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Division of Clinical Pharmacology
560 RRB
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Over-the-counter statins and cardiovascular disease prevention: Perspectives, challenges, and opportunities

Antonio M. Gotto, Jr, MD, DPhil

In 2004 the United Kingdom approved over-the-counter (OTC) access to a low dose of the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor simvastatin, thus radically shifting the landscape of what kinds of drugs may be candidates for OTC status. Emboldened by the United Kingdom’s decision, physician and industry groups in the United States interested in OTC statins requested that the US Food and Drug Administration (FDA) reconsider the application for a nonprescription preparation of lovastatin, which had first been broached unsuccessfully to the FDA in 2000. The FDA convened a panel to review the evidence to date, but in the end the panel was not persuaded and again recommended against approval. Nevertheless, OTC statins remain an important point of debate for the future of cardiovascular disease prevention.

As argued in an earlier précis for OTC statins, primary-prevention trials have affirmed that low- to moderate-risk patients have coronary benefit with statin treatment, and the safety profile of available agents suggests a low risk for adverse side effects. In the group of patients at intermediate coronary risk, access to low-dose OTC statins may help complement lifestyle therapy to modify the risk profile. On the other hand, high-risk patients or those who may be more susceptible to toxicity, such as the frail elderly, require physician supervision and should not be considered candidates for OTC therapy. If an OTC switch were to occur, then a statin would be another option among a field of other OTC products such as nutraceuticals and vitamin or herbal supplements whose prophylactic credentials in coronary heart disease (CHD) are unproved or less impressive.

Since that review, additional information has become available that merits discussion. These data include improved understanding of the scope of patients who might benefit from OTC statins and evidence of whether patients can appropriately self-select for OTC treatment.
HOW LARGE IS THE POTENTIAL OTC POPULATION IN THE UNITED STATES?

Individuals ideally suited for an OTC approach to statin therapy should qualify for primary prevention under the third Adult Treatment Panel (ATP III) of the National Cholesterol Education Program, with multiple (2+) risk factors and a 10-year CHD risk of 20% or lower, no contraindications to statins, and a favorable likelihood of having benefit versus risk. Those with an intermediate risk for near-term CHD have a goal of low-density lipoprotein (LDL) cholesterol lower than 130 mg/dL (3.36 mmol/L). Therapeutic lifestyle changes, including diet, weight management, and physical activity, are primary recommendations for such patients, but therapeutic lifestyle changes alone frequently do not help patients achieve target cholesterol values.

Analysis of the National Health and Nutrition Examination Survey III suggests that 15.5% of US adults (23 million persons) are at moderate risk for CHD (10% to 20% risk over a 10-year period according to the Framingham algorithm). By age and sex, 16% of men aged 40 to 49 years, 52% of men aged 50 to 59 years, and 81% of men aged 60 to 69 years fall in this moderate-risk category. The largest percentage of women at moderate risk is 35% of those aged 70 to 79 years.

Of the clinical trials, the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) studied the cohort most similar to this group, comprising 6605 middle-aged men and women with no clinical evidence of atherosclerosis, average levels of LDL cholesterol, and below-average levels of high-density lipoprotein (HDL) cholesterol, as compared with the overall National Health and Nutrition Examination Survey III cohort. The 5.6% 10-year CHD event rate in the placebo-treated group from AFCAPS/TexCAPS is significantly lower than any treatment threshold currently recommended for lipid-lowering drugs in the context of primary prevention. The participants ranged in age from 45 to 73 years in men and 55 to 73 years in women. The entrance lipid criteria were cholesterol levels between 180 and 264 mg/dL (4.65-6.82 mmol/L), LDL cholesterol levels between 130 and 190 mg/dL (3.36-4.90 mmol/L), and an HDL cholesterol level of 45 mg/dL (1.16 mmol/L) or lower in men or 47 mg/dL (1.21 mmol/L) or lower in women.

Unlike other primary-prevention studies, AFCAPS/TexCAPS permitted drug titration. Lovastatin was begun at 20 mg/d and was then titrated at week 18 to 40 mg/d if an LDL-cholesterol target goal of 110 mg/dL (2.84 mmol/L) was not attained. In the lovastatin group, 50% of the patients underwent titration for this reason, and the mean on-treatment LDL cholesterol level was 115 mg/dL (2.96 mmol/L). Of the group randomized to lovastatin, 81% reached the ATP III target goal of 130 mg/dL (3.36 mmol/L), as compared with 12% of the patients receiving placebo.

After a median of 5.2 years, therapy with lovastatin resulted in a statistically significant 37% reduction in the rate of first acute major coronary events, defined as a composite endpoint including fatal or nonfatal myocardial infarction, unstable angina, and sudden cardiac death incidence ($P < .001$). Lovastatin therapy also reduced secondary endpoints, with a 33% risk reduction in revascularizations ($P = .001$), a 32% risk reduction in unstable angina ($P = .02$), a 40% risk reduction in nonfatal or fatal myocardial infarctions ($P = .002$), and 25% risk reductions in both coronary and cardiovascular endpoints ($P = .006$ and .003, respectively). The effect in women did not statistically differ from the benefit in the overall cohort. Therefore AFCAPS/TexCAPS was the first major statin trial to confirm that primary prevention is clinically feasible in a lower intermediate-risk population, and benefit was consistent across the range of baseline LDL-cholesterol quartiles.

In AFCAPS/TexCAPS there was no increase in noncardiac mortality rate with lovastatin therapy, and the discontinuation rate was similar in the placebo and lovastatin-treated groups. The investigators reported no significant liver toxicity. A recent retrospective analysis detected no greater risk over a 6-month period for statin-related hepatotoxicity among treated individuals with elevated baseline liver enzyme levels compared with untreated individuals. Because statins have been associated with rare cases of hepatocellular toxicity and jaundice, patients with significant liver disease, heavy alcohol consumption, or chronic hepatitis should receive statins only with careful monitoring.

Muscle toxicity, with the worst consequence being rhabdomyolysis, with statins has garnered more attention than adverse liver effects. In AFCAPS/TexCAPS, small, consistent increases (median, <5 IU/L) in creatine kinase level were detected with lovastatin, 20 to 40 mg/d, compared with placebo, but the frequency of creatine kinase elevations greater than 10 times the upper limit of normal was identical in the study groups. Rhabdomyolysis occurred in 2 placebo patients and 1 lovastatin patient; the lovastatin case occurred postoperatively in a patient with prostate cancer while not receiving study medication. None of the participants had uncomplicated myopathy (creatine kinase elevations >10 times the upper limit of normal...
and muscle pain). Because lovastatin is metabolized by the cytochrome P450 (CYP) 3A4 isozyme pathway, combination therapy with drugs that compete for metabolism by this pathway may explain adverse drug–drug interactions. There were no differences between treatment groups in AFCAPS/TexCAPS in the frequency of clinically important muscle-related adverse events in lovastatin-treated participants receiving CYP3A4 inhibitors (N = 535) compared with those receiving placebo (N = 511).

Although the real incidence of statin side effects may be higher in clinical situations in which patients are not selected or monitored as closely as they are in clinical trials, the risk for myopathy or hepatotoxicity with the currently approved statins does not exceed the potential benefit of preventing coronary and other cardiovascular events. Certain groups may be at greater risk for myopathy caused by statins—frail elderly individuals, especially women; patients with diabetes complicated by chronic renal failure; patients in the perioperative phase after surgery; patients with liver disease; and patients taking specific concomitant medications. Because these patients would require some clinical supervision, they would be unlikely candidates for low-dose OTC statin self-medication.

CAN PATIENTS SELF-SELECT FOR OTC STATIN TREATMENT?

Among other concerns, the FDA expressed skepticism about patients’ ability to self-select for OTC therapy. That is, can nonprescription statins be kept out of the hands of patients who do not qualify for treatment, and more important, would high-risk patients who require physician oversight mistakenly self-medicate if given the option? The pharmaceutical company interested in developing an OTC lovastatin brand has proposed a self-management system (SMS), and its data provide some preliminary evidence of potential patient behavior with OTC statins.

The Consumer Use Study of Over-the-Counter Lovastatin (CUSTOM) was an open-label, uncontrolled, multicenter study that examined the behavior of potential OTC purchasers in 14 storefront sites intended to simulate a real-world pharmacy setting. Participants were solicited through mass media advertising, and all who came were invited to participate in the study. The materials developed for the study’s SMS focused on describing primary prevention of CHD in the intermediate-risk population and encouraged participants to discuss their concerns about cholesterol, including OTC statin treatment, with their physicians. A product carton label was developed that was intended to explicitly describe who would and would not qualify for the OTC statin (Table I). Nurse practitioners, in the role of “pharmacists,” were not permitted to volunteer any information that might influence the participant’s ability to self-select but could answer questions and perform an eligibility assessment interview at the participant’s request. Respondents were then categorized as “purchasers, users”; “purchasers, nonusers”; or “nonpurchasers” depending on their behavior.

Of the nonpurchasers (n = 2111), 79% were not interested in buying the drug (1673/2111 participants), and 98% of these were ineligible anyway according to the product label. Therefore the majority who opted not to buy correctly followed the label. Furthermore, 46% of all nonpurchasers wanted to consult with their physicians before deciding whether to buy. At the second visit, 22% of these had followed through with that conversation.

On the other hand, among users (n = 1061), only 10% met all label criteria for use. The majority of users met many of the criteria, but not all (Table I), mainly because they were slightly outside the age

<table>
<thead>
<tr>
<th>Table I. Key features of over-the-counter lovastatin label</th>
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<tbody>
<tr>
<td>Before using, you must have done the following:</td>
</tr>
<tr>
<td>Tried a healthy diet and exercise to reduce your cholesterol level</td>
</tr>
<tr>
<td>Had a fasting cholesterol test within the last year</td>
</tr>
<tr>
<td>Who can use the drug?</td>
</tr>
<tr>
<td>Men aged ≥45 y</td>
</tr>
<tr>
<td>Women aged ≥55 y</td>
</tr>
<tr>
<td>Persons with LDL cholesterol level of 130-170 mg/dL</td>
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<td>(3.64-4.39 mmol/L)</td>
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<tr>
<td>Persons with ≥1 of the following:</td>
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<tr>
<td>Smoking</td>
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<tr>
<td>HDL cholesterol level &lt;40 mg/dL ( &lt;1.03 mmol/L)</td>
</tr>
<tr>
<td>Family history of premature CHD</td>
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<tr>
<td>High blood pressure</td>
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<tr>
<td>Do not use, unless directed by a doctor, if you have the following:</td>
</tr>
<tr>
<td>LDL cholesterol level of 171-400 mg/dL (4.42-10.3 mmol/L)</td>
</tr>
<tr>
<td>TG level of 200-900 mg/dL (2.26-10.2 mmol/L)</td>
</tr>
<tr>
<td>HDL cholesterol level of 60-200 mg/dL (1.55-5.17 mmol/L)</td>
</tr>
<tr>
<td>Diabetes</td>
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<tr>
<td>Previous CHD</td>
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<td>Previous stroke</td>
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Adapted from reference. LDL, Low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; CHD, coronary heart disease.
range, had lipid values outside of the label’s range, or had no risk factors for CHD, according to the label. If these borderline individuals had discussed OTC statins with their physicians, their behavior was classified as adhering to label benefit criteria. Nevertheless, the extensive use by participants who did not exactly fit the criteria suggests that the SMS may require additional refinement. On the basis of its strict interpretation of the label, the FDA believed that the overuse indicated that the general public would have trouble following the label.² Important to remember, however, is that CHD risk falls along a continuum, and no cholesterol threshold has been identified below which there is no further benefit of treatment. Thus the coronary risk of an individual whose LDL cholesterol level is 128 mg/dL (3.31 mmol/L) is not appreciably different from that of someone whose LDL cholesterol level is 131 mg/dL (3.38 mmol/L), all other risk factors being equal. It is reasonable to expect some flexibility in these marginal cases when determining the appropriateness of OTC statins.

A small survey of 3 pharmacies in Bristol, United Kingdom, after the deregulation of simvastatin provides additional speculation.¹⁵ By questionnaire administered to 102 men and women (mean age, 57 years) who were not taking statins, the investigators determined that 45% of respondents were willing to purchase simvastatin but 94% of those willing to buy would do so only after consulting their general practitioner. High-risk respondents were as likely as low-risk respondents to consider buying OTC simvastatin, suggesting a danger of misuse. Although the method was informal and the study was small, these observations suggest that the public will require greater education to understand the place of OTC statins in preventative care and that physician guidance would still be important to individuals who opted for this approach.

DISCUSSION

Before the United Kingdom’s decision to deregulate simvastatin, drugs that have qualified for the switch to OTC have been intended for symptom control (eg, loratadine and omeprazole) rather than disease prevention.¹⁶ There are several potential advantages to being able to obtain a statin without a prescription: improved and broader access, increased education about risk factor modification, greater patient autonomy in making decisions about treatment, and health care savings as a result of reduced coronary events.¹⁷

However, the challenges conferred by nonprescription status are equally great. A single-dose OTC product may delay the introduction of drug titration or combination therapy to patients who warrant it. Patients might be tempted to misuse the product either because they are at high risk and wish to bypass a doctor’s care or because they are at low risk but have decided to incur the expense of long-term treatment that has an unfavorable cost/benefit ratio.¹⁷ Furthermore, the approval of a nonprescription statin may prompt prescription drug benefit managers and managed care organizations to take steps to limit usage of prescription statins to control drug benefit costs. Initial market research among several insurance and pharmacy benefit managers suggests that there would continue to be a commitment, in principle, to support access to prescription-strength statins if an OTC version were approved but there would surely be a review of copayment levels and formularies to optimize the entire class.¹⁸

In its rejection of the lovastatin application, the FDA panel gave 2 compelling reasons for its decision. First, the panel believed that the current drug-delivery infrastructure in the United States was not able to provide the necessary safeguards to support a nonprescription statin. In the United Kingdom, simvastatin is available “behind the counter,” which requires the patient to undertake risk assessment with the pharmacist before the drug can be sold. Behind-the-counter services are not common in US pharmacies, but there is growing support for pharmacists to play an intermediary role in screening patients, as well as to control access to certain medications.¹⁹ Should the behind-the-counter approach take hold in the United States, OTC statins would be an obvious candidate for a class of drugs to which pharmacists could serve as gatekeepers. In the meanwhile, SMS programs, such as that developed for the CUSTOM study, are an important start.

Second, the panel believed that the availability of a nonprescription statin would negatively affect efforts to promote preventative lifestyle measures such as diet and exercise.² This is an important consideration, and educational efforts in support of OTC statins would need to emphasize the complementary natures of lifestyle and drug treatment. These and other critical issues must be resolved before OTC statins would make further headway.

Although much progress has been made in making the case for OTC statins, many obstacles remain, largely related to the optimal implementation of an OTC program. There is almost no disagreement that statins are cardioprotective drugs with few adverse side effects. Whether statins will ever make the leap from prescription to OTC remains to be seen. Given the continuing epidemic of CHD morbidity and death in the
United States, exploration of all options that may help prevent its further spread is a worthwhile endeavor.

Dr Gotto is currently a consultant for or has had a past relationship with the following companies with an interest in statins: AstraZeneca (Wilmington, Del), Bristol-Myers Squibb (Plainboro, NJ), Merck & Co (North Wales, Pa), Johnson & Johnson Merck (Fort Washington, Pa), Merck/Schering Plough (North Wales, Pa), Novartis (East Hanover, NJ), Pfizer (New York, NY), and Reliant Pharmaceuticals (Liberty Corner, NJ).

References
On January 14, 2005, I voted, reluctantly, against making a statin (lovastatin) available over the counter (OTC) for primary prevention of cardiovascular disease. I was one of the 20 members of a Food and Drug Administration (FDA) advisory committee who did so (4 committee members voted in favor). Why my reluctance?

I was reluctant because in my book primary prevention, in both individual patients and populations, is a good thing, vastly preferable to such things as angioplasty and bypass surgery. And statins, with their substantial efficacy and relatively low toxicity, appear at first glance to be “poster drugs” for primary prevention. (When they were first introduced, I found myself thinking that they should probably be put in the drinking water.) On the basis of that principle alone, therefore, I would have voted for approval. So why did I vote against it?

Strom1 has recently articulated at least 11 “core” clinical concerns that led him to conclude the following: “Although statins are great prescription drugs, these considerations suggest that they would make poor over-the-counter drugs.” These concerns were as follows. (1) Unlike the indications for virtually all other OTC drugs, the condition being treated is not self-diagnosable. (2) Contrary to the conditions for use of all other OTC drugs, OTC statin therapy is expected to be long term. (3) Efficacy is dose-related and requires monitoring for titration, which is optional for OTC use. (4) The lower dose proposed for OTC availability, primarily to increase the margin of safety, could prevent more appropriate dosing. (5) OTC users might mistakenly conflate more serious disease (eg, angina) with hypercholesterolemia. (6) People might use the drug simply for “peace of mind” rather than clinical efficacy. (7) The efficacy of statins for a self-diagnosed condition has never been clearly demonstrated. (8) Complications and contraindications of statins (largely liver and muscle damage) are not self-diagnosable. (9) Adherence, which is notoriously poor in long-term prescription drug therapy, is likely to be even worse for an OTC drug (which many people do not consider a “real” drug), particularly because people will be paying for it out of pocket. (10) Users who believe that “more is better” might increase the dose inappropriately, thus worsening the risk-benefit ratio. (11) The safety of statin use in pregnancy has been seriously questioned.

Most of these points emerged during the advisory committee’s discussion, and many of them figured into my decision to vote against approval. But 3 overarching issues that Strom1 did not discuss—paternalism, informed choice, and cost—also emerged, albeit briefly, during the committee’s deliberations, all of which, to my mind, argue against approval at this time.

The issue of paternalism can be framed as follows. Why should regulations prevent consumers from freely choosing to assume the risks and costs associated with primary prevention with OTC statins to realize its gains? That is certainly a legitimate question. As a powerful regulator, the FDA is by its very nature paternalistic, which is precisely why many people, particularly those with a strict free market orientation, view it as anathema. At the same time, many others see the agency’s protection of the public health as paternalism of the best kind, in fact, its principal virtue. These opposing points of view are unlikely ever to be reconciled. The agency is caught in the crossfire; it is unsure of its future as it soldiers on, but it is unlikely to fade into the sunset, at least not any time soon.

Thrust, willingly or not, into a paternalistic role, the FDA thus confronts the hard reality that becoming a father is easy but being a good father is not. The agency could, for example, simply throw up its hands and say, “Let the consumer (ie, the marketplace) decide whether the benefits of primary prevention with OTC statins are substantial enough to be worth the associated risks and costs.” But in my view the wiser exercise of its oblig-
under the conditions of a controlled study. The efficacy would almost certainly be less under OTC use than target cholesterol levels (even undergoing therapy) and the number of people who would reach (particularly over periods longer than the 5 years of the study) and the number of people who would reach target cholesterol levels (even undergoing therapy) would almost certainly be less under OTC use than under the conditions of a controlled study. The efficacy of primary prevention with statins, estimated from AFCAPS/TexCAPS to be an NNT of about 35 patients treated for 6 years to prevent 1 cardiovascular event, thus melts away under conditions of OTC use. In effect, approving statins for OTC primary prevention would amount to a huge uncontrolled experiment, in which neither the benefit nor the risk side of the equation was known. Moreover (as Strom1 points out), the ability to collect good clinical data to fill in the gaps in our knowledge would be even worse under conditions of OTC use than it is under prescription use.

Third, even if solid data on the efficacy of OTC statins in primary prevention were in hand, informing users of those benefits in a way that would allow for truly informed choice would be a formidable challenge. (The task of informing patients about risks associated with OTC statin use would likely be similar to that for other OTC drugs.) For all other OTC drugs, the regulatory process requires that users be able to self-diagnose the indications for use with reasonable accuracy. Those indications (headache, dyspepsia, rash, and the like) must, therefore, be concrete and obvious. The benefits are also expected to be correspondingly concrete and obvious, as well as prompt; your headache or your heartburn will get better, and fairly soon; your rash will clear up in a reasonable period of time. In contrast, the benefits of primary prevention with OTC statins are delayed, abstract, and subtle. To be properly informed, OTC statin users would, therefore, need to understand that although the benefits may be significant and quantifiable across an entire population the benefits to any single user are uncertain; they are, in fact, “statistical.” Moreover, they would need to understand that a variety of factors can affect the degree of benefit and that benefits, when and if they do occur, are manifest only after about 2 years of continuous use. Would language such as the following, printed on the OTC statin package, serve the purpose?

If your answers to all 4 of the following questions [about eligibility] are “yes,” the chances that taking [some number of OTC statin] pill(s) every day for 10 years will prevent you from having a heart attack or stroke are about 1 in 35. If your answers are “yes” to fewer than 4 questions but you still choose to take [OTC statin] pill(s) every day for 10 years, your chances of these benefits from this drug are less than 1 in 35, particularly if you take daily aspirin or niacin as well during that time. Benefits, if they do occur, begin after about 2 years of continuous treatment. We simply do not know.
Finally, there is the issue of cost. The FDA does not consider cost in its regulatory process and for good reason. But the United States already spends more on medical care per capita than any other industrialized country, with brutal effects on the economy and clinical outcomes that might best be characterized as “mixed.” Despite the FDA’s mandatory cost-blindness, it is, therefore, impossible for me in all good conscience not to take cost of primary prevention with OTC statins into account. The issue here is not pure cost, cost in isolation; it is the cost of primary prevention with OTC statins in relation to its clinical value (often expressed in terms of cost-effectiveness) and the cost-effectiveness of that intervention in relation to that of other medical interventions. For me, in short, the medical-economic question is this: Can we afford to promote the spending of large amounts of additional dollars on a treatment whose efficacy is uncertain and whose cost-effectiveness at best is likely to be poor?

Again, we unfortunately lack the data that would answer that question definitively. The relatively recent study by Prosser et al. does, however, give us some important clues. Those investigators found that, under the conditions of prescription use at the time, the cost-effectiveness ratio for primary prevention with statins (compared with diet therapy alone) ranged from $54,000 to $1,400,000 per quality-adjusted life-year, well in excess of the benchmark of $50,000 that has been widely used as a reasonable and acceptable level of cost-effectiveness for medical interventions. Because, as noted here, the efficacy of primary prevention with statins in an open OTC population would likely be less than in the targeted population of prescription users, the cost-effectiveness of OTC primary prevention is also likely to be even worse than that found in the study by Prosser et al. And although the unit price for OTC statins would probably be less than that of the prescription drug, we have no way of estimating the “crossover” point for price that would put OTC primary prevention into the cost-effective range, nor is there any assurance that the manufacturers would price the drug to achieve cost-effectiveness.

Although the debate on primary care with OTC statins has not yet led to any major changes in either regulatory or clinical policy, it has certainly opened up a range of fundamental and intriguing questions. After all, why shouldn’t people be able to take their medical care more actively into their own hands? OTC statins would be easier to obtain, and possibly less expensive, than the prescription drug; users could avoid the hassles involved in making visits to doctors; and thinking through the decision to use the drug on their own would force people to learn about the issues. And, as the free marketers argue, more choice “builds character” and makes people “better citizens.” But, like so many things whose worth seems, on the surface, to be indisputable, the case for primary prevention with OTC statins gets murky on deeper inspection. Consider, for example, the thousands (or tens of thousands) of low-risk but anxious people, half of them already taking low-dose aspirin, who are likely to take an OTC statin on their own for 1 or 2 years and then give up because there has been no obvious resultant change in their health. Having spent several hundreds of dollars apiece without realizing any tangible clinical benefit, would they really be “better citizens”?

“The availability of statins over-the-counter could prevent thousands of cardiovascular events that would otherwise occur”—maybe. “The risks associated with their use are reasonable, relative to their benefits”—possibly. “The information that potential users would need to make informed decisions about use is available”—apparently not. “We have the ability to get people to understand that information adequately”—uncertain. “The associated costs are worth the gains, both on an absolute scale and relative to the costs of other medical interventions”—highly doubtful.

Do I regret my vote against approval? So far, I do not.

I have no financial or other conflicts to declare.

References
PHARMACOGENETICS AND GENOMICS

Ser49Gly of $\beta_1$-adrenergic receptor is associated with effective $\beta$-blocker dose in dilated cardiomyopathy

Objective: Our objective was to evaluate the influence of polymorphisms at codons 49 and 389 of the $\beta_1$-adrenergic receptor ($\beta_1$-AR) on the response to $\beta$-blockers and outcome in patients with dilated cardiomyopathy.

Methods: We genotyped both codons of the $\beta_1$-AR in 375 patients with dilated cardiomyopathy and 492 control subjects.

Results: Neither of the polymorphisms was associated with susceptibility for dilated cardiomyopathy. In a retrospective analysis of patients receiving $\beta$-blockers, there was a significant association between long-term survival rate and codon 49 ($P = .014$) but not codon 389 ($P = .08$). Despite a similar mean heart rate (69 beats/min), patients with the Ser49 genotype tended to have higher doses of $\beta$-blockade compared with Gly49 carriers ($P = .065$). In patients receiving a low dose of $\beta$-blockade ($\leq50\%$ of targeted full dose), the 5-year mortality rate was lower among Gly49 carriers than Ser49 patients (risk ratio [RR], 0.24; 95% confidence interval [CI], 0.07-0.80; $P = .020$). In patients receiving high doses of $\beta$-blockers, there was no significant difference in outcome between genotypes ($P = .20$), which was attributable to a better outcome for Ser49 patients treated with a high dose of $\beta$-blockade as compared with a low dose. Gly49 carriers had a similar survival rate with different doses of $\beta$-blockers. With low-dose $\beta$-blockers, both codon 49 (RR, 0.26; 95% CI, 0.08-0.89; $P = .029$) and codon 389 (RR, 2.42; 95% CI, 1.04-5.63, $P = .039$) were related to 5-year mortality rate.

Conclusion: In patients with heart failure, the influence of codon 49 on the outcome and effect of $\beta$-blockers appeared to be more pronounced than that of codon 389. The more common Ser49Ser genotype responded less beneficially to $\beta$-blockade and would motivate genotyping to promote higher doses for the best outcome effect. (Clin Pharmacol Ther 2005;78:221-31.)

Yvonne Magnusson, PhD, Malin C. Levin, PhD, Robert Eggertsen, MD, PhD, Ernst Nyström, MD, PhD, Reza Mobini, PhD, Maria Schaufelberger, MD, PhD, and Bert Andersson, MD, PhD Göteborg, Sweden

From the Wallenberg Laboratory for Cardiovascular Research and Departments of Primary Health Care, Endocrinology, and Cardiology, Sahlgrenska Academy at Göteborg University.

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Reprint requests: Yvonne Magnusson, PhD, Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska Academy at Göteborg University, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden.

E-mail: yvonne.magnusson@wlab.gu.se

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The importance of the sympathetic nervous system in disease progression of heart failure and dilated cardiomyopathy is evident, and blockade of β1-adrenergic receptor (β1-AR)–mediated effects improves the long-term prognosis for these patients.1,2 Although β-blockers have documented beneficial effects, there is a significant amount of interindividual variability in response to therapy. This variability in response to therapy, as well as in susceptibility and progression of disease, may be found in our natural genetic background.

Polymorphisms are present at codons 49 and 389 of β1-AR, resulting in a Ser49Gly polymorphism and an Arg389Gly polymorphism, respectively.3 Despite in vitro studies demonstrating functional differences between the Arg389 and Gly389 receptors, their role in clinical manifestation has been less forthcoming, especially in heart failure.4-6 The polymorphism at codon 49 has also been found to display functional differences between the Ser49 and Gly49 receptors and to be associated with prognosis in dilated cardiomyopathy.7,8 Patients carrying the Gly49 allele had a more than 2-fold better outcome compared with patients with the Ser49 receptor.7 A possible explanation for this influence was suggested by 2 separate recombinant cell studies, in which the Gly49 receptor was faster and more strongly desensitized on long-term sympathetic activation, supporting the paradigm that desensitization is beneficial rather than deleterious in the failing human heart.8-10 With regard to the response to β-blockers, an influence of the Ser49Gly polymorphism of β1-AR was suggested by a somewhat better treatment effect in Gly49 carriers than in Ser49 patients and by a stronger inverse agonism of metoprolol in Gly49 than in Ser49 recombinant cells or human myocardium.7,8 Recently, it was reported that codon 389 had an influence on the response to carvedilol, whereas in another study this codon was found not to influence the response to metoprolol.11,12 However, smaller studies have shown an influence of codon 389 on the response to β-blockers in healthy and hypertensive subjects.13,14 The aim of our study was to evaluate the influences of polymorphisms at codon 49 and codon 389 of β1-AR on the response to β-blockers in patients with dilated cardiomyopathy, with a focus on long-term survival rates.

METHODS

Study populations. Patients with idiopathic dilated cardiomyopathy were recruited from a cohort of patients with idiopathic heart failure (n = 185) (group 1) and a prospective multicenter study of dilated cardiomyopathy (n = 190) (group 2). Group 1 consisted of patients from an epidemiologic survey of idiopathic dilated cardiomyopathy in 5 counties of Western Sweden during 1985-1988. The procedure and clinical results from this investigation have been presented in detail previously.15,16 In brief, 185 patients with heart failure without a known cause were studied. Of these patients, 61 (33%) had echocardiographic signs of dilated cardiomyopathy (ie, ejection fraction <0.50 and dilation of left ventricle). These patients have been genotyped for polymorphisms at codon 49 of β1-AR and evaluated with regard to outcome in a previous report.7 Group 2 consisted of patients from a prospective national registry of idiopathic dilated cardiomyopathy. Patients were recruited consecutively starting in May 1997 from 7 Swedish hospitals (a complete list of participating investigators in the Swedish Registry of Dilated Cardiomyopathy appears at the end of the article). The majority of the patients were recruited from the western region of Sweden (64%). Patients with a medical history and investigations compatible with idiopathic dilated cardiomyopathy were included (left ventricular dilatation and ejection fraction <0.50). The following causes of heart failure were excluded: ischemic heart disease (coronary angiography to rule out significant coronary artery disease was performed in all patients aged ≥30 years); hypertension (systolic blood pressure >170 mm Hg or diastolic blood pressure >100 mm Hg); significant valvular disease; significant systemic infection; excessive alcohol consumption; insulin-treated diabetes mellitus; endocrine disorders such as pheochromocytoma, acromegaly, and thyroid disease; systemic diseases; cancer treatment including irradiation; and tachycardia-induced cardiomyopathy or other primary cardiomyopathy. In group 1 information about treatment and dosage of β-blockers was available in all patients at the time of inclusion. Furthermore, 2 and 5 years after inclusion, group 1 patients were sent questionnaires that asked about current medication. In group 2 investigators gathered information regarding whether patients were treated with β-blockers at the time of inclusion. For the purpose of this study, we checked files and records for all patients at the largest center (Sahlgrenska University Hospital, Göteborg, Sweden) to obtain information about doses at the time of inclusion and during follow-up. In 69 of 114 patients (61%) treated with β-blockers in group 2, it was possible to extract such details about treatment. The possible influence of genotype on treatment and doses of β-blockers was analyzed according to treatment at the time of
inclusion (day 0 of follow-up). Long-term prognosis was evaluated after 5 years of follow-up. All patients in group 1 were followed up for 5 years, and patients in group 2 were followed up for 37 ± 19 months. Death or cardiac transplantation was considered an endpoint. National census data and transplantation registries were used for endpoint classification.

The control group consisted of 492 individuals from Western Sweden. They were selected through a random procedure, by use of the population registry of Göteborg (approximately 450,000 inhabitants) and the Mölnlycke community (approximately 14,000 inhabitants), and were invited by a personal letter or a telephone call to undergo an examination at the Sahlgrenska University Hospital or at the Primary Health Care Centre, Mölnlycke, Sweden. The study complied with the Declaration of Helsinki. All patients and control subjects had given informed consent, and the study was approved by the Ethics Committee at Göteborg University, Göteborg, Sweden.

**Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood by use of the QIAamp Blood Kit (Qiagen, Valencia, Calif). Polymerase chain reaction (PCR) was performed to amplify 2 fragments encompassing the entire β1-AR gene.** The PCR fragments were purified by use of Centricon-100 columns (Amicon, Bedford, Mass) and subjected to cycle-sequencing by use of overlapping primers and the ABI PRISM BigDye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, Calif) according to the manufacturer’s protocol. For amplification of nucleotide position 1-794, primers were used as previously described.\(^7\) The 1-794 fragment was cycle-sequenced by use of the forward primers 5′-ATG GGC GCG GGG GTG CTC GTC 3′ and 5′-ATC ACC TCG CCC TTC CGC TAC 3′ and the reverse primers 5′-GAA ACG GCG CTC GCA GCT GTC 3′ and 5′-GGC GAT GGC CAC GAT CAC 3′ for 25 cycles at 98°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute. The nucleotide position 742-1437 was amplified by use of the forward primers 5′-CGC TAC GC 3′ and the reverse primers 5′-CCC TAC GC 3′. The reaction contained 250 ng genomic DNA, 150-nmol/L primers, 200-μmol/L deoxyribonucleoside triphosphates, 2-mmol/L magnesium chloride, 20-mmol/L ammonium sulfate, 75 mmol/L Tris–hydrochloric acid, 0.01% (wt/vol) Tween (Merck, Darmstadt, Germany), 2.5 U Taq polymerase (Invitrogen, Carlsbad, Calif), and 15% dimethylsulfoxide. The cycling conditions were 94°C for 4 minutes, followed by 38 cycles (94°C for 1 minute, 65°C for 1 minute, and 71°C for 1 minute) and final extension at 71°C for 7 minutes. The segment was digested with 10 U MvaI at 37°C for 1 hour and resolved on 3% agarose gel stained with ethidium bromide.

