Hepatic Pharmacokinetics of Cationic Drugs in a High-Fat Emulsion-Induced Rat Model of Nonalcoholic Steatohepatitis

Peng Li, Thomas A. Robertson, Camilla A. Thorling, Qian Zhang, Linda M. Fletcher, Darrell H. G. Crawford, and Michael S. Roberts

ABSTRACT:

The hepatic pharmacokinetics of five selected cationic drugs (propranolol, labetalol, metoprolol, antipyrine, and atenolol) was studied in the liver from control rats and from those with high-fat emulsion-induced nonalcoholic steatohepatitis (NASH). Studies were undertaken using an in situ-perfused rat liver and multiple indicator dilution, and outflow data were analyzed with a physiologically based organ pharmacokinetic model. Hepatic extraction (E) was significantly lower in the NASH model, and lipophilicity was the main solute structural determinant of the observed differences in intrinsic elimination clearance (CLint) and permeability-surface area product (PS) with pKs defining the extent of sequestration in the liver [apparent distribution ratio (Kapp)]. The main pathophysiological determinants were liver fibrosis, leading to a decreased PS, liver fat causing an increase in Kapp and an increase in both total liver cytochrome P450 (P450) concentration and P450 isofrom expression for Cyp3a2 and Cyp2d2, causing an increase CLint in NASH rat livers compared with control livers. Changes in hepatic pharmacokinetics (PS, Kapp, CLint, and E ratio) as a result of NASH were related to the physicochemical properties of drugs (lipophility or pKs) and hepatic histopathological changes (fibrosis index, steatosis index, and P450 concentration) by stepwise regression analysis. Thus, it appears that in NASH, counteracting mechanisms to facilitate hepatic removal are created in NASH-induced P450 expression, whereas NASH-induced fibrosis and steatohepatitis inhibit E by decreasing hepatocyte permeability through fibrosis and hepatic sequestration.

Introduction

Nonalcoholic steatohepatitis (NASH) is a fatty inflammation of the liver that arises without excessive alcohol use and is characterized histologically by steatosis, necroinflammation, and fibrosis (Sheth et al., 1997). The fibrosis is characterized by collagen deposition in the sinusoids and space of Disse, which can lead to defenestration and sinusoidal portal hypertension (Ishak et al., 1991; Sheth et al., 1997). Although the histopathological characteristics of NASH are well described, exactly how this syndrome influences drug disposition in the liver is unclear.

The effects of other liver disease and aging on hepatic drug disposition have been recognized for a long time (Williams and Mamelok, 1980; Le Couteur et al., 2005). Our previous research in rats has shown that carbon tetrachloride-induced fibrosis and cirrhosis can significantly alter hepatic pharmacokinetics (Hung et al., 2002a,b). In these studies, changes in hepatic pharmacokinetics were related to alteration in hepatocellular morphology and biochemistry and the nature of the drug. Hepatocyte permeability, intrinsic metabolic clearance, ion trapping, and microsomal binding were all changed in diseased livers (Siebert et al., 2004). An altered hepatic cytochrome P450 (P450) concentration was also seen in adjuvant-induced systemic inflammation (Hung et al., 2006).

There is limited work published on how NASH may affect hepatic pharmacokinetics. Publications have focused on potential changes in hepatic P450, showing, for instance, that liver CYP2E1 and CYP4A levels were increased in patients with NASH (Niemela et al., 2000; Chitoiu et al., 2007). However, Weltman et al. (1998) reported lower liver CYP3A levels in NASH. Lickteig et al. (2007) showed increases in liver transporter mRNA and protein levels as well as a decreased biliary excretion of acetaminophen conjugates in rats fed a methio-
nine- and choline-deficient diet for 8 weeks. Pharmacokinetic studies in NASH patients and the NASH rat model have shown a significant decrease in the hepatic extraction ($E$) ratio (Siepmann et al., 1998; Hung et al., 2005; Schriever et al., 2008). However, the actual effects of steatosis, necroinflammation, and damaged microcirculation in the NASH liver on the individual hepatic pharmacokinetic processes have not, as yet, been well addressed.

