Cationic Drug Pharmacokinetics in Diseased Livers Determined by Fibrosis Index, Hepatic Protein Content, Microsomal Activity, and Nature of Drug

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ABSTRACT

The disposition kinetics of six cationic drugs in perfused diseased and normal rat livers were determined by multiple indicator dilution and related to the drug physicochemical properties and liver histopathology. A carbon tetrachloride (CCl4)-induced acute hepatocellular injury model had a higher fibrosis index (FI), determined by computer-assisted image analysis, than an alcohol-induced chronic hepatocellular injury model. The alcohol-treated group had the highest hepatic\%1-acid glycoprotein, microsomal protein (MP), and cytochrome P450 (P450) concentrations. Various pharmacokinetic parameters could be related to the octanol-water partition coefficient (log $P_{app}$) of the drug as a surrogate for plasma membrane partition coefficient and affinity for MP or P450, the dependence being lower in the CCl4-treated group and higher in the alcohol-treated group relative to controls. Stepwise regression analysis showed that hepatic extraction ratio, permeability-surface area product, tissue-binding constant, intrinsic clearance, partition ratio of influx ($k_{in}$) and efflux rate constant ($k_{out}$), and $k_{in}/k_{out}$ were related to physicochemical properties of drug (log $P_{app}$ or $pK_a$) and liver histopathology (FI, MP, or P450). In addition, hepatocyte organelle ion trapping of cationic drugs was evident in all groups. It is concluded that fibrosis-inducing hepatic disease effects on cationic drug disposition in the liver may be predicted from drug properties and liver histopathology.

It is well established that liver disease alters hepatic microcirculation (Varin and Huet, 1985) and impairs the disposition kinetics of many solutes, including drugs (Reichen et al., 1987; Gariepy et al., 1993). Callaghan et al. (1993) and Morgan and McLean (1995) have suggested that hypoxia, changes in enzyme activity, and alterations in plasma and intrahepatocellular proteins (albumin and $\alpha_1$-acid glycoprotein) during liver disease may influence the hepatic disposition of drugs. Fenyves et al. (1993) have also postulated that the disposition of propranolol in cirrhosis is dependent, in part, on capillarization and intrahepatic shunts, oxygen delivery, and possibly acinar heterogeneity. Furthermore, induced pharmacokinetic changes due to liver disease also depend on drug properties (low or high hepatic extraction, extent of protein binding, etc.) as well as on the severity of liver disease (Rodighiero, 1999). At present, there appears to be no work quantitatively relating hepatic pharmacokinetics to both the drug properties and hepatic pathophysiology (Morgan and McLean, 1995).

In this work, we attempt to define the hepatic pharmacokinetics of a range of cationic drugs in perfused rat livers that had been treated with CCl4, alcohol, or were untreated (controls) using the multiple indicator dilution technique (MID). The pharmacokinetics was then related to drug properties (log $P_{app}$ and $pK_a$) and changes in liver pathophysiology. The latter changes were estimated in terms of: 1) physiological alterations in sinusoidal morphology as expressed by changes in the mean and normalized variance of sinusoidal transit times; 2) a degree of fibrosis index determined by computer-assisted image analysis; 3) the level of intrahepatocellular proteins [e.g., $\alpha_1$-acid glycoprotein (AAG) and microsomal protein]; and 4) cytochrome P450 (P450) concentrations. We anticipated that the fibrosis index (FI) might be a surrogate measure for changes in plasma membrane perme-

ABBREVIATIONS: MID, multiple indicator dilution technique; AAG, $\alpha_1$-acid glycoprotein; CCl4, carbon tetrachloride; $CL_{int}$, intrinsic elimination clearance; P450, cytochrome P450; $E$, hepatic extraction ratio; $F$, availability; $FI$, fibrosis index; $MP$, microsomal protein; $MT$, mean transit time; $PS$, permeability-surface area product; AST, serum aspartate aminotransferase; ALT, alanine aminotransferase; MOPS, 4-morpholinepropanesulfonic acid; HPLC, high-performance liquid chromatography; CR, cytoskeleton residue; CV, normalized variance.
ability due to a decreased diffusivity or an increased diffusion path length. As variations in plasma AAG content in various diseases have been used to account for the fraction unbound in plasma of many drugs (Rowland and Tozer, 1995), we felt it was important to monitor and examine the role of AAG and other proteins in the intrahepatic disposition of drugs. The relationships between the pharmacokinetic parameters (e.g., permeability-surface area product) of a given solute and these histopathological data as well as the physicochemical properties of a solute (e.g., lipophilicity) were then defined by using stepwise regression analysis. Cationic drugs were studied in this work for four reasons: 1) they constitute 70 to 80% of all drugs; 2) they often have a limited therapeutic ratio (e.g., cardiovascular, analgesic, and psychotherapeutic drugs); 3) they constitute the majority of drugs showing a high first-pass effect; and 4) structure-hepatocellular disposition relationships of model cationic drugs with varying lipophilicity exist for normal rat livers.

