Transiently altered acetaminophen metabolism after liver transplantation

Background and Objectives: **Acetaminophen (INN, paracetamol) is metabolized to *N*-acetyl-*p*-benzoquinone imine (NAPQI), a hepatotoxic metabolite, predominantly by cytochrome P450 (CYP) 2E1. Alterations in drug metabolism occur after organ transplantation. This study was designed to characterize acetaminophen disposition during the first 6 months after liver transplantation.**

Methods: **Thirteen liver transplant patients received an oral dose of acetaminophen (500 mg) on days 2, 10, 90, and 180 after transplantation. Serial blood samples were collected for 8 hours, and urine was collected for 24 hours. Liver biopsy specimens were obtained from the donor liver during transplantation (day 0) and on days 10, 90, and 180 after transplantation.**

Results: **There were significant time-dependent changes in acetaminophen metabolism after liver transplantation. When day 2 and day 10 were compared with day 180, the respective mean urinary recovery was 137% and 81% higher for thioether conjugates derived from NAPQI ($P = .0002$ and $P = .01$, respectively); 31% and 22% lower for acetaminophen sulfate ($P = .0006$ and $P = .008$, respectively); and 22% and 27% lower for acetaminophen glucuronide ($P = .05$ and $P = .004$, respectively). Metabolite formation clearances changed in concordance with the fractional urinary recovery. It was surprising that hepatic CYP2E1 content on day 10 after transplantation was only 20% higher, on average, than that found on day 180 (not significant). In contrast, hepatic CYP3A4 content was 984% higher, on average, when tissue from days 10 and 180 was compared after transplantation ($P = .007$).**

Conclusions: **Increased recovery of acetaminophen thioether conjugates during the first 10 days after liver transplantation was a result of impaired glucuronidation and sulfation and enhanced NAPQI formation. (Clin Pharmacol Ther 2003;73:545-53.)**

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Hepatic glucuronidation and sulfation are the major pathways of acetaminophen (INN, paracetamol) elimination, accounting for approximately 50% and 35% of a therapeutic dose, respectively.[1] Less than 10% of a therapeutic dose is oxidized to *N*-acetyl-*p*-benzoquinone imine (NAPQI), a highly reactive quinone imine that is rapidly detoxified by conjugation with glutathione and eventually excreted into urine as thioether metabolites. In situations involving acetaminophen overdose, which saturates the sulfation pathway,[2,3] or induction of the oxidative pathway by ethanol,[4] a greater fraction of the acetaminophen dose is converted to NAPQI. Excessive NAPQI formation can result in glutathione depletion and hepatotoxicity.[5]

Recently, Burckart et al[6] reported that oral chlorzoxazone clearance, a selective in vivo probe of hepatic cytochrome P450 (CYP) 2E1 metabolic activity,[7-9] was increased perioperatively among patients undergoing orthotopic liver transplantation (OLT). Because acetaminophen is commonly recommended to this population for analgesia or fever reduction and because CYP2E1 is the principal catalyst for NAPQI formation in vivo in humans,[10-13] we assessed whether NAPQI formation is increased after OLT. Given the fluctuating

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Supported by National Institutes of Health grants GM32165 and M01-RR-00037.

Received for publication Sept 4, 2002; accepted Feb 21, 2003.

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0009-9236/2003/$30.00 + 0

doi:10.1016/S009-9236(03)00062-6

nature of hepatic function and immunosuppressive regimens in this population, we studied OLT patients on multiple occasions during the first 6-month postoperative period. To verify the mechanistic basis for induction of the NAPQI formation pathway, we measured total CYP2E1 content in liver biopsy tissue collected, per clinical protocol, during the same 6-month period. Hepatic CYP3A4 content was also measured, as it is often the dominant hepatic P450 isozyme,[14] is inducible by some immunosuppressive drugs,[15,16] and can catalyze NAPQI formation.[10,17]

