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Reduced Duodenal Cytochrome P450 3A Protein Expression and Catalytic Activity in Patients with Cirrhosis

Donavon J. McConn II^{1,5,*}, Yvonne S. Lin^{1,*}, Terri L. Mathisen², David K. Blough³, Yang Xu^{1,6}, Takanori Hashizume^{1,7}, Shari L. Taylor^{4,8}, Kenneth E. Thummel¹, and Margaret C. Shuhart²

¹ Department of Pharmaceutics, University of Washington, Seattle, WA

² Department of Medicine, Division of Gastroenterology, University of Washington, Seattle, WA

³ Department of Pharmacy, University of Washington, Seattle, WA

⁴ Department of Pathology, University of Washington, Seattle, WA

Abstract

The small intestine and liver express high levels of cytochrome P450 3A (CYP3A), an enzyme subfamily contributing significantly to drug metabolism. In patients with cirrhosis, reduced metabolism of drugs is typically attributed to decreased liver function, but it is unclear whether intestinal drug metabolism is also compromised. In this study, we compared CYP3A protein expression and *in vitro* midazolam hydroxylation in duodenal mucosal biopsies from subjects with normal liver function (controls; n=20) and subjects with varying severity of cirrhosis (n=23). Compared to samples from controls, duodenal CYP3A expression and total midazolam hydroxylation was reduced by 47% and 34%, respectively in samples from subjects with cirrhosis. Greater decreases in CYP3A expression were seen in subjects with increasing severity of cirrhosis. Thus, patients with advanced cirrhosis may have increased drug exposure following oral dosing as a result of both impaired liver function and decreased intestinal CYP3A expression and activity.

Keywords

Intestine; drug metabolism; CYP3A; protein expression; cirrhosis

INTRODUCTION

Pathophysiological changes in the liver, the main site of drug metabolism, can profoundly alter the clearance of drugs.(1,2) In general, the metabolic clearance of drugs is reduced by liver disease, a phenomenon attributed to a decline in the delivery of portal blood to functional hepatocytes, as well as decreased metabolic and transport function of hepatocytes. (1) The drug metabolism activity in hepatocytes is primarily mediated by the cytochrome

Address correspondence to: Margaret Shuhart, M.D., M.S., Associate Professor of Medicine, Division of Gastroenterology, Harborview Medical Center, 325 Ninth Avenue, Box 359773, Seattle, WA 98104, Phone: 206-341-4641, FAX: 206-731-8698, Email: mshuhart@u.washington.edu.

*These authors contributed equally to the work

⁵Dr. McConn is currently affiliated with Theravance, South San Francisco, CA

⁶Dr. Xu is currently affiliated with Amgen, Thousand Oaks, CA

⁷Dr. Hashizume is currently affiliated with Daiippon Sumitomo Pharma Co., Ltd., Japan

⁸Dr. Taylor is currently affiliated with Caris Diagnostics, Irving, Texas.

CONFLICT OF INTEREST/DISCLOSURE

The authors declared no conflict of interest.

P450 enzymes. In adults, two CYP3A isoforms are expressed: CYP3A4 which is considered to be the dominant drug-metabolizing enzyme in the liver, and CYP3A5, a polymorphic enzyme which is generally considered to play a lesser role.(3) With regard to the mechanism for decreased metabolic clearance, previous investigators reported lower expression of several cytochrome P450 enzymes in cirrhotic liver tissue, including CYP3A4.(4–6) Consistent with this observation, patients with cirrhosis exhibit decreased systemic clearance and an increased half-life of known CYP3A substrates such as midazolam(7), nifedipine(8), nisoldipine(9), and verapamil(10).

For the drugs listed above, patients with hepatic cirrhosis also exhibit an increased systemic bioavailability following oral administration.(8,10,11) Following oral administration of CYP3A substrates in healthy individuals, both the liver and small intestine can contribute significantly to first-pass drug elimination because of the comparable expression of enzymes in hepatocytes and mature enterocytes of the small intestine.(12) Thus, in addition to decreased hepatic CYP3A expression and function, any alterations in oral drug clearance in patients with cirrhosis may be the result of a change in the expression and function of intestinal CYP3A enzymes.