Materials and Methods

Chemicals. Atenolol, antipyrine, prazosin, labetalol, propranolol, diltiazem, ethanol, and carbon tetrachloride were obtained from Sigma-Aldrich (St. Louis, MO) and used without any further purification. [U-14C]Sucrose and [3H]water were obtained from Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK).

CCl4-Induced Liver Disease Rat Model. The CCl4 rat model was established following a procedure outlined in detail previously (Proctor and Chatamra, 1982). Briefly, male Wistar rats (weighing approximately 150 g) were placed in all-wire mesh cages in groups of eight each (two groups) and given sodium phenobarbital (35 mg/dl) (Biotech International Ltd., Rocklea, Australia) in their drinking water ad libitum; the first dose of CCl4 was given 10 days later. Group 1 animals were given CCl4 (Sigma-Aldrich) once a week for 12 weeks. CCl4 was dissolved in corn oil and given by intragastric perfusion. Group 2 animals were treated identical to group 1, except that they were given a control diet (Dyets) that contained no ethanol. For histopathological examination. The Animal Ethics Committee at the University of Queensland approved all animal studies.

Group 1 animals were given CCl4 (Sigma-Aldrich) once a week for 12 weeks. CCl4 was dissolved in corn oil and given by intragastric perfusion. Group 2 animals were treated identical to group 1, except that they were given a control diet (Dyets) that contained no ethanol.

After 12 weeks, the animals were sacrificed for MID studies and histopathological examination. The Animal Ethics Committee at the University of Queensland approved all animal studies.

Liver Biochemistry Determinations. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured on a Hitachi 747 analyzer (Hitachi Ltd., Tokyo, Japan) to assess the severity of disease by taking blood samples from tail vein at any suitable time point.

In Situ Rat Liver Perfusions. The in situ perfused rat liver preparation used in this study has been described previously (Cheung et al., 1996; Hung et al., 2001). Briefly, the portal vein was punctured with a 25-gauge needle and connected to a hydrostatic manometer to measure the in vivo portal venous pressure. The bile duct was cannulated with PE-10 (Clay Adams, Parsippany, NJ). The portal vein was then cannulated using a 16-gauge intravenous catheter, and the liver was perfused via this cannula with 2% bovine serum albumin MOPS buffer, which contains 15% (v/v) prewashed canine red blood cells (School of Veterinary Sciences, The University of Queensland, Brisbane, Australia) at pH 7.4, and oxygenated using a silastic tubing lung ventilated with 100% pure oxygen (BOC Gases, Brisbane, Australia). The perfusion system used was nonrecirculating and used a peristaltic pump (Cole-Parmer Instrument Co., Chicago, IL). After perfusion was effected, the animals were sacrificed by thoracotomy, and the thoracic inferior vena cava was cannulated with PE-240 (Clay Adams). The animal was placed in a temperature-controlled perfusion cabinet at 37°C. Liver viability was assessed by macroscopic appearance, bile production, oxygen consumption, and perfusion pressure as described by Cheung et al. (1996).

Bolus Studies. To reflect in vivo microcirculation, perfusion was initially conducted at a rate of 15 ml/min and was then adjusted to a pressure similar to the in vivo portal venous pressure (Varin and Huet, 1985). After a 10-min perfusion stabilization period, aliquots (50 μl) of perfusion medium containing a particular cationic drug (0.06–0.11 μmol of atenolol/propranolol, 0.09–0.15 μmol of antipyrine, 0.04–0.08 μmol of prazosin/labetalol, or 0.04–0.07 μmol of diltiazem as determined by high-performance liquid chromatography (HPLC) assay), [3H]water (3 × 106 dpm), and [U-14C]sucrose (1.5 × 106 dpm) was injected into the liver with outlet samples collected via a fraction collector over 4 min (1 s × 20, 4 s × 5, 10 s × 5, and 30 s × 5). In each liver, a maximum of six injections was made with the order of injection randomized and no repeat of the same injection in the same rat. A stabilization period of 10 min was afforded between two injections. The total perfusion time for each liver was less than 2 h. The samples were centrifuged at 1500 g (25°C) for 3 min, and aliquots (100 μl) of supernatant (containing [3H]water and [U-14C]sucrose) were taken for scintillation counting using a MINAXI Beta TRI-CARB 4000 series liquid scintillation counter (Packard BioScience, Meriden, CT). The residue was vortexed and prepared for HPLC analysis to determine the outflow concentration of each cationic drug.

