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

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Three physiologically based pharmacokinetic models A—C, incorporating enterohepatic recycling, were developed to predict glycyrrhizin (GLZ) disposition in rat plasma and tissues, and human serum. Model A, which included fourteen compartments (artery, vein, tissues except brain, and gut lumen) with the assumption of direct excretion of GLZ from the liver into the gut lumen gave fairly good agreement between the observed and predicted disposition profiles in rat, but was unsuitable in man, where elimination is very rapid. Models B and C for man were obtained by adding a gallbladder compartment (drug storage organ) for the excretion from the liver into the gut lumen and by assuming continuous transfer from the storage compartment or instantaneous emptying from it during meal ingestion as the excretion process from the gallbladder into the gut lumen, respectively. The agreement between the observed and predicted serum concentration time-course profiles was better with model C than model B, especially in the terminal elimination phase, where secondary peaks appeared. However, it was thought that the observed serum disposition can be sufficiently well predicted by model B. In conclusion, prediction in rat was successful in all compartments except the brain, which shows a negligible distribution. Scale-up of the disposition kinetics of GLZ from rat to man was also successful.

Keywords: glycyrrhizin; physiological pharmacokinetic model; enterohepatic recycling; animal scale-up; rat; man

Glycyrrhizin (GLZ), an active ingredient of Glycyrrhiza (licorice), has frequently been used in the treatment of chronic hepatitis,¹ allergic disorder,² ⁴ inflammation,² ⁵ and gastric ulcers.⁶ Recently GLZ has been reported to have an inhibitory effect on the in vitro infectivity and cytotoxic activity of human immunodeficiency virus,⁷ and produced some improvement in immune function and an objective clinical improvement in patients with human immunodeficiency virus infection.⁸ However, GLZ has been reported to produce the adverse effect of aldosteronism when given in massive doses.⁹ ¹²

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.¹³ Physiologically based pharmacokinetic models have been applied successfully to predict the human plasma disposition of a drug which is subjected to enterohepatic recycling, such as methotrexate¹⁴ or digoxin,¹⁵ by using the data from a laboratory animal.

The purpose of the present study was to predict the time courses of GLZ concentrations in plasma and tissues of rats by using a physiologically based pharmacokinetic model incorporating enterohepatic recycling and also to predict the drug serum disposition in man by using the model and the data obtained from in vivo and in vitro studies in rats.

Experimental

Chemicals GLZ (Minophagen Pharmaceutical Co., Tokyo, Japan) was used as supplied. Inulin was purchased from Wako Pure Chemical Ind. Ltd. (Tokyo, Japan). All other reagents were commercial products of analytical grade.

Animals Male Wistar rats (weighing 240—260 g) that had been fasted for 20 to 24 h prior to the experiments were used throughout. Rats were lightly anesthetized with ether for all surgical procedures. The right femoral vein and left femoral artery were cannulated with PE-50 polyethylene tubing for i.v. drug administration and blood sampling, respectively. After 1 h of recovery from anesthesia, rats with and without bile duct cannulation (PE-10 polyethylene tubing) were given GLZ (100 mg/kg), 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 rats with biliary fistulation was 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.

Plasma and Tissue Disposition After administration of GLZ to rats, with biliary fistulation, blood samples (300 µl each) were collected in heparinized polyethylene centrifuge tubes at 1, 2, 3, and 8 h. In the case of rats without biliary fistulation, after removal of blood samples, the rats were exanguinated via a carotid artery at 1, 2, 3, 8, 16, or 24 h after dosing and perfused with cold saline solution warmed just inferior to the renal veins until the effusate became colorless. The bile (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 for 5 min to obtain plasma. 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 (Kₑ) Blood and biled tissue samples were obtained at 1 h after dosing of the rats with bile fistulas by the same method as described above.

Intestinal Absorption Intestinal absorption was investigated by the single-pass perfusion method.¹⁶ The length of 15 cm from the proximal end of the jejunum was used. The 24-h bile 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). No decomposition of GLZ in the perfused solution was observed for 2 h at 37°C.

