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 cytopathic 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 methotrexate[14] 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 fistulization 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 fistulization, 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 fistulization, after removal of blood samples, the rats were exsanguinated *via* a carotid artery at 1, 2, 3, 8, 16, or 24 h after dosing and perfused with cold 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 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 (K_p) Blood and bled 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 (CL_{int}), 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 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 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 for plasma, blood, perfusate, and tissue homogenate filtrate and 300—500 μl for tissue homogenate were used for GLZ determination. The extraction procedure[17] and high performance liquid chromatography (HPLC) method[18] 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.*[19]

Data Analysis Apparent K_p value was corrected by the method of Chen and Gross.[20] The intestinal absorption clearance (CL_{Abs}) was estimated from Eq. 1:

$$CL_{Abs}=\frac{C_{in}\cdot Q_{in}-C_{out}\cdot Q_{out}}{C_{in}}\times\frac{80.8\,cm}{15.0\,cm} \quad (1)$$

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 Q_{out} was calculated from the inulin concentration difference in the influx (C') and efflux (C'') perfusates as follows: $Q_{out}=Q_{in}\times(C'/C'')$. 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.[21] The hepatic and small intestinal 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 $k_m(min^{-1})=\alpha(min^{-1})/d$, 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 $k_m\times V_T$, where V_T is the volume of liver or small intestine (Table I).

All means are presented with their standard error (mean $\pm$ 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 $CL'_R\times(C_P/C_B)$, where CL'_B and CL'_R are the literature mean values of biliary and renal clearances based on the plasma concentration data, respectively.[13] C_P/C_B is the plasma-to-blood concentration ratio determined in this study. Fecal clearance (CL_F) was estimated from Eq. 2.

$$CL_F=\frac{\text{small intestinal contents volume}}{\text{transit time}} \quad (2)$$

Where transit time (100 min) is the literature value.[14] The conjectural bacterial metabolic clearance (CL_{Bm}) value to create the best fit to plasma concentration data was computed by using the Multi–Runge program.[22] Unbound rat plasma concentration (C_f) was calculated from the equation defined in the previous report[13] and the binding parameters (Table III) by using the Hitchcock–Bairstow program[23] on a personal computer (NEC PC-9801 vm2). As the calculated C_f was 0.383 mM even at the highest plasma concentration (the initial concentration, *ca.* 1.60 mg/ml[13]) after i.v. dosing, and the estimated $K_2\cdot C_f$ (0.04) was a negligible value, the defined equation[13] was simplified as follows:

$$C_{tot}=C_f+\frac{n_1(p)\cdot K_1\cdot C_f}{1+K_1\cdot C_f}+n_2(p)\cdot K_2\cdot C_f \quad (3)$$

where C_{tot} is the total concentration of GLZ in plasma; K_1 and K_2 are the association constants for primary and secondary binding sites, respectively. $n_1(p)$ and $n_2(p)$ are the binding capacities for primary and secondary binding sites, respectively. Consequently, $n_2(p)\cdot K_2$ is expressed as the linear binding coefficient (Table III) and the plasma unbound fraction (f_u) is given by Eq. 4.

$$f_u=\frac{C_f}{C_{tot}}=\frac{(-X+\sqrt{X^2+4Y\cdot C_{tot}})/2Y}{C_{tot}} \quad (4)$$

where:

$$X=n_2(p)\cdot K_2+1+(n_1(p)-C_{tot})\cdot K_1$$

$$Y=(n_2(p)\cdot K_2+1)\cdot K_1$$

Man: The human serum-to-blood concentration ratio (C_S/C_B) was estimated from $C_S/C_B=1/(1-H_t)$. H_t is the hematocrit value (0.42) taken from the literature.[24] The K_p value for each tissue was estimated by using the mean K_p value (Table II) and C_P/C_B (Table III) in the rats as follows: mean $K_p\times(C_S/C_B)/(C_P/C_B)$. The CL_{Abs} was estimated from $CL_{Abs}=k_{Abs}\times$ small intestinal contents volume, where k_{Abs} is the intestinal absorption rate constant (0.00203 min^{-1}) calculated by the feathering method[25] from the mean plasma drug concentration data after an oral GLZ administration (64 mg/man) in five subjects.[26] The small intestinal contents volume (Table I) was taken from the literature.[21] The CL_B or CL_R was calculated from Eq. 5.

