Liver Fibrosis Impairs Hepatic Pharmacokinetics of Liver Transplant Drugs in the Rat Model

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Summary: This study aims to investigate hepatic pharmacokinetics of the four most common drugs (metoprolol, omeprazole, spironolactone, and furosemide) given to patients undergoing liver transplantation before surgery. The investigation was carried out in CCl4-induced fibrotic perfused rat livers and the results were compared to those in normal rat liver. Drug outflow fraction-time profiles were obtained after bolus injection into a single-pass-perfused normal or fibrotic rat liver. The pharmacokinetic parameters were estimated using previously developed barrier-limited and space-distributed models. The results showed a marked increase in the liver fibrosis index for CCl4-treated rats compared to controls (p < 0.05). The extraction ratios (E) for all drugs were significantly lower (p < 0.05) in fibrotic than in normal livers and the decrease in E was consistent with the decrease in intrinsic clearance and permeability–surface area product. In addition, other than for furosemide, the mean transit times for all drugs were significantly longer (p < 0.01) in the fibrotic livers than in normal livers. Pharmacokinetic model and stepwise regression analyses suggest that these differences arise from a reduction in both the transport of drugs across the basolateral membrane and their metabolic clearance and were in a manner similar to those previously found for another group of drugs.

Keywords: hepatic pharmacokinetics; liver fibrosis; omeprazole; spironolactone; metoprolol; furosemide; propranolol

Introduction

Hepatic fibrogenesis is characterised by progressive accumulation of extracellular matrix in the subendothelial space of Disse, causing capillarisation of sinusoids and functional changes in surrounding cell types. Cirrhosis is the end point of the fibrogenic process, and complications from cirrhosis, including portal hypertension and gastrointestinal tract haemorrhage, decompensated liver function including resistant ascites, and hepatocellular carcinoma, are the ultimate causes of death in most patients. Whilst liver transplantation is the only definitive therapy for advanced liver disease, the therapeutic regime of most patients with advanced liver diseases includes a combination of diuretics (furosemide and spironolactone), agents to reduce portal pressure (propranolol), and proton pump inhibitors. However, the dosing regimen and choice of drugs for safety and efficacy is complicated by the declining liver function in the diseased liver.1–6

The liver plays a major role in the absorption and disposition of various plasma substrates, including drugs. In the normal liver, the endothelium is attenuated and there is a minimum amount of collagen in the space of Disse,
with no basement membrane and only minimum barriers to substrate diffusion. Plasma substrates, including drugs, transfer freely in both directions through the endothelial fenestrations and space of Disse. Most substrates transfer freely from the sinusoid to the hepatocyte in the normal liver. Collagen deposits in the space of Disse and defenestration occur during the development of cirrhosis. The normal hepatic lobular architecture is replaced by interconnecting bands of fibrous tissue surrounding nodules of regenerating hepatocytes, resulting in changes in liver structure, haemodynamics, and function. There exists good evidence that the changes in structure invariably lead to alterations in drug handling within the liver.3,5-6 Other changes caused by fibrosis, such as enzyme level or enzyme activity and altered sinusoidal perfusion, can have profound and differing effects on the hepatic clearances of both high- and low-clearance drugs.5) Therefore, the pharmacokinetic behaviour of drugs in patients with fibrosis is different from that in healthy individuals and is influenced by numerous factors, including the severity of the disease. At present, only general rules of rational drug use can be proposed to the clinician treating a patient with liver fibrosis, since there are no readily available laboratory or clinical parameters that provide a practical guide to dosage adjustment in such patients.7,8

Our previous studies have shown that hepatic fibrosis affects hepatic sinusoidal morphology and leads to impaired hepatic uptake of cationic drugs, anionic drugs, and water.8) We showed that the disposition of cationic drugs, such as propranolol, was directly related to the extent of fibrosis caused by liver cirrhosis and the physicochemical properties of the drug.9) Here, we attempt to define hepatic pharmacokinetics of the four most common drugs (metoprolol, omeprazole, spironolactone, and furosemide) given to patients undergoing liver transplantation before surgery.10-14 The investigation was carried out in normal and CCl4-induced fibrotic perfused rat livers using the multiple indicator dilution technique (MID). The rat fibrosis model, obtained by repeated intraperitoneal administration of CCl4, is one of the standard and most widely accepted models of liver disease. The MID technique involves the injection of a mixture of radio-labelled indicators (e.g. sucrose) into the portal vein and the characterization of indicator outflow fraction versus time profiles.