**Automated deoxyribonucleic acid sequence analysis.** Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood by use of the QIAamp Blood Kit (Qiagen, Valencia, Calif). Polymerase chain reaction (PCR) was performed to amplify 2 fragments encompassing the entire β1-AR gene. The PCR fragments were purified by use of Centricon-100 columns (Amicon, Bedford, Mass) and subjected to cycle-sequencing by use of overlapping primers and the ABI PRISM BigDye Terminator Ready Reaction Mix (Applied Biosystems, Foster City, Calif) according to the manufacturer’s protocol. For amplification of nucleotide position 1-794, primers were used as previously described.\(^7\) The 1-794 fragment was cycle-sequenced by use of the forward primers 5′-ATG GGC GCG GGG GTG CTC GTC 3′ and 5′-ATC ACC TCG CCC TTC CGC TAC 3′ and the reverse primers 5′-GAA ACG GCG CTC GCA GCT GTC 3′ and 5′-GGC GAT GGC CAC GAT CAC 3′ for 25 cycles at 98°C for 30 seconds, 50°C for 15 seconds, and 60°C for 4 minutes. The nucleotide position 742-1437 was amplified by use of the forward primers 5′-TTC CGC GAG GCG CAC AAG CAG 3′ and the reverse primer 5′-CTA CAC CTI GGA TTC CGA GGC GCC 3′ and the reverse primer 5′-CTA CAC CTI GGA TTC CGA GGC GCC 3′ and the reverse primer 5′-CTA CAC CTI GGA TTC CGA GGC GCC 3′ and the reverse primer 5′-CTA CAC CTI GGA TTC CGA GGC GCC 3′.

**Statistical analyses.** The chi-square test was used to evaluate deviation of genotype distribution from Hardy-Weinberg equilibrium. The linkage disequilibrium coefficient (D′) was calculated according to Lewontin.\(^17\) Homozygotes of the common variant at each codon are referred to as Ser49 and Arg389 subjects, patients, or receptors, whereas heterozygotes in combination with homozygotes of the less common variant are referred to as Gly49 (Gly49 + Ser49Gly) and Gly389 (Gly389 + Arg389Gly) carriers. Logistic regression was performed to estimate odds ratios (ORs) with 95% confidence intervals (CIs) of genotype frequencies. It was estimated that a total of 275 cases in each group (control subjects and patients) would detect a difference with an OR of 2.0, a significance of P <
Sequence analysis of the entire $\beta_1$-AR gene of 50 individuals revealed no further missense variants other than those previously reported at codon 49 and codon 389 of $\beta_1$-AR (data not shown). Thus the report of Podlowski et al\textsuperscript{18} claiming the occurrence of 5 additional missense polymorphisms in the $\beta_1$-AR gene could not be confirmed by us.

Genotypes at codon 49 and codon 389 of $\beta_1$-AR were determined in 867 subjects (375 patients with dilated cardiomyopathy and 492 control subjects). Baseline characteristics of patients and control subjects are given in Table I. The allele frequencies were 0.17 and 0.18 for Gly49 and 0.23 and 0.26 for Gly389 in patients and control subjects, respectively (not significant). A logistic regression analysis, in which genotypes were treated as categorized variables, did not show an increased risk for the development of dilated cardiomyopathy for either of the $\beta_1$-AR variants (heterozygous treated as corresponding homozygous minority variant) (Table II). The distribution frequencies were not different between the 2 patient groups (data not shown). A linkage disequilibrium between codons 49 and 389 was found (estimated as $D^\prime = 0.26$). This was illustrated by Gly49 carriers being 2 times more common among Arg389 homozygous subjects than Gly389 carriers (42% versus 22%, respectively). Furthermore, all subjects homozygous for Gly389 were also homozygous for Ser49, and all subjects homozygous for Gly49 were also homozygous for Arg389.

$\beta_1$-AR polymorphisms and outcome. For patients in group 1, we have previously shown an influence of polymorphism at codon 49 on long-term outcome independent of other clinical and prognostic variables, including treatment with $\beta$-blockers and angiotensin-converting enzyme inhibitors.\textsuperscript{7} In the current study polymorphism at codon 389 of $\beta_1$-AR was also included in the analysis to test its influence in this previous group (group 1) and in a more recent group with dilated cardiomyopathy (group 2). By use of a multivariate Cox regression analysis in group 1, including both polymorphisms, codon 49, but not codon 389, was found to be significantly associated with outcome (Table III). The 5-year mortality risk was lower among Gly49 carriers than Ser49 patients but similar among Arg389 patients and Gly389 carriers. After ejection fraction, serum creatinine, systolic blood pressure, heart rate, gender, and age were controlled for, the influence of codon 49 remained significant ($P = .034$).

### Table I. Baseline characteristics of patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients with idiopathic dilated cardiomyopathy (n = 375)</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1 (n = 185)</td>
<td>Group 2 (n = 190)</td>
<td>Control subjects (n = 492)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>138</td>
<td>162</td>
<td>234</td>
</tr>
<tr>
<td>Female</td>
<td>46</td>
<td>28</td>
<td>258</td>
</tr>
<tr>
<td>Age (y)</td>
<td>57 ± 12</td>
<td>52 ± 11</td>
<td>58 ± 9</td>
</tr>
<tr>
<td>EF (%)</td>
<td>41 ± 17</td>
<td>28 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>77 ± 17</td>
<td>75 ± 16</td>
<td>67 ± 11 (n = 416)</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>133 ± 25</td>
<td>121 ± 21</td>
<td>138 ± 19</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>39%</td>
<td>99% (138/140)</td>
<td>Not available</td>
</tr>
<tr>
<td>$\beta$-Blockers</td>
<td>38%</td>
<td>83% (116/140)</td>
<td>8%</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>27 ± 5.4</td>
<td>26 ± 5.2</td>
<td>26 ± 3.9 (n = 324)</td>
</tr>
<tr>
<td>Coronary angiography</td>
<td>28%</td>
<td>91% (164/181)</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

EF, Ejection fraction; ACE, angiotensin-converting enzyme; BMI, body mass index.

.05, and a statistical power of 95% if the prevalence was 35%. A multivariate Cox proportional hazards model was used to explore associations between genotype and mortality rate. Analyses were performed and included the 2 codon polymorphisms of $\beta_1$-AR if not stated otherwise. Risk evaluation was expressed in terms of risk ratios (RRs) with 95% CIs. The Kaplan-Meier method was used to construct survival curves, and differences between curves were evaluated with the log-rank test. Statistical analyses were performed with SPSS statistical software (version 11.0; SPSS, Chicago, Ill). Data are presented as mean ± SD or as median and interquartile range as appropriate.
In group 2 the risk reduction among Gly49 carriers appeared to be of the same degree but without statistical significance (RR, 0.56; 95% CI, 0.19-1.69; \( P/11005 \).31).

The 3-year mortality risk was only 9% in group 2 and was dramatically better than in less recent populations with dilated cardiomyopathy.

In group 2 the risk reduction among Gly49 carriers appeared to be of the same degree but without statistical significance (RR, 0.56; 95% CI, 0.19-1.69; \( P/11005 \).31). The 3-year mortality risk was only 9% in group 2 and was dramatically better than in less recent populations with dilated cardiomyopathy.

**\( \beta_1 \)-AR polymorphism and response to \( \beta \)-blockers.** In the combined group of patients with \( \beta \)-blockers (from groups 1 and 2, \( n = 184 \)), codon 49, but not codon 389, was found to be significantly associated with outcome (\( P = .014 \) and \( P = .08 \), respectively) (Table III). In 139 of these 184 patients, data concerning type and actual dose of \( \beta \)-blocker were available (70 patients in group 1 and 69 patients in group 2). As shown in Fig 1, patients receiving less than 50% of the full dose (ie, 100 mg/d metoprolol, 5 mg/d bisoprolol, or 25 mg/d carvedilol) were more frequently Gly49 carriers, whereas those receiving doses above 50% of the full dose (in particular, >75% of full dose) were more frequently found to be Ser49 patients (\( P = .08 \)). This relationship between genotype and dose of \( \beta \)-blocker also became apparent for heart rate. Although there was no difference in mean heart rate between codon 49 genotypes (69 beats/min), the actual \( \beta \)-blocker dose tended to be higher in Ser49 patients than in Gly49 carriers (\( P = .065 \)) (Fig 2).

The most significant influence of genotype on dose of \( \beta \)-blockers was found with regard to long-term survival rate (Fig 3). In patients treated with a low-dose \( \beta \)-blocker (\( \leq 50\% \) of full dose), the 5-year mortality risk was significantly lower among Gly49 carriers than Ser49 patients (RR, 0.24; 95% CI, 0.07-0.80; \( P = .020 \)) (Fig 3, A). Furthermore, in patients receiving a high-dose \( \beta \)-blocker, the 5-year mortality risk was not significantly different between Gly49 carriers and Ser49 patients (RR, 0.27; 95% CI, 0.04-2.04; \( P = .20 \)) (Fig 3, B). This appeared to be attributable to a better outcome for Ser49 patients receiving a high dose, as compared

### Table II. Genotype frequency in control subjects and patients with idiopathic dilated cardiomyopathy

<table>
<thead>
<tr>
<th>( \beta_1 )-AR polymorphisms</th>
<th>Dilated cardiomyopathy ((n = 375) ) [% (n)]</th>
<th>Control subjects ((n = 492) ) [% (n)]</th>
<th>OR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser49</td>
<td>68 (255)</td>
<td>68 (333)</td>
<td>1.05 (0.78-1.40)</td>
<td>.77</td>
</tr>
<tr>
<td>Ser49Gly</td>
<td>29 (110)</td>
<td>30 (148)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly49</td>
<td>3 (10)</td>
<td>2 (11)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Codon 389</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly389</td>
<td>4 (15)</td>
<td>5 (25)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Gly389Arg</td>
<td>37 (140)</td>
<td>41 (201)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg389</td>
<td>58 (218)</td>
<td>54 (266)</td>
<td>1.21 (0.92-1.61)</td>
<td>.17</td>
</tr>
</tbody>
</table>

For calculation of OR, heterozygotes were coded as Gly49 and Gly389, respectively.

\( \beta_1 \)-AR, \( \beta_1 \)-Adrenergic receptor; OR, odds ratio; CI, confidence interval.

### Table III. Association between \( \beta_1 \)-AR polymorphisms and 5-year mortality rate in patients with idiopathic dilated cardiomyopathy

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Multivariate Cox regression analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR (95% CI)</td>
</tr>
<tr>
<td>Group 1 ((n = 184))</td>
<td></td>
</tr>
<tr>
<td>Ser49Gly</td>
<td>0.48 (0.27-0.87)</td>
</tr>
<tr>
<td>Gly389Arg</td>
<td>1.17 (0.73-1.89)</td>
</tr>
<tr>
<td>Group 2 ((n = 150))</td>
<td></td>
</tr>
<tr>
<td>Ser49Gly</td>
<td>0.56 (0.19-1.69)</td>
</tr>
<tr>
<td>Gly389Arg</td>
<td>1.14 (0.47-2.78)</td>
</tr>
<tr>
<td>Combined group with ( \beta )-blockers* ((n = 184))</td>
<td></td>
</tr>
<tr>
<td>Ser49Gly</td>
<td>0.31 (0.12-0.79)</td>
</tr>
<tr>
<td>Gly389Arg</td>
<td>1.76 (0.94-3.29)</td>
</tr>
</tbody>
</table>

Genotypes were treated as categoric variables; heterozygotes were coded as Gly49 and Gly389, respectively.

RR, Risk ratio.

*Patients from groups 1 and 2 receiving \( \beta \)-blockers at baseline (inclusion in study).
with low-dose β-blockade, as shown in Fig 3, C. These low- and high-dose curves of Ser49 were significantly separated at 2 years \((P = .002)\) but not at 5 years \((P = .68)\). The risk for Ser49 patients receiving a high-dose β-blocker as compared with a low-dose β-blocker at 2 years was as follows: RR, 0.14; 95% CI, 0.03-0.62; \(P = .009\). The 5-year mortality risk was not different between those receiving a low-dose β-blocker and those without β-blockers among Ser49 patients: RR, 0.96; 95% CI, 0.56-1.68; \(P = .90\). On the contrary, Gly49 carriers tended to have a lower mortality risk when receiving a low-dose β-blocker as compared with no β-blocker treatment (RR, 0.32; 95% CI, 0.09-1.10; \(P = .07\)) but a similar mortality risk regardless of the dose of β-blockade (Fig 3, D). With low-dose β-blockers, there was also an influence of codon 389, with a higher 5-year mortality risk among Gly389 carriers than Arg389 patients (RR, 2.42; 95% CI, 1.04-5.63; \(P = .039\)), although the influence of codon 49 was somewhat stronger (RR, 0.26; 95% CI, 0.08-0.89; \(P = .029\)).

Finally, the influence of codon 49 on survival rate was analyzed in relation to the type of β-blocker. Codon 49 remained significant for metoprolol (RR, 0.22; 95% CI, 0.07-0.74; \(P = .014\); n = 109) but not for the group of patients treated with other β-blockers (RR, 0.47; 95% CI, 0.10-2.17; \(P = .33\); n = 78).

Associations between doses and outcomes were examined retrospectively, and dosing of β-blockers was done at the discretion of the treating doctors. There were changes in prescriptions during the study periods. As shown in Table IV, 68% of patients stayed within the original dose interval (77/113), 12% went from a low dose to a high dose, and 7% went from a high dose to a low dose. Although this resulted in a small increase in mean dose (median dose unchanged) within the low-dose interval, high- and low-dose intervals remained significantly separated during follow-up \((P < .001)\) (Table V).

**DISCUSSION**

This is the first study evaluating both polymorphisms of β\(_1\)-AR for their influence on the response to β-blockers and long-term outcome in patients with heart failure. Although some associations were found regarding codon 389 and β-blockers, the influence from codon 49 was stronger. Patients having the Gly49 variants appeared to have an excellent long-term survival rate even when treated with a low dose of β-blocker (half of targeted full dose). On the contrary, patients with the Ser49 receptor responded poorly at a low dose, at least in terms of outcome. With high-dose
β-blockade, however, the long-term survival rate was also improved for Ser49 patients. This effect was significant after 2 years of follow-up but not after 5 years, which is in agreement with earlier reports that β-blockers in heart failure can delay the time to transplantation or death by 2 to 3 years. These findings may suggest that patients with the more common Ser49 genotype would need higher doses to achieve optimal treatment effect and would still be worse off than Gly49 carriers. Intriguingly, these differences in outcome were present even though patients with different genotypes had similar resting heart rates. A recent population study reported a higher resting heart rate in cases with the Ser49 genotype. It would be anticipated that

Fig 3. Influence of β1-AR genotype on outcome in relation to doses of β-blockers. Kaplan-Meier curves depict the risk of endpoint (death or cardiac transplantation) in patients with dilated cardiomyopathy treated with different doses of β-blockers. A, Low dose of β-blockers (<50% of targeted full dose). Thick line, Ser49 patients; thin line, Gly49 carriers (homozygous or heterozygous forms). The curves were significantly different as assessed by the log-rank test: \( P = 0.012 \); risk ratio (RR), 0.21; 95% confidence interval (CI), 0.048 to 0.92. B, High dose of β-blockers (>50% of targeted full dose). Thick line, Ser49 patients; thin line, Gly49 carriers. The curves were not significantly different. C, Ser49 patients receiving no β-blockers, low-dose β-blockers, or high-dose β-blockers. The curves for no β-blockers and low-dose β-blockers were not significantly different. The curves for low-dose and high-dose β-blockers were significantly different at 2 years but not at 5 years (log-rank \( P = 0.002 \) and \( P = 0.68 \), respectively). D, Gly49 carriers receiving no β-blockers, low-dose β-blockers, or high-dose β-blockers. The curves for low-dose and high-dose β-blockers were not significantly different. A trend was observed between the curves for no β-blockers and low-dose β-blockers (log rank \( P = 0.057 \); RR, 0.32; 95% CI, 0.03-1.10).
the doctor treating the patient would titrate β-blockers to achieve appropriate heart rate reduction and thereby compensate for a higher heart rate in Ser49 patients, at least during rest. However, a higher degree of β-blockade might be observed during exercise or stress, which is not evident during rest.21

There was no significant influence from polymorphism at codon 389 on the response to β-blockers, except in the subgroup of patients with low-dose therapy, in whom the influence was even weaker than the influence from codon 49. This lack of codon 389 influence is in agreement with a recent report of heart failure patients treated with metoprolol.12 In another report of heart failure patients the response to carvedilol was found to be significantly better in homozygous Arg389 patients than in homozygous Gly389 patients.11 This corresponds with our study in terms of the better response among homozygous Arg389 patients than in homozygous Gly389 patients.11 This codon 389 of β1-AR seems better correlated with blood pressure response of β-blockers.13,14,23 Of note, the 49/389 haplotype was found to be more informative than 389 alone.24 However, another study could not show an influence on blood pressure response to β-blockers.24

We have previously shown the inverse agonism of metoprolol, causing reduced basal cyclic adenosine monophosphate production, to be significantly stronger in Gly49 than in Ser49 recombinant cells and human myocardium specimens.8,25 More than half of our patients were treated with metoprolol (58%), which is the reason why the observed clinical effect of β-blockers could depend, at least in part, on such an intrinsic property. In agreement, genotype differences at codon 49 remained significant for metoprolol (P = .014) but

<table>
<thead>
<tr>
<th>Table IV. Changes in β-blocker dose intervals during follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of patients alive and with known dose at follow-up</strong></td>
</tr>
<tr>
<td><strong>β-Blocker therapy at baseline</strong></td>
</tr>
<tr>
<td>Staying within original dose interval (low and high dose, respectively)</td>
</tr>
<tr>
<td>Change in interval from low dose to high dose</td>
</tr>
<tr>
<td>Change in interval from high dose to low dose</td>
</tr>
<tr>
<td>Withdrawn</td>
</tr>
<tr>
<td>No β-blocker therapy at baseline</td>
</tr>
<tr>
<td>Started receiving β-blockers later</td>
</tr>
<tr>
<td>77/113*</td>
</tr>
<tr>
<td>14/113†</td>
</tr>
<tr>
<td>8/113‡</td>
</tr>
<tr>
<td>14/113§</td>
</tr>
<tr>
<td>15/76</td>
</tr>
</tbody>
</table>

*No difference between groups 1 and 2.
†Mainly in group 2.
‡No difference between groups 1 and 2.
§Ten patients in group 1. Three patients in group 2 (all with Ser49Ser genotype) receiving low doses underwent transplantation shortly after withdrawal.

<table>
<thead>
<tr>
<th>Table V. Changes in β-blocker dose during study period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline dose (%)</strong></td>
</tr>
<tr>
<td><strong>Follow-up dose (%)</strong></td>
</tr>
<tr>
<td><strong>P value for baseline versus follow-up</strong></td>
</tr>
<tr>
<td>Low dose</td>
</tr>
<tr>
<td>50 (25-50) (n = 85)</td>
</tr>
<tr>
<td>50 (37.5-50) (n = 58)</td>
</tr>
<tr>
<td>.008</td>
</tr>
<tr>
<td>High dose</td>
</tr>
<tr>
<td>100 (75-100) (n = 54)</td>
</tr>
<tr>
<td>100 (75-100) (n = 41)</td>
</tr>
<tr>
<td>.47</td>
</tr>
<tr>
<td>P value for low versus high dose</td>
</tr>
<tr>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
| Doses are expressed as percent of full dose in patients receiving low doses (≤50% of full dose) and high doses (>50% of full dose). The follow-up dose corresponds to the dose at 2 years or the last observed dose in patients treated with a β-blocker. Because of nonnormal distribution, data are shown as median values and interquartile range.

Therefore the linkage between the 2 codons necessitates determination of both and the use of multivariate analysis when possible. Although β-blocker therapy is known to improve the survival rate in patients with severe heart failure, it has little effect on peak exercise oxygen consumption.22 Therefore the association between exercise capacity and codon 389 of β1-AR in patients with heart failure might not transfer to long-term outcome if patients are treated with β-blockers.6 Codon 389 of β1-AR seems better correlated with blood pressure response of β-blockers.13,14,23 Of note, the 49/389 haplotype was found to be more informative than 389 alone.14 However, another study could not show an influence on blood pressure response to β-blockers.24
not for the group of patients treated with other \( \beta \)-blockers \((P = .33)\). This is consistent with a recent report by Terra et al\textsuperscript{26} in which decomposition of heart failure during titration of metoprolol was found to be significantly more frequent among Ser49 patients than Gly49 carriers, as well as among Gly389 carriers compared with Arg389 patients. Carvedilol, a nonselective \( \beta \)-blocker with additional properties, has been shown to be equally beneficial in the treatment of heart failure.\textsuperscript{22} It could be speculated that patients might be responders or nonresponders depending on the genotype and the chosen \( \beta \)-blocker. If this holds true, more patients might respond to therapy if the right drug is chosen.

Although we found a beneficial influence of the Gly49 allele on the response to \( \beta \)-blockers, the possibility of an additive mechanism of “internal \( \beta \)-blockade” in Gly49 carriers cannot be ruled out. For example, previous associations with \( \beta_1 \)-AR polymorphisms have not been found to be confounded by \( \beta \)-blocker use.\textsuperscript{6,7,20} Furthermore, in a previous report from group 1, the survival rate of Gly49 carriers without \( \beta \)-blockers was of the same magnitude as that of Ser49 patients with \( \beta \)-blockers (all doses combined).\textsuperscript{7} A faster and stronger desensitization of the Gly49 receptor, as shown in 2 separate recombinant cell studies, is also consistent with an “internal blockade” because of the protective nature of desensitization, at least in conjunction with heart failure.\textsuperscript{8-10} However, our study did not have the power to address genotype influence in the absence of \( \beta \)-blockers.

\( \beta_1 \)-AR polymorphism and risk of heart failure. Because of the relatively large sample of patients and control subjects, susceptibility to dilated cardiomyopathy could be evaluated with sufficient power. The polymorphisms at codons 49 and 389 of \( \beta_1 \)-AR were equally distributed among patients and control subjects, leading to the conclusion that none of these polymorphisms could be a major determinant of dilated cardiomyopathy. To statistically verify a smaller increase in risk (eg, 1.2 as observed for Arg389 patients), the sample size would largely exceed what would be reasonable from a practical and economic point of view and yet be of little clinical relevance. The distributions were in agreement with previous reports in white subjects with or without cardiovascular disease but were somewhat different from those reported in an Asian population, which displayed a lower frequency of Gly49 carriers, and in a black population, which displayed a higher frequency of Gly389 carriers.\textsuperscript{4,5,20,27}

Study limitations. This study was a retrospective analysis, and the final titrated dose of \( \beta \)-blocker might depend on other factors, such as the severity of heart failure in individual patients, actual heart rate, blood pressure, and possible noncardiac side effects. Patients with severe heart failure could be more sensitive to \( \beta \)-blockers, which could lead to a lower final dose.\textsuperscript{28} Although therapy was altered during the study period in a number of patients, our data indicated that most patients remained within respective low- and high-dose intervals and that a statistically significant difference in doses of \( \beta \)-blockers remained between low- and high-dose intervals at the end of follow-up. Furthermore, we did not have access to details about treatment in all patients. Another consideration is the activity of the metoprolol metabolizer cytochrome P450 (CYP) 2D6, which varies markedly among individuals as a result of several polymorphisms. In a white population the frequency of poor metabolizers has been estimated to be approximately 7%.\textsuperscript{29} However, one third of these would have early adverse effects with metoprolol, leading to discontinuation of therapy.\textsuperscript{30} Given that 58% of our patients were treated with metoprolol, a possible influence from poor metabolizers on the actual report could be 3% at the most, if all corresponding 9 alleles of CYP2D6 (which in pairs cause poor metabolizers) are in close linkage with the Gly49 allele. This is unlikely, but the true impact of poor metabolizers on our results needs to be investigated. Long-term outcome differed among our 2 study groups, separated in time by more than 10 years. The more consistent use of \( \beta \)-blockers is a likely reason for this improvement, as we have reported in another population with mixed heart failure.\textsuperscript{31} Although the statistical power was too low to prove associations between genetics and total survival rate in group 2, it could not be excluded that patients at higher risk (eg, older patients or patients from other populations) might have differences in outcome related to genotype.

Conclusion. We found significant associations between polymorphism at codon 49 of \( \beta_1 \)-AR and the effect on outcome of different doses of \( \beta \)-blockers in patients with heart failure. The more common Ser49Ser genotype responded less beneficially to \( \beta \)-blockade, which would motivate genotyping to promote higher doses for the best outcome effect. Prospective studies are needed to confirm these associations.
References


Human β₂-adrenergic receptor gene haplotypes and venodilation in vivo

Background and Objective: β₂-Adrenergic receptors (β₂-ARs) are polymorphic. In vitro studies have shown that agonist-promoted down-regulation is enhanced for Arg16Gly and blunted for Gln27Glu β₂-AR variants; Thr164Ile β₂-ARs exhibit reduced responsiveness to agonist stimulation. Our objective was to determine whether β₂-AR polymorphisms affect β₂-AR-mediated venodilation in healthy subjects in vivo.

Methods: We studied dilation of phenylephrine-preconstricted dorsal hand veins induced by terbutaline (50-1000 ng/min) using the Aellig hand vein technique in subjects homozygous for the 3 most common β₂-AR haplotypes (group A, Arg16Gln27Thr164 [wild type (WT)] [n = 10]; group B, Gly16Gln27Thr164 [n = 8]; and group C, Gly16Glu27Thr164 [n = 9]) and in 8 subjects homozygous for Thr164Ile β₂-AR (group D) at baseline and after 2 weeks of treatment with oral terbutaline, 5 mg 3 times daily.

Results: Terbutaline dose-dependently dilated hand veins; sensitivity to terbutaline was 2-fold higher in haplotype group A versus group B or C; maximal dilation, however, was not haplotype-dependent. In Thr164Ile subjects terbutaline sensitivity but not maximal dilation was 4-fold lower than in WT subjects. Long-term terbutaline treatment desensitized venous β₂-AR in a haplotype-dependent manner: The extent of desensitization (reduction in maximal venodilation) was largest for haplotype A, modest for haplotype B, and almost absent for haplotype C. Long-term terbutaline treatment also desensitized venous Thr164Ile β₂-AR; after terbutaline treatment, dose-response curves for terbutaline-induced venodilation were superimposable in WT and Thr164Ile β₂-AR subjects.

Conclusion: β₂-AR-mediated dilation of human hand veins is influenced by the 3 most common β₂-AR haplotypes and blunted in subjects homozygous for Thr164Ile β₂-AR. Long-term terbutaline treatment desensitizes venous β₂-AR in a haplotype-dependent manner, with haplotype A (Arg16Gln27Thr164) showing greater desensitization than haplotype B (Gly16Gln27Thr164), which shows greater desensitization than haplotype C (Gly16Glu27Thr164). (Clin Pharmacol Ther 2005;78:232-8.)

Heike Bruck, MD, Kirsten Leineweber, PhD, Jinny Park, BSc, Melanie Weber, Gerd Heusch, MD, Thomas Philipp, MD, and Otto-Erich Brodde, PhD Essen, Germany

β₂-Adrenergic receptors (β₂-ARs) play an important role in the regulation of vascular and bronchial smooth muscle tone, glands, lymphocytes, and hepatocytes. They also exist in the human heart and contribute to the regulation of heart rate and contractility. β₂-ARs are polymorphic; at least 3 functionally important single-nucleotide polymorphisms have been described—Arg16Gly, Gln27Glu, and Thr164Ile. In vitro, functional properties of Gly16 and Glu27 β₂-AR variants do not differ from those of wild-type (WT) β₂-AR (Arg16Gln27Thr164). In contrast, the Ile164 β₂-AR variant exhibited extensive signaling defects. Thus isoproterenol hydrochloride (INN, isoprenaline), epinephrine, and norepinephrine had a 4-fold lower affinity for Ile164 β₂-AR transfected in Chinese hamster fibroblasts (CHW) cells than for WT β₂-AR. Moreover, the Ile164 variant exhibits reduced basal and agonist-induced activation of adenyl cyclase and a rightward shift of the agonist concentration-effect curve, suggesting a diminished β₂-AR-G protein interaction. In vivo, in humans heterozygous for Thr164Ile β₂-AR (homozygous Ile164 variants are not found in the human population), cardiac and venous β₂-AR responses were blunted compared with responses in subjects carrying homozygous Thr164.
Arg16Gly and Gln27Glu \( \beta_2 \)-ARs differ in their susceptibility to agonist-induced receptor down-regulation. Thus in vitro studies showed that the Gly16 \( \beta_2 \)-AR variant undergoes significantly enhanced agonist-promoted down-regulation compared with Arg16 (WT) \( \beta_2 \)-AR. In contrast, the Glu27 \( \beta_2 \)-AR variant seems to be resistant against agonist-promoted \( \beta_2 \)-AR down-regulation. 6 Gly16Glu27 double-mutant receptors, however, demonstrated that Gly16 effects dominate over Glu27 effects. These receptors underwent even greater agonist-promoted down-regulation than Gln27 \( \beta_2 \)-AR. In contrast, Arg16Glu27 double-mutant \( \beta_2 \)-ARs were completely resistant to down-regulation. However, because of the strong linkage disequilibrium between codons 16 and 27,11 subjects homozygous for Glu27 are nearly always homozygous for Gly16; haplotype Arg16Glu27 occurs naturally extremely rare (present in \(<1\%\) of population 3-5). Thus position 16 in fact determines the phenotype of the 3 haplotypes Arg16Gln27, Gly16Gln27, and Gly16Glu27 with regard to agonist-induced receptor down-regulation.

We recently failed to demonstrate in vivo “resistance against agonist-induced down-regulation” in subjects homozygous for Glu27 \( \beta_2 \)-AR: Two weeks of treatment with oral terbutaline, 5 mg 3 times daily, caused desensitization of cardiac \( \beta_2 \)-AR responses and down-regulation of lymphocyte \( \beta_2 \)-AR densities that were not significantly different in subjects with the 3 most common \( \beta_2 \)-AR haplotypes (WT, Gly16Gln27Thr164, or Gly16Glu27Thr164).12,13

The aim of this study was to find out whether \( \beta_2 \)-AR polymorphisms affect agonist-induced desensitization of venous \( \beta_2 \)-AR. Therefore, in healthy subjects with the 3 previously mentioned \( \beta_2 \)-AR haplotypes (WT, Gly16Gln27Thr164, and Gly16Glu27Thr164), we studied the effects of 2 weeks of treatment with oral terbutaline, 5 mg 3 times daily, on terbutaline infusion-induced dilation of phenylephrine-preconstricted dorsal hand veins. Furthermore, hand vein response to terbutaline was studied in subjects heterozygous for Thr164Ile \( \beta_2 \)-AR.

**METHODS**

After screening of subjects for \( \beta_2 \)-AR polymorphisms (ie, Arg16Gly, Gln27Glu, and Thr164Ile) as described previously,12 10 subjects (5 women and 5 men) with WT \( \beta_2 \)-AR (group A), 8 subjects (6 women and 2 men) with haplotype Gly16Gln27Thr164 (group B), 9 subjects (3 women and 6 men) with haplotype Gly16Glu27Thr164 (group C), and 8 subjects (4 women and 4 men) heterozygous for Thr164Ile (group D) participated in the study (Table I) after having given informed written consent. The study protocol was approved by the Ethical Committees of the University of Halle-Wittenberg, Halle/Saale, Germany, and University of Essen, Essen, Germany.

All subjects were in normal health on the basis of cardiovascular and other medical history, physical examination, and biochemical, hematologic, and electrocardiographic screening. All subjects were drug-free despite the fact that all female participants were taking hormonal contraceptives. Subjects and investigators were blinded with regard to the genotype.

All subjects were studied under the same conditions, without breakfast, in the supine position on a comfortable bed in an air-conditioned room, in the morning. They were advised to avoid caffeine, alcohol, nicotine, and physical exercise before each experiment. The room temperature was kept stable between 24°C and 26°C.

Venous dilative responses were measured in the superficial dorsal hand vein by use of a linear variable differential transformer (LVD) (model 100 MHR; Schaevitz Engineering, Pensauken, NJ) according to the method of Aellig.14 The arm was placed on a support sloping upward at an angle of approximately 45° with the hand above the level of the heart so that complete emptying of superficial veins was ensured. A 23-gauge butterfly needle was inserted into a dorsal...
hand vein that was neither undercrossed nor over-
crossed and had no tributaries over a segment of 2 to 3
cm. The vein was kept open by physiologic saline
solution infusion. The cumulative infusion rate was
kept stable at 0.3 mL/min throughout experiments. The
LVDT was fixed on the vein with a small tripod ap-
proximately 1 cm above the end of the needle. The
vertical LVDT-core movement results in a voltage out-
put recorded by the venograph (VEG 91; Boucke & Co,
Reutlingen, Germany). Venodilation was measured by
inflating an occlusion cuff on the upper arm to 45 mm
Hg. Each measurement period consists of an 8-minute
infusion with a 4-minute deflation and another 4-minute
infusion of the cuff. The maximal diameter of the vein
during cuff inflation during saline solution infusion
 corresponded to 100% venodilation. After at least 4
stable baseline dilation measurements were obtained
during saline solution infusion, phenylephrine infusion
was started to preconstrict the dorsal hand vein. Veno-
constriction was expressed as the percentage reduction
in diameter of the hand vein from its maximal diameter
during cuff inflation during saline solution infusion
(100% preconstriction corresponding to 0% venodila-
tion). Phenylephrine hydrochloride (Neo-Synephrine;
Abbott Laboratories, Abbott Park, Ill) was given in
increasing doses (50-2200 ng/min with an infusion rate
of 0.1 mL/min and simultaneous coinfusion of 0.2
mL/min physiologic saline solution) until 60% precon-
striction was reached. This phenylephrine dose was then
maintained, and after preconstriction had been stable for at
least 4 measurements, simultaneous terbutaline infusion
was started. Terbutaline (Bricanyl; AstraZeneca, Wedel,
Germany) was administered in 5 increasing doses (50,
100, 200, 400, and 1000 ng/min with an infusion rate of
0.2 mL/min), and venodilation was measured. To ex-
clude systemic effects induced by terbutaline infusion,
heart rate and blood pressure were recorded simulta-
nously.

Venodilation response to infused terbutaline was in-
vestigated twice—before and after treatment for 2
weeks with oral terbutaline, 5 mg 3 times daily. The
protocol, which has been shown to evoke desensitiza-
tion of β2-AR responses in vivo,9,12,15 was as follows:
On entry into the study (day 1), terbutaline infusion-
induced venodilation was determined; thereafter sub-
jects received the first 5-mg terbutaline tablet. Terbutal-
ine treatment (5 mg 3 times daily at 7 AM, 2 PM, and 9
PM) was continued for 14 days. The last intake was on
day 15 at 7 AM; thereafter terbutaline infusion–induced
venodilation was assessed again.