Analytical Procedure. The HPLC method used in this work has been described and validated previously (Hung et al., 2001). The within-day coefficients of variation for all the drugs studied were in the range of 0.6 to 4.4% (n = 3).

Histopathological Examination. For light microscopy, three to five slices of each liver were fixed in 10% neutral buffered formalin then routinely embedded in paraffin. Five-micrometer sections were prepared and stained with H&E for histopathological examination.

Quantitation of Fibrosis. Fibrous tissue was differentially stained pink in 5-μm paraffin sections by the hematoxylin van Gieson method (a solution of 1% aqueous acid fuchsins, saturated aqueous picric acid, and concentrated hydrochloric acid). The degree of fibrosis was 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 FI) as described by Maclntosh et al. (1992).

Determination of Hepatic Tissue AAG Level. Livers were harvested from the rats after MID studies and perfused with a mixed solution of calcium and magnesium-free Hanks’ balanced salt solution, 5 mM EDTA, and 10 mM HEPES (all from Sigma-Aldrich) at 10 ml/min for 10 min to remove the protein and blood from sinusoidal beds. The liver (approximately 1 g) was then homogenized in 1 ml of MOPS buffer using tissue blender and centrifuged at 3000g for 10 min. A quinaldine red (Sigma-Aldrich) fluorometric titration method based on a method described previously was used for tissue AAG level analysis (Imamura et al., 1994).

Determination of Hepatic Cytoskeleton Residue (CR), Microsomal Protein (MP), and P450 Concentrations. The liver (approximately 1 g) was homogenized in 2.5 ml of ice-cold 0.25 M sucrose containing 50 mM Tris-HCl buffer (pH 7.4) for 3 to 5 min. The homogenates were centrifuged at 5,000 rpm for 20 min, and the pellets resuspended in 2.5 ml of Tris buffer as the cytoskeleton.
fraction. The supernatant (approximately 1 ml) from the 5,000 rpm centrifugation was centrifuged again at 50,000 rpm for 1 h. The resulting pellets were resuspended in 2.5 ml of Tris buffer and used as the microsomal fraction. The CR and MP concentrations in the respective fractions were determined by the method of Lowry et al. (1951). P450 content in MP was estimated from the dithionite-reduced difference spectrum of CO-bubbled samples using the molar extinction difference of 104 mM⁻¹ cm⁻¹ in absorption at peak position (about 450 nm) (Matsubara et al., 1976).

**Investigation of Cationic Drug Binding to Hepatic Components.** These experiments were carried out in 1) blank MOPS buffer (pH 7.4); 2) buffer containing 0.35 mg/ml rat AAG (Sigma-Aldrich); 3) buffer containing 0.35 mg/ml MP from normal livers; or 4) buffer containing 0.35 mg/ml CR from normal livers. The unbound fraction of cationic drug in each buffer solution (f_u) was estimated using an ultrafiltration method. A known concentration of the cationic drug stock solution was added to 500 µl of each buffer solution to make a final concentration of 0.05 µM and placed in a centrifugal filter device (Microcon YM-30; Millipore Corp., Bedford, MA) and then centrifuged at 3000g for 10 min. The ultrafiltrate (in triplicate) was assayed by HPLC. The f_u was determined as the ratio of the free concentration to total concentration of solute. Estimation of association constant K_a,AAG (K_a,MP, or K_a,CR) for binding to a given hepatic component (MP, AAG, or CR) to an individual cation was assumed unbound in the perfusate, estimated previously by Hung et al. (2000, 2001). The CCl_4-treated group had a significantly higher in vivo perfusion pressure (1.02 ± 0.08 cm of H_2O) than that of the alcohol-treated (0.92 ± 0.11 cm of H_2O, p < 0.05) and normal control (0.75 ± 0.09 cm of H_2O, p < 0.05) groups. The CCl_4-treated group also had a significantly lower bile flow (0.61 ± 0.05 µl·min⁻¹·g⁻¹·liver) than that of the alcohol-treated (0.83 ± 0.11 µl·min⁻¹·g⁻¹·liver, p < 0.05) and normal control (1.41 ± 0.15 µl·min⁻¹·g⁻¹·liver, p < 0.01) groups. The normal control group had significantly higher liver oxygen consumption (1.13 ± 0.19 mmol·min⁻¹·g⁻¹·liver) than that of CCl_4-treated (0.93 ± 0.11 mmol·min⁻¹·g⁻¹·liver, p < 0.05) and alcohol-treated (0.97 ± 0.06 mmol·min⁻¹·g⁻¹·liver, p < 0.05) groups. The liver biochemistry differed greatly between the three animal models. The CCl_4-treated group has a significantly higher mean ALT (1,027.40 ± 778.60 IU/l) and AST (740.11 ± 301.64 IU/l) than those for the normal controls. The CCl_4-treated group had a significantly higher liver level than those of the alcohol-treated (ALT, 109.60 ± 14.19 IU/l) and normal control (ALT, 54.00 ± 7.70 IU/l and AST, 46.39 ± 7.50 IU/l) groups (p < 0.001). Two other controls used in this study for completeness, CCl_4 control (phenobarbitone treatment only) and alcohol control (liquid diet only), yielded experimental parameters not significantly different than those for the normal control group (data not shown).