In this study, we report that the hepatic extraction for all drugs studied was greatly impaired in the fibrotic liver. The decrease in the drug extraction ratio was consistent with a decrease in intrinsic clearance (CLint) and permeability–surface area product (PS) for fibrotic livers.

Materials and Methods

Chemicals: Omeprazole, spironolactone, metoprolol, furosemide, propranolol, and CCl4 were obtained from Sigma-Aldrich (St. Louis, MO) and used without any further purification. [14C]-sucrose and [1H]-water were obtained from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK).

CCl4-induced liver fibrosis rat model: The animal studies adhered to the Principles of Laboratory Animal Care (NIH publication #85–23, revised 1985) and were approved by the Animal Ethics Committee of the University of Queensland. The CCl4-induced liver fibrosis model was established following a previously reported procedure.15) Briefly, male Wistar rats (weighing approximately 150 g) were placed in all-wire-mesh cages in groups of six each (two groups), given sodium phenobarbital (35 mg/dL) (Biotech International Ltd., Rocklea, Australia) in their drinking water ad libitum, and the first dose of CCl4 was given 10 days later. Liver fibrosis was induced by giving CCl4 once a week for 12 weeks. CCl4 was dissolved in corn oil and given by intragastric gavage. The initial dose of CCl4 was 0.04 mL in 0.96 mL corn oil. Rat body weight was monitored daily, and each subsequent dose was adjusted depending on the weight loss associated with the preceding dose. All doses were multiples of the original doses, and the total dose volume was 1.0 mL. Normal control rats were treated identically to the liver fibrosis rats, except that they were given a 1.0 mL dose of corn oil weekly without CCl4.

In situ rat liver perfusions: In situ rat liver perfusions were conducted using a standard procedure as outlined previously.16,17) Briefly, laparotomy was performed after the rat was anaesthetised by intraperitoneal injection of ketamine hydrochloride 80 mg.kg-1 (Parnell Laboratories, Australia) and xylazine 10 mg.kg-1 (Bayer Australia, Pymble NSW, Australia). The rat was heparinised (200 units heparin sodium; David Bull Laboratories Australia, Mulgrave, Victoria, Australia) via the inferior vena cava. The bile duct was cannulated using polyethylene tubing (PE-10; Clay Adams, Franklin Lakes, NJ). The portal vein was then cannulated using an intravenous catheter and the liver was perfused via this cannula with MOPS buffer (pH = 7.4) with 2% bovine serum albumin and 15% (v/v) prewashed canine RBCs (Veterinary Specialist Service, Brisbane, Australia). The perfusate was oxygenated using a silastic tubing lung ventilated with oxygen. The perfusion system used was non-recirculating and used a peristaltic pump (Cole-Parmer, Vernon Hills, IL). After perfusion was effectuated, the animals were killed by thoracotomy and the thoracic inferior vena cava was cannulated with PE-240 tubing (inner diameter, 1.67 mm; outer diameter, 2.42 mm; length, 10 cm; Clay Adams). The animals were placed in a temperature-controlled perfusion cabinet maintained at 37°C.

Bolus studies: After a 10-min perfusion-stabilization period, an injectate aliquot (50 µL) of perfusion medium containing one of the drugs (propranolol/metoprolol 5 mM, furosemide 1 mM, omeprazole 10...
mM, or spironolactone 30 mM), [3H]-water (3×10^6 dpm), and [14C]-sucrose (3×10^6 dpm) was injected into the liver with outlet samples collected via a fraction collector over 4 min as previously reported. A maximum of six injections were made with the order of injection randomized and no repeat of the same injection in the same rat liver. The total perfusion period in each liver was less than 2 h; a stabilization period of 10 min was afforded between consecutive injections. Liver perfusion outlet samples were centrifuged at 3000 g (25°C) for 3 min, and aliquots of supernatant were taken for scintillation counting or high-performance liquid chromatography (HPLC) analysis as appropriate. The outflow [3H]-water, [14C]-sucrose, and drug concentrations were determined by the MINAXI Beta TRI-CARB 4000 series liquid scintillation counter (Packard BioScience, Meriden, CT) or HPLC analysis, respectively.

