Comparative Pharmacokinetics of Acetohexamide and Its Metabolite, Hydroxyhexamide in Laboratory Animals

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The pharmacokinetic profiles of the hypoglycemic agent, acetohexamide (AH) and its major active metabolite, hydroxyhexamide (HH) were studied in three species of laboratory animals after intraperitoneal (ipl) administration in comparison with those after intravenous (iv) administration of AH and of the preformed metabolite HH. Reductive biotransformation of AH to HH was reversible in rats and guinea pigs, while it was irreversible in rabbits. The parameters of reversible drug-metabolite pharmacokinetics were calculated, including essential clearances of reversible and irreversible elimination, volumes of distribution at the steady state and sojourn times or turnover rates of the metabolite pair.

An interconversion model, which incorporated a first-pass metabolism, was applied to the disposition kinetics of AH and HH, and the available fractions of AH and generated metabolite HH in each species were elucidated.

Keywords metabolite pharmacokinetics; acetohexamide; hydroxyhexamide; reversible metabolite; laboratory animal; pharmacokinetic parameter; first-pass metabolism; interconversion model

After oral or intraperitoneal administration a drug enters the splanchnic circulation and crosses the liver prior to reaching the systemic circulation and undergoing distribution to the various tissues of the body. The role of first-pass elimination in explaining the low oral bioavailability of many drugs is well known. On the other hand, little attention has been paid to the first-pass formation of metabolites which may contribute to the observed pharmacological response after oral drug administration.[1]

(−)-Hydroxyhexamide (HH), the major metabolite of acetohexamide (AH), used as an effective hypoglycemic agent, is thought to contribute significantly to the hypoglycemic response which follows administration of AH.[2]

Previously,[3] the authors have reported that bio-oxidation of preformed HH to AH was reversible in the rat. The present investigation was undertaken to elucidate the reversible drug-metabolite pharmacokinetics by evaluating the time courses of plasma concentration of both AH and HH after intravenous (iv) and intraperitoneal (ipl) administration of AH in three species of laboratory animals.

Experimental

Materials AH was of pharmaceutical grade. HH was obtained by reduction of AH with $NaBH_4$ in methanolic solution. Other reagents were all of special reagent grade.

Animal Experiments AH or HH (250 mg) was dissolved in 50 ml of 1/15 M phosphate buffer solution (pH 7.3), and these solutions were administered intravenously (iv) or intraperitoneally (ipl) as mentioned below to laboratory animals (purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan).

a) Rats: A heparin-treated polyethylene cannula was surgically introduced into the jugular vein of anesthetized male Wistar rats, weighing 250—350 g, to obtain blood samples. The rats (4—5 animals/group) were injected iv or ipl with 0.046 mmol/kg of AH or HH. After dosing, blood samples were drawn at appropriate time intervals, and the plasma was separated immediately by centrifugation and stored in a refrigerator until assayed.

b) Guinea Pigs: Three anesthetized male Hartley guinea pigs per group, weighing about 350 g, were injected through the femoral vein with 0.046 mmol/kg of AH or HH and ipl at 0.139 mmol/kg dose of AH, respectively. After dosing, blood samples collected into a heparinized syringe were treated in the same manner as in the case of rats.

c) Rabbits: To determine the serial plasma concentrations, 2—4 male Japanese white rabbits per group, weighing 2.5—3.1 kg, were injected through the right auricular vein or ipl with 0.041 mmol/kg of AH or HH. Blood (*ca.* 1 ml) was taken from the left marginal auricular vein, and treated in the same manner as in the case of rats. Urine samples were made by addition of one-fifth volume of water to the urine excreted during 48 h after iv dosing of AH or HH.

