Prediction of Glycyrrhizin Disposition in Rat and Man with Liver Failure by a Physiologically Based Pharmacokinetic Model

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Two physiologically based pharmacokinetic models A and B incorporating enterohepatic recycling, which succeeded previously in predicting the disposition of glycyrrhizin (GLZ) in normal rats and subjects,1) were applied to predict GLZ disposition in plasma and tissues of chronically CCl4-intoxicated rats, and serum of humans with hepatitis after i.v. dosing. The prediction by model A with the direct excretion of GLZ from liver into gut lumen gave fairly good agreement with the observed time courses of GLZ concentrations in blood and tissues in the intoxicated rats. The human serum disposition was predicted by model B, to which was added a gallbladder for the excretion from liver into gut lumen to model A by assuming continuous delaying transfer from the gallbladder. An attempt to predict the serum dispositions in five human subjects by considering individual differences in serum free fraction, biliary excretion ratio, and intestinal absorption clearance was successful in model B. Thus, scale-up of the disposition kinetics of GLZ from rat to man with liver failure was successful.

Keywords — glycyrrhizin; physiological pharmacokinetic model; enterohepatic recycling; CCl4 intoxication; liver failure; animal scale-up; rat; man

Introduction

Massive dose i.v. administration of glycyrrhizin (GLZ) has frequently been used in the treatment of chronic hepatitis.2) However, GLZ produces the adverse effect of aldosteronism when given in massive doses.3)–6) We previously confirmed the existence of enterohepatic recycling of GLZ following i.v. administration of 100 mg/kg to rats and suggested that enterohepatic recycling of the drug may also occur in humans.7) Such enterohepatic recycling may result in a long residence time of the drug in the human body. An even longer residence time owing to a decrease of biliary excretion might occur in patients with hepatic disorder. It is desirable, therefore, to be able to predict the human serum disposition of GLZ by using a physiological pharmacokinetic model based on in vivo and in vitro pharmacokinetic data from a laboratory animal in order to use the drug safely in clinical therapeutics.

The purpose of the present study was to predict the concentration time profiles of GLZ in blood and tissues of CCl4-intoxicated rats and the drug serum disposition of humans with hepatitis by using the physiologically based pharmacokinetic models A and B (Fig. 1), incorporating enterohepatic recycling, which succeeded previously in predicting GLZ disposition in normal rats and humans.1)
tubing for i.v. drug administration and blood sampling, respectively. For the biliary and urinary excretion studies, a bile fistula cannula (PE-10 polyethylene tubing) and a urinary bladder cannula (PE-60 polyethylene tubing) were used to collect samples of bile and urine, respectively. After 1 h of recovery from anesthesia, GLZ (100 mg/kg) in 1.0 ml of 5% glucose solution was injected, followed by 0.5 ml of 5% glucose solution. The dosed rats were kept in restraining cages with free access to water under normal housing conditions. The non-dosed normal rats with biliary fistulization were also kept under the same housing conditions to collect the 24-h bile, which was used for the drug intestinal absorption study. The body temperature was kept at 37 °C throughout the experiments by using a heat lamp.

**Blood and Tissue Disposition** — Blood Disposition: After administration of GLZ to the intoxicated rats with and without biliary fistulization, blood samples (300 μl each) were collected in heparinized polyethylene centrifuge tubes at 0.5, 1, 2, 3, 5, 8, and 12 h. In those without bile fistulas, further samples were taken at 16 and 24 h. The rats were injected with fresh blood (1.5 ml), obtained from normal rats, through the femoral vein cannula immediately after sampling at 5 h.

Tissue Disposition: After removal of blood samples, the intoxicated rats were exsanguinated via a carotid artery at 3, 8, or 16 h after dosing and perfused with cold physiological saline via the venous trunk just inferior to the renal veins until the effusate became colorless. The bled tissues (brain, heart, lung, liver, kidney, spleen, pancreas, stomach, small intestine, muscle, skin, and adipose tissue) were excised, rinsed well with cold saline, blotted, and weighed. The small intestinal contents were removed before rinsing. A portion of blood was centrifuged and plasma was separated. Blood, plasma, and tissue samples were stored at −20 °C until required. Each tissue sample was homogenized with two volumes of physiological saline before analysis.

**Tissue-to-Blood Concentration Ratio (K_p)** — Blood and bled tissue samples were obtained at 3 h after dosing of the control and intoxicated rats with bile fistulas by the same method as described above, but in the former, only the bled liver was excised. Blood and tissue samples were stored at −20 °C until required. Each tissue sample was homogenized with two volumes of physiological saline before analysis.

**Biliary and Urinary Excretion** — Bile and urine samples were obtained at 0—12, 12—36, and 36—48 h after dosing of the intoxicated rats. Bile was also sampled at 0—48 h after dosing of the control rats. Bile and urine volumes were measured and stored at −20 °C until required.

**Intestinal Absorption** — In the intoxicated rats, intestinal absorption was investigated by the single-pass perfusion method.9) The length of 15 cm from the proximal end of the jejunum was used. The 24-h bile obtained from normal rats (approximately 50 ml) containing GLZ (1 mg/ml) and inulin (0.32 mg/ml) was perfused at the rate of 0.6 ml/min. The perfused solution was collected every 10 min for 60 min after the lag time (10 min).

