Kinetic Studies on Drug Disposition in Rabbits. III. Effect of Tolbutamide on Renal Excretion of Sulfamethizole

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The effect of tolbutamide (TB) on the urinary excretion of sulfamethizole (SMZ) under constant infusion of SMZ at 100 mg/h was studied. Intravenous administration of TB (50 mg/kg) caused a decrease in the urinary excretion rate of SMZ but an increase in the unbound concentration of SMZ in plasma. The total concentration of SMZ in plasma decreased rapidly after TB injection and then increased gradually to a level higher than the control. The slope of the terminal phase of the unbound concentration of TB in plasma in the presence of SMZ was significantly smaller than that of TB alone. The analysis using the perfusion limited model showed that the elimination kinetics of SMZ in the presence of TB could be described by the mutual displacement of plasma protein binding of both drugs and the competitive inhibition of the tubular secretion of SMZ by the unbound concentration of TB in renal vein. Further, the inhibitor constant was in good agreement with that for the *in vitro* uptake by renal cortex slices.

Keywords — tolbutamide; sulfamethizole; renal clearance; tubular secretion; perfusion limited model; plasma protein binding; drug interaction; competitive inhibition; mutual displacement; renal cortex slice

Introduction

The interaction of tolbutamide (TB), an oral antidiabetic, with sulfonamides has been extensively studied. Displacement of plasma protein binding and inhibition of hepatic oxidation of TB by sulfonamides have been proposed as possible mechanisms of drug interactions.¹⁻⁵⁾ However, these proposed mechanisms were primarily based on the effect of a second drug on TB disposition. Inversely, studies of the effect of TB on the elimination of sulfonamides have been limited.⁶⁾ When TB also influences elimination kinetics of other drugs, potentiation of the hypoglycemic effect is probably more complicated than that in the situation where the inhibition of TB oxidation is only exerted.

The present study deals with the effect of TB on renal excretion of sulfamethizole (SMZ) in rabbits. This report, using a pharmacokinetic model adopted in the previous report,^{7,8)} demonstrates the approaches that can be taken to quantitate drug-drug interactions, *i.e.*, displacement of plasma protein binding and inhibition of tubular secretion of SMZ by TB.

Materials and Methods

Materials — The supply and assay procedures for SMZ and TB were described previously.⁷⁾ Hydroxytolbutamide (HTB) and carboxy-tolbutamide (CTB) were kindly supplied from Dr. Shibasaki (Faculty of Pharmaceutical Sciences, Nagasaki Univ., Japan).⁹⁾ Sulfisoxazole (SIX) was purchased from Yamanouchi Pharmaceutical Ind., Co., Tokyo. Sulfadiazine (SDZ) and sulfamethoxypyridazine (SMP) from Sigma Chem. Co., St. Louis, *p*-aminohippuric acid (PAH) and sulfanilamide (SA) from Nakarai Tesque, Kyoto and sodium phenolsulfonphthalein (PSP) from Tokyo Chem. Ind., Tokyo were purchased commercially and used without further purification. All other chemicals were reagent grade and were also obtained commercially.

Renal Cortex Accumulation *in Vitro* — Renal cortex accumulation studies of SMZ and PAH with or without TB were performed in a manner similar to that described in the previous report,⁷⁾ except for the composition of suspending medium. The basic suspending medium was

composed of NaCl 88.7 mM, KCl 40 mM, sodium phosphate buffer (pH 7.3) 7.4 mM, sodium acetate 11.1 mM and CaCl_2 0.74 mM. Finally, an isotonic solution of suspending medium containing drug(s) was prepared with the addition of NaCl.

Clearance Studies — Clearance studies were performed by the method described previously.⁷⁾ In well hydrated rabbits, the drug solution was infused through the marginal ear vein at a constant flow rate of 100 ml/h. The infusion rate (mg/h) of each drug was the following: SMZ (100), SIX (100), SMP (50), SDZ (50), SA (50), PAH (50), and PSP (200 as sodium salt). A priming dose was given at the start of a constant infusion to immediately attain the steady-state plasma levels. After 3–4 h, two or three control clearance studies at 30 min intervals were carried out. After control studies, a bolus dose of TB (50 mg/kg) was given, and then blood and urine samples were collected for 2.5–5 h.

