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 did an alcohol-induced chronic hepatocellular injury model. The alcohol-treated group had the highest hepatic α1-acid glycoprotein, microsomal protein (MP), and cytochrome P450 (P450) concentrations. Various pharmacokinetic parameters could be related to the octanol-water partition coefficient (log Papp) 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 (ka), and efflux rate constant (kout) were related to physicochemical properties of drug (log Papp or pK4) 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.

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

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ABBREVIATIONS: MID, multiple indicator dilution technique; AAG, α1-acid glycoprotein; CCl4, carbon tetrachloride; CIout, 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; CV², 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 the majority of drugs showing a high first-pass effect; 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-hepatic 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 Chatamara, 1992). 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) 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 fuchsin, 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 MacIntosh 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 2 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 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 (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 (f₁uᵦ) 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 ultrafilter (in triplicate) was assayed by HPLC. The f₁uᵦ was determined as the ratio of the free concentration to total concentration of solute. Estimation of association constant Kᵦᵦᵦ (Kᵦᵦᵦ, Kᵦᵦᵦ, or Kᵦᵦᵦ) for binding to a given hepatic component (MP, AAG, or CR) to an individual cation was deduced using the one of these hepatic components.

Hepatic Extraction Ratio and Mean Transit Time. Nonparametric estimates of hepatic extraction ratio (E), mean transit time (MET), and normalized variance (CV²) were determined from the outflow concentration versus time profiles for the model cationic drugs as described previously (Hung et al., 2001). The recovery for each drug was defined by its availability, which is defined by 1−E (Roberts and Rowland, 1986).

Estimation of Pharmacokinetic Parameters by Modeling and Data Fitting of the Outflow Concentration-Time Profiles of Extracellular and Cellular References after Impulse Dosing. A mixture of two inverse Gaussian density functions with correction for catheter effects was used to estimate the extracellular space (Vₑ), determined by [U-¹⁻¹C]sucrose (Weiss et al., 1997). A barrier-limited plus space-distributed liver model with correction for catheter effects was used to estimate the total water volume (Vₑ), determined by [³¹H]water, Vₑ then being used to estimate the cellular water volume, Vₑ, defined as (Vₑ − Vₑ) (Weiss et al., 2000).

Modeling and Data Fitting of the Outflow Concentration-Time Profiles of Model Cationic Drugs. A heterogeneous (barrier-limited and space-distributed) transit time model (the “two-phase stochastic model”) was used to estimate the pharmacokinetic parameters of hepatocellular influx, efflux, binding, and elimination for the permeating solutes (Hung et al., 2001). Briefly, the model (Fig. 1) assumes unbound drug transfer across the permeability barrier (plasma membrane) with influx and efflux rate constants hᵢᵦ and hᵢᵦ, respectively. An apparent distribution ratio (Kᵦ) for an unbound solute between the cellular and extracellular space is defined by hᵢᵦ/hᵢᵦ. Kᵦ is the equilibrium amount ratio for slowly equilibrating binding sites as defined by an and off rate constant (Hung et al., 2001). Kᵦ for a given drug, in theory, should be related to the product of the association constant Kᵦᵦᵦ of the drug for protein p and the concentration of protein [P] i.e., Kᵦᵦᵦ = Kᵦᵦᵦ · [P] (Rowland and Tozer, 1995). The permeability-surface area product (PS) is given by hᵢᵦ · Vₑ/hᵢᵦ, where Vₑ is the extracellular space and fᵢᵦ is the fraction unbound in the perfusate, estimated previously by Hung et al. (2001). The intrinsic clearance, CLᵦᵦᵦ, was estimated as the product of the elimination rate constant kᵦ and the cellular distribution volume for water, Vₑ, following the approach of Pung et al. (1995). The stochastic approach represents the transit of a molecule through the organ as a series of sojourns in one of the two regions described by density functions. The sojourn time distribution fᵢᵦ(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, fᵢᵦ(s) = L⁻¹[fᵢᵦ(t)], as described earlier (Weiss et al., 2000; Hung et al., 2001).

Statistical Analysis. All data are presented as mean ± standard deviation unless otherwise stated. Stepwise regression analysis was performed using the program SPSS 10.1 for Windows (SPSS, Inc., Chicago, IL). Statistical analysis was performed with two-way analysis of variance, Student’s t test, and regression analysis where appropriate. A p < 0.05 was taken as significant. Linear regression equations have been only considered when r² > 0.5.