**Histopathology.** Sections of normal rat livers showed typical architecture under light microscopy, i.e., cords of hepatocytes one cell wide radiating out from hepatic venules toward portal tracts. Livers from rats fed an alcohol diet showed perivenular macrovesicular steatosis with minor fibrosis. The CCl_4-treated livers showed severe fibrosis, and fibrosis was perivascular with venous-venous fibrous linkage. FI, estimated by computer-assisted image analysis, for each of the animal models is shown in Table 1. The CCl_4-treated group had a significantly higher FI value than the alcohol-treated (p < 0.05) and control groups (p < 0.001).

**Hepatic AAG, MP, and P450 Concentrations.** Table 1 also shows that the alcohol-treated group has a significantly higher hepatic AAG, MP, and P450 content than those of the control (AAG, p < 0.05; MP, p < 0.05; and P450, p < 0.01) and CCl_4-treated groups (AAG, p < 0.01; MP, p < 0.001; and P450, p < 0.001). The AAG, MP, and P450 levels found in the CCl_4 control (phenobarbitone treatment only) and alcohol control (liquid diet only) were not significantly different than those for the normal controls.
Cationic Drug Binding to Hepatic Components. This study shows that the unbound fraction ($f_{uT}$) of cationic drug in the blank MOPS buffer containing MP ($f_{u,MP}$) or AAG ($f_{u,AAG}$) decreases directly with the lipophilicity of drug. Values of 0.98 and 0.96 for atenolol, 0.97 and 0.96 for antipyrine, 0.43 and 0.31 for prazosin, 0.25 and 0.17 for labetalol, 0.11 and 0.06 for propranolol, and 0.09 and 0.05 for diltiazem were found for MP (0.35 mg/ml) and AAG (0.35 mg/ml), whereas $f_{u,CR}$ for various cationic drugs in the buffer of cytoskeleton residue remain relatively constant (=0.97). The relationships between log $f_{uT}$ and

\[ K_S \] is the equilibrium amount ratio characterizing the slowly equilibrating binding sites; $f_{uB}$ is the fraction of drug unbound in perfusate; $V_B$ is the extracellular volume (vascular + Disse space); $V_C$ is the cellular water volume; and $k_{in}$, $k_{out}$, and $k_e$ represent the permeation, efflux, and elimination rate constant, respectively (modified from Hung et al., 2001).
log $P_{app}$ are 1) MP, log $f_{a,MP}$, 0.998 - 0.307 log $P_{app}$ ($p = 0.001$, $r^2 = 0.953$, and $n = 6$) and 2) AAG, log $f_{a,AAG}$, 0.012 + 0.8 log $P_{app}$ ($p = 0.0001, r^2 = 0.999$, and $n = 6$). Although both MP and AAG bind lipophilic cationic drugs (prazosin, labetalol, propranolol, and diltiazem) in hepatocytes, MP may be more dominant given its 10-fold greater concentration in the hepatocyte than AAG for all models (Table 1).

**Hepatic Extraction Ratio and Mean Transit Time.**
Table 2 shows that hepatic extraction ratio ($E$) increases with the lipophilicity ($log P_{app}$) of drug in various animal models. The CCl$_4$-treated group has a significantly lower $E$ than that of the normal control and alcohol-treated groups for the same lipophilic drug but not for the same polar drug. Interestingly, the alcohol-treated group has a significantly higher $E$ than that of the normal control and CCl$_4$-treated groups for the same lipophilic drug but not for the same polar drug. Stepwise regression analysis examining relationships between hepatic extraction ratio ($E$) with lipophilicity of drug ($log P_{app}$) and P450 yielded a high correlation of predicted and observed $E$ (Fig. 2A; $log E = -0.35 + 0.07 P450 + 0.027 log P_{app}; p = 0.0001, r^2 = 0.932$, and $n = 30$). FI was excluded from this regression as nonsignificant. It is also poorly correlated with $P450$ ($r^2 = 0.174$, and $n = 30$).