Changes in intestinal CYP3A first-pass extraction of drugs like midazolam may involve decreased intestinal CYP3A activity, faster drug absorption or decreased intestinal exposure (15). Previous investigations of the effect of liver disease on the metabolic disposition of orally administered moderate to high intrinsic clearance CYP3A substrates have yielded conflicting results. While some studies indicated increased oral bioavailability,(8–11) other studies suggested no change in the absence of a portal shunt.(13,14). Although not completely congruent, the available data suggest that intestinal CYP3A first-pass activity may be unchanged in patients with compensated liver disease who have not received a portacaval shunt, and that any observed change in oral bioavailability is the result of impaired hepatic first-pass extraction. In this study, we sought to test this hypothesis by characterizing duodenal CYP3A expression and function in subjects with normal liver function and in subjects with cirrhosis who were undergoing diagnostic esophagogastroduodenoscopy. To our knowledge, this is the first study to directly measure CYP3A expression and activity in duodenal biopsies from patients with cirrhosis.

RESULTS

In general, control and cirrhotic subjects were well matched for age (range 35 to 65 years) and gender (Table 1). Both men and women were represented. The majority of subjects in the control (80%) and cirrhotic (65%) groups were of self-identified White ancestry. Other clinical information, including etiology of liver disease for patients with cirrhosis and final diagnosis from the endoscopic exam are also summarized in Table 1. Indications for endoscopy in the control group included gastroesophageal reflux disease, diarrhea, and epigastric pain. Screening for esophageal varices was the most common indication for endoscopy in the patients with cirrhosis. The etiology of cirrhosis in these patients consisted of alcoholic liver disease, HCV infection, or both, and one subject was diagnosed with nonalcoholic steatohepatitis. The Child-Pugh scores at the time of study ranged from 5 to 14 (median 9), with the following Child-Pugh class distribution: A, n=5; B, n=8; and C, n=10. All of the subjects exhibited normal duodenal histology.

Intestinal CYP3A Content

Of the study samples collected, one from a control and four from cirrhotic subjects (one CPC-B and three CPC-C) had detectable, but non-quantifiable CYP3A protein and were assigned a value of 4 pmol/mg protein (the limit of quantification). As seen in Table 2, the mean level of CYP3A expression in the samples from cirrhotic subjects was significantly

lower than that of control samples (13.2 ± 8.4 vs. 24.7 ± 12.8 pmol/mg protein, $p = 0.0011$). CYP3A content was related to the severity of liver disease when data were segregated by the Child-Pugh classification of the subjects (Figure 1 and Table 3). On average, subjects with the most advanced liver disease (CPC-C) had significantly lower (2.8-fold) duodenal CYP3A content than that of control subjects. However, one subject (Cirrhotic #15), with the highest Child-Pugh score of 14, exhibited a high level of CYP3A expression compared to the other nine subjects in the CPC-C group. There were no other unusual demographic or clinical features for this subject compared to others in the CPC-C group, including recent medication history, serum albumin and bilirubin concentrations (data not shown).

CYP3A Metabolic Activity

We observed significantly decreased total midazolam hydroxylation activity in biopsy homogenates prepared from cirrhotic subjects, compared to controls ($p = 0.026$, Table 2). Mean total midazolam hydroxylation activity was reduced by approximately 34% in cirrhotic samples compared to control samples. When activity data were stratified according to the Child-Pugh class, subjects with the most advanced liver disease (CPC-C) had significantly lower CYP3A midazolam hydroxylation activity compared to control subjects ($p = 0.019$, Figure 2 and Tables 2 and 3). Similar results were obtained when formation of the major metabolite, 1'-OH midazolam, was evaluated separately (data not shown). When normalized to pmol of CYP3A expression, CYP3A midazolam hydroxylation activity did not differ between cirrhotic and control subjects ($p = 0.34$, Table 2). Thus, it would suggest that the intrinsic CYP3A activity does not differ between control and cirrhotic patients and that cirrhosis may affect the amount of CYP3A expressed per mg of tissue.