**Blood and Tissue Elimination and Tissue Binding** — Blood and blood-free tissue samples of the intoxicated rats were obtained by the same method as that in the tissue disposition study without drug administration. Immediately, each tissue was homogenized with two or five volumes of 0.05 M Tris-HCl buffer solution containing 0.25 M sucrose (pH 7.4) in an ice-bath. After preincubation for 2 min at 37 °C, 20, 40, 60, 100, 150, or 200 µg of GLZ was added to the blood or tissue homogenate (1 ml each) and the mixture was shaken for 5, 10, 15, or 20 min at 37 °C. The reaction was then immediately stopped by freezing the sample in an ice-acetone bath. The control experiment was carried out by the same procedure using only the buffer solution. Spontaneous degradation of GLZ did not occur in the buffer solution. The tissue binding of GLZ was examined in the liver and small intestine homogenates (16.7 and 33.3%) by an ultrafiltration technique using an ultrafiltration membrane (Amicon Micropartition system, MPS-1, Danvers, MA). One milliliter of the homogenate containing GLZ (the same amount as in the case of elimination) was applied to the filtration membrane after incubation at 4 °C for 10 min, and then was centrifuged for 40 min at
1000 g and 4 °C.

**Plasma Protein Binding** — The plasma protein binding of GLZ in the intoxicated rats was determined by an ultrafiltration technique using the same ultrafiltration membrane as in the tissue binding study. One milliliter of plasma containing 0.8 to 6.1 mg of GLZ was applied to the filtration membrane after incubation at 37 °C for 5 min. Ultrafiltration of samples was accomplished by centrifugation (1000 g) at 37 °C for a period (approximately 10 min) sufficient to produce an ultrafiltrate volume of approximately 20% of the initial sample. The adsorption of the drug on the membrane and the leakage of macromolecular components of plasma into the filtrate were negligible.

**Analytical Method** — Samples of 100 μl for plasma, blood, perfusate, and plasma and tissue homogenate filtrates and 300–600 μl for tissue homogenate were used for GLZ determination. The extraction procedure and high-performance liquid chromatographic (HPLC) method for GLZ were the same as those described previously. Inulin in the influx and efflux perfusates (50 μl each) was determined by the method of Tsuji et al. Patho-physiological changes were determined by using plasma in the control and intoxicated rats as follows: glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were determined by the pyruvate oxidase-p-chloro-phenol method using a commercial kit (Wako Pure Chemical Ind. Ltd., Tokyo). Albumin and total protein were determined by the bromocresol green method and the biuret method, respectively. Total bile acids and total bilirubin were determined by an enzymatic fluorometric method and the method of Doumas et al., respectively.

**Data Analysis** — The area under the blood concentration–time curve (AUC) and the mean residence time (MRT) were calculated by trapezoidal integration with extrapolation to infinite time. The slope of the terminal log-linear phase was calculated by linear regression analysis for the blood concentration data after 3 and 12 h in rats with and without bile fistulas, respectively. The steady-state distribution volume (Vds) and total body blood clearance (CLint) were calculated from dose/MRT/AUC and dose/AUC, respectively. The biliary (CLβ) and renal (CLR) clearances were calculated by dividing the total amount of GLZ excreted in bile or urine by AUC. The metabolic clearance (CLM) was calculated from CLM = CLtot − CLB − CLR. Apparent Kp value was corrected by the method of Chen and Gross. The intestinal absorption clearance (CLAbs) was estimated from Eq. 1:

\[
CL_{Abs} = \frac{C_{in} \cdot Q_{in} - C_{ef} \cdot Q_{ef}}{C_{in}} \times \frac{80.8 \text{ cm}}{15.0 \text{ cm}}
\]

where C_{in} and C_{ef} are the drug concentrations in the influx and efflux perfusates, respectively; Q_{in} and Q_{ef} are the influx and efflux flow rates, respectively. The Q_{ef} was calculated from the inulin concentration difference in the influx (C'_{in}) and efflux (C'_{ef}) perfusates as follows:

\[
Q_{ef} = Q_{in} \times \left( C'_{in}/C'_{ef} \right)
\]

Fifteen centimeters of small intestine is the length used in the absorption study and 80.8 cm is its total length according to the literature. The hepatic and small intestinal intrinsic clearances for metabolism (CL_{int}) were calculated as follows: (1) the elimination rate (v, μg/ml/min) for initial drug concentration in the homogenate was calculated from the slope of the linear plot of drug concentration versus incubation time using the least-squares method. (2) The elimination rate constant (k_m) was calculated from k_m (min⁻¹) = α (min⁻¹)/d, where α is the slope of the plot of v value versus initial free drug concentration in the homogenate. The line passed through the origin. d is the dilution factor of the homogenate (0.167 or 0.333). (3) CL_{int} (ml/min) was calculated from k_m × V_T, where V_T is the tissue volume (Table I). The plasma binding parameters (the association constants K_1 and K_2 for the primary and secondary binding sites, respectively, and the binding capacities n_1(p) and n_2(p) for the primary and secondary binding sites, respectively) were calculated from Eq. 2 by a nonlinear iterative least-squares method using the MULTI program.
Where $C_f$ and $C_b$ are free and bound drug concentrations in plasma, respectively.

All means are given with their standard error (mean ± S.E.). Student’s $t$ test was utilized to determine the significance of differences between the control and intoxicated groups and between the intoxicated rats with and without biliary fistulization.

