Effect of Sulfaphenazole on Tolbutamide Distribution in Rabbits: Analysis of Interspecies Differences in Tissue Distribution of Tolbutamide

OSAMU SUGITA, YASUFUMI SAWADA, YUICHI SUGIYAMA, TATSUJI IGA[x], and MANABU HANANO

Received October 4, 1982 from the *University of Tokyo, Faculty of Pharmaceutical Sciences, Department of Pharmaceutics, 3-1, 7-Chome, Hongo, Bunkyo-ku, Tokyo 113, Japan.* Accepted for publication April 5, 1983.

Abstract □ The effect of sulfaphenazole on the distribution of tolbutamide was examined by comparing the change in the steady-state volume of distribution (Vd_{ss}) determined from *in vivo* plasma elimination with the tissue-to-plasma concentration ratio of various tissues (K_p) in rabbits; this effect was compared with that previously reported in rats. In rabbits, the K_p values of six tissues studied (*i.e.*, brain, heart, spleen, small intestine, muscle, and skin) increased in the presence of sulfaphenazole; except for brain, lung, and adipose tissue, the tissue-to-plasma unbound concentration ratio ($K_{p,f}$) of other tissues did show a significant decrease. This suggested that both the tissue and plasma protein binding of tolbutamide were affected by sulfaphenazole and that the increase in K_p was due mainly to the displacement of plasma protein binding of tolbutamide by sulfaphenazole, which was greater than that of tissue binding, while no change in K_p was due to a parallel change in both the plasma protein binding and tissue binding of tolbutamide. In both rabbits and rats, the Vd_{ss} calculated from plasma concentration *versus* time curve was very close to that calculated from the K_p values and volumes of various tissues in the presence and absence of sulfaphenazole, respectively. The interspecies difference of the effect of sulfaphenazole on the tissue distribution of tolbutamide between rabbits and rats was elucidated from both *in vivo* tissue distribution and *in vitro* plasma protein binding studies.

Keyphrases: □ Sulfaphenazole—effect on tissue distribution of tolbutamide, rabbits, interspecies comparison □ Tolbutamide—tissue distribution in rabbits, effect of sulfaphenazole, interspecies comparison □ Tissue distribution—tolbutamide in rabbits, effect of sulfaphenazole, interspecies comparison

In previous papers (1, 2) a blood flow rate-limited pharmacokinetic model was developed to study the effect of sulfonamide on the plasma elimination and tissue distribution of tolbutamide in rats. The tissue-to-plasma concentration ratio (K_p) of all tissues studied increased in the presence of sulfaphenazole, but the tissue-to-plasma unbound concentration ratio ($K_{p,f}$) did not show a significant alteration. This suggested that the tissue binding of tolbutamide is not affected by sulfaphenazole and that the increase of K_p is due mainly to the displacement of plasma protein binding of tolbutamide by sulfaphenazole. The purpose of this study was to investigate an interspecies difference in the effect of sulfaphenazole on the tissue distribution of tolbutamide in rabbits and rats, and to determine whether this finding would encompass other species.

EXPERIMENTAL

Sodium tolbutamide[1] and sulfaphenazole[2] were used. [^{14}C-carbonyl]tolbutamide[3] (48.09 mCi/mmol), which was found to be ≥98–99% pure by TLC, was used as the radioactive compound. All other reagents were commercially available and analytical grade.

Animal Experiments—Adult male albino rabbits[4], weighing 2.6–2.9 kg, were used. Under light ether anesthesia, the femoral vein and artery were cannulated with polyethylene tubing[5]. Cannulated rabbits were kept in a supine position on restraining plates. After a loading dose of 53.6 mg of sulfaphenazole/kg, 57.1 mg/kg/h was infused through the femoral vein cannula for 8 h with a constant-rate infusion pump[6]; with this dosage, steady-state concentrations of sulfaphenazole (153.3 ± 3.5 μg/mL, n = 4) and N^4-acetyl sulfaphenazole (201.7 ± 9.6 μg/mL, n = 3) were obtained at 2 h after the initiation of infusion, as reported previously (3). The rabbits were then given 80 mg/kg of tolbutamide containing 3.33 μCi/kg of [^{14}C]tolbutamide in physiological saline through the other femoral vein cannula. The control rabbits were given physiological saline instead of sulfaphenazole. The body temperature was kept at 37°C by a heat lamp.