Table 2 also shows that the drug MT increases with the lipophilicity of drug in various animal models. The CCl$_4$-treated group has a significantly lower MT value than that of the normal control and alcohol-treated groups for the same lipophilic drug. The CV$^2$ for the drugs did not appear to be related to lipophilicity in various animal models. $E$, MT, and CV$^2$ for CCl$_4$ control (phenobarbitone treatment only) and alcohol control (liquid diet only) obtained for the various cationic drugs was not significantly different than those for the normal controls, although there was a trend for the lipophilic drugs to show a longer MT in the CCl$_4$ control group (data not shown).

**Modeling and Data Fitting of the Outflow Concentration-Time Profiles.** Figure 1, A and B, shows typical logarithms of normalized outflow concentration versus time data for atenolol (most hydrophilic) and diltiazem (most lipophilic) in various animal models. Also shown are the corresponding regression line fits using a heterogeneous (barrier-limited and space-distributed) transit time model and a data weighting of $1/y_{obs}^2$. All MID data appeared to be adequately fitted by the models. The estimated model parameters for extracellular volume $V_B$ and cellular water volume $V_C$ were 1) $V_B = 0.49 \pm 0.15$ ml g$^{-1}$ of liver, $V_C = 1.30 \pm 0.44$ ml g$^{-1}$ of liver for the normal control group ($n = 6$); 2) $V_B = 0.53 \pm 0.19$ ml g$^{-1}$ of liver, $V_C = 1.38 \pm 0.39$ ml g$^{-1}$ of liver for the alcohol-treated group ($n = 6$); and 3) $V_B = 0.57 \pm 0.21$ ml g$^{-1}$ of liver, $V_C = 1.34 \pm 0.41$ ml g$^{-1}$ of liver for the CCl$_4$-treated group ($n = 6$). Although the $V_B$ obtained for the CCl$_4$ control (phenobarbitone treatment only) and alcohol control (liquid diet only) were similar to the normal controls, the $V_C$ for the CCl$_4$ control was larger (data not shown).

The pharmacokinetic parameters for each of the cationic drugs are shown in Table 3. It is evident that $K_s$ increases with increasing $pK_a$ of the drug in the various animal models. Except for antipyrine and prazosin, the CCl$_4$-treated group has a significantly lower $K_s$ than that of the alcohol-treated and normal control groups for the same drug. Table 3 also shows that $Cl_{out}$, $PS$, and $K_s$ all increase with the lipophilicity ($log P_{app}$) of drug in various animal models. The CCl$_4$-treated group has a significantly lower $PS$, $Cl_{out}$, and $K_s$ than those of the alcohol-treated and normal control groups for the same lipophilic drug. The alcohol-treated group has a significantly higher $Cl_{out}$ and $K_s$ than those of the CCl$_4$-treated normal control groups for the same lipophilic drug. In contrast, no significant differences in $Cl_{out}$ and $K_s$ were found between normal control, CCl$_4$-treated, or alcohol-treated rats for the same polar drug. The pharmacokinetic parameters found in the CCl$_4$ control (phenobarbitone treatment only) and alcohol control (liquid diet only) were not significantly different than those for the normal controls (data not shown). Further stepwise regression analysis examining relationships between pharmacokinetic parameters ($log PS, log K_{st}, log K_s, or log Cl_{out}$) with nature of drug ($log P_{app}$ or $pK_a$) and histopathological results ($FI, MP,$ and P450) yielded the following relationships: 1) log PS = $1.647 - 0.028 F + 0.08 log P_{app}$ ($p = 0.001, r^2 = 0.844$, and $n = 30$); 2) log $K_s = -0.017 + 0.242 MP + 0.022 log P_{app}$ ($p = 0.0001, r^2 = 0.926$, and $n = 30$); 3) log $Cl_{out} = 1.938 + 0.31 P450 + 0.112 log P_{app}$ ($p = 0.011, r^2 = 0.799$, and $n = 30$); and 4) log $K_s = 0.596 - 0.021 F + 0.073 P_{app}$ ($p = 0.0001, r^2 = 0.992$, and $n = 30$). Figure 2 shows a comparison of the predicted and observed parameter values. A high correlation between AAG and MP content ($r^2 = 0.731$) precluded an examination of AAG as a determinant of $K_s$ in this work.