The aim of this work was to characterize the hepatic pharmacokinetics of cationic drugs in NASH and to relate the changes in individual pharmacokinetic processes such as membrane permeability, metabolic clearance, and sequestration to the pathophysiology caused by NASH and the nature of drug studied. The hepatic disposition of five selected cationic drugs, including propranolol, labetalol, metoprolol, antipyrine, and atenolol, was studied both in healthy (control) rats and in a high-fat emulsion-induced rat model of NASH. The high-fat diet-induced NASH model has been reported to replicate the key pathological features of human NASH and the typical diagnostic aspects of human NASH such as obesity, abnormal aminotransferase, hyperlipidemia, hyperinsulinemia, hyperglycemia, and insulin resistance (Zou et al., 2006). To appropriately characterize the pharmacokinetic processes without recirculation effects, studies were undertaken in an in situ-perfused rat liver (IPRL) in which the drug and multiple indicators were coadministered as a bolus, and their outflow profiles were used to define the hepatic pharmacokinetics (commonly described as multiple indicator dilution (MID)) (Roberts and Anissimov, 1999; Hung et al., 2001). The IPRL-coupled MID method has been applied previously to studies of cationic drug disposition in control and other diseased livers (Hung et al., 2001, 2002a). The perfusate outflow data were analyzed by a two-phase physiologically based organ pharmacokinetic model that recognized the presence of vascular dispersion, transfer across the hepatocyte membrane, intracellular distribution, and metabolic clearance (Weiss et al., 1997). The extent of fibrosis, steatohepatitis, and P450 levels were determined in both control and diseased rat livers and used to interrelate changes in hepatic pharmacokinetics with NASH-induced changes in hepatocellular histopathology. The results should contribute to a better understanding of whether and how fat deposition, collagen deposition, and alteration in P450 expression will affect the hepatic pharmacokinetics of cationic drugs.

Materials and Methods

Chemicals. Propranolol, labetalol, metoprolol, antipyrine, and atenolol were all obtained from Sigma-Aldrich (St. Louis, MO). Physicochemical properties of these five cationic drugs are summarized in Table 1. [$^{14}$C]Sucrose and [3H]water were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). All other chemicals used in this research were obtained from Sigma-Aldrich.

Rat Model of NASH. The rat model of NASH was induced in rats using the high-fat emulsion method following a procedure outlined previously (Zou et al., 2006). In brief, male Wistar rats (approximately 250 g) were assigned to two groups: control group and NASH group. All rats were fed with standard rat chow and water. In addition, they were allowed free access to saccharose in water (18%). The NASH group was gavaged with the high-fat emulsion (10 ml/kg), which contains corn oil, saccharose, cholesterol, and so on, once a day for 6 weeks (Zou et al., 2006). The control group was gavaged with saline instead of the high-fat emulsion. Body weight, food intake, and water intake were monitored daily for rats in both groups for the entire duration of the experiment. After 6 weeks, the animals were sacrificed for IPRL studies and histopathological examination. All animal studies were carried out according to protocols approved by the University of Queensland Animal Ethics Committee.

Liver Biochemistry Determination. Blood lipid and liver function tests of the control and disease rats were performed at the Pathology Laboratory of the Princess Alexandra Hospital (Brisbane, QLD, Australia). Serum aspartate transaminase, alanine transaminase, alkaline phosphatase, triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), total bilirubin, and albumin were measured on a Synchro LX20 Clinical System (Beckman Coulter, Fullerton, CA).

IPRL. The IPRL preparation used in this study was prepared as described previously (Cheung et al., 1996; Hung et al., 2001). Wistar rats were anesthetized by intraperitoneal injection of xylazine at 10 mg/kg (Bayer Australia, Pymble, NSW, Australia) and ketamine-hydrochloride at 80 mg/kg (Parnell Laboratories, Alexandria, NSW, Australia). After laparotomy, the bile duct was cannulated with PE-10 tubing (BD, Sparks, MD). The animal was heparinized by a bolus injection of 200 units of heparin sodium (David Bull Laboratories, Kewdale, WA, Australia) via the inferior vena cava. The portal vein was cannulated with a 16-gauge intravenous catheter. Through this cannula, the liver was then perfused with MOPS buffer, which contained 2% bovine serum albumin (w/v) and 15% prewashed canine red blood cells (v/v) (Veterinary Specialist Services Pty Ltd., Brisbane, QLD, Australia) at pH 7.4 (Cheung et al., 1996). The perfusion was conducted at 15 ml/min in each liver to reflect in vivo microcirculation. The perfusion medium was pumped with a peristaltic pump (Cole-Palmer, Veteran Hills, IL) and oxygenated using a stainless tubing artificial lung ventilated with 100% pure oxygen (BOC Gas Australia, Brisbane, QLD, Australia). After the perfusion commenced, the animal was sacrificed by thoracotomy, and the thoracic inferior vena cava was cannulated within a PE-240 tubing (BD). The animal was placed in a temperature-controlled cabinet with the temperature set at 37°C. Liver viability was evaluated by oxygen consumption, portal vein pressure, bile flow, and macroscopic appearance as previously described by Cheung et al. (1996).

Bolus Injection Study. After a 10-min perfusion stabilization period, the MID study was performed by bolus injection into the liver of aliquots (50 μl) of perfusion medium that contained [$^{14}$C]Sucrose (1.5 × 10$^{5}$ dpm) and [3H]water (3 × 10$^{6}$ dpm). The outflow samples were collected via a fraction collector at short 4-min intervals. Aliquots (50 μl) of cationic drugs (8 mM propranolol, 6 mM labetalol, 3 mM metoprolol, 4 mM antipyrine, and 4 mM atenolol) were bolus injected separately into the perfused liver. In each liver, the order of injection was randomized, and there was no repeat of the same injection in the same liver. A washout and a stabilization period of approximately 10 min were applied after every injection. The total perfusion time for each liver was less than 2 h. All outflow samples were centrifuged, and aliquots (100 μl) of supernatant were taken for analysis.