After removal of arterial blood samples, the animals were sacrificed 6 h after tolbutamide administration by an injection of saturated potassium chloride solution into the carotid artery. The brain, heart, lung, liver, kidney, spleen, pancreas, small intestine, adipose tissue (around the kidneys and bladder), muscle (abdominal), and skin (dorsal) were quickly excised, rinsed well with cold saline, blotted, and weighed. The wet weights of these tissues were not altered in the presence of sulfaphenazole. All tissues and plasma were stored at −20°C until assayed.

The separation of the metabolites from tolbutamide was carried out according to Shibasaki *et al.* (4) as described in a previous paper (2). With this method, unchanged tolbutamide could be separated from its metabolites, *i.e.*, hydroxy- and carboxytolbutamides (4). Briefly, tissue samples except for skin were homogenized[7] with a threefold excess volume of 0.5 M phosphate buffer (pH 5.0). Four milliliters of each homogenate or 50 μL of the plasma was shaken for 30 min and then extracted twice with 6 mL of *n*-heptane–chloroform (4:1 v/v). The extracts were combined and shaken with 1 mL of 0.5 M NaOH for 15 min, and the aqueous phase was used for the determination of tolbutamide. The concentration of ^{14}C-labeled tolbutamide was determined[8] after addition of a 0.5 mL of 0.5 M HCl and 10 mL of scintillation fluid[9].

The skin was oxidized to $^{14}CO_2$ in a sample oxidizer[10], and then the radioactivity was determined as described before. The value of the plasma free fraction of rats was obtained from a previous paper (2), but that of rabbits was determined by an ultrafiltration method at 37°C as reported previously (1, 3).

Statistical Analysis—All means are presented with their standard error (mean ± SE). Statistical analysis was performed using the Student's t test, with p = 0.05 as the minimal level of significance.

RESULTS

The tissue-to-plasma concentration ratios (K_p) in rabbits 6 h after intravenous bolus administration of tolbutamide in the presence and absence of sulfaphenazole are listed in Table I. In the presence of sulfaphenazole, the K_p values of six tissues, *i.e.*, brain, heart, spleen, small intestine, muscle, and skin, increased significantly. The relationships between the K_p values in the presence and those in the absence of sulfaphenazole are shown in Fig. 1a and are compared with that of rats (Fig. 1b), which were reported previously (2). The plasma free fractions (f_p) of tolbutamide in the absence and presence of sulfaphenazole are listed in Table II. The f_p increased significantly in the presence of sulfaphenazole. The tissue-to-plasma unbound concentration ratios ($K_{p,f}$) were calculated by the following equation and are also listed in Table II:

$$K_{p,f} = \frac{K_p}{f_p} \qquad \text{(Eq. 1)}$$

Significant decreases in $K_{p,f}$ were shown in the heart, liver, kidney, spleen,

[1] Japan Hext, Tokyo, Japan.
[2] Dainippon Pharm. Co., Tokyo, Japan.
[3] New England Nuclear Co., Boston, Mass.
[4] Ichikawaya, Tokyo, Japan.
[5] Type PE-50, Clay Adams; Becton, Dickinson Co., Parsippany, N.J.
[6] Model KN, Type 12H; Natsume Seisakusho Co., Tokyo, Japan.
[7] Teflon glass homogenizer.
[8] Aloka Tri-Carb counter; Aloka Instruments Co., Tokyo, Japan.
[9] 0.1 g of 1,4-bis[2-(4 methyl-5-phenyloxazo-lyl)]-benzene, 4.0 g of 2,5 diphenyloxazole, and 500 mL of Triton X-100/L of toluene.
[10] Model 306; Packard Instruments Corp., Downers Grove, Ill.