The effect of TB on the renal excretion of drugs was expressed with the change of renal clearance, in which the renal clearance referenced to the total drug concentration in plasma (CL_R)

and the renal clearance referenced to the unbound drug concentration in plasma ($\text{CL}_R(f)$) were employed.

Analytical Method — PAH, SIX, SA, SDZ and SMP were determined spectrophotometrically by methods similar to those described in a previous paper.⁷⁾ PSP was determined spectrophotometrically at 560 nm after alkalization.

Data Analysis — All of the calculations were carried out by using the nonlinear least squares method⁷⁾ with the aid of a digital computer (μ -VAX-II, Digital Equipment Corp., Maynard, Mass.).

Statistical significance was evaluated by the *t*-test.

Results

In Vitro Renal Cortex Accumulation

Figure 1 showed renal cortex accumulation of PAH and SMZ with and without TB. The slice/medium ratios of PAH and SMZ in the presence of TB were significantly depressed under aerobic conditions. Under anaerobic conditions, however, TB did not affect the uptake of SMZ. Therefore, it indicated that TB inhibited energy-

Fig. 1. Effect of TB on PAH and SMZ Uptakes by Renal Cortex Slices

The renal cortex slices were incubated at 25 °C for 2 h under aerobic condition (A) and under anaerobic condition (B). The initial PAH or SMZ concentration in the medium was 74 μM and that of TB was 0 or 370 μM . Open bars and closed bars represent the slice/medium ratio without TB and with TB, respectively. The data is given as the mean $\pm$ S.D. for $n=4$ or 5. *a)* Significantly different ($p < 0.01$) from the control.

Fig. 2. Competitive Inhibition for SMZ Uptake by TB under Aerobic Condition

The initial concentrations of SMZ in the suspending medium were 37–296 μM and that of TB was 0 or 18.5 μM . The data represents the mean of two determinations as the Lineweaver-Burk plot of SMZ uptake after correction for the nonspecific physical binding from the overall uptake by renal slices. Apparent inhibitor constant of TB was 8.8 ± 1.0 μM . Open symbol: SMZ without TB. Closed symbol: SMZ with TB.

Fig. 3. Effect of TB on Time Course of Plasma Concentration and Urinary Excretion Rate of SMZ

SMZ was infused at a constant rate of 100 mg/h from 3 or 3.5 h before TB injection, while TB was administered intravenously at a dose of 50 mg/kg at zero time. The plotted points represent the observed data (closed circle, urinary excretion rate; open circle, total plasma concentration; open square, unbound plasma concentration) and the solid lines represent calculated values. Each experimental point is shown as the mean $\pm$ S.D. ($n=6$).

dependent active accumulation of SMZ by renal slices.

The Lineweaver-Burk plot of SMZ uptake after correction for the nonspecific physical binding from the overall uptake by renal slices was shown in Fig. 2. The results indicated that TB inhibited competitively the specific active accumulation of SMZ. Apparent inhibitor constant of TB was $8.8 \pm 1.0 \mu\text{M}$ at $18.5 \mu\text{M}$ of the inhibitor concentration in the initial medium.

Effect of TB on Renal Clearance of SMZ

Time course of SMZ disposition during a constant rate infusion (100 mg/h) before and after intravenous administration of 50 mg/kg of TB is shown in Fig. 3. The unbound SMZ concentration in plasma increased about fourfold from 4.1 ± 1.85 to $15.4 \pm 2.0 \mu\text{g/ml}$ 15 min after injection. Despite the decline in TB concentration following intravenous administration, there was not a temporary increase in the unbound SMZ concentration immediately after injection, but a gradual one. The total SMZ concentration in plasma decreased rapidly with TB injection from $56.5 \pm 7.4 \mu\text{g/ml}$ (mean $\pm$ S.D.) to $46.9 \pm 5.1 \mu\text{g/ml}$ 15 min after injection, but thereafter increased gradually to a level higher than that at preinjection. Thus, TB produced not only the displacing effect on plasma protein binding but

Fig. 4. Effect of TB on Renal Clearance of Some Drugs

TB was administered intravenously at a dose of 50 mg/kg at steady state in plasma level of each drug (detail see the text). Each bar represents the change of the clearance ratio during 0–0.5 h after TB injection relative to control ($n=1-6$). Right panel: change of CL_R/GFR . Left panel: change of $\text{CL}_R(f)/\text{GFR}$. a) Control value of CL_R/GFR . b) Control value of $\text{CL}_R(f)/\text{GFR}$. As no appreciable binding of PAH and SA to plasma protein was detected,¹⁸⁾ only CL_R/GFR was shown.