Analytical Procedure. The MID samples, which contain [$^{14}$C]Sucrose and [3H]water, were taken for scintillation counting of disintegrations per minute using a MINAXI beta TRICARB 4000 series liquid scintillation counter (Packard Instruments). The outflow samples of cationic drugs were determined with established high-performance liquid chromatography methods that have been described and validated previously (Hung et al., 2001).

Histopathology Examination and Quantitation of Histology Index. Three to five slices of tissue were randomly selected from each liver and fixed in 10% neutral buffered formalin and then embedded in paraffin. Sections of 5-μm thickness were prepared and stained with hematoxylin and eosin, Masson’s trichrome, and Sirius red to determine the degree of steatosis or inflammation, respectively. The light microscopy digital images were acquired with a ScanScope digital slide scanner (Aperio Technologies, Vista, CA) at the Pathology Laboratory of the Queensland Institute of Medical Research. The collagen and fat deposition were expressed as fibrosis index (FI) and steatosis index (SI), respectively, which were quantified with computer-assisted image analysis (Image Scope version 10; Aperio Technologies) of the stained sec-

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**TABLE 1**

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>Molecular Weight</th>
<th>logP_{ow}</th>
<th>$f_a$</th>
<th>$p_k$</th>
<th>Metabolism Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol</td>
<td>259.34</td>
<td>3.10</td>
<td>0.69</td>
<td>9.45</td>
<td>CYP2D2</td>
</tr>
<tr>
<td>Labetalol</td>
<td>328.32</td>
<td>2.69</td>
<td>0.57</td>
<td>7.4</td>
<td>Glucuronosyl transferase</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>267.36</td>
<td>1.79</td>
<td>0.76</td>
<td>9.17</td>
<td>CYP2D2/3A2</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>188.23</td>
<td>0.33</td>
<td>1.00</td>
<td>1.45</td>
<td>CYP3A2</td>
</tr>
<tr>
<td>Atenolol</td>
<td>266.34</td>
<td>0.14</td>
<td>0.47</td>
<td>9.6</td>
<td>CYP2D2</td>
</tr>
</tbody>
</table>

* Log octanol/water partition coefficient at pH 7.4 values (Hung et al., 2001).

$^b$ Fraction unbound in perfusate acquired with microfiltration method.

$^c$ Negative logarithm of the ionization constant.
ions. For each rat, the area of stained fibrotic tissue and fat deposition in five randomly selected fields was measured on the binary image. The FI was quantified as the total area of fibrosis divided by the total area of the section, as described previously (O’Brien et al., 2000). The SI was quantified as the total area of fat deposition divided by the total area of the selected section, as described previously (Marsman et al., 2004).

**Determination of Liver P450 Concentration and Fat Weight.** Rat livers were harvested from the sacrificed rat and perfused through the portal vein with a mixed solution of calcium and magnesium-free Hank’s balanced salt solution (5 mM EDTA and 10 mM HEPES) at 15 ml/min for 5 min to remove protein and blood from the sinusoid bed. The liver was then snap frozen in liquid nitrogen and stored at -80°C until required for analysis. Liver tissue (1 g) was then thawed and homogenized in 2.5 ml of ice-cold sucrose (0.25 M) containing 50 mM Tris-HCl buffer (pH 7.4) using a tissue homogenizer. To separate the liver, a microparticle fraction, gradient centrifugation was performed on the liver homogenates as described previously (Hung et al., 2002b). The P450 concentration in the microsome fraction was then determined by the molar extinction difference at peak position (approximately 450 nm), as described previously (Matsubara et al., 1976). As an independent measure of fat deposition, the total fat weight in the rat liver was determined by the Folch method (Folch et al., 1957).

**RNA Extraction, cDNA Synthesis, and Real-Time Reverse Transcription-Polymerase Chain Reaction.** The RNA was extracted from liver tissue using TRIzol reagent (Invitrogen, Mt. Waverley, VIC, Australia). The concentration of RNA per sample was measured by spectrophotometry. DNA within the RNA sample was removed by DNase treatment. Then, first-strand cDNA was synthesized from 1 g of total RNA using SuperScript III Reverse Transcriptase (Invitrogen) and oligo(dT)15, according to the manufacturer’s instructions.

Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed to measure gene expression of P450 enzymes and a control housekeeping gene (Gapdh). Primers were designed using Primer3 software (Whitehead Institute for Biomedical Research, Cambridge, MA), and the gene sequences for each forward and reverse primer used for the PCR are listed in Table 2. A Corbett Rotor Gene 3000 (QIAGEN, Doncaster, VIC, Australia) was used for real-time RT-PCR amplification. The reactions were performed in a total volume of 20 μl, with 400 nM forward primer, 400 nM reverse primer, 12.5 ng of cDNA, and 10 μl of QuantiTect SYBR Green PCR master mix (QIAGEN, Clifton Hill, VIC, Australia). Semiquantification of gene expression was performed using the standard curve method, and gene expression was normalized with the housekeeping gene expression as external standard. The result for each sample was expressed as a percentage compared with gene expression in the liver from control rats.

**Modeling and Data Fitting of the Outflow Concentration-Time Profiles.** Modeling and data fitting were performed by using the dispersion model coupled two-phase physiologically based organ pharmacokinetic model, as described previously (Hung et al., 2001). This dispersion model describes drug sinusoidal mixing (Roberts and Rowland, 1986; Roberts et al., 1988), transfer across the hepatocyte membrane, intracellular distribution, and elimination kinetics in the liver (Weiss et al., 1997; Hung et al., 2001, 2002a). In brief, the stochastic approach represents the transit of a molecule through the organ as a series of sojourns in one of two regions described by density functions, as shown in Fig. 1. The apparent distribution ratio ($K_D$) between cellular and extracellular spaces for the unbound solute was defined by influx rate constant ($k_{in}$) and extracellular space for the unbound solute. The intrinsic elimination clearance per cellular water volume ($V_c$). The density of cellular residence times $f_i(s)$ describes the hepatocellular distribution and elimination kinetics. The sojourn time distribution $f_i(s)$ of a molecule after a single excursion in the cellular space for the resulting two-compartment cell model can be obtained by standard methods in the Laplace domain: $f_i(s) = L^{-1}[f_i(x)]$, as described previously (Weiss, 1999, 2000) (eq. 1):

$$f_i(s) = \frac{k_{o_{in}}(s + k_{off})}{s[1 + K_0] + s[k_{off} + k_{in} + k_e + k_{on} + k_{out}] + k_{out}(k_e + k_{out})}$$

(1)

The hepatic transit time density function $f_i(s)$ of solutes can be evaluated in terms of the extracellular transit time density of a nonpermeating reference.

**TABLE 2**

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Accession Number</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A2</td>
<td>NM_053312</td>
<td>CAGCAAGACACACACAGGATG</td>
<td>TGGGTCTCGAACTCGGTAGAG</td>
</tr>
<tr>
<td>CYP2D2</td>
<td>NM_017730</td>
<td>CAGACACCTTCTFCACACAG</td>
<td>ACAAATATGATATTTCTGAG</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>NM_031543</td>
<td>TGGCAACAGGATAAATGAGAG</td>
<td>GTGCTGAAATTGGCAAGG</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM_017008</td>
<td>GATGTTGAGATGTTGAGG</td>
<td>ATGAGGATGCTCCTGAGG</td>
</tr>
</tbody>
</table>

**Comparison of liver perfusion and biochemistry parameters between control and NASH rats (mean ± S.D., n = 6)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control Rats</th>
<th>NASH Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final rat body weight (g)</td>
<td>335 ± 22</td>
<td>425 ± 36**</td>
</tr>
<tr>
<td>Liver wet weight (g)</td>
<td>10.36 ± 1.31</td>
<td>13.84 ± 0.67**</td>
</tr>
<tr>
<td>Liver fat weight (g)</td>
<td>0.33 ± 0.10</td>
<td>2.08 ± 0.40**</td>
</tr>
<tr>
<td>Perfusion rate (ml · min⁻¹ · g liver⁻¹)</td>
<td>1.47 ± 0.19</td>
<td>1.09 ± 0.06**</td>
</tr>
<tr>
<td>Perfusion pressure (cmH₂O)</td>
<td>26.00 ± 2.21</td>
<td>28.67 ± 3.56</td>
</tr>
<tr>
<td>Bile flow (µl · min⁻¹ · g liver⁻¹)</td>
<td>0.83 ± 0.13</td>
<td>0.34 ± 0.12**</td>
</tr>
<tr>
<td>O₂ consumption (µmol · min⁻¹ · g liver⁻¹)</td>
<td>2.32 ± 0.37</td>
<td>1.49 ± 0.25*</td>
</tr>
<tr>
<td>Cellular water space (µl · g liver⁻¹)</td>
<td>528 ± 52</td>
<td>508 ± 100</td>
</tr>
<tr>
<td>Vascular space sucrose (µl · g liver⁻¹)</td>
<td>300 ± 28</td>
<td>262 ± 32*</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>81.2 ± 11.4</td>
<td>131.7 ± 29.6**</td>
</tr>
<tr>
<td>Aspartate transaminase (IU/l)</td>
<td>119.0 ± 7.8</td>
<td>154.1 ± 17.1**</td>
</tr>
<tr>
<td>Alanine transaminase (IU/l)</td>
<td>28.8 ± 3.0</td>
<td>39.0 ± 3.00**</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>0.18 ± 0.12</td>
<td>0.32 ± 0.12**</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>0.32 ± 0.12</td>
<td>0.3 ± 0.2*</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>0.6**</td>
<td>0.5 ± 0.2*</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>0.6**</td>
<td>0.5 ± 0.2*</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01.

