Right Heart Failure Impairs Hepatic Elimination of \( p \)-Nitrophenol without Inducing Changes in Content or Latency of Hepatic UDP-Gluconuronosyltransferases

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ABSTRACT

Congestive heart failure has been shown to affect oxidative drug metabolism, however, there has been little study of its effects on drug conjugation. Using the isolated perfused livers from rats with right ventricular failure (RVF) due to pulmonary artery constriction, we studied the effects of RVF on hepatic elimination of \( p \)-nitrophenol (PNP) under controlled flow and oxygen delivery conditions. Hepatic clearance of the drug was found to be significantly impaired in RVF as compared with the sham group (0.80 ± 0.23 versus 1.28 ± 0.26 ml/min/g of liver). The impairment of PNP clearance in RVF occurred in parallel with significant reduction in metabolic formation clearance of \( p \)-nitrophenyl-\( \beta \)-D-glucuronide; the major metabolite of PNP. The intrinsic drug-glucuronidation capacity of livers was evaluated by measuring the microsomal content and activity of the UDP-glucuronosyltransferase(s) (UDP-GT) toward \( p \)-nitrophenol. There was no significant difference between sham and the RVF groups in either the content or the activity of the UDP-GT. The latency of the UDP-GT enzymes in microsomes was measured and was found to be similar between the two groups. The results of this study show that RVF impairs hepatic elimination of PNP and that this appears to be independent of changes in hepatic perfusion and oxygenation or alterations in hepatic content, activity, and latency of the UDP-GT.

Clinical and experimental studies of drug elimination in congestive heart failure have demonstrated that biotransformation of drugs may be impaired (Rissam et al., 1983; Shammas and Dickstein, 1988; Patel et al., 1990; Dunselman and Edgar, 1991; Ng et al., 1995). However, the majority of these studies have concentrated on the effects of heart failure on hepatic oxidative metabolism, and little is known of its effects on the capacity of the liver to eliminate drugs via the conjugation pathways.

Our previous studies have suggested that the impaired oxidative drug metabolism in heart failure is predominantly due to the effects of right heart failure and hepatic congestion. The aim of the current study was to determine whether right heart failure also has significant effects on hepatic drug conjugation, and the mechanisms responsible for any changes observed, using a well characterized experimental model of isolated right ventricular failure (RVF) in the rat (Ng et al., 1995).

We studied the effects of RVF on the elimination by the isolated perfused rat liver (IPRL) of \( p \)-nitrophenol (PNP), a drug which is eliminated predominantly via hepatic glucuronidation (Ghabrial et al., 1995). The IPRL model was chosen for these experiments to eliminate the possible effects of other factors that might affect drug clearance in vivo such as changes in extrahepatic clearance, regional blood flow, and protein binding. We also measured the activity and content of drug glucuronidation enzymes in liver microsomes obtained from rats with RVF. Enzyme latency is described as the degree of restriction of UDP-glucuronosyltransferase(s) (UDP-GT) enzyme activity in their natural phospholipid environment (Zakim and Dannenberg, 1992). Because it has been shown that the latency of UDP-GT may change in certain disease states, resulting in changes in the capacity of these enzymes to metabolize drugs (Desmond et al., 1994), we also examined the latency of hepatic UDP-GT in hepatic microsomes.

Materials and Methods

Chemicals

PNP, \( p \)-nitrophenyl-sulfate (potassium salt), sodium taurocholate, bovine serum albumin, uridine diphosphoglucuronic acid (UDP-GA), uridine diphosphoglucuronosyltransferase(s).

ABBREVIATIONS: RVF, right ventricular failure; IPRL, isolated perfused rat liver; PAC, pulmonary artery constriction; PLPC, palmitoyl-lysophosphatidylcholine; PNP, \( p \)-nitrophenol; PNPG, \( p \)-nitrophenyl-\( \beta \)-D-glucuronide; UDP-GA, uridine diphosphoglucuronic acid; UDP-GT, uridine diphosphoglucuronosyltransferase(s).
palmitoyl-lysophosphatidylcholine (PLPC), and tetrabutylammonium hydrogen sulfate were purchased from Sigma Chemical Co. (St Louis, MO). p-Nitrophenyl-β-D-glucoronide (PNPG) was obtained from Roche Diagnostics (Mannheim, Germany). All other chemicals used were of analytical grade. All solvents were HPLC grade (Mallinckrodt, Phillipsburg, NJ).