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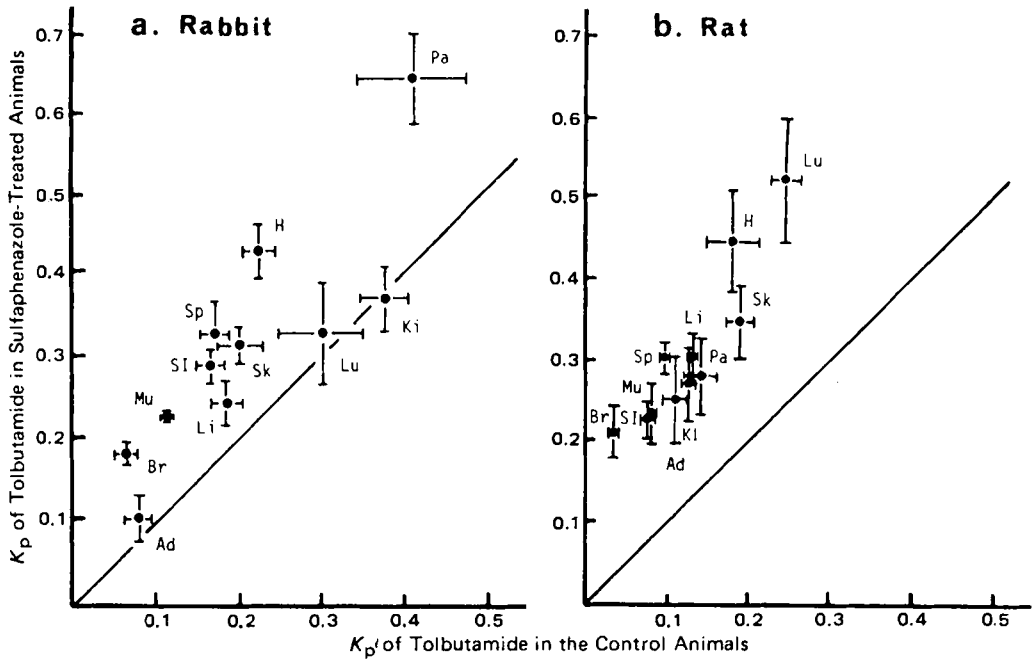

Figure 1—*Relationship between the tissue-to-plasma concentration ratios* (K_p) *of tolbutamide in the presence and absence of sulfaphenazole in rabbits* (*a*) *and rats* (*b*). *The abbreviations, Li, Ki, SI, Lu, H, Mu, Br, Pa, Sp, Ad, and Sk denote liver, kidney, small intestine, lung, heart, muscle, brain, pancreas, spleen, adipose tissue, and skin, respectively. The data for rats were obtained from a previous paper* (*2*). *The line shows that of unity* (*slope = 1*).

pancreas, small intestine, muscle, and skin of the sulfaphenazole-treated rabbits, but there did not appear to be significant changes in the brain, lung, and adipose tissues. The relationships between the $K_{p,f}$ values in the presence and those in the absence of sulfaphenazole are shown in Fig. 2a, and are compared with those of rats (Fig. 2b) as reported previously (2).

The volume of distribution at steady state (Vd_{ss}) calculated by Eq. 5 in the *Appendix* and that based on the unbound drug except for the blood space ($Vd_{T,f}$) calculated by Eq. 7 in the *Appendix I* are listed in Table III along with those for rats, which were calculated from the data reported previously (2). In both the sulfaphenazole-treated rabbits and rats, a significant increase was observed in $\overline{Vd}_{ss}$, whereas in $\overline{Vd}_{T,f}$ a significant decrease was observed only in rabbits. The extent of decrease in $Vd_{T,f}$ for rabbits in the presence of sulfaphenazole was smaller than that of the bound fraction ($1 - f_p$) calculated by f_p in Table II. Using the values of K_p, $K_{p,f}$, and volumes of various tissues listed in Tables I, II, and IV, the Vd_{ss} and $Vd_{T,f}$ were calculated by Eqs. 8 and 10 from the *Appendix I*, respectively, and are listed in Table III with those of rats, which were calculated from the data reported previously (2). The Vd_{ss} significantly increased in the presence of sulfaphenazole in rabbits as well as in rats. A significant decrease in $Vd_{T,f}$ was shown in rabbits in the presence of sulfaphenazole, but in rats no alteration was shown. The extent of changes in $\overline{Vd}_{ss}$ and $\overline{Vd}_{T,f}$ calculated by Eqs. 5 and 7 in the *Appendix I* was comparable with that of those parameters (Vd_{ss} and $Vd_{T,f}$) calculated by Eqs. 8 and 10 in the *Appendix I*, respectively.