Other pharmacokinetic parameters were calculated as follows. Rat: Fecal clearance ($CL_F$) was estimated from Eq. 3.

$$CL_F = \frac{\text{small intestinal contents volume}}{\text{transit time}} \quad (3)$$

Where transit time (100 min) is the literature value. Bacterial metabolic clearance ($CL_{Bm}$) was the conjectural value used for GLZ prediction in normal rat. The $C_f$ was calculated by introducing the parameters (Fig. 2) into the defined equation reported previously. The calculation was carried out by the Hitchcock-Bairstow program on a personal computer (NEC PC-9801 vm2). The calculated $C_f$ value was 0.328 mM at the initial plasma concentration (blood concentration × plasma-to-blood concentration ratio, $C_p/C_B$) after i.v. dosing (ca. 1.3 mg/ml) and the $K_2 \cdot C_f$ was 0.008, regarded as negligible. Therefore, the defined equation can be simplified as follows:

$$C_{tot} = C_f + \frac{n_1(p) \cdot K_1 \cdot C_f}{1 + K_1 \cdot C_f} + n_2(p) \cdot K_2 \cdot C_f \quad (4)$$

where $C_{tot}$ is the total plasma concentration of GLZ. Consequently, $n_2(p) \cdot K_2$ is expressed as the linear binding coefficient (Table III) and the plasma unbound fraction ($f_{u,p}$) is given by Eq. 5.

$$f_{u,p} = \frac{C_f}{C_{tot}} = \frac{(-X + \sqrt{X^2 + 4Y \cdot C_{tot}})/2Y}{C_{tot}} \quad C_{tot} \quad Y = (n_2(p) \cdot K_2 + 1) \cdot K_1$$

Man: The area under the serum concentration-time curve ($AUC'$) was individually calculated by using the serum concentration data after the slow i.v. injection (for 30 min) of 200 mg/man into five subjects with hepatitis (5 men; age 35 to 72 years) (Fig. 4) as follows: the $AUC_{0-6h}$ was determined by using the trapezoidal method. The residual area beyond 6 h ($AUC'_{6-\infty}$) was estimated as $C'/k$. $C'$ is the serum concentration at 6 h that is calculated from $C' = C_0 \exp(-k \times 6)$, where $-k$ is the slope estimated from the log-serum data from 1 to 6 h using linear regression analysis and $C_0$ is the serum concentration at time 0 that was obtained by extrapolating the line with slope $-k$. The $AUC'$ was obtained by adding $AUC_{0-6h}$ to $AUC'_{6-\infty}$.

$CL_{tot}$ expressed as $(\text{Dose} / AUC') \times C_S / C_B$, where $C_S / C_B$ is serum-to-blood concentration ratio calculated as follows: $1/(1 - H_i) \cdot H_i$ is the hematocrit value (0.42) taken from the literature. The $CL_B$ or $CL_R$ was calculated from the following equation:

$$CL_B \ or \ CL_R = \frac{\text{Dose} \times ER_B \ or \ ER_U}{AUC'} \times (C_S / C_B) \quad (6)$$

where $ER_B$ is the biliary excretion ratio (0.51 in the intoxicated rat or 0.80 in the control rat) of the total biliary excretion amount to the dose. $ER_U$ is the urine excretion ratio (0.012) of the total urinary amount to the dose taken from the literature. As in the case of rats, GLZ was assumed to be metabolized only in hepatic and small intestinal tissues and the total metabolic clearance ($CL_{M,tot}$) was similarly calculated. Equation for the hepatic and small intestinal intrinsic clearances ($CL_{int,j}$) was derived as follows:

$$CL_{M,j} = CL_{M,tot} \times \frac{k_{m,j} \cdot V_{T,j}}{\sum_{j} k_{m,j} \cdot V_{T,j}} \quad (7)$$

Where subscript $j$ represents liver or small intestine and $V_T$ is the tissue volume in man (Table I). The $CL_{M,j}$ is expressed by Eq. 8.

$$CL_{M,j} = \frac{Q_j \cdot f_{u,s} \cdot (C_S / C_B) \cdot CL_{int,j}}{Q_j + f_{u,s} \cdot (C_S / C_B) \cdot CL_{int,j}} \quad (8)$$
Where $Q$ is the tissue blood flow rate (Table I) and $f_{u,s}$ is the serum free fraction calculated from Eq. 5 by using serum protein binding parameters (Table III). Rearrangement of Eq. 8 gives

$$CL_{int,j} = \frac{Q_j \cdot CL_{M,j}}{(Q_j - CL_{M,j}) \cdot f_{u,s} \cdot (C_S/C_B)}$$

(9)

As the $CL_{M,tot}$ values (9.6–26.0 ml/min/man) are much smaller than $Q_j$, Eq. 9 can be simplified to Eq. 10.

$$CL_{int,j} = \frac{CL_{M,j}}{f_{u,s} \cdot (C_S/C_B)}$$

(10)

Consequently, $CL_{int}$ in liver and small intestine was calculated from Eq. 10 using the $CL_{M,j}$ value by Eq. 7. The $CL_{Abs}$ was estimated from $k_{Abs} \times$ small intestinal contents volume (Table I), where $k_{Abs}$ is the intestinal absorption rate constant (0.00203 min$^{-1}$) calculated by the feathering method from the mean plasma drug concentration data after an oral GLZ administration (64 mg/man) in five subjects (Table I). $CL_F$ was estimated from Eq. 3, in which the transit time (1000 min) is the literature value. (22) $CL_{Bm}$ is the conjectural value used for GLZ prediction in normal subjects (1). The $K_p$ values for each tissue are also listed in Table II. The $K_p$ was estimated as follows: $K_p \times (C_S/C_B)/(C_P/C_B)$, where $K_p$ and $C_P/C_B$ are the mean $K_p$ and $C_P/C_B$ values in the intoxicated rats (Tables II and III, respectively). However, simulations based on these estimates resulted in higher or lower GLZ concentrations than the observed values. $Vd_{ss}$ was calculated by the same equation as in the case of rats. The $Vd_{ss}$ values varied among five patients (4.0–7.2 l). This variation may be caused by $f_{u,s}$ difference. Therefore, a suitable $f_{u,s}$ for $Vd_{ss}$ was corrected from $f_{u,s} = f'_{u,s} \times (Vd_{ss} \cdot (C_S/C_B))/(\Sigma K_p \cdot V_T + \text{serum volume})$. Where $f'_{u,s}$ is the value (0.004) estimated from Eq. 5 using the binding parameters for normal human serum (28) $Vd_{ss}$ is the value in individual subjects, and $V_T$ and $K_p$ used are shown in Tables I and II, respectively. The $f_{u,s}$ was used for the calculation of $CL_{int}$ in liver and small intestine (Eq. 10). The $f_{u,s}$ values showed as the $K_i$ value in Table III. After testing several estimates, the suitable $CL_{Abs}$ values were individually selected, considering the difference of the elimination rate in five patients (Fig. 4).