Right Heart Failure Model
Thirty-one male Sprague-Dawley rats (age 4–5 weeks, weight 90–120 g) were randomized into two groups [pulmonary artery constriction (PAC) or sham]. Rats in the PAC group (n = 22) underwent constriction of the pulmonary artery via a left-sided thoracotomy as previously described (Ng et al., 1995). Briefly, the pulmonary artery was dissected free of the aorta, a blunt needle (18 gauge, 0.5-cm long) was placed against the pulmonary artery. A sterile silk suture was tied around both the needle and vessel, followed by rapid removal of the needle, resulting in restriction of the pulmonary artery to the size of the needle’s diameter. The chest was closed with a silk suture under negative pressure, and antiseptic was applied. Rats in the sham group (n = 9) underwent the same surgery but without the PAC. Thirteen PAC rats died within 16 weeks of the surgery leaving 9 for the perfusion study. All sham-operated rats survived for the duration of the experimental period. There was no significant difference (P < 0.05) between the initial body weight of the sham (n = 9) (mean weight 104 ± 8 g) and the PAC groups (n = 9) (mean weight 101 ± 9 g).

IPRL Study
Experimental Preparation. 15 to 17 weeks after PAC or sham operation, rats of the two experimental groups were anesthetized with pentobarbitone (60 mg/kg). Before removal of the liver, the left jugular vein was cannulated, and the mean central venous pressure was measured using an electronic pressure monitor unit (model 78205A; Hewlett-Packard, Palo Alto, CA). The common bile duct, the portal vein, and the inferior vena cava were cannulated using standard surgical techniques. The liver was dissected free from the abdominal cavity and weighed before it was connected to the perfusion circuit.

Experimental Design. The liver was perfused at a constant flow rate via the portal vein in a closed cabinet maintained at 37°C. Similar flow rates were used in both groups with an average of 1.5 ml/min/g of liver (see Table 2). The perfusion was initially in a recirculating mode (the equilibrium phase), which lasted for about 20 min to allow the liver to stabilize and its viability to be assessed. Then the experiment was commenced by switching the circuit to a single pass mode. The perfusates in the recirculating and the single pass modes were identical except that PNP was added to the perfusate in the single pass mode. The perfusate consisted of Krebs-Henseleit buffer containing 20% (v/v) washed human red blood cells, 1% (w/v) bovine serum albumin, 0.1% (w/v) glucose, and 30 μM sodium taurocholate, had a pH of 7.2 to 7.4, and was maintained at 37°C. Bile was collected throughout the experiment into preweighed vials in two 30-min aliquots. The viability of the liver preparation was assessed by its macroscopic appearance, oxygen consumption, and perfusion pressure. All perfused liver preparations described in this study were homogeneously perfused, as indicated by an even color of the liver lobes during the perfusion, had oxygen consumption of greater than 3 μmol/min/g of liver, had perfusion pressure of no greater than 12 cm H₂O, and had less than 2 cm H₂O difference in the perfusion pressure between the beginning and the end of the experiment. Inflow perfusate samples (Cᵢ) were collected at 15, 30, 45, and 60 min. Outflow perfusate samples (Cₒₒ) were collected at 5-min intervals from 15 min onward. Cᵢ and Cₒₒ samples were centrifuged to remove the red cells, and the supernatant was stored at −20°C until drug concentration was analyzed by HPLC. At the completion of the perfusion experiment, the liver was weighed, and a small section of the liver was stored in buffered formalin for subsequent histological study (S. T. Chou, Department of Anatomical Pathology, Austin and Repatriation Medical Center, Melbourne, Victoria, Australia). Histological analysis was performed in the absence of knowledge of hemodynamic and pharmacokinetic parameters. The remaining liver tissue was snap-frozen in liquid nitrogen, stored at −70°C, and later used to prepare hepatic microsomes (Aito and Vainio, 1976). The heart was removed from the thoracic cavity of the rat, and its right ventricle (including the interventricular septum) was isolated and weighed.