Furthermore, Tables 1 and 2 show that AAG makes only a small apparent contribution to $K_s$, e.g., 14% for propranolol in normal controls ($K_{a,MP} = K_{a,MP} \cdot MP = 218$, and $K_{a,AAG} = K_{a,AAG}$, AAG = 32 when AAG is expressed as milligram per

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>FI</th>
<th>AAG$^a$</th>
<th>MP</th>
<th>CR</th>
<th>P450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.51 ± 0.23</td>
<td>0.35 ± 0.04</td>
<td>9.40 ± 0.27</td>
<td>186.40 ± 24.18</td>
<td>0.56 ± 0.25</td>
</tr>
<tr>
<td>CCl$_4$ control</td>
<td>0.87 ± 0.24</td>
<td>0.38 ± 0.05</td>
<td>8.16 ± 1.09</td>
<td>195.95 ± 19.93</td>
<td>0.68 ± 0.05</td>
</tr>
<tr>
<td>Alcohol control</td>
<td>0.43 ± 0.17</td>
<td>0.32 ± 0.03</td>
<td>7.82 ± 0.57</td>
<td>177.60 ± 24.83</td>
<td>0.55 ± 0.11</td>
</tr>
<tr>
<td>CCl$_4$-treated</td>
<td>6.07 ± 1.41$^{bc}$</td>
<td>0.22 ± 0.04</td>
<td>5.42 ± 1.31</td>
<td>129.33 ± 36.89</td>
<td>0.14 ± 0.08</td>
</tr>
</tbody>
</table>
| Alcohol-treated | 3.11 ± 1.32 | 0.55 ± 0.06$^{d,e}$ | 11.06 ± 0.38$^{d,e}$ | 219.63 ± 22.38$^{a}$ | 1.02 ± 0.14$^{d,f}$

$^a$ Homogenate of 1 g of liver and 1 ml of buffer.
$^b$ $p < 0.05$ versus alcohol-treated rats.
$^c$ $p < 0.001$ versus controls.
$^d$ $p < 0.05$ versus controls.
$^e$ $p < 0.01$ versus CCl$_4$-treated rats.
$^f$ $p < 0.05$ versus controls.
$^g$ $p < 0.01$ versus CCl$_4$-treated rats.
$^h$ $p < 0.001$ versus CCl$_4$-treated rats.
$^i$ $p < 0.05$ versus CCl$_4$-treated rats.
$^j$ $p < 0.01$ versus controls.
$^k$ $p < 0.001$ versus CCl$_4$-treated rats.

Hepatic Disposition of Cationic Drugs in Diseased Livers 1083

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This work has shown that the pharmacokinetics of cationic drugs in cirrhotic livers can be related to the liver histopathology and solute physicochemical properties. Care was taken to accurately define the vascular dispersion in the liver (Roberts and Rowland, 1986) using a heterogeneous (barrier-limited and space-distributed) transit time model as described in our previous work (Hung et al., 2001). In this work, robust and good fits were also found for cationic drugs in diseased rat livers (Fig. 1, A and B). An implicit assumption in this model is linearity of kinetics as defined by hepatocellular unbound concentrations being below the Michaelis constants for the enzymes metabolizing each drug. The modeling has also assumed that a rapid equilibrium occurs between erythrocytes and the plasma, and that the efflux of the cationic drugs from erythrocytes is sufficiently rapid so as to not lead to an erythrocyte carriage effect (Goresky et al., 2000).

The experimental parameters associated with the normal and CCl4-treated perfusion studies, reflecting liver viability, are also comparable with previous reports (Varin and Huet, 1985; Hung et al., 2001). CCl4 produces acute hepatocellular injury with centrilobular necrosis and stenosis (Hong et al., 1985; Hung et al., 2001). In contrast, alcohol produces chronic hepatocellular injury with inflammation and perivenular macrosolvent steatosis (Sherlock, 1993; Takeyama et al., 1996). These observations were confirmed in this work.

This work has shown that hepatic extraction ratio E and primary parameters PS, Kp, and CLint depend on the lipophilicity of the cationic drug, irrespective of the disease state (Table 3). This work has also shown that a good correlation exists between the predicted and observed values for E and the parameter values (Fig. 2). E or availability F (= 1 – E) can be related to fub, PS, perfusate flow rate, and CLint in a number of models describing hepatic elimination (Pang and Rowland, 1977; Roberts and Rowland, 1986; Roberts and Anissimov, 1999; Roberts et al., 2000), including the dual inverse Gaussian model (Weiss et al., 1998). These models show E is related to fub, PS, CLint, and perfusate flow rate, which are, in turn, related to log Papp (PS, and CLint), FI (PS), and P450 (CLint). The good relationship between log E with log Papp and P450 (Fig. 2A) is therefore consistent with the E being defined by PS, CLint, etc. A high correlation was found for E with log Papp and P450 (r² = 0.932) with the stepwise regression excluding FI as a determinant.

