HEPATOLOGY *Elsewhere*

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CYCLOSPORINE AND LIVER TRANSPLANTATION: WILL THE MIDAZOLAM TEST MAKE BLOOD LEVEL MONITORING OBSOLETE?

Thummel KE, Shen DD, Podoll TD, Kunze KL, Trager WF, Bacchi CE, Marsh CL, et al. Use of midazolam as a human cytochrome P450 3A probe: II. Characterization of inter- and intraindividual hepatic CYP3A variability after liver transplantation. J Pharmacol Exp Ther 1994;271:549-556.

ABSTRACT

Immunosuppression therapy with cyclosporine is often hampered by significant interindividual variability in the metabolic clearance of the drug. It has been suggested that much of the variability in cyclosporine clearance is due to differences in the cytochrome P450 3A4 (CYP3A4) content in the liver and intestinal mucosa. A study was conducted in liver transplant recipients to characterize hepatic CYP3A4 variability during the first 10 days after surgery. The formation of 1′-hydroxymidazolam (1′-OH MDZ) was followed in the plasma after i.v. midazolam (MDZ) administration to 21 multiple-organ donors and to recipients of 10 of the 21 donor livers. Liver biopsy tissue was obtained from donors and recipients after the *in vivo* pharmacokinetic test. For liver donors, the plasma 1′-OH MDZ/MDZ concentration ratio 30 min after the i.v. MDZ dose was well correlated with the hepatic CYP3A4 content ($r = 0.87$, $P < .001$). Much of the variability in the two parameters was attributed to the administration of enzyme-inducing drugs before organ procurement. The mean hepatic CYP3A4 content and plasma 1′-OH MDZ/MDZ concentration ratio in six inducer-treated donors was 4.7-fold and 2.3-fold higher than the respective mean value for all other donors. The hepatic CYP3A4 content and plasma 1′-OH MDZ/MDZ ratio for liver recipients, studied on postoperative day 10, was negatively correlated with the respective parameter measured in donors on day 0 ($r = -0.60$ for CYP3A4 and $r = -0.79$ for 1′-OH MDZ/MDZ; $P < .05$ and $P < .01$). The dynamic changes in hepatic CYP3A4 expression during the perioperative period, some of which appear to be due to the effect of enzyme-inducing drugs, help explain the difficulties often encountered in the achievement and maintenance of therapeutic cyclosporine blood levels after liver transplantation.

COMMENTS

The vast majority of cyclosporine metabolism that occurs in human liver is catalyzed by a single drug-metabolizing enzyme (a cytochrome P450), termed CYP3A4. This discovery has appeared to explain most clinically significant drug interactions involving cyclosporine. For example, cyclosporine dosing requirements generally increase when transplant recipients are treated with antiseizure drugs or the antibiotic rifampin, treatments known to increase (induce) hepatic CYP3A4 catalytic activity.[1] Conversely, cyclosporine-treated patients who begin treatment with known CYP3A4 inhibitors, such as ketoconazole and erythromycin,[1] generally have significant reductions in their cyclosporine requirement. This has led to the increasingly accepted hypothesis that catalytic activity of hepatic CYP3A4 is rate-limiting in the metabolism and elimination of cyclosporine.

It is also appreciated that there are significant interpatient differences in the liver content and catalytic activity of CYP3A4, which can exist in the absence of treatment with known inducers or inhibitors of CYP3A4.[2] This variation, which can exceed 30-fold in certain patient populations, probably reflects complex genetic factors, but may also reflect nongenetic factors such as dietary differences and concomitant diseases. In a simultaneously published companion manuscript,[3] Thummel et al determined clearance of intravenously administered cyclosporine and, on the same day, performed liver biopsies in 10 liver transplant recipients. The liver biopsy content of CYP3A4, which varied more than 20-fold in these patients, correlated well with the intravenous cyclosporine clearance measurements ($r = .81$; $P < .01$). This provided the most direct evidence to date that differences in liver CYP3A4 activity are substantial and largely determine differences in systemic clearance of cyclosporine observed among liver transplant recipients.

In the current studies, the investigators directly address two hypotheses. First, the investigators propose that the intravenous sedative midazolam, a known CYP3A4 substrate, can be used to estimate liver catalytic activity of CYP3A4 in patients, obviating the need for liver biopsy. In support of this hypothesis, the investigators found in 10 liver recipients an impressive correlation between the liver biopsy specimen content of CYP3A4 and the systemic clearance of midazolam.[3] In 19 liver donors they also found a good correlation between the liver content of CYP3A4 and the ratio of 1′-OH midazolam (the major midazolam metabolite produced by CYP3A4) and parent midazolam determined

in a single blood collection obtained 30 minutes after the intravenous dose. This suggests that a full kinetic study may not be necessary and that an estimate of liver CYP3A4 activity using midazolam may require only 30 minutes at the bedside.

