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

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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 (C_Lint) and permeability-surface area product (PS) with p_K defined the extent of sequestration in the liver [apparent distribution ratio (Kv)]. The main pathophysiological determinants were liver fibrosis, leading to a decreased PS, liver fat causing an increase in K_v, and an increase in both total liver cytochrome P450 (P450) concentration and P450 isoform expression for Cyp3a2 and Cyp2d2, causing an increase CL_int in NASH rat livers compared with control livers. Changes in hepatic pharmacokinetics (PS, K_v, CL_int, and E ratio) as a result of NASH were related to the physicochemical properties of drugs (lipophilicity or p_K) 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 Mamlok, 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; Chitoui 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; Schriebner 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, intra-cellular 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. [14C]Sucrose were all obtained from Sigma-Aldrich (St. Louis, MO). Physicochemical properties of these five cationic drugs are summarized in Table 1.

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>Molecular Weight</th>
<th>logPo/water</th>
<th>f_u</th>
<th>pK_a</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>328.41</td>
<td>2.69</td>
<td>0.57</td>
<td>7.4</td>
<td>Glucuronosyltransferase</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>CYP1A2</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>

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

b Fraction unbound in perfusate acquired with microfiltration method.

Negative logarithm of the ionization constant.

**Analytical Procedure.** The MID samples, which contain [14C]Sucrose and [3H]Water, 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 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.

**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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tions. 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 Hanks’ 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-Cl buffer (pH 7.4) using a tissue homogenizer. To separate the liver microsome 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 dithionite-reduced difference spectrum of carbon monoxide-bubbled samples using 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 space for the unbound solute is defined by influx rat constant ($k_{on}$) and efflux rat constant ($k_{off}$). The cellular binding processes were assumed to be one rapid and one slow dissociation process, respectively. The rapid binding process was simplified to an instantaneous equilibrium process characterized by $K_d = k_{on}/k_{off}$ because the dissociation rate constant of the rapid process was extremely high. The slow binding process contains the intracellular binding rate constant ($k_{on}$) and intracellular unbinding rate constant ($k_{off}$), respectively. The elimination rate constant $k_e = CL_{int}/V_e$ is the intrinsic elimination clearance per cellular water volume ($V_e$). The density of cellular residence times $f(t)$ 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, $f(t) = L^{-1}[f(s)]$, as described previously (Weiss, 1999, 2000) (eq. 1):
molecule (in this study, sucrose) \( f_{d}(s) \) (see eq. 2 and the density function of successive sojourn times \( f_{s}(s) \) of the drug molecules in the cellular space):
\[
 f(s) = f_{d}[s + k_{o}(1 - f_{s}(s))]
\]
(2)
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.** Non-compartmental 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:
\[
 F = \frac{Q \cdot AUC}{D}
\]
(3)
\[
 AUC = \int_{0}^{t} C(t) \, dt
\]
where \( AUC \) 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 were expressed in molar equivalents. \( E \) equals 1 - \( F \):
\[
 MTT = \frac{\int_{0}^{t} C(t) \, dt}{AUC}
\]
(4)

**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 (where 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 (Matsui 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 noncompartmental 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<sub>app</sub>). 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<sub>obs</sub>)<sup>2</sup>. 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 lower permeability-surface area product (logPS), logarithm of apparent distribution ratio (logK<sub>a</sub>), and logarithm of intrinsic elimination clearance (logC<sub>int</sub>) than the control livers. In the present analysis, we found that the CL<sub>int</sub> 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 glucuronyltransferase. An examination of the mRNA expression of Cyp2e1 (Cyp3a2, and 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 Mae 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 palmiote 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<sub>int</sub> (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<sub>app</sub>, is a key predictor for CL<sub>int</sub> and PS, whereas solute pK<sub>a</sub> defines ion-trapping in the cells as defined by K<sub>a</sub>. 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

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### Table 5

Nonparametric estimates of cationic drug pharmacokinetic parameters in perfused rat livers (mean ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>E</th>
<th>MTT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NASH</td>
<td>Control</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>0.08 ± 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

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### Table 6

Kinetic parameters derived from two-phase stochastic model fitting for cationic drugs in control and NASH model rat livers (mean ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Cationic Drugs</th>
<th>PS (ml · min&lt;sup&gt;-1&lt;/sup&gt; · g liver)</th>
<th>K&lt;sub&gt;S&lt;/sub&gt;</th>
<th>K&lt;sub&gt;R&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></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>
</tr>
<tr>
<td>Labetalol</td>
<td>16.22 ± 1.46</td>
<td>8.97 ± 1.13**</td>
<td>6.45 ± 1.13</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>13.43 ± 1.24</td>
<td>7.59 ± 1.40**</td>
<td>9.64 ± 1.07</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>10.01 ± 0.90</td>
<td>6.97 ± 0.96**</td>
<td>2.67 ± 0.22</td>
</tr>
<tr>
<td>Atenolol</td>
<td>5.91 ± 0.67</td>
<td>2.60 ± 0.45**</td>
<td>9.90 ± 0.92</td>
</tr>
</tbody>
</table>

* p < 0.05

** p < 0.01

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HEPATIC PHARMACOKINETICS OF CATIONIC DRUGS IN NASH

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 (log \( P_{\text{app}} \)) for the cationic drugs and logPS. B, the logarithm of octanol/water partition coefficient (log \( P_{\text{app}} \)) for the cationic drugs and log\( \text{Cl}_{\text{int}} \). C, the \( pK_a \) for the cationic drugs and log\( K_v \). The line represents the linear regression line. The data are represented as the mean ± S.D.

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

be expressed in terms of their determinants, the lipophilicity of the drugs, log\( P_{\text{app}} \), 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 (Weltlman 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 log\( \text{Cl}_{\text{int}} \) (\( r^2 = 0.819 \)) (Fig. 5).

The MT Ts for all five cationic drugs were increased in the NASH rat livers compared with the control rat livers (Table 5); 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 \( K_v \), 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 \( \text{Cl}_{\text{int}} \) because of enhanced P450 expression. Therefore, a reduction in dosing may be required for patients with NASH who have been given heptically 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 \( E \) 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, $K_v$, $CL_{int}$, and $E$) could be related to the physicochemical properties of cationic drugs ($P_{app}$ or $pK_a$) and liver histopathological changes (IF, 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.

References


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