The underlying relationship between PS with FI and log Papp is consistent with the definition of PS, namely PS = Km×Dm/hm, where Km is the plasma membrane-unbound perfusate concentration coefficient, Dm is the solute diffusivity in the plasma membrane, S is the surface area, and hm is the membrane path length for diffusion. Hence, in the absence of transporters, Km is defined by the solute lipophilicity and log Papp as a surrogate. An impaired uptake of solute across the capillarized endothelium in cirrhosis (Huet et al., 1985) is consistent with a slower solute diffusivity and longer diffusion path length as a consequence of collagenization of the Disse space. The FI is a surrogate measure for changes in Dm and hm as a determinant of PS.

The extent of solute uptake into normal and cirrhotic livers.
Fig. 2. Comparison of predicted and observed pharmacokinetic parameters of the model cationic drugs in various animal models. A, E; B, PS; C, tissue-binding constant, \( K_s \); D, \( CL_{int} \); and E, partition ratio of influx and efflux rate constant (\( K_{i} = k_{in}/k_{out} \)). The open circles (○) represent the normal group. The filled triangles (▼) represent the alcohol-treated group. The filled circles (●) represent the CCl₄-treated group.
depends on both tissue binding as well as ion trapping by organelles such as the mitochondria and lysosomes. The main measure of tissue uptake is slow binding as expressed by the equilibrium amount ratio for slowly equilibrating binding sites, \( K_a \). In this work, we found that the alcohol-treated group has the highest intracellular AAG level and MP concentration, whereas the CCl4-treated group has the lowest AAG level and MP concentration among various animal models (Table 1). One source for these binding sites is the soluble microsomal proteins, MP. The regression between \( \log K_S \) with the determinants of the contribution of MP to \( K_S \), its association constant \( K_{a,MP} \) (defined from in vitro binding, Table 2), and the concentration of MP in the liver, i.e., \( K_{S,MP} = K_{a,MP} \), \( MP \) is \( \log K_S = 0.027 + 0.618 \log K_{a,MP} + 0.295 \log MP (p = 0.001, r^2 = 0.947, and n = 30) \). Earlier, it was shown that the unbound fraction (and thus, \( K_{a,MP} \)) of cationic drug in perfusate or buffers containing MP or AAG also correlated with the octanol-water partition coefficient \( \log P_{app} \). Hence, the derived expression \( \log K_S = -0.017 + 0.242 \log P_{app} \) (\( p = 0.0001, r^2 = 0.926, and n = 30 \)), Fig. 2C) suggests that hepatic tissue binding can be predicted from a knowledge of the lipophilicity of the solute as defined by \( \log P_{app} \), or of protein content as defined by MP. As discussed earlier, AAG, one of the acute-phase proteins that increases in the liver more than in other organs in various conditions including inflammatory diseases (Brinkman-van der Linden et al., 1996), has concentrations in the liver too low to make a substantial contribution to \( K_S \).

The \( K_a \), or distribution ratio of a solute between the tissue and vascular space, is mainly dependent on ion trapping in organelles and asymmetric transport across the plasma membrane (Hung et al., 2001). We have previously shown that \( K_a \) increases with drug \( pK_a \) and an ion-trapping effect for cationic drugs relative to the volume of circulation and inorganelle pH in the hepatic cytoplasm, mitochondria, and lysosomes (Hung et al., 2001). We have now shown that \( K_a \) is related to both \( pK_a, FI, \) and \( P_{app} \), enabling a lesser dependence of \( K_a \) on \( pK_a \) for the alcohol-treated and, even less so, for the CCl4-treated group to be described. The decreased dependence is most likely due to a higher intracellular pH and a reduction in the ion trapping of the cations as a consequence of a reduction in ionization. Hence, the higher cytosolic pH in cirrhosis rats (7.45) than in normal rats (7.27), due to the activation of hepatocellular \( Na^+/{H}^+ \) exchange in cirrhosis (Elsing et al., 1994), would explain a lower \( K_a \) for the CCl4-treated group relative to the normal control group as well as a loss of dependence of \( K_a \) on \( pK_a \) for the CCl4-treated group. How fibrosis affected the organelle volumes (and potential ion-trapping volume) is less certain. For instance, CCl4-induced cirrhosis reduces rat liver mitochondrial volume (Krähenbühl et al., 2000) (normally about 20% of hepatocyte volume; Hung et al., 2001) but increases the activity and numbers of lysosomes (Dufour et al., 1994) (normally about 1% of hepatocyte volume; Hung et al., 2001).