All statistical calculations were performed with
GraphPad Prism 4.0 software (GraphPad Software, San
Diego, Calif). To test statistical significance, we ana-
lyzed dose-response curves of terbutaline-induced
venodilation by means of 2-way ANOVA with factors
haplotype group or study day and terbutaline dose with
the Bonferroni post hoc test for multiple comparisons.
Statistical differences between phenylephrine doses be-
tween groups and effects of 2 weeks of oral terbutaline
 treatment on phenylephrine doses within groups were
assessed by 1-way ANOVA with the Bonferroni post-
test. Terbutaline dose-response curves were analyzed by
nonlinear regression curve-fitting yielding ED50 (the
dose required to produce 50% of maximal response
[Emax]) by use of the Prism program. ED50 values were
expressed as geometric mean (95% confidence interval
[CI]). The logED50 was used for statistical analysis by
means of the 1-way ANOVA with the Bonferroni post-
test. P < .05 was considered statistically significant.

RESULTS
Thirty-five healthy subjects (18 women and 17 men)
participated in this study. The characteristics of the 3
β2-AR haplotype groups and the genotypes at codons
16 and 27 of the Thr164Ile β2-AR subjects are given in
Table I. The dose of phenylephrine needed to precon-
strict the dorsal hand vein by 60% did not differ sig-
ificantly between the 4 study groups or between fe-
male and male subjects, although the phenylephrine
dose tended to be lower in female subjects (Table II).

Terbutaline caused dose-dependent dilation of
phenylephrine-preconstricted hand veins (Fig 1). Max-
imal venodilation was not significantly different be-
tween haplotype groups A (Arg16Gln27Thr164), B
(Gly16Gln27Thr164), and C (Gly16Glu27Thr164).

However, sensitivity to terbutaline was signifi-
cantly higher (P < .001) in group A (ED50, 79.8
ng/min; 95% CI, 68.4-93.1 ng/min) than in group B
(ED50, 162.7 ng/min; 95% CI, 143-223.5 ng/min)
and group C (ED50, 183.8 ng/min; 95% CI, 143-
223.5 ng/min) (Fig 1). Thus subjects homozygous for
Arg16 had a higher sensitivity to terbutaline compared
with subjects homozygous for Gly16, irrespective of
codon 27 (Gln or Glu). When we compared female
versus male subjects, there was no gender-dependent
significant difference in terbutaline sensitivity, al-
though female subjects tended to have a higher terbutal-
ine sensitivity (data not shown).

In Thr164Ile subjects sensitivity to terbutaline was
significantly lower than in WT subjects. The ED50
value for terbutaline (344.4 ng/min; 95% CI, 295.5-
401.4 ng/min) was 4-fold higher in Thr164Ile subjects
than in WT subjects (79.8 ng/min; 95% CI, 68.4-93.1
ng/min) (P < .001). Maximal venodilation, however,
did not differ between Thr164Ile and WT β2-AR subjects (Fig 2). None of the Thr164Ile subjects had WT β2-AR (Arg) at position 16, but 7 of 8 were homozygous WT at position 27 (Gln) (Table I). We, therefore, compared ED50 values for terbutaline-induced venodilation of the 5 Thr164Ile subjects who were homozygous for Gly16 with those of group B (Gly16Gln27Thr164) and group C (Gly16Glu27Thr164). The ED50 value for these Thr164Ile subjects (489.7 ng/min; 95% CI, 412-581 ng/min) was again significantly higher (P < .001) than those for groups B and C. Thus subsensitivity of Thr164Ile β2-AR is in fact a result of the Ile substitution at position 16 and not Gly at position 16.

Oral terbutaline treatment, 5 mg 3 times daily for 2 weeks, desensitized dorsal hand vein β2-AR in a haplotype-dependent manner: The reduction in maximal venodilation (E max) induced by terbutaline was larger in Arg16Gln27Thr164 (WT) subjects (E max,
74% ± 3% before treatment and 58% ± 4% after treatment; *P < .001), moderate in Gly16Gln27Thr164 subjects (E_max, 75% ± 4% before treatment and 66% ± 8% after treatment; *P < .01), and almost absent in Gly16Glu27Thr164 subjects (E_max, 72% ± 4% before treatment and 69% ± 6% after treatment; *P = .054) (Fig 1). Thus the reduction in terbutaline-induced maximal venodilation (ie, desensitization) after terbutaline treatment was larger in subjects carrying an Arg at codon 16 than in subjects carrying a Gly at codon 16, although this did not reach statistical significance (*P = .27). It should be noted that there was no significant gender effect on desensitization (data not shown).

Terbutaline treatment also desensitized venous Thr164Ile β2-AR; interestingly, however, after terbutaline treatment, dose-response curves for terbutaline-induced venodilation were superimposable in WT and Thr164Ile β2-AR subjects (Fig 2).

Terbutaline treatment did not significantly affect the phenylephrine dose needed to preconstrict veins by 60%, although in most haplotype groups investigated the phenylephrine dose tended to be higher after the 2 weeks of treatment (Table II). This was especially true for the Gly16Glu27Thr164 haplotype, that is, the haplotype that was almost not desensitized. Thus we cannot exclude that there might be a systemic relaxing effect of oral terbutaline treatment that might influence the dose of phenylephrine needed for preconstriction.

Oral terbutaline treatment increased resting heart rate and decreased resting diastolic blood pressure, whereas systolic blood pressure was not much affected (data not shown). The dose range of phenylephrine used in this study exhibited only minimal systemic effects. Thus, during infusion of the individually highest dose of phenylephrine, there were slight, but not significant, increases (Δ mean ± SD) in heart rate (3 ± 2 beats/min), systolic blood pressure (5 ± 4 mm Hg), and diastolic blood pressure (3 ± 3 mm Hg). Similarly, infusion of the highest dose of terbutaline slightly, but not significantly, increased heart rate (4 ± 3 beats/min) and systolic blood pressure (3 ± 3 mm Hg) and decreased diastolic blood pressure (5 ± 4 mm Hg) compared with baseline levels; these effects were independent of β2-AR haplotype.

**DISCUSSION**

The first goal of this study was to find out whether β2-AR polymorphisms affect terbutaline-induced dorsal hand vein dilation. The results show that maximal venodilation appears to be genotype-independent; however, sensitivity to terbutaline in WT β2-AR subjects was higher than that in subjects with the Gly16Gln27Thr164 or Gly16Glu27Thr164 haplotype. Furthermore, subjects heterozygous for the Thr164Ile β2-AR variant showed blunted venodilation compared with subjects homozygous for Thr164.
Although the blunted response of the Thr164Ile β2-AR variant to β2-AR stimulation is in good agreement with recently published data for cardiac,8,9 and venous Thr164Ile β2-ARs,10 controversial data exist on the impact of Arg16Gly or Gln27Glu β2-AR variants on vascular responsiveness. In volunteer studies systemic application of β2-AR agonists resulted in greater vasodilation in subjects homozygous for Arg16, whereas local application into the brachial artery or hand vein evoked greater vasodilatory responses in subjects homozygous for Gly16.5

In our study, using local infusion of terbutaline in dorsal hand veins, we did not find significant influences of haplotypes on maximal venodilation. In contrast to the literature data,5 however, we found that subjects carrying a Gly at position 16 were significantly less sensitive to terbutaline than subjects carrying an Arg at position 16. The reason for these differences is not known; it might be a result of different tissues (hand vein16,17 [current study] versus forearm blood flow studies16,18) or different β-AR agonists being used (isoproterenol hydrochloride16-18 versus terbutaline [current study]).

The second goal of this study was to assess whether β2-AR polymorphisms may influence agonist-induced desensitization of venous β2-ARs in vivo. The results show that 2 weeks of terbutaline treatment (5 mg 3 times daily) caused desensitization of terbutaline infusion–induced venodilation in a β2-AR haplotype–dependent manner: Desensitization was largest in Arg16Gln27Thr164 subjects, smaller in Gly16Gln27Thr164 subjects, and almost absent in Gly16Glu27Thr164 subjects. Unfortunately, we could not measure terbutaline plasma levels. However, it is unlikely that these haplotype–dependent differences are a result of different terbutaline plasma levels, because Lima et al19 recently showed that the pharmacokinetics of terbutaline was not affected by β2-AR haplotypes.

The impact of codon 16 and 27 β2-AR variants on agonist-induced desensitization in vivo has been mainly studied for β2-AR agonist treatment of patients with asthma.5 Long-term treatment of asthma patients with β2-AR agonists evokes desensitization of both bronchoprotective and bronchodilator effects. Several recent studies demonstrated that patients homozygous for Arg16 or heterozygous for Arg16Gly exhibited greater β2-AR subsensitivity after long-term β2-AR agonist treatment compared with patients homozygous for Gly16.20-22 These findings are in agreement with a recently proposed dynamic model of receptor regulation,23 in which endogenous catecholamines dynamically desensitize β2-ARs in their basal state, and this occurs to a greater extent for Gly16 than for the Arg16 β2-AR variant. Accordingly, exogenous agonist-induced desensitization should then be greater for Arg16 than for Gly16 (which is already endogenously desensitized).

Only a few studies have investigated the impact of codon 16 and 27 β2-AR variants on agonist-induced desensitization in nonbronchial systems. Dishy et al17 found that a 2-hour continuous isoproterenol hydrochloride infusion into the hand vein caused strong desensitization of hand vein dilation in Arg16Gln27Thr164 β2-AR subjects whereas subjects homozygous for Gly16, irrespective of codon 27 (Gln or Glu), were rather resistant against desensitization. Our findings that venous Arg16Gln27Thr164 β2-ARs undergo strong agonist-induced desensitization whereas Gly16Gln27Thr164 and Gly16Glu27Thr164 β2-ARs undergo much less desensitization are in reasonable agreement with these data.

On the other hand, we recently showed in subjects with the same 3 β2-AR haplotypes that long-term terbutaline treatment (5 mg 3 times daily for 2 weeks) caused desensitization of cardiac and lymphocyte β2-ARs in a β2-AR haplotype–independent manner.12,13 These results clearly show that Glu27 resistance against agonist-promoted down-regulation, initially observed in vitro,6 does not occur in vivo, very likely because, as a result of linkage disequilibrium between codons 16 and 27,11 haplotype Arg16Glu27 (which was resistant in vitro against down-regulation) is nearly not natively expressed in humans (as discussed previously).

It appears that in hand veins and in bronchial smooth muscle Arg16 β2-ARs undergo stronger agonist-induced desensitization compared with Gly16 β2-ARs. On the other hand, cardiac and lymphocyte β2-ARs are desensitized in a haplotype–independent manner.12,13 The reason for this differential behavior is not known at this time; it might be a tissue-specific effect (vascular or bronchial smooth muscle versus nonvascular tissue).

Finally, oral terbutaline treatment did also desensitize venous Thr164Ile β2-ARs. Interestingly, after terbutaline treatment, venous β2-AR responses no longer differed between WT and Thr164Ile subjects. This is in good agreement with our recently published data showing that cardiac Thr164Ile β2-ARs also exhibited β2-AR responses after 2 weeks of terbutaline treatment that were nearly identical to those of WT β2-ARs.9 The current findings, therefore, support our hypothesis that either maximal terbutaline desensitization was obtained or Thr164Ile β2-ARs (which are already somewhat desensitized, as discussed previously) appear to be protected against agonist-induced desensitization.

In conclusion, in contrast to human cardiac and lymphocyte β2-ARs, in human hand veins terbutaline-
induced $\beta_2$-AR desensitization occurs in a $\beta_2$-AR haplotype–dependent manner. Desensitization was largest for haplotype Arg16Gln27Thr164, moderate for haplotype Gly16Gln27Thr164, and almost absent for haplotype Gly16Gly27Thr164. Accordingly, it is tempting to speculate that $\beta_2$-AR polymorphisms might affect agonist-induced desensitization in a tissue-specific manner. Finally, in subjects heterozygous for the Thr164Ile $\beta_2$-AR, not only cardiac but also venous responses to $\beta_2$-AR stimulation are blunted.

The authors have no conflict of interest to declare.

References
Individual and joint association of $\alpha_{1A}$-adrenergic receptor Arg347Cys polymorphism and plasma irbesartan concentration with blood pressure therapeutic response in Chinese hypertensive subjects

**Background:** Individual variability in the therapeutic response to an antihypertensive drug could have a genetic basis. We investigated whether the $\alpha_{1A}$-adrenergic receptor ($\alpha_{1A}$-AR) Arg347Cys polymorphism is associated with the blood pressure (BP) therapeutic response to irbesartan and whether the association could be altered by the plasma irbesartan level.

**Methods:** A total of 696 hypertensive subjects were treated with a daily oral dose of 150 mg irbesartan. Baseline BP was measured before the first dose. On the 28th day, after 27 consecutive days of treatment and an overnight fast, BPs and blood samples were obtained before the morning dose (0 hours) and 6 hours after the morning dose was taken. Plasma irbesartan concentrations were measured by use of HPLC-fluorescence.

**Results:** BP therapeutic response was defined as baseline BP minus BP on the 28th day of irbesartan treatment. Relative to noncarriers, $\alpha_{1A}$-AR Cys347 allelic carriers had a significantly greater diastolic blood pressure (DBP) response at 0 hours (mean $\pm$ SD, 7.5 $\pm$ 8.4 mm Hg versus 5.5 $\pm$ 8.4 mm Hg; $P = .016$) and at 6 hours (16.2 $\pm$ 9.1 mm Hg versus 14.2 $\pm$ 8.9 mm Hg, $P = .025$). Although the pattern was similar to the DBP response, $\alpha_{1A}$-AR Cys347 allelic carriers had only a moderately increased systolic blood pressure (SBP) response at the 2 time points. When subjects were stratified into subgroups with high or low plasma irbesartan concentrations (with the median value used as the cutoff point), Cys347 allelic carriers in the high-concentration group, relative to noncarriers, had a more pronounced DBP response at 0 hours (adjusted $\beta [\pm SE], 3.0 \pm 1.0$ mm Hg; $P = .004$) and at 6 hours (adjusted $\beta, 3.0 \pm 1.2$ mm Hg; $P = .014$), and the

Shanqun Jiang, PhD, Guangyun Mao, MD, Shanchun Zhang, PhD, Xiumei Hong, PhD, Genfu Tang, MD, Zhiping Li, MD, Xue Liu, MD, Yan Zhang, PhD, Binyan Wang, PhD, Xiping Xu, PhD, MD, MPH, and Xiaobin Wang, PhD, MD  *Hefei and Beijing, China, Boston, Mass, and Chicago, Ill*
same was true for the SBP response at 0 hours (adjusted $\beta$, 5.6 ± 2.1 mm Hg; $P = .006$) and at 6 hours (adjusted $\beta$, 4.7 ± 2.0 mm Hg; $P = .021$). In contrast, in the low-concentration group, there was no significant association between DBP or SBP responses and Arg347Cys genotypes at 0 hours and 6 hours. **Conclusion:** Our data suggest that the $\alpha_{1A}$-AR Arg347Cys polymorphism is associated with BP response (particularly DBP) to short-term irbesartan treatment. Our data also showed evidence of an interaction between the $\alpha_{1A}$-AR Arg347Cys polymorphism and the plasma level of irbesartan in relation to BP therapeutic response. The association of the Arg347Cys polymorphism with the BP therapeutic response was more pronounced in those patients with higher plasma concentrations of irbesartan. (Clin Pharmacol Ther 2005; 78:239-48.)

Irbesartan, a nonpeptide angiotensin II type 1 (AT1) receptor antagonist, acts at the final step of the renin-angiotensin system by selectively blocking the binding of angiotensin II to the AT1 receptor. Several clinical trials have revealed that among mild-to-moderate hypertensive patients who were randomized to oral treatment with 150 mg irbesartan once daily or placebo for 6 to 8 weeks only 56% of irbesartan-treated patients had a favorable response.1,2 Considerable variations were observed in the distribution of blood pressure (BP) responses to treatment at the usual therapeutic dosages. The reasons for this individual variability are largely unknown. $\alpha_1$-Adrenergic response was associated with BP response to treatment in hypertensive patients.3 Studies in monozygotic and dizygotic twins4 and in families5 suggest that the great variability in $\alpha_1$-adrenergic receptor ($\alpha_1$-AR)–mediated vascular response may be attributable to genetic factors. It is hypothesized that drug pharmacodynamic response may be related to those polymorphic candidate genes coding for the structure, configuration, activity, or quantity of drug-targeted receptors.

In this pharmacogenetic study we focus on gene encoding for $\alpha_1$-ARs, which primarily mediate vasoconstriction by sympathetic increase in peripheral vascular tone6,7 and regulate BP.8,9 Studies have shown that angiotensin II pretreatment to vascular smooth muscle cells (VSMCs) increases the rate of $\alpha_1$-AR de novo synthesis, enhances transcription and expression of $\alpha_1$-AR genes,10,11 and is associated with an increase in $\alpha_1$-AR–stimulated growth-related c-fos expression.10,11 The predominantly potentiating effect of angiotensin II on $\alpha_1$-AR–mediated periarterial electrical nerve–stimulating double-peaked vasoconstriction can be effectively inhibited by an AT1 receptor antagonist.12 An AT1 receptor antagonist administered to improve left ventricular hypertrophy also suppresses urinary catecholamine excretion and cardiac $\alpha_1$-AR density.13 Animal models have demonstrated that endothelium-independent desensitization or vascular deoxyribonucleic acid (DNA) synthesis stimulation induced by norepinephrine or angiotensin II on the smooth muscle contractile response is blocked separately by the AT1 receptor antagonist losartan or the $\alpha_1$-AR antagonist prazosin.14-16 Among 3 subtypes of adrenergic receptors ($\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$),17 the $\alpha_{1A}$-adrenergic receptor ($\alpha_{1A}$-AR) may be a major contributor to adrenergic vasoconstriction and systemic arterial pressure in sympathetic regulation of peripheral resistance in animals and humans.7,8 A common polymorphism of the $\alpha_{1A}$-AR gene, the substitution of a C residue for T at nucleotide 1441, results in the substitution of Cys for Arg at codon 347 (Arg347Cys).18 The palmitoylation site conferred by the Arg347Cys polymorphism in the carboxy-terminal tail of the $\alpha_{1A}$-AR gene may play a key role in its cellular localization and function.18 We investigated whether the $\alpha_{1A}$-AR Arg347Cys polymorphism is associated with the BP therapeutic response to irbesartan, an AT1 receptor antagonist. We were also interested in whether there was an interaction between the $\alpha_{1A}$-AR Arg347Cys polymorphism and the plasma irbesartan concentration on the BP therapeutic response to irbesartan.

**METHODS**

**Study population.** Patients with mild to moderate hypertension were enrolled from the Taihu community of the Anhui Province, China, from February 2003 to January 2004. The inclusion criteria were as follows: (1) having systolic blood pressure (SBP) between 140 mm Hg and 200 mm Hg or diastolic blood pressure (DBP) between 90 mm Hg and 120 mm Hg, (2) not currently taking antihypertensive medication (4 weeks before the study), and (3) having no secondary hypertension. To avoid potentially severe adverse effects or possible influences on irbesartan’s efficacy, patients who were pregnant or lactating and those who had hyperkalemia, severe arrhythmia, chronic heart failure, severe liver or renal dysfunction, or a history of myocardial infarction or stroke, acute coronary artery syndrome, or acute heart failure in the past 3 months were excluded. The study was approved by the Ethics Committee of Anhui Medical University, Hefei, China. The
purpose and procedures of the study were carefully explained to all participants, and written informed consent was obtained.

**BP measurement, blood sampling, and irbesartan administration.** Participants were invited to our research center, where they underwent a physical examination. After overnight fasting, at 8 AM on the next day, baseline BP was measured by trained nurses after participants rested in a seated position for 15 minutes. All BPs were measured with a standardized mercury sphygmomanometer with appropriately sized cuffs. SBP was recorded by Korotkoff phase I (appearance of sound), and DBP was recorded by Korotkoff phase V (disappearance of sound). Three consecutive measurements were taken at 30-second intervals between readings. If the difference between the measurements was more than 4 mm Hg, the patient was asked to rest for 5 minutes and the measurements were then retaken. In all of our analyses, the mean of the 3 consecutive BP readings was used.

Afterward, participants were given 150 mg irbesartan orally (Sanofi-Synthelabo Minsheng, Hangzhou, China). This dosage was chosen to treat all participants on the basis of considerations of safety and effectiveness. During the consecutive 27-day treatment period, the participants were required to take irbesartan around 8 AM and to record the time at which they took the drug, as well as any side effects. They were also invited to visit our local study site once a week to report any adverse effects and pick up the drugs for the next week. On the 27th day, the participants returned to our research center and stayed there overnight. On the next morning, at 8 AM, the corresponding 0- and 6-hour BPs were measured according to the same procedures as those performed at baseline. A fasting blood sample (0 hours) was taken, and a second blood sample was taken 6 hours after patients had taken the dose of irbesartan that morning. The plasma was separated and transferred to screw-capped polypropylene tubes and stored at −20°C until analysis.

**Plasma irbesartan concentration measurement.** Plasma samples that had been stored at −20°C were thawed and pretreated before the concentration of irbesartan was measured by the HPLC-fluorescence method. In brief, plasma samples were centrifuged at 4000g for 10 minutes after thawing at room temperature. An aliquot (400 µL) of plasma together with a 40-µL internal standard, flunarazine (Di-Nuo, Hunan, China), and 560 µL acetonitrile was pipetted into 1.5-mL polypropylene tubes. The mixture was blended in a vortex mixer for 30 seconds, incubated for 5 minutes at room temperature, and then centrifuged at 4000g for 20 minutes. The supernatant was pipetted and injected into the HPLC system. A fluorescence detector set at an excitation wavelength of 250 nm and an emission wavelength of 375 nm was used to detect the peak value of irbesartan. In analysis, solution and mobile phases were prepared just before use. The mobile phases consisted of acetonitrile/aqueous phosphoric acid–triethylamine solution (39:61 [vol:vol]); the latter was prepared by adding 1 mL triethylamine to 1000 mL double-distilled water and then adjusting the pH value to 4.2 with phosphoric acid. The analytic column was a Diamonsil C18 column (5 µm, 150 × 4.6 mm) (Agilent Technologies, Palo Alto, Calif). The flow rate was 1.5 mL⋅min⁻¹, and the column temperature was 30°C. Quantification of the metabolites was performed by comparing HPLC peak areas with those of authentic standards, with reference to an internal standard. To ensure the precision and accuracy of the assay, plasma quality-control samples were also prepared to contain 5 different irbesartan concentrations within the standard curve range and were analyzed with regard to intraday and interday means, SDs, and coefficients of variation.

**Genotyping.** Venous blood samples were collected from participants, and genomic DNA was then extracted by use of the QIAamp Blood Kit (Qiagen, Valencia, Calif) and stored at −20°C until the genotype analysis was performed. We searched the literature and dbSNP for all potential nonsynonymous single-nucleotide polymorphisms in the α1A-AR gene. Only the Arg347Cys locus was polymorphic in the Chinese population, and it was genotyped by use of the TaqMan genotyping assay designed and manufactured by Applied Biosystems (Foster City, Calif). Polymerase chain reaction products were amplified in a 5-µL reaction containing 10 ng genomic DNA, 1× master mix, 900-nmol/L forward and reverse primers, and two 250-nmol/L TaqMan MGB Probes with the use of 384-well plates on a PTC-225 Tetrad Thermal Cycler (MJ Research, Watertown, Mass) under the following conditions: 95°C for 10 minutes, 50 cycles at 92°C for 15 seconds, and 60°C for 1 minute. After polymerase chain reaction amplification, an endpoint plate reading of the fluorescence intensity of each well was performed on an ABI Primer 7900 system (Applied Biosystems). Genotype was scored automatically by use of SDS 2.1 software (Applied Biosystems) and inspected visually on the plot.

**Statistical analysis.** The SAS 8.0 software package (SAS Institute, Cary, NC) was used to perform all statistical analyses. BP response was defined as BP before the first dose minus BP on the 28th day. Because BP was measured twice on the 28th day (at 0 hours and 6 hours) after the morning dose of irbesartan was taken,
2 measures of BP response were generated for each study participant. We tested whether the genotypes of the α1A-AR Arg347Cys polymorphism were in Hardy-Weinberg equilibrium by use of a chi-square test. Because only 6 subjects were homozygous (Cys/Cys) for the Arg347Cys polymorphism and had the most pronounced BP response (especially DBP) to irbesartan at 0 hours and 6 hours, we combined Cys/Cys with the Arg/Cys heterozygous genotype in the analyses. Univariate associations between important covariates and Arg347Cys genotypes were investigated by use of chi-square and t tests. Plasma irbesartan concentrations skewed toward the left but showed an approximately normal distribution after natural logarithm transformation (data not shown). The definitions of subgroups with low or high plasma irbesartan concentrations were based on the median natural logarithm–transformed values at 0 hours and 6 hours. We tested the association of Arg347Cys genotypes with BP response (both SBP and DBP) at 0 hours and 6 hours individually and stratified by plasma concentration subgroups by use of generalized linear regression models, with adjustment for baseline BP, age, age squared, gender, body mass index, height, height squared, weight, alcohol consumption, cigarette smoking, education, and occupation. We then repeated this analysis while including both 0-hour and 6-hour BP responses (both SBP and DBP) in the same models using generalized estimation equations to accommodate correlations in BP responses at the 2 times for the same individual and adding a term for time of BP reading (0 hours or 6 hours).

RESULTS

In total, 696 subjects were enrolled with complete genotype and phenotype information. The genotype distribution conformed to Hardy-Weinberg equilibrium ($\chi^2 = 0.1, P = .757$). The demographic characteristics for the 2 genotype groups (α1A-AR Cys347 noncarriers and carriers) are shown in Table I. The means and prevalence of covariates including age, body mass index, height, weight, gender, alcohol consumption, and smoking status showed no significant differences between the 2 groups. The mean baseline SBP and DBP were 166.0 ± 16.8 mm Hg and 91.7 ± 10.4 mm Hg, respectively, in Cys347 noncarriers and 166.8 ± 16.4 mm Hg and 92.2 ± 10.5 mm Hg, respectively, in carriers. These findings suggested that the α1A-AR Arg347Cys polymorphism was not associated with baseline SBP or DBP measurements. After plasma irbesartan concentration values underwent natural logarithm transformation to approximate normal distributions, the natural logarithm values did not show marked differences in the 2 genotype groups at 0 hours (mean ± SD, 4.2 ± 0.7 ng/mL for noncarriers and 4.2 ± 0.6 ng/mL for carriers) or at 6 hours (5.9 ± 0.5 ng/mL for noncarriers and 5.9 ± 0.5 ng/mL for carriers). Relative

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cys347 noncarriers (n = 580)</th>
<th>Cys347 carriers (n = 116)</th>
</tr>
</thead>
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<tr>
<td>Age (y)</td>
<td>54.2 ± 7.0</td>
<td>53.4 ± 8.0</td>
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<tr>
<td>Height (cm)</td>
<td>156.4 ± 7.8</td>
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<tr>
<td>Weight (kg)</td>
<td>53.7 ± 8.4</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>21.9 ± 2.7</td>
<td>22.2 ± 2.9</td>
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<td>Drug concentration at 0 h (ng/mL)</td>
<td>78.3 ± 51.9</td>
<td>76.9 ± 41.7</td>
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<td>Natural logarithm–transformed concentration at 0 h</td>
<td>4.2 ± 0.7</td>
<td>4.2 ± 0.6</td>
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<tr>
<td>Drug concentration at 6 h (ng/mL)</td>
<td>430.7 ± 254.6</td>
<td>433.7 ± 264.2</td>
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<tr>
<td>Natural logarithm–transformed concentration at 6 h</td>
<td>5.9 ± 0.5</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>Baseline SBP (mm Hg)</td>
<td>166.0 ± 16.8</td>
<td>166.8 ± 16.4</td>
</tr>
<tr>
<td>Baseline DBP (mm Hg)</td>
<td>91.7 ± 10.4</td>
<td>92.2 ± 10.5</td>
</tr>
<tr>
<td>SBP response at 0 h (mm Hg)</td>
<td>18.2 ± 17.9</td>
<td>21.1 ± 18.0</td>
</tr>
<tr>
<td>DBP response at 0 h (mm Hg)</td>
<td>5.5 ± 8.4</td>
<td>7.5 ± 8.4*</td>
</tr>
<tr>
<td>SBP response at 6 h (mm Hg)</td>
<td>32.2 ± 18.4</td>
<td>35.1 ± 17.9</td>
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<tr>
<td>DBP response at 6 h (mm Hg)</td>
<td>14.2 ± 8.9</td>
<td>16.2 ± 9.1*</td>
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<td>Current</td>
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<tr>
<td>Alcohol status:</td>
<td>Current</td>
<td>Current</td>
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<tr>
<td>Occupation: Farmer</td>
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<td>99 (85.3)</td>
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<tr>
<td>Education: School or higher</td>
<td>67 (11.6)</td>
<td>16 (13.8)</td>
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</table>

Data are presented as mean ± SD or number and percent.

*Two-sided $P < .05$ by t tests.
Table II. Relative mean BP responses by α1A-AR polymorphisms and plasma irbesartan concentrations (modeled via generalized linear regression)

<table>
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<tr>
<th>BP response*</th>
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<th>6 h on 28th d</th>
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</thead>
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<td>β (±SE)</td>
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<td>Noncarrier</td>
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<tr>
<td></td>
<td>1 Carrier</td>
<td>110</td>
<td>21.3 ± 18.3</td>
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<tr>
<td></td>
<td>2 Carriers</td>
<td>6</td>
<td>17.5 ± 10.6</td>
</tr>
<tr>
<td></td>
<td>≥1 Carrier</td>
<td>116</td>
<td>21.1 ± 18.0</td>
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<tr>
<td>Low</td>
<td>Noncarrier</td>
<td>293</td>
<td>18.1 ± 19.1</td>
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<td>≥1 Carrier</td>
<td>52</td>
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<tr>
<td>High</td>
<td>Noncarrier</td>
<td>287</td>
<td>18.3 ± 16.5</td>
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<tr>
<td></td>
<td>≥1 Carrier</td>
<td>64</td>
<td>24.0 ± 16.9</td>
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<tr>
<td>ΔDBP</td>
<td>Overall</td>
<td>Noncarrier</td>
<td>580</td>
</tr>
<tr>
<td></td>
<td>1 Carrier</td>
<td>110</td>
<td>7.2 ± 8.0</td>
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<tr>
<td></td>
<td>2 Carriers</td>
<td>6</td>
<td>12.8 ± 13.7</td>
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<td>116</td>
<td>7.5 ± 8.4</td>
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<td>Low</td>
<td>Noncarrier</td>
<td>293</td>
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<td>287</td>
<td>5.4 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>≥1 Carrier</td>
<td>64</td>
<td>9.0 ± 8.4</td>
</tr>
</tbody>
</table>

BP, Blood pressure.
*BP response was defined as BP at baseline minus BP at 0 hours and 6 hours on 28th day.
†Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 0 hours and 6 hours.
‡Adjusted for baseline DBP and SBP, age, age squared, gender, body mass index, height, height squared, weight, alcohol consumption, cigarette smoking, education, and occupation.

To noncarriers, the DBP response was significantly greater in Cys347 allelic carriers at 0 hours (7.5 ± 8.4 mm Hg versus 5.5 ± 8.4 mm Hg, P = .016) and at 6 hours (16.2 ± 9.1 mm Hg versus 14.2 ± 8.9 mm Hg, P = .025), but the SBP response was only moderately higher in Cys347 allelic carriers at 0 hours (21.1 ± 18.0 mm Hg versus 18.2 ± 17.9 mm Hg, P = .112) and at 6 hours (35.1 ± 17.9 mm Hg versus 32.2 ± 18.4 mm Hg, P = .117).

We further used multiple linear regression models to estimate the association of Arg347Cys genotypes with BP responses (both SBP and DBP) at 0 hours and 6 hours in total subjects and in subjects stratified by plasma irbesartan concentration (Table II). Without consideration of plasma concentration, the mean DBP responses for genotype subgroups with no copies, 1 copy, and 2 copies of the Cys347 allele were 5.5 ± 8.4, 7.2 ± 8.0, and 12.8 ± 13.7 mm Hg, respectively, at 0 hours and 14.2 ± 8.9, 15.8 ± 8.5, and 23.7 ± 16.0 mm Hg, respectively, at 6 hours. Only a moderate association was observed for SBP responses in these genotype subgroups, as follows: 18.2 ± 17.9, 21.3 ± 18.3, and 17.5 ± 10.6 mm Hg, respectively, at 0 hours and 32.2 ± 18.4, 35.1 ± 17.9, and 35.8 ± 20.9 mm Hg, respectively, at 6 hours. The group of homozygous 347Cys/Cys carriers, who showed the most pronounced BP response (especially DBP) to irbesartan at 0 hours and 6 hours, consisted only of 6 subjects. In the remaining analyses we combined Arg/Cys and Cys/Cys genotype groups because of the sample-size issue. After stratification of plasma concentration by its median values, Cys347 allelic carriers in the high-concentration group, relative to noncarriers, had a more pronounced DBP response at 0 hours (adjusted β [±SE], 3.0 ± 1.0 mm Hg; P = .004) and 6 hours (adjusted β, 3.0 ± 1.2 mm Hg; P = .014), and the same was true for the SBP response at 0 hours (adjusted β, 5.6 ± 2.1 mm Hg; P = .006) and 6 hours (adjusted β, 4.7 ± 2.0 mm Hg; P = .021). In contrast, in the low-concentration group, there was no significant association between either DBP or SBP response and Arg347Cys genotypes at 0 hours and 6 hours. These results are shown graphically in Fig 1.

As shown in Table III, we evaluated the potential interaction between Arg347Cys genotypes and plasma irbesartan concentration on SBP and DBP therapeutic responses. We used a generalized estimation equation model to adjust for intraclass correlation within subjects for multiple BP measures. We also adjusted for important covariates including a term for timing of BP measurement (0 hours or 6 hours). The interaction term
Fig 1. A, Systolic blood pressure (SBP) response at 0 hours on 28th day by \(\alpha_{1A}\)-adrenergic receptor (\(\alpha_{1A}\)-AR) genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. Each column shows mean values (± SE). Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 0 hours. 1 Asterisk, \(P < .01\) for Cys347 carriers versus noncarriers in high-concentration group. B, Diastolic blood pressure (DBP) response at 0 hours on 28th day by \(\alpha_{1A}\)-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. 1 Asterisk, \(P < .05\) for Cys347 carriers versus noncarriers in overall population. 2 Asterisks, \(P < .01\) for Cys347 carriers versus noncarriers in high-concentration group. C, SBP response at 6 hours on 28th day by \(\alpha_{1A}\)-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. Low and high groups were defined by median value of natural logarithm–transformed plasma concentration at 6 hours. 1 Asterisk, \(P < .05\) for Cys347 carriers versus noncarriers in high-concentration group. D, DBP response at 6 hours on 28th day by \(\alpha_{1A}\)-AR genotype in overall population and low versus high groups stratified by plasma concentration of irbesartan. 1 Asterisk, \(P < .05\) for Cys347 carriers versus noncarriers in overall population and high-concentration group.
of Plasma irbesartan concentration × Arg347Cys genotypes was significant for SBP response ($P = .0106$) and DBP response ($P = .0343$).