METHODS

Standards and analytic reagents

Synthetic unlabeled acetaminophen metabolite standards (glucuronide, sulfate, and *S*-methyl) were kindly provided by Dr Sid Nelson, Department of Medicinal Chemistry, University of Washington, Seattle, Wash. Other unlabeled thioether metabolites were isolated from mouse urine, as described below. Unlabeled acetaminophen was purchased from Sigma Chemical Co (St Louis, Mo). $^{2}H_3$-Labeled acetaminophen was synthesized as follows. In brief, 2.9 g of *p*-aminophenol (Aldrich, Milwaukee, Wis) was stirred into 100 mL of acetonitrile in a 200-mL round-bottom flask. To this was added 3.0 mL of deuterium-labeled $^{2}H_6$-acetic anhydride (Aldrich). The flask was sealed and stirred overnight in the dark at room temperature. The resulting crystalline slurry was chilled to −20°C for 2 hours, and acetaminophen crystals of high purity were isolated by filtration. The recovered molar yield was 60%. Isotopic purity of the final product was assessed by mass spectrometry and found to consist of a 100:5:<0.1 mole ratio of $^{2}H_3$:$^{2}H_2$:$^{2}H_1$-acetaminophen. Male Swiss-Webster mice, weighing 25 to 35 g, were purchased from Animal Tech (Kent, Wash). β-Glucuronidase/sulfatase enzyme, a crude extract from *Helix pomatia*, was purchased from Sigma Chemical Co. All other reagents were of analytic grade or better.

Human subjects

This study was approved by the Institutional Review Board at the University of Washington, Seattle, Wash, and written informed consent was obtained from all subjects. Fifteen patients receiving an OLT at the University of Washington Medical Center (UWMC) were enrolled. Liver transplantation operations were performed according to standard protocols for the UWMC Department of Transplant Surgery. The common bile duct was anastamosed during surgery with or without a T tube. In subjects with a T tube, the external port was closed for at least 48 hours after each dose of acetaminophen.

All 15 study subjects received a standard posttransplantation immunosuppressive regimen. Of these, 12 received a quadruple immunosuppressive regimen (cyclosporine [INN, ciclosporin], azathioprine, prednisone, and daclizumab induction therapy). For the other 3, azathioprine was omitted from the regimen of 2 subjects, because of preexisting hepatocellular carcinoma in the cirrhotic organ, and 1 subject received tacrolimus and sirolimus instead of cyclosporine as part of the maintenance regimen.

Study design

Acetaminophen, as a 500-mg oral tablet (McNeil Consumer Products Co, Fort Washington, Pa) or 500-mg oral solution (Roxane Laboratories, Inc, Columbus, Ohio), was given to each subject on four occasions: approximately 2, 10, 90, and 180 days after transplantation. On each study day, serial blood samples were collected at 0, 0.5, 3, and 8 hours after administration of the acetaminophen dose. Blood-sampling frequency was limited for patient safety considerations during the early perioperative period. Plasma was separated from blood cells by centrifugation and stored at −80°C until analyzed for acetaminophen. Urine was collected for 24 hours after acetaminophen ingestion. It was stabilized with 3 g of ascorbic acid and maintained at 4°C during the collection period. Total 24-hour urine volume was recorded and an aliquot stored at −80°C until analyzed for acetaminophen and its metabolites.

A core needle biopsy specimen of the liver was collected according to clinical protocol during the transplantation operation (day 0) and approximately 48 hours after the acetaminophen kinetic experiments on days 10, 90, and 180. When available, approximately 10 to 30 mg of each biopsy specimen in excess to that needed for diagnostic purposes was used for research. The biopsy tissue was placed in 100 to 300 μL of homogenization buffer (10 mmol/L potassium phosphate, pH 7.4; 0.25 mol/L sucrose; Complete protease inhibitor cocktail with ethylenediaminetetraacetic acid [Boehringer Mannheim, Germany]), frozen immediately on dry ice, and stored at −80°C until Western blot analysis was performed. Just before analysis, the tissue/buffer mixture was thawed on ice and homogenized with the use of a 1-mL glass pestle-glass homogenization tube. Approximately 20 complete strokes were applied by hand.