$$CL_B \text{ or } CL_R=\frac{\text{dose}\times ER_B \text{ or } ER_U}{AUC}\times(C_S/C_B) \quad (5)$$

Where ER_B 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_{0-6h} 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/man).[27] The residual area beyond 6 h ($AUC_{6-\infty}$) was estimated as C'/k. C' is the serum concentration at 6 h that was estimated from $C'=C'_0\exp(-k\times6)$, where $-k$ is the slope (-3.26×10^{-3} min^{-1}) estimated from the log-serum data from 1 to 6 h using linear regression analysis and C'_0 (11.10 μg/ml) is the serum concentration at time 0 that was obtained by extrapolating the line with slope $-k$. The $AUC_{0-\infty}$ was calculated by adding AUC_{0-6h} to $AUC_{6-\infty}$. The mean $AUC_{0-\infty}$ value was used for AUC in Eq. 5. The distribution volume at the steady state (Vd_{ss}) was calculated by $Vd_{ss}=\text{dose}\cdot AUMC/(AUC)^2$, 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_{int} in liver and small intestine was calculated from Eq. 6.

$$CL_{int,j}=\frac{CL_M}{f_{u,s}\times(C_S/C_B)}\times\frac{k_{m,j}\cdot V_{T,j}}{\sum_j k_{m,j}\cdot V_{T,j}} \quad (6)$$

Where CL_M is metabolic clearance, given by $CL_M=CL_{tot}-CL_B-CL_R$. CL_{tot} is total blood clearance expressed as $(\text{dose}/AUC)\times C_S/C_B$. $f_{u,s}$ is serum free fraction calculated from the equation defined in the previous report[28] by using serum protein binding parameters (Table III). The subscript j represents liver and small intestine. CL_F was estimated from Eq. 2, in which the transit time (1000 min) is the literature value.[14] CL_{Bm} is a conjectural 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 weighed 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 I. Physiological Parameters for Modeling in a 0.25 kg Rat and a 70 kg Man

Tissue	Volume (ml)		Blood flow rate (ml/min)	
	Rat	Human	Rat	Human
Lung	1.3[a]	600[f]	41.3[h]	3920[h]
Heart	1.1[a]	300[f]	3.5[i]	240[f]
Liver	12.3[a]	3900[f]	14.7[b]	1580[f]
Kidney	2.2[a]	300[f]	11.4[b]	1240[f]
Muscle	125.0[b]	30000[f]	6.8[b]	600[f]
Skin	44.7[c]	3000[f]	4.5[c]	60[f]
Adipose tissue	17.5[d]	10000[f]	0.4[d]	200[f]
Spleen	0.5[a]	200[f]	0.4[i]	200[f]
Pancreas	0.9[a]	100[g]	1.2[i]	200[j]
Stomach	1.3[a]	150[g]	0.6[i]	136[k]
Small intestine	13.8[b]	640[g]	12.0[i]	391[k]
Small intestinal contents	3.1[a]	400[g]		
Blood Artery	7.5[e]	1700[e]	41.3[h]	3920[h]
Vein	15.1[e]	3390[e]	41.3[h]	3920[h]

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*) $Q_{He}+Q_{Li}+Q_{Ki}+Q_{Mu}+Q_{Sk}+Q_{Ad}$; for nomenclature, see Appendix I. *i*) Obtained from ref. 32. *j*) Assumed to be equal to the value for spleen. *k*) See the text for details.