**Analytical procedure:** HPLC analysis was generally carried out using a system consisting of a Shimadzu LC-10AD pump; a Shimadzu SIL-6B auto injector; a Symmetry C18 5 μm 3.9×150 mm column with C18 guard column; a Shimadzu RF-10 AXL fluorescence detector for furosemide, propranolol, and metoprolol (excitation 268 nm, 300 nm, and 225 nm; emission 410 nm, 375 nm, and 310 nm, respectively); and a Shimadzu UV detector for omeprazole and spironolactone (wavelength 302 and 238 nm, respectively). The mobile phases used for furosemide, propranolol, and metoprolol were 10 mM KH₂PO₄ buffer with 30%, 25%, and 20% acetonitrile at pH 3.0 (flow rate 1.0 mL/min), respectively. The mobile phase used for spironolactone was water with 50% acetonitrile at pH 3.0 (flow rate 1.0 mL/min). The mobile phase used for omeprazole was water with 28% acetonitrile (flow rate 1.0 mL/min). The standards and samples were prepared for HPLC analysis according to the following extraction procedure. 1) Furosemide, metoprolol, and propranolol: 50-μL aliquots of sample were mixed with 100 μL of 0.1 M ZnSO₄ and 100 μL of methanol in a 1.5-mL Eppendorf tube; 2) Omeprazole: 50-μL aliquots of sample were mixed with 50 μL of 0.1 M ZnSO₄ and 100 μL of methanol in a 1.5-mL Eppendorf tube; 3) Spironolactone: 100-μL aliquots of sample were mixed with 100 μL of 0.1 M ZnSO₄ and 100 μL of methanol in a 1.5-mL Eppendorf tube. The tubes were vortexed and centrifuged and 50 μL of the resulting supernatant was injected into the HPLC column.

**Perfusion medium binding:** These experiments were performed following the previously reported method in 2% BSA MOPS buffer (pH 7.4), which contained 15% (v/v) prewashed canine RBCs. The unbound fraction (f_{ub}) of tested drugs was determined as the ratio of the free concentration to total concentration of drug.

**Data analysis:** A mixture of two inverse Gaussian density functions with correction for catheter effects was used to estimate the extracellular space (V_B, determined by [14C]-sucrose). A barrier-limited plus space-distributed liver model with correction for catheter effects was used to estimate the total water volume (V_W, determined by [3H]-water); V_W was then used to estimate the cellular water volume, V_C, defined as (V_W–V_B). A heterogeneous (barrier-limited and space-distributed) transit time model (the two-phase stochastic model), as shown in Figure 1, was used to estimate the pharmacokinetic parameters for the permeating solutes. Briefly, the model assumes drug transfer into and out of hepatocytes with influx and efflux rate constants k_{in} and k_{out}, respectively. Within the cell, drug disposition is described by equilibrium amount ratio K_R, characterising the rapidly equilibrating binding sites; the binding and unbinding rate constants k_{on} and k_{off}, characterising the slowly accessible binding sites; and elimination rate constant k_e. Permeability-surface area product (PS) established from the expression: PS = k_{in}V_B/f_{ub}. The intrinsic elimination clearance (CL_{int}) is obtained from the expression: CL_{int} = k_eV_C. The stochastic approach represents the transit of a drug molecule through the organ as a series of sojourns in one of the two regions described by density functions. The distribution of successive sojourn times in the tissue region, i.e.,

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**Fig. 1.** Schematic overview of hepatocellular drug transport in the stochastic space-distributed liver model (modified from Hung et al. 17.)
the density of cellular residence times \( \hat{f}(s) \) describes the hepatocellular distribution and elimination kinetics. The sojourn time distribution \( f(t) \) 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, \( \hat{f}(s) = L^{-1}[f(t)] \), as described previously.\(^{19} \)

\[
\hat{f}(s) = \frac{(s + k_{in})k_{in}}{s^2(k_{in}k_{out})(1 + K_s) + s(k_{in}k_{out})k_{eff} + K_sk_{eff} + k_s + k_{out}} \tag{1}
\]

The transit time density function \( \hat{f}(s) \) of drug molecules across the liver can then be derived in terms of the extracellular transit time density of non-permeating reference molecules \( \hat{f}(s) \), in this study sucrose, \( \hat{f}_{sucrose}(s) \), and the density function of successive sojourn times \( f(s) \) of the drug molecules into the cellular space:

\[
\hat{f}(s) = \hat{f}_{sucrose}(s + k_{out}(1 - \hat{f}(s))) \tag{2}
\]