**DISCUSSION**

In clinical settings, considerable individual variability in therapeutic responses to antihypertensive drugs has been observed. We hypothesize that such individual variability may be in part a result of genetic variability. This study investigated individual and joint association of the $\alpha_{1A}$-AR Arg347Cys polymorphism and plasma irbesartan concentrations with BP therapeutic responses. The most important findings of our study were that the $\alpha_{1A}$-AR Arg347Cys polymorphism might alter

**Table III.** Parameter estimates for models of BP response to irbesartan treatment including interaction terms for $\alpha_{1A}$-AR genotype and plasma drug concentration*  

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<th>Variable</th>
<th>Parameter estimates</th>
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<td>.7182</td>
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<td>0.4942</td>
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<tr>
<td>Interation: Cys347 carrier × High plasma concentration</td>
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<td>.0106</td>
<td>2.5635</td>
<td>1.2112</td>
<td>.0343</td>
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*Modeled by use of generalized estimation equations to accommodate correlations within BP responses at the 2 times for the same individual.

†BP response was defined as BP at baseline minus BP on 28th day.
BP response (particularly DBP) to short-term irbesartan treatment. Our data also provided evidence of an interaction between the \( \alpha_{1A}-AR \) Arg347Cys polymorphism and plasma irbesartan concentrations in relation to BP therapeutic response. The association of Arg347Cys polymorphism with BP response was more pronounced in those patients with higher plasma concentrations of irbesartan. The antihypertensive steady-state effect of irbesartan was usually seen within 2 weeks of initiation of therapy, with maximum effects occurring between 2 and 6 weeks.\(^2,21\) Thus our major analytic traits, such as BPs and drug concentrations, were precise and reliable because they were measured separately at 0 hours and 6 hours on the 28th day (4 weeks) after the initiation of irbesartan administration.

Although the exact biologic mechanisms remain to be elucidated, our findings appear to be biologically plausible. Studies have demonstrated that there are important interactions between AT1 and \( \alpha_{1A}-ARs \) in activating a common signal system inducing potential pathophysiologic significance.\(^1,10,15,16\) Thus functional polymorphic variants in the \( \alpha_{1A}-AR \) gene might mediate various arterial responses and, to a certain extent, alter the therapeutic effectiveness of the AT1 receptor antagonist irbesartan. The \( \alpha_{1A}-AR \) is widely distributed and expressed in human vasculature, including resistance arteries and veins.\(^6\) Rat hemodynamic studies have suggested that the vascular \( \alpha_{1A}-AR \) is the major subtype involved in the sympathetic regulation of peripheral resistance and systemic arterial pressure.\(^22,23\) In vivo transgenic mice, it was shown that in heterozygous animals the level of \( \alpha_{1A}-AR \) overexpression is directly related to marked enhancement of cardiac contractility.\(^24\) Most recently, an 8% to 12% reduction in BP dependent on \( \alpha_{1A}-AR \) gene copy number was shown in \( \alpha_{1A}-AR \) knockout mice.\(^8\) These findings suggested that the \( \alpha_{1A}-AR \) is primarily responsible for mediating vasoconstriction to regulate BP by sympathetic vascular tone. A previous study has identified a common polymorphism in the \( \alpha_{1A}-AR \) gene.\(^18\) The substitution of Cys347 for Arg347 can confer a palmitoylation site and play a key role in its cellular localization and function.

There has been observational evidence that an interaction between angiotensin-converting enzyme (ACE) insertion/deletion polymorphism and ACE inhibitor can result in a difference in AT1 receptor messenger ribonucleic acid expression.\(^25\) The Swedish Irbesartan Left Ventricular Hypertrophy Investigation (SILVHIA) trial evaluated the role of ACE insertion/deletion polymorphism as a potential predictor of BP response to treatment with irbesartan.\(^26\) The result identified that individuals with the insertion/insertion genotype had a significantly greater DBP-lowering effect compared with those with either the deletion/deletion or the insertion/deletion genotype. Further studies are needed to evaluate the role of other potentially important candidate genes, as well as gene-gene interactions, in relation to BP therapeutic response to irbesartan.

Previous studies have shown interactions between AT1 and \( \alpha_{1A}-ARs \) in VSMCs.\(^10,15,16\) Angiotensin II facilitates neurotransmitter release from the presynaptic nerve terminals, mostly mediated by \( \alpha_{1A}-AR \), to cause vasoconstriction and myocardial damage.\(^27\) The \( \alpha_{1A} \)-adrenergic contractile response to phenylephrine is significantly potentiated by angiotensin II. With the use of phenoxybenzamine to pretreat cells, \( \alpha_{1A}-ARs \) numbers decrease to around 15% of control values and then partially recover after reincubation of the cells in the absence of phenoxybenzamine.\(^10\) However, activation of the angiotensin II receptor also stimulates a marked increase in de novo synthesis of the \( \alpha_{1A}-AR \) in phenoxybenzamine-pretreated VSMCs, which strongly indicates that a lower number of \( \alpha_{1A}-ARs \) may in return stimulate the formation of many more activated AT1 receptors that potentiate de novo synthesis of the \( \alpha_{1A}-AR \). Therefore we can rationally postulate that Cys347 allelic carriers could harbor lower activities or densities of \( \alpha_{1A}-ARs \) embedded on the surfaces of VSMCs’ membranes, which would cause a decreased pressor response in regulating arterial pressure. In return, a systemically negative feedback would stimulate many more activated AT1 receptors to compensate for the loss of arterial pressure and maintain a normal BP level.

Our study results, similar to those in a previous study,\(^28\) revealed that the \( \alpha_{1A}-AR \) Arg347Cys polymorphism was not associated with baseline SBP and DBP levels in these hypertensive subjects. In Cys347 allelic carriers, however, higher levels of plasma irbesartan might adequately bind and block those activated AT1 receptors’ actions and result in greater SBP and DBP decreases with irbesartan treatment. Several earlier investigations failed to identify a simple association between drug plasma concentration and BP response.\(^29,30\) Our LOWESS smoothing curves of BP therapeutic responses by plasma irbesartan concentration also did not exhibit a linear relationship (data not shown). However, the relationship appeared to differ by \( \alpha_{1A}-AR \) genotypes. In Cys347 allelic noncarriers, there was little correlation between the steady-state drug level and BP-lowering effect at 0 hours and 6 hours. An explanation may be that 4 weeks of antihypertensive therapy alters the hypertensive disease process and decreases peripheral resistance as a result of a regression of
vascular structural damage. Thus it would be difficult to observe an association between steady-state drug levels and BP-lowering effects. In Cys347 allelic carriers, however, a positive association of drug level with BP response was observed as a result of more stimulations of receptor-mediated vascular sensitivity, which would suggest the physiologic significance of the functional variant.

It appears that pharmacodynamic and pharmacokinetic mechanisms each may play a role in determining interindividual variation in BP responses to the antihypertensive drug. In vitro studies have shown that cytochrome P450 (CYP) 2C9 plays a major part in the metabolism of irbesartan. Therefore genetic variants of CYP2C9 affecting the metabolic enzyme functionality may alter the pharmacokinetic profile of irbesartan, which in turn may influence BP therapeutic responses.

Considerable work remains to be done in this research area. Our findings remain to be replicated in other ethnic populations. The frequency of the Arg347Cys polymorphism is differently distributed among different races, with the Cys347 allele being more common in white subjects (53.8%) than in black (29.5%), Japanese (10%), and Chinese subjects (8.8%). To date, there have been no substantial functional studies on the Arg347Cys genetic polymorphism. An in vivo study in small-sized healthy Americans (N = 74) did not show that the Arg347Cys polymorphism in the α1A-AR gene alters the agonist-mediated vasoconstriction response to phenylephrine. The 2 polymorphic recombinant α1A-ARs expressed stably by Chinese hamster ovary cells in an in vitro study also exhibit similar pharmacologic properties in their binding affinities and calcium signal transduction. However, we cannot directly apply this finding to humans because the regulation of the α1-AR messenger ribonucleic acid level and receptor densities by agonists or antagonists is tissue- and cell-specific, such as differential sensitivity of venular and arteriolar α1-AR constriction to inhibitors (ie, nifedipine and hyponxia). In vivo functional variant study in human populations is needed. Furthermore, we could not exclude the possibility that Arg347Cys may be in linkage disequilibrium with another causal variant nearby.

In summary, our study suggests that the α1A-AR Arg347Cys polymorphism is associated with BP therapeutic responses to short-term irbesartan treatment and such an association is more pronounced at higher levels of plasma irbesartan. Our study represents one of the first steps in our goal to achieve individualized therapy based on an individual’s genetic characteristics and plasma drug level.

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References


The G–113A polymorphism in CYPIA2 affects the caffeine metabolic ratio in a Chinese population

Objective: This study was designed to better understand genetic variation in the cytochrome P450 (CYP) gene CYPIA2 and its impact on CYPIA2 activity in Chinese subjects.

Methods: CYPIA2 genetic polymorphisms were screened by direct sequencing in 27 selected Chinese subjects. Plasma 1,7-dimethylxanthine/caffeine ratios 5 hours after a 100-mg caffeine administration, used as an index of CYPIA2 in vivo activity, were determined in 422 healthy subjects. Five single-nucleotide polymorphism markers, including G/H11546860A (CYPIA2*1C), T/H115463594G, G/H115463113A, A/H11546163C (CYPIA2*1F), and C5347T (CYPIA2*1B), were selected and genotyped by either polymerase chain reaction–restriction fragment length polymorphism or direct sequencing.

Results: Thirteen polymorphisms and 2 linkage disequilibrium blocks with a boundary around H115462467 were identified at this locus. The allele frequency for G/H115463860A, G/H115463594G, G/H115463113A, A/H11546163C, and 5347T was 0.21, 0.15, 0.10, 0.36, and 0.14, respectively, in the CYPIA2-phenotyped cohort. A significant difference in CYPIA2 activity was observed among genotypes of polymorphism G/H115463113A (P = .038), and CYPIA2 activity in subjects carrying the AA genotype was lower than that in those carrying the GA (P = .096) and GG genotypes (P = .036): −0.45 ± 0.05 (mean ± SD), −0.32 ± 0.16, and −0.29 ± 0.16, respectively. Further analysis based on haplotype pairs found a 1.92-fold variation (95% confidence interval, 1.13-2.71) in mean CYPIA2 activity between haplotype pairs 13 and 15, and the difference was significant (−0.19 ± 0.15 versus −0.45 ± 0.05, P = .016). As compared with haplotype pair 10, haplotype pairs 9 and 15 and most haplotype pairs heterozygous for the haplotype with an A allele at H115463113, including pairs 5, 8, and 12, also showed significantly lower CYPIA2 activity (P = .015, .048, .008, .024, and .014 for pairs 5, 8, 9, 12, and 15, respectively). In addition, haplotype pairs 5, 9, and 12 also showed significantly lower CYPIA2 activity than pair 13 (P = .034, .020, and .037 for pairs 5, 9, and 12, respectively).

Conclusions: The G–3113A polymorphism is associated with decreased CYPIA2 activity, haplotype pairs 10 and 13 are responsible for high CYPIA2 activity, and haplotype pairs 5, 8, 9, 12, and 15 are responsible for low CYPIA2 activity in Chinese subjects. (Clin Pharmacol Ther 2005;78:249-59.)

Xiaoping Chen, MD, PhD, Liqing Wang, MD, Lianteng Zhi, MD, Gangqiao Zhou, MD, PhD, Haijian Wang, MD, PhD, Xiumei Zhang, BD, Bingtao Hao, MD, Yunping Zhu, MD, Zeneng Cheng, MD, PhD, and Fuchu He, MD, PhD Beijing, Shanghai, and Changsha, China

CYPIA2 is a smoking-inducible isozyme of cytochrome P450 (CYP), a superfamily that plays important roles in the metabolism of numerous structurally di-

From the Department of Genomics and Proteomics, Beijing Institute of Radiation Medicine, and Chinese National Human Genome Center at Beijing, Beijing; Institutes of Biomedical Sciences, Fudan University, Shanghai; and School of Pharmaceutical Sciences, Central South University, Changsha. Xiaoping Chen, MD, PhD, and Liqing Wang, MD, contributed equally to this work.

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dine. CYP1A2 is also involved in the activation of various toxic compounds and carcinogens such as food-derived aflatoxin B1 and heterocyclic or aromatic amines found in tobacco smoke.

Considerable interindividual differences in CYP1A2 expression, exceeding 40-fold in messenger ribonucleic acid levels, have been observed both in vivo and in vitro. Interethnic differences in CYP1A2 activity have also been reported, with Chinese women and black subjects demonstrating lower CYP1A2 activity than white subjects. These remarkable interindividual and interethnic differences in constitutive or inducible CYP1A2 activity contribute greatly to variation in drug responses, as well as an individual’s predisposition to common diseases such as colon cancer, bladder cancer, testicular cancer, and primary hepatocellular carcinoma.

Although environmental exposures such as cigarette smoking, caffeine intake, and cruciferous vegetable consumption and several therapeutic drugs affect CYP1A2 activity to some degree, the results of a twins study in white subjects indicate that CYP1A2 in vivo activity is determined mainly by genetic factors, with a heritability estimate of 0.725. The CYP1A2 gene is located on 15q22 and spans about 7.8 kilobases (kb). Several studies have been devoted to the identification of the functional polymorphisms in CYP1A2. Three single-nucleotide polymorphisms (SNPs), namely, −3860A (CYP1A2*1C), C−163A (CYP1A2*1F), and C−729T, seem to be associated with CYP1A2 activity and inducibility. However, these polymorphisms could not completely explain the large interindividual variation in CYP1A2 activity. The overall nucleotide diversity pattern in CYP1A2 and the major genetic basis for the variation of CYP1A2 expression remain largely unexplored.

To better understand the nucleotide diversity pattern at the CYP1A2 locus and its impact on CYP1A2 activity, we first performed SNP screening of all exons, relevant exon-intron boundaries, and approximately a 3-kb promoter region of CYP1A2 by polymerase chain reaction (PCR) direct sequencing in 27 selected Chinese individuals. Then, by using caffeine as the probe drug, we evaluated the associations of those common genetic polymorphisms with CYP1A2 activity in vivo in this study.

**METHODS**

**Subjects.** A total of 422 healthy unrelated Chinese volunteers, comprising 238 men and 184 women aged 18 to 33 years (mean age, 20 ± 2 years), participated in the study. Each subject was in good health on the basis of medical history, physical examination, and laboratory evaluation. All subjects were students from Central South University, Changsha, China, and had similar foodstuffs provided by an eatery at the university 1 week before and during phenotyping. In addition, all subjects were self-stated nonsmokers and were asked to abstain from coffee, tea, cola, chocolate, or any caffeine-containing drinks within 1 week before and during phenotyping. None of the subjects was taking any medications including oral contraceptives 1 week before and during phenotyping. This study was approved by the Ethics Committee of the School of Pharmaceutical Science, Central South University, and all subjects gave written informed consent before commencing the study.

**SNP discovery.** SNP screening of all exons, exon-intron boundaries, and approximately 3-kb promoter region of CYP1A2 (referred to as GenBank accession no. NT_010194.16) was performed by PCR direct sequencing. The screening panel included 27 unrelated individuals selected randomly from the 422 volunteers. The sample size yielded 95% probability of detecting alleles with a minimal frequency of 5.4%. In brief, the primers for the target regions were designed by use of Web-based software (Primer3.0). Deoxyribonucleic acid (DNA) samples from the 27 Chinese individuals were amplified and purified. The PCR products were then sequenced by use of the ABI PRISM Dye Terminator Sequencing Kit with AmpliTaq DNA polymerase and loaded onto an ABI 3700 sequencer (Applied Biosystems, Foster City, Calif). SNP candidates were identified by the PolyPhred program and inspected by 2 observers. SNP positions and individual genotypes were confirmed by reamplifying and resequencing the SNP site from the opposite strand.

**Caffeine phenotyping.** The caffeine plasma metabolic ratio (MR) indicative of CYP1A2 in vivo activity was determined according to the method of Carrillo et al. In brief, a 100-mg oral dose of a caffeine capsule (Hunan Pharmaceutical, Changsha, China) was given at 7 to 8 AM after overnight fasting. Five-milliliter venous blood samples were drawn into ethylenediaminetetraacetic acid–containing tubes at 0 hours and 5 hours after caffeine administration. Plasma and the peripheral leukocytes were isolated immediately and stored separately at −20°C until analysis. Molar concentrations of caffeine (137X) and its metabolite 1,7-dimethylxanthine (paraxanthine) (17X) were determined by HPLC as described elsewhere.

**SNP genotyping.** DNA samples were extracted from peripheral leukocytes by standard phenol/chloroform protocols. DNA samples were diluted to 8 ng/μL and
distributed to 96-well plates; each 96-well plate contained 94 samples and 2 DNA-free water controls. Five SNPs (ie, CYP1A2*1C, T–3594G, A–3113G, CYP1A2*1F, and CYP1A2*1B) were genotyped in the phenotyped population. The polymorphisms CYP1A2*1C, T–3594G, and CYP1A2*1F were genotyped by direct sequencing. PCR amplification of the target fragments consisted of the following 2 sets of primers (forward/reverse), with the forward primers in each set being the sequencing primers: 5′-GAA-CACAACGGGACCTTCTTG-3′/5′-GGATTACGCTC-CTTCTCTTT-3′ for CYP1A2*1C and T–3594G and 5′-TCTCAAGACACCTGCCTCTAC-3′/5′-AGGG-AACAGACTGGGACAAT-3′ for CYP1A2*1F. A–3113G and CYP1A2*1B were genotyped by PCR–restriction fragment length polymorphism. The A–3113G polymorphism results in the formation of an HpyCHIV restriction site, and the CYP1A2*1B polymorphism abolishes an Eco57I restriction site. For G–3113A, a 441–base pair (bp) fragment was amplified by use of primers 5′-AAGGAGAAGGACCGTGAATCC-3′/5′-GTTCCAGGACCCATTGGA-3′ (forward/reverse). Five microliters of the PCR products was digested overnight at 37°C in a 10-μL reaction mixture with 1 U of HpyCHIV (New England Biolabs, Beverly, Mass). For CYP1A2*1B, a 438-bp fragment was amplified with primers 5′-AGGTCCTAATCTCTCTGTC-3′ (forward) and 5′-GCACCTGTGCTAAAGCTGGA-3′ (reverse). Five microliters of the PCR products was digested overnight at 37°C in a 10-μL reaction mixture with 1 U of Eco57I (MBI Fermentas, St Leon-Rot, Germany). The digested products were subsequently separated on 3% agarose. For A–3113G polymorphism, HpyCHIV digestion gave 2 bands of 398 bp and 43 bp for the GG genotype, 1 band of 443 bp for the AA genotype, and all 3 bands for the AG genotype. In contrast, for CYP1A2*1B polymorphism, Eco57I digestion gave 2 bands of 269 bp and 169 bp for the CYP1A2*1A/CYP1A2*1A genotype, 1 band of 438 bp for the CYP1A2*1B genotype, and all 3 bands for the CYP1A2*1A/CYP1A2*1B genotype. An additional DNA panel including 94 subjects was also genotyped by direct sequencing for the functional SNP T3613C; and 1.9% for C63G (0.9% in white subjects), and 29.6% for A3594G, G2159A, and C5347T (0.4% in white subjects). All haplotypes except haplotype 1 could be inferred unambiguously. Among these polymorphisms, 2 coding SNPs were identified. CYP1A2*2 was a singleton that resulted in an amino acid change from phenylalanine to leucine, and CYP1A2*1B was a synonymous mutation.

RESULTS

SNP discovery. Resequencing of 7262-bp genomic DNA in the 27 Chinese subjects identified 13 biallelic polymorphisms, yielding a mean density of 1 polymorphism per 605 bp (Table I and Fig 1). Twelve of these polymorphisms were common SNPs with a minor allele frequency (MAF) of approximately 10% or higher. MAF was 50.0% for T–2467 delT; 29.6% for A–163C (CYP1A2*1F); 22.2% for G–3860A (CYP1A2*1C); 20.4% for T–3594G, G2159A, and C5347T (CYP1A2*1B); 9.3% for G–3598T, G–3113A, T–2847C, T–1708C, T–739G (CYP1A2*1E), and T3613C; and 1.9% for C63G (CYP1A2*2). Among these polymorphisms, 2 coding SNPs were identified. CYP1A2*1B was a singleton that resulted in an amino acid change from phenylalanine to leucine, and CYP1A2*1B was a synonymous mutation. In comparison with those in white subjects,15 allele frequencies for CYP1A2*1C (0.9% in white subjects), −739G (0.4% in white subjects), and CYP1A2*1B (61.8% in white subjects) varied significantly between populations (P < .001) (Table I). The C–729T polymorphism was not observed either in the SNP screening panel or in the additional 94 subjects.

Haplotype structure and LD pattern at CYP1A2 locus. Construction of haplotypes was based on genotype data of all SNPs identified. Eleven haplotypes were identified, 5 of which were common haplotypes with a frequency greater than 5% (Fig 1, A). All haplotypes except haplotype 1 could be inferred unambiguously. Among the haplotypes inferred, haplotype 1 was most distinctive at the nucleotide level and showed unique differences at 6 sites from all other haplotypes.

Lewontin’s D’ was calculated to evaluate the pairwise LD between polymorphisms.

SPSS 10.0 for Windows was applied for statistical analysis of the data (SPSS, Chicago, Ill). Frequency distribution and probit plot were used in analysis of phenotype distribution. Normal distribution of CYP1A2 activity was assessed by the Kolmogorov-Smirnov test. Comparison of CYP1A2 activity (expressed as mean ± SD) between genders was carried out by the Mann-Whitney U test. Comparisons of allele frequencies between populations were carried out by chi-square test or Fisher exact test. Comparisons of CYP1A2 activity (indicated by the log transformation of 17X/137X ratios) among genotypes and haplotype pairs were analyzed by ANOVA. Statistical significance was regarded as P < .05.
from 0.20 to 1.0, with a mean value of 0.96. Two LD blocks with a threshold of absolute value of |D’| equal to 0.8 were identified (Fig 1, B). The boundary between the 2 blocks existed around −2467. The 5′ upstream block (block 1) and 3′ downstream block (block 2) spanned roughly 1.6 kb and 5.7 kb, respectively. Among the SNPs identified, G−3598T, A−3113G, C−2847T, C−1708T, G−739T (CYP1A2*1E), and T3613C were in absolute LD; G2159A and CYP1A2*1B were also in absolute LD. A−3113G, a polymorphism located in a region containing a potential binding site for positive regulatory transcription factor,10 the synonymous mutation CYP1A2*1B, and other 3 haplotype tag SNPs (including CYP1A2*1C, T−3594G, and CYP1A2*1F) were selected for further phenotype-genotype association study (Fig 1, A). These 5 substitutions described more than 90% of the haplotype diversity in the sequences.

**Phenotyping with caffeine.** No endogenous interfering peaks were found in any of the 0-hour plasma samples. Caffeine MR was not available for 3 samples (2 for women and 1 for men) because of a lapse during plasma sample treatment. Caffeine MR ranged from 0.18 to 1.51 (log-transformed, −0.74 to 0.18), with a mean value of 0.54 ± 0.21 (log-transformed, −0.29 ± 0.16 [mean ± SD]). In men CYP1A2 activity was slightly higher than in women (P = .028) — 0.56 ± 0.21 (n = 236) and 0.52 ± 0.21 (n = 183), respectively. Normal distribution was indicated in the overall population (P = .075) by the Kolmogorov-Smirnov test. Neither the frequency distribution nor the probit plot of caffeine MRs showed any obvious antimode (Fig 2). After stratification by gender, normal distribution was detected in men (P = .200) but not in women (P = .049).

**Association of individual SNPs and haplotype pairs with caffeine MR.** All SNPs were found to be in compliance with Hardy-Weinberg equilibrium in the CYP1A2-phenotyped cohort. A significant difference in CYP1A2 activity was shown among genotypes at the G−3113A polymorphic site (P = .038). Individuals carrying the AA genotype at −3113 showed significantly lower CYP1A2 activity (−0.45 ± 0.05) as compared with those carrying either the GG (−0.29 ± 0.16) or AG (−0.32 ± 0.16) genotype (P = .036 for AA versus GG and P = .096 for AA versus AG) (Table II). Individuals carrying the CYP1A2*1C/*1C genotype also showed marginally lower CYP1A2 activity as compared with both CYP1A2*1A/*1A and CYP1A2*1A/*1C genotypes (P = .064 and .078, respectively) (Table II). In addition, CYP1A2*1B homozygotes showed marginally significantly higher CYP1A2 activity as compared with CYP1A2*1A/*1A homozygotes (−0.19 ± 0.15 versus −0.30 ± 0.16, P = .052) (Table II). No differ-

![Table I. Single-nucleotide polymorphisms identified in CYP1A2 in Chinese subjects and comparison with white subjects](image)

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<td>4646427</td>
<td>—</td>
</tr>
<tr>
<td>C5347T</td>
<td>Exon 7</td>
<td>T</td>
<td>20.4</td>
<td>61.8</td>
<td>2470890</td>
<td>CYP1A2*1B</td>
</tr>
</tbody>
</table>

†Reference data reported by Sachse et al13 in 114 white subjects.
‡rs (reference SNP ID) in dbSNP database. Dashes indicate polymorphisms not studied in the reference or not deposited in the dbSNP database or CYP allele nomenclature Web site.20b
§The numbers indicate the SNP locations relative to the start codon ATG according to National Center for Biotechnology Information genomic contig NT_010194.16.

P < .001, significantly different from those in white subjects.

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**From the image:**

- Table I summarizes single-nucleotide polymorphisms identified in CYP1A2 in Chinese subjects and comparison with white subjects.
- The table lists SNPs, their locations, minor allele frequencies, and their associations with caffeine MRs.
- The table includes polymorphisms such as G−3860A, G−3598T, T−3594G, G−3113A, and T−2847C, among others.
- The minor allele frequencies are compared between Chinese and white subjects, with notable differences observed in some SNPs.
- The table also includes rs IDs for each SNP, which are crucial for genetic research.
- The CYP nomenclature is used to identify the genetic variations.

---

**From the text:**

- The study found that the boundary between LD blocks was around −2467, with upstream and downstream blocks spanning 1.6 kb and 5.7 kb, respectively.
- Among the SNPs identified, G−3598T, A−3113G, C−2847T, C−1708T, G−739T (CYP1A2*1E), and T3613C were in absolute LD, while G2159A and CYP1A2*1B were also in absolute LD.
- A−3113G, a polymorphism located in a region containing a potential binding site for a transcription factor, was selected for further study.
- The study also identified 3 haplotype tag SNPs (CYP1A2*1C, T−3594G, and CYP1A2*1F) for phenotype-genotype association analysis.
- Significant differences in CYP1A2 activity were observed among genotypes at the G−3113A polymorphic site, with the AA genotype showing lower activity compared to the GG and AG genotypes.
- Individuals carrying the CYP1A2*1C/*1C genotype showed marginally lower activity compared to both the CYP1A2*1A/*1A and CYP1A2*1A/*1C genotypes.
- CYP1A2*1B homozygotes had marginally higher activity compared to CYP1A2*1A/*1A homozygotes.

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**From the table:**

- The table provides a detailed view of single-nucleotide polymorphisms, their locations, and frequencies in Chinese and white subjects.
- The table includes polymorphisms such as G−3860A, G−3598T, T−3594G, G−3113A, and T−2847C, among others.
- The minor allele frequencies are listed for each SNP, with variations observed between Chinese and white subjects.
- The table also includes rs IDs for each SNP, which are essential for genetic research.
- The CYP nomenclature is used to identify the genetic variations.

---

**From the analysis:**

- The study found significant differences in CYP1A2 activity among genotypes at the G−3113A polymorphic site.
- Individuals carrying the AA genotype showed lower activity compared to those carrying the GG or AG genotypes.
- The study also identified significant differences in the activity of individuals carrying the CYP1A2*1C/*1C genotype compared to both CYP1A2*1A/*1A and CYP1A2*1A/*1C genotypes.
- CYP1A2*1B homozygotes had marginally higher activity compared to CYP1A2*1A/*1A homozygotes.

---

**From the conclusion:**

- The study provides valuable insights into the genetic variations in CYP1A2 and their implications on caffeine metabolism.
- The findings can aid in understanding the genetic basis of inter-individual differences in caffeine metabolism.
- The study highlights the importance of identifying genetic markers that influence drug response.
Fig 1. Gene structure, regions resequenced, polymorphisms discovered, and haplotype (Hap) and linkage disequilibrium (LD) pattern at CYP1A2 locus. A, Gene structure, regions resequenced, and locations of all 13 polymorphisms identified at CYP1A2 locus. Coding exons are marked with solid boxes, and 5'- and 3'-untranslated regions are marked by hatched boxes. The locations of each polymorphism (relative to the first nucleotide of the open reading frame) are shown under the exon-intron structure of CYP1A2 (accession number of reference sequence NT_010194.16). Haplotypes and their frequencies in 27 unrelated Chinese individuals can be inferred. Locations of 5 single-nucleotide polymorphisms (SNPs) selected for further phenotype-genotype association study were boxed. bp, Base pairs. B, Pairwise LD between polymorphisms identified at CYP1A2 locus. The absolute values of D' for each pair of polymorphisms are indicated. Two LD blocks (block 1 and block 2) with low haplotype diversities are also shown.
ences in CYP1A2 activity among genotypes of T−3594G or CYP1A2*1F polymorphisms were observed (Table II).

Haplotypes were constructed for 403 individuals whose genotype data were complete. A total of 9 haplotypes and 27 haplotype pairs (data not shown) were observed. Differences in CYP1A2 activity among haplotype pairs were also compared. In addition, for the purposes of analysis, the difference in CYP1A2 activity was compared only among homozygous haplotype pairs and heterozygous pairs with frequency higher than 3%. Fifteen pairs representing 92% of the cohort

**Table II.** Association of CYP1A2*1C, T−3592G, G−3113A, CYP1A2*1F, and CYP1A2*1B polymorphisms with plasma caffeine metabolic ratio in Chinese subjects

<table>
<thead>
<tr>
<th>SNP locus</th>
<th>Genotype</th>
<th>No.†</th>
<th>Log-transformed 17X/137X ratio</th>
<th>Total P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G−3860A</td>
<td>*1A/*1A</td>
<td>n = 256‡</td>
<td>−0.29 ± 0.16</td>
<td>.176</td>
</tr>
<tr>
<td></td>
<td>*1C/*1A</td>
<td>n = 140§</td>
<td>−0.29 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1C/*1C</td>
<td>n = 20</td>
<td>−0.36 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>T−3594G</td>
<td>TT</td>
<td>n = 299</td>
<td>−0.30 ± 0.17</td>
<td>.341</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>n = 106</td>
<td>−0.28 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>n = 9</td>
<td>−0.35 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>G−3113A</td>
<td>GG</td>
<td>n = 332</td>
<td>−0.29 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>n = 79</td>
<td>−0.32 ± 0.16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>n = 3</td>
<td>−0.45 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>A−163C</td>
<td>*1A/*1A</td>
<td>n = 173</td>
<td>−0.30 ± 0.14</td>
<td>.636</td>
</tr>
<tr>
<td></td>
<td>*1F/*1A</td>
<td>n = 175</td>
<td>−0.30 ± 0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1F/*1F</td>
<td>n = 59</td>
<td>−0.28 ± 0.17</td>
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<tr>
<td>C5347T</td>
<td>*1A/*1A</td>
<td>n = 302</td>
<td>−0.30 ± 0.16</td>
<td>.068</td>
</tr>
<tr>
<td></td>
<td>*1B/*1A</td>
<td>n = 104</td>
<td>−0.28 ± 0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*1B/*1B</td>
<td>n = 8</td>
<td>−0.19 ± 0.15</td>
<td></td>
</tr>
</tbody>
</table>

SNP, Single-nucleotide polymorphism; 17X/137X, 1,7-dimethylxanthine/caffeine ratio.
†Because of polymerase chain reaction failure, the total number of individuals was less than 422 for all of the polymorphisms.
‡P = .064 and §P = .078, compared with corresponding *1C/*1C genotype.
¶P = .036 and ‡P = .096, compared with corresponding AA genotype.
#P = .052, compared with corresponding *1A/*1A genotype.
met this criterion (Table III). Individuals with the haplotype pair 15 (homozygous for −3860G/−3594T/−3113A/−163A/5347C, n = 3) and the haplotype pair 13 (homozygous for −3860G/−3594T/−3113G/−163A/5347T, n = 8) demonstrated the lowest and highest mean plasma 17X/137X ratios, respectively, in the population (Table III). To delineate which pairs differ from one another with regard to CYP1A2 activity, comparisons were made for pair 13 and pair 15 versus the other haplotype pairs. The results showed a significant difference in caffeine log-transformed MR between pairs 13 and 15 (−0.45 ± 0.05 versus −0.19 ± 0.15, P = .016) (Table III). A 1.92-fold variation (95% confidence interval, 1.29-2.71) in mean plasma 17X/137X ratio was observed between pairs 13 and 15. A significant difference in CYP1A2 activity was also shown between pair 5 (log-transformed 17X/137X ratio, −0.33 ± 0.19; n = 27) and pair 13 (P = .034), pair 9 (homozygous for −3860A/−3594T/−3113G/−163A/5347C; log-transformed 17X/137X ratio, −0.35 ± 0.16; n = 18) and pair 13 (P = .020), and pair 12 (log-transformed 17X/137X ratio, −0.34 ± 0.15; n = 12) and pair 13 (P = .037). When pairwise tests were performed on the remaining pairs, significant differences in CYP1A2 activity were also observed between pairs 9 and 10 (−0.35 ± 0.16 versus −0.21 ± 0.16, P = .008) and pairs 15 and 10 (−0.45 ± 0.05 versus −0.21 ± 0.16, P = .014). In addition, most pairs heterozygous for the haplotype −3860G/−3594T/−3113A/−163A/5347C (including pairs 5, 8, and 12) also showed decreased CYP1A2 activity as compared with pair 10 (P = .015 for pair 5 versus pair 10, P = .048 for pair 8 versus pair 10, and P = .024 for pair 12 versus pair 10) (Table III).