Microsomal Study
Preparation of Hepatic Microsomes. Microsomes were prepared from six livers randomly chosen from the sham group and from the livers of all animals that developed RVF (n = 5, see Table 2). Two grams of each liver was homogenized with a Potter-Elvenjem homogenizer in 20 ml of prechilled buffer (pH 7.25) containing 0.25 M phosphate, 0.15 M KCl, and 1 mM EDTA. The 10% (w/v) homogenate was then centrifuged at 9000g for 30 min at 4°C, and the resultant supernatant was centrifuged at 105,000g for 90 min at 4°C. The microsomal pellet obtained after the second centrifugation was suspended in 2 ml of 0.25 M phosphate buffer containing 30% w/v glycerol, pH 7.25, and stored at −70°C until use. Microsomal protein content was measured by standard Lowry assay (Lowry et al., 1951) using bovine serum albumin as the protein standard.

Measurement of UDP-GT Activity in Native and Activated Microsomes. Microsomal UDP-GT activity was assessed by measuring the amount of PNPG formed from PNP. Total UDP-GT activity (i.e., in the activated state) can only be detected after disruption of that restriction with the addition of detergent or introduction of an excess of phospholipid such as PLPC (Yokota and Yuasa, 1992). UDP-GT enzyme latency was determined by comparing the amount of PNPG formed from PNP in native and activated microsomes, at saturating concentrations of both UDP-GA and PNP, which was achieved by a modification of a previously described method (Desmond et al., 1994). The incubation mixture (final volume = 0.25 ml) consisted of PNP (4 mM: 80 μl of 12.5 mM), MgCl₂ (5 mM: 12.5 μl of 100 mM), Tris-HCl (0.1 M, pH 7.4: 10 μl of 2.5 M), microsomal protein (0.1 mg: 20 μl of 5 mg/ml), UDP-GA (10 mM: 50 μl of 50 mM), and deionized and distilled water (77.5 μl). UDP-GA was added after 2-min preincubation of all other components under air at 37°C. The reaction was allowed to run for 10 min before it was stopped by addition of 300 μl of acetonitrile. The concentration of PNPG in the incubation medium at the end of the incubation was measured by a sensitive HPLC method as described under Analytical Assays. The formation rate of PNPG was calculated per minute of incubation and per milligram of microsomal protein present. Before normalizing the activity data to time of incubation and amount of microsomal protein, preliminary experiments were conducted to check the linearity of the reaction with respect to time of incubation and microsomal protein concentration. The results showed that the reaction rate was linear for incubation time up to at least 20 min and protein concentration up to 1 mg/ml. Based on these findings, 10 min of incubation and 0.4 mg/ml microsomal protein concentration were the conditions used in the present study. All incubations were carried out in duplicate.

UDP-GT activity in its activated state was measured under the same conditions as described above for native microsomes, but PLPC was incorporated in the incubation mixture during the preincubation to activate the microsomes. The optimal concentration of PLPC was determined in preliminary experiments by adding varying amounts of PLPC in the incubation medium during the incubation and monitoring the amount of PNPG formed. Maximal enzyme activities were observed at a PLPC/protein ratio of about 0.3 (w/w) for both experimental groups; therefore, 0.03 mg of PLPC (previously dissolved in deionized and distilled water) was added to the incubation mixture to activate the microsomes. Preliminary experiments with the activated microsomes showed that the reaction rate was linear over the first 20 min of incubation and linear up to a protein concentration of 1 mg/ml. 4 mM PNP and 10 mM UDP-GA were used in the experi-
ments for determination of UDP-GT latency. At this concentration of PNP and UDP-GA the UDP-GT(s) were saturated and glucuronida-
tion was at maximum velocity. All incubations were carried out in
duplicate. Utilization of PNP and UDP-GA was less than 10% at the
end of the reaction in each experiment.