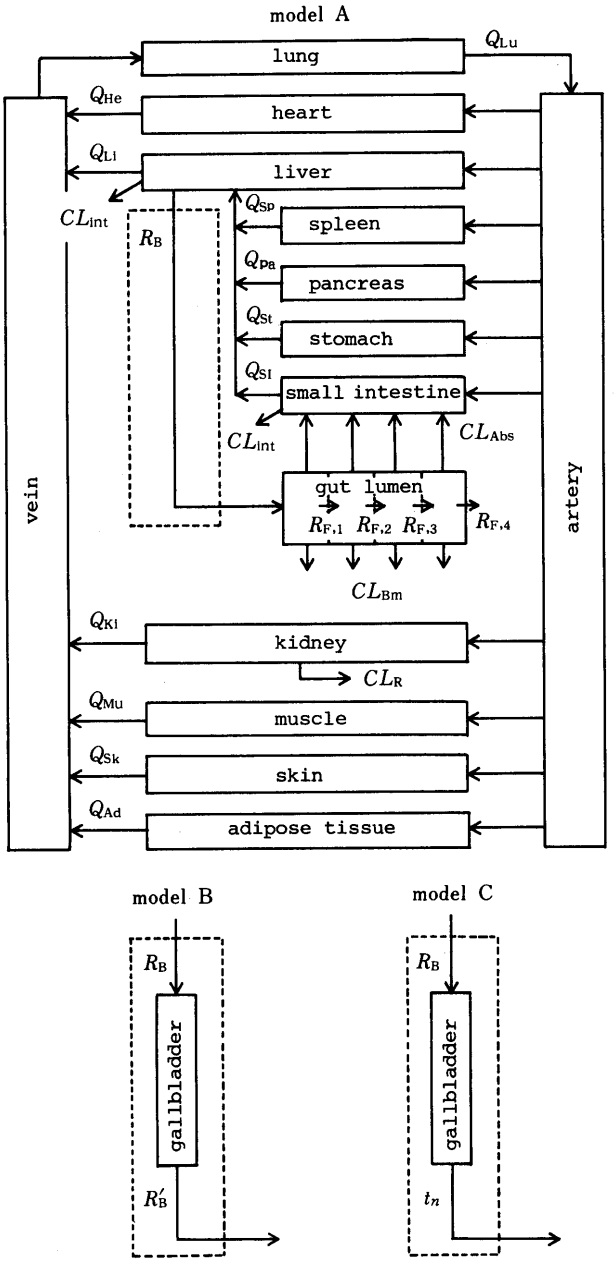

Fig. 1. Pharmacokinetic Model for the Disposition of GLZ in the Rat (Model A) and Man (Models A, B, and C)

For nomenclature, see Appendix I.

$$V_{plasma} = 44 \times (\text{body weight, kg})^{0.99} \quad (7)$$

$$V_B = V_{plasma}/(1 - H_t) \quad (8)$$

The H_t 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.[31] 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 in the same way as in the rat and those of stomach and small intestine were estimated by applying to the gastrointestinal blood flow rate in man (850 ml/min)[34] 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_B was calculated from Eqs. 7 and 8.

Model Development Physiological pharmacokinetic models A—C, incorporating enterohepatic 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_B (Appendix II) with a multicompartment description of the gut lumen based on physiological principles, as reported by Bischoff *et al.*[14] 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 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'_B with the holding time τ (Appendix II), where τ is a conjectural value to create the best fit to human serum concentrations using the Multi-Runge program.[22] Model C incorporated instantaneous empting from a storage compartment (gallbladder) during meal ingestion.[35-37] The human serum concentration data used for the prediction were taken from the literature.[27] The drug i.v. administration time (09:00 a.m.) was available,[27] but no specific information was available about the meal ingestion times on each day. Therefore, meal ingestion times were assumed to be at 07:00 a.m. and 12:00 and 18:00 p.m. These models assume that (a) each tissue acts as a well-stirred compartment, (b) intercompartmental transport occurs by blood flow, (c) distribution of the drug is a blood flow-limited and linear process, (d) only free drug is available for tissue distribution, (e) only free drug is metabolized in only hepatic and small intestinal tissues, and the drug is also metabolized by bacteria in the small intestinal contents, and (f) the drug is excreted in bile, urine, and feces. The mass balance-blood flow equations were written for 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 (Tables II and III) were used for the simulation.