### DISCUSSION

In this study we began with the investigation on nucleotide diversity pattern at the CYP1A2 locus in Chinese subjects. Twelve of the polymorphisms were common with MAF ranging from 9.3% to 50.0%. As compared with the allele frequencies observed in white subjects, some polymorphisms showed remarkable ethnic difference. No common nonsynonymous polymorphism (with MAF >5%) was identified in our study. The only rare nonsynonymous SNP identified in our study, CYP1A2*2, has been reported to be nonfunctional. Most of the polymorphisms are located in the 5′-flanking regulatory region and introns. A similar set of polymorphisms across the CYP1A2 locus was also observed recently in Japanese subjects. This pattern of polymorphism distribution at the CYP1A2 locus suggests that the remarkable interindividual variation in CYP1A2 activity in Chinese subjects is not a result of a structural or functional difference in CYP1A2 itself, whereas SNPs located in noncoding regions may play a role. A functional polymorphism located in intron 1, C−729T, has been reported to decrease both CYP1A2 activity and its inducibility in Ethiopians. However,
this SNP was not observed in 121 unrelated Chinese DNA samples. Several other rare coding SNPs reported recently in Japanese subjects also have not been identified in our study.22

We found significantly lower CYP1A2 activity in subjects with the AA genotype at the −3113 polymorphic site in this Chinese population. As has been reported previously, G−3113A is located in a 32-bp region, −3115 to −3084 bp, containing a cis element for CYP1A2 transcription.30 An in vitro study with transfection systems has suggested that the entire 32 bp of this region is necessary for CYP1A2 promoter activity.31 However, because this SNP is in complete LD with 5 other SNPs (ie, G−3598T, C−2847T, C1708T, G−739T, and T3613C) observed in CYP1A2, it is difficult, in this study, to determine separately the functional relevance of A−3113G. Previous studies using either transient transfection systems or electrophoretic mobility shift assay analysis have excluded the possibility of G−3598T134 and G−739T (CYP1A2*1E)10 to be functional.

Much attention has also been focused on the functional significance of other SNPs, especially for CYP1A2*1C and CYP1A2*1F, in recent years. Both CYP1A2*1C and CYP1A2*1F were initially found to be associated with CYP1A2 inducibility in smokers in white and Japanese subjects, respectively.19,20 However, contradictory results have been obtained in some other clinical investigations.35,39 In our study we found marginally lower CYP1A2 activity in CYP1A2*1C homozygotes, and this is in accordance with the previous finding that CYP1A2*1C is associated with lower theophylline clearance in Japanese patients with asthma.35 In agreement with other studies performed in schizophrenic patients in Japanese and white populations,36,39 we did not find any association between CYP1A2*1F polymorphism and caffeine MRs in this Chinese population. The lack of association between the T−3594G mutation and CYP1A2 activity observed in our study further supports the result of an in vitro study using transient transfection systems.34 As for C5347A (CYP1A2*1B), a negative result has also been obtained in white subjects.15 Because of the lack of cigarette smokers recruited in the study, whether these SNPs affect CYP1A2 inducibility deserves further investigation.

The relevance of combinations of multiple SNPs or haplotypes in CYP1A2 for predicting CYP1A2 activity was also assessed in our study. About a 2-fold difference in mean CYP1A2 activity was demonstrated between individuals carrying haplotype pair 13 (homozygotes of the haplotype −3860G/−3594T/−3113G/−163A/5347T) and pair 15 (homozygotes of the haplotype −3860G/−3594T/−3113A/−163A/5347C), the haplotype pairs that demonstrated the highest and lowest CYP1A2 activity in the overall population. In addition, most haplotype pairs heterozygous for the haplotype −3860G/−3594T/−3113A/−163A/5347C are associated with significantly lower CYP1A2 activity as compared with haplotype pair 10. Because the A allele at −3113 is included within the haplotype −3860G/−3594T/−3113A/−163A/5347C, these results further support the finding that G−3113A is associated with decreased CYP1A2 in vivo activity. Because the −3113A polymorphism is common, with an MAF of about 10%, in Chinese subjects, this polymorphism may play a role in the interindividual variations in drug response in this population. Because CYP1A2 also plays important roles in the metabolism of environmental carcinogens such as aflatoxin B1,7 the functional relevance of the G−3113A polymorphism observed in our study makes this SNP an important marker for association studies for relevant diseases such as the aflatoxin B1−associated hepatocellular carcinoma,6 a malignancy prevalent in China.

The genotype-phenotype association based on haplotype pairs also suggests that the constitutive expression of CYP1A2 in Chinese subjects is regulated by several SNPs simultaneously and that the contribution of individual SNPs is limited. Similar genotype-phenotype associations based on haplotype pairs have also been reported in other drug response genes such as the β2-adrenergic receptor gene.40 According to mean caffeine MRs, haplotype pairs 10 and 13, with a frequency that adds up to 6.2% in the overall population, account for high CYP1A2 activity, whereas haplotype pairs 5, 8, 9, 12, and 15, with a total frequency of 20.3%, account for low CYP1A2 activity in this Chinese population. Most other individuals (73.5%) with inherited haplotypes show intermediate CYP1A2 activity in this population. In addition, it is obvious that polymorphisms including CYP1A2*1C, G−3113A, and CYP1A2*1B are necessary to discriminate individuals with either low or high CYP1A2 activity in the Chinese population. Because both of the haplotype pairs associated with either low or high CYP1A2 activity are common in Chinese subjects, they are suggested to be relevant to pharmacogenetics and xenobiotic metabolism in this population.

Of note, phenotype-genotype association studies based on haplotypes in CYP1A2 have also been carried out in other populations. In a study in Ethiopians, subjects with haplotype CYP1A2*1K (−739G/−729T/−163A) in intron 1 showed significantly decreased
CYP1A2 in vivo activity. However, this study observed haplotypes limited to intron 1 rather than the entire gene region. In addition, the CYP1A2*1K haplotype was not observed either in our study or in a Japanese population as reported recently. Another study has also suggested that there is no haplotype responsible for interindividual variation in CYP1A2 activity in white subjects. Because both frequency spectrums of SNPs and haplotype structures at CYP1A2 are different between Asian and white subjects, this result is not necessarily contradictory to ours. Because the nucleotide diversity pattern in CYP1A2 is similar in Chinese and Africans or other Asian populations, our observations would be convincing if they can be replicated in those populations.

With regard to phenotype distribution, polymorphic distribution in CYP1A2 activity is not indicated in the overall population we studied, and this is in disagreement with the former findings of trimodal or bimodal distribution observed in Chinese and African subjects. When stratified by gender, polymorphic distribution is observed in female, but not male, subjects, and a reduction in population size in the subgroups may account for this difference. Differences in population size may also explain the discordance between our results and those of other reports. Other confounding factors such as cigarette smoking and environmental exposure to inhibitory or inducible agents to CYP1A2 may also contribute to this difference.

In conclusion, our study indicates that A–3113G is associated with decreased CYP1A2 activity in vivo in Chinese subjects. Comparing CYP1A2 activity among genotypes that are based on haplotype pairs, we find that haplotype pairs 10 and 13 are responsible for high CYP1A2 activity whereas haplotype pairs 5, 8, 9, 12, and 15 are responsible for low CYP1A2 activity in Chinese subjects. Our observation provides some explanations for interindividual variation in CYP1A2 activity, although other genetic and environmental factors such as passive smoking may also play a role. When drugs that are substrates of CYP1A2 are used, dosages should be adjusted in individuals with inherited genotypes or haplotype pairs that are associated with both high and low CYP1A2 activity.

None of the authors has a conflict of interest.

References


Transporters and drug therapy: Implications for drug disposition and disease

The efficacy of drug therapy results from the complex interplay of multiple processes that govern drug disposition and response. Most studies to date have focused on the contribution of drug-metabolizing enzymes to the drug disposition process. However, over the past decade, it has become increasingly apparent that carrier-mediated processes, or transporters, also play critical roles in the overall disposition of numerous drugs in clinical use. In addition to their roles in xenobiotic transport, drug transporters often mediate important physiologic functions via transport of endogenous substrates such as amino acids, bile acids, and hormones that are critical for maintenance of normal homeostasis. In this review, we focus on the emerging field of transporter proteins in relation to the drug disposition process, with particular emphasis on clinical implications of transporters to drug-drug interactions and subsequent development of adverse effects, inter-individual variability in drug response, and human disease. (Clin Pharmacol Ther 2005;78:260-77.)

Richard H. Ho, MD, and Richard B. Kim, MD Nashville, Tenn

Drug efficacy represents the observed interplay among multiple processes that regulate drug disposition (pharmacokinetics) and response (pharmacodynamics). For orally administered drugs, their pharmacologic action is dependent on adequate intestinal absorption and distribution to sites of action, before their elimination by metabolic and excretory pathways. Most studies to date have focused on the role of drug-metabolizing enzymes as the key determinants of drug disposition. Drug metabolism reactions are generally grouped into 2 phases, phase I and phase II. Phase I reactions involve changes such as oxidation, reduction, and hydrolysis and are primarily mediated by the cytochrome P450 (CYP) family of enzymes. Phase II reactions use an endogenous compound, such as glucuronic acid, glutathione, or sulfate, for conjugation to the drug or its phase I-derived metabolite to produce a more polar end product that can be more readily excreted. Although the physicochemical properties of a drug, such as pKa, size, and lipophilicity, can often affect the extent of drug absorption and access to target tissue compartments, it is now increasingly apparent that carrier-mediated processes, or transporters, have a significant impact on the overall drug disposition process through their targeted expression in organs such as the intestine, kidney, and liver.

Membrane transporters have long been recognized to be an important class of proteins for regulating cellular and physiologic solute and fluid balance. With the initial sequencing of the human genome, it has been estimated that approximately 500 to 1200 genes encode transport proteins. A number of those transporters appear to facilitate or, in some cases, prevent drug...
passage through membrane barriers. These drug transporter proteins tend to be multifunctional and often have normal physiologic roles in terms of transporting endogenous substances such as sugars, lipids, amino acids, bile acids, steroids, and hormones. In this review we focus on the emerging field of transporter proteins in relation to the drug disposition process, with particular emphasis on the clinical implications of transporters to drug-drug interactions, drug toxicity, interindividual variability in drug response, and human disease.

TRANSPORTERS AND DRUG DISPOSITION

Drug transporters can be generally separated into 2 major classes—uptake and efflux transporters (Table I). Uptake transporters act by facilitating the translocation of drugs into cells. Included within this class of transporters are members of the organic anion transporting polypeptide (OATP, \( SLCO \)) family, organic anion transporter (OAT, \( SLC22A \)) and organic cation transporter (OCT, \( SLC22A \)) family, organic cation/carnitine transporter (OCTN, \( SLC22A \)) family, and peptide transporter (PEPT, \( SLC15A \)) family. By contrast, efflux transporters function to export drugs from the intracellular to the extracellular milieu, often against high concentration gradients. Most efflux transporters are members of the adenosine triphosphate (ATP)–binding cassette (ABC) superfamily of transmembrane proteins, which use energy derived from ATP hydrolysis to mediate substrate translocation across biologic membranes. Included within this class of transporters are members of the P-glycoprotein (\( ABCB \)) family, such as multidrug resistance protein 1 (MDR1) and the bile salt export pump (BSEP), the multidrug resistance–associated (MDR1A) protein family, and the breast cancer resistance protein (BCRP, \( ABCC \)).

Certain transporters have been shown to exhibit both influx and efflux properties. For example, the OATPs are primarily considered uptake transporters that mediate transport via sodium-independent mechanisms, in contrast to sodium-dependent uptake transporters such as sodium-taurocholate cotransporting polypeptide (NTCP).\(^5\) The mechanism of transport appears to be a result of anion exchange as supported by bidirectional organic anion transport polypeptide 1 (Oatp1)–mediated taurocholate/bicarbonate exchange.\(^6\) Further evidence from studies of rat Oatp1 and Oatp2 would suggest that physiologic glutathione (GSH) efflux may be the driving force for Oatp-mediated transport activity.\(^7\)\(^9\) However, for drugs studied to date, the observed phenotype is that of either uptake or efflux but not both.

Important to our understanding of transporter-mediated drug disposition is the dynamic interplay between uptake and efflux transporters within any given epithelial cells, in which the translocation of drugs across such cells may be impeded or facilitated by the localization of transporters on apical or basolateral membranes (Fig 1). Therefore, for many drugs, the combined and often complementary actions of transporters expressed within specific membrane domains of epithelial cells determine the extent and direction of drug movement across organs such as the liver, kidney, and brain. Moreover, consideration must also be given to the fact that transporters with overlapping substrate capabilities are often expressed in organs of importance for drug disposition. From an evolutionary perspective, this redundancy of transport systems may serve as a protective mechanism whereby multiple pathways exist for the elimination of potentially harmful xenobiotic substances. Therefore synergistic or additive function of multiple transport systems may need to be considered to account for the apparent disposition profile of a drug.

Transporters and tissue drug distribution. Targeted localization of transporters appears to facilitate the intestinal absorption and urinary or biliary excretion of many drugs (Table I). In the small intestine, enterocytes possess a number of transporters critical for absorption of dietary constituents and drugs. Among these proteins, MDR1 (P-glycoprotein) has been determined to be a particularly important efflux transporter that can actively extrude or pump drugs back into the intestinal lumen, effectively limiting the extent (bioavailability) of substrate drug absorption (Fig 1). The key role of MDR1 to drug absorption has been exemplified by studies in knockout mice with disruption of the homologous \( mdr1a \) gene.\(^10\) The oral bioavailability of paclitaxel,\(^11\) digoxin,\(^12\) and human immunodeficiency virus (HIV) 1 protease inhibitors\(^13\) is markedly increased in \( mdr1a \) knockout mice in comparison with wild-type mice, indicating that MDR1-mediated drug efflux by enterocytes limits the bioavailability of many drugs. In humans the extent of intestinal MDR1 expression and activity has been shown to influence drug levels after administration of cyclosporine (INN, ciclosporin)\(^14\) and digoxin,\(^15\) further supporting its role in determining the bioavailability of commonly administered drugs.

In the liver efficient extraction of drugs from the portal blood into hepatocytes is often mediated by uptake transporters expressed on the sinusoidal (basolateral) membrane. These include members of the OATP and OCT transporter families. For example, hepatic uptake of the 3-hydroxy-3-methylglutaryl–co-
### Table I. Human drug transporters

<table>
<thead>
<tr>
<th>Family</th>
<th>Member</th>
<th>Tissue distribution</th>
<th>Cellular localization</th>
<th>Examples of typical substrates</th>
<th>Important roles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SLCO</strong></td>
<td>OATP1A2</td>
<td>Brain, kidney, liver</td>
<td>Basolateral</td>
<td>Fexofenadine, indomethacin (INN, indometacin), bile salts, hormone conjugates, eicosanoids</td>
<td>CNS distribution</td>
<td>5, 126</td>
</tr>
<tr>
<td></td>
<td>OATP2B1</td>
<td>Liver, intestine, placenta</td>
<td>Basolateral</td>
<td>Bile salts, digoxin, benzylpenicillin, hormone conjugates</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>OATP1B1</td>
<td>Liver</td>
<td>Basolateral</td>
<td>Benzylpenicillin, pravastatin, rifampin, methotrexate, bilirubin, bile salts, hormone conjugates, eicosanoids</td>
<td>Hepatic uptake</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OATP1B3</td>
<td>Liver</td>
<td>Basolateral</td>
<td>Digoxin, methotrexate, rifampin, bile salts, hormone conjugates, eicosanoids</td>
<td>Hepatic uptake</td>
<td></td>
</tr>
<tr>
<td><strong>SLC22</strong></td>
<td>OAT1</td>
<td>Kidney, brain</td>
<td>Basolateral</td>
<td>Cidofovir, PAH, acyclovir (INN, aciclovir), tetracycline</td>
<td>Renal uptake</td>
<td>127</td>
</tr>
<tr>
<td></td>
<td>OAT3</td>
<td>Kidney, brain</td>
<td>Basolateral</td>
<td>Cimetidine, PAH, methotrexate, salicylate, valacyclovir, tetracycline</td>
<td>Renal uptake</td>
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</tr>
<tr>
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<td>OAT4</td>
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<td>Apical</td>
<td>PAH, tetracycline</td>
<td>Renal secretion</td>
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<tr>
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<td>OCT1</td>
<td>Liver, brain, small intestine</td>
<td>Basolateral</td>
<td>Cimetidine, corticosteroids, quinidine, quinine, midazolam, verapamil</td>
<td>Hepatic/renal uptake</td>
<td></td>
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<tr>
<td></td>
<td>OCT2</td>
<td>Kidney, brain, small intestine</td>
<td>Basolateral</td>
<td>Amantadine, choline, dopamine, histamine, norepinephrine, serotonin</td>
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<td>OCT3</td>
<td>Placenta, liver, adrenal</td>
<td>ND</td>
<td>Epinephrine, cimetidine, histamine, norepinephrine</td>
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<tr>
<td><strong>ABCB</strong></td>
<td>MDR1 (P-glycoprotein)</td>
<td>Kidney, liver, brain, small intestine</td>
<td>Apical</td>
<td>Digoxin, cyclosporine, paclitaxel, vinca alkaloids, doxorubicin, loperamide, erythromycin, HMG-CoA reductase inhibitors, HIV-1 protease inhibitors</td>
<td>Oral absorption, Renal secretion, Biliary excretion</td>
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</tr>
<tr>
<td></td>
<td>BSEP</td>
<td>Liver</td>
<td>Apical</td>
<td>Bile salts, vinblastine, tamoxifen citrate (INN, tamoxifen)</td>
<td>Biliary excretion</td>
<td></td>
</tr>
<tr>
<td><strong>ABCC</strong></td>
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<td>Ubiquitous</td>
<td>Basolateral</td>
<td>Vinca alkaloids, methotrexate, etoposide</td>
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<tr>
<td></td>
<td>MRP2</td>
<td>Liver, kidney, small intestine</td>
<td>Apical</td>
<td>Vinca alkaloids, methotrexate, pravastatin, ampicillin, ceftriaxone, cisplatin, irinotecan, hormone conjugates</td>
<td>Biliary excretion, Renal secretion</td>
<td></td>
</tr>
</tbody>
</table>
enzyme A (HMG-CoA) reductase inhibitor pravastatin is dependent on OATP1B1 (OATP-C), and its activity is thought to be the rate-limiting step in pravastatin hepatic clearance.16 Once a drug gains access into hepatocytes, it often undergoes metabolism mediated by phase I and II enzymes or may be secreted unchanged. Efflux transporters localized on the canicular (apical) membrane of the hepatocyte, such as MDR1, MRP2, and BCRP, represent the final step in the vectorial transport of drugs from portal circulation into bile. In addition to MDR1, a transporter previously referred to as the canalicular multispecific organic anion transporter, now referred to as MRP2, is responsible for the biliary excretion of numerous endogenous organic anions including bilirubin glucuronides, as well as drugs such as methotrexate,17 irinotecan (CPT-11),18 and pravastatin.19

In the kidney drug secretion also represents the coordinate function of uptake and efflux transporters localized to the basolateral and apical membranes of proximal tubular cells. Members of the OAT family appear to be important renal transporters for uptake of organic anions. OAT substrates include a wide variety of clinically important anionic drugs, such as β-lactam antibiotics, diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), nucleoside antiviral drugs, and anticancer agents.20 Renal efflux transporters have not been as extensively studied, but members of the MRP and OATP families of transporters have been localized to the apical membrane of proximal tubular cells and likely contribute to the urinary elimination of substrate drugs.

In organs such as the brain, targeted transporter expression is critical to the maintenance of barrier function. For example, the blood-brain barrier (BBB) serves a protective function by limiting access of drugs and toxic substances into the central nervous system (CNS). The BBB function is maintained by brain capillary endothelial cells, whose closely sealed, tight junctions effectively limit entry of drugs via the paracellular route.21 Whereas expression of certain uptake transporters facilitates CNS entry of essential endogenous substances such as glucose, amino acids, and nucleosides, efflux transporters such as MDR1, localized to the luminal side of the BBB endothelial cells, prevent CNS entry of substrate drugs.22 The importance of MDR1 expression at the level of the BBB has been shown in studies with mdr1a knockout mice.10 These mice are viable and fertile, with no apparent phenotypic abnormalities. However, mdr1a knockout mice have been shown to be 50- to 100-fold more sensitive to the neurotoxic pesticide ivermectin, and the accumulation of this drug in the brain tissue of mdr1a (−/−) mice was noted to be 80- to 100-fold greater when compared with control mice.10 Additional studies have demonstrated that CNS entry of a number of MDR1 drug substrates, such as digoxin, quinidine, tacrolimus, and HIV-1 protease inhibitors, is profoundly limited by MDR1 expression at the BBB.13,23,24

**TRANSPORTERS AND DRUG-DRUG INTERACTIONS**

As the number of newly approved drugs increases, medication errors and drug-drug interactions have become a significant source of patient morbidity and, in some cases, death. Indeed, it has been estimated that drug interactions account for nearly 3% of all hospital admissions.25 Factors such as age, diet, nonprescription drugs, and herbal and alternative therapies may also influence the development and extent of drug interactions. Moreover, inhibition of drug-metabolizing enzymes, as well as transporters (Table II), is now rec-

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**Table I—Cont’d**

<table>
<thead>
<tr>
<th>Family</th>
<th>Member</th>
<th>Tissue distribution</th>
<th>Cellular localization</th>
<th>Examples of typical substrates</th>
<th>Important roles</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP3</td>
<td></td>
<td>Liver, kidney, small intestine</td>
<td>Basolateral</td>
<td>Doxorubicin, vincristine, methotrexate, cisplatin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCG</td>
<td>BCRP</td>
<td>Placenta, liver, small intestine</td>
<td>Apical</td>
<td>Mitoxantrone, doxorubicin, topotecan, methotrexate, irinotecan (SN-38)</td>
<td>Oral absorption</td>
<td>Fetal exposure</td>
</tr>
</tbody>
</table>

OATP, Organic anion transporting polypeptide; CNS, central nervous system; OAT, organic anion transporter; PAH, para-aminobiphenylic acid; OCT, organic cation transporter; ND, not determined; ABC, adenosine triphosphate–binding cassette; MDR, multidrug resistance protein; HMG-CoA, 3-hydroxy-3-methylglutaryl-Coenzyme A; HIV, human immunodeficiency virus; BSEP, bile salt export pump; MRP, multidrug resistance–associated protein; BCRP, breast cancer resistance protein.
ognized as the likely mechanism accounting for many drug-drug interactions, because transporters and CYP enzymes often share overlapping tissue expression and substrate capacities. For example, CYP3A4 and MDR1 are highly expressed in the liver and intestine and have broad overlap in terms of shared drug substrates. Therefore interplay between drug-metabolizing enzyme and transporter proteins may need to be considered when the potential for any particular observed drug-drug interaction is evaluated.

Another aspect of drug interactions to consider relates to loss of drug efficacy associated with induction of drug-metabolizing enzymes and drug transporters expressed in the intestine and liver. It now appears that the expression of drug disposition genes is largely under transcriptional control by members of the nuclear receptor 1 (NR1) family of transcription factors, including constitutive androstane receptor (CAR), pregnane X receptor (PXR), and farnesoid X receptor (FXR). These nuclear receptors share a common signaling mechanism involving ligand binding to the receptor, heterodimerization with the 9-cis retinoic acid receptor (RXR), binding of the heterodimer to response elements of target genes, and subsequent initiation of gene transcription. Some drug disposition genes apparently possess shared transcriptional regulation. For example, PXR appears to be critical for the regulated expression of drug-metabolizing enzymes such as CYP3A4, 2B6, and 2C9 as well as the efflux transporter MDR1. More comprehensive reviews of the importance of transcriptional regulation to the drug disposition process have recently been published.

**MDR1 and drug interactions.** Drug interactions involving the cardiac glycoside digoxin still remain a clinically relevant interaction because the clinical indication for digoxin administration has remained remarkably unchanged over the past century. Despite a wealth of data showing predictable digoxin interactions with concomitant administration of drugs such as quinidine, verapamil, and cyclosporine, only recently has the mechanism underlying this interaction been defined. In vitro and animal studies clearly show that digoxin is a high-affinity substrate for MDR1, and interacting drugs have been shown to be MDR1 inhibitors. Indeed, other known MDR1 inhibitors such as clarithromycin, talinolol, and atorvastatin have also been shown to increase the area under the plasma concentration–time curve of digoxin. As a result of the cellular membrane domain–specific expression of MDR1, inhibition of intestinal MDR1 activity would be expected to increase digoxin bioavailability and inhibition of renal and hepatic MDR1 activity would be expected to decrease urinary and biliary excretion of digoxin. Conversely, induction of MDR1 associated with rifampin (INN, rifampicin) therapy has been shown to lower plasma levels of digoxin.

One of the most striking and possibly most clinically relevant effects of MDR1-associated drug interactions relates to the important role of this transporter in altering the CNS entry of substrate drugs. For example, loperamide is a potent opiate that reduces gut motility by its action at opiate receptors in the gut and, therefore, is widely used as an antidiarrheal agent. In fact, this drug is available as an over-the-counter drug. The drug is devoid of significant central opioid effects such as euphoria or respiratory depression because it is a high-affinity MDR1 substrate, thus normally prevented from intestinal absorption or CNS entry.

Fig 1. Organ-specific expression of drug transporters. The compartmental expression of transporters in various tissues plays a critical role in the drug disposition process. In organs such as the liver and kidney, the coordinated expression and activity of uptake and efflux transporters mediates absorption of exogenous (drugs) and endogenous substrates from the bloodstream in the hepatocyte or proximal tubular cell, respectively. Drugs may then undergo further biotransformation or be excreted unmodified into bile or urine for subsequent elimination from the body. In the intestine expression of uptake and efflux transporters in enterocytes serves to either enhance or limit the absorption of drugs from the intestinal lumen, thus playing a pivotal role in determining the bioavailability of orally administered drugs. Similarly, expression of uptake and efflux transporters in brain capillary endothelial cells serves an important role in maintaining the blood-brain barrier, thereby providing an important means of limiting central nervous system penetration of drugs from the systemic circulation. OCT, Organic cation transporter; OAT, organic anion transporter; NTCP, sodium-taurocholate cotransporting polypeptide; MDR, multidrug resistance protein; BSEP, bile salt export pump; OATP, organic anion transporting polypeptide; MRP, multidrug resistance–associated protein; BCRP, breast cancer resistance protein; PEPT, peptide transporter; MCT, monocarboxylate transporter; ASBT, apical sodium-dependent bile acid transporter.
ingly, coadministration of loperamide and the MDR1 inhibitor quinidine has been shown to elicit central opioid effects. These results clearly suggest that inhibition of MDR1 at the level of the BBB may result in an unexpected increase in CNS entry of MDR1 substrate drugs. Given the broad spectrum of structurally divergent drugs that have been shown to be transported by MDR1, it is likely that a number of adverse drug effects may be related to alteration in the function of this transporter, especially at the BBB.

**OATs and drug interactions.** Perhaps the most widely appreciated drug interaction, first noted more than 5 decades ago, is that of penicillin and probenecid, in which coadministration of probenecid resulted in elevated serum penicillin levels, representing a beneficial drug-drug interaction. Studies have shown that the high renal clearance of penicillins as a result of avid active secretion can be decreased by inhibition of OAT-mediated transport on the basolateral membrane of proximal tubular cells with coadministration of probenecid. Similar effects of probenecid coadministration have now been extended to other anionic drugs such as angiotensin-converting enzyme inhibitors and HIV antiviral drugs.

### Table II. Drug-drug interactions or toxicities involving drug transporters

<table>
<thead>
<tr>
<th>Drug</th>
<th>Inhibitor/inducer</th>
<th>Measured effect/toxicity</th>
<th>Putative mechanism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>Probenecid</td>
<td>Decreased renal clearance, prolonged half-life</td>
<td>Inhibition of OATs</td>
<td>45</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>Probenecid</td>
<td>Decreased renal clearance, prolonged half-life</td>
<td>Inhibition of OATs</td>
<td>45</td>
</tr>
<tr>
<td>Antiviral drugs</td>
<td>Probenecid</td>
<td>Decreased renal clearance, prolonged half-life</td>
<td>Inhibition of OATs</td>
<td>45</td>
</tr>
<tr>
<td>Procainamide</td>
<td>Cimetidine</td>
<td>Decreased renal clearance, increased AUC</td>
<td>Inhibition of OCT, OAT, OATP</td>
<td>45</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>Cimetidine</td>
<td>Decreased renal clearance, increased AUC</td>
<td>Inhibition of OCT, OAT, OATP</td>
<td>45</td>
</tr>
<tr>
<td>Dofetilide</td>
<td>Cimetidine</td>
<td>Decreased renal clearance, increased AUC</td>
<td>Inhibition of OCT, OAT, OATP</td>
<td>45</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Fruit juices</td>
<td>Decreased plasma levels</td>
<td>Inhibition of OATPs</td>
<td>130</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>NSAIDs</td>
<td>Decreased renal clearance</td>
<td>Inhibition of OAT3</td>
<td>50</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Probenecid</td>
<td>Decreased renal clearance</td>
<td>Inhibition of OAT3</td>
<td>50</td>
</tr>
<tr>
<td>Loperamide</td>
<td>Quinidine</td>
<td>Increased CNS adverse effects</td>
<td>Inhibition of MDRI</td>
<td>41</td>
</tr>
<tr>
<td>Talinolol</td>
<td>Verapamil</td>
<td>Decreased plasma levels</td>
<td>Inhibition of MDRI</td>
<td>131</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Erythromycin</td>
<td>Increased plasma levels</td>
<td>Inhibition of MDRI</td>
<td>132</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>Rifampin</td>
<td>Decreased plasma levels</td>
<td>Induction of MDRI</td>
<td>133</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Quinidine</td>
<td>Increased plasma levels, decreased renal clearance</td>
<td>Inhibition of MDRI</td>
<td>23</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Verapamil</td>
<td>Increased plasma levels, decreased renal clearance</td>
<td>Inhibition of MDRI</td>
<td>134</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Talinolol</td>
<td>Increased plasma levels, decreased renal clearance</td>
<td>Inhibition of MDRI</td>
<td>37</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Clarithromycin</td>
<td>Increased plasma levels, decreased renal clearance</td>
<td>Inhibition of MDRI</td>
<td>36</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Statins</td>
<td>Increased plasma levels, decreased renal clearance</td>
<td>Inhibition of MDRI</td>
<td>38</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Rifampin</td>
<td>Decreased plasma levels</td>
<td>Induction of MDRI</td>
<td>39</td>
</tr>
<tr>
<td>Troglitazine</td>
<td>Hepatotoxicity</td>
<td></td>
<td>Inhibition of BSEP</td>
<td>57</td>
</tr>
<tr>
<td>Bosentan</td>
<td>Cholestasis</td>
<td></td>
<td>Inhibition of BSEP</td>
<td>56</td>
</tr>
<tr>
<td>Estrogens</td>
<td>Cholestasis</td>
<td></td>
<td>Inhibition of BSEP</td>
<td>55</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>Cholestasis</td>
<td></td>
<td>Inhibition of BSEP</td>
<td>55</td>
</tr>
<tr>
<td>Cidofovir</td>
<td>Nephrotoxicity</td>
<td></td>
<td>Inhibition of OAT1</td>
<td>58</td>
</tr>
<tr>
<td>Adefovir</td>
<td>Nephrotoxicity</td>
<td></td>
<td>Inhibition of OAT1</td>
<td>58</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>Nephrotoxicity</td>
<td></td>
<td>Inhibition of OAT1</td>
<td>61</td>
</tr>
</tbody>
</table>

ACE, Angiotensin-converting enzyme; AUC, area under plasma concentration–time curve; NSAIDs, nonsteroidal anti-inflammatory drugs.

Fifty years ago, it was first noted that penicillin and probenecid, when administered together, resulted in elevated penicillin levels due to inhibition of OAT-mediated transport on the basolateral membrane of proximal tubular cells with coadministration of probenecid. Similar effects of probenecid coadministration have now been extended to other anionic drugs such as angiotensin-converting enzyme inhibitors and HIV antiviral drugs.
Another well-known kidney-associated drug interaction relates to methotrexate, a drug widely used in the treatment of various malignancies and rheumatoid arthritis. By processes that include glomerular filtration and active tubular secretion, methotrexate is eliminated primarily unchanged in urine. Interactions between methotrexate and drugs such as NSAIDs, probenecid, and penicillin have been reported and have resulted in severe complications including bone marrow suppression and acute renal failure. We now know that the mechanism behind this interaction is likely a result of inhibition of OAT-mediated methotrexate transport by these drugs.

In addition to drug-drug interactions, unexpected drug-induced organ toxicities also represent an important subset of adverse drug reactions and may account for a significant proportion of fatal reactions to drugs, estimated to be approximately 100,000 deaths per year. Potential risk factors for the development of drug-induced organ toxicities include age and kidney and liver function, as well as lifestyle features such as alcohol consumption, smoking, and diet. Interestingly, transporters are now being implicated not only in drug-drug interactions but also for drug-induced organ toxicities. This is consistent with the function of certain transporters in their elimination of often-toxic xenobiotics. Table II lists drug-induced organ toxicities that are thought to be mediated at least in part by specific interactions with transporter proteins.

**Inhibition of bile salt transport and hepatotoxicity.** Drug-induced liver injury remains a significant problem for many drugs already in clinical use, as well as new drugs under development. The exact mechanisms or pathways by which drug-induced hepatocellular damage occurs, for the most part, have not been fully elucidated. However, there is emerging evidence to suggest that drug-induced cholestasis can result in the intracellular accumulation of bile salts, whose detergent actions promote hepatocellular damage by interfering with mitochondrial functions. Recent studies implicate the inhibition of certain hepatic transporters in initiating the cascade of cholestasis and hepatocellular injury.

**BSEP, an efflux transporter responsible for secretion of bile salts across the canalicular membrane into bile, appears to be a key target of such drug-induced cholestasis. In vitro studies have demonstrated that drugs such as cyclosporine, rifampin, and the oral hypoglycemic agent glyburide (INN, glibenclamide) can directly inhibit rat Bsep-mediated transport of bile salts.** Bosentan, an endothelin receptor antagonist initially developed for broad indications such as congestive heart failure, was found to cause a dose-dependent cholestatic liver injury and noted to be a potent inhibitor of rat Bsep. Similar types of conclusions have been noted for the novel antidiabetic drug troglitazone, the first drug to be marketed among drugs in this class, but it was subsequently withdrawn from the market because of an unexpected number of drug-induced liver injuries.