Fig. 5. Time Course of Plasma Concentration and Urinary Excretion Rate of TB Following TB Administration with and without SMZ

TB was administered intravenously at a dose of 50 mg/kg without SMZ or during the constant infusion of SMZ at 100 mg/h. The plotted points represent the mean $\pm$ S.D. The solid lines represent the calculated values. Open symbol: TB alone ($n=4$). Closed symbol: TB with SMZ ($n=6$). $\circ$ $\bullet$: the total concentration of TB in plasma, $\triangle$ $\blacktriangle$: the unbound concentration of TB in plasma, and $\square$ $\blacksquare$: the urinary excretion rate of TB.

also the marked decrease of the systemic clearance of SMZ. As the urinary excretion rate of SMZ was markedly decreased, this indicated that the decrease of the systemic clearance of SMZ was mainly attributable to that of its renal clearance.

The effects of TB on renal clearances of some drugs was summarized in Fig. 4. CL_R of PAH, PSP and SIX as well as SMZ were both decreased with treatment of TB. The renal clearance of SA, which is not secreted by renal tubules,¹⁰⁾ remained constant. For SMP and SDZ with low clearance values, $CL_R(f)$ were decreased, but CL_R were rather increased, although the change of CL_R were slightly smaller than that of $CL_R(f)$.

Effect of SMZ on TB Disposition

The time courses of plasma concentration and the urinary excretion rate of TB without and with SMZ infusion at 100 mg/h were shown in Fig. 5. The slope of the terminal phase of total TB concentration in plasma with SMZ seemed

Fig. 6. Relationship between Renal Clearance of TB and Plasma Unbound Concentration of TB

TB was administered intravenously at a dose of 50 mg/kg without SMZ or during the constant infusion of SMZ at 100 mg/h. Each data represents the clearance ratio ($CL_R(f)/GFR$) against the unbound concentration of TB in plasma. Open symbol: TB alone. Closed symbol: TB with SMZ.

to be larger than that without SMZ though there was not a significant difference between them. The slope of the terminal phase of unbound TB concentration in the presence of SMZ was significantly smaller than that in the absence of SMZ. In this way, the fact of not only the displacement of SMZ by TB as mentioned above but the increase of unbound fraction of TB by SMZ, *i.e.*, the mutual displacement of TB and SMZ, was revealed.

Figure 6 shows the profile of the renal clearance ratio of TB *versus* the unbound TB concentration in plasma. $CL_R(f)/GFR$ is only presented as the reliable marker of renal handling of a drug with low renal clearance.¹¹⁾ The renal clearance ratio of TB did not vary significantly with the unbound TB concentration irrespective of SMZ coadministration and gave mean values ($\pm$ S.D.) of $3.67 \pm 0.99\%$ without SMZ and $3.54 \pm 0.96\%$ with SMZ. However, the urine pH was 7.3 ± 0.2 under these experimental conditions and about 10% of filtered water was excreted in the urine. The fact indicated that TB was more effectively reabsorbed by the tubules than water and the reabsorption of TB could not be explained by the simple passive diffusion based on the concentration gradient across the tubular lumen and the capillary bed.

Simulation of Drug-Drug Interaction

The pharmacokinetic model was constructed to describe quantitatively SMZ disposition accompanied with the displacement of the plasma protein binding and the change in renal excretion of SMZ by TB. The decay of the unbound TB concentration in plasma ($C_{f, BP}^T$) was expressed as follows:

$$C_{f, BP}^T = C_1 \exp(-\lambda_1 t) + C_2 \exp(-\lambda_2 t) \quad (1)$$

where C_i and λ_i ($i=1, 2$) are the coefficients and the corresponding exponents of bi-exponential equation. It was assumed that TB distributed immediately through the body except for the kidneys after injection.