Table II. Tissue-to-Blood Concentration Ratios (K_p) of GLZ Used for Prediction in Rat and Man

Tissue	K_p	
	Rat[a]	Human[b]
Lung	0.083±0.012	0.071
Heart	0.031±0.006	0.026
Liver	0.291±0.024	0.247
Kidney	0.173±0.024	0.147
Muscle	0.092±0.018	0.078
Skin	0.407±0.026	0.345
Adipose tissue	0.086±0.022	0.072
Spleen	0.018±0.004	0.015
Pancreas	0.018±0.004	0.015
Stomach	0.079±0.008	0.067
Small intestine	0.051±0.014	0.043

a) Results are given as the mean±S.E. of three rats with biliary fistulization at 1 h after i.v. administration of 100 mg/kg. Each value was corrected according to the method of Chen and Gross.[20] *b*) Calculated by using the mean K_p values in the rats. See the text for details.

Table III. Pharmacokinetic Parameters of GLZ Used for Prediction

Parameter	Rat	Human
CL_{Abs} (ml/min)	0.310±0.021[a]	0.812
CL_{int} (ml/min)		
Liver	1.314±0.115[a]	763.34
Small intestine	0.702±0.035[a]	59.60
CL_B (ml/min)	0.367	24.13
CL_R (ml/min)	0.016	0.361
CL_F (ml/min)	0.031	0.400
CL_{Bm}[b]	0.120	1.350
C_P/B_B	2.036±0.035[c]	
C_S/C_B		1.724
Rat plasma and human serum protein binding		
K_1 (mM^{-1})	124.2[d]	136.1[f]
$n_1(p)$ (mM)	1.35[d]	1.87[f]
$n_2(p) \cdot K_2$	0.647[e]	0.429[f]

Results are given as the mean±S.E. of six *a*) and eighteen *c*) rats. *b*) Conjectural value to create the best fit to rat plasma or human serum concentration data. For details, see the text. *d*) Obtained from the previous report.[18] *e*) For details, see the text. *f*) Obtained from the previous report.[28] For nomenclature, see the text.

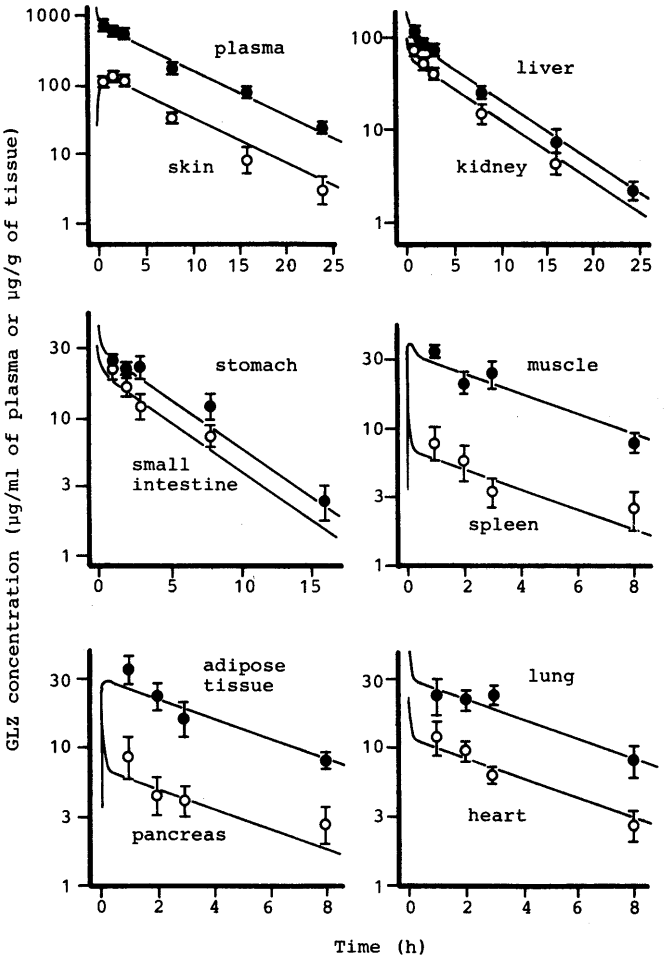

Fig. 2. Predicted (Lines) and Observed (Points) Log Concentrations of GLZ in Plasma and Tissues after i.v. Administration of 100 mg/kg to Rats

Each point and vertical bar represent the mean and S.E. of three rats. The prediction was performed by using model A in Fig. 1.