Measurement of UDP-GA $V_{max}$ Toward PNP. The incubation
mixture was the same as used with the activated microsomes except
that the microsomes were spiked with 50 $\mu$l of varying concentra-
tions of UDP-GA (1.25, 2.5, 7.5, 15, 25, 50, and 100 mM) to give
UDP-GA concentrations of 0.25, 0.5, 1.5, 3, 5, 10, and 20 mM, re-
spectively. The concentration of UDP-GA was varied rather than
that of PNP, because PNP is known to be a substrate of multiple
UDP-GT isoenzymes, hence complicating the kinetics. By choosing a
saturating concentration of PNP (4 mM) and varying the UDP-GA
centration, we were able to detect the total UDP-GT activity in
the microsomal preparation. The apparent $V_{max}$ and $K_m$ toward
UDP-GA were determined by fitting a Michaelis-Menten equation to
the velocity versus concentration data by nonlinear least-squares
regression analysis (Minim 3.0.8; R. D. Purves, University of Otago,
New Zealand). Utilization of PNP and UDP-GA were less than 10% at
the end of the reaction in each experiment (Segal, 1975).

Analytical Assays
Concentration of PNP ($C_m$ and $C_{out}$) and its metabolites PNPG
and p-nitrophenyl-sulfate in the supernatants of the IPRL perfu-
sate samples were measured simultaneously by a specific and sensitive
HPLC method (Ghabrial et al., 1995). The measured concentration of
PNP and its metabolites in the red cell free samples (supernatant)
was found to be identical with those obtained from the whole blood
samples. In the microsomal experiments, after the reaction was
stopped with 300 $\mu$l of acetonitrile, the mixture (total volume: 0.55
ml) was vortexed, centrifuged, and processed as follows. 400 $\mu$l of the
supernatant was evaporated under vacuum for 2 h. The residue was
reconstituted in 0.4 ml of mobile phase comprising 15% (v/v) ace-
nitrite, 10% (v/v) MeOH, 75% (v/v) KH$_2$PO$_4$, 0.2% (v/v) triethylamine,
and 5 mM tetrabutylammonium hydrogen sulfate at pH 6.0 (adjust-
ed with orthophosphoric acid). Microsomal PNPG concentration was
then measured using the HPLC method of Ghabrial et al. (1995) with
minor modifications. Elution times for UDP-GA, PNPG, and PNP in the
assay were approximately 2.3, 3.7, and 10.4 min, respectively. The co-efficient of variation for repeated measures ($n = 6$) of PNPG
was 1% for 5 $\mu$g/ml and 2% for 10, 80, and 150 $\mu$g/ml. Inaccuracy of
PNP concentration in the microsomes was determined from a sev-
en-point standard curve constructed from PNPG concentrations of 5,
10, 40, 80, 100, and 150 $\mu$g/ml.

Calculations and Statistics
Steady-state extraction ($E$) of PNP in the IPRL experiments was
calculated as

$$E = \frac{C_{in} - C_{out}}{C_{in}}$$  \hspace{1cm} (1)

Hepatic clearance (CL) was calculated as

$$\text{Clearance} = \frac{E \cdot Q}{Q}$$  \hspace{1cm} (2)

where $Q$ is the perfusate flow rate.

Metabolic formation clearance (CL$_{f, a}$) was calculated as

$$\text{CL}_{f, a} = \frac{A_{m, b} \cdot t_{1-2} + \frac{(Q \cdot C_{in} \cdot t_2 - t_1)}{(Q \cdot C_{in} \cdot t_2 - t_1)} \cdot CL}{Q}$$  \hspace{1cm} (3)

where $A_{m, b}$ is the amount metabolite in bile (molar units) from
time $t_1$ to time $t_2$, $C_{in}$ is the metabolite concentration in the hepatic
venous outflow at steady state in $\mu$M, and $Q$ is the perfusate flow
rate. The times $t_1$ and $t_2$ were at steady state (20–60 min).

Data in the tables and graphs are presented as mean ± S.D.

Statistical comparison between the sham and the RVF groups in the
IPRL and the microsomal studies were performed by the Student
unpaired $t$ test. All statistical tests were performed using the Stat-
View SE package (v1.4; Abacus Concepts Inc., Berkeley, CA), and $P$
values of less than .05 were accepted as significant.