Quantitation of acetaminophen and metabolites

Plasma acetaminophen concentration and acetaminophen sulfate and glucuronide concentrations in unhydrolyzed urine were quantified by HPLC, as described previously[18] with minor modifications. The concentration of acetaminophen thioether metabolites derived from NAPQI conjugation with glutathione (3-cysteinyl, 3-mercapturic acid, and 3-*S*-methyl) in hydrolyzed urine (β-glucuronidase/sulfatase treated for 24 hours at 37°C) was quantified by liquid chromatography-mass spectrometry. Because of the instability of 3-cysteinyl and 3-mercapturic acid metabolites with prolonged storage, fresh metabolites were isolated by HPLC (repeated injections of urine from a mouse and human dosed with acetaminophen), just before the analysis of study samples, following the general elution method described by Chien et al.[18] Synthetic 3-*S*-methyl metabolite was repurified by the same HPLC method. Metabolite concentrations in the purified stock solution were determined by comparison of the ultraviolet absorbance at 254 nm for metabolites and an acetaminophen stock solution of known concentration, with correction for differences in the molar absorptivity coefficients of the metabolites and acetaminophen.

Deuterated internal standards for the thioether metabolites were generated biologically. In brief, 200 mg/kg $^{2}H_{3}$-labeled acetaminophen was administered intraperitoneally to Swiss-Webster mice. Urine (0-12 hours) was collected, diluted 1:5 with 12.5 mmol/L sodium acetate (pH 5.0), aliquoted, and stored at −80°C. Analysis for thioether metabolite concentrations in the hydrolyzed urine of study subjects was performed on an Agilent Technologies 1100 Series Liquid Chromatograph/Mass Selective Detector (Palo Also, Calif), operated in the positive ion electrospray mode with selective ion monitoring. A Phenomenex Luna 3-μm, C_8 (100 × 2-mm) column (Phenomenex Inc, Torrance, Calif) was used for nominal chromatographic separation. The mobile phase was 20 mmol/L acetic acid (pH 3.5) delivered at a flow rate of 0.22 mL/min. The following mass spectrometry conditions were used: capillary and fragmentor voltages of 3300 and 100 V, respectively, and nitrogen (N_2) as a drying gas at a flow rate of 10 L/min, a temperature of 300°C, and a nebulizer gas pressure of 25 psi. The following pairs of ions (unlabeled and deuterated internal standard) were detected by selective ion monitoring: mass-to-change ratio (*m/z*) 198 and 201 (3-*S*-methyl), *m/z* 271 and 274 (3-cysteinyl), and *m/z* 313 and 316 (3-mercapturic acid).

Quantitation was performed by comparison of peak area ratios for unknown samples to standard curves for each metabolite. For measurement of acetaminophen and its metabolites in urine, the respective interday and intraday variability (coefficient of variation for repeated measures) was as follows: glucuronide, 11.1% and 0.9%; sulfate, 5.7% and 1.2%; *S*-methyl, 14.3% and 7.9%; cysteine, 13.9% and 3.2%; mercapturate, 7.4% and 4.0%; and acetaminophen, 3.4% and 2.2%. Limits of quantitation were not rigorously assessed. All measured urine metabolite concentrations were above that of the lowest standard: glucuronide, 17 μmol/L; sulfate, 19 μmol/L; *S*-methyl, 0.8 μmol/L; cysteine, 3 μmol/L; and mercapturate, 7.5 μmol/L.

Pharmacokinetic analysis

The area under the acetaminophen plasma concentration-time curve (AUC) for the 0- to 8-hour interval was calculated with the use of the log-linear trapezoidal rule. The AUC from 8 hours to infinity was calculated as the ratio $C_{8\ hours}/k_e$, in which $C_{8\ hours}$ is the acetaminophen plasma concentration at 8 hours after dosing and k_e is the terminal elimination rate constant obtained from the 3- and 8-hour plasma concentration data. Apparent oral clearance (CL/F) was calculated as dose/total AUC. The molar amount of each thioether metabolite (3-mercapturate, 3-cysteinyl, and 3-*S*-methyl) recovered in urine was summed and the total divided by the acetaminophen dose for calculation of the fraction of acetaminophen dose metabolized to NAPQI. The fraction of dose metabolized to acetaminophen glucuronide and acetaminophen sulfate was calculated in an analogous manner.