The fractional outflow versus time data were fitted in the time domain using a numerical inverse Laplace transformation of the appropriate transit time density function applying the non-linear regression program SCIENTIST (MicroMath Scientific Software, Salt Lake City, UT). Data were analysed by a sequential procedure: first, the fractional outflow curve of the extracellular marker \( ^{14}\text{C}sucrose \) was modelled using the function:

\[
C_{inj}(t) = \frac{Dose}{Q} L^{-1}[\hat{f}(s)] \hat{f}(s) \tag{3}
\]

where dose is the amount of sucrose injected; \( Q \) is the perfusion flow rate; \( \hat{f}(s) \) accounts for the catheter and \( \hat{f}_{sucrose}(s) \) includes the large vessel transit time. The transit time density (TTD) of the non-permeating indicator sucrose \( \hat{f}_{sucrose}(s) \) is given by:

\[
f(s) = p\hat{f}(s) + (1 - p)\hat{f}(s) \tag{4}
\]

with

\[
\hat{f}(s) = \exp \left\{ \frac{1}{CV^2} - \left[ \frac{MT}{CV^2/2} \left( s + \frac{1}{2MTCV^2} \right) \right]^{1/2} \right\}
\]

\( i = 1, 2 \) \tag{5}

Equation 5 represents the Laplace transform of the inverse Gaussian density function with mean \( MT \), and relative dispersion \( CV^2 \). Second, utilising this information, the outflow concentration data of the permeating drugs, \( C(t) \), were analysed, i.e. the parameters \( MT, CV^2 \)\( (i = 1, 2) \), and \( p \) of the individual fits of \( ^{14}\text{C}sucrose \) data were substituted as fixed parameters in \( \hat{f}(s) \) of the model (eq. 2) and the pharmacokinetic parameters were estimated.

\[
C(t) = \frac{Dose}{Q} L^{-1}[\hat{f}_{sucrose}(s) \hat{f}(s)] \tag{6}
\]

Nonparametric estimates of hepatic extraction ratio (E), mean transit time (MTT), and normalized variance (CV\(^2 \)) were determined from the outflow fraction versus time profiles for the drugs as described previously.\(^{17} \)

**Histopathologic analyses:** Rat liver specimens were fixed in 10% neutral formalin and embedded in paraffin. Haematoxylin and eosin (HE) staining was performed according to the standard procedure. In the evaluation of the extent of liver fibrosis, Sirius red was used to specifically stain collagen fibres. The degree of fibrosis was then quantified by computer-assisted image analysis (Image-Pro Plus version 3.0 for Windows; Media Cybernetics, Inc., Silver Spring, MD). For each rat, the area of stained fibrous tissue in five randomly selected fields was measured, and the average was expressed as fibrosis per unit area of liver tissue (termed the fibrosis index, FI) as described previously.\(^{20} \)

**Statistical analysis:** Data were expressed as mean ± S.D. unless otherwise stated. Stepwise regression analysis was performed using the program SPSS 10.1 for Windows. Statistical analysis was performed with two-way analysis of variance, Student’s t test, and regression analysis where appropriate. \( p < 0.05 \) was considered statistically significant. Linear regression equations were considered only when \( r^2 > 0.5 \).

**Results**

**Liver physiology:** CCl\(_4\)-induced fibrotic rat livers had a significantly higher in vivo perfusion pressure (1.42 ± 0.12 cm of H\(_2\)O) than control rat livers (0.84 ± 0.11 cm of H\(_2\)O, \( p < 0.01 \)). Liver bile flow in CCl\(_4\)-treated rat liver (0.53 ± 0.06 \( \mu \)L.min\(^{-1}.g\(^{-1} \) of liver) was significantly lower than that in the control rat liver (0.86 ± 0.09 \( \mu \)L.min\(^{-1}.g\(^{-1} \) of liver, \( p < 0.05 \)). The liver oxygen consumption in the control group (1.24 ± 0.20 \( \mu \)mol.min\(^{-1}.g\(^{-1} \) of liver) was significantly lower than that in the CCl\(_4\)-treated group (0.98 ± 0.22 \( \mu \)mol.min\(^{-1}.g\(^{-1} \) of liver, \( p < 0.05 \)). The liver index (liver weight/body weight) in control livers (2.56 ± 0.28%) was significantly lower than that in fibrotic livers (5.43 ± 0.69%, \( p < 0.05 \)).

