ABSTRACT:
The purpose of this investigation was to examine the effects of surgery and anesthesia on in vivo CYP3A activity and portal venous blood flow. Midazolam, a CYP3A probe for both rats and humans, was administered orally (2.7 mg), intravenously (0.57 mg), or via the portal vein (0.57 mg) to rats 4 h after anesthesia with ketamine/xyazine and surgery for placement of indwelling vascular and duodenal catheters and 3 days after surgery (chronic). The systemic clearance of midazolam was 51 ± 4 ml/min/kg in the chronic animals, and this was significantly decreased (29 ± 1 ml/min/kg, P = 0.0024) in acute rats studied 4 to 6 h after anesthesia and surgery. The hepatic availability (F_H), directly determined from the aortic and hepatic venous concentration gradient, was significantly higher in the acute animals (0.57 ± 0.05) compared with the chronic animals (0.33 ± 0.07, P = 0.001). Hepatic availability was determined using a classical approach in which F_H was calculated from the area under the plasma concentration versus time curve ratio after portal venous or intravenous administration. F_H was higher in the acute rats (0.48) compared with the chronic animals (0.27 ± 0.03). Portal venous blood flow was significantly lower in the acute animals (5.0 ± 0.4 ml/min/100 g body weight) compared with the chronic animals (9.1 ± 0.9 ml/min/100 g body weight, P = 0.015). The effect of surgery and anesthesia was confirmed using the indicator dye dilution method after infusion of [14C]polyethylene glycol 4000 into the superior mesenteric artery. Our data suggest that anesthesia and surgery decreases both hepatic CYP3A activity and hepatic blood flow in rats. Studies performed in rats within 3 days of surgery and anesthesia are conducted under nonphysiologic conditions and therefore provide inaccurate assessment of drug disposition, in particular, clearance and bioavailability.

During drug development, in vivo drug absorption and hepatic first-pass metabolism studies commonly are performed in rats, dogs, and primates after surgical manipulation to implant vascular and gastrointestinal catheters. The absorption and bioavailability of orally administered drugs is dependent on intestinal and liver function, including metabolism. The ability to quantify the extent and variability of first-pass metabolism and the inhibition of metabolism in vivo is important for the development of oral dosage forms. To separately determine the effects of the liver on hepatic first-pass metabolism from the contribution by the gut, the area under the plasma concentration versus time curve (AUC) following drug administration via the portal vein is compared with the AUC obtained after oral and intravenous drug administration. These experiments are typically conducted using rodents and are often conducted shortly after the animal has regained its righting reflex. Consciousness and mobility, not gastrointestinal or liver function, are therefore frequently used as parameters of surgical recovery. Although studies have clearly demonstrated that surgery and anesthesia alter gastrointestinal function (Cohn et al., 1995; Elfant et al., 1995; Schuitmaker et al., 1999; Ueda et al., 1999), no studies have directly examined whether pharmacokinetic parameters determined within 4 to 6 h of a surgical procedure accurately reflect normal physiologic conditions.

The cytochromes P450 are the most important enzyme system for the metabolism of drugs and the turnover of endogenous substrates. CYP3A is the most abundant subfamily within the cytochromes P450 in human liver and catalyzes the metabolism of a wide variety of structurally diverse endogenous substrates and exogenous chemicals (Nebert and Russell, 2002). Due to the considerable overlap in affinity for several substrates and inhibitors, rat hepatic CYP3A2, the predominant form in male rats, has been used as a surrogate for studying several aspects of human hepatic CYP3A4 activity, including induction, inhibition, and drug-drug interactions (Thummel and Wilkinson, 1998).

We have developed a catheterized rat model in which portal venous, hepatic venous, and aortic blood samples were obtained simultaneously following duodenal infusion of drug. We examined the effects of surgery and anesthesia on the absorption and disposition of midazolam, a probe for hepatic CYP3A in both rats and humans. Drug clearance, intestinal drug absorption rate, portal venous blood flow, bioavailability (F), and hepatic availability (F_H) were determined after a single oral dose of midazolam using this newly developed model.