DISCUSSION

When metabolism is the rate-determining step of plasma drug elimination (1, 3), the Vd_{ss} can be expressed as follows (10) (see *Appendix I*):

$$Vd_{ss} = \frac{V_B}{s} + \sum_j K_{p_j} \cdot V_j = \frac{V_B}{s} + \sum_j f_p \cdot K_{p,f_j} \cdot V_j \qquad \text{(Eq. 2)}$$

Previously, the effects of sulfaphenazole on the plasma free fraction and intrinsic clearance of tolbutamide in rats (2) and rabbits (3) have been reported. The plasma protein binding showed a nonlinearity and was decreased in the presence of sulfaphenazole in both animals. But little has been reported for the effect of sulfaphenazole on the tissue distribution of tolbutamide (1).

Although the K_p values for many tissues studied in the presence of sulfaphenazole significantly increased in rabbits, the $K_{p,f}$ values calculated by Eq. 1 showed a significant decrease. From these findings, it was suggested that the tissue binding of tolbutamide in rabbits may depend on the plasma tolbutamide concentration or may be decreased in the presence of sulfaphenazole or N^4-acetylsulfaphenazole. The increase in Vd_{ss} (1.2-fold of that for the control rabbits) might be explained by a simultaneous decrease in the plasma protein binding of tolbutamide (0.45-fold of that for the control; Table II) and in the tissue binding (0.53-fold of that for the control; Table III). The decrease in the tissue binding of tolbutamide can be seen in the decrease in $Vd_{T,f}$ (Table III).

In sheep, Thiessen and Rowland (5) reported that although the plasma free fraction of tolbutamide increased, the distribution volume did not show an alteration in the presence of sulfadimethoxine. Accordingly, in sheep a parallel change in both the plasma protein binding and the tissue binding of tolbutamide might minimize any change in Vd as a function of the plasma free fraction. However, in rats, although the K_p values for most of the tissues

Table I—Tissue-to-Plasma Concentration Ratios (K_p) of Tolbutamide in Rabbits [a]

Tissue	Control, $n = 4$	Sulfaphenazole, $n = 3$
Brain	0.062 ± 0.011	0.186 ± 0.008[b]
Heart	0.230 ± 0.014	0.420 ± 0.033[b]
Lung	0.297 ± 0.054	0.325 ± 0.064
Liver	0.184 ± 0.009	0.240 ± 0.033
Kidney	0.382 ± 0.028	0.368 ± 0.028
Spleen	0.171 ± 0.009	0.329 ± 0.025[b]
Pancreas	0.405 ± 0.074[c]	0.629 ± 0.047
Small intestine	0.169 ± 0.016	0.287 ± 0.022[b]
Adipose tissue	0.080 ± 0.015	0.107 ± 0.021
Muscle	0.111 ± 0.004	0.229 ± 0.007[b]
Skin	0.200 ± 0.026	0.316 ± 0.014[b]

[a] Results are given as the mean ± *SE*; at 6 h after bolus injection of tolbutamide. [b] Significantly different ($p < 0.05$) from the control. [c] $n = 2$.

Table II—Tissue-to-Plasma Unbound Concentration Ratios ($K_{p,f}$) of Tolbutamide in Rabbits [a]

Tissue	Control, $n = 4$	Sulfaphenazole, $n = 3$
C_p, g/mL[b]	118.6 ± 11.3	94.8 ± 1.5[d]
f_p[c]	0.094 ± 0.003	0.273 ± 0.001[d]
Brain	0.706 ± 0.135	0.682 ± 0.030
Heart	2.474 ± 0.229	1.543 ± 0.124[d]
Lung	3.210 ± 0.663	1.195 ± 0.236
Liver	1.971 ± 0.158	0.880 ± 0.150[d]
Kidney	4.117 ± 0.439	1.353 ± 0.104[d]
Spleen	1.821 ± 0.079	1.212 ± 0.091[d]
Pancreas	4.369 ± 1.020[e]	2.310 ± 0.174[d]
Small intestine	1.812 ± 0.205	1.055 ± 0.081[d]
Adipose tissue	0.868 ± 0.184	0.393 ± 0.078
Muscle	1.188 ± 0.072	0.841 ± 0.031[d]
Skin	2.139 ± 0.278	1.160 ± 0.050[d]

[a] Results are given as the mean ± *SE*; at 6 h after bolus injection of tolbutamide. [b] Plasma concentration of tolbutamide. [c] Plasma free fraction of tolbutamide. [d] Significantly different ($p < 0.05$) from the control. [e] $n = 2$.