**Physiological Constants** — The physiological constants were based on a 0.27 kg rat and a 70 kg man.

**Rat:** The $V_T$ values except for muscle, skin, adipose tissue, and blood were determined experimentally from the wet tissue weight by assuming a density of 1.0 for each tissue. The weighed contents of the small intestine were similarly treated. The $V_T$ of muscle was assumed to be half of the body weight. (22) The other tissue volumes were taken from the literature. (31, 32)

The blood volume ($V_B$) was calculated by the method of Bischoff et al. (23) The $H_I$ value used for $V_B$ calculation was determined to be 0.47 in this study. The blood volume ratio of artery to vein was assumed to be the same as that of humans, i.e., 0.5. (33) The blood flow rate of the lung was assumed to be the same as that of an artery or vein. The $Q$ values of other tissues except for liver, spleen, pancreas, stomach, and small intestine were the literature values for normal rats. (22, 31) The hepatic blood flow rate ($Q_{Li}$) was the literature value in CCl$_4$-intoxicated rats. (35) The $Q$ values of spleen, pancreas, stomach, and small intestine were calculated from 0.527 $\times$ $Q'$, where 0.527 is the $Q_{Li}$ ratio of the intoxicated rat to normal rat (22) and $Q'$ is blood flow rate of each tissue in normal rat (34).

**Man:** The $V_T$ and $Q$ values in 70 kg adult standard human were taken from the literature, but the $Q$ of the lung was assumed in the same way as in the rat. The $Q$ values of stomach and small intestine were estimated by applying to $Q$ of the gastrointestinal in man (850 ml/min) (37) each organ $Q$ ratio (0.16 in stomach and 0.46 in small intestine) to $Q$ of the gastrointestinal in monkey. (33) The $V_B$ was calculated by the method of Bischoff et al. (22) Physiological constants in the rat and man used for the simulation are listed in Table I. The mean experimental values of tissue volume were used for the simulation.

**Model Development** — The physiological pharmacokinetic models A and B, incorporating
enterohepatic circulation, are shown in Fig. 1. These models, which succeeded previously in predicting the GLZ disposition in normal rat (model A) and subject (model B), were applied to GLZ pharmacokinetics for rat and man in the present study, respectively. Namely, model A assumes that the drug excreted from the liver is directly transferred into the gut lumen at the rate \( R_B \) (Appendix II) with a multicomartment description of the gut lumen based on physiologic principles. Further, the rate \( (R_F) \) of transfer of drug down the small intestinal lumen was handled similarly, and the intestinal absorption and bacterial metabolism are assumed to be the same for all segments. For model B, the gallbladder was added to model A. It was assumed that the drug in the gallbladder is excreted in the gut lumen at the rate of \( R'_B \) with the holding time \( \tau \) (Appendix II), where \( \tau \) (540 min) is a conjectural value used for the prediction of GLZ serum concentrations in normal subjects. The human serum concentration data used for the prediction were taken from the literature. The other program used for the models A and B was the same as that used in the previous study. The mass balance-blood flow equations were written for
TABLE I. Physiological Parameters for Modeling in a 0.27 kg Rat and a 70 kg Man

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Volume (ml)</th>
<th>Blood flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rat</td>
<td>Human</td>
</tr>
<tr>
<td>Lung</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>600&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heart</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Liver</td>
<td>12.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3900&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>300&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Muscle</td>
<td>135.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30000&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Skin</td>
<td>48.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>17.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1000&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>200&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>100&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>150&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestine</td>
<td>14.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>640&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestinal contents</td>
<td>2.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>400&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blood Artery</td>
<td>7.7&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1700&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vein</td>
<td>15.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3390&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined experimentally from the wet tissue weight by assuming a density of 1.0. <sup>b</sup> Ref. 22. <sup>c</sup> Ref. 31. <sup>d</sup> Ref. 32. <sup>e</sup> Calculated according to the report of Bischoff et al. <sup>f</sup> Ref. 36. <sup>g</sup> Ref. 20. <sup>h</sup> Q<sub>H</sub> + Q<sub>L</sub> + Q<sub>K</sub> + Q<sub>M</sub> + Q<sub>S</sub> + Q<sub>Acc</sub> for nomenclature, see Appendix I. <sup>i</sup> Ref. 34. <sup>j</sup> Ref. 35. <sup>k</sup> See the text for details. <sup>l</sup> Assumed to be equal to the value for spleen.

The concentration in each compartment as shown in Fig. 1. The complete set of differential equations is given in Appendix II and was solved numerically by the Runge-Kutta-Gill method using a DEC Micro Vax II digital computer. Physiological constants (Table I) and pharmacokinetic parameters (Table II and III) were used for the simulation.

**Results**

Patho-physiological Consequences of Chronic CCl<sub>4</sub> Intoxication

Parameters of patho-physiological changes caused by chronic CCl<sub>4</sub> intoxication<sup>8</sup> are shown in Table IV. No significant difference was found in body weight, plasma albumin, or total plasma protein between the control and intoxicated rats, but the GOT and GPT activities, total bile acids, and total bilirubin were increased significantly (<i>p</i> < 0.01) and bile flow rate was decreased significantly (<i>p</i> < 0.01) by the intoxication.