ABBREVIATIONS: AUC, area under the plasma concentration versus time curve; IVC, inferior vena cava; PV, portal vein; HV, hepatic vein; A, aorta; D, duodenum; Q_IVC, portal venous blood flow; Q_PV, hepatic venous blood flow; Q_A, hepatic artery blood flow; Q_HV, total hepatic blood flow; PEG, polyethylene glycol.
performing at 4 days after surgery and anesthesia, and these were designated as the “chronic” group. In the first series of experiments, the classical approach was used to assess the effects of surgery and anesthesia on hepatic drug extraction. Midazolam (0.6 mg dissolved in 1 ml of normal saline) was administered via all routes by a constant infusion over 5 min. In the acute phase animals, midazolam was infused into the inferior vena cava (0.57 mg, \( \frac{n}{n} = 4 \)), or via the portal vein (0.57 mg, \( n_1 = 3 \)) or via duodenal catheter (2.7 mg, \( n_2 = 5 \)). Aortic blood samples (0.2 ml) were obtained from the animals at 2.5, 4, 6, 8, 10, 15, 20, 30, and 60 min after the start of each drug infusion. In the chronic studies (\( n = 6 \)), 4 days after surgery, midazolam (0.57 mg) was administered into the inferior vena cava followed by collection of aortic blood samples. Five days later, 0.57 mg of midazolam was administered to the same animals via the portal vein catheter followed by collection of aortic blood samples. In a separate group of rats studied 4 days after surgery and anesthesia, midazolam (2.7 mg) was administered via a duodenal catheter followed by aortic blood sample withdrawal.

For the novel five-catheter rats (\( n_1 = 4 \)), 2.7 mg of midazolam (3 mg dissolved in 1 ml) was administered by constant infusion over 5 min via the duodenal catheter. At 2.5, 5, 10, 15, 20, 25, 30, 45, and 90 min after the start of the duodenal infusion, three blood samples (0.2 ml) were simultaneously obtained from the aortic, portal, and hepatic venous catheters. There are several advantages to this approach. The five-catheter method ensures paired data. Moreover, the ability to obtain simultaneous aortic and hepatic venous blood samples allowed determination of the instantaneous hepatic gradients and, thus, the time course of \( F_{PV} \) during drug absorption and elimination. Using the classical approach, animals received two doses of midazolam on different occasions. Possible confounding factors included the potential interday variability in parameters such as hepatic blood flow or CYP3A activity. In addition, occasional catheter failure between study periods sometimes prevented the use of an animal as its own control.

In all studies, after each blood sample withdrawal, animals were immediately transfused with an equal volume of blood obtained from other healthy, chronically catheterized male Sprague-Dawley rats. The hematocrit of all study animals remained stable over the time course of drug administration and blood sample withdrawal. For the midazolam assays, plasma was separated and stored at −80°C.

**Pharmacokinetic Analysis.** It was assumed that the systemic blood clearance is equivalent to the hepatic blood clearance (\( CL_{HCA} \)). Preliminary data (not shown) indicated the lack of a portal venous/aortic gradient for midazolam, indicating that the intestine did not contribute to the systemic clearance. The AUC in aortic blood up to the last measured time point was estimated using the linear trapezoidal rule. The extrapolated area under the concentration versus time curve from the last sample time to infinity was determined as the quotient of the last measured serum concentration and the terminal elimination rate constant. The elimination rate constant was obtained from the terminal slope of the log-linear portion of the serum concentration time curves using least-squares regression. The systemic blood clearance (\( CL \)) of midazolam was calculated as the quotient of dose, administered via the inferior vena cava catheter (\( Dose_{IVC} \)) and aortic AUC from zero to infinity (\( AUC_{A} \)).

Areas under the curve, from zero to infinity, are reported as AUC\(_{\text{SAMPLE LOCATION}}\) (ROUTE), where sample locations are aortic (A), PV, or hepatic venous (HV) and routes are duodenal (D), inferior vena cava (IVC), or PV. For example,\( AUC_{A}(D) \) indicates aortic samples obtained after duodenal administration. The AUC is expressed as \( \mu g \cdot ml/\text{min} \).