Blood and Tissue Elimination and Tissue Binding Blood and blood-free tissue samples were obtained by the same method as in the plasma and 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 (over the range of tissue concentrations after i.v. dosing) 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 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. The spontaneous degradation of GLZ did not occur in the buffer solution. For the determination of tissue intrinsic clearance for metabolism (Clₑ(tissue)), the tissue binding of GLZ was examined in the liver or small intestine homogenates (16.7 and 33.3%) by an ultrafiltration technique using the MPS-1 Starter Kit (Amicon Co., Mass.). One millilitre 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 the filtrate was obtained by centrifuging for 40 min at 1000 g and 4°C in a
centrifugal separator (KR/200B, Kubota Co., Tokyo, Japan). The adsorption of the drug on the membrane and the leakage of macromolecular components of tissue homogenate into the filtrate were negligible.

**Determination Method** Samples of 100 μl each for plasma, blood, perfusate, and tissue homogenate filtrate and 300–500 μl for tissue homogenate were used for GLZ determination. The extraction procedure17 and high performance liquid chromatography (HPLC) method20 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.18

**Data Analysis** Apparent $K_a$ value was corrected by the method of Chen and Gross.20 The intestinal absorption clearance ($CL_{IA}$) was estimated from Eq. 1:

$$CL_{IA} = \frac{C_{in} - C_{in} - C_{out} \times 80.8 \text{ cm}}{C_{in}} \times 150 \text{ cm}$$

where $C_{in}$ and $C_{out}$ are the drug concentrations in the influx and efflux perfusates, respectively; $Q_{in}$ and $Q_{out}$ are the influx and efflux flow rates, respectively. The $C_{in}$ was calculated from the inulin concentration difference in the influx ($C_{in}$) and efflux ($C_{out}$) perfusates as follows: $C_{in} = Q_{in} \times C_{in} / (C_{in} / C_{out})$. Fifteen centimeters of small intestine was the length used in the absorption study and 80.8 cm is its total length according to the literature.27 The hepatic and small intestinal $CL_{IA}$ were calculated as follows: (1) the elimination rate ($r$, 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 ($k_E$) was calculated from $k_E (\text{min}^{-1}) = x (\text{min}^{-1}) d$, where $x$ is the slope of the plot of r 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) $CL_{IA}$ (ml/min) was calculated from $k_E \times V_I$, where $V_I$ is the volume of liver or small intestine (Table I).

**Means** are presented with their standard error (mean ± S.E.).

Other pharmacokinetic parameters were derived from the literature values as follows. Rat: The biliary ($CL_B$) and renal ($CL_R$) clearances based on blood concentration data were obtained from $CL_B$ or $CL_R \times (C_{in}/C_{out})$, where $CL_B$ and $CL_R$ are the literature mean values of biliary and renal clearances based on the plasma concentration data, respectively.16 $CL_B$ or $CL_R$ is the plasma-to-blood concentration ratio determined in this study. Fecal clearance ($CL_F$) was estimated from Eq. 2.

$$CL_F = \frac{\text{small intestinal contents volume}}{\text{transit time}}$$

Where transit time (100 min) is the literature value.14 The conjectural metabolic clearance ($CL_{MCA}$) value to create the best fit to plasma concentration data was computed by using the Multi-Runge program.22 Unbound rat plasma concentration ($C_{P,un}$) was calculated from the equation defined in the previous report15 by using the binding parameters (Table III) by using the Hitchcock–Bairstow program20 on a personal computer (NEC PC-9801VM2). As the calculated $C_{P,un}$ was 0.383 min even at the highest plasma concentration (the initial concentration, ca. 1.60 mg/ml)17 after i.v. dosing, and the estimated $K_t; C_t (0.04)$ was a negligible value, the defined equation13 was simplified as follows:

$$C_{un} = C_t + n_p (p) \times K_t \times C_t \times 1 + 1 + n_p (p) \times K_t \times C_t$$

where $C_{un}$ is the total concentration of GLZ in plasma; $K_t$ and $K_p$ are the association constants for primary and secondary binding sites, respectively; $n_p (p)$ and $n_p (p)$ are the binding capacities for primary and secondary binding sites, respectively. Consequently, $n_p (p) \times K_t$ is expressed as the linear binding coefficient (Table III) and the plasma unbound fraction ($f_{un}$) is given by Eq. 4.