In this analysis, we estimated the intrinsic metabolic clearance \( CL_{int} \) as a product of \( k_a \) and \( V_C \). This approach, used previously by Pang et al. (1995), ensures that solutes iontrapped in organelles or bound to rapidly equilibrating binding sites are not determinants of \( CL_{int} \). The good correlation of observed and predicted \( CL_{int} \) (Fig. 2D) derived from the expression \( \log CL_{int} = 1.938 + 0.31 P_{450} + 0.112 \log P_{app} \) (\( p = 0.011, r^2 = 0.799, \) and \( n = 30 \)) suggests that intrinsic elimination clearance of a solute in the diseased liver can be

### Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>( V_C )</th>
<th>( k_a )</th>
<th>( CL_{int} )</th>
<th>( P_{app} )</th>
<th>( pK_a )</th>
<th>( K_{a,MP} )</th>
<th>( K_{S,MP} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>3.10</td>
<td>1.37</td>
<td>1.83</td>
<td>9.45</td>
<td>0.31</td>
<td>0.24</td>
<td>3.10</td>
</tr>
<tr>
<td>Propranolol</td>
<td>6.30</td>
<td>1.33</td>
<td>3.20</td>
<td>9.45</td>
<td>0.31</td>
<td>0.24</td>
<td>6.30</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>3.53</td>
<td>1.35</td>
<td>2.53</td>
<td>9.45</td>
<td>0.31</td>
<td>0.24</td>
<td>3.53</td>
</tr>
</tbody>
</table>

\( V_C \) denotes the volume of distribution of free drug. \( k_a \) denotes the apparent rate constant for an unbound solute between the cellular and vascular spaces. \( CL_{int} \) denotes the elimination clearance of a solute in the diseased liver. \( P_{app} \) denotes the apparent permeability-surface area product (milliliters per minute per gram). \( pK_a \) denotes the negative logarithm of the ionization constant. \( K_{a,MP} \) denotes the association constant for the derived expression \( \log CL_{int} \).
Hepatic Disposition of Cationic Drugs in Diseased Livers

Predicated by lipophilicity of the solutions and P450 activity of the liver tissue. It should be noted that P450 consists of a number of isozymes that, in response to a stimulant, may be differentially expressed. Better correlations may therefore be expected by relating CLint to the individual isozymes responsible for the metabolism of a given drug. Relevant to this work, Kono et al. (1999) showed chronic exposure to alcohol caused induction of CYP3A4, CYP2C12, CYP1A2, and CYP2B, and CYP2E1 expression in mouse livers. Bastien et al. (2000) showed CCl4-induced cirrhosis was associated with reduction of CYP1A1, CYP2C, CYP2E1, and CYP3A expression in rat livers. Given that P450 accounted for a low and variable fraction of MP (0.076 normal group; 0.093 alcohol-treated group; and 0.03 CCl4-treated group), caution should be exercised in using MP as a surrogate for P450. It is therefore apparent, as noted by Obach (1997) and McLure et al. (2000), that much of the drug binding to MP is nonspecific and not necessarily a determinant of CLint.

In summary, the disposition of cationic drugs may be directly related to the extent of fibrosis caused by the liver disease and the physicochemical properties of the drugs. The changes in the extraction ratio E are consistent with a reduction in PS, CLint, and perfusate flow rate, each of which define E. These reductions arise from alterations in sinusoidal morphology and in the hepatic microcirculation and a decreased enzymatic activity due to loss of normal metabolic zonation and microsomal activity in the liver. In addition, hepatic distribution is reduced in cirrhosis due to reduced tissue binding (lower Keo) as a consequence of a lower synthesis of intracellular proteins as well as a loss of an ion-trapping effect (lower kint/hout) due to an elevation of intracellular pH and changes in organelle volumes. Drug lipophilicity is a key determinant of cation drug extraction.

There were good correlations between the predicted and observed pharmacokinetic parameters (E, PS, Keo, CLint, or Keo) of the model cationic drugs based on nature of drug (log Pag or Papp) and the histopathological results (FL, MP, and P450). This prediction of E using various determining variables such as log Pag and P450 is likely to be improved by the use of larger data (drugs and severity of disease state). These results should be relevant in better understanding how changes in fibrosis-induced hepatic diseases quantitatively affect hepatic drug pharmacokinetics and in assisting preclinical drug development through predictions of the first-pass effect.

Acknowledgments

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References


Krahenbuhl L, Ledermann M, Lang C, and Krahenbuhl S (2000) Relationship between hepatic mitochondrial zonation and microsomal activity in the liver. In addition, hepatic distribution is reduced in cirrhosis due to reduced tissue binding (lower Keo) as a consequence of a lower synthesis of intracellular proteins as well as a loss of an ion-trapping effect (lower kint/hout) due to an elevation of intracellular pH and changes in organelle volumes. Drug lipophilicity is a key determinant of cation drug extraction.

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