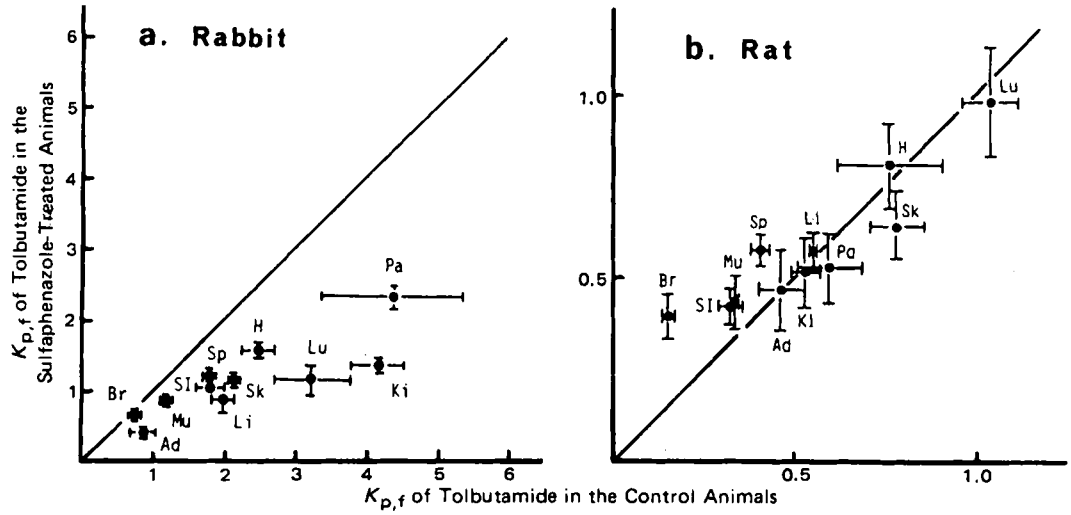

Figure 2—*Relationship between the tissue-to-plasma unbound concentration ratios ($K_{p,f}$) of tolbutamide in the presence and absence of sulfaphenazole in rabbits (a) and rats (b). Abbreviations are the same as those in Fig. 1. The data for rats were obtained from a previous paper (2). The line shows that of unity (slope = 1).*

studied in the presence of sulfaphenazole and at a higher steady-state concentration of tolbutamide than that of the control, significantly increased, the $K_{p,f}$ values calculated by Eq. 1 did not show an alteration (Figs. 1b and 2b). Consequently, the tissue binding of tolbutamide may not depend on the plasma tolbutamide concentration and may not be decreased in the presence of sulfaphenazole, whereas the plasma protein binding of tolbutamide showed a nonlinearity and was decreased in the presence of sulfaphenazole (2). From these findings, it was suggested that in rats the increase in K_p might be explained mainly by an alteration of the plasma protein binding of tolbutamide.

The difference in the effect of sulfaphenazole on the tissue distribution of tolbutamide between rats and rabbits may be due to the presence of a higher concentration of N^4-acetylsulfaphenazole in rabbits than in rats. As previously reported, the displacement of thiopental by sulfadimethoxine was not revealed in the tissue binding using rat muscle, liver, and adipose tissue homogenates (6). Moreover, the tissue binding of tolbutamide was not significantly influenced by phenylbutazone using rabbit muscle homogenates (7). Wardell (8) also reported that phenylbutazone did not affect significantly the tissue binding of sulfadimethoxine using rat muscle, liver, and kidney homogenates. Consequently, the increase in Vd_{ss} of tolbutamide in rats in the presence of sulfaphenazole might be explained by the unchanged tissue binding of tolbutamide and the increase in the plasma free fraction of tolbutamide, caused by the displacement of the plasma tolbutamide binding by sulfaphenazole. As shown in Table IV, the difference between $\overline{Vd}_{ss}$ and Vd_{ss}, calculated by Eqs. 5 and 8, respectively, in both the control rabbits and rats may result from the nonlinearity of the plasma protein binding (2, 3), since the plasma concentration of tolbutamide 6 h after intravenous bolus administration was lower than the *in vivo* mean plasma concentration for 6 h (see footnote in Table III). On the other hand, the close agreement between $\overline{Vd}_{ss}$ and Vd_{ss} in the presence of sulfaphenazole may be due to the linearity of the plasma protein binding of tolbutamide, which was observed only in the presence of sulfaphenazole (2, 3).

