Hepatic pharmacokinetics of propranolol in rats with adjuvant-induced systemic inflammation

Daniel Y. Hung, Gerhard A. Siebert, Ping Chang, Michael W. Whitehouse, Linda Fletcher, Darrell H. G. Crawford, and Michael S. Roberts. Hepatic pharmacokinetics of propranolol in rats with adjuvant-induced systemic inflammation. *Am J Physiol Gastrointest Liver Physiol* 290: G343–G351, 2006. First published September 15, 2005; doi:10.1152/ajpgi.00155.2005.—Systemic inflammation is known to affect drug disposition in the liver. This study sought to relate and quantitate changes in hepatic pharmacokinetics of propranolol with changes in hepatic architecture and physiology in adjuvant-treated rats. Transmission electron microscopy was used to assess morphological changes in mitochondria and lysosomes of adjuvant-treated rat livers. The disposition of propranolol was assessed in the perfused rat liver using the multiple indicator dilution technique. Hepatic extraction and mean transit time were determined from outflow-concentration profiles using a nonparametric method. Kinetic parameters were derived from a two-phase physiologically based organ pharmacokinetic model. Possible relationships were then explored between the changes in hepatic drug disposition and cytochrome P-450 activity and iron concentration. Adjuvant treatment induced the appearance of mitochondrial inclusions/tubularization and irregularly shaped lysosomes in rat livers. Livers from adjuvant-treated rats had (relative to normal) significantly higher α1-acid glycoprotein (orosomucoid) and iron tissue concentrations but lower cytochrome P-450 content. The hepatic extraction, metabolism, and ion trapping of propranolol were significantly impaired in adjuvant-treated rats and could be correlated with altered iron store and cytochrome P-450 activity. It is concluded that adjuvant-induced systemic inflammation alters hepatocellular morphology and biochemistry and consequently influences hepatic disposition of propranolol.---acidic vesicles; iron overload; ion trapping

ADJUVANT-INDUCED ANIMAL MODELS of rheumatoid arthritis have been used to characterize some of the immunopathogenic mechanisms for chronic inflammatory synovitis (1, 2, 29). The development of systemic inflammation and self-perpetuating synovitis with joint destruction in these experimental models are similar to that in human rheumatoid arthritis.

Symptoms developed after adjuvant treatment are, however, not restricted to local inflammatory response and distal joint destruction. A number of hepatic events follow adjuvant treatment and consequent cytokine activation. They range from a marked decrease of xenobiotic metabolism to alterations in the hepatic architecture and increased iron stores (26, 47). Metabolism changes are largely due to impairment of microsomal enzymes, decreased activities of cytochrome P-450 (CYP) and glutathione transferases, reduced glucuronidation of some substrates, and perhaps also intoxicating mechanisms caused by the elevated iron stores (24, 33, 34). Prominent targets for iron-induced injury include mitochondria and lysosomes (26) and involve several mechanisms such as free radical-mediated peroxidative reactions at different subcellular sites damaging essential lipids, proteins, or nucleic acids (4). Other mechanisms of iron intoxication involve destabilization of lysosomes (leaking digestive enzymes into the cell cytoplasm) and decreasing intracellular ion trapping, eventually in apoptotic or necrotic cell death (10, 11, 27).

We (43) have previously described the relative contributions of ion trapping, intracellular binding, and distribution of unbound solutes and retention of basic drugs in the perfused normal rat liver. In this study, we evaluated changes in 1) hepatocellular morphology (histology), 2) hepatic biochemistry (e.g., CYP content and iron storage), and 3) hepatic pharmacokinetics of propranolol after adjuvant-induced systemic inflammation. Kinetic parameters were derived from a two-phase physiologically based organ pharmacokinetic model (43). These results could contribute to better understanding of how changes in cytokine-induced systemic inflammation quantitatively affect hepatic drug pharmacokinetics (with propranolol used as a model probe).

MATERIALS AND METHODS

Materials. All chemicals were purchased from Sigma (Castle Hill, New South Wales, Australia). [3H]water and [U-14C]sucrose were purchased from New England Nuclear (Boston, MA). Animals and treatment. Animal studies were carried out according to protocols approved by the University of Queensland Animal Ethics Committee. Female dark Agouti rats (150 ± 10 g) were given 0.5 mg heat-killed and delipidated *Mycobacterium tuberculosis* (mixed human strains, Ministry of Agriculture, Weybridge, UK) dispersed in 0.1 ml squalane (Sigma) by a subcutaneous injection into the tail base to elicit adjuvant-induced polyarthritis (33, 47). Animals were killed after 15 days, when the polyarthritis was fully expressed.