Individual differences in duodenal CYP3A expression were strongly correlated with midazolam metabolic activity irrespective of the liver function status (Figure 3, Pearson's correlation coefficient = 0.80, $p < 0.0001$). CYP3A expression in the duodenal biopsies explained approximately 61% of the variability in total midazolam hydroxylation. Regressions of combined data from control and cirrhotic groups were comparable to those generated separately for each group.

DISCUSSION

We found that duodenal CYP3A expression and activity are reduced in patients with cirrhosis of the liver. Moreover, the reduction in CYP3A specific content, and thus the potential reduction in first-pass drug metabolism, was associated with the severity of liver disease. Generally, subjects with the most advanced liver disease (CPC-C) had significantly lower duodenal CYP3A expression compared to those with normal liver function or less severe liver disease. Indeed, there was no significant difference in mean duodenal CYP3A expression and catalytic activity between subjects with mild or moderate liver disease (CPC-A or CPC-B) and the control group. These data offer a plausible explanation for the discordant clinical findings for drugs like midazolam, verapamil and many dihydropyridine calcium channel blockers, where intestinal CYP3A-dependent first-pass metabolism contributes to an incomplete oral bioavailability.

Using midazolam as a CYP3A probe, Chalasani et al.(13) applied a pharmacokinetic modeling approach to evaluate changes in hepatic and intestinal first-pass metabolism in patients with cirrhosis and the effect of transjugular intrahepatic portosystem shunts (TIPS). In patients with cirrhosis but without TIPS, Chalasani et al. determined that the oral bioavailability of midazolam was unchanged compared to healthy controls ($27 \pm 14\%$ and $30 \pm 10\%$, respectively). Moreover, they reported no change in the calculated gut wall bioavailability in cirrhotic patients ($32 \pm 16\%$) compared to healthy controls ($42 \pm 15\%$). In contrast, patients who had a portal-caval shunt exhibited a marked increase of oral

bioavailability and intestinal availability ($76 \pm 20\%$ and $83 \pm 17\%$, respectively). Similarly, the oral bioavailability of nifedipine was increased more extensively in patients with TIPS, compared to those without a shunt.(8) In addition, Chalasani and colleagues(13) re-evaluated published data on the disposition of isradipine(16), which is also a CYP3A substrate, in cirrhotic patients and found that only those who had undergone a TIPS procedure exhibited a significant increase in the oral bioavailability. However, not all study results with CYP3A substrates are fully consistent with these observations. For example, Regårdh et al.(14) studied felodipine disposition in patients with cirrhosis and healthy volunteers and found no change in the oral bioavailability of felodipine in cirrhotic patients with (14 and 19 %) or without TIPS (17 %), compared to healthy control data (15 and 16% reported from different studies). In contrast, Van Harten et al.(9) reported that the bioavailability of nisoldipine was increased from 3.7 ± 2.1 to $14.7 \pm 10.1\%$ in patients with cirrhosis. Although the effect appeared to be greater for those patients who had a meso-caval shunt ($F = 16$ and 36%), the mean bioavailability for the rest of the subjects ($9.1 \pm 2.5\%$) was still significantly higher than control.(9)

In general, most of these studies had a relatively small sample size and few patients with advanced liver disease. Thus, in consideration of our data, increased systemic drug exposure following oral administration of a high first-pass CYP3A substrate may only be evident and clinically significant in patients with the most severe liver disease. For example, there was no difference in the oral bioavailability of triazolam in healthy volunteers and patients with mild cirrhosis (5/6 were CPC-A and 1/6 was CPC-B).(17) Similarly, Regårdh et al. reported no difference in the oral bioavailability of felodipine in their study of healthy volunteers and patients with mild cirrhosis (7/9 were CPC-A and 2/9 were CPC-B).(14) In a study by Chalasani et al., they observed no change in the oral and intestinal bioavailability of midazolam in the absence of a TIPS procedure in patients with predominantly moderate cirrhosis (7/9 were CPC-B and 2/9 were CPC-C).(13) In contrast, nearly half of cirrhotic subjects recruited to this study (10/23) were CPC-C. Based on our endoscopy results, one might expect such patients to exhibit 50 – 100 % increases in the oral bioavailability of high first-pass CYP3A substrate drugs.