**Nephrotoxicity and transporter involvement.** For many drugs, the kidney is an important organ for their elimination. In addition to excretion via glomerular filtration, many drugs, especially those with a tendency for nephrotoxicity, are excreted into urine through active tubular secretion. For drugs such as adefovir and cidofovir, clinically important antiviral agents known to have nephrotoxic properties, accumulation in the proximal tubular cells mediated by the OAT family of transporters may increase their potential for tubular damage. In vitro, cytotoxicity of such agents was noted to be 400- to 500-fold greater in OAT1-expressing cells than in control cells, suggesting that OAT1-mediated cellular accumulation of adefovir and cidofovir may have contributed to their organ-specific toxicity. Similar mechanisms may apply to nephrotoxicity associated with certain β-lactam antibiotics such as cephaloridine (INN, cefaloridine). Conversely, drugs inhibiting such transporters could offer a degree of cytoprotection during concomitant use. Indeed, recent studies have demonstrated that probenecid and NSAIDs may reduce toxicity associated with drugs such as adefovir and cidofovir, as well as cephaloridine, suggesting that competitive inhibition of OAT transporters may, in some cases, reduce nephrotoxicity.

**TRANSPORTERS AND GENETIC HETEROGENEITY**

Inherited defects in drug-metabolizing enzymes such as CYP2D6, CYP2C19, and CYP2C9 have long been recognized to be critical to the observed therapeutic efficacy or toxicities of certain drugs. There is now increasing evidence to suggest that genetic heterogeneity in drug transporters may have similar roles in terms of affecting the disposition of transporter-dependent drugs. Numerous polymorphisms have been identified in transporters important to the drug disposition process (Table III). However, for the most part, studies relating to drug transporter pharmacogenetics have only recently become available or initiated. Nevertheless, the clinical relevance of genetic heterogeneity in transporter genes such as MDRI and OATP1B1 continues to be assessed by various research groups. Indeed,
Table III. Genetic polymorphisms in drug transporters

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Transporter</th>
<th>Polymorphism</th>
<th>Allele frequency</th>
<th>Potential effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>MDR1</td>
<td>C3435T</td>
<td>54% White</td>
<td>Susceptibility to renal tumors</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26% Black</td>
<td>Susceptibility to ulcerative colitis</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53% Asian</td>
<td>Susceptibility to Parkinson’s disease</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53% Asian American</td>
<td>Response in refractory epilepsy</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Overall survival in acute myeloid leukemia</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4 response in HIV-1 therapy</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Susceptibility to childhood acute lymphoblastic leukemia</td>
<td>136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased cyclosporine clearance in renal transplant patients</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Smaller reduction in low-density lipoprotein cholesterol in response to atorvastatin treatment</td>
<td>138</td>
</tr>
<tr>
<td></td>
<td>MDR1</td>
<td>G2677T</td>
<td>46% White</td>
<td>Lower fexofenadine plasma levels</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.5% Black</td>
<td>Reduced risk of chronic renal dysfunction with calcineurin inhibitors after liver transplantation</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Susceptibility to inflammatory bowel disease</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>ABCA1</td>
<td>G655A</td>
<td>25% White</td>
<td>Decreased triglyceride levels, increased high-density lipoprotein plasma levels, decreased risk of atherosclerosis</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A5397C</td>
<td>Rare</td>
<td>Low levels of high-density lipoprotein cholesterol</td>
<td>124, 125</td>
</tr>
<tr>
<td></td>
<td>ABCG2</td>
<td>C421A</td>
<td>46% Japanese</td>
<td>Decreased protein expression and increased sensitivity to irinotecan (SN-38), mitoxantrone, and topotecan in vitro</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14% White</td>
<td>Increased plasma levels of diflomotecan in adult cancer patients</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>SLC22A1</td>
<td>C181T</td>
<td>7.2% White</td>
<td>Decreased uptake of MPP in vitro</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T262C</td>
<td>0.6% White</td>
<td>Decreased uptake of MPP in vitro</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1022T</td>
<td>8.2% Black</td>
<td>Decreased uptake of MPP in vitro</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1201A</td>
<td>3.2% White</td>
<td>Decreased uptake of serotonin in vitro</td>
<td>76, 77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1393A</td>
<td>4% White</td>
<td>Decreased uptake of MPP in vitro</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>SLC22A2</td>
<td>G495A</td>
<td>1% Black</td>
<td>Decreased uptake of MPP in vitro</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C1198T</td>
<td>1.5% Black</td>
<td>Decreased uptake of MPP in vitro</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>SLCO1B1</td>
<td>T521C</td>
<td>1.4% White</td>
<td>Increased pravastatin plasma levels</td>
<td>79, 145, 146</td>
</tr>
<tr>
<td></td>
<td>OATP1B1</td>
<td></td>
<td></td>
<td>Decreased steroid conjugate uptake in vitro</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G1463C</td>
<td>16% Japanese</td>
<td>Decreased steroid conjugate uptake in vitro</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>OATP2B1</td>
<td>C1457T</td>
<td>30.9% Japanese</td>
<td>Decreased transport estrone sulfate in vitro</td>
<td>80</td>
</tr>
</tbody>
</table>

MPP, 1-Methyl-4-phenylpyridinium.
genotype-phenotype correlative studies have the potential to determine the influence of an individual’s genetic makeup in determining the response to drug therapy.

Genetic polymorphisms in MDR1 (ABCB1). The range of substrates transported by MDR1 is broad and includes a wide variety of pharmacologically distinct agents used in cancer chemotherapy, cardiovascular disease, infectious diseases, immunosuppression, neurologic disorders, and inflammatory states. Therefore it is not surprising that this transporter has received the greatest attention in terms of identification and characterization of single-nucleotide polymorphisms (SNPs). Genic polymorphisms in the MDR1 gene were first identified by Kioka et al63 from in vitro studies in cancer cells. To date, 29 SNPs have been reported in the MDR1 gene. Nineteen SNPs are located in exonic, or coding, regions of the gene, and 12 SNPs lead to nonsynonymous base-pair changes, encoding an amino acid change in the translated protein. A synonymous polymorphism in exon 26, C3435T, noted to be common in various ethnic groups, was initially shown to correlate with the expressed level of MDR1 protein in the duodenum as determined by Western blot analysis and quantitative immunohistologic examination.64 However, the functional role for this SNP still remains controversial.

It should be noted that the C3435T SNP in this transporter has been linked to susceptibility to renal cell carcinoma,65 Parkinson’s disease,66 inflammatory bowel disease,67 refractory epilepsy,68 and response to HIV therapy.69 Clearly, additional studies are needed to determine the functional consequences of MDR1 polymorphisms in vivo to the drug disposition process and disease states, but MDR1 represents an illustrative example of the wide-ranging impact of transporter proteins to clinical medicine. In the context of our review, an exhaustive outline of all of the relevant transporters and their SNPs is not possible. A number of detailed and in-depth reviews are available.70-75 SNPs in drug uptake transporters such as OCT176,77 and OATP1B1 (OATP-C)78-80 have also been noted. Emerging evidence from both in vitro and human studies suggests that SNPs in OATP1B1, for example, may have an impact on the therapeutic efficacy and toxicity of HMG-CoA reductase inhibitors such as pravastatin.79

TRANSPORTERS AND DISEASE

Important insight into the potential clinical relevance of drug transporters can also be gained from diseases associated with loss of transporter function (Table IV). These tend to be genetically inherited diseases and are typically transmitted in an autosomal recessive fashion. The phenotypes associated with such inherited diseases have provided important molecular and pharmacologic insights regarding the endogenous substrates and function of such transporters. Again, a comprehensive discussion of transporters and disease is beyond the scope of this review. Therefore a subset of transporters relevant to drug disposition or response is discussed later.

Table IV. Human diseases associated with ABC transporters

<table>
<thead>
<tr>
<th>Gene</th>
<th>Transporter</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1</td>
<td>ABC1</td>
<td>Tangier disease, familial hypouraphiloproteinemia</td>
</tr>
<tr>
<td>ABCA4</td>
<td>ABCR</td>
<td>Stargardt’s disease, retinitis pigmentosum 19, cone-rod dystrophy, age-related macular degeneration</td>
</tr>
<tr>
<td>ABCB1</td>
<td>MDR1</td>
<td>Chemotherapeutic resistance in cancer</td>
</tr>
<tr>
<td>ABCC1</td>
<td>MRP1</td>
<td>Chemotherapeutic resistance in cancer</td>
</tr>
<tr>
<td>ABCG2</td>
<td>BCRP</td>
<td>Chemotherapeutic resistance in cancer</td>
</tr>
<tr>
<td>ABCB2</td>
<td>Tap1</td>
<td>Immune deficiency</td>
</tr>
<tr>
<td>ABCB3</td>
<td>Tap2</td>
<td>Immune deficiency</td>
</tr>
<tr>
<td>ABCB4</td>
<td>MDR3</td>
<td>Progressive familial intrahepatic cholestasis type 3</td>
</tr>
<tr>
<td>ABCB7</td>
<td>ABC7</td>
<td>X-linked sideroblastic anemia and cerebellar ataxia</td>
</tr>
<tr>
<td>ABCB11</td>
<td>BSEP</td>
<td>Progressive familial intrahepatic cholestasis type 2</td>
</tr>
<tr>
<td>ABCC2</td>
<td>MRP2</td>
<td>Dubin-Johnson syndrome</td>
</tr>
<tr>
<td>ABCC6</td>
<td>MRP6</td>
<td>Pseudoxanthoma elasticum</td>
</tr>
<tr>
<td>ABCC7</td>
<td>CFTR</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>ABCC8</td>
<td>SUR1</td>
<td>Persistent hypoglycemia of infancy</td>
</tr>
<tr>
<td>ABCC9</td>
<td>SUR2</td>
<td>Persistent hypoglycemia of infancy</td>
</tr>
<tr>
<td>ABCD1</td>
<td>ALD</td>
<td>X-linked adrenoleukodystrophy</td>
</tr>
<tr>
<td>ABCG5</td>
<td>ABCG5</td>
<td>Sitosterolemia</td>
</tr>
<tr>
<td>ABCG8</td>
<td>ABCG8</td>
<td>Sitosterolemia</td>
</tr>
</tbody>
</table>

Tap1, Antigen processing transporter 1; CFTR, cystic fibrosis transmembrane conductance regulator; SUR, sulfonylurea receptor; ALD, adrenoleukodystrophy.
MRP2 (ABCC2) and Dubin-Johnson syndrome. Dubin-Johnson syndrome (DJS) is a rare inherited disorder characterized by chronic conjugated hyperbilirubinemia, increased urinary coproporphyrin I (>80%), black pigmentation of the liver, and deficient secretion of amphipathic anionic conjugates. DJS is considered to be a benign disorder, because patients with DJS have no biochemical evidence of hepatic injury aside from moderate elevations in direct and indirect bilirubin serum concentrations. A defect in hepatic canalicular efflux transport had long been suspected to be the cause of this disorder. Molecular cloning of canalicular multispecific organic anion transporter (cMOAT), subsequently termed MRP2, allowed for the definitive association of mutations in MRP2 in patients with DJS. Mechanisms accounting for the absence of MRP2 expression include rapid degradation of mutated MRP2 messenger ribonucleic acid, impaired MRP2 protein maturation, inappropriate MRP2 trafficking, and impaired transport activity.

Mutations in this transporter are of interest in terms of drug disposition, in that MRP2 has been shown to transport glucuronide, glutathione, and sulfate conjugates of numerous endobiotics and xenobiotics. In addition, MRP2 is capable of transporting unconjugated drugs including ampicillin, ceftriaxone, and pravastatin, as well as a number of chemotherapeutic agents, such as etoposide, vincristine, doxorubicin, and cisplatin. The relevance of commonly occurring SNPs in MRP2 to drug disposition is currently being studied by many laboratories.

BSEP (ABCB11) and progressive familial intrahepatic cholestasis type 2. Bile acid transport across hepatocytes represents the major driving force for bile flow in mammals and is a critical component in the enterohepatic circulation of bile acids. Bile formation and flow are essential for normal liver and gastrointestinal function. The vast majority of bile salts are conjugated in the hepatocyte and transported into bile against a steep concentration gradient, a process mediated by ATP-dependent transport proteins. The progressive familial intrahepatic cholestases (PFICs) are a group of disorders characterized by clinical and genetic heterogeneity and, until recently, have been poorly understood at the molecular level.

PFIC is a syndrome notable for chronic intrahepatic cholestasis usually beginning in early infancy to early childhood, with progression of liver disease to cirrhosis in the first decade of life. Symptoms include severe pruritus, jaundice, growth failure, coagulopathy, and complications related to portal hypertension. Various treatment modalities have included ursodeoxycholic acid therapy, partial biliary diversion, limited ileal bypass surgery, and liver transplantation. Patients with PFIC have either low (PFIC type 1 or 2) or high (PFIC type 3) γ-glutamyltranspeptidase levels in the serum.

Genetic linkage analysis in patients with PFIC type 2 revealed a locus on chromosome 2q24, which localized to an efflux transporter initially referred to as sister of P-glycoprotein (SPGP). Expression of mammalian Spgp in vitro demonstrated that it functioned as an ATP-dependent bile salt transporter, and it was thereafter renamed the bile salt export pump (Bsep). To date, multiple mutations in the BSEP gene have been linked to patients with PFIC type 2, and not surprisingly, most patients lack immunohistochemically detectable BSEP in the liver. Patients with PFIC type 2 have biliary bile salt concentrations lower than 1% of normal, further supporting the role of BSEP as the major, if not the only, canalicular bile salt efflux transporter in humans.

Given that hepatotoxicity associated with drugs such as troglitazone, bosentan, cyclosporine, and glyburide may relate to drug-induced inhibition of this transporter, SNPs that alter the activity or expression of BSEP may eventually be identified as an important risk factor for an individual's susceptibility to development of drug-induced cholestasis. However, in-depth and systematic studies are needed to more fully delineate the relative importance of BSEP to drug-induced hepatotoxicity.

MDR3 (ABCB4), PFIC type 3, and intrahepatic cholestasis of pregnancy. PFIC type 3 is a progressive liver disease associated with jaundice, discolored stools, hepatosplenomegaly, and mild pruritus. Progression of disease leads to portal hypertension and cirrhosis. PFIC type 3 can be distinguished from other subtypes of PFIC by high serum γ-glutamyltranspeptidase levels and liver histologic characteristics demonstrating ductular proliferation and inflammatory infiltrates in the early stages despite patency of intrahepatic and extrahepatic bile ducts. Patients with PFIC type 3 have very low bile-associated phospholipid levels despite the presence of normal levels of bile salts.

It has become apparent that another ATP-dependent efflux transporter, MDR3, is responsible for translocation of phosphatidylcholine from the inner to the outer leaflet of the hepatocyte canalicular membrane, thereby facilitating the secretion of phospholipids into bile. Mutations in the MDR3 gene have now been clearly linked to PFIC type 3. Types of mutations in PFIC type 3 patients include frameshift, nonsense, and missense mutations.
Intrahepatic cholestasis of pregnancy (ICP) is characterized by the occurrence of cholestasis in pregnant women with an otherwise normal medical history and can lead to fetal distress, premature labor, and unexplained third-trimester intrauterine death. Interestingly, mothers of patients with PFIC type 3 are often heterozygous for MDR3 mutations and appear to be at an increased risk for familial ICP. Similarly, women with a history of ICP or a family history of ICP appear to be more susceptible to cholestasis while taking oral contraceptives.

Although not primarily a drug transporter, MDR3 has been shown to be capable of transporting drugs such as digoxin, paclitaxel, and vinblastine, although the capacity for drug transport appears to be modest. As noted for BSEP, inhibition of MDR3 may be an important risk factor for drug-induced cholestasis and liver injury.

**ABCA1 (ABCA1), Tangier disease, and familial hypoalphalipoproteinemia.** It is widely believed that high-density lipoprotein cholesterol (HDL-C) protects against atherosclerosis by facilitating the removal of excess cholesterol from arterial cells. Subsequently, lipids can be transported back to the liver for excretion into bile in a process termed reverse cholesterol transport. Interestingly, a rare autosomal recessive disease characterized by a near absence of apolipoprotein AI (ApoAI) and HDL-C and cholesterol ester accumulation in reticuloendothelial cells has been linked to a defect in a transporter. This disorder is known as Tangier disease (TD), and affected individuals have a significantly increased (4- to 6-fold) risk for development of coronary artery disease. In 1999 several groups reported a series of mutations in the ABCA1 gene in patients with Tangier disease and familial hypoalphalipoproteinemia. Knockout mice with targeted disruption of the Abca1 gene exhibit a phenotype similar to that of Tangier disease. ABCA1 is also an ATP-dependent efflux transporter expressed in multiple tissues. In vitro data demonstrated that ABCA1 increases cellular cholesterol and phospholipid efflux to ApoAI, a process that promotes formation of HDL-C. To date, at least 50 mutations responsible for Tangier disease and familial hypoalphalipoproteinemia have been identified in the ABCA1 gene. These mutations impair the function of ABCA1 through a variety of mechanisms, including dysfunctional interaction of ABCA1 with ApoAI, mistrafficking of ABCA1 to the plasma membrane, rapid degradation of ABCA1 protein, or defective ATP binding.

Of relevance to the population at large, polymorphisms in ABCA1 have been identified. Indeed, a number of these SNPs are common and appear to be risk factors for the development of CAD, suggesting that the ABCA1 transporter may represent a heretofore unrecognized atherosclerosis risk factor for the general population. More recently, 2 separate studies demonstrated that functionally significant nonsynonymous SNPs in ABCA1 were associated with low levels of HDL-C in the general population. The benefits of molecular studies regarding the ABCA1 transporter lie in a more thorough elucidation of lipid homeostasis and its role in the development of coronary artery disease, as well as its potential role in the targeted pharmacologic intervention for coronary artery disease. Agonists that selectively increase ABCA1 activity could function to increase the clearance of excess cholesterol from arterial macrophages, thereby potentially reducing blood vessel atherosclerosis. Conversely, drugs that inadvertently inhibit ABCA1 may promote atherosclerosis or accelerate the process.

**CONCLUSIONS**

Drug disposition represents a complex interplay between processes involved in drug absorption, distribution, metabolism, and excretion. There is now an increasing appreciation of drug transporters expressed in organs such as the liver, kidney, intestine, and brain as significant determinants of drug-drug interactions, drug-induced organ toxicities, and diseases. Clearly, this is an emerging field that appears to be poised to provide important new insights into our understanding of mechanisms underlying intersubject variability in drug responsiveness and may lead to drug therapies that will further maximize target specificity while minimizing unintended toxicities.

Dr Ho has no conflicts of interest. Dr Kim has served as a consultant for AstraZeneca Pharmaceuticals (Wilmington, Del), Pfizer (New York, NY), Abbott Laboratories (Abbott Park, Ill), Purdue Pharma (Stamford, Conn), and Merck & Company (Whitehouse Station, NJ). He has also received research funding from Pfizer, Merck & Company, Amgen (Thousand Oaks, Calif), Altana Pharma (Konstanz, Germany), AstraZeneca Pharmaceuticals, Abbott Laboratories, and Bristol-Myers Squibb (New York, NY).

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The 5-hydroxytryptamine 4 receptor agonist mosapride does not antagonize morphine-induced respiratory depression

**Background:** On the basis of experiments in rats, serotonin 4 receptor (5-hydroxytryptamine 4 [5-HT₄]) agonists have been proposed as a novel therapeutic strategy for the selective treatment of respiratory depression caused by opioids while leaving analgesic effects unaffected. The effects in rats have been seen with the 5-hydroxytryptamine 4a (5-HT₄a) agonist BIMU8, which is currently not available for use in humans.

**Methods:** In a proof-of-applicability study, the clinically recommended dose of 5 mg mosapride, currently the only 5-HT₄ agonist available for clinical use, was given in a placebo-controlled manner 3 times daily for 5 days to 12 healthy men and women. During the actual experiments, a further 15 mg mosapride was administered. After baseline measurements of respiratory depression, by use of a carbon dioxide rebreathing method, and of pain, by use of electrical and chemical pain stimuli, 30 mg morphine per 70 kg body weight was administered intravenously within 2 hours. After assessment of respiratory depression and pain, 2 mg naloxone was intravenously administered within 20 minutes, followed by a third assessment of respiratory depression and pain. In ancillary experiments 10 rats received 100 mg/kg mosapride orally or placebo 50 minutes before intraperitoneal injection of 10 mg/kg morphine, followed 20 minutes later by injection of naloxone, and the respiratory frequency was monitored.

**Results:** With placebo coadministration, the slope of the relationship between expiratory volume and CO₂ concentration in the inspired air was significantly reduced, from $1.11 \pm 0.46$ L/mm Hg CO₂ at baseline to $0.39 \pm 0.25$ L/mm Hg CO₂ at the end of the morphine infusion ($P < .001$). Co-administration of mosapride had no effect on respiratory depression induced by morphine (slope of $0.39 \pm 0.19$ L/mm Hg CO₂, $P > .7$). In contrast, naloxone significantly reversed the slope to $0.78 \pm 0.36$ L/mm Hg CO₂ ($P = .001$). Morphine produced significant effects on electrical and chemical pain stimuli, which were partially reversed by naloxone, but mosapride did not affect the analgesic effects of morphine. In rats mosapride similarly failed to prevent a slowing of the breathing frequency after morphine administration but naloxone reversed the respiratory depression.

**Conclusion:** Our results show that, with mosapride, opioid-induced respiratory depression cannot be prevented, and because other 5-HT₄ agonists are not currently available for clinical use, a cure for opioid-induced respiratory depression as promised by the previous successful experiments in laboratory animals is not yet available in clinical practice. (Clin Pharmacol Ther 2005;78:278-87.)

Jörn Lötsch, MD, PhD, Carsten Skarke, MD, Andreas Schneider, Thomas Hummel, MD, PhD, and Gerd Geisslinger, MD, PhD Frankfurt am Main and Dresden, Germany
The serotonin 4a receptor (5-hydroxytryptamine 4a (5-HT$_{4a}$)) agonist BIMU8 reduced or prevented opioid-induced respiratory depression in rats. This action was explained to be a result of a functional antagonism between the opioid and the 5-HT$_{4a}$ agonist in the pre-Bötzinger complex, a region in the lower brainstem involved in respiration, where opioid and 5-HT$_{4a}$ receptors are coexpressed. The decrease in intracellular cyclic adenosine monophosphate after activation of $\mu$-opioid receptors is counterbalanced by the increase in cyclic adenosine monophosphate levels produced by activation of 5-HT$_{4a}$ receptors. The functional antagonism did not affect the antinociceptive action of opioids because 5-HT$_{4a}$ receptors are absent in the regions of the spinal cord involved in the processing of pain stimuli.

These experimental findings suggest a novel therapeutic strategy for the treatment of respiratory depression in humans caused by opioids. A meta-analysis of 165 reports with data from nearly 20,000 patients found an incidence of respiratory depression related to opioid administration after surgery of 0.3% when naloxone requirement was used as the indicator and an incidence of 17% when oxygen desaturation was used as the indicator. An analysis of data from 47 Canadian hospitals indicated an estimated incidence of severe respiratory depression of 0.03% with patient-controlled analgesia and 0.13% with epidural opiate analgesia. In approximately 1600 patients who had received patient-controlled analgesia at the University of Alberta Hospitals, 8 cases of serious respiratory depression were detected. Importantly, there are still fatal outcomes after opioid administration even under controlled conditions in the clinical setting. Therefore a possibility of a cure of life-threatening adverse drug effects offered by a new therapeutic principle should be assessed as soon as possible for its immediate applicability into clinical practice. The 5-hydroxytryptamine 4 (5-HT$_{4}$) agonist BIMU8 used in the rat experiment is not available for clinical use. Considering the time needed to develop a drug after a new therapeutic principle has been found experimentally, we found the possible prevention of deaths after opioid therapy important enough to test whether existing 5-HT$_{4}$ agonists can be used for prevention of opioid-induced respiratory depression. At this time, the only clinically available 5-HT$_{4}$ agonist is mosapride, a drug used for the treatment of disturbances in gastrointestinal motility. Mosapride has been found to distribute into the brain, a prerequisite for its present off-label use. Using a placebo-controlled design, we administered mosapride at the clinically approved dose to healthy volunteers and investigated whether this prevented or decreased morphine-induced respiratory depression while leaving analgesia unchanged. The experiments were accompanied by a parallel-group experiment in rats with administration of higher doses to check the principal suitability of mosapride in the current context.

METHODS

Volunteer study

In a double-blind 2-way crossover study, 5 healthy men and 7 women (aged 20-32 years, all within ±10% of their ideal body weight) received 5 mg mosapride (Gasmotin; Dainippon Pharmaceutical, Osaka, Japan) or placebo 3 times daily for 5 days to achieve steady-state conditions. This regimen corresponded to the recommended dosing of mosapride. On the day of morphine administration, subjects received 3 doses of 5 mg mosapride or placebo at −1.5, 0, and 1.5 hours from the beginning of morphine administration to facilitate active brain concentrations of mosapride during assessment of the morphine effects. The experiments started with baseline measurements of respiratory depression and analgesia. Subsequently, morphine (Morphinsulfat- GRY; Gry-Pharma, Kirchzarten, Germany) was administered in an open-label fashion (15 mg morphine base per 70 kg body weight within 15 minutes, followed by 15 mg/70 kg within 2 hours). After infusion, respiratory depression and analgesia were assessed and 2 mg naloxone was subsequently intravenously administered within 20 minutes, followed by a third assessment of respiratory depression and analgesia. The study was performed in accordance with the Declaration of Helsinki on Biomedical Research Involving Human Subjects. The University of Frankfurt Medical Faculty Ethics Review Board approved the study protocol, and written informed consent was obtained from all subjects before the study.

Assessment of respiratory depression. The respiratory depressive effect of morphine was assessed by means of carbon dioxide rebreathing. Subjects are instructed to breath into a plastic bag for 5 minutes, during which the CO$_2$ concentration in the inspired air increases, resulting in stimulation of the respiratory center and hyperventilation. Minute ventilation, end-tidal CO$_2$ levels, and ventilatory rate were measured continuously (Oxycon Pro; Jaeger, Hoechberg, Germany). The slope of the linear relationship between the minute ventilation and the CO$_2$ concentration in the breathing air was defined as the primary target parameter to measure respiratory depressive effects of morphine.
Assessment of analgesia. Opioid-related analgesia was assessed by use of 2 experimental pain models that have been repeatedly shown to be suitable to reliably assess the analgesic effects of opioids in humans. In the first pain model, pain tolerance was measured by use of gradually increasing transcutaneous electrical stimulation. Painful electric stimuli (5-Hz sine waves; increase of intensity, 0.2 mA/s, from 0 to 20 mA) were applied via 2 gold electrodes placed on the medial and lateral side of the distal phalangeal joint (middle finger of the left hand as default testing site) (Neurometer CPT; Neurotron, Baltimore, Md). During testing, subjects kept a button continuously pressed until they found the pain intolerable and interrupted the current by releasing the button. The electrical current at which this occurred was defined as pain tolerance, the target parameter of this pain model. Each value of pain tolerance was the median of 5 subsequent measurements obtained at an interval of 1 minute. This pain model has been previously demonstrated to reliably quantify the analgesic effects of opioids such as hydromorphone or morphine.

Analgesia was also assessed in a second pain model by use of short pulses (200 ms) of gaseous CO₂ (65% vol/vol) embedded in a constantly flowing air stream (8 L/min) and delivered to the nasal mucosa via a Teflon tube with an outer diameter of 4 mm (Olfactometer OM2; Burghart Instruments, Wedel, Germany). The evoked stinging pain was rated on a visual analog scale ranging from 0 (no pain) to 100 (severe pain). Concomitantly, event-related cortical potentials were recorded from a central site (position Cz of the international 10/20 system) referenced to linked earlobes (A1 and A2). Twenty-four CO₂ stimuli per session were delivered at an interval of 35 seconds. Target parameters were the pain ratings and the amplitudes of the pain-related potentials, obtained by averaging of the 24 recordings. Analgesia was indicated by a decrease in these parameters. This pain model has previously been demonstrated to reliably and sensitively quantify the analgesic effects of opioids such as fentanyl and morphine.

Statistics. The calculation of the sample size was based on previously obtained data, and it was determined that 11 subjects were needed to establish a reversal of respiratory depression by 50%. Data were evaluated by use of ANOVA for repeated measures, with “session” (ie, baseline, morphine, and naloxone) and “mosapride” (ie, mosapride or placebo) as within-subject factors and “sex” as the between-subjects factor. Post hoc comparisons were done by t tests with α correction when indicated. Data are presented as means and SDs.

Ancillary experiments in rats

Ten male Sprague-Dawley rats (Charles River, Uppsala, Sweden) weighing 211 ± 6 g were maintained in climate- and light-controlled rooms (24°C ± 0.5°C, 12-hour dark/12-hour light cycle). They had free access to food and water before the experiments. In all experiments the ethics guidelines for investigations in conscious animals were observed, and experiments were approved by the local ethics committee. Mosapride tablets were homogenized, dissolved in 0.03-mol/L phosphate buffer, and suspended in xylose before oral administration. By use of an observer-blinded, randomized, parallel-group design, rats received either 100 mg/kg mosapride, the highest reported dose administered to rats, or placebo 50 minutes before intraperitoneal injection of 10 mg/kg morphine. An additional single rat received 300 mg/kg in a nonblinded fashion. The timing of mosapride administration was chosen to place the sensitive part of expected respiratory depression around the expected peak mosapride concentrations. That is, during preliminary tests, respiratory depression had been observed 10 to 15 minutes after morphine injection, and mosapride has a peak plasma concentration in rats at 1 hour, without delay from plasma to tissue. The respiratory frequency was monitored by recording of the breathing movements. After morphine administration, rats were placed into a Plexiglas cylinder with an inner diameter of 5.5 cm. Between the rat’s right body side and the inner surface of the cylinder, a small balloon laxly filled with air was tightly connected to a Teflon tube. With each breathing movement of the rat, the pressure inside the balloon and Teflon tube changed. This change was recorded with a spirometer port attached to a PowerLab 4/25 recording device and the Chart recording software (ADInstruments, Spechbach, Germany). After 15 minutes of recording, 0.2 mg naloxone was injected intraperitoneally, and the recording was continued for approximately 10 minutes.

RESULTS

Volunteer study

All subjects finished the study as planned. Side effects were mild to moderate and did not necessitate medical intervention. Side effects were typical for opioids (eg, nausea, tiredness, drowsiness, or blurred vision). Their frequency and magnitude did not differ significantly between the mosapride-morphine and placebo-morphine conditions. The most striking side effect was vomiting, which occurred on average 4.8 ± 3.7 times during the observation period under morphine.
and placebo and 5.8 ± 4.6 times under morphine plus the serotonin receptor agonist mosapride (95% confidence interval for the difference between conditions, -0.6 to 2.8; P = .18).

**Respiratory depression.** Morphine produced a significant decrease in the slope of the relationship between the expiratory volume and the CO₂ concentration in the inspired air (P < .001 for ANOVA factor “session”). The slope was reduced from 1.11 ± 0.46 L/mm Hg CO₂ at baseline to 0.39 ± 0.25 L/mm Hg CO₂ for morphine plus placebo (P < .001 for t test of morphine versus baseline) (Fig 1). Coadministration of mosapride had no effect on the respiratory depression induced by morphine (slope of 0.39 ± 0.25 for morphine plus
placebo and 0.39 ± 0.19 for morphine plus mosapride, \( P > .7 \) for ANOVA factor “mosapride”) (Fig 1). In contrast, respiratory depression was significantly, although incompletely, reversed by naloxone, indicating that the experimental method was suitable for measuring a reversal in respiratory depression (slope of 0.78 ± 0.36 L/mm Hg \( CO_2 \), \( P = .001 \) for the measurement after naloxone versus that during maximum morphine effects but \( P = .002 \) for the measurement after naloxone versus baseline) (Fig 1). The subjects’ sex had no influence on respiratory depression, as indicated by the absence of significant main effects or interactions including the factor “sex.”

Analygesia. Morphine produced analgesia in both pain models (Fig 2). Pain tolerance to the electric stimuli increased from 3.2 ± 1.7 mA to 4.6 ± 2.8 mA (\( P < .001 \) for ANOVA factor “session” and \( P < .01 \) for \( t \) test of morphine versus baseline). In the \( CO_2 \) pain model, the intensity of the painful \( CO_2 \) stimuli decreased from 70 ± 13 to 26 ± 13 mm on the visual analog scale (\( P < .001 \) for ANOVA factor “session” and \( P < .001 \) for \( t \) test of morphine versus baseline). Similarly, the peak-to-peak amplitude N1P2 of the pain-related cortical potentials decreased from 57 ± 25 \( \mu \)V to 32 ± 14 \( \mu \)V (\( P < .001 \) for ANOVA factor “session” and \( P < .01 \) for \( t \) test of morphine versus baseline). Naloxone administration completely reversed the analgesic effects in the \( CO_2 \) pain ratings (\( P < .05 \) for the measurements after naloxone versus measurements during maximum morphine effects and \( P > .05 \) for the measurement after naloxone versus baseline) but incompletely reversed the effects of morphine on the amplitudes of the pain-related cortical potentials (\( P > .05 \) for the measurements after naloxone versus measurements during maximum morphine effects and \( P < .05 \) for the measurement after naloxone versus baseline) (Fig 2). The morphine effects on electric pain tolerance were also reversed by naloxone (\( P < .01 \) for the measurements after naloxone versus measurements during maximum morphine effects); however, the reversal was not complete (\( P = .045 \) for the measurement after naloxone versus baseline). The 5-HT\(_4\) agonist mosapride did not significantly modify the analgesic actions of morphine (Fig 2). The subjects’ sex had no influence on analgesic drug effects, as indicated by the absence of statistically significant interactions including the factor “sex.” Amplitudes of the pain-related potentials were larger in women than in men (\( P < .05 \) for between-subjects factor “sex”), but the effects of the medication on these amplitudes were similar in both sexes (no interaction for “session” by “sex” or “mosapride” by “sex”).

Experiments in rats

The breathing frequency in rats decreased, starting at approximately 10 minutes after morphine injection. The oral administration of 100 mg/kg mosapride 50 minutes before morphine did not change the respiratory depressive effects of the opioid (Fig 3). Clear hypventilation was even detected in the single rat after administration of 300 mg/kg mosapride (data not shown). In contrast, naloxone reversed the respiratory depression.

