Extrahepatic Ischemia-Reperfusion Injury Reduces Hepatic Oxidative Drug Metabolism as Determined by Serial Antipyrine Clearance

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Purpose. All transplanted solid organs experience some degree of ischemia-reperfusion (I-R) injury. This I-R injury can contribute to graft dysfunction which stems in part from the acute phase response and a resultant host of cytokines. Recent evidence suggests that organs remote to the site of I-R injury can be affected by circulating cytokines originating from these I-R injuries. Since many of these acute phase cytokines inhibit hepatic cytochrome P-450 (CYP) enzymes, we chose to investigate whether extrahepatic I-R injuries could influence hepatic oxidative drug metabolism.

Methods. Fifteen dogs were divided into three surgical groups: (I) sham I-R; (II) bilateral normothermic renal I-R; and (III) normothermic intestinal I-R. Antipyrine (AP) was selected as a model substrate and administered intravenously at a dose of 10 mg/kg. AP serum concentrations were determined by HPLC and cytokine activity (IL-1, IL-6, and TNFα) was measured via bioassay. Serial AP clearance and serum cytokine concentrations were determined 3 days prior to and at 4 hr, 24 hr, 3 days and 7 days after surgery. Hematology and blood chemistries were monitored throughout the study period.

Results. AP clearance was significantly reduced in groups II and III at 4 and 24 hrs post-I-R injury, while AP binding and apparent volume of distribution were unaffected. Peak levels of TNF and IL-6 activity occurred at 1 and 4 hours, respectively. IL-1 activity was not detected in any group. AP clearance correlated strongly to circulating levels of IL-6 ($r = -0.789$, $p = 0.0002$).

Conclusions. Our findings indicate that extrahepatic I-R injury can affect hepatic oxidative drug metabolism and this effect is mediated in part by circulating cytokines.

KEY WORDS: ischemia-reperfusion injury; hepatic clearance; antipyrine; cytokines; interleukin-6; tumor necrosis factor.

INTRODUCTION

In the transplant recipient, transplanted graft dysfunction is often the result of pathological processes unrelated to rejection. Dysfunction frequently stems from microvascular damage accrued during the ischemic period of preservation followed by reperfusion of the graft with whole blood. This injury is designated an ischemia-reperfusion (I-R) injury, and all transplanted organs experience it to some degree.

Microvascular endothelial cells are particularly susceptible to I-R injury, and their destruction initiates a cascade of events known as the acute phase response. A hallmark of this response is the accumulation of polymorphonuclear leukocytes both in affected tissues and the systemic circulation. Activated polymorphonuclear leukocytes in turn release acute phase cytokines [tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6)] which act on target cell receptors leading to a systemic reaction. During this systemic reaction, marked fluctuations in specific plasma proteins (acute phase proteins) occur. Acute phase proteins exhibiting increased synthesis include C-reactive protein, serum amyloid A, fibrinogen, and α_1-acid glycoprotein, while production of albumin, transferrin, and α_1-lipoprotein are reduced (1).

Because liver is the major organ for acute phase protein synthesis, much attention has been focused on its role in regulating these proteins (1). Emerging evidence now points to hepatic cytochrome P-450 (CYP) enzymes as being among those proteins whose synthesis and activity are down-regulated during the acute phase response. Since hepatic CYP enzymes are central to the biotransformation of exogenous compounds, a reduction in CYP activity may affect the pharmacokinetics of many drugs.

In the reperfused ischemic liver, hepatic CYP content and enzyme activity are severely diminished (2). Diminution of CYP activity is also evident when acute phase responses arise from injuries far removed from the liver. Remote sites of infection (3), trauma (4), and acute inflammation (5) have been shown to affect hepatic CYP-mediated drug elimination. Elevations in circulating plasma levels (6) coupled with their ability to reduce CYP activity (7) provide strong evidence that TNFα, IL-1, and IL-6 are mediators of altered drug elimination. Several factors, however, point to IL-6 as the key mediator of remote hepatic CYP regulation. First, liver is the major target organ for IL-6 (1). Second, hepatocytes express an IL-6 receptor on their surface (1). Third, IL-6 is the major regulator of the acute phase response in human hepatocytes (1). And finally, administration of IL-6 suppresses hepatic CYP activity (8,9). With this in mind, regulation of hepatic CYP synthesis by IL-6 of nonhepatic origin is not unexpected.