Results

Liver Biochemistry and Physiology. The CCl₄-treated group has a significantly lower perfusion rate (0.75 ± 0.02 ml · min⁻¹ · g⁻¹ of liver) than that of the alcohol-treated (0.89 ± 0.04 ml · min⁻¹ · g⁻¹ of liver, p < 0.05) and normal control (1.25 ± 0.09 ml · min⁻¹ · g⁻¹ of liver, p < 0.01) groups. Accordingly, the CCl₄-treated group had a significantly higher in vivo perfusion pressure (1.02 ± 0.08 cm of H₂O) than that of the alcohol-treated (0.92 ± 0.11 cm of H₂O, p < 0.05) and normal control (0.75 ± 0.09 cm of H₂O, p < 0.01) groups. The CCl₄-treated group also had a significantly lower bile flow (0.61 ± 0.05 ml · min⁻¹ · g⁻¹ of liver) than that of the alcohol-treated (0.83 ± 0.11 ml · min⁻¹ · g⁻¹ of liver, p < 0.05) and normal control (1.41 ± 0.15 ml · min⁻¹ · g⁻¹ of liver, p < 0.01) groups. The normal control group had significantly higher liver oxygen consumption (1.13 ± 0.19 µmol · min⁻¹ · g⁻¹ of liver) than that of CCl₄-treated (0.93 ± 0.11 µmol · min⁻¹ · g⁻¹ of liver, p < 0.05) and alcohol-treated (0.97 ± 0.06 µmol · min⁻¹ · g⁻¹ of liver, p < 0.05) groups. The liver biochemistry differed greatly between the three animal models. The CCl₄-treated group has a significantly higher mean ALT (1,027.40 ± 778.60 IU/l) and AST (740.11 ± 301.64 IU/l) and normal Liver (109.60 ± 30.12 IU/l) than levels of the alcohol-treated (ALT, 109.60 ± 13.10 IU/l and AST, 90.94 ± 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, CCl₄ 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 CCl₄-treated livers showed severe fibrosis, and fibrosis was perivenular with venous-venous fibrous linkages. FI, estimated by computer-assisted image analysis, for each of the animal models is shown in Table 1. The CCl₄-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 CCl₄-treated groups (AAG, p < 0.01; MP, p < 0.001; and P450, p < 0.001). The AAG, MP, and P450 levels found in the CCl₄ 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

Fig. 1. Typical outflow profiles for model cationic drugs in various animal models (data weighted $1/y^2$) in the regressions. A, atenolol (most hydrophilic) and B, diltiazem (most lipophilic). The open circles (○) represent the normal group. The filled triangles (▼) represent the alcohol-treated group. The filled circles (●) represent the CCl₄-treated group. The lines are the fits of the profiles to a heterogeneous ( barrier-limited and space-distributed) transit time model. Inset, $K_S$ 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).
Hepatic Disposition of Cationic Drugs in Diseased Livers

Comparison of fibrosis (FI), AAG tissue level, MP concentration, CR, and P450 content in various animal models (mean ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>FI</th>
<th>AAG*</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>CCl4 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>CCl4-treated</td>
<td>6.07 ± 1.41bc</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>
<tr>
<td>Alcohol-treated</td>
<td>3.11 ± 1.32</td>
<td>0.55 ± 0.06d,e</td>
<td>11.06 ± 0.38f,e</td>
<td>219.63 ± 22.38h</td>
<td>1.02 ± 0.14f</td>
</tr>
</tbody>
</table>

* Homogeneous of 1 g of liver and 1 ml of buffer.
bc p < 0.05 versus alcohol-treated rats.
p < 0.001 versus controls.
p < 0.05 versus controls.
p < 0.01 versus CCl4-treated rats.
p < 0.05 versus controls.
p < 0.01 versus CCl4-treated rats.
p < 0.05 versus CCl4-treated rats.
p < 0.01 versus controls.
p < 0.001 versus CCl4-treated rats.

log $P_{app}$ are 1) MP, log $f_{u,MP}$ = 0.998 - 0.307 log $P_{app}$ (p = 0.001, $r^2 = 0.953$, and n = 6) and 2) AAG, log $f_{u,AAG}$ = 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, labetolol, 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 CCl4-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 CCl4-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). $E = -0.35 + 0.07 P_{450} + 0.027 log P_{app}$: 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 ($r^2 = 0.35$, and n = 30). Table 2 also shows that the drug MT increases with the lipophilicity of drug in various animal models. The CCl4-treated group has a significantly lower MT value than that of the normal control and alcohol-treated groups for the same lipophilic drug. The CV2 for the drugs did not appear to be related to lipophilicity in various animal models. E, MT, and CV2 for CCl4 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 CCl4 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/$v_{obs}$. 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) $V_B = 0.49 ± 0.15$ ml g$^{-1}$ of liver, $V_C = 1.30 ± 0.44$ ml g$^{-1}$ of liver for the normal control group (n = 6); 2) $V_B = 0.53 ± 0.19$ ml g$^{-1}$ of liver, $V_C = 1.38 ± 0.39$ ml g$^{-1}$ of liver for the alcohol-treated group (n = 6); and 3) $V_B = 0.57 ± 0.21$ ml g$^{-1}$ of liver, $V_C = 1.34 ± 0.41$ ml g$^{-1}$ of liver for the CCl4 control group (n = 6). Although the $V_B$ obtained for the CCl4 control (phenobarbitone treatment only) and alcohol control (liquid diet only) were similar to the normal controls, the $V_C$ for the CCl4 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 CCl4-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 Clout, PS, and $K_s$ all increase with the lipophilicity (log $P_{app}$) of drug in various animal models. The CCl4-treated group has a significantly lower PS, Clout, 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 Clout and $K_s$ than those of the CCl4-treated and normal control groups for the same polar drug. The pharmacokinetic parameters found in the CCl4 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 $K_s$, log $K_v$, or log Clout) with nature of drug (log $P_{app}$ or p$K_a$) and histopathological results (FI, MP, and P450) yielded the following relationships: 1) log PS = 1.647 – 0.028 FI + 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 Clout = 1.938 + 0.31 P450 + 0.112 log $P_{app}$ (p = 0.011, $r^2 = 0.799$, and n = 30); and 4) log $K_v$ = 0.596 - 0.073 FI + 0.073 pK_a (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 $K_a$, 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...
Discussion