$$f_{un} = \frac{C_{un} - \frac{\sqrt{X + \sqrt{X^2 + 4Y}} - X}{2}}{C_{un}}$$

where:

$$X = n_p (p) \times K_t \times 1 + 1 + n_p (p) \times C_{un}$$

$$Y = (n_p (p) \times K_t \times 1 + 1) \times K_t$$

Man: The human-skin-to-blood concentration ratio ($C_{S,h}, C_{l,un}$) was estimated from $C_{S,un} C_{l,un} \times 1 + H, C_{l,un}$ is the hematocrit value (0.42) taken from the literature.24 $K_a$ for each tissue was estimated by using the mean $K_a$ value (Table II) and $C_{S,h}, C_{l,un}$ (Table III) in the rats as follows: mean $K_a \times (C_{S,h} C_{l,un})/C_{l,un}$ (Table III). The $CL_{IA}$ was estimated from $CL_{IA} \times \text{small intestinal contents volume, where } K_{AB} \text{ is the intestinal absorption}$

rate constant (0.00203 min$^{-1}$) calculated by the feathering method25 from the mean plasma drug concentration data after an oral GLZ administration (64 mg/m in five subjects).26 The small intestinal contents volume (Table I) was taken from the literature.21 The $CL_{IA}$ or $CL_{FA}$ was calculated from Eq. 5.

$$CL_{IA} = \frac{\text{dose} \times ER_{U} \times ER_{U}}{AUC_{\text{IA}}} \times (C_{in}/C_{un})$$

Where $ER_{U}$ is the biliary excretion ratio (0.8) of the total cumulative excretion amount to the dose, assumed to be the same as in the case of rat. $ER_{U}$ is the urinary excretion ratio (0.012) of the total cumulative excretion amount to the dose taken from the literature.27 The $AUC_{\text{IA}}$ was determined by using the trapezoidal method from the human serum concentration data in individual humans ($n$ = 3, Fig. 3) after i.v. dosing (80 mg/m).27 The residual area beyond 6 h ($AUC_{\text{IA}} - \text{area}$) was estimated as $C_{in} \times (C_{in} / C_t)$ in the serum concentration at 6 h that was estimated from $C_{in} \times \exp (-k) \times 6$, where $k$ is the slope ($-3.26 \times 10^{-5}$ min$^{-1}$) estimated from the log-serum data from 1 to 6 h using linear regression analysis and $C_{in} (11.10 \mu g/ml)$ is the serum concentration at time 0 that was obtained by extrapolating the slope with slope $-k$. The $AUC_{\text{IA}} - \text{area}$ was calculated by adding $AUC_{\text{IA}} - \text{area}$ to $AUC_{\text{IA}} - \text{area}$. The mean $AUC_{\text{IA}} - \text{area}$ was used for $AUC_{\text{IA}}$ in Eq. 5. The distribution volume at the steady state ($V_d$) was calculated by $V_d = \frac{\text{dose}}{AUC_{\text{IA}}}$, where $AUMC$ is the area under the first moment curve. GLZ was assumed to be metabolized only in hepatic and small intestinal tissues, as in the case of rats. $CL_{IA}$ in liver and small intestine was calculated from Eq. 6.

$$CL_{L,d} = \frac{V_d \times (C_{P,un})}{\text{sum of } C_{P,un} \times V_I}$$

Where $CL_{L,d}$ is the metabolic clearance, given by $CL_{L,d} = CL_{IA} - CL_{B} - CL_{R}$. $CL_{L,d}$ is total blood clearance expressed as (dose$/AUC_{\text{IA}}$)$\times C_{P,un}$, $f_{un}$ is serum free fraction calculated from the equation defined in the previous report20 by using serum protein binding parameters (Table III). The subscript $j$ represents liver and small intestine. $CL_{L,d}$ was estimated from Eq. 2, in which the transit time (1000 min) is the literature value.44