Results
Right Heart Failure Model
At 15 weeks, five of the nine rats that underwent the PAC had developed RVF as evidenced by a 10-fold increase in the mean central venous pressure (Table 1) and an engorged appearance of the liver at laparotomy. In these animals, there was evidence of cardiac hypertrophy with a mean in-
crease in right ventricular weight of 57% (Table 1). Although the other four rats that underwent the PAC also developed cardiac hypertrophy (mean right ventricular weight: 1.18 ± 0.13 g), there was no evidence of RVF as indicated by the near normal mean central venous pressure (3 ± 1 mm Hg) and the absence of hepatic congestion. The RVF rats did not have increased lung weight to suggest pulmonary edema due to impairment of left ventricular function (Table 1). There was no significant difference in the mean body weight between the sham and the RVF groups (Table 1).

Evidence of Hepatic Congestion. The livers from the five animals with RVF showed macroscopic evidence of hep-
ic congestion. When examined under the light microscope, livers from these animals showed sinusoidal dilatation and congestion, but hepatic fibrosis was absent. None of the livers from the sham group showed hepatic congestion under light
microscopy. The mean liver weight of the RVF rats was not signif-
ificantly different from those of the sham group (Table 1).

Elimination by the IPRL
Viability of the IPRLs. Physiological parameters in the
isolated perfused livers of the sham and RVF rats are sum-
marized in Table 2. The data illustrate that liver prepara-
tions in both groups were perfused at similar flow rates and
that oxygen delivery and perfusate pH were similar. All
preparations were viable, with oxygen extraction and con-
sumption not being different between livers in the two
groups. However, the mean perfusion pressure of the livers
from the RVF rats was slightly higher from that in shams (11
versus 9 cm H$_2$O, $P = .047$).

Extraction and Hepatic Clearance of PNP in Isolated
Perfused Livers. Perfuse outflow concentration of PNP in
the perfused livers of the RVF animals was significantly
elevated compared with those in sham rats. Extraction and hepatic clearance of PNP in the isolated perfused livers are
shown in Table 3. Extraction and hepatic clearance of PNP
were reduced by approximately 40% in RVF compared with
the sham-operated livers. In keeping with the reduced drug

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Physiological parameters in sham and RVF rats</th>
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<tbody>
<tr>
<td>Values are means ± S.D.</td>
<td></td>
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<tr>
<td>Sham ($n = 9$)</td>
<td>RVF ($n = 5$)</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>460 ± 58</td>
</tr>
<tr>
<td>Right ventricular weight (g)</td>
<td>0.76 ± 0.13</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>12.0 ± 2.1</td>
</tr>
<tr>
<td>Lung weight (g)</td>
<td>2.01 ± 0.44</td>
</tr>
<tr>
<td>Central venous pressure (mm Hg)</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>

$^*$Significantly different from the values of the sham group, $P < .001$. |
TABLE 2
Physiological parameters in isolated perfused livers of sham and RVF rats

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 9)</th>
<th>RVF (n = 5)</th>
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<tbody>
<tr>
<td>Perfusion rate (ml/min/g liver)</td>
<td>1.52 ± 0.26</td>
<td>1.55 ± 0.22</td>
</tr>
<tr>
<td>O₂ delivery (µmol/min/g liver)</td>
<td>5.69 ± 0.99</td>
<td>5.87 ± 0.85</td>
</tr>
<tr>
<td>O₂ consumption (µmol/min/g liver)</td>
<td>4.65 ± 0.47</td>
<td>4.18 ± 0.70</td>
</tr>
<tr>
<td>O₂ extraction (%)</td>
<td>83 ± 6</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>Perfusion pressure (cm H₂O)</td>
<td>9 ± 1</td>
<td>11 ± 2*</td>
</tr>
<tr>
<td>Perfusate pH</td>
<td>7.32 ± 0.07</td>
<td>7.30 ± 0.07</td>
</tr>
<tr>
<td>Bile flow (ml/h)</td>
<td>1.29 ± 0.33</td>
<td>1.33 ± 0.01</td>
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</tbody>
</table>

Significantly different from the values of the sham group, *P < .05.