**Fig. 1.** Schematic overview of the two compartment convection dispersion model applied to cationic drugs disposition in the liver. $k_i/k_{off}$, the $K_i$ between cellular and extracellular space for the unbound solute; $k_{in}$, elimination rate constant; $K_e$, equilibrium amount ratio characterizing the fast binding process; $k_{on}$ and $k_{off}$, rate constant for drug transport from cytosol into slow binding site, which determines the equilibrium amount ratio characterizing the slow binding process ($K_i = K_e + k_{off}$).
molecule (in this study, sucrose) \( \hat{f}_2(s) \) (see eq. 2 and the density function of successive sojourn times \( f_1(s) \) of the drug molecules in the cellular space):

\[
\hat{f}(s) = \hat{f}_1(s + k_s(1 - \hat{f}(s))]
\]

The fractional outflow is fitted in the time domain by applying a numerical inverse Laplace transformation of the appropriate transit time density function using the nonlinear regression program SCIENTIST (MicroMath Inc., Salt Lake City, UT).

**Estimation of Noncompartmental Pharmacokinetic Parameters.** Noncompartimental estimates of hepatic pharmacokinetic parameters including hepatic availability and mean transit time (MTT) were determined from the outflow concentration-time profiles for reference from eqs. 3 and 4 using the trapezoidal method (extrapolated to infinity) with exponential tail approximation:

\[
F = \frac{Q \cdot AUC}{D}
\]

\[
MTT = \frac{\int C(t)dt}{AUC}
\]

**Statistical Analysis.** All data are presented as mean ± S.D. unless otherwise stated. Statistical analysis was performed with two-way analysis of variance, Student’s \( t \) test, and regression analysis (when appropriate). Stepwise regression analysis was performed with SPSS 14.1 for Windows (SPSS Inc., Chicago, IL), and \( p < 0.05 \) was taken as significant. Linear regression equations have only been considered when \( r^2 > 0.5 \). Correlation analysis between two parameters was performed with SPSS 14.1 for Windows (SPSS Inc.) to assess the Pearson’s product-moment correlation coefficient, and \( p < 0.05 \) was taken as a significant correlation.

**Results**

Table 3 shows a comparison of various physiological changes found in normal rats and in those fed with a high-fat emulsion to induce NASH. NASH rats had a significantly higher final body weight, liver weight, and liver fat weight than the control rats and are comparable to those reported previously (Zou et al., 2006). The NASH rats had impaired bile excretion, and their bile flow was significantly lower than that of the control group. Hepatic oxygen consumption in the NASH group was also lower than that of the control group. The NASH rat livers had significantly decreased vascular sucrose space as shown in Table 3. However, there was no significant change with the cellular water space in the NASH rat livers. Furthermore, also shown in Table 3, the serum biochemistry from NASH rats was abnormal compared with control rats. All biochemistry parameters, other than TG, are significantly higher, indicating that NASH is associated with substantial impairment in liver function.

Representative liver sections of Masson’s trichrome-stained liver slides from control and NASH rats are shown in Fig. 2. Sections of control livers show typical architecture under light microscopy, such as the cords of hepatocytes (one cell wide) radiating out from each hepatic venule toward the portal tract. The NASH liver sections show substantial fat deposition, ballooning hepatocytes, and inflammatory damage, which are accepted as diagnostic features of NASH (Matteoni et al., 1999). Collagen deposition was also found within the sinusoids of the NASH model rats. The FI and SI estimated by computer-assisted image analysis for control and NASH rats are listed in Table 4. The NASH model rats show significantly higher FI and SI than control rats. The total P450 concentrations in NASH livers are higher than that in control livers (Table 4).

Typical outflow perfusate concentration-time profiles for each cationic drug in control and NASH rats are shown in Fig. 3. Each profile is characterized by a rapid throughput to yield a peak within seconds of administration, followed by a fast and then a slower decline in concentration over time. Table 5 shows noncompartimental pharmacokinetic parameters for the cationic drugs derived from these profiles in control and NASH rat livers as well as some physicochemical properties of the drugs. The extraction ratio, \( E \), had decreased significantly in NASH livers for all cationic drugs other than propranolol and was higher for the more lipophilic drugs, as defined by an

**Table 4**

Comparison of FI, SI, and liver CYP450 concentration between control and NASH rat liver (mean ± S.D., \( n = 6 \))

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Rats</th>
<th>NASH Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>FI (%)</td>
<td>0.42 ± 0.11</td>
<td>7.32 ± 1.40**</td>
</tr>
<tr>
<td>SI (%)</td>
<td>0.73 ± 0.22</td>
<td>7.23 ± 1.17**</td>
</tr>
<tr>
<td>CYP450 (nmol/g liver)</td>
<td>110 ± 8</td>
<td>135 ± 10**</td>
</tr>
</tbody>
</table>

**p < 0.01.
Fig. 3. Representative outflow profile for the sucrose and cationic drugs in control (●) and NASH (△) rats.
increased apparent partition coefficient [logarithm of octanol/water partition coefficient (logP_{app})]. The MTTs of all five cationic drugs were longer in NASH livers relative to the control livers.