**Calculation of Hepatic and Systemic Bioavailability.** The bioavailable fraction (\( F \)) following a duodenal dose was calculated as

\[
F = \frac{AUC_{A}(D) \cdot Dose_{IVC}}{Dose(D) \cdot AUC_{A}(IVC)}
\]

(1)

The hepatic availability (\( F_{PV} \)) was calculated as the fraction of drug entering the liver that escaped extraction. In the novel five-catheter animal model, after duodenal administration, the rate of midazolam entry into the liver (\( Rate_{PV} \)) was estimated as the sum of the amounts of midazolam entering from the portal vein and the hepatic artery, represented by the following equation:

\[
Rate_{PV} = [AUC_{PV}(D) \cdot Q_{PV} + AUC_{A}(D) \cdot Q_{HA}]
\]

(2)

Based upon microsphere studies (Richter et al., 2001), we assumed that portal venous blood flow (\( Q_{PV} \)) and hepatic artery flow (\( Q_{HA} \)) account for 75 and
25%, respectively, of total hepatic blood flow (Qh). The rate of midazolam leaving the liver (Rate_{OUT}) is represented by

\[ \text{Rate}_{OUT} = \frac{\text{AUC}_{PV} \cdot Q_h}{\text{R}} \]  

(3)

Hepatic availability (F_{h}) was calculated as

\[ F_h = \frac{\text{Rate}_{OUT}}{\text{Rate}_{IN}} \]  

(4)

Using the classical approach, F_{h} was also calculated as

\[ F_h = \frac{\text{AUC}_{PV}}{\text{AUC}_{PV} + \text{AUC}_{AV}} \]  

(5)

We also estimated the fraction of the orally administered drug that reached the portal circulation as intact drug (F_{G}) from the quotient of systemic bioavailability and hepatic availability. We assumed that 100% of the dose is absorbed from the gastrointestinal lumen (F_{ABS} = 1). F_{G} is determined from

\[ F = F_h \cdot F_G \]  

(6)

From the experiments using the five-catheter method, the portal venous-aortic concentration gradients of midazolam were determined at each sampling time point. The absorption rate of midazolam absorption from the intestinal lumen into the portal vein was calculated as the product of portal venous blood flow and the aortic-portal venous concentration difference during absorption. We assumed that portal venous blood flow (Q_{PV}) remained constant during the time course of an individual study. Q_{PV} was estimated from the five-catheter animals using a mass-balance relationship for the portal vein as shown below

\[ Q_{PV} = \frac{(F_G \cdot \text{Dose}(D))}{[\text{AUC}_{PV}(D) - \text{AUC}_{AV}(ID)]} \]  

(7)

Preliminary data (not shown) indicated that gut wall availability for midazolam was close to unity in both the acute and chronic rats determined from the five-catheter model or the classical approach. This is consistent with the report of Cummins et al. (2003) that first-pass intestinal extraction of midazolam is negligible in the rat. Accordingly, in subsequent calculations, F_{G} was assumed to be unity.

**Estimation of Portal Venous Blood Flow.** To verify that estimates of portal venous blood flow (Q_{PV}) were accurate (eq. 7), we separately measured the effect of surgery and anesthesia on the time course of Q_{PV} (immediately after surgery, 4 h after surgery, and on postoperative days 1, 2, and 3) using the indicator dye dilution method of Fick (Guyton et al., 1973; Corbic et al., 1984; Richter et al., 2001). Briefly, portal venous and aortic catheters were surgically implanted in a separate group of animals as previously described. An additional catheter was placed in the superior mesenteric artery in retrograde fashion approximately 1 cm from its bifurcation from the aorta. [14C]PEG 4000 was constantly infused into the superior mesenteric artery at a rate of 0.25 ml/min using an infusion pump (Razel Scientific Instruments, Stamford, CT). At 1.5 min after the end of the infusion, serial blood samples (0.25 ml) were simultaneously obtained from the portal venous and aortic catheters and radioactivity was determined by liquid scintillation. Q_{PV} was calculated as the quotient of infusion rate and the portal venous-aortic concentration difference.