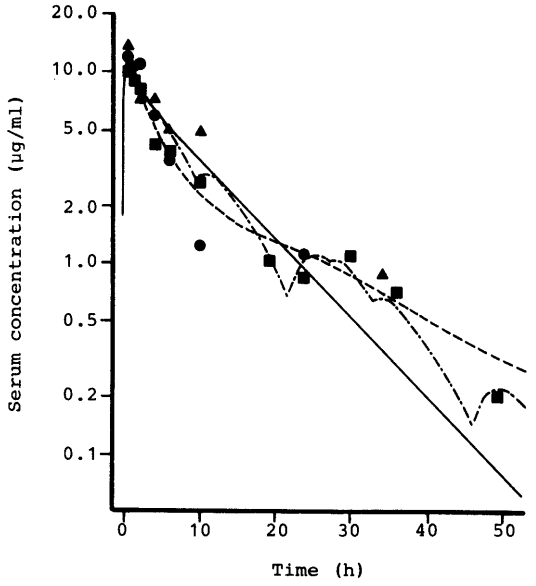

Fig. 3. Predicted (Lines) and Observed (Points) Log Serum Concentrations of GLZ after Slow i.v. Administration (for 30 min) of 80 mg/man to Man

The predictions were performed by using models A, B, and C in Fig. 1. The observed values were obtained from the literature.[27] Body weight was presumed to be 70 kg. Key: (●, ▲, ■) data of each normal subject; ——, model A; ------, model B; – – – –, model C.

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/man 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 ($\sum$ mean $K_p \cdot V_T/(C_P/C_B)$) and V_{plasma}[14] in rat is 28.90 ml/250 g, which corresponds well to the Vd_{ss} 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 $K_p \cdot V_T/(C_P/C_B)$ values to the human serum volume (3.0 l/70 kg man)[14] corresponded well to the calculated Vd_{ss} value (7.0 l/70 kg man).[27] 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 hepatitis[1] and allergic skin disorder,[2-4] 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.[38] 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.[39] When CL_{Bm} 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_{Bm} 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]

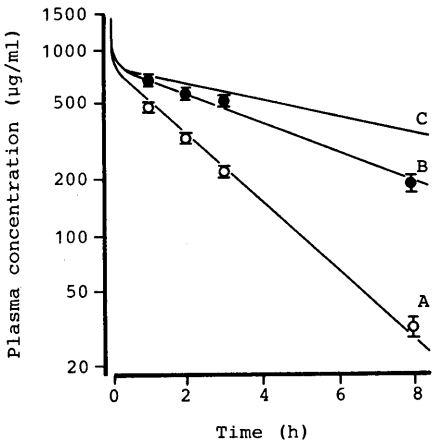

Fig. 4. Predicted (Lines) and Observed (Points) Log Concentrations of GLZ in Plasma after i.v. Administration of 100 mg/kg to Rats with (A) and without (B and C) Biliary Fistulization

The lines A and C were simulated by setting CL_{Abs} and CL_{Bm} equal to zero by model A using the Runge-Kutta-Gill method, respectively. B is the same as the results in Fig. 2. Each point and vertical bar represent the mean and S.E. of three rats.