Also shown for each profile in Fig. 3 are the nonlinear regression lines obtained using a heterogeneous (barrier-limited and space-distributed) transit time model and a data weighting of 1/(y_{obs})^2. The pharmacokinetic parameters derived from the model fitting for each cationic drug are summarized in Table 6. The NASH livers had significantly lower permeability-surface area product (PS) for each cationic drug than the control livers. In contrast, cationic drugs had a significantly higher Cl_{int} and K_s in NASH livers than in control livers. Larger PS and Cl_{int} values were observed for the more lipophilic cationic drugs, as defined by logP_{app}, whereas the K_s values were related to their pK_a values (Fig. 4). Associated with the increased Cl_{int} of cationic drugs was an increase in total P450 concentration. Stepwise regression analysis, used to examine the potential relationships among the pharmacokinetic parameters [logarithm of permeability-surface area product (logPS), logarithm of apparent distribution ratio (logK_d), and logarithm of intrinsic elimination clearance (logCl_{app})], the cationic drug physicochemical properties (logP_{app} and pK_a), and the histopathological results (FI, SI, and P450 concentration), yielded the following relationships:

\begin{align*}
1. \logPS &= 0.721 - 0.186^{*}\logFI + 0.211^{*}\logP_{app} \quad (r^2 = 0.762, n = 30, p < 0.001)
2. \logK_d &= 0.382 + 0.163^{*}\logSI + 0.066^{*}\logP_{app} \quad (r^2 = 0.928, n = 30, p < 0.001)
3. \logCl_{int} &= -4.220 + 1.757^{*}\logCYP + 0.453^{*}\logP_{app} \quad (r^2 = 0.819, n = 30, p < 0.001)
4. \logE &= -1.460 + 0.230^{*}\logCYP - 0.123^{*}\logFI + 0.365^{*}\logP_{app} \quad (r^2 = 0.867, n = 30, p < 0.001).
\end{align*}

A comparison of the observed and predicted pharmacokinetic parameters derived from the stepwise regression analysis are shown in Fig. 5. The correlations suggest that SI, a measure of liver steatosis levels, is a predictor for cationic drug trapping in the hepatocytes, and it also suggests that the Cl_{int} is related to the P450 levels. However, it needs to be emphasized that labetalol is metabolized by glucuronidation, and the reduced labetalol clearance might be due to the decreased expression of glucuronidtransferase. An examination of the mRNA expression of Cyps (Cyp3a2, Cyp2d2, and Cyp2e1) in NASH rat livers showed that the mRNA expression of Cyp3a2, Cyp2d2, and Cyp2e1 was significantly increased in NASH rat liver than in the control rat liver (Fig. 6).

**Discussion**

In this work, we studied the hepatic pharmacokinetics of cationic drugs in rat livers that had NASH induced by a high-fat emulsion diet. This high-fat diet-induced NASH model has the same key pathological features as those reported for other rat NASH models (Koteish and Mao Diehl, 2002). The presence of substantial fat deposition, inflammatory damage, and collagen deposition in NASH livers (Fig. 2) is a typical feature of a NASH syndrome in rat livers (Ishak et al., 1991; Sheth et al., 1997). Significantly increased serum levels of HDL, LDL, and vLDL (Table 3) are most likely caused by the long-term high-fat diet. The NASH rat livers were significantly larger than that in normal rats, arising mainly from an increase in fat content, which, in turn, is reflected by significantly increased serum lipid levels (Table 3), and this result is consistent with previous research (Zou et al., 2006). Because the liver is larger in NASH rats, the perfusion rate per gram of liver is lower in the NASH rats than in the control rats (Table 3). This might cause decreased vascular sucrose space in the NASH rat livers (Table 3), as recognized in previous work (Cheung et al., 1996). However, the cellular water space in the NASH rat livers was not affected (Table 3).

The E for four cationic drugs was decreased significantly in NASH rat livers than in control rat livers, with the exception of propranolol (Table 5). This finding is consistent with the results reported for the extraction of other compounds in human and rat NASH livers. For instance, silymarin has a significantly increased area under the curve in human NASH (Schrieber et al., 2008), whereas propiverine has a small but insignificant change (Siepmann et al., 1998). We have shown that palmitate E is lower in NASH rat livers (Hung et al., 2005). The minimal effects of NASH on propiverine and on propranolol may reflect the dependence of E for these highly hepatic extracted drugs on hepatic blood flow, which has been shown to determine the E of compounds with a high Cl_{int} (Pang and Rowland, 1977).