The distribution of SMZ in the body is fundamentally identical with our previous report⁸⁾ except for the mutual displacement of plasma protein binding. For the plasma protein binding of both drugs, SMZ and TB compete for the same high affinity binding site and then the total concentrations of SMZ and TB in plasma (C_p^S and C_p^T , respectively) are given as follows:

$$C_p^S = C_f^S + \frac{N_1(p) C_f^S}{K_1^S(1 + C_f^T/K_1^T) + C_f^S} + \frac{N_2(p)^S C_f^S}{K_2^S + C_f^S} \quad (2)$$

$$C_p^T = C_f^T + \frac{N_1(p) C_f^T}{K_1^T(1 + C_f^S/K_1^S) + C_f^T} + \frac{N_2(p)^T C_f^T}{K_2^T + C_f^T} \quad (3)$$

where $N_1(p)$ and $N_2(p)$ are the capacities for high and low affinity sites, K_1 and K_2 are the dissociation constants for these sites, and subscripts (S and T) denote SMZ and TB. The binding parameters were adopted from the previous report.⁷⁾ The each binding capacity in the renal capillary was F times as much as the corresponding capacity in the renal artery, in which F was the ratio of the protein concentration in the capillary to that in the artery, *i.e.*, $Q_{p, KI}/(Q_{p, KI} - UR)$.

The reabsorption of TB was assumed to be directly proportional to the TB concentration in tubular lumen as shown in Fig. 7. Therefore, the mass balance equations for TB in the capillary bed, tubular lumen and urine compartment are given as follows:

$$V_{KI} F B_{KI} (1 - Hct_{KI}) \frac{dC_{p, KI}^T}{dt} = (Q_{p, KI} C_{p, BP}^T - GFR C_{f, BP}^T) - (Q_{p, KI} - UR) C_{p, KI}^T + CL_{re} C_u^T \quad (4)$$

$$V_{KI} F L_{KI} \frac{dC_u^T}{dt} = GFR C_{f, BP}^T - CL_{re} C_u^T - UR C_u^T \quad (5)$$

$$\frac{dX_u^T}{dt} = UR C_u^T \quad (6)$$

where $C_{p, BP}$, $C_{p, KI}$ and C_u are drug concentrations in the arterial plasma, capillary plasma and tubular lumen, respectively; X_u is the cumulative amount of urinary excretion; $Q_{p, KI}$ and UR

Fig. 7. Schematic Representation of Model for Renal Excretion of TB

$$V_{KI}FB_{KI}(1-Hct_{KI})\frac{dC_{p,KI}^S}{dt} = (Q_{p,KI}C_{p,BP}^S - GFR C_{f,BP}^S) - (Q_{p,KI} - UR)C_{p,KI}^S \quad (7)$$

$$- \frac{V_m C_{f,KI}^S}{K_m(1 + C_{f,KI}^S/K_I) + C_{f,KI}^S} + CL_{re}(C_u^S - C_{f,KI}^S)$$

$$V_{KI}FL_{KI}\frac{dC_u^S}{dt} = GFR C_{f,BP}^S - UR C_u^S + \frac{V_m C_{f,KI}^S}{K_m(1 + C_{f,KI}^S/K_I) + C_{f,KI}^S} - CL_{re}(C_u^S - C_{f,KI}^S) \quad (8)$$

$$\frac{dX_u^S}{dt} = UR C_u^S \quad (9)$$

are the renal plasma flow and urine flow rate, respectively; CL_{re} is renal reabsorption clearance of TB; V_{KI} is the volume of kidneys; FB_{KI} and FL_{KI} are the fractions of capillary bed and tubular lumen; Hct_{KI} is hematocrit in the capillary bed.

For SMZ, assuming the competitive inhibition of TB for the tubular secretion of SMZ, the mass balance equations are given as follows:

where V_m and K_m are the maximal transport capacity of secretion for SMZ and the corresponding Michaelis-Menten constant of the drug-carrier complex; CL_{re} is the reabsorption clearance for passive diffusion of SMZ; K_I is the apparent inhibitor constant of TB for the secretion of SMZ.