$CL_{IA}$ is a conjunctival value to create the best fit to human serum concentrations by model A using the Multi-Runge program.22

**Physiological Constants** The physiological constants were based on a 0.25 kg rat and a 70 kg man. Rat: Tissue volumes 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 weighted contents of the small intestine were similarly treated. The muscle volume was assumed to be half of the body weight.14 The other tissue volumes were taken from the literature.29,30 The blood volume ($V_B$) was calculated by the method of Bischoff et al.14 as follows:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Volume (ml)</th>
<th>Blood flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>1.3±0.1</td>
<td>600±0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>1.1±0.1</td>
<td>300±0.1</td>
</tr>
<tr>
<td>Liver</td>
<td>1.2±0.1</td>
<td>3900±0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.2±0.1</td>
<td>300±0.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>125±0.1</td>
<td>300±0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>4.7±0.1</td>
<td>300±0.1</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>17.5±0.1</td>
<td>10000±0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.5±0.1</td>
<td>200±0.1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>0.9±0.1</td>
<td>100±0.1</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>1.3±0.1</td>
<td>150±0.1</td>
</tr>
<tr>
<td>Small intestine</td>
<td>13.8±0.1</td>
<td>640±0.1</td>
</tr>
<tr>
<td>Small intestinal contents</td>
<td>3.1±0.1</td>
<td>400±0.1</td>
</tr>
<tr>
<td>Blood Artery</td>
<td>7.5±0.1</td>
<td>1700±0.1</td>
</tr>
<tr>
<td>Vein</td>
<td>15.1±0.1</td>
<td>3390±0.1</td>
</tr>
</tbody>
</table>

a) Determined experimentally from the wet tissue weight by assuming a density of 1.0.

b) Obtained from ref. 14. c) Obtained from ref. 30. d) Obtained from ref. 29.

e) Calculated according to the report of Bischoff et al.14 f) Obtained from ref. 33. g) Obtained from ref. 21. h) Obtained from ref. 16. i) Obtained from ref. 20. j) Assumed to be equal to the value for spleen. k) See the text for details.
Fig. 1. Pharmacokinetic Model for the Disposition of GLZ in the Rat (Model A) and Man (Models B, C, and D). For nomenclature, see Appendix I.

\[ V_{\text{plasma}} = 44 \times \text{(body weight, kg)}^{0.99} \]

\[ V_p = V_{\text{plasma}} \times (1 - H) \]

The \( H \) value was determined to be 0.509 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. Blood flow rates of other tissues or organs were the literature values.\(^{14,29,30,32}\) Man: Tissue volumes and tissue blood flow rates were taken from the literature.\(^{21,33}\) But the blood flow rate of the lung was assumed to be the same as that of the rat and those of stomach and small intestine were estimated by applying to the gastrointestinal blood flow rate in man (850 ml/min)\(^{40}\) each organ blood flow ratio (0.16 in stomach and 0.46 in small intestine) to gastrointestinal blood flow rate in monkey.\(^{31}\) The \( V_p \) was calculated from Eqs. 7 and 8.

**Model Development**

Physiological pharmacokinetic models A–C, incorporating entericographic circulation, are shown in Fig. 1. Model A assumes that the drug excreted from the liver is directly transferred into the gut lumen at rate \( R_h \) (Appendix II) with a multiparameter description of the gut lumen based on physiological principles, as reported by Bischoff et al.\(^{16}\) Further, the rate (\( R_h \)) 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 models B and C, the gallbladder was added to model A. For model B, it was assumed that the drug in the gallbladder is excreted in the gut lumen at the rate of \( R_h \) with the holding time \( t \) (Appendix II), where \( t \) is a conjunctural value to create the best fit to rat plasma or human serum concentration data. For details, see the text. b) Obtained from the previous report.\(^{16}\) c) For details, see the text. f) Obtained from the previous report.\(^{34}\) For nomenclature, see the text.
Results

Simulation in Rat  The GLZ concentration profiles in plasma and tissues after 100 mg/kg i.v. dosing were simulated by model A. Fairly good agreements were observed between the predicted and observed GLZ concentration profiles in all cases (Fig. 2).