Western blot analysis

Western blot analysis for CYP2E1 content in individual liver homogenate samples was performed with the use of specific goat polyclonal antihuman CYP2E1 serum (Daiichi Pure Chemical Co, Tokyo, Japan) as the primary antibody and antigoat immunoglobulin G alkaline phosphatase conjugate (Sigma Chemical Co) as the secondary antibody. CYP2E1 protein levels were quantified by densitometry, with purified human CYP2E1 used as a reference standard.[19] CYP3A4 content in liver homogenates was determined by a similar method, as described previously.[20]

Statistical analysis

One-way repeated-measures ANOVA, followed by paired comparisons testing, was conducted to evaluate pharmacokinetic and enzyme expression differences among the four study days. Data are reported as mean ± SD.

Table I. Study subject demographics and relevant drug therapy

Subject No.	*Age (y)*	*Sex*	*Ethnicity*	*Liver disease*	*Relevant concomitant medications**
L01	60	F	White	Hepatitis C	Clotrimazole (d 10, 90), omeprazole (d 90)
L02	48	M	White	Hepatitis C/ethanol	Ranitidine (d 2), clotrimazole (d 10), ciprofloxacin (d 180)
L03	64	M	African American	Hepatitis C	Clotrimazole (d 10, 90), ranitidine (d 90)
L04	52	M	White	Hepatitis C/ethanol	Clotrimazole (d 2, 10), ranitidine (d 10, 90)
L05	53	F	White	Hepatitis C	Dapsone (d 10, 90, 180), clotrimazole (d 10), omeprazole (d 10, 90, 180)
L06	51	M	White	Ethanol	Vancomycin (d 2), ranitidine (d 2), clotrimazole (d 10), nefazodone (d 10, 90), insulin (d 180)
L07	57	F	White	α_1-Antitrypsin deficiency	Levothyroxine (d 2, 10), clotrimazole (d 10), ranitidine (d 10), omeprazole (d 90), ursodiol (INN, ursodeoxycholic acid) (d 90)
L08	54	M	White	Hepatitis C	Clotrimazole (d 10), insulin (d 10, 90, 180), ranitidine (d 10, 90)
L09	57	M	Hispanic-Latino	Primary sclerosing cholangitis	Clotrimazole (d 2, 10), ranitidine (d 2, 10, 90, 180), insulin (d 180)
L10	49	M	White	Hepatitis C	Clotrimazole (d 2, 10), ranitidine (d 2, 10), insulin (d 180), DHEA (d 180)
L11	47	M	White	Hepatitis C	Metoclopramide (d 2, 10), clotrimazole (d 10, 90), ranitidine (d 10)
L12	48	F	White	Hepatitis C	Omeprazole (d 2, 10, 90), clotrimazole (d 10), insulin (d 10), vitamin D (d 90, 180)
L13	50	M	White	Hepatitis C	Dapsone (d 2, 10), clotrimazole (d 10, 90), ranitidine (d 10, 90), insulin (d 10, 90, 180), ursodiol (d 90, 180)
L14	44	F	White	Hereditary telangiectasia	Ranitidine (d 2), clotrimazole (d 10), fluoxetine (d 10, 90, 180)
L15	47	M	White	Hepatitis C	Clotrimazole (d 2, 10, 90), paroxetine (d 90, 180), insulin (d 90, 180)

DHEA, Dehydroepiandrosterone.

*List of concomitant medications that might alter hepatic P450 or uridine diphosphate–glucuronosyltransferase expression and function and acetaminophen metabolism, in addition to standard immunosuppressive or prophylaxis therapies described in Methods section. Study dates in parentheses indicate concomitant drug therapy on day of acetaminophen administration.

RESULTS

The majority of study subjects were white men aged approximately 50 years. Additional demographic and clinical information is presented in Table I. The major cause of liver disease in study subjects was hepatitis C infection. Recurrent infection after transplantation is common and might have affected hepatic enzyme expression. However, there was no evidence that this occurred on the basis of the acetaminophen metabolic profile for the study group. Similarly, we identified a number of concomitant drugs that might alter acetaminophen metabolism (Table I) but found no evidence for an association between use of these drugs and urine metabolite recovery.