The present study was conducted to determine whether extrahepatic I-R injury (renal or intestinal) could affect the pharmacokinetics of intravenously administered antipyrine, and if so, whether this effect was cytokine-mediated. Antipyrine is a model compound for hepatic CYP oxidative biotransformation studies (10) and was selected for this study based on the following criteria: negligible binding to plasma proteins; a low hepatic extraction ratio with virtually complete metabolism via CYP enzymes; negligible renal elimination of the parent compound (11); an absence of CYP autoinduction with infrequent administration; and a half-life of less than two hours in the dog (12).

MATERIALS AND METHODS

Chemicals

Antipyrine (AP) sterile solution (400 mg/mL) was purchased from the Parenteral Medications Laboratory, University of Tennessee, Memphis.

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Animals

Fifteen adult male coonhounds, conditioned, heartworm-free, and weighing 26.7 ± 2.3 kg, were used in these experiments. Dogs were randomly assigned to one of three groups (five dogs per group): (I) surgery controls (sham I-R), (II) bilateral renal ischemia-reperfusion, and (III) intestinal ischemia-reperfusion. Animal care was supervised by the Division of Laboratory Animal Medicine, University of Arkansas for Medical Sciences and complied with federal standards.

Anesthesia and Intraoperative Monitoring

Dogs were fasted 12 hours before surgery but allowed free access to water. Anesthesia was induced with intravenous administration of tiletamine and zolazepam (10 mg/kg, Telazol®, Fort Dodge Laboratories, Fort Dodge, IA). Each animal was then intubated with a cuffed endotracheal tube and placed on a veterinary anesthesia machine (Veta-Flex, Ohio Medical Products, Madison, WS). Inhalation anesthesia was maintained with isoflurane, nitrous oxide, and oxygen. A 20-gauge intravenous catheter was inserted percutaneously into the left cephalic vein for heparin administration and blood withdrawal.

Canine Renal Ischemia Model

The abdomen was shaved and swabbed with iodophor solution and then draped in a sterile fashion. A midline celiotomy was performed starting at the xiphoid and extending below the umbilicus to expose the intra-abdominal organs. The intestines were easily retracted and maintained within the abdomen to expose the kidneys bilaterally in the retroperitoneum. The retroperitoneum above and below the right and left renal vascular pedicles was sharply opened and the pedicles encircled with a right angle clamp. A silastic vessel loop (Maxi size: Oxboro-Medical, Ham Lake, MN) was then double-looped (Potts technique) around each renal vascular pedicle including both the renal artery and vein. A second upper or lower polar renal artery, which is more common on the left kidney, was encircled separately when encountered. An intravenous heparin bolus (2500 units, Solopak, Elk Grove Village, IL) was then administered. The renal pedicles were then occluded bilaterally by carefully applying tension on the silastic loops to occlude blood without injuring the vessels. Blood flow occlusion was confirmed with direct visualization of the kidneys and a hand-held doppler probe (Parks Medical Electronics, Aloaba, OR). After 1 hour of renal ischemia, the loops were removed, and reperfusion was confirmed both by direct visualization of the kidneys and by the hand-held doppler probe. The midline celiotomy was closed with a running 0-size monofilament polyglyconate suture (Maxon®, Davis and Geck, Manati, PR) starting at each end of the wound. The skin was closed with a running subcuticular suture of 3-0 braided polyglycolic acid suture (Dexon®, Davis and Geck, Manati, PR). Once the anesthesia was stopped, the dog was extubated and allowed to recover.

Canine Intestinal Ischemia Model

Surgical preparation and celiotomy were identical to those described for canine renal ischemia. Beginning at the junction of the duodenum and jejunum, the proximal 100 cm of jejunum was identified. The mesentery above and below the branches of the vascular archaid suppling only that segment of intestine was sharply opened. The vessels including both the artery and vein were encircled with a double-looped silastic vessel loop (Potts technique) (Maxi size: Oxboro-Medical, Ham Lake, MN). Any collateral circulation at the edges of the 100 cm intestinal segment was identified at the mesenteric border of the intestine and encircled with a silastic vessel loop. An intravenous bolus of heparin (2500 units) was administered prior to mesenteric occlusion. After applying controlled tension to the vessel loops, blood flow occlusion was confirmed with visual ischemic changes, loss of mesentery pulsations, and with the hand-held doppler probe positioned in the mesentery and along the antimesenteric margin of the bowel. After 1 hour of ischemia the loops were removed and restoration of blood flow was confirmed by visual reperfusion of the intestine, palpation of the mesenteric pulse, and by the hand-held doppler probe. Wound closure was identical to the renal ischemia model with the exception that mesentery defects were closed with 6-0 polypropylene suture (Surgilene®: Davis and Geck, Manati, PR). Following the cessation of anesthesia, the dog was extubated and allowed to recover.