Serum biochemistry measurement. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels were assessed on a Hitachi 747 analyzer (Tokyo, Japan) by taking blood samples from the tail vein before (day 0) and after adjuvant treatment (day 15).

In situ rat liver perfusions. Perfusion of the isolated rat liver in this study was performed as previously described (6). Briefly, normal and adjuvant-induced systemic inflammatory female dark Agouti rats were anesthetized by an intraperitoneal injection of xylazine (10

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The outlet samples were centrifuged at 1,500 g for 4 s balanced salt solution (Sigma Chemical), were injected into the liver, and outlet samples were collected (1 s/H9262 1 min. Hepatic oxygen consumption (measured with an OSM3 analyzer (Radiometer) operating in the dog mode. Oxygen consumption, bile flow, and macroscopic appearance were used to assess the system used was nonrecirculating and employed a peristaltic pump using a Silastic tubing lung ventilated with oxygen. The perfusion medium was oxygenated with 5% carbon dioxide (5% CO2/95% air) and an extrapolated initial concentration being much less than the reported Michaelis-Menten constant for propranolol (17) was used to determine the free concentration to total concentration. A Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu; Kyoto, Japan) was used for absorption spectrophotometric analysis.

Investigation of propranolol binding to hepatocellular components. These experiments were carried out in 1) blank 50 mM Tris-HCl buffer (pH 7.4), 2) buffer containing 0.35 mg/ml MP from normal or adjuvant-treated livers, or 3) buffer containing 0.35 mg/ml CR from normal or adjuvant-treated livers. The fraction unbound of propranolol in each hepatocellular component (fuT) was estimated using an ultrafiltration method. A known concentration of the propranolol stock solution was added to 500 μl of each buffer solution to make a final concentration of 0.05 μM and placed in a centrifugal filter device [Microcon YM-30 (30,000 MWCO), Millipore; Bedford, MA] and then centrifuged at 3,000 g for 10 min at 37°C. The ultrafiltrate (in triplicate) was assayed by HPLC. Protein leakage in the ultrafiltration process was shown not to be occurring by showing undetectable protein levels in the ultrafiltrate using the Lowry method. A control study has been conducted to measure hydroxylation metabolites generated by propranolol in the upper chamber of the YM-30 device under identical conditions, and it has been confirmed that propranolol metabolism was negligible. fuT was determined as the ratio of the free concentration to total concentration of solute.

In vitro metabolism of MP. The impact of adjuvant treatment on hepatic drug metabolism was assessed by incubation of 0.05 μM propranolol with MP (0.35 mg/ml) from normal or adjuvant-treated rat livers at 37°C. Samples were then collected at 0, 5, 10, and 20 min. The concentration in the supernatant after centrifugation was determined by HPLC, and the logarithm of the concentration remaining in the solution was plotted against time (t) to obtain a slope and an extrapolated initial concentration (at time 0). Linearity of the relationship and an extrapolated initial concentration being much less than the reported Michaelis-Menten constant for propranolol (17) was used to confirm linear kinetics. The intrinsic elimination clearance (CLint) was estimated as the product of the slope and the dose divided by the extrapolated initial concentration.

Determination of hepatic iron. Hepatic iron concentrations were measured colorimetrically using a modification of the method of Torrance and Bothwell (44). Tissue (5–10 mg) was oven dried at 65°C for at least 72 h. Dried liver tissue (1–3 mg dry wt) was digested in 150 μl concentrated nitric acid (Aristar grade) on a sand bath in a fume hood for 4 h. After being cooled, the solution was adjusted to 1 ml with deionized water. Five milliliters of chromogen reagent (0.1% bathophenanthroline sulphate and 1% thioglycollic acid) was added to 0.1 ml of sample. Four milliliters of chromogen reagent was also added to 1 ml of standard iron solution (2 μg/ml, Fluka Chemie; Buchs, Switzerland). After the samples were mixed, they were allowed to stand for 30 min, and absorbance was read in a spectrophotometer at 535 nm.

Data analysis. A mixture of two inverse Gaussian density functions with correction for catheter effects was used to estimate the sinusoidal area.

