\infty ((\mu\text{g} \cdot \text{L}^{-1} \cdot \text{h}))</td>
<td>1461.9 ± 341.9</td>
<td>1877.8 ± 508.7†</td>
</tr>
<tr>
<td>t(\text{max}) (h)</td>
<td>2.1 ± 0.6</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>C(\text{max}) ((\mu\text{g} \cdot \text{L}^{-1}))</td>
<td>260.3 ± 81.5</td>
<td>272.9 ± 86.6</td>
</tr>
<tr>
<td>CL(\text{oral}) (L⋅h(^{-1}))</td>
<td>3.0 ± 0.6</td>
<td>2.3 ± 0.4†</td>
</tr>
<tr>
<td>CL(\text{form}) (L⋅h(^{-1}))</td>
<td>1.3 ± 0.3</td>
<td>0.8 ± 0.2†</td>
</tr>
</tbody>
</table>

\(t_{\text{half}},\) Half-life; AUC0-48, area under plasma concentration–time curve from 0 to 48 hours; AUC0-\infty, area under plasma concentration–time curve from time 0 to infinity; t\(\text{max},\) time to maximum plasma concentration; C\(\text{max},\) maximum plasma concentration; CL\(\text{oral},\) oral clearance; CL\(\text{form,}\) formation clearance.

†\(P<0.05\) for CYP2C9*1/*3 versus CYP2C9*1/*1 (Dunnett test).

*CYP2C9*3/*3 subjects were included in the phenotype phase of the study. Blood and urine samples were collected after 4 mg glimepiride was administered orally. Plasma glimepiride and urine M1 and M2 concentrations were determined by HPLC.

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To the Editor:

Recently, De Smet discussed the health risks of herbal remedies. He pointed out the importance of comprehensive, reliable, and easily accessible reference sources for disseminating and evaluating the risks, as well as therapeutic effects, of herbs. Because of increased use of traditional medicines, there is also a need for resources that provide comprehensive information about these medicines, which are otherwise not easily accessible and are sometimes not retrievable from the conventional literature resources. The ability to evaluate the beneficial and risk effects of herbs can be enhanced if information about herbal constituents is also provided.

An initiative for collecting and evaluating toxicologic data about traditional Chinese medicine (TCM) has recently been launched. More comprehensive data about TCM are needed for fully assessing the beneficial and risk effects and for facilitating scientific and clinical study of TCM. The TCM Information Database (TCM-ID) has been introduced as a Web resource to provide free-of-charge information about all aspects of TCM including prescriptions, constituent herbs, and herbal ingredients, as well as their respective therapeutic effects and clinical indications and applications. The structure and functional properties of active ingredients are also provided.

Data were obtained from reputable Chinese TCM books and relevant Western and Chinese journals including the Journal of Ethnopharmacology, Planta Medica, Journal of Pharmaceutical Sciences, Phytochemistry, Complementary Therapies in Medicine, Journal of Alternative and Complementary Medicine, The American Journal of Chinese Medicine, Chinese Traditional Herbs and Drugs, Acta Pharmacologica Sinica, Journal of Chinese Medicine Mat, and Chinese Journal of Medicine and Chemistry. TCM-ID currently contains 1197 TCM prescriptions covering 4111 disease conditions, 1104 herbs, and 9862 ingredients (4500 of these with 3-dimensional structure provided). Each prescription/ingredient can be retrieved through multiple methods including prescription name, herb name in 3 languages (Latin, English, and Chinese), name of herbal ingredient, therapeutic effect, and symptom.

This work was supported in part by grants from the Shanghai Commission for Science and Technology (04DZ19850, 04QMX1450, 04DZ14005) and the “973” National Key Basic Research Program of China (2004CB720103, 2004CB715901).

The authors have no conflicts of interest to disclose.

References


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Traditional Chinese medicine information database

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References

Celecoxib is often combined with cytochrome P450 2D6 substrates in general clinical practice

To the Editor:

Tirkkonen and Laine recently reported in the Journal that prodrugs such as codeine are often combined with inhibitors of cytochrome P450 (CYP)–activating enzymes in hospital inpatients. Celecoxib (cyclooxygenase 2 inhibitor) was one of the most frequently combined CYP2D6 inhibitors with codeine. We have measured the concurrent use of celecoxib with codeine or other typical CYP2D6 substrates in general practice.