Sample Analysis Analytical procedures were undertaken as reported previously.[3] A sample of 200 μl for plasma or diluted urine was shaken with 2.0 ml of 0.1 M phosphate buffer solution (pH 5.0) and 5.0 ml of benzene–ethyl acetate (1 : 1) containing cyclohexamide as the internal standard. After centrifugation at 3000 rpm for 20 min, the supernatant fluid (4 ml, organic phase) was evaporated *in vacuo* at 40 °C, the residue was dissolved in 100 μl of the mobile phase, and 20 μl of this solution was subjected to high-performance liquid chromatography (HPLC). HPLC was carried out using a Shimadzu LC-6A apparatus equipped with a Cosmosil 5 C_{18} column (0.46 i.d. × 15 cm) and a Shimadzu SPD-6A UV monitor (240 nm). Acetonitrile–0.2% AcOH (1 : 1) was employed as a mobile phase at a flow rate of 1.2 ml/min.

Pharmacokinetic Calculation The area under the plasma concentration *vs.* time curve (AUC) and mean residence time (MRT) were calculated by applying the trapezoidal rule with extrapolation to infinity (Eqs. 1 and 2).[4] The terminal elimination rate constant (λ) was determined by least-squares linear regression of the logarithm of the plasma concentration profile. The values are given as mean values of data with standard deviation (S.D.)

$$AUC=\sum_{i=1}^{n-1}(C_{i+1}+C_i)(t_{i+1}-t_i)/2+C_n/\lambda \tag{1}$$

$$MRT=\left[\sum_{i=1}^{n-1}(t_{i+1}C_{i+1}+t_iC_i)(t_{i+1}-t_i)/2+t_nC_n/\lambda+C_n/\lambda^2\right]\Big/AUC \tag{2}$$

The pharmacokinetic parameters in the interconversion moment analysis were calculated using the respective AUC and MRT values produced following iv dosing of AH or preformed HH as follows,[5] where superscripts indicate the administration routes of AH (D) and HH (M). The administered drug doses are indicated in molar unit.

Fundamental clearance;

$$CL_{10}=\frac{AUC_M^{Miv}\,\mathrm{dose}^{Div}-AUC_M^{Div}\,\mathrm{dose}^{Miv}}{AUC_D^{Div}AUC_M^{Miv}-AUC_M^{Div}AUC_D^{Miv}} \tag{3}$$

$$CL_{12}=\frac{AUC_M^{Div}\,\mathrm{dose}^{Miv}}{AUC_D^{Div}AUC_M^{Miv}-AUC_M^{Div}AUC_D^{Miv}} \tag{4}$$

$$CL_{20}=\frac{AUC_D^{Div}\,\mathrm{dose}^{Miv}-AUC_D^{Miv}\,\mathrm{dose}^{Div}}{AUC_D^{Div}AUC_M^{Miv}-AUC_M^{Div}AUC_D^{Miv}} \tag{5}$$

$$CL_{21}=\frac{AUC_D^{Miv}\,\mathrm{dose}^{Div}}{AUC_D^{Div}AUC_M^{Miv}-AUC_M^{Div}AUC_D^{Miv}} \tag{6}$$

Apparent plasma clearance;

$$CL_{app,D}=\mathrm{dose}^{Div}/AUC_D^{Div}=CL_{10}+CL_{12}CL_{20}/(CL_{20}+CL_{21}) \tag{7}$$

$$CL_{app,M}=\mathrm{dose}^{Miv}/AUC_M^{Miv}=CL_{20}+CL_{21}CL_{10}/(CL_{10}+CL_{12}) \tag{8}$$

Real plasma clearance;

$$CL_{\text{real,D}} = CL_{10} + CL_{12} \quad (9)$$

$$CL_{\text{real,M}} = CL_{20} + CL_{21} \quad (10)$$

Apparent distribution volume at the steady state;

$$V_{\text{ss,app,D}} = CL_{\text{app,D}} MRT_{\text{D}}^{\text{D}_{\text{iv}}} \quad (11)$$

$$V_{\text{ss,app,M}} = CL_{\text{app,M}} MRT_{\text{M}}^{\text{M}_{\text{iv}}} \quad (12)$$