Pharmacokinetic Aspects

The blood disappearance of GLZ after i.v. administration of 100 mg/kg in the intoxicated rats with and without biliary fistulas followed a biexponential curve. The pharmacokinetic parameters of GLZ in both groups are shown in Table V. The mean AUC and CL<sub>tot</sub> values were decreased and increased significantly by the bile
**TABLE III.** Pharmacokinetic Parameters of GLZ Used for Prediction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intoxicated rat</th>
<th>Patient A, B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>$CL_{Abs}$ (ml/min)</td>
<td>0.321 ± 0.025a)</td>
<td>3.2d)</td>
<td>1.6d)</td>
<td>4.1d)</td>
<td>0.81d)</td>
</tr>
<tr>
<td>$CL_{in}$ (ml/min)</td>
<td>0.923 ± 0.125a)</td>
<td>1428e)</td>
<td>1745e)</td>
<td>3393e)</td>
<td>2640e)</td>
</tr>
<tr>
<td>Liver</td>
<td>778d)</td>
<td>527d)</td>
<td>1106d)</td>
<td>2801d)</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.710 ± 0.065a)</td>
<td>276e)</td>
<td>194e)</td>
<td>377e)</td>
<td>294e)</td>
</tr>
<tr>
<td></td>
<td>87d)</td>
<td>59d)</td>
<td>123d)</td>
<td>312d)</td>
<td></td>
</tr>
<tr>
<td>$CL_B$ (ml/min)</td>
<td>0.096</td>
<td>11.8e)</td>
<td>14.4e)</td>
<td>28.0e)</td>
<td>21.5e)</td>
</tr>
<tr>
<td>$CL_R$ (ml/min)</td>
<td>0.018</td>
<td>0.28e)</td>
<td>0.51e)</td>
<td>0.34e)</td>
<td>0.66e)</td>
</tr>
<tr>
<td>$CL_F$ (ml/min)</td>
<td>0.031</td>
<td>0.4b)</td>
<td>1.35j)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$CL_{BM}$ (ml/min)</td>
<td>0.12d)</td>
<td>1.913 ± 0.010b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_P/C_B$</td>
<td></td>
<td>1.72b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat plasma/dn human serum protein binding $K_1$ (mm⁻¹)</td>
<td>42.3</td>
<td>136.1d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_1(p)$ (mm)</td>
<td>1.25</td>
<td>181k)</td>
<td>139k)</td>
<td>100ª)</td>
<td>111ª)</td>
</tr>
<tr>
<td>$n_2(p) \cdot K_2$</td>
<td>0.167</td>
<td>1.87j)</td>
<td>0.43j)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are given as the mean ± S.E. of three a) and nine b) rats. c) Calculated from the mean plasma data in five normal subjects. d) The selected values. e) and f) Calculated by using the biliary excretion ratio in $CCl_4$-intoxicated (0.51) and control (0.8) rats, respectively. g) Calculated by using the urinary excretion ratio (0.012) in normal subjects. h) Conjectural values in the normal rat and human. i) The values in normal human serum. j) The corrected values. k) The corrected values. l) and m) Assumed to be equal among the subjects. For nomenclature and the calculation methods, see the text.

product cannulation ($p < 0.05$), respectively. This suggests an existence of enterohepatic cycling. While the mean $Vd_{ss}$ and $CL_R$ values showed no significant difference between the two groups, the biliary excretion ratio of the dose decreased more in the intoxicated rats ($51.2 \pm 8.7\%$, $n = 3$) than that in the control rats ($79.9 \pm 10.1\%$, $n = 3$), but with no significant difference. The excretion of GLZ in bile and urine was no longer detectable at 36 h after dosing.

**TABLE IV.** Patho-Physiological Changes in Rats after Chronic $CCl_4$ Intoxication

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Intoxication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>279 ±15</td>
<td>270 ±10</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GOT (Karmen's unit/ml)</td>
<td>81.2 ± 2.2</td>
<td>398.4 ± 26.7a)</td>
</tr>
<tr>
<td>GPT (Karmen's unit/ml)</td>
<td>24.8 ± 0.9</td>
<td>165.3 ± 13.3a)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.6 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.2 ± 0.4</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Total bile acids (µmol/l)</td>
<td>7.9 ± 0.5</td>
<td>137.5 ± 8.6a)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.24 ± 0.06</td>
<td>0.45 ± 0.05a)</td>
</tr>
<tr>
<td>Bile flow rate (10⁻⁵ ml/min)</td>
<td>9.3 ± 1.8</td>
<td>5.6 ± 0.7a)</td>
</tr>
</tbody>
</table>

Results are given as the mean ± S.E. of five control and eleven intoxicated rats. a) Significant difference from the control ($p < 0.01$).
Table V. Pharmacokinetic Parameters of GLZ in CCl₄-Intoxicated Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without biliary fistulation</th>
<th>With biliary fistulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>( AUC ) (min·mg/ml)</td>
<td>230.13 ±28.89</td>
<td>144.80 ±8.50(b)</td>
</tr>
<tr>
<td>( Vd_{ss} ) (ml/kg)</td>
<td>239.94 ±32.75</td>
<td>211.20 ±6.94</td>
</tr>
<tr>
<td>( k_1 \times 10^5 ) (min⁻¹)</td>
<td>1.32 ±0.23</td>
<td>3.13 ±0.16(c)</td>
</tr>
<tr>
<td>( CL_{int} ) (ml/min/kg)</td>
<td>0.428±0.042</td>
<td>0.695±0.041(b)</td>
</tr>
<tr>
<td>( CL_B ) (ml/min/kg)</td>
<td>0.356±0.021</td>
<td></td>
</tr>
<tr>
<td>( CL_R ) (ml/min/kg)</td>
<td>0.079±0.008</td>
<td>0.068±0.004</td>
</tr>
<tr>
<td>( CL_M ) (ml/min/kg)</td>
<td>0.267±0.013</td>
<td></td>
</tr>
</tbody>
</table>

a) Results given as the mean ± S.E. of three rats. For nomenclature and the calculation methods, see the text. b) and c) represent a significant difference from the rats without biliary fistulation \((p < 0.05)\) and \(p < 0.01\), respectively.