**Measurement of Plasma Midazolam Concentrations.** A total of 50 µl of 1 M NaOH was added to 100 µl of plasma and vortexed for 20 s. Then, 2.0 ml of pentane/dichloromethane (1:1) was added, and the sample was vortexed for 40 s and centrifuged for 5 min at 4000g. Prazepam (200 ng) was added as an internal standard. The organic layer was evaporated to dryness and the sample was reconstituted with 100 µl of mobile phase and injected onto the high-performance liquid chromatograph. Midazolam and the internal standard were separated under isocratic conditions using reversed-phase chromatography (Symmetry C18 column, 3.0 × 150 mm, 5 µm; Waters, Milford, MA). The mobile phase [40% 25 mM KH2PO4, pH 7.4 and 60% methanol/acetonitrile (1:1)] was pumped at 0.6 ml/min (Rainin Dynamax SD-200; Rainin Instruments, Woburn, MA). Midazolam was quantified by ultraviolet absorption at 232 nm. The coefficient of variation at the highest standard concentration (5 µg/ml) was 3.4% and at 150 ng/ml was 10.3%. Sample concentrations above the 5 µg/ml were diluted before assay. The relative error at 5 µg/ml was less than 1% and at 80 ng/ml was 14%.

**Statistical Analysis.** Data are expressed as mean ± S.E.M. Statistical analyses were performed using a paired or unpaired t test using JMP 5.1 statistics software (SAS Institute, Inc., Cary, NC). Differences were not attributed to chance variation when P < 0.05.

**Results**

We characterized the effects of surgery and anesthesia on the time course of portal venous blood flow using [14C]PEG 4000 and determined that blood flow had returned to baseline 3 days after surgery and anesthesia (data not shown). Based upon these preliminary data, we undertook more extensive studies in animals at two time points, namely 4 to 6 h or 4 days after surgery and anesthesia. The time course of midazolam concentrations in aorta, portal, and hepatic veins following duodenal infusion of 2.7 mg of midazolam are shown in Fig. 1. In the acute animals (Fig. 1A), the hepatic vein concentrations peaked at 4.2 ± 0.3 µg/ml. The peak aortic midazolam concentration was 2.0 ± 0.9 µg/ml at 10 min and declined to 1.0 ± 0.4 µg/ml by 45 min. The portal vein midazolam peaked at 7.0 ± 1.3 µg/ml and declined to 1.9 ± 0.5 µg/ml by 30 min. In the chronically catheterized animals (Fig. 1B), the midazolam concentration difference across aorta and hepatic vein was greater compared with that in the acute group. The peak hepatic vein and aortic midazolam concentrations, 1.2 ± 0.3 and 1.7 ± 0.3 µg/ml, respectively, occurred at 10 min. Hepatic availability of midazolam was 73% greater (P = 0.032) in the acute compared with the chronic rats using the five-catheter approach (Table 1).

The time course of midazolam concentrations in aorta following separate portal venous or intravenous administration (classical approach) is shown in Fig. 2. In the acute animals (Fig. 2A), the aortic concentrations were higher compared with the chronic animals. Be-

![Fig. 1](image_url)
Chronic animals received midazolam 4 days after implantation of vascular and duodenal catheters. Acute animals received midazolam after regaining their righting reflex, typically 4 to 6 h after surgery and anesthesia.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chronic</th>
<th>Acute</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCC/Duodenal infusion</td>
<td>6.6 ± 1.3 (4)</td>
<td>19.5 ± 4.5 (3)</td>
<td>0.024</td>
</tr>
<tr>
<td>AUCC/IVC infusion</td>
<td>20.0 ± 1.3 (6)</td>
<td>34.3 ± 1.5 (4)</td>
<td>0.001</td>
</tr>
<tr>
<td>AUCC/PV infusion</td>
<td>5.3 ± 0.5 (6)</td>
<td>16.4 ± 2.2 (3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Systemic clearance (ml/min/kg)</td>
<td>51 ± 4 (6)</td>
<td>29 ± 1 (4)</td>
<td>0.018</td>
</tr>
<tr>
<td>Hepatic availability (Fph, five-catheter)</td>
<td>0.33 ± 0.07 (4)</td>
<td>0.57 ± 0.05 (3)</td>
<td>0.032</td>
</tr>
<tr>
<td>Hepatic availability (Fph, classical)</td>
<td>0.27 ± 0.03 (6)</td>
<td>0.48 (4)</td>
<td>—</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.E.M., with n in parentheses.