The physiological parameters and the parameters of SMZ for distribution in the body and elimination from the body were adopted from the previous report.^{7,8)} For the simulation, Eqs. 1 and 4–6 were fitted to the data of the un-

Fig. 8. Influence of Changes in Plasma Protein Binding, Secretion Clearance and Reabsorption Clearance on Renal Clearance

Simulation was carried out using Eqs. A-6 and A-7. The solid line and dashed line represent CL_R/GFR and $CL_R(f)/GFR$, respectively. Reabsorption parameter is (A): $CL_{re}/UR=0.1$, (B): $CL_{re}/UR=10.0$. CL_{se}/GFR for the curves from upward to downward in the figures are 1000, 100, 10, 1, 0.1 and 0.01, respectively. Other parameters were set at $Q_p=4.0$ l/h, $GFR=1.0$ l/h and $UR=0.1$ l/h.

TABLE I. Disposition Parameters of TB during SMZ Infusion and Inhibitor Constant of TB for SMZ Secretion^{a)}

$C_1^{b)}$ ($\mu\text{g/ml}$)	$C_2^{b)}$ ($\mu\text{g/ml}$)	$\lambda_1^{b)}$ (h^{-1})	$\lambda_2^{b)}$ (h^{-1})	$\text{CL}_{\text{re}}^{b)}$ (l/h)	$K_I^{c)}$ (μM)
10.2 ± 2.0	38.3 ± 2.6	1.68 ± 0.87	0.152 ± 0.018	2.67 ± 0.10	7.56 ± 0.48

^{a)} Values are presented as mean $\pm$ S.D. ^{b)} Disposition parameters were estimated by fitting the unbound plasma and urine data of TB. ^{c)} Inhibitor constant was estimated by fitting the total and unbound plasma, and urine data of SMZ with fixed values for other parameters.

bound concentrations in plasma and the urinary excretion rates of TB at first and next the inhibitor constant (K_I) was estimated from the observed values of the total and unbound SMZ concentration in plasma, and the urinary excretion rates of SMZ after TB injection. The calculated values were shown as the solid lines in Figs. 3 and 8. The estimated parameters are shown in Table I. The inhibitor constant (K_I) of TB was in good agreement with that for the uptake of SMZ by renal cortex slices (Fig. 2).

Discussion

In the present study, the renal clearance of SMZ was significantly depressed when TB was administered. TB has been shown to be metabolized to HTB and further to CTB, which is mainly excreted in the urine.^{12,13)} Under HTB infusion at 150 mg/h, in which the total HTB and CTB concentrations in plasma were equivalent to, or greater than, the peak levels of those when 50 mg/kg of TB was given intravenously, both renal clearances of HTB and CTB were large and the values corresponded to renal effective plasma flow, indicating that the contribution of tubular secretion to the overall urinary excretion of both materials was extremely large (data not shown). The renal clearance of SMZ, however, remained fundamentally constant when HTB was given. Therefore, it has been confirmed that the inhibition of renal excretion of SMZ was not due to the metabolites of TB but due to TB alone.

For a drug like TB, which might be exclusively reabsorbed as described above, $C_{p,KI}$ would be virtually equal to $C_{p,BP}$. C_u was proportional to $C_{f,BP}$ because of the concentration inde-

pendent reabsorption. Furthermore, the tissue concentration of TB in the kidney was proportional to $C_{f,BP}$ (data not shown). In a such case, to quantitate the SMZ kinetics in the presence of TB, it does not seem to matter whether $C_{f,KI}$, $C_{f,BP}$, C_u or the tissue concentration of TB is used, although there are some differences in the estimated inhibitor constant. It is recognized that secretion for the organic anion transport system involves the active transport across the basolateral membrane into the tubular cell and the passive process across the brush-border membrane into the tubular fluid.¹⁴⁾ Thus, we employed the unbound concentration of TB in the renal vein, which was thought to be capable of access to the basolateral membrane. The estimated inhibitor constant (K_I) of TB was found to be in reasonable agreement with that for the uptake by renal cortex slices, suggesting that the proposed model can accurately describe the drug-drug pharmacokinetic interactions.