**Histopathology:** Histopathologic examination showed no abnormalities in the normal liver (Fig. 2A). Liver tissue from CCl\(_4\)-injured rats displayed higher rates of steatosis, cell necrosis, and inflammatory infiltration than tissue from control rats. Fibrous septa encompassing regenerating hepatocytes in pseudo-lobules and higher liver collagen content were also present, fulfilling the diagnostic standard for irreversible hepatitis fibrosis (Fig. 2B and C). Computer-assisted image analysis results showed that the fibrotic livers had a significantly higher FI value than normal livers (\( p < 0.01 \) (Table 2).

**Hepatic extraction ratio and mean transit time:** Table 1 shows that the fibrotic livers have a significantly lower E than that of the normal livers for the same drug. The MTT values of metoprolol, propranolol, omeprazole, and spironolactone in fibrotic livers were significantly
higher than those of the normal livers for the same drug; however, this was not the case for furosemide. The change of CV² for the five drugs did not appear to follow the same trend as E in the control group and CCl₄-treated group. The CV² of omeprazole and spironolactone were significantly higher in the CCl₄-treated rat livers than that in normal control livers. However, the CV² of the other three drugs (metoprolol, propranolol and furosemide) did not differ significantly between fibrotic and control rat livers.

Data fitting of outflow fraction-time profiles and modelling: Figure 3 shows typical plots of logarithmic normalized outflow concentrations versus time data for the five drugs in normal control and CCl₄-treated fibrotic rat livers. All MID data appeared to be adequately fitted by the models. Table 2 shows that the estimated model parameters for extracellular volume Vₛ and cellular water volume V_C in fibrotic rat livers were significantly higher than those in normal control rat livers (p < 0.01). The pharmacokinetic parameters for each drug are shown in Table 3. The kₛ and PS of the five drugs in the fibrotic livers were significantly lower than those in normal livers. The kₛ and CL_{int} of omeprazole, spironolactone, metoprolol, and propranolol were significantly lower than those in normal control groups. Furthermore, the predicted log PS of the five drugs studied here as defined by the nature of the drug (log P) and the histopathological results (FI) using a previously developed equation for a different set of drugs (log PS = 1.647 − 0.028 FI + 0.08 log P)9 showed a good correlation with the observed values. Figure 4 shows the predicted versus observed PS values for the drugs studied here as well as those reported previously.9

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**Table 1. Nonparametric pharmacokinetic parameters for commonly used liver transplantation drugs in normal and fibrotic rat livers (mean ± S.D., n = 6)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Hepatic extraction ratio (E)</th>
<th>Mean transit time (MIT, sec)</th>
<th>Normalized variance (CV²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>CCl₄</td>
<td>Normal</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>0.98 ± 0.02</td>
<td>0.87 ± 0.07**</td>
<td>55.56 ± 10.45</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>0.97 ± 0.03</td>
<td>0.83 ± 0.12*</td>
<td>17.46 ± 4.06</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.99 ± 0.01</td>
<td>0.93 ± 0.02**</td>
<td>36.20 ± 6.24</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>0.99 ± 0.01</td>
<td>0.95 ± 0.02**</td>
<td>8.06 ± 2.29</td>
</tr>
<tr>
<td>Furosemide</td>
<td>0.31 ± 0.07</td>
<td>0.18 ± 0.03**</td>
<td>16.55 ± 3.19</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01, compared with control group.

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**Fig. 2.** Histological images of liver tissues from normal control rat and a rat with CCl₄-induced liver fibrosis
(A) Normal lobular architecture with central veins and radiating hepatic cords in normal control rat. (B) and (C) show markedly fatty degeneration, necrosis, infiltration of inflammatory cells, and nodular formation of fibrotic septa, encompassing regenerated hepatocytes into pseudo-lobules and accompanied by increased collagen content. (A) and (B) were stained with HE; (C) was stained with Sirius red. The magnification for A and B was × 100, and for C was × 40.