Simulation in Man  The prediction of GLZ serum disposition was simulated by using models A—C. Figure 3 shows the observed and predicted serum GLZ concentration profiles following the slow i.v. injection (for 30 min) of 80 mg/men into three normal subjects.27) The predicted and observed serum concentration profiles agreed well until 23 h after dosing in each model. However, after 23 h, the prediction by model A fell markedly below the observed values. On the other hand, the predictions by models B and C agreed well with the observed values as compared with the case of model A.

Discussion

Models A—C (Fig. 1), incorporating enterohepatic recycling, for predicting the disposition of GLZ in rat and man have been developed. The $K_p$ value in brain could not be evaluated, because the brain drug concentration at 1 h after dosing (the $K_p$ determination time) was under the detection limit (2 μg/g wet weight). Therefore, the brain was not incorporated in the models. The sum of the total tissue distribution volume (∑ $K_p$; $V_T/(C_P/(C_B))$) and $V_{plasma}$14) in rat is 28.90 ml/250 g, which corresponds well to the $V_{dav}$ value (27.92 ml/250 g).13) The mean $K_p$ values in rats were used for the prediction in man. The total tissue distribution volume in man was calculated by using the mean $K_p$ values in rats. The total value (7.5 l/70 kg man) obtained by adding the sum of the $K_p$; $V_T/(C_P/(C_B))$ values to the human serum volume (3.0 l/70 kg man)14) corresponded well to the calculated $V_{dav}$ value (7.0 l/70 kg man).23) This suggests that the human tissue distribution profile of GLZ may be similar to that of rats. GLZ has frequently been used for the treatment of chronic hepatitis13) and allergic skin disorder,2,4)—8) so it is interesting to note that although all $K_p$ values were lower than 1.0 (Table II), the $K_p$ values in liver and skin were higher than those in other tissues.

Nakao reported that GLZ is eliminated in the rat liver homogenate.29) In this study, GLZ was eliminated linearly only in liver and small intestine homogenates, so that these tissues were also taken as the drug-eliminating tissues in man, and each tissue $CL_{int}$ was calculated by using $k_m$ in the corresponding rat tissue from Eq. 6. It has been reported that GLZ is metabolized by human intestinal bacteria.29) When $CL_{int}$ was excluded, the predicted values in the rat plasma and tissues by model A were markedly higher than the observed values (Fig. 4). Therefore, a conjectural $CL_{int}$ value to create best fit to the rat plasma concentrations was used in the simulation as a parameter, as in the case of human.

Previously we reported that GLZ is absorbed from the rat intestinal tract after the duodenal administration of bile containing GLZ and ca. 80% of GLZ excreted in the bile after a 100 mg/kg i.v. dose is reabsorbed during each enterohepatic cycling.13) In this study, $CL_{abs}$ was determined by using the single-pass perfusion technique.16)
GLZ was not decomposed in the perfusion solution for 2 h at 37°C, but no study on metabolism of GLZ in the intestinal tract was carried out. Therefore, the $CL_{AB}$ value (Table III) might involve metabolic clearance in the intestinal tract, and consequently the $CL_{AB}$ value might be overestimated. Accordingly the $CL_{BM}$ value might be unreliable.

When enterohepatic recycling is excluded from model A, the predicted rat plasma line fell markedly below the observed values with enterohepatic recycling and further, agreed well with the observed values in rats with biliary fistulation (Fig. 4). This indicates the existence of enterohepatic cycling in rat, as reported previously.13)

Russell and Klaassen have reported on species difference in the biliary excretion of ouabain, digoxin, and digitoxin among rats, rabbits, and dogs, i.e., the biliary excretion ratio tended to decrease with increase of body weight, but relatively minor species difference was observed in the case of digitoxin.40) There is no report on species difference in the biliary excretion of GLZ. When the biliary excretion of GLZ in man is assumed to be 1.3—13.7% /h as in the cases of ouabain and digoxin in dogs, the predicted serum concentrations by models B and C were underestimated relative to the observed values. When the biliary excretion of GLZ in man is assumed to be the same as that in rat (80%/h), the prediction was successful. This suggests a similar biliary excretion ratio between rat and human.