We acknowledge some limitations with our data and its interpretation. Although Watkins and colleagues have used villin to adjust for the biopsy depth,(18) we did not use the relative villin content to control for potential differences in biopsy depth because of a trend for decreased duodenal villin expression in samples from cirrhotic subjects (data not shown). When normalized for total cellular protein, the mean villin content in samples from cirrhotic subjects was 1.4-fold lower than the mean villin content in samples from controls. Given that the same investigator collected all of the study samples, the biopsy depth among all subjects (cirrhotic and controls) should be relatively uniform. Moreover, we would expect any variation in biopsy depth to be random and unrelated to the absence or presence of liver disease. Due to the limited duodenal sample collected, we were unable to repeat the Western Blot procedure to probe for another marker for normalization. However, all of our CYP3A expression and activity measurements were normalized to total protein content of the duodenal biopsy homogenate. Based on our data, it appears that duodenal villin expression may be altered along with CYP3A in patients with severe cirrhosis. The fact that villin and CYP3A were both decreased in samples from cirrhotic patients suggest that there may be a generalized effect on transcription or protein degradation which is not associated with observable pathology.

There was a significant degree of inter-subject variability in duodenal CYP3A content in samples from both the control and cirrhotic subjects. This finding is consistent with previously published reports that suggest substantial differences in CYP3A4 gene expression.(19–21) In addition, some of the variability in the measurement of CYP3A

specific content and catalytic activity might be attributed to the presence of CYP3A5, which has been shown to be polymorphically expressed as a result of alternative, aberrant mRNA splicing.(22) Due to the limited duodenal sample collected, we were unable to quantitate CYP3A4 and CYP3A5 separately. Migration of the two proteins under our SDS-PAGE conditions was nearly overlapping and not clearly resolvable. However, because the antibody employed favors the detection of CYP3A4, the resulting protein expression value assigned for samples with some CYP3A5 represents predominantly CYP3A4. Moreover, we do not believe that the presence or absence of CYP3A5 confounded our interpretation of the effect of cirrhosis on intestinal CYP3A expression. Based on demographics of the study subjects, we do not expect many of the samples to have detectable CYP3A5 as the frequency of expression is low in Caucasians.(22)

Rather than recruiting healthy volunteers to undergo small intestinal biopsy, the controls were selected to be comparable to the patients with cirrhosis in that both sets of subjects were undergoing diagnostic esophagogastroduodenoscopy. While it is possible that control subjects undergoing an upper endoscopy for diarrhea may have decreased intestinal CYP3A expression due to inflammation or other small bowel pathology, duodenal histology was normal in all subjects. Furthermore, this would have biased our results toward the null. Finally, the use of a healthy volunteer population as a control group could in fact increase confounding due to a lesser use of medications when compared to patients with cirrhosis of the liver.

If an elevation in total bilirubin reflects a general decline in the hepatic biliary transport of endogenous molecules, the absence of endogenous factors that are positive regulators of duodenal CYP3A might result in a reduction in CYP3A protein expression. A possible regulatory factor is $1\alpha,25$ -dihydroxyvitamin D_3 which is decreased in patients with alcoholic liver disease and/or cirrhosis,(23–25) and has been shown to activate CYP3A4 transcription *in vitro*(26–28) and *in vivo*.(29,30) Other possible factors include lithocholic acid and related metabolites, which have similar CYP3A inductive effects.(31)