In this work, we sought to dissect the mechanisms by which NASH was associated with altered cationic solute pharmacokinetics. Both linear regression and stepwise regression analysis showed that solute lipophilicity, logP_{app}, is a key predictor for Cl_{int} and PS, whereas solute pK_a defines ion-trapping in the cells as defined by K_s. These findings are consistent with the relationships we have reported previously for other liver conditions (Hung et al., 2001, 2002a). In addition, we showed that the observed decrease in PS for NASH rat livers could be related to a significantly increased FI in the livers, as we have also shown for other liver conditions (Hung et al., 2002a,b). We had previously used FI as a predictor for PS control, fibrotic, and cirrhotic livers (Hung et al., 2002a). In the present analysis, we found that the

**Table 5**

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>E</th>
<th>MTT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NASH</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.98 ± 0.02</td>
<td>0.96 ± 0.03</td>
</tr>
<tr>
<td>Labetalol</td>
<td>0.86 ± 0.02</td>
<td>0.79 ± 0.03**</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.84 ± 0.05</td>
<td>0.72 ± 0.05**</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>0.16 ± 0.03</td>
<td>0.88 ± 0.02**</td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.16 ± 0.03</td>
<td>0.09 ± 0.02**</td>
</tr>
</tbody>
</table>

*p < 0.05.
**p < 0.01.

**Table 6**

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>PS (mL · min⁻¹ · g liver)</th>
<th>K_d</th>
<th>Cl_{int} (mL · min⁻¹ · g liver)</th>
<th>K_r</th>
<th>K_s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>NASH</td>
<td>Control</td>
<td>NASH</td>
<td>Control</td>
</tr>
<tr>
<td>Propranolol</td>
<td>37.28 ± 2.43</td>
<td>25.94 ± 2.12**</td>
<td>10.76 ± 1.40</td>
<td>16.31 ± 1.25**</td>
<td>7.50 ± 1.26</td>
</tr>
<tr>
<td>Labetalol</td>
<td>16.22 ± 1.46</td>
<td>8.97 ± 1.13**</td>
<td>6.46 ± 1.13</td>
<td>10.94 ± 1.02**</td>
<td>3.34 ± 0.69</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>13.43 ± 1.24</td>
<td>7.59 ± 1.40**</td>
<td>9.64 ± 1.07</td>
<td>11.97 ± 0.76**</td>
<td>1.13 ± 0.20</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>10.01 ± 0.90</td>
<td>6.97 ± 0.96**</td>
<td>2.67 ± 0.22</td>
<td>4.50 ± 0.57**</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Atenolol</td>
<td>5.91 ± 0.67</td>
<td>2.60 ± 0.45**</td>
<td>9.90 ± 0.92</td>
<td>12.51 ± 1.09**</td>
<td>0.65 ± 0.18</td>
</tr>
</tbody>
</table>

*p < 0.05.
**p < 0.01.
HEPATIC PHARMACOKINETICS OF CATIONIC DRUGS IN NASH

A

\[
\text{logPS}_{\text{Control}} = 0.199 \times \text{logP}_{\text{app}} + 0.816
\]
\[r^2 = 0.826\]

\[
\text{logPS}_{\text{NASH}} = 0.222 \times \text{logP}_{\text{app}} + 0.543
\]
\[r^2 = 0.706\]

B

\[
\text{logCL}_{\text{int}, \text{NASH}} = 0.395 \times \text{logP}_{\text{app}} - 0.286
\]
\[r^2 = 0.919\]

\[
\text{logCL}_{\text{int}, \text{Control}} = 0.452 \times \text{logP}_{\text{app}} - 0.647
\]
\[r^2 = 0.824\]

C

\[
\text{logK}_{\text{app}} = 0.060 \times pK_a + 0.572
\]
\[r^2 = 0.942\]

\[
\text{logK}_{\text{Control}} = 0.073 \times pK_a + 0.312
\]
\[r^2 = 0.988\]

Fig. 4. Relationship between the physicochemical properties and the hepatic pharmacokinetic parameters for the cationic drugs in control and NASH rats. A, the logarithm of octanol/water partition coefficient (logP_{app}) for the cationic drugs and the logPS. B, the logarithm of octanol/water partition coefficient (logP_{app}) for the cationic drugs and logCL_{int}. C, the pK_a for the cationic drugs and logK_{app}. The line represents the linear regression line. The data are represented as the mean ± S.D.

be expressed in terms of their determinants, the lipophilicity of the drugs, logP_{app}, and changes in FI and P450 concentration, and much of the variability in the data can be explained by these parameters (\(r^2 = 0.876\)) (Fig. 5). The predicted E values acquired from this regression showed a good correlation with the observed E values. The changes in overall total P450 concentration in the NASH rat livers were reflected by an increase in expression of the individual hepatic P450 isoforms Cyp3a2 and Cyp2d2 (Fig. 6). These results are consistent with other studies that have shown a significantly increased expression of Cyp2e1 and Cyp1a1 in NASH rat models (Weltman et al., 1996; Zou et al., 2006; Fisher et al., 2008) and in CYP2E1 and CYP4A in NASH patient livers (Niemela et al., 2000; Chtioui et al., 2007; Prompila et al., 2008). In this work, we showed that the logarithm of P450 was a good predictor for logCL_{int} (\(r^2 = 0.819\)) (Fig. 5).