The $C_p/C_b$ values were almost constant (Table III) over the range of plasma concentration (20—640 µg/ml) following i.v. dosing, indicating that GLZ is not well taken into erythrocytes ($H_i$ was 0.51 in this study).

A physiologically based pharmacokinetic model, incorporating enterohepatic cycling, for the prediction of methotrexate disposition in mice, rat, dog, and man has been reported by Bischoff et al.14) In the model, multicompartment models for the biliary secretion process and for the gut lumen were used in these mammalian species. In this study, a multicompartment model for only the gut lumen was incorporated in models A—C, though with the bacterial metabolic clearance. Model A was suitable for the prediction of the drug disposition in rat, but was unsuitable for that in man, especially for the prediction of the terminal elimination, when human serum concentrations may show secondary peaks owing to enterohepatic recycling. The predictions by models B and C, which incorporate the gallbladder, both gave good agreement with the observed values, especially for the secondary peaks after 23 h following i.v. dosing, as compared with that in the case of model A without the gallbladder, but model C gave the best fit, especially for the secondary peaks, though the meal ingestion times were simulated. It was thought that the human serum disposition can be sufficiently well predicted by model B, considering that few serum concentration data after 23 h were available. Thus, the prediction was successful in all compartments in rat, and scale-up of the disposition kinetics of GLZ from rat to man was also successful.

**Appendix**

**I: Nomenclature** General: $V$, volume of tissue, blood, plasma, or serum, ml; $Q$, blood flow rate through tissue, ml/min; $C$, tissue, blood, plasma, or serum concentration of drug, µg/ml or µg/ml; $A_{inj}$, amount of drug in gallbladder, µg; $K_{p,t}$, tissue-to-blood concentration ratio; $C_p/C_b$, plasma-to-blood concentration ratio; $f_{p,t}$, plasma unbound fraction; $f_{u,t}$, serum unbound fraction; $CL_{AB}$, intestinal absorption clearance, ml/min; $CL_b$, biliary clearance based on the blood concentration, ml/min; $CL_{BM}$, renal clearance based on the blood concentration, ml/min; $CL_{BMr}$, bacterial metabolic clearance, ml/min; $CL_f$, fecal clearance, ml/min; $CL_{inr}$, intrinsic clearance for metabolism, ml/min; $R_b$, biliary excretion rate, µg/min; $R_b$, rate of drug transfer down the gut lumen, µg/min; $t$, holding time, min; $t_i$, gallbladder emptying time, min; $θ$, reciprocal of injection time, min$^{-1}$; $I(t)$, 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 pharmaco kinetic model shown in Fig. 1.

**Rat: Model A**

Artery blood:

$$V_{Ar} \frac{dC_{Ar}}{dt} = Q_{Ar} \left( \frac{C_{Lu}}{K_{p,Lu}} - C_{Ar} \right)$$  \hspace{1cm} (A-1)

Venous blood:

$$V_{Ve} \frac{dC_{Ve}}{dt} = Q_{Hs} \left( \frac{C_{Ha}}{K_{p,Ha}} + \frac{C_{Li}}{K_{p,Li}} + \frac{C_{Ki}}{K_{p,Ki}} + \frac{C_{Me}}{K_{p,Me}} \right) + Q_{Sa} \left( \frac{C_{Sa}}{K_{p,sa}} + \frac{C_{Ad}}{K_{p,Ad}} \right) - Q_{Ve} C_{Ve} + I(t)$$ \hspace{1cm} (A-2)

where the injection function, $I(t)$, can be expressed as follows.43)

$$I(t) = \frac{\text{dose} \cdot \theta(\theta)}{\theta(\theta - 1)} (1 - \theta)^2 \left( \theta \approx 2 \text{ min}^{-1} \right)$$ \hspace{1cm} (A-3)

**Lung:**

$$V_{Lu} \frac{dC_{Lu}}{dt} = Q_{Lu} \left( \frac{C_{Ve}}{K_{p,Lu}} \right)$$ \hspace{1cm} (A-4)