The use of therapeutic agents in patients with liver disease is quite challenging, given a myriad of uncertainties about constitutive and disease-related differences in drug disposition and pharmacological effects. This includes drugs that are excellent substrates for CYP3A and that exhibit low oral bioavailability, such as some of the statins (lovastatin and simvastatin), calcium channel blockers (verapamil, nifedipine and felodipine), benzodiazepines (midazolam and triazolam) and HIV protease inhibitors (saquinavir, ritonavir and lopinavir). Based on our findings, further studies should be performed in patients with cirrhosis to confirm and determine the mechanism of reduced intestinal CYP3A expression and other $1\alpha,25$ -dihydroxyvitamin D_3 -sensitive markers. In addition, we need to more rigorously evaluate whether the severity of liver disease correlates with changes in the intestinal bioavailability (and hence, systemic availability) of CYP3A substrates. For example, additional *in vivo* studies in subjects with varying severity of liver disease could be performed to correlate observed CYP3A changes in the duodenum with midazolam clearance. Although there was a significant trend for decreasing CYP3A activity with an increasing severity of liver dysfunction, one individual subject with severe cirrhosis exhibited CYP3A activity that was well above that of others in the group and within the range found for control subjects. Thus, possible inter-individual effects of cirrhosis on CYP3A activity may need to be taken into consideration. Overall, our results suggest that in addition to the pharmacokinetic effect from an expected decline in hepatic drug metabolism, patients with advanced cirrhosis (CPC-C) may have substantially increased systemic drug exposure following oral drug administration as a result of reduced intestinal first-pass metabolism.

MATERIALS AND METHODS

Chemicals

Midazolam, 1'-hydroxy midazolam, 4-hydroxy midazolam and $^{15}\text{N}_3$ -midazolam were kindly provided by Roche Laboratories (Nutley, NJ). $^{15}\text{N}_3$ -labeled 1'-OH and 4-OH midazolam were generated enzymatically from $^{15}\text{N}_3$ -midazolam as previously described. (21) Acetonitrile and ethyl acetate were purchased from Fisher Scientific (Santa Clara, CA). N-methyl-N-(*t*-butyl-dimethylsilyl) trifluoroacetamide was obtained from Pierce Chemical (Rockford, IL). Phenylmethylsulfonyl fluoride, benzamidine, β -nicotinamide adenine dinucleotide phosphate, and aprotinin were purchased from Sigma Chemical Co. (St. Louis, MO). Complete protease inhibitor cocktail tablets were obtained from Roche Applied Science (Indianapolis, IN). Nitrocellulose was purchased from Schleicher and Schuell (Keene, NH). Appropriate secondary antibodies were purchased from Sigma-Aldrich. Alkaline phosphatase development reagents (5-bromo-4-chloro-3-indoyl phosphate and nitroblue tetrazolium) were purchased from Kirkegaard and Perry (Gaithersburg, MD). All other reagents were of the highest grade commercially available.

Study Population

This study was approved by the University of Washington Institutional Review Board. Patients undergoing diagnostic esophagogastroduodenoscopy at Harborview Medical Center were eligible. We enrolled a total of 43 subjects: twenty with normal liver function and twenty-three with a diagnosis of non-biliary cirrhosis of the liver. Subjects with a pre-endoscopic diagnosis of biliary cirrhosis were excluded, as previous investigators have shown that they behave differently with respect to changes in hepatic CYP expression,(4) possibly due to altered excretion of regulatory factors in the bile. Inclusion criteria included non-emergency endoscopic exam, stable mental status and normal renal function. Exclusion criteria included platelet count < 60,000, INR > 2.0, diagnosis of small bowel disease, chronic or recent (within 2 weeks) treatment with known inducers or inhibitors of CYP3A enzymes, and recent consumption of grapefruit-containing products. In addition, the control group had laboratory tests and a physical exam on the day of the endoscopy that revealed no evidence of liver disease. Recruitment from the eligible control population was conducted to match subjects with cirrhosis with respect to gender, age (± 5 yrs) and race/ethnicity. Subjects with cirrhosis were classified as Child-Pugh Class A, B or C(32).