In rabbits, the $K_{p,f}$ values were greater than unity in most of the tissues studied (Fig. 2 and Table II) and were different from those of rats, which were not greater than unity in all tissues (1). The comparison of $Vd_{T,f}$ and f_p among rabbits, rats, sheep, and humans is summarized in Table V. It was shown that the distribution of tolbutamide to the tissues was approximately two times higher in rabbits and sheep than that in humans and rats. A remarkable interspecies difference was also shown in f_p between humans and rats, whereas only a small difference was shown in the tissue distribution of tolbutamide between the two species. This suggests the possibility of the animal scale-up from rats to humans for the prediction of Vd of humans using the data of the tissue distribution of tolbutamide obtained in the rat study.

In the present study, the interspecies difference of the tissue distribution of tolbutamide between rabbits and rats was demonstrated from both the *in vivo* tissue distribution and *in vitro* plasma protein binding.

APPENDIX I: Calculation of the Volume of Distribution

The volume of distribution at steady state (Vd_{ss}) is given by:

$$Vd_{ss} = Vd_B + Vd_T \qquad \text{(Eq. 3)}$$

The volume of distribution for the blood space (Vd_B) can be expressed by:

$$Vd_B = \frac{V_B \cdot C_B}{C_p} = \frac{V_B}{s} \qquad \text{(Eq. 4)}$$

The Vd_{ss} is calculated by two methods as follows:

Method 1—The Vd_{ss} calculated by plasma concentration *versus* time curve ($\overline{Vd}_{ss}$) is given by (9):

$$\overline{Vd}_{ss} = \frac{\text{Dose} \cdot \text{AUMC}}{(\text{AUC})^2} \qquad \text{(Eq. 5)}$$

From Eq. 3 (10), the following equation is obtained:

$$\overline{Vd}_T = \overline{Vd}_{ss} - Vd_B = f_p \cdot \overline{Vd}_{T,f} \qquad \text{(Eq. 6)}$$

Table III—Effect of Sulfaphenazole on Volume of Distribution of Tolbutamide [a]

Animal	Volume of Distribution, mL/kg	Control, $n = 4$	Sulfaphenazole, $n = 3$
Rabbit	$\overline{Vd}_{ss}$ [b]	222.84 ± 1.89	268.90 ± 13.97[f]
	$Vd_{T,f}$ [c]	1336.54 ± 14.88	703.42 ± 46.72[f]
	Vd_{ss} [d]	152.19 ± 4.91	237.41 ± 3.82[f]
	$Vd_{T,f}$ [e]	1061.01 ± 74.68	656.00 ± 13.23[f]
Rat	$\overline{Vd}_{ss}$ [b]	216.76 ± 2.13	297.79 ± 6.46[f]
	$\overline{Vd}_{T,f}$ [c]	597.39 ± 7.95	436.73 ± 11.83
	Vd_{ss} [d]	146.79 ± 3.50	273.89 ± 26.82[f]
	$Vd_{T,f}$ [e]	381.90 ± 13.99	405.59 ± 53.82

[a] Results are given as the mean ± *SE*. [b] Calculated by Eq. 5 in *Appendix I*: Dose·AUMC/(AUC)^2 (9). [c] Calculated by Eq. 7 in *Appendix I*: $(\overline{Vd}_{ss} - V_B/s)/f_p$. The f_p values calculated from the C_f of the *in vivo* mean plasma concentration (C_t) for 360 min were used. The C_t was calculated mathematically by: $C_t = \text{AUC}_{0\ 360\ min}/360$ min. In rabbits, the mean f_p values were 0.127 in the absence and 0.299 in the presence of sulfaphenazole, respectively, while in rats the mean f_p values were 0.286 in the absence and 0.546 in the presence of sulfaphenazole, respectively. [d] Calculated by Eq. 8 of *Appendix I*: $Vd_B + \Sigma K_{p,j} \cdot V_j$ (10). [e] Calculated by Eq. 10 of *Appendix I*: $\Sigma_j K_{p,j} \cdot V_j / f_p$. The f_p values at 360 min after intravenous bolus administration of tolbutamide were used (see Table II). [f] Significantly different ($p < 0.05$) from the control.