For SMZ, PSP and SIX, CL_R and $\text{CL}_R(f)$ both were decreased when TB was given, suggesting that the results were caused by the direct inhibition of the secretion process by TB. Whereas, for SMP or SDZ, $\text{CL}_R(f)$ was decreased as well. However, CL_R was rather increased, though the magnitude of change in CL_R was smaller than that in $\text{CL}_R(f)$. The result could not be explained by the inhibition of the secretion process. It is well known that plasma protein binding affects renal clearance. The increase of the unbound fraction of drug results in the increase of CL_R and the decrease of $\text{CL}_R(f)$, though the magnitude of change in CL_R or $\text{CL}_R(f)$ depend on the efficiency of the secretion. In the cases of SMP and SDZ, however, it could not just be explained by the displace-

ment of plasma protein binding.

By using Eqs. A-6 and A-7 (see Appendix), these circumstances are illustrated in Fig. 8. For a drug with low extraction ratio, regardless of the presence or the absence of passive reabsorption, CL_R varies in proportion to the unbound fraction of drug. But the change of $CL_R(f)$ is relatively small. On the other hand, when the secretion clearance is altered, the change of renal clearances (CL_R and $CL_R(f)$) is pronounced for a drug with an intermediate extraction ratio but is relatively small for a drug with a low or high extraction ratio. Therefore, for SMP and SDZ with low extraction ratios, it was suggested that both effects, *i.e.*, enhancement by the displacement of plasma protein binding and depression by inhibition of the secretion process by TB, contributed to CL_R , eventually producing the increase of CL_R as net effect. As both effects of the displacement and the inhibition would induce the decrease in $CL_R(f)$, the relative change of CL_R should be smaller than that of $CL_R(f)$.

The inhibition mechanism in the tubular secretion by TB, that is, whether TB shares with a specific organic anion transport system or has the metabolic effect for the tubular secretion, is not clear. The urinary excretion of TB was not affected in the presence of SMZ (Fig. 6). The *in vitro* renal cortex accumulation of TB was scarcely affected with SMZ as well (data not shown). Even though TB would be subject to the organic anion transport system, the inhibition of TB secretion by SMZ might be concealed behind the exclusive reabsorption of TB. In the view of metabolic effect, Beer *et al.*¹⁵⁾ showed that TB had an uncoupling effect on oxidative phosphorylation. It was shown that dinitrophenol, a representative uncoupler of phosphorylation, inhibited competitively PAH uptake by isolated tubules.¹⁶⁾ However, Ross *et al.*¹⁷⁾ demonstrated that dinitrophenol acted both as a metabolic inhibitor and as a competitive inhibitor, in which the latter shared with a specific organic anion transport system. Therefore, whether the uncoupling effect proceeds apparently in competitive fashion is not clear at present. Further studies on the relation between uncoupling potency and activity in inhibiting

transport of TB should also be performed.

The duration of the effect of TB on the renal clearance of PAH or PSP was more temporary than that on SMZ (data not shown). This might be due to the differences in TB elimination. The unbound TB concentration in plasma in the presence of SMZ declined more slowly than in the absence of SMZ (Fig. 5). It seemed to be due to the inhibition of hepatic metabolism of TB by SMZ, which has been pointed out by Lumholtz *et al.*³⁾ The present study was restricted to the effect of TB on the renal excretion of SMZ. To quantitate the drug-drug pharmacokinetic interactions, some more comprehensive aspects involving hepatic elimination and the distribution of TB should be done. This is now under investigation.

In conclusion, the effects of TB on the renal excretion of some anionic drugs were examined. The change of the renal excretion of SMZ by TB was quantitatively explained by the perfusion-limited renal model, assuming the displacement of plasma protein binding and the competitive inhibition of the secretion process of SMZ by TB as the mechanism of drug-drug interactions.

Appendix

The renal clearance of a drug which incorporates with glomerular filtration, passive tubular reabsorption and saturable tubular secretion in the presence of a competitive inhibitor at the steady state is expressed according to the previous report as follows:⁷⁾

$$CL_R = \frac{[(Q_p - UR)f_{p_{in}}GFR + f_{p_{out}}Q_p \left(\frac{V_m}{K_{app} + C_{f,KI}} + CL_{re} \right)] \left[1 - \frac{CL_{re}}{UR + CL_{re}} \right]}{(Q_p - UR) + f_{p_{out}} \left(\frac{V_m}{K_{app} + C_{f,KI}} + CL_{re} \right) \left(1 - \frac{CL_{re}}{UR + CL_{re}} \right)} \quad (A-1)$$