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**Table 2.** Extracellular space (Vₛ, mL·g⁻¹ of liver), cellular distribution volume of water (V_C, mL·g⁻¹ of liver), and fibrosis index changes in CCl₄-induced rat fibrotic livers (mean ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Vₛ</th>
<th>V_C</th>
<th>Fibrosis index (FI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.26 ± 0.05</td>
<td>0.70 ± 0.10</td>
<td>0.62 ± 0.25</td>
</tr>
<tr>
<td>CCl₄</td>
<td>0.38 ± 0.03*</td>
<td>0.88 ± 0.13*</td>
<td>9.87 ± 3.34**</td>
</tr>
</tbody>
</table>

*p < 0.05; **p < 0.01, compared with normal control group.
Discussion

In this study, a CCl4-induced rat liver fibrosis model was established to study the hepatic disposition of omeprazole, spironolactone, metoprolol, and furosemide, as they are the most widely used pre-operative drugs in liver transplantation and little data appears to be available on their disposition in fibrotic livers. Consistent with previous findings, CCl4 treatment was found to induce severe fibrosis with centrilobular necrosis and stenosis, and this was confirmed by an increased estimated fibrosis index (determined by computer-assisted image analysis) and histological examination.21) Increased liver extracellular (Vb) and cellular (Vc) volumes (obtained through model fitting) were observed in the CCl4-induced fibrotic rat liver.5,9) The PS of each drug in the diseased rat liver was significantly lower than the PS in the control rat livers, as demonstrated by impaired uptake of solute across the capillarised endothelium. These changes mimic the pathological changes in hepatic circu-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Log P a</th>
<th>$f_{ua}$ b</th>
<th>$k_{in}$ c Normal</th>
<th>$k_{in}$ CCl4</th>
<th>$k_{id}$ Normal</th>
<th>$k_{id}$ CCl4</th>
<th>Cl int e Normal</th>
<th>Cl int CCl4</th>
<th>PS f Normal</th>
<th>PS f CCl4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metoprolol</td>
<td>1.789</td>
<td>0.61</td>
<td>0.93±0.30</td>
<td>0.28±0.08**</td>
<td>0.67±0.12</td>
<td>0.26±0.06**</td>
<td>0.28±0.08</td>
<td>0.13±0.04**</td>
<td>0.41±0.16</td>
<td>0.19±0.06*</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>2.173</td>
<td>0.34</td>
<td>0.89±0.15</td>
<td>0.39±0.11**</td>
<td>1.08±0.40</td>
<td>0.15±0.28**</td>
<td>0.44±0.13</td>
<td>0.02±0.00**</td>
<td>0.69±0.15</td>
<td>0.43±0.09*</td>
</tr>
<tr>
<td>Propranolol</td>
<td>3.097</td>
<td>0.66</td>
<td>1.02±0.20</td>
<td>0.68±0.16*</td>
<td>0.63±0.13</td>
<td>0.30±0.07**</td>
<td>0.26±0.07</td>
<td>0.16±0.06**</td>
<td>0.42±0.08</td>
<td>0.33±0.03**</td>
</tr>
<tr>
<td>Spironolactone</td>
<td>3.124</td>
<td>0.26</td>
<td>0.91±0.11</td>
<td>0.61±0.13**</td>
<td>1.64±0.36</td>
<td>0.31±0.07**</td>
<td>0.69±0.22</td>
<td>0.18±0.05**</td>
<td>0.98±0.20</td>
<td>0.55±0.11**</td>
</tr>
<tr>
<td>Furosemide</td>
<td>3.001</td>
<td>0.02</td>
<td>0.03±0.01</td>
<td>0.01±0.00*</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.40±0.14</td>
<td>0.18±0.12*</td>
</tr>
</tbody>
</table>

a Log octanol/water partition coefficient values at pH 7.4.
b Fraction unbound in perfusate.
c Influx rate constant across the permeability barrier (plasma membrane) (s⁻¹).
d Elimination rate constant (s⁻¹).
e Intrinsic elimination clearance (mL·s⁻¹·g⁻¹ of liver).
f Permeability–surface area product (mL·s⁻¹·g⁻¹ of liver).
*p < 0.05; **p < 0.01 compared with normal control group.