mg/kg, Bayer) with ketamine hydrochloride (80 mg/kg, Parnell Laboratories). After a laparotomy, animals were heparinized (200 units heparin sodium, David Bull Laboratories) via the inferior vena cava. The bile duct was cannulated with polyethylene (PE)-10 tubing (Clay Adams). The portal vein was then cannulated using an intravenous catheter, and the liver was perfused via this cannula with 25 mM MOPS-buffered Ringer solution (pH 7.4) containing 2% bovine serum albumin and 15% (vol/vol) prewashed canine red blood cells (obtained from the School of Veterinary Sciences, University of Queensland, Brisbane, Queensland, Australia). This medium was oxygenated using a Silastic tubing lung ventilated with oxygen. The perfusion system used was nonrecirculating and employed a peristaltic pump (Cole-Parmer). After liver perfusion was initiated, animals were killed by a thoracotomy. After the thoracic inferior vena cava was cannulated using PE-240 tubing (Clay Adams). Oxygen consumption, bile flow, perfusion pressure, and macroscopic appearance were used to assess liver viability. PO2 and hematocrit of the collected perfusate were determined with an ABL 520 analyzer (Radiometer; Copenhagen, Denmark), and total hemoglobin and O2 content (vol%) were measured using an OSM3 analyzer (Radiometer) operating in the dog mode. Hepatic oxygen consumption (μmol min−1·g liver−1) was determined by the following formula:

\[
\text{Inflow O}_2 \text{ content - outflow O}_2 \text{ content} \times \text{Flow rate} \times \frac{\text{Liver wet weight}}{(J)}
\]

Livers were perfused at a rate of 15 ml/min. After a 10-min stabilization period, aliquots (50 μl) of perfusate plasma containing propranolol (0.075 μmol), [3H]water [3 × 10⁶ disintegrations/min (dpm) or 5 × 10⁴ Bq], and [U-14C]sucrose (1.5 × 10⁴ dpm or 2.5 × 10⁴ Bq) were injected into the liver, and outlet samples were collected (1 s × 20, 4 s × 5, 10 s × 5, and 30 s × 5) via a fraction collector for 4 min. The outlet samples were centrifuged at 1,500 g (25°C) for 3 min, and aliquots (75 μl) of the supernatant were taken for scintillation counting to determine [3H]water and [U-14C]sucrose using a MINAXI beta TRI-CARB 4000 series liquid scintillation counter (Packard Instruments; Meriden, CT) and “Emulsifier-Safe” scintillation fluid (Packard BioScience; Groningen, The Netherlands). The residue was vortexed and prepared for HPLC analysis to determine the outflow concentration of propranolol (15). The dose of propranolol (0.075 μmol) used in this work has been confirmed to exhibit linear kinetics (13, 15).

Analytic procedures. The HPLC method has been described and validated previously (15).

Histopathological analyses. For transmission electron microscopy, diced liver tissue was fixed in 5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). The tissue was then washed in cacodylate buffer, postfixed in 1% osmium tetroxide, washed in distilled water, stained “en bloc” in 5% aqueous uranyl acetate, dehydrated through a series of graded alcohols, cleared in propylene oxide, and finally embedded in an Epon/Araldite mixture. An equal number of sections selected from perportal and perivenous areas was cut with a Leica Ultracut ultratome (Leica Microsystems; Wetzlar, Germany) and stained with toluidine blue for detailed study. Ultrathin sections (1 μm) were picked up on uncoated copper grids, stained with aqueous lead citrate [−0.09 M (37)] for 1–2 min, and examined with a JEOL 1200EXII transmission electron microscope (JEOL; Tokyo, Japan).

Determination of intrahepatic α₁-acid glycoprotein levels. Livers were harvested from the rats after in situ hepatic perfusion and washed with a mixed solution of 1) calcium and magnesium-free Hanks’ balanced salt solution (Sigma Chemical), 2) 5 mM EDTA (pH 7.0), and 3) 10 mM HEPES (pH 7.0) at 10 ml/min for 10 min to remove protein and blood from the sinusoidal beds. Approximately 1 g of liver was then homogenized in a Kinematic PT 10/35 tissue blender (Kinematica; Kreins, Switzerland) with 1 ml of 25 mM MOPS (pH 7.4) buffer and centrifuged at 3,000 g for 10 min. A quininaline red (Sigma) fluorometric titration method was used to measure tissue α₁-acid glycoprotein (AAG) levels (16).