Norwegian pharmacists (44 pharmacists from 35 pharmacies) who had been trained on managing drug interactions systematically checked for concurrent substrate use during a 2-month period in 2004 (tramadol not included). An electronic popup notice appeared when celecoxib was registered. Combined substrate use was disclosed by checking the prescription database and consulting the patient. Information about drug doses, prescription history, and any unwanted symptoms declared by the patient was recorded.

Celecoxib was dispensed to 764 patients, and 192 events (25%) of concurrent substrate use were found. Codeine, metoprolol, and amitriptyline were the most frequent substrates and represented more than 90% of the findings (Table I). In 50% of cases the prescribing physicians were informed about the interacting potential. One third of the physicians changed the prescription immediately. A similar fraction wished to consider the interaction at the next patient consultation, whereas the remainder did not consider the issue as relevant.

Celecoxib at a dosage of 400 mg daily for 7 days has been shown to increase metoprolol exposure by a factor of 1.4 and 2.0 in heterozygous and homozygous extensive CYP2D6 metabolizers, respectively. The most common celecoxib dose in our material was 200 mg daily (Table I), and a study investigating the inhibitory effect of this dosage would have been useful. Nevertheless, about 25% of the patients received 400 mg or more daily (pooled data), which is likely to produce relevant interactions in extensive metabolizers. Pharmacokinetics of codeine and amitriptyline has not been studied with celecoxib, but the exposure of their active form(s) could change substantially during CYP2D6 inhibition.

The clinical use of celecoxib is extensive, and it is important to be aware of the high rate of concurrent CYP2D6 substrates. Although the future role of cyclooxygenase 2 inhibitors seems more uncertain after the withdrawal of rofecoxib, further research on celecoxib interactions is warranted.

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References
ation in the total propofol concentration in blood during cardiopulmonary bypass. The increase in the unbound fraction was caused mainly by a lower concentration of albumin. Because propofol is a drug with a high clearance and is extensively bound to plasma albumin, the increase in unbound fraction is clinically important. The increase in unbound propofol might occur as a result of the loss of plasma albumin accompanying hemorrhage, especially when followed by crystalloid resuscitation. The purpose of this study was to estimate the changes in unbound concentration of propofol during hemorrhage.

After institutional approval, informed consent was obtained from 10 patients (6 men and 4 women; mean age, 57 ± 6.4 years; mean height, 162 ± 12 cm; mean weight, 59 ± 13 kg) undergoing elective surgery. Anesthesia was maintained by 60% nitrous oxide in oxygen, 10 to 20 μg/kg fentanyl, and an infusion of propofol at 4 mg·kg⁻¹·h⁻¹ until the end of the operation. Radial arterial samples were collected for measurement of propofol concentrations just before the start of the operation and at the point when blood loss was greater than 10, 20, and 30 mL/kg. Patients were resuscitated with lactated Ringer’s solution and dopamine infusion to maintain a mean arterial blood pressure that was ±20% of that before hemorrhage. Three-fold the volume of blood loss was replaced by lactated Ringer’s solution. Total and unbound propofol concentrations were determined by use of HPLC as reported previously.

Mean blood pressure and heart rate were well maintained during the operation in all patients. There were no significant differences in total propofol concentrations across the time points. The unbound propofol concentration was increased in conjunction with the extent of hemorrhage (Fig 1).

It has been reported that hemorrhagic shock increases the potency of propofol. However, the mechanism of this effect remains uncertain. We report that the unbound concentration of propofol increases during hemorrhage. Thus the increased potency of propofol during hemorrhage might be explained by the changes in unbound propofol. Further study regarding the pharmacodynamics of propofol during hemorrhage is required.

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None of the authors has financial or personal relationships that could potentially be perceived as influencing the described research.
References


NOTES OF THE AMERICAN SOCIETY FOR CLINICAL PHARMACOLOGY AND THERAPEUTICS

A MESSAGE FROM THE ASCPT MEMBERSHIP COMMITTEE CHAIRPERSON

On behalf of the Membership Committee, I am pleased to welcome the new members accepted into the Society in April 2005. Please take a moment to review their names and welcome those in your geographic area. Thank you also to the current ASCPT members who have sponsored these applicants.

Please continue your recruitment efforts on behalf of ASCPT and take an active role in ensuring new members can both contribute to and benefit from the Society. Again, welcome to our new members!

John T. Sullivan, MD
Chairperson, Membership Committee

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