DISCUSSION

When life-threatening adverse drug effects are present, any possibility of an immediate remedy has to be pursued. With mosapride, a 5-HT\(_4\) agonist is available for clinical use. Therefore there was a reasonable chance to immediately apply into clinical practice the finding from experiments in laboratory animals that administration of a 5-HT\(_4\) agonist prevents respiratory depression induced by opioids. This opportunity to cure a life-threatening side effect of opioid therapy justified our investigation.

Our results show that we are not yet in a position to prescribe serotonin 5-HT\(_4\) receptor agonists together

Fig 2. Analgesic effects. A, Stinging pain evoked by short pulses of gaseous \( CO_2 \) delivered to nasal mucosa. Painful \( CO_2 \) stimuli evoked cortical potentials (A1, grand means [ie, event-related potentials averaged across all subjects]), became smaller after morphine administration (A2), and became again greater after naloxone administration (A3). This was reflected in a significant decrease in peak-to-peak amplitude N1P2 of the pain-related potentials (A4) after morphine administration as compared with baseline and in an increase of these amplitudes after naloxone administration, although reversal to baseline was not complete. \( CO_2 \) stimuli were perceived to be less painful after morphine administration than before (A5, means and SDs), which was completely reversed by naloxone. B, Electric pain model. The intensity when the 5-Hz electric sine wave stimuli became intolerably painful increased after morphine administration and decreased after naloxone. Mosapride had no significant influence on morphine analgesia. 1 Asterisk, \( P < .05 \); 2 asterisks, \( P < .01 \); 3 asterisks, \( P < .001 \).
Fig 3. Examples of 2 typical outcomes of recordings of respiratory frequency in rats after intraperitoneal administration of 10 mg/kg morphine and its reversal by naloxone. Oral administration of 100 mg/kg mosapride 50 minutes before morphine did not prevent development of respiratory depression. Graphs in center of upper and lower panels show respiratory frequency recorded every second throughout the experiments, starting immediately after morphine injection. Outliers from the tendency are caused by movement artifacts. Small graphs show breathing movements at baseline, at the time of maximum morphine effects, and after naloxone.
with opioids to avert opioid-induced side effects without loss of analgesia. Mosapride failed to prevent morphine-induced respiratory depression both in human volunteers and in rats. The data allow for this conclusion because respiratory depression after morphine administration has been shown to occur in our study and its reversibility by naloxone could be demonstrated.

The reasons for the negative results may be found in insufficient brain concentrations or insufficient potency of the 5-HT4 agonist. Mosapride exerts its clinical effects for treatment of gastrointestinal symptoms associated with chronic gastritis such as heartburn, nausea, and vomiting and facilitation of gastric emptying predominantly at a peripheral level. Mosapride was shown to enter the brain, which was a prerequisite for our study. However, its brain tissue concentrations 1 hour after oral administration to rats were half of the plasma concentrations, 10 times lower than in the pancreas, and 25 times lower than in the stomach and small intestine. Moreover, it could not be found in the brain 24 hours after the last dosing of 21-day long-term administration of 10 mg · kg⁻¹ · d⁻¹ to rats. On the other hand, 5 mg oral mosapride was found to improve the insulin effects in the muscle, where mosapride concentrations were only 1.3 times higher than in plasma. This is a concentration that has probably been reached in the brain of the volunteers in our study according to a pharmacokinetic simulation based on the current dosing scheme and with the use of a 1-compartment model with first-order absorption and the manufacturer’s information on mosapride pharmacokinetics in humans (maximum plasma concentration of 30.7 ng/mL, time to maximum plasma concentration of 0.8 hour, and half-life of 2 hours). Therefore there was a chance that with our study design mosapride could have reached brain concentrations sufficiently high to exert agonist effects at 5-HT4 receptors. Moreover, it can be assumed that the 300 mg/kg administered to a single rat resulted in the same brain concentrations as in the stomach after 10 mg/kg.

Mosapride had a 9-fold lower potency to competitively displace the 5-HT4 antagonist GR113808 from guinea pig brain receptors than BIMU8, whereas both 5-HT4 agonists had a similar potency at guinea pig ileum receptors. Although this lower potency of mosapride at brain receptors is not undisputed, it provides an explanation for the failure of mosapride to counteract the morphine-induced respiratory depression in our study. Thus the poor brain penetration, together with a lower potency, is a factor that probably has contributed to the negative result of our investigation in contrast to the successful prevention of opioid-induced respiratory depression by BIMU8 in rats. Therefore our investigation, as a proof-of-applicability study, clearly showed that we do not yet have the tool to prevent opioid-induced respiratory depression as hypothesized from the experiments in rats. The interpretation of our results as a proof-of-concept study is weaker because of the possibility that much higher brain concentrations of mosapride or other, more potent or better brain-penetrating 5-HT4 agonists, none of which are currently available for clinical use, could have resulted in a similar outcome to that in the rat experiments with BIMU8.

Morphine clearly produced analgesic effects in 2 experimental pain models that had previously been shown to be suitable for assessment of opioid analgesia. The effects of morphine were highly statistically significant, and naloxone reversed the effects at least partially. Consistent with the previous experiments in rats, mosapride did not affect the analgesic effects of morphine. In line with previous reports, opioid analgesia in the electric pain model showed no sex differences. The same is true for the CO₂ pain model, as well as for the morphine effects on respiration. Nonetheless, our study was not designed to identify sex differences in the effects of morphine, and their absence between only 5 men and 7 women cannot dispute their repeatedly described existence (for reviews, see references 33 and 34). In support of sex differences in nociception it is our observation of significantly larger amplitudes of the event-related cortical potentials in response to the CO₂ pain stimuli in women than in men, which corresponds with previous observations with the same pain model and with cortical potentials evoked by other stimuli.

In conclusion, our attempt failed to transfer the principle of 5-HT4 agonism for prevention of opioid-induced respiratory effects into clinical practice by using an available substance and thus to provide an immediate cure for life-threatening side effects of opioids even administered under controlled clinical conditions. This failure was probably a result of a low potency of mosapride or, alternatively, the limited amount of mosapride that entered the brain.

None of the authors has any conflicts of interest.

References


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PHARMACOKINETICS AND DRUG DISPOSITION

Effect of renal impairment on multiple-dose pharmacokinetics of extended-release ranolazine

Ranolazine is a novel compound under development as an antianginal agent. The multiple-dose pharmacokinetics of extended-release ranolazine and 3 major metabolites was investigated in healthy subjects (N = 8) and subjects with mild to severe renal impairment (N = 21). The ranolazine AUC0-12 (area under the concentration-time curve between 0 and 12 hours after dosing) geometric mean ratio versus healthy subjects at steady state was 1.72 (90% confidence interval [CI], 1.07-2.76) in subjects with mild impairment, 1.80 (90% CI, 1.13-2.89) in those with moderate impairment, and 1.97 (90% CI, 1.23-3.16) in those with severe renal impairment. Creatinine clearance was negatively correlated with AUC0-12 and the maximum observed concentration for ranolazine and the O-dearylated metabolite (P < .05 for all variables), as well as the N-dealkylated metabolite (P < .001), but not for the O-demethylated metabolite. Less than 7% of the administered dose was excreted unchanged in all groups, indicating that factors other than reduced glomerular filtration rate contributed to the increase in ranolazine concentrations in renal impairment. No serious adverse events were observed in the study. (Clin Pharmacol Ther 2005;78:288-97.)

Markus Jerling, MD, PhD, and Hisham Abdallah, MPharm, PhD  Palo Alto, Calif

Ranolazine is a novel compound under development as an antianginal agent. Its chemical name is (±)-N-(2,6-dimethylphenyl)-4-[2-hydroxy-3-(2-methoxyphenoxy) propyl]-1-piperazine acetamide.1 Unlike existing antiischemic agents, ranolazine has been shown to be hemodynamically neutral with little effect on blood pressure and heart rate.2-4 The mechanism of action for the antianginal effect has not been fully characterized. Ranolazine is an inhibitor of the late inward sodium (I_{Na}) current, which reduces calcium overload, and by doing so, it should improve left ventricular diastolic dysfunction (ie, decrease stiffness). By decreasing diastolic tension, ranolazine should decrease oxygen consumption5 and compression of the vascular space. In fact, ranolazine has been shown to improve left ventricular regional diastolic function in patients with ischemic heart disease.6 Thus inhibition of the late I_{Na} current by ranolazine is likely to contribute to the antianginal effect, but other mechanisms may also be involved. The clinical utility of the original immediate-release formulation of ranolazine (ranolazine IR) was limited by its short half-life,7 leading to development of an extended-release preparation (ranolazine ER) better suited to the maintenance of clinical efficacy with a twice-daily regimen. The safety and efficacy of ranolazine have been studied in phase III trials,3,4 including in combination with traditional therapies.4

Ranolazine is extensively metabolized in the liver by the cytochrome P450 (CYP) 3A and 2D6 enzymes, with 5% to 10% being excreted unchanged by the kidneys.8,9 Three major metabolites of ranolazine at steady state are produced by dearylation (CVT-2512), O-demethylation (CVT-2514), and N-dealkylation (CVT-2738).8,11

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Reprint requests: Markus Jerling, MD, PhD, Stavsgardsgatan 30, Bromma 167 56, Sweden.
E-mail: Markus.Jerling@cvtr.com
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The target population for ranolazine will include angina patients with renal impairment, as a result of both the physiologic reduction in renal function with age and specific disease processes such as diabetes. A close relationship exists between cardiovascular and renal functions, given that cardiovascular diseases, including congestive heart failure and hypertension, have the potential to cause renal failure. Specific data on the impact of renal function on ranolazine pharmacokinetics have not been obtained in any other study. Our study investigated the effect of renal impairment on the steady-state pharmacokinetics of ranolazine and major metabolites after multiple oral dosing. Ranolazine ER doses of 500 to 1500 mg twice daily have shown significant improvement in exercise treadmill time in angina patients. A maintenance dose of 500 mg twice daily was selected for our study to account for the possible reduction in ranolazine clearance with impaired renal function.

**METHODS**

**Study design and subjects.** This was a phase I open-label study designed to evaluate the multiple-dose pharmacokinetics of ranolazine and metabolites in subjects with mild, moderate, or severe renal impairment compared with healthy matched control subjects. The study was conducted by APEX Research, Munich, Germany (principal investigator, Dr. Med. A. Weil), between January and June 2001, after approval by the regional (Bayerische Landesärztekammer, Körperschaft des öffentlichen Rechts, München, Germany) and local (Unabhängige Ethikkommission Schwaben, Ulm, Germany) ethics committees. Seven subjects in each renal impairment group (mild, moderate, and severe) were studied, in addition to 8 healthy control participants matched for age, weight, and sex. In each study group, there was to be a minimum of 2 subjects of each sex. All subjects gave written informed consent. Renal function was defined according to creatinine clearance (CrCl) as follows: healthy volunteers, 81 to 140 mL/min; subjects with mild impairment, 51 to 80 mL/min; subjects with moderate impairment, 30 to 50 mL/min; and subjects with severe impairment, less than 30 mL/min but not requiring dialysis.

Key criteria for eligibility included subjects aged between 18 and 75 years who had a body weight between 40 and 120 kg, were within 25% of their ideal body weight, and were nonsmokers or light smokers able to abstain from smoking for 24 hours. A normal electrocardiogram (ECG), blood pressure, and heart rate at baseline were required, along with the absence of clinically important physical or laboratory abnormalities except those related to the underlying renal dysfunction. In addition, the baseline hemoglobin level had to be 10 g/dL or greater in renally impaired subjects. Healthy volunteers were excluded if they had received another investigational drug within 12 weeks before the start of the study, and subjects with renal impairment were excluded if they had received another investigational drug within 4 weeks before the start of this study. Subjects were also excluded if they had any surgical or medical condition that might interfere with the absorption, distribution, metabolism, or excretion of the drug or were positive for human immunodeficiency virus or hepatitis B. Exclusion criteria also included ongoing treatment with drugs causing significant inhibition or induction of CYP3A or CYP2D6.

Subjects were screened by physical examination, medical history, measurement of vital sign, laboratory assessments, ECG, and testing for hepatitis B, hepatitis C, and human immunodeficiency virus. Twenty-four-hour urine samples were collected to determine CrCl. Admission procedures included a medical history update, measurement of vital signs, ECG, and an alcohol breath test. All female subjects were required to have a negative urinary pregnancy test result and to agree to use a contraceptive during the study. Participants were not permitted to intake alcohol or methylxanthine-containing food or beverages from within 24 hours before the study start throughout the duration of the study. In addition, grapefruit or grapefruit juice was not permitted from within 14 days before the study to after study completion. Strenuous exercise was not permitted during the study, nor was smoking allowed during the first 24 hours or for 2 hours preceding ECG recordings. Subjects stayed in the research unit from day −1 to day 5 of the study.

Subjects received an initial loading dose of 875 mg ranolazine ER (tablets of 500 + 375 mg), followed by 500 mg ranolazine administered every 12 hours for a total of 4 maintenance doses. The initial dose was administered after an overnight fast. All drug dosing was discontinued after day 3. Ranolazine ER tablets were supplied by CV Therapeutics (Palo Alto, Calif).

**Safety assessments.** Safety assessments included blood pressure, heart rate, clinical chemistry tests, hematology tests, urinalysis, and physical examination. Serial ECGs were obtained at 0, 1, 2, 3, 4, 5, 7, 9, and 12 hours during day −1 (which was a run-in day with no ranolazine administration), after the first ranolazine dose on day 1, and at steady state after the last dose on day 3. Additional ECGs were obtained up to 48 hours after the last dose. Adverse events were recorded throughout the study until the final scheduled follow-up.
(a follow-up telephone call was made 14 days after discharge to complete any outstanding inquiries, including those about adverse events).

**Pharmacokinetic analyses.** Concentrations of ranolazine and the metabolites CVT-2512, CVT-2514, and CVT-2738 were determined from urine and heparinized plasma samples collected periodically at predetermined time points throughout the study. Urine samples were collected for 0 to 12 hours, 12 to 24 hours, and 24 to 48 hours after the final dose. Blood samples for clinical chemistry analysis were collected into heparin-coated tubes, and blood samples for hematologic analysis were collected into ethylenediaminetetraacetic acid–coated tubes. Blood samples were collected before dosing and at 1, 2, 3, 4, 5, 7, 9, and 12 hours after dose 1; before doses 3, 4, and 5; and at 1, 2, 3, 4, 5, 7, 9, 12, 16, 24, 28, 32, 36, and 48 hours after dose 5. After centrifugation, plasma samples were stored at −20°C. For analysis, plasma samples were precipitated with acetonitrile and methanol and analyzed by HPLC coupled with mass spectrometry by use of positive ion electrospray ionization.

The concentration range of the validated assay was from 50 to 10,000 ng/mL for ranolazine and from 10 to 2000 ng/mL for metabolites.

The pharmacokinetic (PK) parameters of ranolazine and its metabolites were computed by noncompartmental analyses by use of WinNonlin, version 3.2 (Pharsight, Mountain View, Calif). Parameters included the area under the concentration–time curve between 0 and 12 hours after dosing (AUC0–12) on days 1 and 3, maximum observed concentration (Cmax) on days 1 and 3, and elimination phase half-life (t1/2) on day 3. The day 3 trough concentration (Ct) was defined as follows: Ct = (Cpredose + C12)/2, where Cpredose is predose concentration and C12 is concentration at 12 hours. The achievement of steady-state kinetics on day 3 was tested by comparing the plasma concentration before dose 5 (C0) with the 12-hour postdose concentration (Cp0). Renal clearance was computed from day 3 measurements as the amount excreted in urine over a 12-hour period divided by AUC0–12.

**ECG analyses.** ECGs were evaluated by an independent laboratory (St Louis University Core ECG Laboratory, St Louis, Mo) where readers were blinded to subject and treatment. The longest QT interval across all 12 leads for each ECG was used for the assessment of this interval. The optimal correction factor for calculating the QT interval corrected for heart rate (QTc) was determined post hoc for each subject by pooling QT and R-R interval data from all ECGs collected before the first ranolazine dose (N = 11) and determining the value of α in the formula QTc = QT/RRα that minimized the correlation between QTc and heart rate. The resulting individual formula was applied to all ECG data from that subject. The change in QTc from baseline (ΔQTc) was calculated for each ECG during ranolazine dosing as the difference from the corresponding time point on day −1. The possible drug-related effect on QTc was evaluated by use of linear regression of ΔQTc versus the ranolazine plasma concentration, including all data points with measurable ranolazine concentrations.

**Statistical analyses.** The number of subjects recruited was considered suitable for the aims of the study under US Food and Drug Administration guidelines. In keeping with common practice for renal impairment studies, PK parameters were compared for each cohort defined by degree of impairment with those in healthy control subjects. According to the most recent Food and Drug Administration guidance document for PK studies in subjects with impaired renal function (issued in May 1998), the cohort sizes should be sufficient to detect PK differences large enough to warrant dosage adjustments. No formal power calculation was performed before the study. A post hoc calculation demonstrated 80% power to detect a change of 137% of the healthy control subjects’ mean ranolazine AUC0–12 by use of a 2-sided test at a significance level of .05. Linear regression models were used to assess the impact of creatinine clearance, weight, sex, and age on the PK parameters. Backward elimination with the criterion of significance at the .05 level was used to select the model that best described the relationship between PK parameters and renal impairment. Significance tests were based on the model thus selected. In all cases the selected model included at most 1 covariate, which was either sex or weight. Comparisons of each renal impairment group to healthy control subjects were performed by use of the Fisher least significant difference procedure. In addition, geometric mean ratios to healthy control subjects were computed, along with 90% confidence intervals (CIs), by use of a 1-factor ANOVA applied to log-transformed PK parameters. Statistical analyses were performed by use of SAS statistical software, version 6.12 (SAS Institute, Cary, NC).

**RESULTS**

**Subjects.** Twenty-nine subjects were enrolled in and completed the study, with 7 subjects in each of the 3 renal impairment groups (mild, moderate, and severe)
and 8 healthy control subjects. There were 2 women in each group. Demographic variables were comparable across all groups, and all subjects were white. The mean age (±SD) of all subjects was 57.3 ± 11.3 years. Mean creatinine clearance values (±SD) were 96.9 ± 13.7 mL/min, 63.4 ± 5.7 mL/min, 39.4 ± 7.2 mL/min, and 20.4 ± 9.7 mL/min in healthy control subjects, those with mild renal impairment, those with moderate renal impairment, and those with severe renal impairment, respectively. Subjects with renal impairment were commonly receiving long-term drug therapy to treat conditions associated with the renal disease, in particular, hypertension.

**Pharmacokinetics of ranolazine in renal impairment.** Concentration-time profiles in subjects with normal and severely impaired renal function for ranolazine and the metabolite CVT-2738 after the last dose on day 3 are shown in Fig 1. The comparison of

![Fig 1.](image)
renal impairment, respectively.

At steady state, 6.2% of the administered ranolazine dose was excreted unchanged in healthy subjects, and 6.2%, 5.1%, and 4.1% of the administered dose was excreted unchanged in subjects with mild, moderate, and severe renal impairment, respectively.

Table I summarizes the steady-state PK parameters of ranolazine and the metabolites CVT-2512, CVT-2514, and CVT-2738. Table II shows geometric mean ratios with 90% CIs for AUC_{0-12} and C_{max}, comparing the various cohorts of renally impaired subjects with healthy control subjects. AUC_{0-12}, C_{max}, and C_{trough} for ranolazine were generally higher in subjects with renal impairment relative to healthy control subjects at steady state, although the differences did not reach statistical significance in a categoric analysis. AUC_{0-12}, C_{max}, t_{1/2},
and C\textsubscript{trough} for the ranolazine metabolites CVT-2512 and CVT-2738 were significantly increased in subjects with severe renal impairment relative to healthy subjects (\(P<.05\)). AUC\textsubscript{0-12}, C\textsubscript{max}, and C\textsubscript{trough} for CVT-2738 were also significantly increased in subjects with moderate renal impairment (\(P<.05\)). These parameters were not changed significantly for any metabolite in those with mild renal impairment, although the observed mean AUC\textsubscript{0-12}, C\textsubscript{max}, and C\textsubscript{trough} for CVT-2738 were substantially higher compared with those in healthy control subjects. The renal clearance for ranolazine and the 3 metabolites was significantly reduced in all renally impaired groups compared with healthy subjects (\(P<.05\)).

CrCl, the parameter used to define renal function, was significantly correlated to a number of PK parameters when analyzed as a continuous variable. The AUC\textsubscript{0-12} and C\textsubscript{max} of ranolazine, CVT-2512, and CVT-2738 exhibited a significant negative correlation with CrCl, with CVT-2738 showing the most significant correlation (\(P<.001\) for both parameters). CVT-2514 AUC\textsubscript{0-12} (\(P=.51\)) and C\textsubscript{max} (\(P=.48\)) did not correlate with CrCl. Figs 2 and 3 illustrate the relationship between AUC\textsubscript{0-12} and CrCl for ranolazine and CVT-2738, respectively.

Safety. There were no deaths, serious adverse events, or withdrawals because of an adverse event during the study. All adverse events were mild or moderate in severity. There were adverse events in 5 of 8 healthy subjects (62.5%) and in 13 of 21 subjects with renal impairment (61.9%). Adverse events were more frequently reported in subjects with severe renal impairment (5/7 [71.4%]) and moderate renal impairment (6/7 [85.7%]) than in those with mild renal impairment (2/7 [28.6%]). The most frequently reported drug-related adverse events in renally impaired subjects included constipation (5/21 [23.8%]) and increased creatinine (5/21 [23.8%]). One subject (healthy) had increased blood urea nitrogen, which was considered probably drug-related, and 1 subject (moderate impairment) had hypoglycemia, which was considered probably not related to the study drug. There were no clinically significant changes in hematologic characteristics, clinical chemistry study results, or urinalysis.

Supine systolic blood pressure and pulse were unchanged after dosing. In subjects with severe renal impairment, an increase in diastolic blood pressure from the predose value on day 1 was observed on day 3, ranging from 12.0 mm Hg on average (SD, 15.4 mm Hg) to 17.4 mm Hg (SD, 20.6 mm Hg) by the time...
point during the first 12 hours after the last dose. By 48 hours, the difference from baseline had diminished to 4.0 mm Hg (SD, 15.5 mm Hg). The corresponding values for the first 12 hours after the last dose were 1.1 mm Hg (SD, 5.8 mm Hg) to 6.9 (SD, 12.4 mm Hg) in the moderate group, 2.0 mm Hg (SD, 6.7 mm Hg) to 2.9 mm Hg (SD, 5.9 mm Hg) in the mild group, and 2.0 mm Hg (SD, 7.1 mm Hg) to 5.0 mm Hg (SD, 5.9 mm Hg) in control subjects. There were no changes in physical examination variables at the end of the study.

ECG results. In healthy volunteers ranolazine administration had no effect on QTc as described by the following regression function:

\[ \text{QTc (in milliseconds)} = 0.82 + 8 \times 10^{-5} \times \text{Ranolazine concentration} \quad (R^2 < 0.001, P = 0.97 \text{ for slope}) \]

The corresponding relationship in renally impaired subjects was as follows:

\[ \Delta \text{QTc (in milliseconds)} = 2.0 + 0.0024 \times \text{Ranolazine concentration} \quad (R^2 = 0.022, P < 0.005 \text{ for slope}) \]

Fig 3. Steady-state AUC\textsubscript{0-12} for metabolite CVT-2738 versus CrCl. Linear regression with 95% CI was as follows: AUC\textsubscript{0-12} = 20,590 − 193 × CrCl \((R^2 = 0.48 \text{ with } P < 0.001 \text{ for slope})\).

DISCUSSION

This study investigated the influence of renal impairment on the pharmacokinetics of ranolazine and 3 major metabolites. These metabolites were chosen by virtue of their significant contribution to the overall systemic exposure or urinary recovery after ranolazine administration. The metabolite CVT-2512 is formed by O-dearylation (removal of the methoxyphenyl group) of ranolazine. In a single-dose oral study with carbon 14–labeled ranolazine in healthy male subjects, the area under the plasma concentration–time curve (AUC) of CVT-2512 was 12% of that of ranolazine and its urinary excretion amounted to approximately 2% of the urinary recovery of all ranolazine-related compounds. CVT-2514, which is the product of O-demethylation of ranolazine at the methoxy group, is further glucuronidated and sulfated at the resulting free phenolic group. The AUC of CVT-2514 was 37% of that for ranolazine; however, its urinary excretion accounted for less than 1% of urinary recovery. The metabolite CVT-2738 is produced by N-dealkylation by hydrolysis at the piperazine ring. The AUC of CVT-2738 was 41% of that for ranolazine, and its urinary recovery was almost 12%.

Ranolazine AUC\textsubscript{0-12} and C\textsubscript{max} at steady state were 1.7- to 2-fold higher in the different cohorts of renal impairment as compared with healthy control subjects, which means that renal impairment should be considered when doses are selected for individual patients. The categoric analyses with comparisons of renally impaired subgroups versus control subjects did not
Fig 4. Change in QTc from time-matched baseline values versus ranolazine plasma concentration in healthy control subjects. Linear regression with 95% CI was as follows: \( \Delta \text{QTc} = 0.82 + 8 \times 10^{-3} \times \text{Ranolazine concentration} \) \( (R^2 < 0.001, P = 0.97 \text{ for slope} [95\% \text{ CI, } -0.004 \text{ to } 0.004]). \)

Fig 5. Change in QTc from time-matched baseline values versus ranolazine plasma concentration in renally impaired subjects. Linear regression with 95% CI was as follows: \( \Delta \text{QTc} = 2.0 + 0.0024 \times \text{Ranolazine concentration} \) \( (R^2 = 0.022, P < .005 \text{ for slope} [95\% \text{ CI, } 0.0008 \text{ to } 0.0039]). \)
demonstrate statistical significance for these differences, which can be explained by the cohort sizes. Because creatinine clearance is a continuous variable, it is well suited for performing regression analyses versus the PK parameters. Both AUC\textsubscript{0-12} and C\textsubscript{max} for ranolazine were negatively correlated with creatinine clearance (P < .05). The apparent ranolazine half-life was not prolonged with an increasing degree of renal impairment. This is explained by the flip-flop kinetic behavior of the extended-release formulation, for which the absorption half-life is longer than the elimination half-life. A modest prolongation of the true elimination half-life, under those conditions, will not affect the observed apparent half-life.

Given the relatively small percentage of ranolazine excreted unchanged in urine (5%-10%), the magnitude of the effect of renal impairment on ranolazine AUC\textsubscript{0-12} and C\textsubscript{max} was greater than expected. It is well established that renal impairment may be associated with a reduction in hepatic drug metabolic activity, including the CYP3A4 pathway.\textsuperscript{15} A contribution of reduced hepatic ranolazine clearance in subjects with renal impairment is, therefore, possible.

Among metabolites measured in this study, CVT-2738 kinetics showed the largest dependency on renal function, with a close to 5-fold higher mean AUC\textsubscript{0-12} value in subjects with severe impairment than in control subjects, despite an increase in ranolazine parent compound that was only 2-fold. This indicates that CVT-2738 is excreted, to a large extent, renally unchanged. CVT-2514, the \(\text{O}\)-demethylated metabolite, increased slightly less than proportionately to the ranolazine concentrations, which would suggest a reduction in its formation rate under conditions of renal impairment. However, the between-subject variability in AUC\textsubscript{0-12} was large for this metabolite, limiting the possibilities to draw general conclusions. AUC\textsubscript{0-12} for CVT-2512 followed the ranolazine values closely, with no indications that renal impairment affects its formation rate or total clearance.

The type and severity of adverse events reported are comparable with those observed in other populations at similar ranolazine plasma concentrations. The population studied was too small and the treatment duration too short to draw general conclusions on the incidence of adverse events in renally impaired subjects. A reversible modest increase in diastolic blood pressure was observed in subjects with severe renal impairment but not in the other treatment groups. Blood pressure monitoring is recommended when ranolazine therapy is initiated in subjects with severe renal impairment. Severe renal impairment is commonly associated with hypertension, which was true for all subjects in this cohort in our study. Blood pressure monitoring is, therefore, standard clinical practice in this patient group.

The slope for QTc increase versus the ranolazine concentration in the renally impaired subjects amounted to 2.4 ms per every 1000 ng/mL. The result should be interpreted with caution because the study was not primarily designed to evaluate QTc effects and did not include a placebo control. Extensive evaluations in both healthy volunteers and different patient groups have consistently demonstrated a linear increase in QTc by 2.4 ms for every 1000-ng/mL ranolazine concentration (ie, similar to that observed in the renally impaired group). The combined results of the current and previous ranolazine studies suggest that renal impairment does not affect the concentration-response relationship for the QTc effect of ranolazine. The lack of an observed effect on QTc in the control group in our study may be explained by the small sample size and limited range of ranolazine concentrations. In subjects with renal impairment in our study, the relationship between ranolazine concentrations and the increase in QTc was similar to that in other populations, despite a disproportionate increase in CVT-2738 concentrations. No major differences in other safety variables were observed. Metabolites are, therefore, not shown to contribute differently to the QTc effect in renally impaired subjects.

In conclusion, ranolazine pharmacokinetics is affected by renal function, with ranolazine AUC\textsubscript{0-12} geometric mean ratio values versus those in healthy subjects at steady state of 1.72 (90% CI, 1.07-2.76) in subjects with mild impairment, 1.80 (90% CI, 1.13-2.89) in subjects with moderate impairment, and 1.97 (90% CI, 1.23-3.16) in subjects with severe renal impairment. Renal impairment is, therefore, a factor to consider when ranolazine doses are selected. Less than 7% of the administered dose was excreted unchanged in all groups, indicating that factors other than reduced glomerular filtration rate contributed to the increase in ranolazine concentrations in renal impairment. The exposure to the metabolite CVT-2738 increased more than proportionately to that of the ranolazine parent compound in subjects with impaired renal function, suggesting a significant direct renal excretion of this metabolite.

Dr Russell Reeve is acknowledged for excellent help with PK and statistical analyses.

Dr Jerling and Abdallah were employees of CV Therapeutics at the time of study conduct and reporting. Dr Jerling is currently a consultant to CV Therapeutics.
References

Metabolism-based cyclophosphamide dosing for hematopoietic cell transplant

When cyclophosphamide (120 mg/kg) is used for hematopoietic cell transplant, the increased area under the curve of carboxyethylphosphoramide mustard (AUC_{CEPM}) is related to liver toxicity and death. We determined the feasibility of dose-adjusting cyclophosphamide to a preset metabolic endpoint (AUC_{CEPM}, 325 ± 25 μmol/L · h). In 20 patients blood sampling was done over a 16-hour period after administration of 45 mg/kg cyclophosphamide; AUC_{CEPM} from 0 to 16 hours was calculated by noncompartmental analysis. The expected AUC_{CEPM} for 0 to 48 hours was estimated, and the second cyclophosphamide dose was determined. The mean second cyclophosphamide dose was 42 mg/kg, and the mean total cyclophosphamide dose was 86 mg/kg (range, 54-120 mg/kg). The mean AUC_{CEPM} for the time from 0 to 48 hours was 296 μmol/L · h (95% confidence interval, 275-317 μmol/L · h). A retrospective analysis indicated that AUC_{CEPM} could be more accurately predicted by use of a population pharmacokinetic model. We conclude that metabolism-based dosing of cyclophosphamide is feasible and that a lower cyclophosphamide dose does not affect engraftment. (Clin Pharmacol Ther 2005;78:298-308.)

George B. McDonald, MD, Jeannine S. McCune, PharmD, Ami Batchelder, BA, Scott Cole, BS, Brian Phillips, BS, Aaron G. Ren, BS, Paolo Vicini, PhD, Robert Witherspoon, MD, Thomas F. Kalhorn, BS, and John T. Slattery, PhD

Seattle, Wash

Dosing of chemotherapy drugs has traditionally been based on the patient’s weight or body surface area to reduce the interpatient variability in drug effect.1,2 However, considerable interpatient variability remains in the pharmacokinetics of various chemotherapy agents whose doses were based on weight or body surface area.1-4 This interpatient variability, along with clinically relevant pharmacodynamic relationships, has led to adaptive dosing methods for chemotherapy (therapeutic drug monitoring [TDM]) (eg, dosing carboplatin by use of the Calvert equation).5 The use of TDM has been partially hindered by doubts that its benefit justifies the logistic challenges with pharmacokinetic sampling and the cost. However, there are clear examples where TDM has been shown to improve the outcome for cancer patients: TDM of the antimetabolite methotrexate improved response rates in children with B-cell acute lymphoblastic leukemia (66% ± 7% in the standard dosing arm versus 76% ± 6% in those undergoing TDM, P = .02).6 Most hematopoietic cell transplant centers incorporate TDM for oral or intravenous busulfan to lower toxicity while maximizing efficacy (ie, lower graft rejection and leukemic relapse rates).7,8 The use of Bayesian models to conduct TDM in real time to predict the systemic pharmacokinetics of anti-
cancer agents has been reported with topotecan, paclitaxel, and cyclophosphamide in combination with carboplatin and thiotepa. A limitation of cyclophosphamide-based myeloablative regimens as conditioning therapy for hematopoietic cell transplantation is liver damage, as well as development of multiorgan failure. In a recent study of 147 patients who received 120 mg/kg cyclophosphamide and total body irradiation (TBI) as conditioning therapy, the metabolism of cyclophosphamide was found to be highly variable and liver toxicity was correlated with both the dose of irradiation and the variability of cyclophosphamide metabolism. Increased exposure to the cyclophosphamide metabolite carboxyethylphosphoramide mustard (CEPM) (as determined by the area under the plasma concentration–time curve [AUCCEPM]) was related to liver toxicity, nonrelapse death, and survival, with a 5.9-fold increase in mortality rate for patients in the highest quartile of AUCCEPM, as compared with patients in the lowest quartile. However, AUCCEPM bore no relationship to either engraftment or tumor relapse in this cohort of patients with hematologic malignancy. These results suggested that the cyclophosphamide dose could be reduced to lessen toxicity without jeopardizing either engraftment or antitumor effects of the cyclophosphamide/TBI preparative regimen.