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., 2000). In contrast, alcohol produces chronic hepatocellular injury with inflammation and perivenular macrovesicular steatosis (Sherlock, 1993; Takeyama et al., 1996). These observations were confirmed in this work.

This work has shown that hepatic extraction ratio 

\[ E = \frac{f_{ub}}{f_{ub} + P_{450}} \]

and primary parameters \( K_a \), \( K_{MP} \), and \( CL_{int} \) 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 \( f_{ub} \), \( PS \), perfusate flow rate, and \( CL_{int} \) in a number of models describing hepatic elimination (Pang and Anissimov, 1999; Roberts et al., 2000), including the dual inverse Gaussian model (Weiss et al., 1998). These models show \( E \) is related to \( f_{ub} \), \( PS \), \( CL_{int} \), and perfusate flow rate, which are, in turn, related to \( \log P_{app} \) (PS, and \( CL_{int} \) (PS), and \( P_{450} \) (CLint)). The good relationship between \( \log E \) with \( \log P_{app} \) and \( P_{450} \) (Fig. 2A) is therefore consistent with the \( E \) being defined by \( PS \), \( CL_{int} \), etc. A high correlation was found for \( E \) with \( \log P_{app} \) and \( P_{450} \) (r² = 0.932) with the stepwise regression excluding \( FI \) as a determinant.

The underlying relationship between \( PS \) with \( FI \) and \( \log P_{app} \) is consistent with the definition of \( PS \), namely \( PS = K_m D_m / h_m \), 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. Hence, in the absence of transporters, \( K_m \) is defined by the solute lipophilicity and \( \log P_{app} \) 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 \( D_m \) and \( h_m \) 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 ($K_v = k_{in}/k_{out}$). The open circles (○) represent the normal group. The filled triangles (▼) represent the alcohol-treated group. The filled circles (●) represent the CCl$_4$-treated group.
Table 3

Kinetic parameters derived from the two-phase stochastic model fitting for model cationic drugs in various animal models (mean ± S.D., n = 6)

<table>
<thead>
<tr>
<th>Drug</th>
<th>CLint</th>
<th>PS</th>
<th>fV</th>
<th>CLapp</th>
<th>AI</th>
<th>Kapp</th>
<th>Kdis</th>
<th>KMP</th>
<th>Kc</th>
<th>KMP</th>
<th>Kas</th>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Atenolol</td>
<td>0.14</td>
<td>1.00</td>
<td>0.67</td>
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<td>0.67</td>
<td>0.23</td>
<td>0.67</td>
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<td>0.23</td>
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From Table 2.

Apparent elimination clearance of a solute in the diseased liver can be predicted from a knowledge of the lipophilicity of the solute as defined by log P app and binding protein content as defined by MP. As discussed earlier, AAG, one of the acute-phase proteins that increases 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 Ks.

The Ks, 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 Ks increases with drug pKs and an ion-trapping effect for cationic drugs related to the volume of and intraorganelle pH in the hepatic cytoplasm, mitochondria, and lysosomes (Hung et al., 2001). We have now shown that Ks is related to both pKs and FL, enabling a lesser dependence of Ks on pKs 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 Ks for the CCl4-treated group relative to the normal control group as well as a loss of dependence of Ks on pKs 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 (Krahenbühl 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 CLint as a product of k, and Vc. This approach, used previously by Pang et al. (1995), ensures that solutes ion-trapped in organelles or bound to rapidly equilibrating binding sites are not determinants of CLint. The good correlation of observed and predicted CLint (Fig. 2D) derived from the expression log CLint = 1.938 + 0.31 P450 + 0.112 log P app (p = 0.011, r2 = 0.799, and n = 30) suggests that intrinsic elimination clearance of a solute in the diseased liver can be...
predicted from lipophilicity 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. (1997) showed chronic exposure to alcohol caused induction of CYP3A, CYP2A12, CYP1A1, 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; 0.095 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 Kapp) as a consequence of a lower synthesis of intrahepatocellular proteins as well as a loss of an ion-trapping effect (lower kint/kout) 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, Kapp, CLint, or Kout) of the model cationic drugs based on nature of drug (log Papp or pKof), and the histopathological results (FI, MP, and P450). This prediction of E using various determining variables such as log Papp and P450 is likely to be improved by the use of larger data set (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.

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 References

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