### Figures

**Fig. 2.** Mean (±S.E.M.) aortic serum midazolam concentrations following separate infusions of 0.57 mg into the inferior vena cava (○) or the portal vein (●) over 5 min. Three catheters were implanted in each animal as described under Materials and Methods. Panel A represents animals studied 4 to 6 h after regaining consciousness and mobility (acute; n = 4). Panel B represents chronic animals studied 4 days after catheter implantation (chronic; n = 6).

**Fig. 3.** Hepatic availability (Fph) in acute animals studied 4 to 6 h after regaining consciousness and mobility and in chronic animals studied 4 days after catheter implantation. Fph was estimated using the classical method from data in four acute animals and six chronic animals. Using the five-catheter method, Fph was calculated from data in three acute animals and four chronic animals. Data are mean ± S.E.M. for the chronic animals. *, P < 0.05

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Chronic (n = 4)</th>
<th>Acute (n = 3)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCC(D) (μg · min/ml)</td>
<td>56 ± 12*</td>
<td>132 ± 31</td>
<td>0.048</td>
</tr>
<tr>
<td>AUCP(D) (μg · min/ml)</td>
<td>153 ± 6</td>
<td>269 ± 21</td>
<td>0.002</td>
</tr>
<tr>
<td>Dose/body weight (mg/kg)</td>
<td>8.5 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>0.023</td>
</tr>
<tr>
<td>QPV (ml/min/100 g b.wt.)</td>
<td>9.1 ± 0.9</td>
<td>5.0 ± 0.4</td>
<td>0.015</td>
</tr>
</tbody>
</table>

* Data are expressed as mean ± S.E.M.

### Discussion

The purpose of the present study was to investigate quantitatively the influence of surgery and anesthesia on the pharmacokinetics of midazolam, an in vivo probe for hepatic CYP3A activity. Over the past few decades, clearance and bioavailability have been identified as key sources of variability in the response to drug therapy. Increasingly, drug disposition studies have adopted a physiological approach to characterizing the pharmacokinetics of drugs. For example, oral bioavailability (the fraction of a dose that reaches the systemic circu-
4 to 6 h after regaining consciousness and mobility and in chronic animals (4) studied 4 days after surgery and anesthesia. Bioavailability was increased during pentobarbital femoral artery. Bioavailability was increased during pentobarbital anesthesia in rats simultaneously catheterized in the portal vein and cardiac surgery. Ueda et al. (1999) characterized oxacillin bioavailability was decreased 50% in patients after the postoperative phase. Schuitmaker et al. (1999) reported that postoperatively. Plasma concentrations were significantly lower during the postoperative phase. Surgery and anesthesia change several physiological factors that can alter drug disposition. Surgery and anesthesia alter intestinal and hepatic blood flow (Gumbleton et al., 1990a,b; Colombato et al., 1991). Alterations in intestinal blood flow have been shown to alter intestinal nutrient and drug absorption (Winne et al., 1979), leading to alterations in portal venous drug concentrations and in the drug concentration versus time course. Surgery and anesthesia also alter the disposition of endogenous compounds. Intestinal glucose and amino acid absorption do not return to baseline for at least 24 to 48 h after surgery (Uhung and Kimura, 1995; Uhing and Arango, 1997). Singh et al. (1991) also showed that intestinal d-xylose absorption was decreased after surgery and anesthesia. In this current study, we showed that surgery and anesthesia adversely affected several of the principal processes (intestinal absorption, intestinal blood flow, and hepatic metabolism) controlling drug absorption and elimination. Separate experiments confirmed that a decrease in hepatic extraction occurred in the acute model, reflecting that surgery and anesthesia compromised both hepatic blood flow and drug-metabolizing (CYP3A) activity. In studies of intestinal amino acid and xylene absorption, intestinal absorptive function was not normal until 2 days postoperative (Singh et al., 1991; Uhing and Arango, 1997). Bile acid excretion, an endogenous index of hepatocyte function, is decreased 64 to 71% from 0 to 6 h after laparotomy, compared with 7 days postoperative. The present study confirms that the physiology of the intestine and liver has not returned to a relevant baseline for at least 2 to 3 days after surgery. These data suggest that using consciousness and mobility as metrics of surgical recovery is not reliable.