The MTTs for all five cationic drugs were increased in the NASH rat livers compared with the control rat livers (Table 3); this result is consistent with the NASH livers being significantly larger (Table 3), and it is also consistent with our earlier finding of a higher MTT of palmitate in NASH rat livers (Hung et al., 2005). The increase in liver size is likely to be due to a combination of increased fat, collagen, and P450-containing organelles in the NASH livers. SI is a surrogate measurement for fat deposition in the hepatocyte during NASH and can be used to explain the increase in cationic drug partitioning, as defined by K_a, within NASH livers (Fig. 5).

Clinical studies indicate that patients with NASH have greater hepatic fibrosis and increased hepatic P450 expression (Niemela et al., 2000; Chtioui et al., 2007; Prompila et al., 2008). However, the relationships between hepatic clearance and changes in liver morphology and biochemistry in NASH have not been studied previously. The present work suggests that the E of drugs with lower E is likely to be severely reduced in patients with NASH because of collagen deposition, even though there is a balancing increase in CL_{int} because of enhanced P450 expression. Therefore, a reduction in dosing may be required for patients with NASH who have been given heptatically cleared drugs. However, the measurement of collagen deposition normally entails a liver biopsy, which can be painful and has a significant morbidity. An alternative measure of collagen deposition is noninvasive elastography (Wang et al., 2009). The stiffness of the liver measured with this method correlates well with the liver fibrosis level. The fibrosis score is another noninvasive estimate of liver fibrosis that can give a semiquantitative evaluation of the fibrosis level in patients through analysis of routinely measured and readily available clinical and laboratory data (Wong et al., 2008). The observed relationship between FI and E, as reported in this work, provides another possibility, namely, the use of hepatic drug extraction data as a measure of the extent of fibrosis in NASH patients. The difficulty in using drugs for such a purpose is that a decrease in drug extraction could also arise from alterations in hepatic blood flow, other clearance mechanisms, and altered hepatic metabolism.

The IPRL model provides controlled and reproducible conditions under which the disposition of various drugs in liver diseases can be studied. It also allows the effects of other organs and recirculation from those organs to be avoided, as well as the ability to study toxic drugs or drugs in excess of their usual therapeutic concentrations. NASH in human livers appears to be similar to NASH in rat livers (Zou et al., 2006), and, therefore, the results obtained here should be applicable to patients with NASH. However, it does have a limitation in that it does not simulate all in vivo conditions and especially events occurring in humans. For instance, NASH may also cause significantly increased serum levels of cholesterol and lipoproteins (Table 3). These, in turn, can affect the binding of lipophilic drugs in the
blood and, hence, their elimination in the liver (Yeganeh and McLachlan, 2002). Other drugs, such as some of the β-blockers studied here, can induce hemodynamic effects resulting in a change in hepatic blood flow (Sabba et al., 2001). The function of the human liver also differs from that of the rat liver in its morphology (e.g., bile from rat livers does not drain into a gall bladder) and P450 expression. Furthermore, a limitation in the rat liver disease model used here is that it does not precisely represent conditions in humans. NASH in humans also contains heterogeneity in terms of both morphology and function, which the liver disease model lacks here.

In summary, this study has shown that the hepatic pharmacokinetics of cationic drugs are significantly changed when NASH has been induced by a high-fat diet. We showed that the individual hepatic pharmacokinetic parameters (PS, \(K_v\), C\(_{L_{int}}\), and \(E\)) could be related to the physicochemical properties of cationic drugs (log \(P_{app}\) or pK\(_a\)) and liver histopathological changes (FI, SI, and P450 concentration) in the NASH livers. This work adds to our overall knowledge on how liver diseases and aging affect hepatic drug disposition, as also studied by other groups (Le Couteur et al., 2005; Fisher et al., 2009; Makino et al., 2009).

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Authorship Contributions
Participated in research design: Li, Fletcher, Crawford, and Roberts.
Conducted experiments: Li, Thorling, and Zhang.
Contributed new reagents or analytic tools: Robertson and Fletcher.
Performed data analysis: Li and Roberts.
Wrote or contributed to the writing of the manuscript: Li, Robertson, Zhang, Crawford, and Roberts.

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