Table IV—Volumes of Blood and Tissues in Rabbits and Rats [a]

Tissue	Rabbits, mL/kg	Rats, mL/kg
Blood	73.8[b]	75.4[b]
Gastrointestinal tract[c]	51.5	44.3
Lung	7.3	4.6
Brain	2.6	4.6
Heart	2.6	4.3
Liver	42.8	44.3
Kidney	6.4	7.9
Muscle	500.0[d]	500.0[d]
Skin	100.0	175.0
Adipose tissue	51.5	40.0
Pancreas	0.4	3.6
Spleen	0.4	3.6

[a] Determined experimentally from the wet tissue weight by assuming a density of 1.0, except for muscle and blood volume. [b] The blood volume was calculated according to Bischoff *et al.* (11) as: $V_{plasma} = 44 \times (\text{body weight, kg})^{0.99}$ and $V_{blood} = V_{plasma}/(1 - H_t)$, where H_t is the hematocrit value and was determined to be 0.41 in this study. Body weights used were 2.8 kg for rabbits and 280 g for rats. [c] Includes the stomach, small intestine, and large intestine; the K_p of the small intestine was used to calculate the distribution volume (Vd) of tolbutamide for this tissue. [d] The muscle volume was assumed to be half of the body weight (12).

The volume of distribution based on the measurement of the unbound drug in plasma except for the blood space ($\overline{Vd}_{T,f}$) is given by:

$$\overline{Vd}_{T,f} = \frac{\overline{Vd}_{ss} - V_B/s}{f_p} \quad \text{(Eq. 7)}$$

In this paper, the $\overline{Vd}_{T,f}$ of humans and sheep were calculated by using the data reported previously (4, 12), respectively. The mean value of 1.36 (1.33 for rats and 1.39 for rabbits) was used as the value of s. The V_B was calculated by the equation in footnote b of Table IV.

Method 2—The Vd_{ss} calculated by tissue-to-plasma concentration ratios at 6 h after intravenous administration of tolbutamide is given by the following (10):

$$Vd_{ss} \simeq Vd_B + \sum_j K_{pj} \cdot V_j \quad \text{(Eq. 8)}$$

The subscript j represents all tissues studied. From Eqs. 3 and 8, the following equation is obtained:

$$Vd_T = \sum_j K_{pj} \cdot V_j \quad \text{(Eq. 9)}$$

The volume of distribution based on the measurement of the unbound drug in plasma except for the blood space ($Vd_{T,f}$) is given by:

$$Vd_{T,f} = \sum_j K_{p,f_j} \cdot V_j = \sum_j K_{pj} \cdot V_j/f_p \quad \text{(Eq. 10)}$$

Appendix II: Glossary

Vd_{ss} = volume of distribution at steady state (mL)
Vd_B = total amount of the drug in the blood compartment divided by the concentration of the drug in plasma (mL)
Vd_T = total amount of the drug in all tissue compartments divided by the concentration of the drug in plasma (mL)
$Vd_{T,f}$ = total amount of the drug in all tissue compartments divided by the concentration of the unbound drug in plasma (mL)
K_p = tissue-to-plasma concentration ratio
$K_{p,f}$ = tissue-to-plasma unbound concentration ratio
V_B = blood volume (mL)
V = volume of the tissue (mL)
s = plasma-to-blood concentration ratio
C_p = plasma concentration (μg/mL)
C_B = blood concentration (μg/mL)
AUC = area under the plasma concentration *versus* time curve [μg/(ml·min)]
AUMC = area under the moment curve (μg/[mL·(min)^2])
f_p = plasma free fraction

Table V—Interspecies Difference in the Total Amount of Drug in All Tissue Compartments Divided by the Concentration of the Unbound Drug in Plasma ($Vd_{T,f}$) and the Plasma Free Fraction (f_p)

Species	$Vd_{T,f}$, mL/kg	f_p
Rabbit	1336.5	0.127
Rat	597.4	0.286
Sheep	1341.2[a]	0.146
Human	639.2[b]	0.093

[a] Calculated by Eq. 7 of *Appendix I* using the reported value (5). [b] Calculated by Eq. 7 of *Appendix I* using the reported value (13).

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