Thirteen of fifteen enrolled subjects completed all four of the pharmacokinetic study days. One subject discontinued study participation after the day 90 study because of the need for retransplantation. Day 180 study data for another subject were excluded from statistical analysis because of apparent acetaminophen use immediately before the study.

The apparent total body clearance (CL/F) and half-life of acetaminophen varied significantly over the 180-day posttransplantation study period ($P = .02$ and .0007 by ANOVA, respectively) (Table II). Moreover, mean clearance and half-life parameters for day 180 were similar to those we have reported previously for healthy volunteers.[4,18] On the basis of these findings, postoperative day 180 was chosen as a reference time point for longitudinal comparisons of urinary recovery and formation clearance data from subjects.

The urinary recovery of thioether conjugates varied significantly over the 6-month study period: 15.4% ± 4.3%, 12.5% ± 5.2%, 6.3% ± 2.8%, and 7.7% ± 3.5% for postoperative days 2, 10, 90, and 180, respectively ($P < .0001$ by ANOVA) (Table III). These parameters

Table II. Acetaminophen pharmacokinetic parameters

Parameter	*Day 2*	*Day 10*	*Day 90*	*Day 180*
CL/F (mL/h)	19.6 ± 6.41 (.07)	28.4 ± 6.77 (NS)	26.5 ± 7.24 (NS)	24.2 ± 7.84
$t_{1/2}$ (h)	4.50 ± 2.46 (0.02)	2.19 ± 0.35 (.02)	2.34 ± 0.48 (.001)	2.62 ± 0.47

Data are presented as mean ± SD, with *P* values in parentheses representing paired comparisons for day 2, 10, or 90 versus day 180. For ANOVA, $P = .02$ for CL/F and $P = .0007$ for $t_{1/2}$.
CL/F, Apparent oral clearance; $t_{1/2}$, half-life; NS, not significant.

Table III. Urinary recovery and formation clearances for acetaminophen metabolites

Parameter	*Day 2*	*Day 10*	*Day 90*	*Day 180*
*Urinary recovery (% dose)**				
Thioether conjugates	15.4 ± 4.29 (.0002)	12.2 ± 5.18 (.01)	6.30 ± 2.81 (NS)	7.67 ± 3.49
Sulfate	27.2 ± 7.94 (.0006)	30.1 ± 6.82 (.008)	37.7 ± 9.00 (NS)	40.6 ± 9.66
Glucuronide	27.7 ± 14.7 (.05)	26.8 ± 8.55 (.004)	34.7 ± 9.08 (NS)	38.5 ± 9.71
Formation clearance (L/h)†				
Thioether conjugates	2.87 ± 0.90 (.004)	3.33 ± 1.30 (.0008)	1.59 ± 0.61 (NS)	1.78 ± 0.81
Sulfate	5.14 ± 1.96 (.0006)	8.50 ± 2.46 (NS)	9.87 ± 3.05 (NS)	9.69 ± 3.60
Glucuronide	5.67 ± 3.53 (.02)	7.95 ± 4.15 (NS)	9.36 ± 3.82 (NS)	9.56 ± 4.37

Data are presented as mean ± SD, with *P* values in parentheses representing paired comparisons for day 2, 10, or 90 versus day 180.
*For ANOVA, $P < .0001$ for thioether conjugates, $P = .0004$ for sulfate, and $P = .02$ for glucuronide.
†For ANOVA, $P < .0001$, $P = .0003$, and $P = .06$, respectively.

represent the minimum fraction of the acetaminophen dose converted to NAPQI. Compared with day 180, thioether recovery was, on average, 137% and 81% higher on days 2 and 10 after transplantation, respectively ($P = .0002$ and .01, respectively) (Table III, Fig 1). Interestingly, for one individual (subject L14), thioether conjugate recovery remained elevated (approximately 13% of the dose) over the entire 180-day study period (Fig 1).