or

$$CL_R = \frac{(Q_p - UR)f_{p_{in}}GFR + f_{p_{out}}Q_p \left(\frac{V_m}{K_{app} + C_{f,KI}} + CL_{re} \right)}{(Q_p - UR) + \frac{f_{p_{out}}V_m}{K_{app} + C_{f,KI}} + [Q_p - UR(1 - f_{p_{out}})] \frac{CL_{re}}{UR}} \quad (A-2)$$

$$K_{app} = K_m(1 + C_{f,KI}^T/K_I) \quad (A-3)$$

where fp_{in} and fp_{out} are the unbound fractions of drug in the renal artery and the renal vein, *i.e.*, $C_{f,BP}/C_{p,BP}$ and $C_{f,KI}/C_{p,KI}$, respectively, and K_{app} is the apparent Michaelis-Menten constant for tubular secretion. In Eq. A-2, as the urine flow rate is much smaller than the renal plasma flow, $Q_p - UR$ and $Q_p - UR(1 - fp_{out})$ reduce to Q_p and one obtains

$$CL_R = \frac{fp_{in}GFR + fp_{out}\left(\frac{V_m}{K_{app} + C_{f,KI}} + CL_{re}\right)}{1 + \frac{fp_{out}V_m}{Q_p(K_{app} + C_{f,KI})} + \frac{CL_{re}}{UR}} \quad (A-4)$$

If K_{app} is greater than $C_{f,KI}$, the substitution of CL_{se} for V_m/K_{app} yields

$$CL_R = \frac{fp_{in}GFR + fp_{out}(CL_{se} + CL_{re})}{1 + \frac{fp_{out}CL_{se}}{Q_p} + \frac{CL_{re}}{UR}} \quad (A-5)$$

The unbound fraction in plasma is dependent on the drug concentration, the dissociation constant, the concentrations of the binding sites, and further the concentration of displacer and its affinity. As the concentrations of the binding sites in the renal vein are $Q_p/(Q_p - UR)$ times those in the renal artery, there seems to be only a few differences in both the binding capacities in the artery and the vein. For drugs with the very low extraction ratio at the secretion sites, the difference in fp_{in} and fp_{out} is small, because of the small changes of drug concentration in the renal passage. There might be some differences in fp_{in} and fp_{out} only when $C_{f,BP}$ of a drug with high extraction ratio is greater than the dissociation constant for the drug-plasma protein complex and $C_{p,KI}$ is much smaller than $C_{p,BP}$ because of a large fall in drug concentration at the secretion sites. If the displacer would be subject to predominant secretion, the venous concentration of the displacer might be much smaller than that in the artery and the similar change in apparent dissociation constant for the

drug-plasma protein complex might occur. For a drug like TB with predominant reabsorption and virtually no difference in the concentration in the artery and the vein it should produce no change in the apparent dissociation constants within the kidney.

If there are only a few differences in the unbound fractions in the renal artery and the renal vein ($fp_{out} = fp_{in}$), Eq. A-5 reduces to

$$CL_R = \frac{fp_{in}(GFR + CL_{se} + CL_{re})}{fp_{in}\frac{CL_{se}}{Q_p} + 1 + \frac{CL_{re}}{UR}} \quad (A-6)$$

or

$$CL_R(f) = \frac{GFR + CL_{se} + CL_{re}}{fp_{in}\frac{CL_{se}}{Q_p} + 1 + \frac{CL_{re}}{UR}} \quad (A-7)$$

Eqs. A-6 and A-7 indicate the relationship between the plasma protein binding and the renal clearance, which are determined by the relative magnitude of CL_{se} to Q_p and/or that of CL_{re} to UR . The effects of the magnitude of the unbound fraction (fp_{in}) and secretion clearance (CL_{se}) on the renal clearance (CL_R or $CL_R(f)$) are illustrated in Fig. 8.