Surgery Controls

Surgical preparation and celiotomy were identical to those described for canine renal and intestinal ischemia. Both kidneys and the jejunum were exposed in a manner similar to that described except that neither organ was subjected to ischemia. Following celiotomy and organ exposure, control animals remained anesthetized for 1 hour at which time the midline celiotomy was closed with a running 0-size monofilament polyglyconate suture (Maxon®) starting at each end of the wound. The skin was then closed, anesthesia was stopped, and the animal allowed to recover.

Antipyrine Administration and Pharmacokinetic Analysis

AP was administered to each dog at 3 days prior to surgery and at 4 hours, 24 hours, 3 days and 7 days post-surgery. This schedule allowed each dog to act as its own control. On the day of AP administration, dogs were weighed, rectal temperatures obtained, and 20 gauge intravenous catheters were placed in the right and left cephalic veins. A 10 mg/kg dose of AP was administered as an intravenous bolus into the left cephalic vein, and the catheter flushed with 2 mL of sterile water for injection. Following AP administration, serial blood samples were obtained from the right cephalic vein at 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 360 minutes. Serum was harvested and samples stored at −70°C until analysis. Three additional blood samples (3 mL each) were obtained prior to surgery, and at 1 hour (intestinal I-R only), 4 hours, 24 hours, 3 days, and 7 days post-surgery for hematology, blood chemistry, and serum cytokine (IL-1, IL-6, TNFα) determination. AP serum concentrations were analyzed by a direct injection high performance liquid chromatography (HPLC) method developed in this laboratory (13). Serum AP concentration-time profiles were fitted to select polyexponential equations using a nonlinear least squares fitting computer program, and pharmacokinetic parameters were calculated from computer-generated coefficients and exponents via compartmental analysis (14).

In addition to pharmacokinetic analysis, AP free fraction was determined for each dose. A 1 mL aliquot of the 2 hour serum sample was divided and used for total and unbound AP determinations. Total AP concentrations were determined in the first aliquot by direct serum injection HPLC (13). The second 500 μL aliquot of each divided sample was analyzed for unbound AP. Separation of bound and unbound AP fractions was accomplished by centrifugation (Biofuge Model 17-R, Baxter Scientific Products, McGaw Park, IL, USA) at 1726g and 37°C for 10 minutes using a fixed angle rotor and the Centrifree® micropartition system (Amicon, Beverly, MA, USA). The serum ultrafiltrate was then analyzed for AP concentration. Antipyrine free fractions were calculated from the quotient of unbound and total AP concentration.

Cytokine Bioassay

IL-1, IL-6, and TNF-like activity in canine serum samples was determined via bioassay per the method of Yamashita et al. (15).

Statistical Analysis

Significance of observed differences in mean values of pharmacokinetic parameters, hematology, and blood chemistries were determined by multiple comparison testing with a one-way analysis of variance (ANOVA) procedure using the Least Significant Difference post hoc test. The relationship between AP clearance, leukocytes and serum cytokines were correlated using regression analysis and F test. Statistical significance was defined as $P > 0.05$ for all tests.

RESULTS

All animals survived their respective surgical procedures and remained in good physical condition until pharmacokinetic studies were completed. At the end of the study period all dogs were adopted out to local citizens. During the seven day period no significant alterations in body weight or rectal temperature were observed at the time of AP administration. No significant differences in hematology or blood chemistry data were noted between intestinal I-R recipients and sham-operated animals. However, when compared to shams, aspartate aminotransferase (AST) was significantly higher in renal I-R recipients at 4 hours (27.5 ± 7.9 U/L vs 55.3 ± 17.2, $p = 0.033$) and 24 hours (28.8 ± 11.4 U/L vs 60.7 ± 21.4, $p = 0.048$). Serum creatinine was also higher ($p < 0.05$) at each postoperative time point for renal I-R recipients.