Tissue-to-Blood Partition Coefficient \((K_p)\)

The \( K_p \) values of GLZ determined at 3 h after a 100 mg/kg i.v. dose in the intoxicated rats with bile fistulas are shown in Table II. The \( K_p \) of brain could not be evaluated, because the drug concentration was under the detection limit (\( > 2 \mu g/g \) wet weight). The sum of the total tissue distribution volume (\( \Sigma K_p \cdot V_T \) and plasma volume\(^{22} \)) is 54.9 ml/270 g, which corresponds well to the mean \( Vd_{ss} \) values (Table V).

The mean \( K_p \) value of liver in the intoxicated rats was similar to that (0.277 ± 0.021, \( n = 3 \)) in the control rats. The mean \( C_P/C_B \) values determined at 3, 8, and 16 h after i.v. dosing were almost constant (Table III) and the \( H_t \) value was 0.47, indicating that GLZ is not well taken into erythrocytes.

Tissue Metabolic Clearance

GLZ was eliminated only in hepatic and small intestinal homogenates of the intoxicated rats. The \( CL_{int} \) values in those tissues are shown in Table III.

Plasma Protein Binding

Figure 2 shows a Rosenthal plot of the plasma binding data of GLZ in the intoxicated rats. The data fitted the case of two classes of binding sites reasonably well. The calculated parameters are listed in Fig. 2. The \( K_1 \) was larger than \( K_n \), indicating that plasma protein binding of GLZ is mainly determined by the binding to the primary binding sites.

Simulation in Rat

The GLZ concentration time-courses in blood and tissues after a 100 mg/kg i.v. dose in the intoxicated rats were simulated by using model A shown in Fig. 1. Fairly good agreement was observed between the predicted and observed GLZ concentration profiles in all cases (Fig. 3).

Simulation in Man

The prediction of GLZ serum disposition following the slow i.v. injection (for 30 min) of 200 mg/man into five humans with hepatitis\(^{24} \) was made by using model B. The prediction fell markedly below the observed values in four patients \( A-D \), but in patient E, the prediction
Fig. 3. Predicted (Lines) and Observed (Points) log Concentrations of GLZ in Blood and Tissues after i.v. Administration of 100 mg/kg to CCl₄-Intoxicated Rats
Each point and vertical bar represent the mean and S.E. of three rats.
agreed well with the observed values (not shown). Therefore, the predictions for patients A—D were tested by changing respectively the $CL_{Abs}$ and $K_1$ values using the biliary excretion ratio 80% in control rats. The test was carried out by using the 2—5 times larger $CL_{Abs}$ and 0.7—1.3 times larger $K_1$ values than those in normal subjects (Table III). After testing several estimates, the pharmacokinetic parameters were selected (Table III). As the selected values were almost identical between patients A and B, the mean values were used for both patients. Figure 4 shows the predicted and observed serum GLZ concentration profiles. The predicted and observed serum disposition agreed reasonably in all cases.

**Discussion**

Repeated intoxication by CCl$_4$ is often used as a model of chronic liver disease. Cameron and Karunaratne reported that intoxication by CCl$_4$ for 8—10 weeks (16 or 20 doses) produces histological changes typical of chronic liver disease. In the present study, rats intoxicated by the repeated injection of CCl$_4$ for 8 weeks (16 doses) were used. It is well known that administration of CCl$_4$ to rats depresses various drug-metabolizing activities. The $CL_M$ (Table V) was just 80% of that in the normal rats (0.335 ± 0.176 ml/min/kg) reported previously, but the difference was not statistically significant. This suggests that GLZ-metabolizing enzyme ($\beta$-glucuronidase) activity was not greatly affected by CCl$_4$ hepatotoxication. $\beta$-Glucuronidase is present mainly in liver and small intestine.

In this study, GLZ was metabolized only in the liver and small intestine. The biliary excretion ratio and bile flow rate (Table IV) decreased approximately 36 and 40% by the intoxication, respectively. However, the $K_p$ of liver in the intoxicated rats (Table II) was similar to that in the control rats. This might suggest the suppression to only the transport of GLZ from liver into bile by the intoxication, but with no effect on the uptake from plasma into liver. We previously reported the binding of GLZ to plasma protein in normal rats. It was concluded that there are two classes of binding sites, primary and secondary, and the binding to the primary sites mainly determines the plasma binding of GLZ. These results are similar to those in the present study. However, the $K_p$ in the intoxicated rats (Fig. 2) was only one third of that in the normal rats (124.2 mM$^{-1}$), but with no change in $n_i$ ($p$) between the two groups. Such a decrease of $K_p$ might be caused by competitive displacement of the drug from binding sites by endogenous substances, because it has been reported that drug binding to rat plasma and human serum protein is decreased by the increase of bilirubin and bile acids in blood caused by liver failure. Plasma bilirubin and bile acids were certainly increased significantly by CCl$_4$ hepatotoxication (Table IV).

Good agreements were obtained between the predicted and observed time courses of GLZ concentrations in blood and many tissues in CCl$_4$-intoxicated rats by using model A incorporating enterohepatic recycling. As reported previously, model A gave a successful prediction of GLZ in normal rats after an i.v. dose (100 mg/kg), and the prediction by model B, which incorporates the gallbladder, gave good agreement with the observed values in normal sub-
jects. In these models, the conjectural $CL_{Bm}$ value was used, as GLZ is metabolized by human intestinal bacteria. The $CL_{Bm}$ values of normal rat and man were used in the present study.