TABLE 3
p-Nitrophenol extraction, hepatic clearance, and metabolic formation clearance in grams

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 9)</th>
<th>RVF (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNP extraction ratio</td>
<td>0.84 ± 0.09</td>
<td>0.50 ± 0.10**</td>
</tr>
<tr>
<td>PNP hepatic clearance (ml/min/g liver)</td>
<td>1.28 ± 0.26</td>
<td>0.80 ± 0.23*</td>
</tr>
<tr>
<td>PNP glycine (mg/min/g liver)</td>
<td>1.03 ± 0.57</td>
<td>0.51 ± 0.12*</td>
</tr>
<tr>
<td>Metabolic formation clearance (µmol/min/g liver)</td>
<td>1.03 ± 0.25</td>
<td>0.08 ± 0.03</td>
</tr>
</tbody>
</table>

Significantly different from the values of the sham group, *P < .05.

Microsomal UDP-GT Metabolism and Content

Effect of RVF on the Content of PNP UDP-GT. When the microsomal glucuronidation rate of PNP was plotted against the variable concentration of UDP-GA, a typical Michaelis-Menten relationship was found (Fig. 1). Analysis of this plot in the sham and the RVF groups showed that there was no significant difference the Vₘₐₓ of the reaction between the two groups (Fig. 2. However, the Michaelis-Menten constant of the reaction (Kₘ) was slightly reduced in the RVF group (P < .05, Fig. 2).

Discussion

There has been very little study of the effects of congestive cardiac failure on hepatic drug conjugation. A previous study in patients with congestive heart failure demonstrated impaired clearance of paracetamol (Ochs et al., 1983), a process dependent on hepatic glucuronidation and sulfation. However, the mechanisms responsible for this finding were not elucidated. The present study in the isolated liver demonstrates that, in the presence of normal hepatic perfusion and oxygenation, hepatic elimination of PNP is significantly impaired in animals with RVF. This suggests that in congestive heart failure there is impairment of the intrinsic capacity of the liver to conjugate drugs.

Hepatic elimination of PNP is expected to be flow-dependent, because the metabolic capacity of the liver to eliminate the drug is large (extraction being >70%). Thus, if hepatic perfusion was reduced in heart failure, it might be expected to result in impairment of hepatic clearance of the drug (Wilkinson and Shand, 1975). The fact that hepatic clearance of the drug was significantly impaired in perfused livers from animals with RVF, when perfusate flow rates were the same as those in controls, suggests that there is reduced intrinsic clearance of the drug in RVF and that this is likely to affect hepatic clearance of the drug, independent of the effects of changes in blood flow.

Consistent with previous studies, in the current study elimination of PNP was predominantly via the glucuronidation pathway (Table 3) (Ghahrial et al., 1995). The impaired elimination of PNP in RVF in the present study primarily reflects a reduction in the glucuronidation capacity of the liver.
liver, because there was a commensurate reduction in metabolic formation clearance of PNPG.

Drug glucuronidation may be impaired if hepatic oxygenation is reduced in heart failure, because oxygen is consumed for the generation of ATP, which is subsequently utilized for the synthesis of cofactors required for glucuronidation such as the UDP-GA (Angus et al., 1987; Wu et al., 1990; Aw et al., 1991). Another factor that may make glucuronidation reactions sensitive to hypoxia is that UDP-GTs are predominantly localized in the centrilobular zone of the acinus (Knapp et al., 1988), where oxygen concentration is lowest (Matsumura et al., 1986). Indeed, previous studies have shown that hepatic elimination of PNP was impaired by minor reductions in hepatic oxygen supply that are likely to occur in vivo (Aw et al., 1991). The present finding of markedly reduced hepatic clearance of PNP in livers from animals with RVF, in which the levels of hepatic oxygenation delivery and consumption were the same as controls, suggests that impairment of enzyme function by hypoxia is not likely to be the sole cause of reduced drug elimination.