The urinary recovery of acetaminophen sulfate also varied significantly over the 6-month study period: 27.2% ± 7.9%, 30.1% ± 6.8%, 37.7% ± 9.0%, and 40.6% ± 9.7% for postoperative days 2, 10, 90, and 180, respectively ($P = .0004$ by ANOVA) (Table III). Compared with day 180, acetaminophen sulfate recovery was, on average, 31% and 22% lower on days 2 and 10 after transplantation, respectively ($P = .0006$ and .008, respectively) (Table III). There were also differences in the mean urinary recovery of acetaminophen glucuronide over the 6-month study period: 27.7% ± 14.7%, 26.7% ± 8.6%, 34.7% ± 9.1%, and 38.5 ± 9.7% for postoperative days 2, 10, 90, and 180, respectively ($P = .02$). Compared with day 180, acetaminophen glucuronide recovery was, on average, 22% and 27% lower on days 2 and 10 after transplantation, respectively ($P = .05$ and .004, respectively) (Table III).

The formation clearance of thioether conjugates, acetaminophen sulfate, and acetaminophen glucuronide (calculated as the product of clearance and urinary recovery fraction) varied significantly during the first 180 days after transplantation ($P < .0001$, $P = .0003$, and $P = .06$ by ANOVA, respectively) (Table III). The thioether formation clearance was, on average, 97% and 122% higher on postoperative days 2 and 10, respectively, compared with day 180 ($P = .004$ and .0008, respectively). In contrast, the acetaminophen sulfate and glucuronide formation clearances were, on average, 42% and 20% lower on day 2 after transplan-

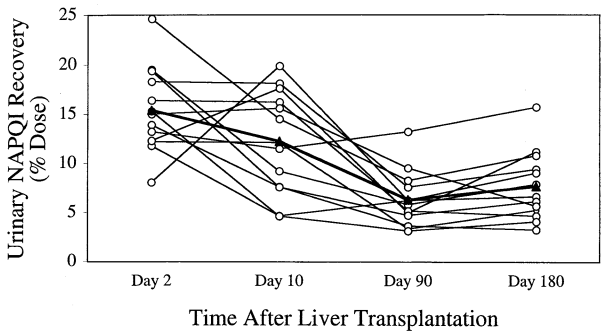

Fig 1. Time course of change in fraction of acetaminophen dose converted to *N*-acetyl-*p*-benzoquinone imine (NAPQI). Each *data point* represents the sum of all acetaminophen thioether metabolites recovered in urine divided by the administered dose. The mean urinary recovery from 13 subjects for each time period is shown as *solid triangles*.