Determination of hepatic microsomal protein, cytoskeleton residue, and CYP levels. The liver (1g) was homogenized in 2.5 ml of ice-cold 0.25 M sucrose containing 50 mM Tris-HCl buffer (pH 7.4) for 3–5 min. Homogenates were centrifuged at 5,000 rpm for 20 min, and pellets were resuspended in 2.5 ml Tris buffer as the cytoskeleton fraction. The supernatant (~1 ml) from the 5,000-rpm centrifugation was centrifuged again at 50,000 rpm for 1 h. The resulting pellets were resuspended in 2.5 ml Tris buffer and used as the microsomal fraction. The cytoskeleton residue (CR) and microsomal protein (MP) concentrations in the respective fractions were determined by the method of Lowry et al. (20). CYP content in MP was estimated from the dithionite-reduced difference spectrum of CO-bubbled samples using the molar extinction difference of 104 mM⁻¹·cm⁻¹ in absorption at peak position (~450 nm) (23). A Shimadzu RF-5301PC spectrofluorophotometer (Shimadzu; Kyoto, Japan) was used for absorption spectrophotometric analysis.
sucrose space ($V_{\text{suc}}$; determined by [U-$^{14}$C]sucrose, which was used as a surrogate for water and propranolol sinusoidal distribution volumes). A barrier-limited plus space-distributed liver model with correction for catheter effects was used to estimate the total water space ($V_W$; determined by [3H]water), with $V_W$ then being used to estimate the cellular water space ($V_C$; defined as $V_W - V_{\text{suc}}$) as described previously (14, 15). Kinetic parameters of propranolol were derived from a two-phase physiologically based organ pharmacokinetic model, which accounts for vascular dispersion and has been described in detail elsewhere (43). A schematic representation of hepatocellular propranolol transport, metabolism, ion trapping, and cytosolic binding is shown in Fig. 1. The underlying equations are as follows (43):

$$
\hat{f}(s) = \hat{f}(s + k_{in}[1 - \hat{f}(s)])
$$

$$
\hat{f}(s) = \frac{s(1 + K_b) + s(k_{in} + k_{out} + k_{cv})}{s(1 + K_b) + s(k_{in} + k_{out} + k_{cv}) + k_{in}(k_{in} + k_{out})}
$$

where $\hat{f}(s)$ is the transit density for the nonpermeating reference, $\hat{f}_c(s)$ is the sojourn time of the solute into the cellular space, and $k_{in}$ is the permeation rate constant [$k_{in} = f_{up}PS/V_{\text{suc}}$, where $PS$ is the permeability-surface area product and $f_{up} = 0.45$ is the fraction unbound in the perfusate, as estimated previously by Hung et al. (15)], $k_{out}$ is the efflux rate constant. The equilibrium amount ratio ($K_v = k_{cv}/k_{vc}$) characterizes the slowly accessible pool for ion trapping [$k_{cv}$ and $k_{vc}$ represent the rate constants for transport from the cytosol into acidic vesicles (lysosomes and mitochondria) or from acidic vesicles into the cytosol, respectively]. $K_b$ is defined as rapidly equilibrating intracellular binding sites (microsomal and nonspecific binding). The elimination rate constant ($k_e$) is $CL_{\text{int}}/V_C$ and is $CL_{\text{int}}$ normalized per cellular volume ($V_C$) (32). Data were fitted and calculated using a Scientist program (Micromath Scientist; Salt Lake City, UT).

Nonparametric estimates of hepatic availability ($F$), mean transit time (MTT), and normalized variance ($CV^2$) were determined from the outflow concentration ($C$) versus time profiles for the reference from Eqs. 4–7 using the parabolas through the origin method (extrapolated to infinity) with the assistance of the Moments Calculator 2.2 program for Macintosh (35).

$$
F = \frac{Q \times AUC}{D}
$$

where $AUC = \int_0^\infty C(t)dt$ is the area under the solute concentration versus time curve, $Q$ is the perfusate flow rate, and $D$ is the dose of solute administered. All concentrations used were expressed in molar equivalents. The hepatic extraction ratio ($E$) equals $1 - F$.

$$
MTT = \frac{AUMC}{AUC}
$$

where $AUMC = \int_0^\infty tC(t)dt$ is the area under the first moment curve.
DISPOSITION OF PROPRANOLOL IN THE LIVER

\[ CV^2 = \frac{\sigma^2}{MTT^2} \]

where \( \sigma^2 \) is the variance and is given by

\[ \sigma^2 = \frac{\int C(t)dt}{C(t)dt} - MTT^2 \]

**RESULTS**

Table 1 shows a comparison of liver perfusion parameters between normal and adjuvant-treated rats. There were significant differences between the adjuvant-treated and normal animals in liver wet weight, final body weight, and bile flow \((P < 0.05)\). The hepatic O₂ consumption, perfusion rate, and perfusion pressure for all animals were in the range of 1.33–1.83 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{g liver}^{-1} \), 1.11–1.27 ml·min \(^{-1} \cdot \text{g liver}^{-1} \), and 7.9–11.8 cmH₂O, respectively. These parameters were comparable to those reported previously (6, 14).