AP serum concentrations were measurable for up to 6 hours post i.v. administration, and all concentration-time profiles were best fit to a two compartment open model. Mean values of pharmacokinetic parameters for each group are presented in Table I. Sham I-R injury had no effect upon AP pharmacokinetics, yet significant reductions in AP clearance were observed at 4 hours post-surgery for dogs receiving renal (46%, $p = 0.009$) and intestinal (26%, $p = 0.016$) I-R injury. Although not significantly different from sham I-R controls, clearance at 24 hours was 23% and 13% lower in renal and intestinal I-R recipients, respectively. Such reductions were transient, however, as clearance returned to preoperative levels by 72 hours.

Due to the variability of AP clearance in each group, plots of the change in clearance versus time were also evaluated (Figure 1). AP clearance change was calculated for each animal based on the difference between presurgery determinations and those obtained at 4, 24, and 72 hours, respectively. These difference plots corroborated the drop in AP clearance at 4 hours and further revealed that I-R injury remained influential in reducing clearance at 24 hours after surgery ($p = 0.01$, renal I-R; $p = 0.05$ intestinal I-R) (Figure 1). By 72 hours, however, this effect had dissipated. No significant change in apparent volumes of distribution were noted for any of the groups (Table I). Mean AP free fraction remained unchanged (0.990 ± 0.011) among all three groups throughout the study period.

Serum cytokine determinations revealed measurable concentrations of IL-6 and TNFα in animals from each of the three groups, while IL-1 concentrations were consistently below assay detection limits. Despite the presence of TNF in many serum samples, complete serial cytokine-time profiles were obtainable only for IL-6. Serial IL-6-time profiles presented in Figure 2 revealed that peak serum concentrations of this cytokine occurred at 4 hours post-surgery, and were significantly higher in those animals receiving renal or intestinal I-R. Moreover, regression analysis of AP clearance and corresponding serum IL-6 concentrations yielded a significant negative correlation ($r = -0.789$, $p = 0.0002$) (Figure 3). For those dogs in which TNFα concentrations were quantifiable, no significant changes were noted at either 4, 24, or 72 hours; however, samples obtained 1 hour after intestinal I-R were significantly elevated (Figure 4). Due to an insufficient number of pairings for TNFα and AP clearance, no correlation was determined.

DISCUSSION

Only recently has an appreciation developed for the immunopathological repercussions of organs remote to the site of I-R injury. Intestinal I-R injury has been shown to induce both leukocyte-mediated liver and cardiac dysfunction (16,17) while hepatic I-R injury engendered a leukocyte-dependent pulmonary microvascular injury (18). In the present study, 60 minutes of renal or intestinal ischemia followed by whole blood reperfusion produced significant although short-lived reductions in AP clearance indicative of diminished hepatic oxidative drug metabolism. In turn, these reduced AP clearances correlated well with circulating IL-6 concentrations (see Figure 3). Published serum concentration-time profiles for IL-6 have noted that peak concentrations occur 3 to 6 hours after initiation of an inflammatory response (6,19,20). Our results agreed with those above and demonstrated that endogenous IL-6 levels stemming from an extrahepatic I-R injury affect hepatic oxidative metabolism in a time-dependent fashion.

While the AP clearance nadir corresponded to the IL-6 concentration maximum (see Figure 2) contributions from TNFα or other cytokines could not be ruled out. Reduced CYP activity in response to TNFα has been noted (10). However, these results may simply reflect the activity of IL-6 since IL-6 biosynthesis is induced by TNFα and IL-1, and these in turn are inhibited by IL-6 (1). Serum TNFα concentrations typically peak at 1 to 2 hours after tissue injury while maximum IL-6 concentrations are reached several hours later (6,19,20). This observation was confirmed in the present study (see Figure 4). Although TNFα and AP clearance pairings were insufficient to perform regression analysis, data from the intestinal I-R group showed TNFα concentrations returning to presurgery

Table I. Serial Determinations of Antipyrine Pharmacokinetic Parameters (Mean ± SD) in Dogs Receiving Sham, Renal, and Intestinal I-R Injury