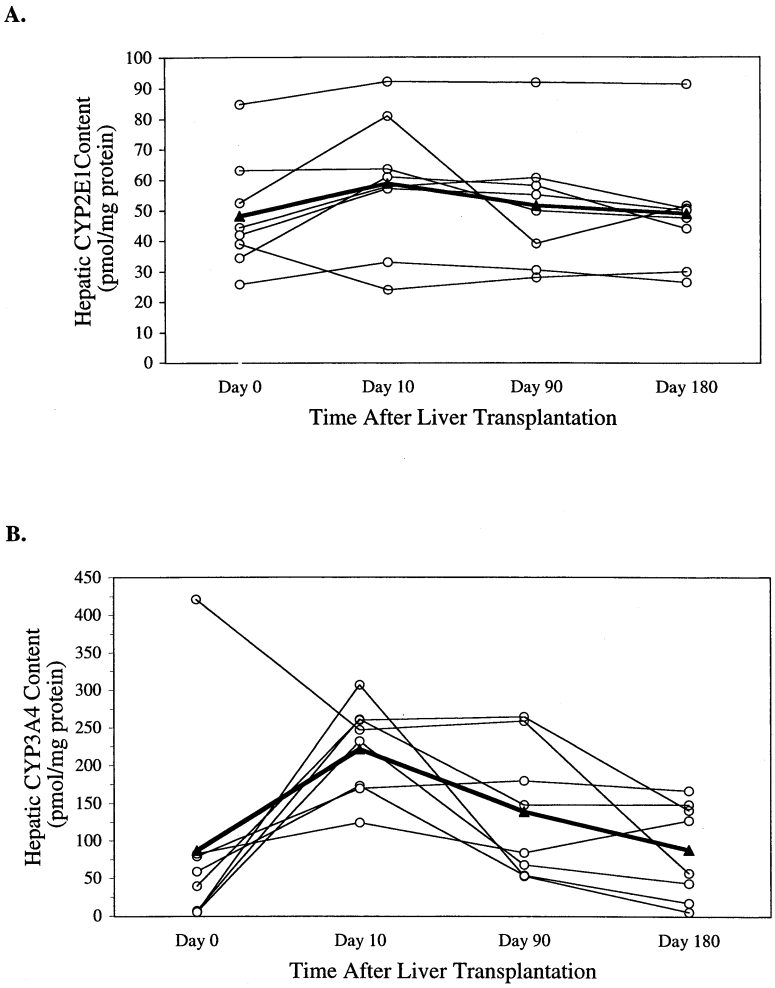

Fig 2. CYP2E1 content (**A**) and CYP3A4 content (**B**) in liver biopsy specimens from 8 subjects, as estimated by Western blot immunodetection, during first 6 months after liver transplantation. Mean values for each posttransplantation period are shown as *solid triangles*.

tation, respectively, as compared with day 180 after transplantation ($P = .0006$ and .02, respectively) (Table III). Acetaminophen sulfate and glucuronide formation clearances were also lower on postoperative day 10, but the differences compared with day 180 were not significant.

For 8 of 15 subjects, a portion of liver biopsy specimens collected on days 0, 10, 90, and 180 after transplantation were made available for research. Although hepatic CYP2E1 content varied between subjects, the ANOVA analysis indicated no significant change over the 180-day study period (Fig 2, *A*). A paired comparison revealed only a 20% higher level of CYP2E1 in the day 10 biopsy specimen, on average, compared with the day 180 biopsy specimen. However, the mean formation clearance to thioether conjugates for these same 8 subjects was 81% higher on day 10, on average, compared with day 180 after transplantation ($P = .006$).

In contrast to the generally invariant apparent CYP2E1 expression, hepatic CYP3A4 levels varied significantly over the 180-day study period ($P = .02$ by ANOVA) (Fig 2, *B*). Hepatic CYP3A4 content increased several-fold between day 0 and day 10 and declined gradually back to baseline by day 180 after transplantation. CYP3A4 content in day 10 biopsy tissue was, on average, 984% higher than that found in the matched day 180 specimens ($P = .007$).

The oxidation of acetaminophen by liver homogenates could not be measured because of insufficient tissue mass and limited assay sensitivity.

DISCUSSION

The mean fraction of a single 500-mg acetaminophen dose converted to the protoxic metabolite NAPQI was enhanced by approximately 100% during the first 10 days after liver transplantation. For some OLT patients, urinary recovery of thioether metabolites represented as much as 25% of the administered dose. This metabolic change appeared to be transient, and by 3 to 6 months after OLT, thioether recovery had returned to a mean value similar to that which we have reported for healthy volunteers.[4,18] The mean increase in NAPQI formation observed in this study population was modest but greater than that reported after short-term ethanol ingestion[4] and long-term isoniazid treatment,[18,21] drugs for which an increased risk of hepatotoxicity has been suggested.[22-25] Individual thioether conjugate urinary recovery fractions of 15% to 25% are comparable to recovery values reported for poisoned patients with severe liver injury.[1-3]

Although we had expected to find elevated NAPQI formation in our subjects, on the basis of published chlorzoxazone clearance data in OLT patients,[6] an increase in hepatic CYP2E1 content during the same early perioperative period was also anticipated. However, significant postoperative changes in CYP2E1 content were not evident (Fig 2, *A*). Hepatic CYP2E1 content was 20% higher on day 10, as compared with day 180, but this effect was modest in comparison to the 81% higher NAPQI formation clearance (day 10 versus day 180) for the same subset of subjects. CYP2E1 content was not measured at 2 days after OLT, a time when NAPQI formation was also enhanced. It is possible that there was a shift in the ratio of CYP2E1 holoenzyme (heme present) to apoprotein (inactive enzyme, heme lost) during the early perioperative period, such that the pool of active enzyme actually increased, whereas there was only a minimal increase in total CYP2E1 protein detected by Western blot analysis. Discrimination between the two protein pools in future studies may help to resolve the discrepancy between posttransplantation changes in protein content and metabolic activity.