Marked alterations in liver histology were evident in the adjuvant-treated animals. Figure 2 shows transmission electron micrographs of mitochondria \((A \text{ and } B)\) and lysosomes \((C \text{ and } D)\) from livers of normal and adjuvant-treated rats. The invaginations and cristae tubulization of mitochondria in the adjuvant-treated rat livers are clearly discernible (Fig. 2B). Micrographs of hepatic lysosomes from these adjuvant-treated rats showed pronounced irregularity in shape (Fig. 2D). Adjuvant-treated rats also showed significantly higher serum biochemistry markers \((\text{AST, ALT, and ALP})\) than normal rats (Table 2). Biochemical data for some hepatic components \((\text{AAG, MP, CR, and iron tissue concentrations})\) in normal and adjuvant-treated rats are also summarized in Table 2. The adjuvant-treated group had significantly higher intrahepatic AAG (one of the acute phase proteins that increases in inflammatory diseases \((P < 0.05)\) and iron concentrations \((P < 0.001)\) but a significantly lower CYP \((P < 0.05)\) level. However, the levels of MP and CR did not differ between treated and untreated animals. There were no significant differences between adjuvant-treated and normal animals for the in vitro propranolol tissue binding (Table 3). Table 3 also shows the results of the in vitro propranolol metabolism study. The adjuvant treatment affected the metabolic activity of propranolol in vitro. The calculated \( \text{CL}_{\text{int}} \) values for propranolol in adjuvant-treated rats were significantly lower \((P < 0.01)\) than those of normal rats.

Table 4 shows a comparison of kinetic parameters for propranolol between normal and adjuvant rats. The adjuvant-treated group showed a significantly decreased E \((P < 0.001)\) and MTT \((P < 0.001)\) of propranolol compared with the normal group. No significant difference in \( CV^2 \) values was found between these two groups.

Figure 3 shows a comparison of typical measured and predicted (fitted data) outflow perfusion concentration versus time profiles for propranolol in isolated perfused normal and adjuvant-induced systemic inflammation rat livers using coadministered \([U-14C] \text{sucrose and } [3H] \text{water for estimation of sinusoidal and cellular volumes. Data regression lines predicted by the two-phase physiologically based organ pharmacokinetic model appeared adequately fitted. It is evident that adjuvant treatment increased the peak propranolol outflow concentration and enhanced the decline of tail section in outflow concentrations (lower MTT). Figure 3 also demonstrates that the AUC for adjuvant-treated animals was significantly larger (lower E) than that of the normal animals. Also shown in Table 4 is a comparison of kinetic parameters derived from the two-phase physiologically based pharmacokinetic organ model for propranolol between normal and adjuvant-treated rats. There were significantly smaller values of \( K_r \) \((P < 0.001)\) and \( \text{CL}_{\text{int}} \) \((P < 0.001)\) in adjuvant-treated animals compared with normal animals, whereas adjuvant-treated animals showed a significantly larger \( V_{\text{in}} \) value \((P < 0.05)\). However, there were no significant differences of PS, \( K_h \), and \( V_C \) values between the two groups. The uncertainties (asymptotic CVs) associated with the estimated propranolol parameter values derived from modelling did not exceed 36%, and all correlation coefficients between parameters were <65%. The propranolol model estimates were also affected by using sucrose as a suitable reference for the propranolol sinusoidal space. This latter space can be estimated to be ~6.5% less than the estimated sucrose space (using the known spaces, hematocrit, red blood cell perfusate partition coefficient, and propranolol perfusate protein binding).

**DISCUSSION**

Extrahepatic adjuvant treatment invades the local lymph node, causing proinflammatory cytokine (e.g., TNF-α, IL-6, and IL-1β) release, and consequently triggers systemic responses (e.g., liver, adrenal, stomach, and articular joints) (46, 47). This study has shown that an immunological adjuvant (delipidated \( M. \text{ tuberculosis} \) dispersed in squalane) administered to rats alters hepatic histology (Fig. 2), biochemistry (Table 2), and pharmacokinetics of propranolol in the liver (Table 4). The changes of solute distribution in the adjuvant-
induced systemic inflammation liver can be attributed to a complex array of factors such as decreased hepatic metabolism (7, 36); changes in hepatic architecture such as defenestration, capillarization, or intrahepatic shunts (45); and toxic effects impacting on mitochondrial energy production or lysosomal membrane stability caused, at least in part, by elevated hepatic iron stores (4, 47). The highly detailed electron micrographs of the hepatocytes along a single plane show that the most obvious effects of extrahepatic adjuvant treatment on liver cells are in the acidic subcellular organelles, mitochondria, and lysosomes. Hepatic mitochondria from adjuvant-treated animals are characterized by changes in shape, the presence of inclusions, and cristae tubulization (Fig. 2B). Hepatic lysosomes from adjuvant-treated animals are recognized by an obvious abnormality in shape (Fig. 2D).