Time	CL (L/hr)	Sham I-R Injury CL (L/hr/kg)	Vdss (L/kg)	T1/2 (hr)
Presurg.	9.74 ± 0.98	0.371 ± 0.054	0.668 ± 0.020	1.24 ± 0.189
4 hour	8.55 ± 0.80	0.337 ± 0.025	0.635 ± 0.025	1.35 ± 0.075
24 hour	9.68 ± 1.45	0.377 ± 0.041	0.602 ± 0.050	1.19 ± 0.061
3 days	9.89 ± 1.49	0.387 ± 0.036	0.567 ± 0.076	1.08 ± 0.059
7 days	10.21 ± 1.65	0.427 ± 0.037	0.598 ± 0.045	1.07 ± 0.180
Time	CL (L/hr)	Renal I-R Injury CL (L/hr/kg)	Vdss (L/kg)	T1/2 (hr)
Presurg.	10.22 ± 2.76	0.379 ± 0.110	0.676 ± 0.056	1.46 ± 0.623
4 hours	5.55 ± 2.20[a,b]	0.205 ± 0.066[a,b]	0.626 ± 0.122	1.96 ± 0.449[a,b]
24 hours	7.56 ± 1.38	0.292 ± 0.070	0.678 ± 0.150	1.73 ± 0.541[a,b]
3 days	10.39 ± 0.29	0.413 ± 0.032	0.722 ± 0.212	1.24 ± 0.282
7 days	10.01 ± 0.51	0.414 ± 0.032	0.703 ± 0.129	1.24 ± 0.310
Time	CL (L/hr)	Intestinal I-R Injury CL (L/hr/kg)	Vdss (L/kg)	T1/2 (hr)
Presurg.	9.42 ± 1.70	0.365 ± 0.070	0.613 ± 0.092	1.26 ± 0.288
4 hours	6.86 ± 1.27[a,b]	0.271 ± 0.037[a,b]	0.616 ± 0.067	1.71 ± 0.222[a,b]
24 hours	8.01 ± 1.19	0.318 ± 0.048	0.648 ± 0.036	1.50 ± 0.231[a,b]
3 days	9.34 ± 1.56	0.372 ± 0.055	0.583 ± 0.030	1.24 ± 0.166
7 days	9.62 ± 1.75	0.384 ± 0.071	0.612 ± 0.050	1.21 ± 0.229

[a] $P < 0.05$ ANOVA, compared to presurgery (presurg.) samples.
[b] $P < 0.05$ ANOVA, compared to corresponding sham I-R injury samples.

levels at the time of AP clearance nadir (see Figure 4). This suggests that the influence of TNFα on CYP activity was in all likelyhood mediated by IL-6.

Why we failed to detect IL-1 activity is unclear, although lack of assay sensitivity may have been a factor. Previously this method was successful in measuring endogenous IL-1 activity (15), however, in a subsequent study of acute inflammation no IL-1 activity was detected (21). Despite our inability to detect IL-1, its influence on AP clearance cannot be ruled out. IL-1's ability to selectively suppress not only CYP activity but constitutive gene expression of specific isoforms is well documented (8,9,22).

Plasma clearance for drugs exhibiting low hepatic extraction ratios (i.e. AP) is best described as a function of plasma protein binding and intrinsic clearance by the following equa-

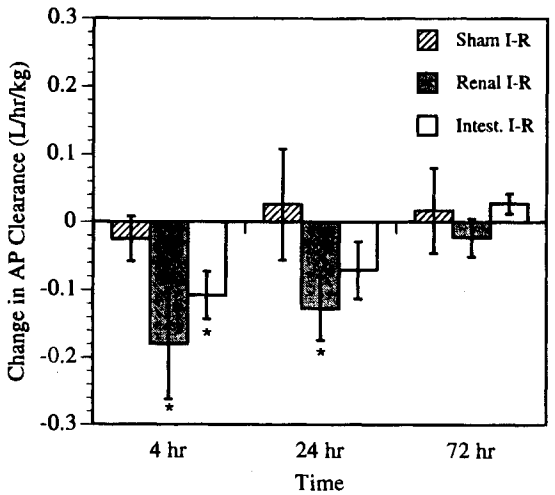

Fig. 1. Plots of the change in AP clearance were calculated as the difference in presurgery AP clearance and that determined at 4, 24, and 72 hours postoperatively. The results are expressed as mean ± S.D. (standard deviation) of five dogs. Significance (*) was defined as $p < 0.05$.