The variations in human serum concentration time courses following an i.v. injection of GLZ (80 mg/man) to normal subjects were small. However, the serum concentrations were more varied among the five individual patients, especially for 9 to 24 h (Fig. 4). Therefore, $AUC'$ was individually calculated. For the $AUC'$ calculation, the elimination rate constant $k$ was estimated by using the serum concentration data from 1 to 6 h in five patients in order to avoid the effect of enterohepatic recycling on the elimination rate. The obtained $Vd_{ss}$ values varied widely among the patients (4.0–7.2 l). This variation may be caused by $f_{u,s}$ difference. Therefore, a suitable $f_{u,s}$ for $Vd_{ss}$ was individually calculated by changing only $K_1$ (0.7–1.3 times larger than that in normal subjects) (see Data Analysis), considering that only $K_1$, except for $n_1 (p)$ and $n_2 (p) \cdot K_2$, changed between the normal and intoxicated rats, as described already, and the serum free fraction changes by liver disease. The species difference in biliary excretion ratio has been reported, but there is no report on species difference in the biliary excretion of GLZ. In the previous study, the biliary excretion ratio in normal rat (80%) was also used in normal subjects. In this study, the biliary excretion ratio in the intoxicated rats (51%) was used for patients. But the predicted lines by model B fell markedly below as compared with the observed values in patients A–D, except E. Therefore, the ratio in patients A–D was assumed to be the same as that (80%) in control rats, but the predictions again fell below. Considering these results, the $CL_{Abs}$ was selected in patients A–D after testing several estimates, but the $CL_{Abs}$ in patient E was the same as that in normal subjects (Table III). The prediction gave reasonable agreement with the observed values in all patients (Fig. 4). The difference in biliary excretion ratio between patients A–D and E may be caused by the nature and/or extent of liver injury. Unfortunately, the information was not available. The need for higher $CL_{Abs}$ estimates (2–5 times larger than that in normal subjects) suggests individual differences in the transit of fluid through the gut lumen and bacterial metabolism which contribute to elimination of GLZ from the gastrointestinal tract.

Thus, the prediction was successful in all compartments in CCl$_4$-hepatotoxicated rat, and a scale-up of the disposition kinetics of GLZ from CCl$_4$-hepatotoxicated rat to humans with hepatitis was also successful, although the selected pharmacokinetic parameters were used.

**Appendix**

1. Nomenclature

<table>
<thead>
<tr>
<th>General</th>
<th>$V$</th>
<th>Volume of tissue, blood, plasma, or serum (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Q$</td>
<td>Blood flow rate through tissue (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$C$</td>
<td>Tissue, blood, plasma, or serum concentration of drug ($\mu$g/g or $\mu$g/ml)</td>
<td></td>
</tr>
<tr>
<td>$A_{Ga}$</td>
<td>Amount of drug in gallbladder ($\mu$g)</td>
<td></td>
</tr>
<tr>
<td>$K_p$</td>
<td>Tissue-to-blood concentration ratio</td>
<td></td>
</tr>
<tr>
<td>$C_p/C_B$</td>
<td>Plasma-to-blood concentration ratio</td>
<td></td>
</tr>
<tr>
<td>$C_{S/C_B}$</td>
<td>Serum-to-blood concentration ratio</td>
<td></td>
</tr>
<tr>
<td>$f_{u,p}$</td>
<td>Plasma unbound fraction</td>
<td></td>
</tr>
<tr>
<td>$f_{u,s}$</td>
<td>Serum unbound fraction for the patient</td>
<td></td>
</tr>
<tr>
<td>$f'_{u,s}$</td>
<td>Serum unbound fraction for the normal subject</td>
<td></td>
</tr>
<tr>
<td>$CL_{Abs}$</td>
<td>Intestinal absorption clearance (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$CL_B$</td>
<td>Biliary clearance based on the blood concentration (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$CL_R$</td>
<td>Renal clearance based on the blood concentration (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$CL_{Bm}$</td>
<td>Bacterial metabolic clearance (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$CL_F$</td>
<td>Fecal clearance (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$CL_{int}$</td>
<td>Intrinsic clearance for metabolism (ml/min)</td>
<td></td>
</tr>
<tr>
<td>$R_M$</td>
<td>Metabolic rate in the tissue ($\mu$g/min)</td>
<td></td>
</tr>
<tr>
<td>$R_B$</td>
<td>Biliary excretion rate ($\mu$g/min)</td>
<td></td>
</tr>
<tr>
<td>$R_F$</td>
<td>Rate of drug transfer down the gut lumen ($\mu$g/min)</td>
<td></td>
</tr>
<tr>
<td>$\tau$</td>
<td>Holding time (min)</td>
<td></td>
</tr>
<tr>
<td>$\theta$</td>
<td>Reciprocal of injection time (min$^{-1}$)</td>
<td></td>
</tr>
</tbody>
</table>
\( I(t) \) Injection function

**Subscripts**
- Ar: Artery
- Ve: Vein
- Lu: Lung
- He: Heart
- Li: Liver
- Ki: Kidney
- Mu: Muscle
- Sk: Skin
- Ad: Adipose tissue
- Sp: Spleen
- Pa: Pancreas
- St: Stomach
- SI: Small intestine
- SIC: Small intestinal contents
- Ga: Gallbladder
- T: Tissue

**II. Model Equations**

The following mass balance blood flow equations describe the concentration in each compartment of the pharmacokinetic model shown in Fig. 1.