Duodenal Biopsy Collection

We collected duodenal mucosal biopsies at the end of the diagnostic endoscopic exam. An identical biopsy procurement procedure was implemented for all subjects and was performed by the same clinician (MS). A total of five samples (~ 2–5 mg each) from the mucosal villi of each subject was collected. Two samples were submitted for histological examination and were reviewed by a single gastrointestinal pathologist (ST) who was blinded to all other data. The remaining three samples were placed in a tube and immediately frozen on dry ice. Samples were subsequently homogenized in 1 ml of solution D (0.05 M Tris-HCl, 20% glycerol and 2 mM ethylenediaminetetraacetic acid)(33) supplemented with a protease inhibitor mixture (aprotinin, benzamidine and 0.1 mM phenylmethylsulfonyl fluoride) or Complete protease inhibitor cocktail tablets, aliquoted and stored at -80°C until analyzed for CYP3A content and metabolic activity. The protein concentration of the homogenate was measured by the method of Lowry et al.(34)

Quantitation of Duodenal CYP3A Protein Expression

We determined the specific CYP3A content of each homogenate sample (15 μg) using Western immunoblot analysis as described by Lin et al.(35) An Integrated Optical Density

(IOD) for each protein band was generated using a Bio-Rad ChemiDoc and Quantity One program (Hercules, CA). CYP3A protein levels in biopsy homogenates were estimated by comparison of the unknown band IOD with that generated from a purified CYP3A4 enzyme standard curve (0.125 to 1 pmol loaded). The CYP3A specific contents are presented as pmol CYP3A/mg protein. Although we did not quantify CYP3A5 expression, minor contributions from CYP3A5 protein may be included in the detectable CYP3A4 band because of incomplete separation of the two CYP3A isoforms in the Western blot procedure. However, the antibody is more selective for CYP3A4(36) and based on demographics of the study subjects, we do not expect many of the samples to have detectable CYP3A5 as the frequency of expression is low in Caucasians.(22)

Quantitation of Duodenal CYP3A Catalytic Activity

We conducted all incubations with duodenal homogenate in an aqueous buffer containing 100 mM potassium phosphate and 1 mM ethylenediaminetetraacetic acid, pH = 7.4. After preheating to 37°C for 5 min, reactions were initiated with the addition of β -nicotinamide adenine dinucleotide phosphate (1 mM, final concentration) and incubated at 37°C for the indicated length of time. Incubations were performed in duplicate.

We conducted a 4-minute incubation of midazolam (4 μ M) with human duodenal homogenate (0.1 mg protein; 0.5 mL final volume) following published procedures.(35) After the reactions were quenched, 100 μ l of internal standard solution ($^{15}\text{N}_3$ -labeled 1'-OH and 4-OH midazolam) was added to all tubes, followed by extraction with 5 ml of ethyl acetate. The extracted molecules were derivatized using N-methyl-N-(*t*-butyl-dimethylsilyl) trifluoroacetamide. We used gas chromatography-negative chemical ionization mass spectrometry to analyze the derivatized 1'-OH (m/z = 455 and 460) and 4-OH (323 and 328) midazolam metabolites. The amounts of 1'-OH and 4-OH midazolam metabolites produced in the incubations were summed to calculate a total midazolam hydroxylation rate.

Statistical Methods

Data are presented as mean $\pm$ standard deviation. We compared the CYP3A expression and activity between the control and cirrhotic groups (severity of cirrhosis) using ANOVA and Dunnett's t-test after adjusting for gender, age and race (white or non-white). We assessed the correlation of duodenal CYP3A expression with total midazolam hydroxylation using Pearson's correlation coefficient. All statistical analyses were performed using SAS (SAS Institute Inc., version 9), and comparisons differing at the level of *P* less than 0.05 were considered significant.

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Figure 1.

CYP3A expression in duodenal biopsies from 20 control subjects and 23 subjects with cirrhosis. CYP3A protein content was measured by Western blot analysis of tissue homogenates and normalized for total protein content of the homogenate. Duodenal CYP3A expression is affected by the severity of liver cirrhosis (CPC, Child-Pugh class). Overall, subjects with cirrhosis had decreased CYP3A expression compared to control subjects ($P = 0.0018$) as determined by ANOVA.

Figure 2.

Subjects with liver cirrhosis have decreased midazolam hydroxylation activity compared to control subjects. Total midazolam hydroxylation activity was measured in duodenal biopsy homogenates. Subjects with advanced cirrhosis, classified by Child-Pugh class (CPC), differ in total midazolam hydroxylation activity compared to controls ($P = 0.019$).

Figure 3.