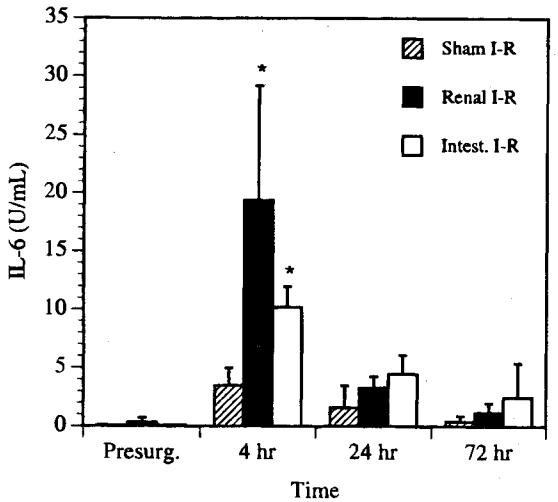

Fig. 2. Concentration-time profile of IL-6 activity following sham, renal, and intestinal I-R injury. The results are expressed as the mean ± S.D. (standard deviation) of four dogs. Significance (*) was defined as $p < 0.05$. Comparisons were based on sham I-R means at each time point.

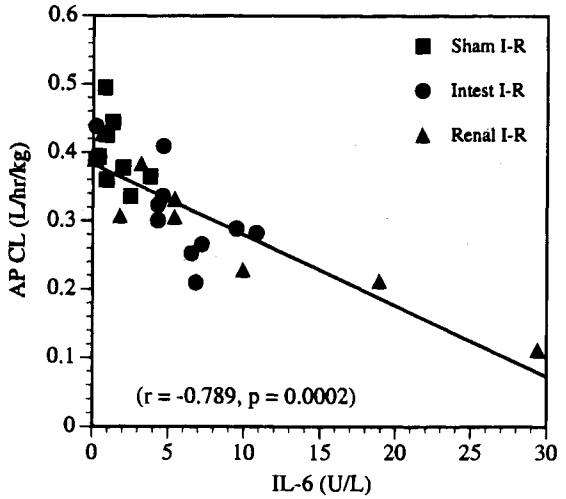

Fig. 3. Regression plot of AP clearance (AP CL) and IL-6 activity.

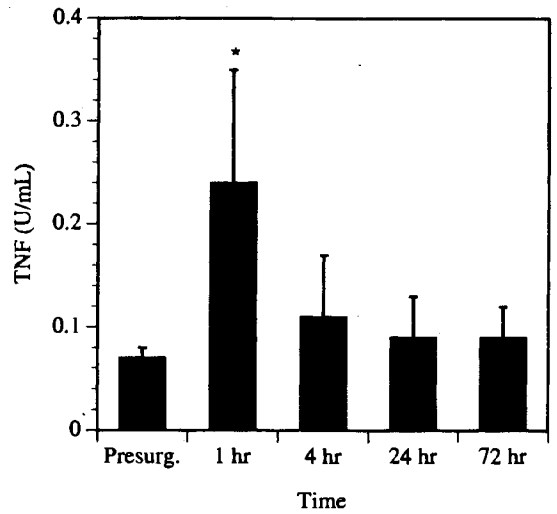

Fig. 4. Concentration-time profile of TNFα activity following intestinal I-R injury. The results are expressed as the mean ± S.D. (standard deviation) of five dogs. Significance (*) was defined as $p < 0.05$.

tion: $CL = f_{up} * CL_{int}$, where f_{up} is the fraction unbound in plasma and CL_{int} is a function of CYP activity. Therefore, reduced AP clearance could have resulted from decreases in either f_{up} (increased binding to acute phase proteins), CL_{int}, or both. While AP is generally considered not to bind to plasma proteins, until now no studies had addressed whether its binding properties were affected by the acute phase response. Drug binding to plasma proteins has been shown to increase in parallel with the acute phase protein, α1-acid glycoprotein (23); however, our AP binding studies disproved that decreased f_{up} was accountable for the observed changes in clearance.

An additional point that deserves mention was whether extrahepatic I-R altered liver blood flow. While this should have had little impact on AP clearance *per se*—owing to its low extraction ratio—reduced liver blood flow may have affected CYP activity. Although hepatic blood flow was not measured in the present study, the premise that transient extrahepatic ischemia induces liver ischemia is inconclusive. Utilizing the superior mesenteric artery (SMA) occlusion model—a more aggressive approach to intestinal I-R than the isolated pedicle technique employed by our group—Poggetti et al. concluded that hepatocellular injury in the rat was not due to liver ischemia but rather the result of an undefined gut-liver signaling mechanism (16). Conversely, recent intravital microscopy studies documented the sequestration of leukocytes in hepatic sinusoids following SMA occlusion, and it was felt that leukocyte adherence engendered localized hypoxia (24). Based on these studies, our findings suggest that IL-6 is at least a component of the undefined signaling mechanism proposed by Poggetti, and activated sequestered leukocytes may serve as a source of its production.