We performed our acute experiments at 4 to 6 h after laparotomy to mimic the conditions under which many drug studies in rats are performed. The results clearly demonstrate that surgery and anesthesia markedly decrease both CYP3A activity and portal venous blood flow. Our data using the classical approach validated using the five-catheter method for measuring the hepatic extraction of midazolam. CYP3A2 and CYP2C11 are the major P450 proteins found in male rat liver, and midazolam is almost exclusively metabolized by CYP3A2 (Shaw et al., 2002), undergoing hepatic metabolism to form primarily 4-hydroxymidazolam. Matsubara et al. (2004) recently reported a new intestinal CYP3A form, CYP3A62, in the rat and characterized the expression and activity of all of the known rat CYP3A genes. Importantly, mRNA expression and activity toward formation of 6β-hydroxy-testosterone by the intestinal forms of CYP3A (recombinant CYP3A9 and 3A62) were much lower compared with hepatic CYP3A2. This is consistent with our data indicating that midazolam does not undergo significant intestinal first-pass metabolism during oral absorption in rats. The systemic clearance reported in our chronic animals is in the range reported by others (Clenton et al., 1998; Visser et al., 2003) and indicates that midazolam is an "intermediate" clearance drug. As such, alterations in systemic clearance would therefore reflect changes in either organ blood flow or CYP3A activity or both (Pang and Rowland, 1977). Changes in hepatic extraction during first-pass metabolism more closely reflect changes in hepatic intrinsic clearance, namely CYP3A activity.

Surgery and anesthesia change several physiological factors that can alter drug disposition. Surgery and anesthesia alter intestinal and hepatic blood flow (Gumbleton et al., 1990a,b; Colombato et al., 1991). Alterations in intestinal blood flow have been shown to alter intestinal nutrient and drug absorption (Winne et al., 1979), leading to alterations in portal venous drug concentrations and in the drug concentration versus time course. Surgery and anesthesia also alter the disposition of endogenous compounds. Intestinal glucose and amino acid absorption do not return to baseline for at least 24 to 48 h after surgery (Uhung and Kimura, 1995; Uhing and Arango, 1997). Singh et al. (1991) also showed that intestinal d-xylose absorption was decreased after surgery and anesthesia. In this current study, we showed that surgery and anesthesia adversely affected several of the principal processes (intestinal absorption, intestinal blood flow, and hepatic metabolism) controlling drug absorption and elimination. Separate experiments confirmed that a decrease in hepatic extraction occurred in the acute model, reflecting that surgery and anesthesia compromised both hepatic blood flow and drug-metabolizing (CYP3A) activity. In studies of intestinal amino acid and xylose absorption, intestinal absorptive function was not normal until 2 days postoperative (Singh et al., 1991; Uhing and Arango, 1997). Bile acid excretion, an endogenous index of hepatocyte function, is decreased 64 to 71% from 0 to 6 h after laparotomy, compared with 7 days postoperative. The present study confirms that the physiology of the intestine and liver has not returned to a relevant baseline for at least 2 to 3 days after surgery. These data suggest that using consciousness and mobility as metrics of surgical recovery is not reliable.
calculated for discrete time intervals. The concentration differences across the liver were measured at the same time in the same rat. This eliminated the effect of possible changes in hepatic blood flow that can occur when studying an animal on more than one occasion. It also eliminated interindividual variability. Although surgery and anesthesia changed the absorption rate of midazolam, there was no difference between the five-catheter and the classical method with respect to hepatic extraction.

In summary, drug disposition studies conducted within 2 days of surgery and anesthesia do not accurately characterize drug clearance and bioavailability because the studies are performed under abnormal physiologic conditions. The alterations in \( C_{PLV} \), intestinal absorption, and hepatic intrinsic clearance directly determine the assessment of drug bioavailability. These data are in agreement with studies examining the effect of anesthesia and surgery on drug pharmacokinetics in humans (Cohn et al., 1995; Elfant et al., 1995; Schuitmaker et al., 1998). The current practice of measuring drug bioavailability under acute conditions will produce misleading data and could compromise decision making during the early stages of drug development.

References


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