CYP3A-dependent midazolam hydroxylation is correlated with CYP3A expression in control subjects and subjects with cirrhosis. Control subjects (open circles), CPC-A (filled boxes), CPC-B (filled triangles), CPC-C (filled circles) are shown. Individual differences in duodenal CYP3A expression were strongly correlated with midazolam metabolic activity irrespective of the liver function status (Pearson's correlation coefficient = 0.80, $p < 0.0001$).

Table 1

Subject characteristics according to liver disease status.

	Control Subjects N = 20	Subjects with Cirrhosis N = 23
Age, years [median (range)]	46 (37–62)	48 (35–65)
Sex [n (%) male]	12 (60)	14 (61)
Race/Ethnicity [n (%)]		
White	16 (80)	15 (65)
Black	2 (10)	2 (9)
Hispanic	1 (5)	3 (13)
Asian	1 (5)	1 (4)
Native American	0	2 (9)
Indication for Endoscopy [n (%)]		
GERD	15 (75)	--
Diarrhea	2 (10)	--
Barrett's esophagus	1 (5)	--
Dysphagia	1 (5)	--
Ulcer	1 (5)	--
Screening for varices	--	12 (52)
Surveillance of known varices	--	11 (47)
Etiology of Liver Disease [n (%)]		
ALD	--	10 (43)
HCV	--	3 (13)
ALD and HCV	--	9 (39)
NASH	--	1 (4)

GERD, gastroesophageal reflux disease; ALD, alcoholic liver disease; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis

Table 2
Summary of CYP3A expression and activity in duodenal biopsies from control and cirrhotic subjects.^a

Measure	Control Subjects		Subjects with Cirrhosis			Controls vs. Cirrhotics		Controls, CPC-A, B, C ^b	
	N = 20	Total N = 23	CPC-A N = 5	CPC-B N = 8	CPC-C N = 10	t-test P-value	ANOVA P-value ^f		
Albumin (g/dL) ^c	3.9 ± 0.5	3.0 ± 0.9	4.1 ± 0.5	3.0 ± 0.9	2.5 ± 0.4	<0.0001	<0.0001		
Bilirubin (mg/dL) ^c	0.7 ± 0.2	2.7 ± 4.0	0.9 ± 0.3	1.1 ± 0.4	4.8 ± 5.5	0.0227	0.0048		
CYP3A (pmol/mg protein)	24.7 ± 12.8	13.2 ± 8.4	20.4 ± 6.5	14.2 ± 6.7	8.8 ± 8.2	0.0011	0.0018		
MDZ OH (pmol/min/mg) ^d	87.4 ± 43.2	57.7 ± 41.4	95.3 ± 18.6	53.3 ± 35.3	42.4 ± 44.7	0.026	0.019		
MDZ OH (pmol/min/pmol CYP3A) ^e	3.8 ± 1.2	4.2 ± 1.7	4.9 ± 1.2	3.7 ± 1.3	4.3 ± 2.2	0.34	0.74		

^aMeans ± standard deviations are presented

^bCPC: Child-Pugh class

^cOnly 19 observations available for the control group

^dTotal midazolam hydroxylation determined at 4 μM midazolam normalized by protein content

^eTotal midazolam hydroxylation determined at 4 μM midazolam normalized by estimated CYP3A content

^fAdjusting for age, sex and race

Table 3

Impact of severity of cirrhosis on CYP3A expression and activity in duodenal biopsies. Values presented are the difference between mean CYP3A or midazolam hydroxylation activity with controls (95% confidence limits).

Comparison ^a	CYP3A (pmol/mg protein)	MDZ OH (pmol/min/mg) ^b
CPC-A vs. control	-4.3 (-17.4, 8.8)	7.9 (-43.8, 59.5)
CPC-B vs. control	-10.5 (-21.4, 0.4)	-34.1 (-77.3, 9.1)
CPC-C vs. control	-15.9 (26.0, 5.8) ^c	-45.1 (-85.0, -5.1) ^c

^a Assessed by Dunnett's t-test

^b Total midazolam hydroxylation

^c Significant at the 0.05 level