According to Hung et al. (15), the model-predicted hepatocellular unbound concentrations of propranolol in normal and adjuvant-treated rat livers are 0.06 ± 0.01 and 0.04 ± 0.01 μM, respectively. These values are below the estimated unbound Michaelis constant for the main metabolic 4-hydroxylation pathway (high affinity 0.11 ± 0.02 μM, low affinity 125 ± 28 μM) in whole rat livers (17). Thus an implicit assumption in this work is linearity of kinetics. In addition,

Table 2. Comparison of serum biochemistry levels of ALT, AST, and ALP and tissue concentrations of AAG, MP, CR, CYP, and iron between normal and adjuvant-treated rats

<table>
<thead>
<tr>
<th>Serum and Tissue Markers</th>
<th>Animal Model</th>
<th>Normal</th>
<th>Adjuvant treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT, U/l</td>
<td></td>
<td>38.5±7.42</td>
<td>91.8±23.03‡</td>
</tr>
<tr>
<td>AST, U/l</td>
<td></td>
<td>61.5±8.62</td>
<td>76.8±13.5*</td>
</tr>
<tr>
<td>ALP, U/l</td>
<td></td>
<td>319±14.9</td>
<td>385±32.1†</td>
</tr>
<tr>
<td>AAG, mg/g liver</td>
<td></td>
<td>0.17±0.05</td>
<td>0.23±0.04*</td>
</tr>
<tr>
<td>MP, mg/g liver</td>
<td></td>
<td>9.40±0.27</td>
<td>8.73±2.98</td>
</tr>
<tr>
<td>CR, mg/g liver</td>
<td></td>
<td>143±31.5</td>
<td>129±21.7</td>
</tr>
<tr>
<td>CYP, nmol/mg protein</td>
<td></td>
<td>0.19±0.07</td>
<td>0.11±0.05*</td>
</tr>
<tr>
<td>Iron, μmol/g dry liver</td>
<td></td>
<td>3.82±2.11</td>
<td>24.0±3.88‡</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 animals. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; AAG, α1-acid glycoprotein; MP, microsomal protein; CR, cytoskeleton residue; CYP, cytochrome P=450. *P < 0.05; †P < 0.01; ‡P < 0.001.

Table 3. Comparison of in vitro propranolol to hepatic components (MP and CR) and metabolism between normal and adjuvant-treated rats

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>f_{uT,MP}</th>
<th>f_{uT,CR}</th>
<th>CL_{int}, ml/min/mg MP^{-1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.51±0.17</td>
<td>0.91±0.04</td>
<td>0.15±0.03*</td>
</tr>
<tr>
<td>Adjuvant treated</td>
<td>0.46±0.14</td>
<td>0.87±0.09</td>
<td>0.09±0.02</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 6 animals. f_{uT,MP}, drug fraction unbound in MP; f_{uT,CR}, drug fraction unbound in CR; CL_{int}, intrinsic elimination clearance. *P < 0.01.
linearity is confirmed by the logarithm of the propranolol concentration remaining versus time being linear after incubation with 0.05 μM propranolol in both normal and adjuvant rat liver microsomes in vitro.

E of propranolol depends on perfusate flow, hepatic metabolism (CL_int), and permeability (13). The decrease in E (Table 4) and CL_int (Table 4) of propranolol corresponded to a decrease in hepatic CYP level (Table 2) and is consistent with the significant decrease in hepatic propranolol metabolism observed after turpentine-induced inflammation (7). The hepatic extraction of propranolol was obviously compromised by the adjuvant-induced systemic inflammation.

Propranolol is a widely used nonselective β-blocking agent highly extracted by the liver (42). Intrahepatic propranolol in the liver is mainly oxidized to 4-hydroxypropranolol and 5-hydroxypropranolol (30) by CYP2D6 (22). The significant differences in CYP levels between the two groups (Table 2) indicate a general decrease in enzymatic activity, including phase I xenobiotic detoxification. Given that only 70% of MP homogenized from liver tissue is metabolically active and the average production of MP from 1 g liver tissue amounts to about 50 mg (39), the calculated CL_int values obtained from the in vitro MP metabolism study (Table 3) can be converted to total liver tissue CL_int values for propranolol (normal: 10.7 ± 2.14 ml/min−1·g liver−1; adjuvant treated: 6.42 ± 1.28 ml/min−1·g liver−1). The in vitro results are similar to the CL_int values (normal: 11.0 ± 2.08 ml/min−1·g liver−1; adjuvant treated: 6.01 ± 1.68 ml/min−1·g liver−1) derived from the two-phase organ model used in this work for propranolol fitting in an in situ isolated perfused liver study (Table 4).