Table 3. Kinetic parameters derived from the two-phase stochastic model fitting in normal control and CCl4-induced rat fibrotic livers (mean ± S.D., n = 6)
PS is defined as $K_mD_mS/h_m$, where $K_m$ is the plasma membrane-unbound perfusate concentration coefficient, $D_m$ is the solute diffusivity in the plasma membrane, $S$ is the surface area, and $h_m$ is the membrane path length for diffusion. In the absence of transporters, $K_m$ is defined by the solute lipophilicity (Log $P$ as a surrogate). An impaired uptake of solute in fibrotic liver is consistent with a slower solute diffusivity ($D_m$) and longer diffusion path length ($h_m$) as a consequence of collagenization of Disse’s space.\(^{22}\) Our previous study showed that $F_l$ was a surrogate measure for changes in $D_m$ and $h_m$.\(^{9}\) In addition, we derived an expression by stepwise regression that enabled PS of the drugs studied to be estimated from $\log P$ and $FI$.\(^{9}\) This study has shown that the expression accurately estimates PS values for the group of solutes studied here that are consistent with the observed PS values obtained by experimentation. The severity of fibrosis in this study, as defined by the perfusion pressure associated with the normal and fibrotic livers and respective measures of liver viability (bile flow, oxygen consumption), was similar to that previously described.\(^{23}\)

This study focused on liver disposition kinetics of the pre-operative drugs omeprazole, spironolactone, metoprolol, and furosemide used in liver transplantation. Propranolol, used in our earlier study,\(^{9}\) was used as a control. Good regression fits were found for each of the five drugs in fibrotic and normal rat livers using a heterogeneous (barrier-limited and space-distributed) transit time model (Fig. 3), consistent with our earlier study.\(^{9}\) The results show that the influx rate constant $k_{in}$ for all tested drugs was significantly decreased in fibrotic rat livers relative to normal rat livers. The parameter $k_{in}$ is determined by the clearances into the hepatocyte via the sinusoidal membrane and by the distribution spaces for the unbound drugs between the hepatocyte and the perfusate. The decreased $k_{in}$ in fibrotic livers, as opposed to healthy liver, is most likely due to the increased barrier to solute entry to the cells resulting from collagenisation of the space of Disse. Our previous study in fibrotic rat livers also showed that $k_{in}$ mainly depends on $F_l$; thus, the increased $F_l$ in fibrotic rat liver would explain the decreased $k_{in}$ in CCl4-treated rats.\(^{17}\) In this study, these findings were confirmed using drugs with a range of pharmacological effects, i.e., diuretics, a proton pump inhibitor, and $\beta$-receptor blockers.

We also estimated the intrinsic clearance (CL\textsubscript{int}) for the four drugs in fibrotic and healthy rat livers and found that CL\textsubscript{int} was lower in fibrotic rat livers compared to controls. Drug hepatic CL\textsubscript{int} is usually determined by metabolic and/or biliary excretion enzyme activity. Small molecules (low molecular weight) and lipophilic drugs, as studied here, are normally cleared by hepatic metabolism,\(^{24}\) and a decreased cytochrome P450 (CYP) concentration in the liver tissue has been reported in fibrotic rat liver.\(^{25}\) The highly bound furosemide has very low $k_{in}$, $k_r$, and CL\textsubscript{int} values (data not shown).

The present work has shown that the hepatic extraction ratio ($E$) for all tested drugs decreases in fibrotic rat livers. In general, hepatic extraction is a function of CL\textsubscript{int}, PS, $F_{int}$, and flow rate ($Q$).\(^{26–30}\) The observed decrease in $E$ in the diseased liver is consistent with the decrease in CL\textsubscript{int} and PS for the fibrotic livers. We previously showed that $E$ and CL\textsubscript{int} are highly correlated with CYP activity of the liver tissue, and better correlations may be expected by relating CL\textsubscript{int} to individual CYP isozymes responsible for the metabolism of a given drug.\(^{9}\) CYP isozyme regulation and expression in liver fibrosis has been reviewed.\(^{25}\) It is well known that the hepatocyte quantity in fibrotic livers is lower than in normal liver, and that the amounts of drug-metabolizing enzymes are mainly decreased due to loss of liver tissue.\(^{25}\) Hence, the reduction in hepatic intrinsic clearance of propranolol in fibrosis far exceeds the reduction in content of CYP1A2 and CYP2D1 observed in hepatocytes.\(^{31}\)

In conclusion, the main finding in this work is that the disposition of omeprazole, spironolactone, metoprolol, and furosemide in the fibrotic liver is markedly impaired in a manner similar to that previously found for propranolol. The drug dosing regimens in patients with liver fibrosis should take into account changes in both the uptake into and elimination of drugs from the liver. For example, the dosage of orally administered drugs might need to be reduced when drug hepatic extraction is decreased in the diseased state. Changes in hepatic pharmacokinetics in the diseased liver appear to be defined by the drug properties and the degree of liver fibrosis.

References

Drug Pharmacokinetics in Fibrotic Rat Liver