**Liver:**

$$V_{Li} \frac{dC_{Li}}{dt} = \left( C_{Li} - Q_{Sp} - Q_{Sp} - Q_{Sp} - Q_{Sp} \right) C_{Ar} + \frac{C_{Sp}}{K_{p,Sp}}$$

$$+ Q_{Ps} \frac{C_{Ps}}{K_{p,Ps}} + Q_{Ps} \frac{C_{Ps}}{K_{p,Ps}} + Q_{Ps} \frac{C_{Ps}}{K_{p,Ps}}$$

$$- f_d \left( C_{Sp} / C_{Ps} \right) C_{Li}$$ \hspace{1cm} (A-5)

where:

$$R_b = CL_{bm} \left( \frac{C_{Li}}{K_{p,Li}} \right)$$ \hspace{1cm} (A-6)
January 1990

Kidney:

\[ V_{dK1} = \frac{dC_{K1}}{dt} = Q_h \left( C_{A1} - C_{K1} \right) K_{P,K1} - C_L = C_{K1} \]

Small intestine:

\[ V_{dC_{SI}} = -Q_h \left( C_{A1} - C_{SI} \right) + \sum_{i=1}^{4} \left( \frac{1}{4} CL_{ASi} C_{SCi} \right) \]

\[ -f_{d}(C_{P1}/C_{P})CL_{in,t_{SI}} \frac{dt}{K_{P,SI}} \]

Gut lumen:

\[ \frac{dC_{S1}}{dt} = -C_S \left( \sum_{i=1}^{4} \frac{dC_{SCi}}{dt} \right) \]

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

\[ V_T = \frac{dC_T}{dt} = \left( C_{A1} - C_T \right) K_{P,T} \]

Man: Model A

Liver:

\[ V_{dC_{Li}} = \left( Q_h - Q_p - Q_R - Q_S \right) C_{Li} K_{P,Sp} + \]

\[ + Q_S C_{A1} + Q_R C_{SI} + \]

\[ -Q_p C_{P1} K_{P,Sp} - C_{Li} \]

\[ -f_{d}(C_{P1}/C_{P})CL_{in,t_{Li}} \frac{dt}{K_{P,Pl,1}} \]

Small intestine:

\[ V_{dC_{SI}} = -Q_h \left( C_{A1} - C_{SI} \right) + \sum_{i=1}^{4} \left( \frac{1}{4} CL_{ASi} C_{SCi} \right) \]

\[ -f_{d}(C_{P1}/C_{P})CL_{in,t_{SI}} \frac{dt}{K_{P,SI}} \]

\( l(t) \) can be expressed as follows:

\[ R(t) = 80 \text{ mg} / 30 \text{ min} \] (0 < t ≤ 30 min)

Mass balance equations for the other compartments are the same as those of the rat.

Model B

Gallbladder:

\[ \frac{dR_B}{dt} = R_B - R_{GB} \quad (t = 540 \text{ min}) \]

Gut lumen: Mass balance equations are the same as those in rats except for Eq. A-10. Equation A-10 is expressed as:

\[ V_{dC_{SCi}} = -K_{P,SI} - \frac{1}{4} CL_{ASi} C_{SCi} - R_{F1} \]

Mass balance equations for all compartments are the same as those of model A in man.

Model C

Gallbladder:

\[ \frac{dA_{GB}}{dt} = R_B \]

The drug is stored in the gallbladder at the time intervals of \( t_n - t_{n-1} \) or \( t_n - t_{n+1} \) (n = 1-6), where \( t_n \) is the drug administration time, \( t_0 \) is 0 min, and \( t_n \) is the gallbladder emptying time at each meal ingestion, \( t_1 = 180 \), \( t_2 = 540 \), \( t_3 = 1200 \), \( t_4 = 1500 \), \( t_5 = 1860 \), and \( t_6 = 2520 \), and \( t_1 = 3180 \text{ min} \) (see the model development section).

Acknowledgments

This work was supported in part by a Grant-in-Aid from the Shimabara Science Promoting Foundation.

References