References

- 1) L. K. Christensen, J. M. Hansen and M. Kristensen: Sulfaphenazole-induced hypoglycemic attacks in tolbutamide treated diabetics, *Lancet*, ii, 1298-1301 (1963).
- 2) J. J. Thiessen and M. Rowland: Kinetics of drug-drug interactions in sheep: Tolbutamide and sulfadimethoxine, *J. Pharm. Sci.*, **66**, 1063-1070 (1977).
- 3) B. Lumholtz, K. S. Nielsen, L. Skovsted, J. Kampmann and J. M. Hansen: Sulfamethizole-induced inhibition of diphenylhydantoin, tolbutamide, and warfarin metabolism, *Clin. Pharmacol. Ther.*, **17**, 731-734 (1975).
- 4) J. Shibasaki, R. Konishi and K. Yamasaki: Tolbutamide-sulfaphenazole interaction in rabbits, *J. Pharmacokin. Biopharm.*, **5**, 277-290 (1977).
- 5) O. Sugita, Y. Sawada, Y. Sugiyama, T. Iga and M. Hanano: Effect of sulphaphenazole on tolbutamide disposition in rabbits: Analysis of interspecies in tissue distribution of tolbutamide, *J. Pharm. Sci.*, **73**, 631-634 (1984).

- 6) S. S. Ghosh, S. K. Garg and V. S. Mathur: Effect of tolbutamide treatment on the pharmacokinetics of intravenously administered sulphamethoxazole in rabbits, *Pharmacology*, **35**, 61—64 (1987).
- 7) K. Katayama, H. Ohtani, T. Kawabe, H. Mizuno, M. Endoh, M. Kakemi and T. Koizumi: Kinetic studies on drug disposition in rabbits. I. Renal excretion of iodopyracet and sulfamethizole, *J. Pharmacobio-Dyn.*, **13**, 97—107 (1990).
- 8) K. Katayama, H. Ohtani, A. Murai, M. Kakemi and T. Koizumi: Kinetic studies on drug disposition in rabbits. II. Dose dependent pharmacokinetics of sulfamethizole, *J. Pharmacobio-Dyn.*, **13**, 108—119 (1990).
- 9) J. Shibasaki R. Konishi, T. Ueki and T. Morishita: Drug absorption, metabolism, and excretion. VIII. Determination of tolbutamide and its metabolites in blood and urine, *Chem. Pharm. Bull.*, **21**, 1747—1753 (1973).
- 10) R. Hori, K. Sunayashiki and A. Kamiya: Tissue distribution and metabolism of drugs. I. Quantitative investigation on renal handling of pheolsulfonphthalein and sulfonamides in rabbits, *Chem. Pharm. Bull.*, **26**, 740—745 (1978).
- 11) G. Levy: Effect of plasma protein binding on renal clearance of drugs, *J. Pharm. Sci.*, **69**, 482—483 (1980).
- 12) J. Shibasaki, R. Konishi, T. Morishita and T. Ueki: Drug absorption, metabolism, and excretion. IX. Some new aspects of pharmacokinetics on tolbutamide in rabbits, *Chem. Pharm. Bull.*, **21**, 1754—1763 (1973).
- 13) S. J. Gee, C. E. Green and C. A. Tyson: Comparative metabolism of tolbutamide by isolated hepatocytes from rat, rabbit, dog, and squirrel monkey, *Drug Metab. Dispos.*, **12**, 174—178 (1984).
- 14) B. M. Tune, M. B. Burg and C. S. Patlak: Characteristics of *p*-aminohippurate transport in proximal renal tubules, *Am. J. Physiol.*, **217**, 1057—1063 (1969).
- 15) L. De Beer and P. J. De Schepper: Metabolic effects of hypoglycemic sulfonylureas-II. *In vitro* effect of sulfonylureas on cell-free protein synthesis and energy metabolism in rat tissues, *Biochem. Pharmacol.*, **16**, 2355—2367 (1967).
- 16) K. C. Huang and D. S. T. Lin: Kinetic studies on transport of PAH and other organic acids in isolated renal tubules, *Am. J. Physiol.*, **208**, 391—396 (1965).
- 17) R. Ross and I. M. Weiner: Adenine nucleotides and PAH transport in slices of renal cortex: Effects of DNP and CN⁻, *Am. J. Physiol.*, **222**, 356—359 (1972).
- 18) K. Koshi, Y. Shirai, K. Katayama, M. Kakemi, M. Ueda and T. Koizumi: A note on saturable and inhibitory kinetics of *p*-aminohippurate renal transport in rabbits, *J. Pharmacobio-Dyn.*, **6**, 161—169 (1983).