Also related to the issue of induced liver ischemia was the elevated serum AST observed in renal I-R recipients. Although elevated serum AST is often indicative of hepatocellular injury, Maessen et al. demonstrated that renal I-R injury increased circulating AST in a time-dependent fashion identical to that seen in the present study (25). Furthermore, they concluded that renal and skeletal muscle tissue were the principal sources of AST. Taken together, Maessen's results and the fact that AST concentrations were within the normal range for canines (19–66 U/L), liver ischemia seemed an unlikely result of either I-R technique.

AP clearance has long been recognized as an effective though nonspecific technique for assessing hepatic CYP activity. Only recently have the specific AP metabolizing CYP isoforms been identified in humans (10). They include CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C18, and CYP3A4. The specific isozymes responsible for AP biotransformation in the dog have yet to be described, however, it has been noted that CYP1A, 2B, 2C, and 3A subfamilies constitute 60% of CYP content in the canine liver (26). Compared to man, intrinsic AP clearance is greater in the dog (27). This interspecies difference in AP metabolism may stem either from enhanced activity of conserved CYPs, distinct canine isoforms, or both. Nevertheless, cytokine-mediated alterations in AP clearance subsequent to extrahepatic I-R injury may reflect potential changes in the pharmacokinetics of other hepatically eliminated drugs.

Virtually all solid organ transplant recipients receive the immunosuppressant cyclosporine (CsA) to control rejection and all transplanted organs experience I-R injury to some degree. Hepatic I-R injury was primarily responsible for reduced CsA clearance following orthotopic liver transplantation in the dog (28). Therefore, based on the current study, it stands to reason that I-R injuries of nonhepatic origin may in some degree account for the altered pharmacokinetic profiles observed in many transplant recipients. Evidence from recent studies tend to substantiate this assumption. In a group of bone marrow transplant recipients, Chen et al. noted that blood levels of CsA increased twofold in response to elevated serum IL-6 concentrations, and that peak CsA concentration correlated with the time of peak IL-6 (29). In another study, Karim et al. noted that 90 minutes of renal ischemia followed by reperfusion produced significant elevations in trough CsA concentrations on postoperative days 1 and 2 (30). Even though cytokine levels were not monitored in the Karim study, their time table documenting the rise and fall in CsA trough levels appears to parallel the apparent IL-6-mediated results of the present study.

Since a number of different CYP isoforms are involved in the biotransformation of AP, caution must be excerised when

extrapolating the results of AP clearance studies to those drugs metabolized by a specific CYP subfamily. Based on our results and those of Karim (30), it is tempting to speculate that extrahepatic I-R injury would similarly affect CsA elimination. CYP3A is the major gene subfamily responsible for CsA metabolism and, at least in humans, it contributes to AP bioconversion. Furthermore, IL-6 is a potent suppressor of CYP3A activity (8,9). Thus, a causal relationship between IL-6 and reduced CsA clearance in response to extrahepatic I-R injury could be implied. Assuming CsA clearance is affected in a manner similar to AP, a transient rise in CsA blood concentrations immediately post-transplant would not be unexpected and dose adjustments may be warranted; however, the general approach to therapeutic drug monitoring among non-liver transplant recipients would in all likelyhood remain unchanged. In short, while the clinical implications of our findings are potentially minor, they do strengthen the premise that hepatic oxidative drug metabolism can be modulated by immunopathological events in extrahepatic tissues.

In conclusion, ischemia-reperfusion injury to an organ other than the liver reduces hepatic oxidative drug metabolism as determined by serial AP clearance monitoring. Circulating IL-6 concentrations correlated well with reduced AP clearance and thus appear to mediate the down-regulation of hepatic CYP activity. While the temporal effects observed in this study are restricted to AP, further studies are warranted in order to effectively discern the clinical relevance of extrahepatic I-R injury on the pharmacokinetics of specific agents administered to the transplant recipient.

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