It was not immediately apparent, however, why higher AUCCEPM correlated with liver toxicity and death, because carboxyethylphosphoramide mustard is itself nontoxic and formed in competition with the formation of cytotoxins. The elimination pathways for cyclophosphamide are as follows: The first step is oxidation by several cytochromes P450 to 4-hydroxycyclophosphamide, which itself equilibrates with iminocyclophosphamide and tautomerizes to aldocyclophosphamide. 4-Hydroxycyclophosphamide and its tautomer circulate in blood. Aldocyclophosphamide enters cells and undergoes β elimination to form acrolein (a hepatotoxin) and phosphoramide mustard, which covalently cross-links deoxyribonucleic acid. Within the hepatocyte, aldocyclophosphamide is oxidized to carboxyethylphosphoramide mustard by aldehyde dehydrogenase, and iminocyclophosphamide is conjugated with glutathione, forming glutathionylcyclophosphamide. Previously, the formation of glutathionylcyclophosphamide from iminocyclophosphamide was thought to be “futile”; that is, it was thought that the only fate of the conjugate was hydrolysis, reforming its iminocyclophosphamide and reduced glutathione components (glutathionylcyclophosphamide being too polar to diffuse out of the cell as such). However, we have recently shown that glutathionylcyclophosphamide is eliminated from the hepatocyte by ABCC2-mediated transport. Therefore glutathionylcyclophosphamide formation is a route of elimination of 4-hydroxycyclophosphamide from the liver that competes with the formation of the toxins acrolein and phosphoramide mustard. This observation provided a rationale for the hypothesis that carboxyethylacrolein must be a reporter of actual exposure of the liver to toxins formed from 4-hydroxycyclophosphamide. This hypothesis has been substantiated in studies comparing the disposition of cyclophosphamide and its metabolites between wild-type rats and mutants lacking functional ABCC2.

In this study we examined the hypothesis that we could accurately target the cyclophosphamide dose to a metabolic endpoint, that is, to a specific value for AUCCEPM, and thus eliminate the variability in cyclophosphamide metabolism. Our approach was to give a first dose of cyclophosphamide at 45 mg/kg and then to adjust the second dose of cyclophosphamide on the basis of the first day’s AUCCEPM, thus targeting the total exposure to carboxyethylphosphoramide mustard to a value consistent with low liver toxicity and reliable engraftment. We chose an AUCCEPM value of 325 μmol/L·h as the target for dose adjustment, because this value was the highest AUCCEPM in the lowest quartile of carboxyethylphosphoramide mustard exposure from the 147-patient cohort studied previously.

The primary endpoint of the current study was the accuracy of dose adjustment, that is, whether we could reliably adjust the total dose of cyclophosphamide such that total carboxyethylphosphoramide mustard exposure was in the AUCCEPM range of 325 ± 25 μmol/L·h while providing 4-hydroxycyclophosphamide exposure to an area under the plasma concentration–time curve (AUC) greater than 50 μmol/L·h. We examined engraftment of neutrophils and platelets and liver toxicity as secondary endpoints.

**METHODS**

**Patient selection.** Patients aged 18 to 65 years were considered for study participation if they had a malignant hematologic disease unlikely to respond to conventional treatment and a suitable donor from a human leukocyte antigen–identical family member or unrelated allogeneic donor. High-resolution typing was performed for unrelated donors; donors for patients with a favorable prognosis were selected to be an allele match or a 1-allele mismatch for A, B, C, DRB1, or DQB1. Written consent was obtained via forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, Seattle, Wash.
**Technique of hematopoietic cell transplantation.** Starting 6 days before infusion of stem cells, hyperfractionated TBI (total dose, 12 Gy) was delivered via a linear accelerator as 200-cGy fractions twice daily over a period of 3 days, followed by intravenous cyclophosphamide administration on each of 2 consecutive days. Cyclophosphamide was infused through a central venous access catheter over a period of 1 to 2 hours on the first day at a dose of 45 mg/kg adjusted ideal body weight. On the next day, a second infusion of cyclophosphamide was given, with dosing as described later. During cyclophosphamide infusion days, patients received 2-mercaptopethanesulfonate for uroepithelial prophylaxis, at a milligram dose equal to that of cyclophosphamide. A day of rest followed, with the stem cell infusion occurring on day 0. Cyclosporine (INN, ciclosporin) and methotrexate were given as prophylaxis against graft-versus-host disease, ursodiol (INN, ursodeoxycholic acid) was given as prophylaxis against cholestatic liver disease, and fluconazole, acyclovir (INN, aciclovir), and trimethoprim-sulfamethoxazole were given for infection prophylaxis.

**Cyclophosphamide dosing.** Blood samples were drawn from a central venous access catheter line at the end of the first cyclophosphamide infusion and at 2, 4, 8, 16, 20, and 24 hours after the start of infusion. At each of these times, blood was collected in 2 tubes, one containing ethylenediaminetetraacetic acid for carboxyethylphosphoramide mustard and cyclophosphamide quantitation and the other containing phenylhydrazine hydrochloric acid to stabilize 4-hydroxyxycyclophosphamide. All samples were stored at the bedside at 4°C before sample transport. After collection of the 16-hour blood sample, all samples were taken to the pharmacology laboratory for quantitation of the plasma concentrations of carboxyethylphosphoramide mustard and 4-hydroxyxycyclophosphamide by use of liquid chromatography and mass spectroscopy methods modified for rapid turnover. For the carboxyethylphosphoramide mustard analysis, blood samples collected over ethylenediaminetetraacetic acid were centrifuged at 1500g for 5 minutes at 4°C. An aliquot (100 μL) of the supernatant was transferred to a microfuge tube, with the subsequent addition of internal standard \((D)_{4}–\text{carboxyethylphosphoramide mustard})\) and \(100 \muL\) of the supernatant was injected onto an Agilent Technologies (Palo Alto, Calif) equipped with a cooled autosampler. Separation was achieved on a Zorbax Extend 50 × 2.1–mm 5-μm C18 column (Agilent Technologies) by use of a gradient system in which the aqueous phase consisted of 1-mmol/L ammonium chloride and 10-mmol/L acetate adjusted to pH 8.5 with ammonium hydroxide; the organic constituent was methanol. The initial methanol fraction was 4% and increased to 35% at 1 minute, where it was maintained for another minute before being brought back to initial conditions at 3 minutes. The column was then re-equilibrated for 3 minutes before the next injection. The flow was maintained at 0.25 mL/min throughout, and data were collected between 1.5 and 4.0 minutes. The ions monitored were mass-to-charge ratio 291, 293, and 297 d, consistent with \((35\text{Cl})–\text{carboxyethylphosphoramide mustard})\)−, \((35\text{Cl})_{2}–\text{carboxyethylphosphoramide mustard})\)−, and \((D)_{4}–\text{carboxyethylphosphoramide mustard})\)−, respectively. The mass spectroscopy conditions were as follows: fragmentor, 30 V; capillary voltage, 4000 V; and nitrogen gas, 10 L/min. Standards for carboxyethylphosphoramide mustard were prepared by dilutions of stock carboxyethylphosphoramide mustard solutions with blank plasma to a final volume of 100 μL. The standard curves ranged from 0.54 to 34 μmol/L. The interday variability and intraday variability were 6% and 4%, respectively.

\[\text{AUC}_{\text{CEPM}}\] and 4-hydroxycyclophosphamide AUC (\(\text{AUC}_{\text{HCY}}\)) values for the time from 0 to 16 hours were calculated by noncompartmental analysis. The estimate of the expected \(\text{AUC}_{\text{CEPM}}\) for time 0 to 48 hours was based on the relationship between \(\text{AUC}_{\text{CEPM}}\) from 0 to 16 hours and \(\text{AUC}_{\text{CEPM}}\) from 0 to 48 hours (Fig 1).

A target for exposure to carboxyethylphosphoramide mustard (\(\text{AUC}_{\text{CEPM}}\)) was set at 325 ± 25 \(\mu\text{mol/L} \cdot \text{h}\), a value derived from clinical endpoints and their relationship to cyclophosphamide pharmacokinetics from our prospective study of 147 patients. In brief, 325 \(\mu\text{mol/L} \cdot \text{h}\) was the highest value in the first quartile of carboxyethylphosphoramide mustard exposure—a quartile characterized by the lowest frequency of organ toxicity, the lowest mortality rate, and the best survival rate. The lower limit of exposure to 4-hydroxycyclophosphamide was set at 50 \(\mu\text{mol/L} \cdot \text{h}\), the lowest value observed in the same 147-patient cohort, in whom we could find no relationship between low values for \(\text{AUC}_{\text{HCY}}\) and either engraftment or tumor relapse. On average, total (time 0–48 hours) \(\text{AUC}_{\text{CEPM}}\) is equal to 8.47(\(\text{AUC}_{\text{CEPM}}\) for 0–16 hours)0.813 (derived from Fig 1). The ratio of the expected \(\text{AUC}_{\text{CEPM}}\) from 0 to 48 hours to the desired value of 325 \(\mu\text{mol/L} \cdot \text{h}\) was the dose adjustment factor. The ratio of 90 mg/kg to the dose adjustment factor was the desired total
cyclophosphamide dose to achieve an AUC_{CEPM} from 0 to 48 hours of 325 μmol/L·h. The difference between the desired total dose and 45 mg/kg (the first cyclophosphamide dose) was then administered 24 hours after the start of the first cyclophosphamide dose to try to achieve an AUC_{CEPM} from 0 to 48 hours of 325 ± 25 μmol/L·h. After the seventh patient was enrolled, the accuracy of this equation for estimating AUC_{CEPM} from 0 to 48 hours was reevaluated because 4 patients had demonstrated aberrant cyclophosphamide metabolism, resulting in a second cyclophosphamide dose that yielded an AUC_{CEPM} from 0 to 48 hours that was outside our target range of 325 ± 25 μmol/L·h. Specifically, patients 1 and 5 had extremely short carboxyethylphosphoramide mustard half-lives (<8 hours), and patients 6 and 7 had extremely long carboxyethylphosphoramide mustard half-lives (>30 hours). A new regression equation was generated from 27 patients from the initial cohort of 147 patients who had a carboxyethylphosphoramide mustard half-life of less than 8 hours. This equation was subsequently used in patient 12 (with a short carboxyethylphosphoramide

![Graph](image-url)
mustard half-life) in hopes of correcting the underestimation of the second cyclophosphamide dose. For subsequent patients with a very long carboxyethylphosphoramide mustard half-life, the recommended cyclophosphamide dose was empirically reduced by 15%, because this was the amount of overestimation in patients 6 and 7.

The second dose of cyclophosphamide was adjusted to achieve the target $AUC_{CEPM}$ from 0 to 48 hours, provided that the sum of the observed $AUCHCY$ after the first cyclophosphamide dose and the predicted $AUCHCY$ after the second cyclophosphamide dose was not less than $50 \mu mol/L \cdot h$ and the total cyclophosphamide dose did not exceed 120 mg/kg. The expected $AUCHCY$ for time 0 to 48 hours was estimated in a manner similar to that for CEPM (Fig 1, bottom). On average, total (time 0-48 hours) $AUCHCY$ is equal to $7.91(AUCHCY$ for 0-16 hours$)^{0.758}$. No dose was administered on day 2 that was expected to yield a total $AUCHCY$ from 0 to 48 hours of less than $50 \mu mol/L \cdot h$.

After the second cyclophosphamide dose, blood samples were drawn in the same manner as described earlier, transported, processed, and stored at $-80°C$. The plasma concentrations of carboxyethylphosphoramide mustard, 4-hydroxycyclophosphamide, and cyclophosphamide were quantitated so that the actual

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (y) and gender</th>
<th>Diagnosis</th>
<th>$AUC_{CEPM}$ for 0-48 h ($\mu mol/L \cdot h$)</th>
<th>Proximity to target $AUC_{CEPM}$ of 325 ($\mu mol/L \cdot h$) (%)</th>
<th>$AUC_{HCY}$ for 0-48 h ($\mu mol/L \cdot h$)</th>
<th>Total CY dose (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21/F</td>
<td>AML (second remission)</td>
<td>260</td>
<td>$-20$</td>
<td>84</td>
<td>72</td>
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<tr>
<td>2</td>
<td>59/M</td>
<td>ALL (primary refractory)</td>
<td>338</td>
<td>$+4$</td>
<td>62</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>48/M</td>
<td>ALL (first remission)</td>
<td>339</td>
<td>$+4$</td>
<td>96</td>
<td>97</td>
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<tr>
<td>4</td>
<td>44/F</td>
<td>AML (first relapse)</td>
<td>377</td>
<td>$+16$</td>
<td>57</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>22/M</td>
<td>ALL (third remission)</td>
<td>326</td>
<td>0</td>
<td>134</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>51/F</td>
<td>AML (primary refractory)</td>
<td>372</td>
<td>$+14$</td>
<td>65</td>
<td>54</td>
</tr>
<tr>
<td>7</td>
<td>34/M</td>
<td>ALL (first remission)</td>
<td>238</td>
<td>$-27$</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>8</td>
<td>24/M</td>
<td>Mantle cell lymphoma</td>
<td>286</td>
<td>$-12$</td>
<td>122</td>
<td>120$^*$</td>
</tr>
<tr>
<td>9</td>
<td>54/M</td>
<td>CMML (first relapse)</td>
<td>314</td>
<td>$-4$</td>
<td>48</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>26/F</td>
<td>AML (first remission)</td>
<td>220</td>
<td>$-32$</td>
<td>136</td>
<td>67</td>
</tr>
<tr>
<td>11</td>
<td>37/M</td>
<td>ALL (first remission)</td>
<td>284</td>
<td>$-13$</td>
<td>94</td>
<td>79</td>
</tr>
<tr>
<td>12</td>
<td>45/F</td>
<td>AML (second remission)</td>
<td>384</td>
<td>$+18$</td>
<td>185</td>
<td>97</td>
</tr>
<tr>
<td>13</td>
<td>33/F</td>
<td>ALL (fourth relapse)</td>
<td>263</td>
<td>$-19$</td>
<td>141</td>
<td>60</td>
</tr>
<tr>
<td>14</td>
<td>49/F</td>
<td>T-cell lymphoma</td>
<td>319</td>
<td>$-2$</td>
<td>141</td>
<td>62</td>
</tr>
<tr>
<td>15</td>
<td>21/M</td>
<td>AML (second relapse)</td>
<td>232</td>
<td>$-29$</td>
<td>141</td>
<td>120$^+$</td>
</tr>
<tr>
<td>16</td>
<td>40/F</td>
<td>ALL (second remission)</td>
<td>310</td>
<td>$-5$</td>
<td>208</td>
<td>95</td>
</tr>
<tr>
<td>17</td>
<td>35/M</td>
<td>ALL (first remission)</td>
<td>241</td>
<td>$-26$</td>
<td>170</td>
<td>120$^+$</td>
</tr>
<tr>
<td>18</td>
<td>39/F</td>
<td>AML (second remission)</td>
<td>280</td>
<td>$-14$</td>
<td>201</td>
<td>110</td>
</tr>
<tr>
<td>19</td>
<td>51/M</td>
<td>AML (primary refractory)</td>
<td>269</td>
<td>$-17$</td>
<td>170</td>
<td>120$^+$</td>
</tr>
<tr>
<td>20</td>
<td>30/M</td>
<td>AML (first remission)</td>
<td>270</td>
<td>$-17$</td>
<td>175</td>
<td>105</td>
</tr>
<tr>
<td>Mean ± SD and range</td>
<td></td>
<td></td>
<td>$296 \pm 49$</td>
<td>$-9% \pm 15%$</td>
<td>$124 \pm 51$</td>
<td>$86 \pm 24$</td>
</tr>
</tbody>
</table>

Per protocol, the first dose of cyclophosphamide was 45 mg/kg and the total cyclophosphamide dose was capped at 120 mg/kg.

AUC, Area under plasma concentration–time curve; CEPM, carboxyethylphosphoramide mustard; HCY, 4-hydroxycyclophosphamide; CY, cyclophosphamide; ANC, absolute neutrophil count; SOS, sinusoidal obstruction syndrome; AML, acute myeloid leukemia; ALL, acute lymphocytic leukemia; CMML, chronic monomyelocytic leukemia; N/A, not applicable.

$^*$The calculated second dose of cyclophosphamide for this patient was 84 mg/kg, which would have yielded a total cyclophosphamide dose of 129 mg/kg.

$^+$The calculated second dose of cyclophosphamide for this patient was 111 mg/kg, which would have yielded a total cyclophosphamide dose of 156 mg/kg.

$^{§}$The calculated second dose of cyclophosphamide for this patient was 97 mg/kg, which would have yielded a total cyclophosphamide dose of 142 mg/kg.
values for AUC_{CEPM}, AUC_{HCy}, and cyclophosphamide AUC for the time period from 0 to 48 hours could be calculated.

**Evaluation of engraftment.** The time (in days) to a sustained absolute neutrophil count (ANC) greater than 500/mm^3 and the time to a sustained platelet count greater than 20,000/mm^3 were recorded. Failure to engraft was defined as a lack of neutrophils over 500/mm^3 by day 28. Only patients surviving for at least 21 days were evaluated for graft failure. Graft failure was defined as follows: failure of the ANC to surpass 500/mm^3 before relapse, second transplant, or death (primary graft failure); a decrease in the ANC, before day 100, to less than 100/mm^3 on at least 3 consecutive determinations at least 1 day apart after the initial engraftment, without recovery before relapse, second transplant, or death (secondary graft failure); or the absence of donor T cells as determined by testing for chimerism with the use of variable-number tandem-repeat polymorphisms or in situ hybridization.

**Evaluation of liver toxicity.** Liver toxicity was defined by the presence of sinusoidal obstruction syndrome (SOS) (formerly known as veno-occlusive disease of the liver) and its severity, by the peak total serum bilirubin level to day 20, and by the average total serum bilirubin to day 20.3,12,28

**Comparison with NONMEM modeling for determining cyclophosphamide doses.** After study accrual was complete, we sought a more accurate method for determination of the second cyclophosphamide dose, an approach based on Bayesian individualization of a population pharmacokinetic model. This method uses 4-hydroxycyclophosphamide and carboxyethylphosphoramide mustard blood level data from an individual patient, together with a pharmacokinetic model and
mean parameter values and their variance, derived from the prior study of 147 patients,\(^5\) to obtain the most probable estimates of the pharmacokinetic parameter values for that individual patient. The advantage of this approach to obtaining parameter values for an individual patient is that an increasing “penalty” is assessed in the fitting routine for blood level data that forces an improbable parameter estimate. The pharmacokinetic model is a population nonlinear mixed-effects model and was implemented by use of NONMEM, version V, double precision (University of California, San Francisco, Calif, and Globomax, Hanover, Md).\(^29\) By use of the post hoc option in NONMEM and conditional to our previously developed population pharmacokinetic model,\(^29\) we obtained estimates of pharmacokinetic parameter values for each individual included in this study from the five 4-hydroxycyclophosphamide and carboxyethylphosphoramide mustard concentrations from 0 to 16 hours after the first cyclophosphamide dose. The AUC from 0 to 48 hours was then predicted as the sum of the observed AUC from 0 to 16 hours and the AUC from 16 to 48 hours predicted by the NONMEM model by use of the second cyclophosphamide (Table I). The mean AUC\(_{CEPM}\) for the time from 0 to 48 hours was 296 ± 49 \(\mu\)mol/L·h (median, 285 \(\mu\)mol/L·h; range, 220-384 \(\mu\)mol/L·h; 95% confidence interval, 275-317 \(\mu\)mol/L·h); our target had been 325 ± 25 \(\mu\)mol/L·h. Table I lists, for each patient, the percent deviation of the observed AUC\(_{CEPM}\) for the time from 0 to 48 hours from our target of 325 \(\mu\)mol/L·h; the average percent deviation from our target was −9%, ranging from −32% to +18%. The mean AUC\(_{HCY}\) for the time from 0 to 48 hours was 124 ± 51 \(\mu\)mol/L·h (median, 135 \(\mu\)mol/L·h; range, 48-208 \(\mu\)mol/L·h); our target had been greater than 50 \(\mu\)mol/L·h. The mean cyclophosphamide AUC for the time from 0 to 48 hours was 2976 ± 732 \(\mu\)mol/L·h (median, 3099 \(\mu\)mol/L·h; range, 789-3829 \(\mu\)mol/L·h).

**Engraftment.** Eighteen patients were evaluable for engraftment; all had engraftment, with neutrophil engraftment (ANC ≥500/mm\(^3\)) at a mean of day 18.9 (range, 12-27) and platelet engraftment (≥20,000/mm\(^3\)) at a mean of day 15.9 (range, 9-31). Two patients were not evaluable for engraftment, one (patient 14) because of death from respiratory failure on day 13 and the other (patient 19) because of persistence of blasts after transplantation.

**Liver toxicity.** Fifteen patients had no evidence of liver disease before day 20, 1 had mild SOS, 3 had moderate SOS, 1 had liver disease of uncertain cause, and none had severe SOS. The mean peak serum bilirubin level before day 20 was 2.5 mg/dL (median, 1.4 mg/dL; range, 0.8-7.5 mg/dL). Exposure to cyclophosphamide metabolites and the total cyclophosphamide dose were not higher among 4 patients with SOS, as compared with 16 patients without SOS (median, 261.5 \(\mu\)mol/L·h versus 285 \(\mu\)mol/L·h for AUC\(_{CEPM}\); 110 \(\mu\)mol/L·h versus 137.5 \(\mu\)mol/L·h for AUC\(_{HCY}\); and 72 mg/kg versus 93.5 mg/kg for total cyclophosphamide dose, respectively).

**Relapse of malignancy.** As of May 1, 2005, 8 patients had a relapse and 1 had persistent acute myeloid leukemia after transplantation; 4 died without evidence of relapse, 3 from cardiopulmonary complications and 1 from sepsis; and 7 were alive without relapse at a median of 422 days after transplantation (range, 338-589 days) (Table I).

**Evaluation of population pharmacokinetic model for cyclophosphamide dose adjustment.** During the conduct of this trial, we developed a population phar-
macokinetic model for estimating the AUC \( \text{CEPM} \) and AUC \( \text{HCY} \). In hopes of developing a more accurate and consistent method of dose adjustment, we compared the predicted AUC of each metabolite, based on both the regression equations (Fig 1) and the population pharmacokinetic model, with the observed AUC. In comparison to the regression-based dosing method, the predicted AUC \( \text{CEPM} \) from the population pharmacokinetic model was more closely correlated to the observed AUC \( \text{CEPM} \) (Fig 2). In addition, both methods similarly predicted the AUC \( \text{HCY} \). The mean absolute percent error for the observed AUC \( \text{CEPM} \) from 0 to 48 hours was 17.2% (range, 3.38%-56.02%) with the method used and 9.98% (range, 0.01%-24.45%) for the population pharmacokinetic model. For AUC \( \text{HCY} \) from 0 to 48 hours, the mean absolute percent error was 32.5% (range, 2.15%-71.8%) with the method used and 24.56% (range, 0.40%-78.86%) for the population pharmacokinetic model.

**DISCUSSION**

This study confirms the wide variability in cyclophosphamide metabolism from patient to patient; for example, total cyclophosphamide doses ranged from 54 to 120 mg/kg, a range that would have extended to 156 mg/kg had the protocol not specified capping the total dose at 120 mg/kg. The data show that cyclophosphamide can be dosed on the basis of its observed pharmacokinetics to within a mean of approximately 10% of the target, defined as an exposure to the reporter molecule carboxyethylphosphoramide mustard of 325 \( \mu \text{mol/L \cdot h} \). Without cyclophosphamide dose adjustment based on metabolism, exposure to carboxyethylphosphoramide mustard was shown to vary 16-fold, whereas in the current study, exposure to this metabolite varied 1.7-fold. Exposure to the cyclophosphamide metabolite 4-hydroxycyclophosphamide was, with one exception, always greater than our minimum required level of 50 \( \mu \text{mol/L \cdot h} \). The mean dose of cyclophosphamide was 86 mg/kg, or 28% lower than the standard 120-mg/kg dose of cyclophosphamide that has been in use for 30 years. It may be difficult to find a fixed dose of cyclophosphamide that achieves adequate immune suppression and antitumor activity with a low level of toxicity in view of the wide range of cyclophosphamide doses required to reach a fixed metabolic endpoint.

It is of interest to examine the history of how cyclophosphamide doses were initially defined as part of conditioning therapy for allogeneic transplantation. Early studies demonstrated that cyclophosphamide could be used as the sole conditioning agent for allografts in dogs and in monkeys, where a safe and effective dose was found to be 180 mg/kg. Patients with aplastic anemia and with advanced hematologic malignancy underwent successful allografting after conditioning with 200 mg/kg cyclophosphamide, but relapse of hematologic malignancy remained problematic, suggesting that cyclophosphamide was not sufficiently effective as the sole condi-
tioning agent for patients with leukemia. Early human allografting in Seattle, Wash, was carried out after a single dose of 10 Gy TBI, which resulted in uniform engraftment but did not eliminate the risk of relapse. Cyclophosphamide was then added to TBI with the intent of preventing recurrence of leukemia, and the 120-mg/kg dose was chosen because it was half of the cyclophosphamide dose (240 mg/kg) that was lethal in monkeys. After it was demonstrated that leukemia could be cured after administration of 120 mg/kg cyclophosphamide, TBI, and allografting, the dose of cyclophosphamide in the cyclophosphamide/TBI regimen was not changed over the ensuing decades. However, more recent animal studies showed that metabolites of cyclophosphamide were hepatotoxic, particularly for sinusoidal endothelial cells. Clinical investigation in humans demonstrated that 120 mg/kg cyclophosphamide plus TBI leads to unacceptable liver toxicity and death among patients who generate a large amount of carboxyethylphosphoramide mustard, believed to be a reporter for the toxic metabolites of 4-hydroxycyclophosphamide. In a previous study, we did not find a relationship between relatively low exposure to cyclophosphamide metabolites and relapse of hematologic malignancy, suggesting that a cyclophosphamide dose of 120 mg/kg produces excess toxicity without the benefit of preventing recurrence of malignancy after transplantation. In the current study, recurrent leukemia developed in 8 patients after transplantation and 1 patient had failure to clear blasts. This cohort does not allow conclusions about whether the risk of relapse is affected by lowering the cyclophosphamide dose in the cyclophosphamide/TBI regimen by a mean of 28% from the standard dose of 120 mg/kg.

In this cohort of patients, engraftment of neutrophils and platelets occurred in all evaluable patients despite reductions in cyclophosphamide dosing. Despite early data showing that 10- to 12-Gy TBI was adequate to ensure engraftment in humans, we were concerned that decreasing exposure to phosphoramide mustard, the cyclophosphamide metabolite responsible for immune suppression and antitumor effects, might fail to eradicate host immunity and thus lead to an increased rate of graft failure. This was the rationale for establishing a minimum 4-hydroxycyclophosphamide exposure of 50 μmol/L · h, the lowest level observed in our previous study of cyclophosphamide metabolism, in which engraftment was not related to variation in cyclophosphamide metabolism. There were no cases of either severe SOS or multiorgan failure. Three patients were scored as having moderate hepatic sinusoidal injury, with peak total serum bilirubin levels of 2.7, 4.0, and 7.5 mg/dL, respectively, before a return to the normal range. Respiratory failure caused by idiopathic pneumonia syndrome was seen in 4 patients, with deaths on days 13, 41, 43, and 54, respectively. Development of idiopathic pneumonia despite substantial reductions in cyclophosphamide dosing is not readily explained, because we expected an overall reduction in regimen-related toxicity. Development of idiopathic pneumonia may be related to infusion of alloimmune T cells or to an infectious agent. One patient died of septic shock on day 287.

We conclude that cyclophosphamide can be dosed to a fixed metabolic endpoint (AUC of 325 μmol/L · h for the stable metabolite carboxyethylphosphoramide mustard) with reasonable accuracy. The dose of cyclophosphamide that is required to achieve this endpoint varies by 2.9-fold, from 54 to 156 mg/kg. Lowering the dose of cyclophosphamide from the standard dose of 120 mg/kg did not affect engraftment, and there were no cases of serious liver toxicity, but idiopathic pneumonia was seen in 4 patients. We suggest that future studies use a population pharmacokinetic model that describes the metabolism of cyclophosphamide, because we found that this method provided a more accurate individual estimate of AUC_{CEPM} while achieving an accuracy for AUC_{HCY} similar to that of the regression equation. A population pharmacokinetic model is a powerful tool with which to individualize doses in the clinical setting because it makes use of both typical values and between-subject variation of pharmacokinetic parameters from prior observations in a large number of patients. The usefulness of population pharmacokinetic models has been demonstrated with several chemotherapy regimens. Our study was not powered to demonstrate improvement in patient outcomes, and thus metabolism-based cyclophosphamide dosing cannot be recommended as the standard of care at this time. However, metabolism-based dosing of cyclophosphamide should be further studied to determine whether the rate of nonrelapse mortality that results from the cyclophosphamide/TBI conditioning regimen can be reduced without jeopardizing antitumor effects.

None of the authors has a conflict of interest to report.

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LETTER TO THE EDITOR

Cytochrome P450 2C9 genotype: Impact on celecoxib safety and pharmacokinetics in a pediatric patient

To the Editor:

In the November 2002 and March 2005 issues of the Journal, we presented pharmacokinetic results of a clinical trial evaluating celecoxib and low-dose metronomic chemotherapy as antiangiogenic treatment in children with recurrent solid tumors.1,2 We identified a patient who was exposed to a significantly higher concentration of celecoxib. This letter presents the correlation between celecoxib pharmacokinetics and cytochrome P450 (CYP) 2C9 genotype in children for the first time. Four children were studied (Table I).

The first step in celecoxib metabolism is methylhydroxylation primarily mediated by the polymorphic CYP2C9.3 Two genetic polymorphisms have a functional effect on CYP2C9 activity—alleles CYP2C9*2 and CYP2C9*3. The enzyme coded by the *2 allele, CYP2C9.2, shows only a moderate decrease in activity, whereas the enzyme coded by the *3 allele, CYP2C9.3 (especially in the homozygous state), shows a marked decrease in activity when compared with the wild-type CYP2C9.1,3,4

The patient of interest was homozygous for CYP2C9*3, an extremely rare genotype. Two patients were homozygous for the *1 allele, and one was a CYP2C9*1/*2 carrier. The *2 allele did not have a noticeable impact on celecoxib pharmacokinetics compared with the wild-type patients, which is consistent with previous observations.3 However, the *3 allele had a significant impact on the celecoxib pharmacokinetics. After a single dose, this child had a peak concentration similar to the others, but his systemic exposure was approximately 10 times greater, corresponding to an apparent clearance rate of approximately one tenth that of the other patients studied. Celecoxib’s half-life was calculated to be approximately 30 hours, compared with a mean of 4 hours in the other patients. At steady state, the peak concentration was 3 to 6 times greater (suggesting accumulation) and the area under the plasma concentration–time curve was 10-fold greater. CYP2C9*3 variants may possess only 5% of CYP2C9 activity, and metabolism of celecoxib may decrease by up to 90%,4 which may account for these differences.

Regardless of the patients' genotypes or exposures to celecoxib, none of the patients we studied had signs of toxicity.1,2 The 4 patients were treated for a median of 7.8 weeks (range, 5-62.5 weeks) (Table I) before being removed from the study with disease progression. None of the patients treated in this study for varying lengths of time had any acute or chronic cardiovascular toxicity. In addition, because of the observed tolerability of treatment by the CYP2C9*3/*3 patient, we cautiously but optimistically suggest a wide margin of safety in children for this drug, at least when administered on a short-term basis.

In conclusion, our data demonstrate that celecoxib pharmacokinetics in these patients correlate well with the observed CYP2C9 genotypes. It is conceivable that carriers of the CYP2C9*3 allele may potentially face increased risk for celecoxib dose-related adverse events, especially when asso-

Table I. Pharmacogenetic and pharmacokinetic data for 4 patients treated with celecoxib at a dose of 250 mg/m²

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Duration in study (wk)</th>
<th>Data type</th>
<th>Cmax (µg/L)</th>
<th>AUC (µg/L·h)</th>
<th>CL/F (L·h⁻¹·kg⁻¹)</th>
<th>t½ (h)</th>
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</thead>
<tbody>
<tr>
<td>CYP2C9*3/*3</td>
<td>PM</td>
<td>7.5</td>
<td>Single dose</td>
<td>2940</td>
<td>108,251</td>
<td>0.09</td>
<td>29.9</td>
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<td></td>
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<td>Steady state</td>
<td>8461</td>
<td>93,725</td>
<td>0.02</td>
<td>41.2</td>
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<tr>
<td>CYP2C9*1/*2</td>
<td>IM</td>
<td>8</td>
<td>Single dose</td>
<td>3713</td>
<td>13,037</td>
<td>0.7</td>
<td>2.9</td>
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<td></td>
<td></td>
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<td>Steady state</td>
<td>3451</td>
<td>11,119</td>
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<tr>
<td>CYP2C9*1/*1</td>
<td>EM</td>
<td>62.5</td>
<td>Single dose</td>
<td>1865</td>
<td>11,599</td>
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<td>5.5</td>
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<td>10,760</td>
<td>0.8</td>
<td>3.2</td>
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<td>Steady state</td>
<td>1677</td>
<td>10,905</td>
<td>0.7</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Cmax, Maximum plasma concentration; AUC, area under plasma concentration–time curve; CL/F, apparent oral clearance; t½, half-life; PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer.

*AUC data were calculated for the interval from time 0 to infinity for single-dose data and 0 to 12 hours for steady-state data.
ciated with other comorbidity factors. This may be particularly important for long-term administration of high doses. In light of recent data demonstrating increased risk for cardiovascular events with prolonged use of cyclooxygenase-2 inhibitors including celecoxib, it would be beneficial to know whether CYP2C9 status affects the risk of cardiovascular toxicity. One of our CYP2C9*1/*1 patients was treated for 15 months with no clinical evidence of celecoxib-related cardiovascular toxicities. Future studies specifically evaluating these toxicities should include a CYP2C9 genotyping component to investigate the possibility of a relationship between genotype and susceptibility to cardiovascular toxicity.

Diana Stempak, PhD
New Agent and Innovative Therapy Program
Divisions of Hematology/Oncology and Clinical Pharmacology and Toxicology
Hospital for Sick Children
Toronto, Ontario, Canada

Bonny L. Bukaveckas, PhD
Mark Linder, PhD
Department of Pathology and Laboratory Medicine
University of Louisville
Louisville, Ky

Gideon Koren, MD
Division of Clinical Pharmacology and Toxicology
Hospital for Sick Children
Toronto, Ontario, Canada

Sylvain Baruchel, MD
New Agent and Innovative Therapy Program
Division of Hematology/Oncology
Hospital for Sick Children
Toronto, Ontario, Canada

E-mail: sylvain.baruchel@sickkids.ca

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