Xenobiotics are oxidized by a number of CYP-containing isozymes that, in response to systemic inflammatory diseases, may be differentially expressed. The loss of CYP during inflammation involves a number of features common to host defence mechanisms including the production of a series of mediators (25, 36). IL-1β depresses the oxidation of benzphetamine, ethoxycoumarin, and debrisoquine (18). TNF-α depresses CYP2C11 and CYP3A2 levels (28). IL-6 appears to induce different responses among CYP isoforms, including a dose-dependent sensitivity often not seen with other cytokines (5).

Adjuvant-treated rats had significantly higher hepatic iron concentrations compared with normal rats (Table 2). Increased uptake of hepatic iron may be mediated by an upregulation of intestinal iron transporters [e.g., divalent metal transporter (DMT1)] during systemic inflammation. Proinflammatory cytokines have been demonstrated to effect an elevated expression of DMT1 in a macrophage cell line (21). Inflammation-induced hemolysis provides an alternative mechanism for increased hepatic iron accumulation (9). However, an investigation of how iron was overloaded in the adjuvant-treated liver is beyond the scope of this study.

It is well documented that increased hepatic iron concentrations have a range of toxic effects on mitochondria and lysosomal membranes alike (11, 27, 41). Lysosomes play an important role in intracellular digestion (19), transcellular transport (12), and intracellular storage (e.g., iron sequestration) (4). They are also responsible for ion trapping of basic drugs (such as propranolol) (15). Preservation of membrane integrity and fluidity is absolutely essential for the maintenance of cellular, and particularly lysosomal, function. Hepatic injury arises from excessive iron sequestration into hepatic lysosomes followed by peroxidation of membrane lipids and alterations in the lysosomal membrane lipid composition (11). This decreases the stability and fluidity of these membranes. These changes also impair the lysosomes’ ability to fuse with the canalicular membrane and other neighboring structures, inhibiting the normal function of the proton pump and raising endolysosomal pH (27). Consequently, lysosomal digestive and excretory functions are greatly impaired. The altered membrane structure allows some leakage of lysosomal contents into the cytosol (26, 27). Lysosomal iron trapping is also abolished, or at least significantly impaired, particularly for highly basic drugs such as propranolol (pKb = 9.45). Lysosomal iron trapping contributes almost equally to hepatic drug distribution, as does intracellular binding (43). The toxic effects of excessive intrahepatic iron accumulation followed by membrane damage and rupture (11) significantly decreases lysosomal iron trapping; this is borne out by the observation that a highly extracted drug like propranolol has a dramatically changed outflow profile, as shown by a significantly larger AUC (Fig. 3). The curve presented in Fig. 3 shows the
similarity of effects by treatment with the ionophore monensin (43). In both events, the pH in the acidic subcellular compartments increases and lysosomal and mitochondrial integrity, function, and ion-trapping capacity is compromised. A reduction in ion trapping appears to be evident as the major reason for the decrease in the MTT for propranolol from 107 s in normal livers to 71 s in livers from adjuvant-treated animals. Support for this mechanism is provided by changes in liver morphology (Fig. 2) and a significantly smaller value for $K_v$ (a kinetic parameter mainly reflecting ion trapping) in livers from adjuvant-treated animals (Table 4). The changes in $K_v$ could be correlated with iron content, suggesting that a higher liver iron content may be reducing sequestration by intracellular organelles such as mitochondria. CYP is not involved as adjuvant treatment led to a decrease in CYP levels and did not affect intracellular hepatic binding of propranolol (similar $f_{av}$ and $K_b$ values; Tables 3 and 4). Furthermore, there was no relationship evident between $K_v$ and hepatic CYP levels.

The relative contribution of ion trapping, intracellular binding, and distribution of unbound propranolol for hepatic sequestration can be estimated as follows:

$$\frac{K_v}{1 + K_v + K_b}, \quad \frac{K_b}{1 + K_v + K_b}, \quad \text{and} \quad \frac{1}{1 + K_v + K_b}$$

respectively (43). Given that the kinetic values have been determined (Table 4), ion trapping, intracellular binding, and unbound drug distribution account for 40%, 55%, and 5% of the sequestration of propranolol in the normal rat liver. In contrast, values of 20%, 75%, and 5%, respectively, were found in the adjuvant-induced systemic inflammation rat liver. These values, reflecting the relative contribution of ion trapping for sequestration of propranolol in the liver, were significantly lower in the adjuvant-treated rats (20%) than normal rats (40%).

Stepwise regression analysis showed that hepatic iron concentration and CYP content were the best predictors of $K_v$ and $C_{L_{int}}$, respectively. Figure 4A shows that a conversely linear relationship existed between $K_v$ values (an indicator of the

Fig. 3. Comparison of corresponding regression line fits [normal scale (A) and logarithmic scale (B)] for propranolol data ($y_{obs}$) using a two-phase physiologically based organ pharmacokinetic model and a data weighting of $1/y_{obs}^2$ for isolated perfused normal and adjuvant-induced systemic inflammation rat liver data.