The second hypothesis tested in these studies was that there are changes in the CYP3A4 activity when the liver is removed from the donor and placed in the recipient. To address this, the investigators obtained biopsy specimens from 10 donor livers and repeated the biopsies in the same livers 10 days after transplantation. The 30-minute midazolam test was also administered at the time of each liver biopsy, and the results closely mirrored the CYP3A4 concentrations obtained directly in the liver biopsy specimens. Donors that had been receiving the known CYP3A4 inducers, phenytoin or phenobarbital,[1] generally had the highest liver levels of CYP3A4. Livers obtained from these donors showed a profound decrease in CYP3A4 activity after transplantation, presumably because the recipients were not receiving antiseizure drugs. In contrast, livers obtained from donors who were not receiving known inducers generally had increases in CYP3A4 content 10 days after the livers were placed in the recipients. The investigators suggest that steroid medications given to the recipients, but generally not received by the donors, may account for this apparent induction. This is plausible because some steroid medications have been shown to induce CYP3A4 in human hepatocytes.[1] The inverse correlation between pretransplantation and posttransplantation values for liver CYP3A4 content found by the investigators (see abstract), therefore, appears to have been predictable based on differences in the medications received by the donors and recipients. The investigators reasonably speculate that such wide swings in liver CYP3A4 activity may largely account for the difficulty in achieving stable cyclosporine blood levels during the early postoperative period. It logically follows that knowledge of the activity of CYP3A4 might be useful to transplantation physicians attempting to individualize dosing of the drug.

Should we start using midazolam to measure CYP3A4 activity in our transplant recipients? Clearly more research needs to be performed because the number of patients studied is quite small and clinical utility was not investigated. However, the use of midazolam as a probe in this way is attractive because the prolonged elimination kinetics of cyclosporine makes dosing based on blood levels alone largely empiric during the first few days after surgery. Moreover, the rapid changes in CYP3A4 that Thummel et al observed during the postoperative period might make it desirable to have frequent (i.e., daily) measurements of CYP3A4 activity. In addition, CYP3A4 has been shown to be the major liver enzyme that catalyzes metabolism of many other medications, including FK506 and rapamycin.[2] Therefore, the data obtained from this test might give useful information concerning dosing of multiple drugs.

Some issues dampen enthusiasm for the midazolam test, however. Probably the major issue is that the assay used by the investigators to measure midazolam and its metabolite is relatively complex, and involves mass spectral analysis. Same day turnaround for the results would probably require a dedicated laboratory and staff—an expensive proposition. Second, the dose of midazolam used (approximately 1 mg) will produce sedative effects in some patients and, therefore, the test may not be entirely without risk.

It should also be noted that the information obtained from this "midazolam test" will probably be of limited value as a guide to oral dosing of cyclosporine (as opposed to the intravenous dosing used in this study). In a recent study,[4] measurements of liver CYP3A4 activity using the intravenous erythromycin breath test significantly predicted variability in oral kinetics of cyclosporine in kidney transplant recipients, but the measurement accounted for less than 30% of this variability. The inability of liver CYP3A4 activity to accurately predict oral cyclosporine kinetics is undoubtedly multifactorial, and would be expected to include impaired bile production in liver transplant recipients. However, an important factor is also likely to be first-pass metabolism of cyclosporine in the small intestine, where CYP3A4 is also quite abundant.[5] Current data suggest that within an individual, the activity of CYP3A4 in intestine does not closely mirror the activity in liver (i.e., a given patient might have relatively high liver CYP3A4 activity while having relatively low intestinal CYP3A4 activity).[6] This should be especially true in a liver transplant recipient in whom the liver and small bowel are of different genetic origin. Hence, tests that involve intravenous administration of CYP3A4 "probe" drugs such midazolam probably do not estimate the extent of intestinal metabolism of orally administered cyclosporine. A variety of investigators, including Dr Thummel's group (Thummel K, Personal communication, March 1995), are now attempting to develop tests based on the oral administration of suitable probe drugs that may be capable of measuring both liver and intestinal CYP3A4 activity.[7]

One final note, lidocaine conversion to the MEGX metabolite (the basis of the "MEGX test") has been shown to be catalyzed by CYP3A4. Some data have recently been presented supporting the idea that the MEGX test result may in part reflect liver CYP3A4 activity, and, therefore, might also be useful in dosing cyclosporine.[8] However, because lidocaine is very rapidly cleared by the liver, variation in liver blood flow, and not just variation in liver CYP3A4 activity, should influence the MEGX test result.