The amount and the activity of UDP-GTs may also influence the rate of glucuronidation of PNP, with the latter also influenced by enzyme latency (Zakim and Dannenberg, 1992). The latency of UDP-GT may be altered if the interaction between the enzyme and the phospholipid is perturbed or disrupted, as occurs with exposure of microsomes to PLPC (Yokota and Yuasa, 1992). The microsomal studies in the present investigation indicated that the latency of UDP-GT is not altered in RVF, because pretreatment of microsomes with PLPC did not alter the percentage of activation of the UDP-GT when compared with the sham group. Thus, in contrast to conditions such as liver cirrhosis where changes in latency of UDP-GTs contribute to the preservation of glucuronidation (Desmond et al., 1994), RVF does not alter the latency of the hepatic UDP-GT enzymes.

There are at least three different forms of UDP-GTs that are responsible for the glucuronidation of PNP (Antoine et al., 1993). The nonavailability of antibodies to the various rat forms of UDP-GTs meant that we were unable to measure their protein content directly. The alternative method available to us was to measure UDP-GT $V_{\text{max}}$ in activated microsomes as a reflection of enzyme content, as previously used by Luquita et al. (1994). Our kinetic studies in vitro with UDP-GA demonstrated that there was no significant difference in the $V_{\text{max}}$ of the reaction between the sham and RVF groups. Hence, the reduced glucuronidation of PNP in the isolated perfused livers in RVF could not be explained by reduction of the hepatic content of UDP-GTs. There was a modest, but statistically significant reduction in the $K_m$ observed in the RVF group. The reduction in $K_m$ and $V_{\text{max}}$ in the RVF did not translate in a significant difference in calculated intrinsic clearance ($V_{\text{max}}/K_m$, 93.8 ± 28.2 ml/min/mg of microsomal protein in the sham group versus 84.3 ± 10.2 ml/min/mg of microsomal protein in the RVF group). Therefore, change in $K_m$ could not explain the difference in the elimination of PNP observed. Thus, it appears that in heart failure changes in factors other than reductions in enzyme content, activity, or latency of the UDP-GTs are involved in impaired glucuronidation.

There has been a number of studies demonstrating that conjugation reactions, including the conjugation of PNP, are sensitive to the level of carbohydrate reserves and cofactor supply (Reinke et al., 1979; Thurman et al., 1981; Qu et al., 1995). Thus, it is possible that the reduced clearance of PNP in RVF in the current study is due to depletion of cofactors within the liver. This may explain why PNP glucuronidation was preserved in microsomes in comparison to the IPRL, because in microsomes cofactors such as UDP-GA are supplemented in excess, whereas no cofactors were supplied to the IPRL.

Histological examination of the perfused livers from the RVF animals showed that there was sinusoidal dilatation and congestion, which was not relieved (hepatic outflow open to atmosphere) after the livers had been removed from the RVF animals and perfused for 80 min. It is possible that this sinusoidal congestion in RVF livers leads to disturbed microcirculation and impaired access of drugs to functional hepatocytes, as has been suggested to occur in cirrhosis (McLean and Morgan, 1991; Gariepy et al., 1993). The higher portal perfusion pressure in the RVF perfused livers than in controls supported the presence of a disturbed microcirculation in RVF livers. Factors such as changes in local oxygen delivery, flow distribution, and shunting in RVF livers might explain in part the decreased clearance of PNP in the IPRL. A previous electron microscopic study demonstrated that chronic hepatic congestion leads to deposition of collagen in the space of Disse and development of a sinusoidal basement membrane (Safran and Schaffner, 1967). It is possible that similar changes occur in RVF and affect hepatic drug uptake. In addition, it has been suggested that congestion of the liver can result in perisinusoidal edema (Brauer et al., 1959; Greenway and Lautt, 1970), which may also lead to impaired drug uptake (Dunn et al., 1973).

In conclusion, the current study shows that right heart failure impairs the ability of the liver to conjugate drugs and
that this cannot be totally explained by reduced hepatic oxygen delivery, reductions in hepatic content or activity of UDP-GT, or changes in the latency of the UDP-GT. Our findings provide further evidence that the elimination of a wide range of drugs may be impaired in heart failure increasing the risk of drug accumulation and toxicity.

Acknowledgment

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