Fig. 4. Relationship between hepatic iron concentration and ion trapping ($K_v$) of propranolol (A) and hepatic cytochrome P-450 (CYP) level and metabolism ($C_{L_{ext}}$) of propranolol (B) in normal and adjuvant-induced systemic inflammation rats.
ion-trapping effect) and hepatic iron concentration, and Fig. 4B shows that a positively linear relationship existed between CLint values (an indicator of metabolism) and hepatic CYP content in normal and adjuvant-treated rats. These strong relationships are consistent with the notion that adjuvant treatment reduced the hepatic extraction of propranolol by reducing metabolism (CYP loss in response to systemic inflammation) and decreased hepatic sequestration of propranolol by reducing ion trapping (instability of mitochondrial and lysosomal membrane in response to elevation of hepatic iron stores).

We (13) have previously shown that hepatic permeability (PS) for propranolol significantly differs between normal rats and those with CCl4-induced fibrosis (13). An impaired uptake of propranolol across the capillarized endothelium in fibrosis is consistent with a slower solute diffusivity and longer diffusion pathlength as a consequence of collagenization of the Disse space (13). In contrast, the PS for propranolol appeared similar between the adjuvant-treated and normal groups in this study, suggesting that hepatic morphology alterations caused by extrahepatic adjuvant treatment are different to those by CCl4-induced fibrosis and have negligible impact on propranolol transmembrane uptake. It is possible that a difference exists with the intrinsic permeability in the adjuvant-treated group being lower, but this is being masked by a larger surface area in adjuvant-induced systemic inflammation as the adjuvant-treated group had a significantly larger \( V_{\text{su}} \) than the normal group, as estimated by kinetic analysis (Table 4).

Ring and Stremmel (38) proposed a series of consequential events of sinusoidal relaxation during sepsis. First, microbes enter the hepatic circulation, where they first activate sinusoidal endothelial cells and Kupffer cells to produce proinflammatory mediators, including TNF-\( \alpha \), IL-1, IL-6, reactive oxygen metabolites, and eicosanoids. Second, leukocytes are targeted to liver sinusoids by chemoattractants and, like platelets, bind to sinusoidal endothelial cells, which are in a procoagulant state of inflammatory activation. Third, obstruction of the sinusoids by these cells leads to a decrease of blood flow through the sinusoids, which is further aggravated by endothelin-1, effectuating constriction of hepatic stellate cells in the sinusoids. Finally, both nitric oxide (NO) and carbon monoxide (CO) act as antagonists of endothelin-1 by mediating relaxation of sinusoidal vessels (both NO and CO are hepatoprotective during the early, hyperdynamic phase of sepsis). However, during the late, hypodynamic phase of sepsis, massive overproduction of NO by inducible NO synthase leads to a breakdown of the liver circulation (sinusoidal overrelaxation) (38). Adjuvant-induced systemic inflammation might also cause an overproduction of NO by inducible NO synthase, resulting in a larger hepatic sinusoidal volume (larger \( V_{\text{su}} \) than normal animals).

A heterogeneous (barrier limited and space distributed) transit time model was used to estimate the rate constants of hepatocellular influx, efflux, binding, and elimination for any permeable solute (including water). In general, models using vascular references as a basis for drug disposition in perfused organs are generally comparable but mathematically not identical. The PS found for water in this study (45.1 ± 5.47 ml·min⁻¹·g liver⁻¹) is similar to what we (14) have found previously (38.3 ± 3.73 ml·min⁻¹·g liver⁻¹). Whereas a barrier limitation is necessary in the heterogeneous model to best describe the data, such a limitation was found to be unnecessary when water transport in the liver was analyzed using the Goresky model and different references (31). A barrier limitation for transport has been reported for a range of other solutes in the liver (8, 40). Adjuvant disease yielded a similar PS value for water (35.6 ± 9.43 ml·min⁻¹·g liver⁻¹) as found for controls and contrasts to the low PS for water found in CCl4-induced fibrotic livers (6.79 ± 1.93 ml·min⁻¹·g liver⁻¹), in which sinusoidal collagen deposition and fibrotic changes alter the barrier (14).

In conclusion, subcutaneous caudal administration of adjuvant to rats changed their liver architecture, biochemistry, and drug disposition/metabolism. Changes included the appearance of mitochondrial inclusions/tubularization, irregularly shaped lysosomes, increased AAG content and iron concentration, and lower CYP level relative to normal rats. The metabolism and ion trapping of propranolol in the liver were significantly impaired in adjuvant-induced systemic inflammation and may be related to changes in hepatic iron concentration and CYP content.

GRANTS
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