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_{Abs} value (Table III) might involve metabolic clearance in the intestinal tract, and consequently the CL_{Abs} 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 fistulization (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%, 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%), 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_t 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 36 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/g or μg/ml; A_{Ga}, amount of drug in gallbladder, μg; K_p, tissue-to-blood concentration ratio; C_P/C_B, plasma-to-blood concentration ratio; C_S/C_B, serum-to-blood concentration ratio; f_u, plasma unbound fraction; $f_{u,s}$, serum unbound fraction; CL_{Abs}, intestinal absorption clearance, ml/min; CL_B, biliary clearance based on the blood concentration, ml/min; CL_R, renal clearance based on the blood concentration, ml/min; CL_{Bm}, bacterial metabolic clearance, ml/min; CL_F, fecal clearance, ml/min; CL_{int}, intrinsic clearance for metabolism, ml/min; R_B, biliary excretion rate, μg/min; R_F, rate of drug transfer down the gut lumen, μg/min; τ, holding time, min; t_n, 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 pharmacokinetic model shown in Fig. 1.

Rat: Model A

Artery blood:

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

Venous blood:

$$V_{Ve}\frac{dC_{Ve}}{dt}=Q_{He}\frac{C_{He}}{K_{p,He}}+Q_{Li}\frac{C_{Li}}{K_{p,Li}}+Q_{Ki}\frac{C_{Ki}}{K_{p,Ki}}+Q_{Mu}\frac{C_{Mu}}{K_{p,Mu}}+Q_{Sk}\frac{C_{Sk}}{K_{p,Sk}}+Q_{Ad}\frac{C_{Ad}}{K_{p,Ad}}-Q_{Ve}C_{Ve}+I(t) \quad \text{(A-2)}$$

where the injection function, $I(t)$, can be expressed as follows:[41]

$$I(t)=\text{dose}\cdot\theta(\theta t)^2(1-\theta t)^2 \quad (\theta=2\,min^{-1}) \quad \text{(A-3)}$$

Lung:

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

Liver:

$$V_{Li}\frac{dC_{Li}}{dt}=(Q_{Li}-Q_{Sp}-Q_{Pa}-Q_{St}-Q_{SI})C_{Ar}+Q_{Sp}\frac{C_{Sp}}{K_{p,Sp}}+Q_{Pa}\frac{C_{Pa}}{K_{p,Pa}}+Q_{St}\frac{C_{St}}{K_{p,St}}+Q_{SI}\frac{C_{SI}}{K_{p,SI}}-Q_{Li}\frac{C_{Li}}{K_{p,Li}}-f_u(C_P/C_B)CL_{int,Li}\frac{C_{Li}}{K_{p,Li}}-R_B \quad \text{(A-5)}$$

where:

$$R_B=CL_B\frac{C_{Li}}{K_{p,Li}} \quad \text{(A-6)}$$

Kidney:

$$V_{Ki}\frac{dC_{Ki}}{dt}=Q_{Ki}\left(C_{Ar}-\frac{C_{Ki}}{K_{p,Ki}}\right)-CL_R\frac{C_{Ki}}{K_{p,Ki}} \quad \text{(A-7)}$$

Small intestine:

$$V_{SI}\frac{dC_{SI}}{dt}=Q_{SI}\left(C_{Ar}-\frac{C_{SI}}{K_{p,SI}}\right)+\sum_{i=1}^{4}\frac{1}{4}CL_{Abs}C_{SIC,i}-f_u(C_P/C_B)CL_{int,SI}\frac{C_{SI}}{K_{p,SI}} \quad \text{(A-8)}$$

Gut lumen:

$$\frac{dC_{SIC}}{dt}=\frac{1}{4}\sum_{i=1}^{4}\frac{dC_{SIC,i}}{dt} \quad \text{(A-9)}$$

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,1}}{dt}=R_B-\frac{1}{4}(CL_{Abs}+CL_{Bm})C_{SIC,1}-R_{F,1} \quad \text{(A-10)}$$

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,i}}{dt}=R_{F,i-1}-\frac{1}{4}(CL_{Abs}+CL_{Bm})C_{SIC,i}-R_{F,i} \quad \text{(A-11)}$$

$(i=2\text{—}4)$

where:

$$R_{F,1}\text{ or }R_{F,i}=CL_FC_{SIC,1}\text{ or }CL_FC_{SIC,i} \quad \text{(A-12)}$$