It is unlikely that the observed induction of hepatic CYP3A4 content during the perioperative period contributed significantly to the enhancement of NAPQI formation and urine thioether metabolite recovery. Treatment of healthy volunteers with the potent CYP3A4 inducer rifampin (INN, rifampicin) failed to significantly alter NAPQI formation clearance,[13] suggesting that the intrinsic clearance for CYP3A4 may be too low to have a clinical impact under either control or induced conditions.

Burckart et al[6] studied CYP2E1 activity in liver transplantation patients using the plasma concentration ratio of 6-hydroxychlorzoxazone/chlorzoxazone at 4 hours after chlorzoxazone administration as an index of CYP2E1 metabolic activity. They reported a significant increase in the metabolic ratio in the first month after liver transplantation and a subsequent decline in the ratio over time to a mean value similar to that seen in healthy control subjects. These results are in agreement with our observed perioperative increase in NAPQI formation clearance in OLT subjects but are again at odds with a failure to demonstrate appreciable induction of hepatic CYP2E1 content. The reason for this discrepancy is unknown, but our suggestion of a change in the CYP2E1 holoenzyme/apoprotein pool also applies. There may also have been significant perioperative changes in chlorzoxazone plasma protein binding (approximately 97% in healthy volunteers) that could confound a mechanistic interpretation of the results of Burckart et al.

In addition to an increase in NAPQI formation clearance, the urinary recovery of thioether metabolites was elevated by the decline in formation clearances for acetaminophen conjugation pathways. Acetaminophen glucuronide and sulfate formation clearances and corresponding urinary metabolite recoveries were reduced on days 2 and 10 after transplantation, as compared with postoperative day 180. Previous investigators have reported that the extent of glucuronidation and sulfation of acetaminophen in clinically stable OLT patients is similar to that seen in healthy control subjects.[26] However, the time after transplantation was not specified in the report, and patients may have been studied 3 months or more after OLT.

The biologic basis for a decrease in glucuronidation and sulfation during the early post-OLT period is not clear. It is possible that the period of brain death preceding liver procurement or the liver transplantation procedure itself resulted in a transient decrease in hepatic pools of the conjugation enzymes (ie, uridine diphosphate-glucuronosyltransferase or sulfotransferase). In addition, it is possible that reduced dietary intake of inorganic sulfate, expected during and after surgery, might have reduced cosubstrate availability (ie, 3'-phosphoadenosine 5'-phosphosulfate) for sulfation. These homeostatic perturbations may have lasted for several days and may account for the observed reduction in acetaminophen conjugation clearances, as well as enhanced thioether recovery.

Acetaminophen hepatotoxicity is an uncommon event that, when it occurs, is associated with the ingestion of doses above the recommended 4-g maximum

daily dose.[2,27] Although we could find no mention in the literature of liver injury from postoperative use of a therapeutic dose of acetaminophen in OLT patients, diagnosis of such an event would be problematic, because there are other known causes for perioperative hepatic injury that might obscure such a clinical diagnosis. Hepatic function immediately after OLT is inevitably compromised to some degree, and in some patients there can be a significant loss of hepatocytes. This acute predisposition for liver injury and the enhancement of NAPQI formation observed in this study suggest a brief period of greater risk for acetaminophen toxicity in OLT patients than in the general population.

In summary, the oxidation of acetaminophen to NAPQI and urinary recovery of thioether metabolites was elevated during the first 10 days after OLT. This change was the result of a reduction in parallel glucuronidation and sulfation clearance pathways and an increase in NAPQI formation clearance. Accordingly, the risk of hepatotoxicity from acetaminophen in the early post-OLT period may be increased, and a reduction in the maximum daily dose of acetaminophen during that time should be considered.

A portion of this work was conducted through the Clinical Research Center Facility at the University of Washington. We thank the clinical staff of the Clinical Research Center and the organ transplant recovery ward for their excellent assistance in the conduct of this study.

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