In summary, Thummel et al have provided the most clear-cut evidence to date supporting the idea that interpatient and intrapatient differences in hepatic CYP3A4 activity largely account for difficulty in arriving at appropriate stable dosing of intravenous cyclosporine in liver transplant patients. The data they have obtained using midazolam highlight the feasibility of developing convenient probe-based tests that could significantly improve the dosing of immunosup-

pressants to the transplant population, and could have wide implications in many other areas of clinical pharmacology.

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IMMUNOSUPPRESSION: NOW WE HAVE CHOICES

The U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994;331:1110-1115.

ABSTRACT

Background. **Tacrolimus (FK 506), a macrolide compound isolated from a bacterium, is a potent immunosuppressant with activity in solid-organ transplants. Most immunosuppressive regimens for liver transplantation are based on cyclosporine.**

Methods. **We conducted an open-label, randomized, multicenter trial to compare the efficacy and safety of tacrolimus-based and cyclosporine-based immunosuppressive regimens for patients receiving a first liver transplant. A total of 478 adults and 51 children (≤12 years of age) were randomly assigned at the time of transplantation to receive tacrolimus (n = 263) or cyclosporine (n = 266) and were followed for one year. The primary end points were patient and graft survival at one year. The secondary end points were the incidence of acute rejection, corticosteroid-resistant rejection, and refractory rejection (continued rejection after two courses of corticosteroids and an intervening course of muromonab-CD3).**

Results. **According to Kaplan-Meier analysis, actuarial patient-survival rates at day 360 were 88 percent for both the tacrolimus and cyclosporine groups ($P = 0.85$; 95 percent confidence interval for the difference, −5.4 to 6.6 percent), and graft-survival rates were 82 percent and 79 percent, respectively ($P = 0.55$; 95 percent confidence interval for the difference, −4.8 to 9.7 percent). Acute rejection occurred in 154 patients in the tacrolimus group and 173 patients in the cyclosporine group ($P < 0.002$), corticosteroid-resistant rejection occurred in 43 and 82 patients, respectively ($P < 0.001$), and refractory rejection occurred in 6 and 32 patients, respectively ($P < 0.001$). Tacrolimus was associated with a higher incidence of adverse events requiring withdrawal from the study, primarily nephrotoxicity and neurotoxicity; 37 patients in the tacrolimus group and 13 in the cyclosporine group discontinued the study because of adverse events ($P < 0.001$).**

Conclusions. **After one year, immunosuppressive regimens based on tacrolimus and cyclosporine were comparable in terms of patient and graft survival. Tacrolimus was associated with significantly fewer episodes of acute, corticosteroid-resistant, or refractory rejection, but substantially more adverse events requiring discontinuation of the drug. (N Engl J Med 1994;331:1110-1115.)**

In the past decade, the surgical and medical aspects of orthotopic liver transplantation have been so refined as to result in excellent patient and graft survival for most indications. The lengthy and costly hospitalizations of the past have been replaced by short postoperative hospital stays and more cost-effective patient management. The question is no longer can the procedure be successfully performed, but rather, what is the most efficient and cost-beneficial method of performing the procedure.

The main reasons for prolonged hospital stays and subsequent readmissions continue to be the complications of too much or too little immunosuppression. These include rejection, infection, medication toxicity, and long-term complications, such as malignancy and renal failure. With a better understanding of the rejection phenomena, immunosuppression can be better tailored to each individual patient, taking into account their specific immunosuppressive needs and liabilities. Tacrolimus is a welcome addition to the immunosuppressive armamentarium, and this important article helps better define the role of this agent in the management of the transplant recipient.

Although the 1-year patient and graft survival remain the same between the two study groups, there is a clear-cut decrease in the number of rejection episodes, the amount of steroid usage, and the number of refractory rejection episodes in the tacrolimus-treated group. Surprisingly, this did not impact on the patient survival, implying that the increased immunosuppressive effect is not at the cost of increased life-threatening infections. Or is tacrolimus toxicity merely counterbalanced by the increased steroid use in the cyclosporine-treated group? Of interest would be a discussion of the infectious complications experienced by both groups as