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

$$V_T\frac{dC_T}{dt}=Q_T\left(C_{Ar}-\frac{C_T}{K_{p,T}}\right) \quad \text{(A-13)}$$

Man: Model A

Liver:

$$V_{Li}\frac{dC_{Li}}{dt}=(Q_{Li}-Q_{Sp}-Q_{Pa}-Q_{St}-Q_{SI})C_{Ar}+Q_{Sp}\frac{C_{Sp}}{K_{p,Sp}}+Q_{Pa}\frac{C_{Pa}}{K_{p,Pa}}+Q_{St}\frac{C_{St}}{K_{p,St}}+Q_{SI}\frac{C_{SI}}{K_{p,SI}}-Q_{Li}\frac{C_{Li}}{K_{p,Li}}-f_{u,s}(C_S/C_B)CL_{int,Li}\frac{C_{Li}}{K_{p,Li}}-R_B \quad \text{(A-14)}$$

Small intestine:

$$V_{SI}\frac{dC_{SI}}{dt}=Q_{SI}\left(C_{Ar}-\frac{C_{SI}}{K_{p,SI}}\right)+\sum_{i=1}^{4}\frac{1}{4}CL_{Abs}C_{SIC,i}-f_{u,s}(C_S/C_B)CL_{int,SI}\frac{C_{SI}}{K_{p,SI}} \quad \text{(A-15)}$$

$I(t)$ can be expressed as follows:

$$I(t)=80\text{ mg}/30\text{ min}\quad(0<t\leqq 30\text{ min}) \quad \text{(A-16)}$$

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

Model B

Gallbladder:

$$\tau\frac{dR'_B}{dt}=R_B-R'_B\quad(\tau=540\text{ min}) \quad \text{(A-17)}$$

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

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,1}}{dt}=R'_B-\frac{1}{4}(CL_{Abs}+CL_{Bm})C_{SIC,1}-R_{F,1} \quad \text{(A-18)}$$

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

Model C

Gallbladder:

$$\frac{dA_{Ga}}{dt}=R_B \quad \text{(A-19)}$$

The drug is stored in the gallbladder at the time intervals of t_0—t_1 or t_n—t_{n+1} $(n=1\text{—}6)$, where t_0 is the drug administration time, t_0 is 0 min, and t_n is the gallbladder emptying time at each meal ingestion, $t_1=180$, $t_2=540$, $t_3=1200$, $t_4=1500$, $t_5=1860$, $t_6=2520$, and $t_7=3180$ min (see the model development section).

Small intestine:

$$V_{SI}\frac{dC_{SI}}{dt}=Q_{SI}\left(C_{Ar}-\frac{C_{SI}}{K_{p,SI}}\right)+\sum_{t=t_n}^{t=t_{n+1}}\left(\frac{1}{4}\sum_{i=1}^{4}CL_{Abs}C_{SIC,i}\right)-f_{u,s}(C_S/C_B)CL_{int,SI}\frac{C_{SI}}{K_{p,SI}} \quad \text{(A-20)}$$

Gut lumen:

$$\frac{dC_{SIC}}{dt}=\sum_{t=t_n}^{t=t_{n+1}}\left(\frac{1}{4}\sum_{i=1}^{4}\frac{dC_{SIC,i}}{dt}\right) \quad \text{(A-21)}$$

When t is t_n,

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,1}}{dt}=A_{Ga}(t_n) \quad \text{(A-22)}$$

When t is t_n—t_{n+1},

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,1}}{dt}=-\frac{1}{4}(CL_{Abs}+CL_{Bm})C_{SIC,1}-R_{F,1} \quad \text{(A-23)}$$

$$\frac{V_{SIC}}{4}\frac{dC_{SIC,i}}{dt}=R_{F,i-1}-\frac{1}{4}(CL_{Abs}+CL_{Bm})C_{SIC,i}-R_{F,i} \quad \text{(A-24)}$$

$(i=2\text{—}4)$

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

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