**Rat**

Model A

**Artery blood:**

\[
V_{Ar} \frac{dC_{Ar}}{dt} = Q_{Ar} \left( \frac{C_{Lu}}{K_{p,Lu}} - C_{Ar} \right) \quad (A-1)
\]

**Venous blood:**

\[
V_{Ve} \frac{dC_{ve}}{dt} = Q_{He} \left( \frac{C_{He}}{K_{p,He}} \right) + Q_{Li} \frac{C_{Li}}{K_{p,li}} + Q_{Ki} \frac{C_{Ki}}{K_{p,Ki}} + Q_{Mu} \frac{C_{Mu}}{K_{p,Mu}} + Q_{Sk} \frac{C_{Sk}}{K_{p,Sk}} + Q_{Ad} \frac{C_{Ad}}{K_{p,Ad}} - Q_{ve} \cdot C_{ve} + I(t) \quad (A-2)
\]

Where \( I(t) \) is the injection function expressed as follows:

\[
I(t) = \text{dose} \cdot \theta^2 (1 - \theta)^2 \quad (\theta = 2 \text{ min}^{-1})
\]  

**Lung:**

\[
V_{Lu} \frac{dC_{Lu}}{dt} = Q_{Lu} \left( C_{Ve} - \frac{C_{Lu}}{K_{p,Lu}} \right) \quad (A-4)
\]

**Liver:**

\[
V_{Li} \frac{dC_{Li}}{dt} = (Q_{Li} - Q_{Sp} - Q_{Pa} - Q_{St} - Q_{SI}) \cdot C_{Ar} + Q_{Sp} \frac{C_{Sp}}{K_{p,Sp}} + Q_{Pa} \frac{C_{Pa}}{K_{p,Pa}} + Q_{St} \frac{C_{St}}{K_{p,St}} + Q_{SI} \frac{C_{SI}}{K_{p,SI}} - Q_{Li} \frac{C_{Li}}{K_{p,Li}} - R_{M,Li} - R_{B} \quad (A-5)
\]

Where \( R_{M,Li} \) and \( R_{B} \) are given by

\[
R_{M,Li} = f_u \cdot \left( C_p / C_B \right) \cdot CL_{int,Li} \cdot C_{Li} / K_{p,Li} \quad (A-6)
\]

\[
R_{B} = CL_B \cdot C_{Li} / K_{p,Li} \quad (A-7)
\]

**Kidney:**

\[
V_{Ki} \frac{dC_{Ki}}{dt} = Q_{Ki} \left( C_{Ar} - \frac{C_{Ki}}{K_{p,Ki}} \right) - CL_R \frac{C_{Ki}}{K_{p,Ki}} \quad (A-8)
\]

**Small intestine:**

\[
V_{SI} \frac{dC_{SI}}{dt} = Q_{SI} \left( C_{Ar} - \frac{C_{SI}}{K_{p,SI}} \right) + \sum_{i=1}^{4} \frac{1}{4} CL_{abs} \cdot C_{SIC,i} - R_{M,SI} \quad (A-9)
\]

Where \( R_{M,SI} \) is given by

\[
R_{M,SI} = f_u \cdot \left( C_p / C_B \right) \cdot CL_{int,SI} \cdot C_{SI} / K_{p,SI} \quad (A-10)
\]

**Gut lumen:**

\[
\frac{dC_{SIC}}{dt} = \frac{1}{4} \sum_{i=1}^{4} \frac{dC_{SIC,i}}{dt} \quad (A-11)
\]
Prediction of Glycyrrhizin Disposition

\[
\frac{V_{\text{SIC}}}{4} \frac{dC_{\text{SIC},1}}{dt} = R_B - \frac{1}{4} (CL_{\text{Abs}} + CL_{\text{Bm}}) C_{\text{SIC},1} - R_{F,1} \quad (A-12)
\]

\[
R_{F,i} = CL_F \cdot C_{\text{SIC},i} \quad (i = 2 - 4) \quad (A-13)
\]

Where \( R_{F,1} \) and \( R_{F,i} \) are given by

\[
R_{F,1} = CL_F \cdot C_{\text{SIC},1} \quad (A-14)
\]

\[
R_{F,i} = CL_F \cdot C_{\text{SIC},i} \quad (A-15)
\]

Non-eliminating tissue (T=He, Mu, Sk, Ad, Sp, Pa, and St):

\[
V_T \frac{dC_T}{dt} = Q_T \left( C_{\text{Ar}} - \frac{C_T}{K_{p,T}} \right) \quad (A-16)
\]

Man

Model B

Mass balance equations are the same as those in model A except for following equations. However, \( K_{p,T} \) replaced \( K_{p,T} f'_{u,s}/f_{u,s} \).

Liver:

Equation A-6 is expressed as:

\[
R_{M,Li} = f_{u,s} \cdot (C_S/C_B) \cdot CL_{\text{int,li}} \cdot C_{\text{Li}}/K_{p,Li} \quad (A-17)
\]

Small intestine:

Equation A-10 is expressed as:

\[
R_{M,SI} = f_{u,s} \cdot (C_S/C_B) \cdot CL_{\text{int,SI}} \cdot C_{\text{SI}}/K_{p,SI} \quad (A-18)
\]

Gallbladder:

\[
\tau \frac{dR'_B}{dt} = R_B - R'_B \quad (\tau = 540 \text{ min})^1 \quad (A-19)
\]

Gut lumen:

Equation A-12 is expressed as:

\[
\frac{V_{\text{SIC}}}{4} \frac{dC_{\text{SIC},1}}{dt} = \frac{V_{\text{SIC}}}{4} \frac{dC_{\text{SIC},i}}{dt} = R'_B - \frac{1}{4} (CL_{\text{Abs}} + CL_{\text{Bm}}) C_{\text{SIC},1} - R_{F,1} \quad (A-20)
\]

\[
I(t) \text{ can be expressed as follows:}
I(t) = 200 \text{ mg/30 min} \quad (0 < t \leq 30 \text{ min}) \quad (A-21)
\]

References

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44. C. J. Bowner, P. G. Donoghue, C. F. Leong, and M. S. Yates: Effect of bile acids on the binding of drugs and