Real distribution volume at the steady state;

$$V_{\text{ss,real,D}} = (V_{\text{ss,app,D}} - K_{\text{d2}}^{1} V_{\text{ss,app,M}})/(1 - K_{\text{d2}}^{1} \cdot K_{\text{d1}}^{2}) \quad (13)$$

$$V_{\text{ss,real,M}} = (V_{\text{ss,app,M}} - K_{\text{d1}}^{2} V_{\text{ss,app,D}})/(1 - K_{\text{d2}}^{1} \cdot K_{\text{d1}}^{2}) \quad (14)$$

where, $K_{\text{d2}}^{1} = CL_{12}CL_{21}/(CL_{20} + CL_{21})^2$ and $K_{\text{d1}}^{2} = CL_{12}CL_{21}/(CL_{10} + CL_{12})^2$

Sojourn time;

$$\bar{S}_{\text{D}} = V_{\text{ss,real,D}}/CL_{\text{real,D}} \quad (15)$$

$$\bar{S}_{\text{M}} = V_{\text{ss,real,M}}/CL_{\text{real,M}} \quad (16)$$

Theoretical

An interconversion model[5,6] which incorporates a first-pass metabolism is illustrated in Chart 1, where both the parent drug and its relevant metabolite have linear dispositions.

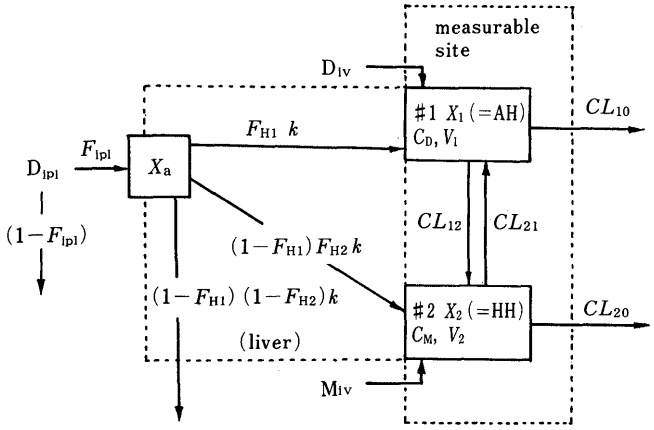

Chart 1. Simple Interconversion Model with First-Order Input Step for Pharmacokinetics of Acetohexamide AH (D) and Its Metabolite HH (M) in Laboratory Animals

D_{iv} and M_{iv}, doses of AH and preformed HH administered intravenously; D_{ipl}, dose of AH administered intraperitoneally; X_a, amount of AH appearing at the absorption site; X_1 and X_2, amounts of AH and HH at the compartments #1 and #2; C_D and C_M, plasma concentrations of AH and HH in the respective compartments; V_1 and V_2, distribution volumes of AH and HH in the respective compartments; CL_{10} and CL_{20}, clearance rates for the irreversible loss of AH and HH; CL_{12} and CL_{21}, clearance rates associated with metabolic interconversion; k, first-order rate constant associated with the drug absorption process; F_{ipl}, fraction of dose of AH appearing in absorption site; F_{H1}, fraction of X_a that enters compartment #1 intact escaping first-pass elimination; F_{H2}, fraction of the generated metabolite HH that enters compartment #2 escaping sequential biotransformation. In rabbits, CL_{21} is exclusive because no reverse metabolism was recognized.

The symbol X_a indicates the amount of drug AH (D) at the absorption site following ipl administration of AH, and F_{ipl} is the fraction of dose of AH that appears in the absorption site. A certain fraction of X_a enters through the tissue compartment (such as mesentery and liver) into the measurable compartment #1 intact (F_{H1}), escaping first-pass elimination. The symbol k indicates the first-order rate constant associated with the drug absorption process. A part of the remaining fraction $(1-F_{H1})$ of X_a enters the systemic circulation (compartment #2) as the metabolite HH (M). The symbol F_{H2} indicates the fraction of the generated metabolite HH which enters compartment #2, escaping sequential hepatic first-pass elimination. When F_{H2} is unity, the drug and/or generated metabolite may not undergo sequential biotransformation to other metabolites or biliary secretion followed by fecal excretion. CL_{12} and CL_{21} are the clearance rates for the metabolic interconversion, and CL_{10} and CL_{20} are those for the irreversible loss of drug and metabolite, respectively. In this system, first-order linear differential equations describing the rates of change across the compartments for the drug and metabolite can be written as follows:

$$dX_a/dt = -[F_{H1} + (1-F_{H1})F_{H2} + (1-F_{H1})(1-F_{H2})]kX_a = -kX_a \quad (17)$$

$$dX_1/dt = F_{H1}kX_a - (CL_{10} + CL_{12})X_1/V_1 + CL_{21}X_2/V_2 \quad (18)$$

$$dX_2/dt = (1-F_{H1})F_{H2}kX_a + CL_{12}X_1/V_1 - (CL_{20} + CL_{21})X_2/V_2 \quad (19)$$

When the Laplace transform[7] of the plasma concentration–time equation is given by Eq. 20, those of the drug and its metabolite after ipl administration of the drug, for instance, are given by Eqs. 21 and 22, respectively.

$$\tilde{C}\left[\tilde{C}(s) = \int_0^{\infty} e^{-st} C(t)\,dt\right] \quad (20)$$

$$\tilde{C}_{\text{D}}^{\text{D}_{\text{ipl}}}(s) = \frac{F_{\text{ipl}}\,\text{dose}^{\text{D}_{\text{ipl}}}}{V_1} \times \frac{k\left[F_{\text{H1}}\left(s + \frac{CL_{20}+CL_{21}}{V_2}\right) + (1-F_{\text{H1}})F_{\text{H2}}\frac{CL_{21}}{V_2}\right]}{(s+k)\left[\left(s + \frac{CL_{10}+CL_{12}}{V_1}\right)\left(s + \frac{CL_{20}+CL_{21}}{V_2}\right) - \frac{CL_{12}CL_{21}}{V_1V_2}\right]} \quad (21)$$

$$\tilde{C}_{\text{M}}^{\text{D}_{\text{ipl}}}(s) = \frac{F_{\text{ipl}}\,\text{dose}^{\text{D}_{\text{ipl}}}}{V_2} \times \frac{k\left[F_{\text{H1}}\frac{CL_{12}}{V_1} + (1-F_{\text{H1}})F_{\text{H2}}\left(s + \frac{CL_{10}+CL_{12}}{V_1}\right)\right]}{(s+k)\left[\left(s + \frac{CL_{10}+CL_{12}}{V_1}\right)\left(s + \frac{CL_{20}+CL_{21}}{V_2}\right) - \frac{CL_{12}CL_{21}}{V_1V_2}\right]} \quad (22)$$

where s is the Laplace variable with respect to time, t.

AUC values are given[8] by

$$AUC_{\text{D}}^{\text{D}_{\text{ipl}}} = \lim_{s\to 0} \tilde{C}_{\text{D}}^{\text{D}_{\text{ipl}}}(s) = \frac{F_{\text{ipl}}\,\text{dose}^{\text{D}_{\text{ipl}}}[F_{\text{H1}}(CL_{20}+CL_{21}) + (1-F_{\text{H1}})F_{\text{H2}}CL_{21}]}{CL_{10}CL_{20} + CL_{10}CL_{21} + CL_{12}CL_{20}} \quad (23)$$

$$AUC_{\text{M}}^{\text{D}_{\text{ipl}}} = \lim_{s\to 0} \tilde{C}_{\text{M}}^{\text{D}_{\text{ipl}}}(s) = \frac{F_{\text{ipl}}\,\text{dose}^{\text{D}_{\text{ipl}}}[F_{\text{H1}}CL_{12} + (1-F_{\text{H1}})F_{\text{H2}}(CL_{10}+CL_{12})]}{CL_{10}CL_{20} + CL_{10}CL_{21} + CL_{12}CL_{20}} \quad (24)$$

The general solutions for the AUC under different administration routes of the drug and preformed metabolite are summarized in Table I (Eqs. 23—28).

Using the AUC values given in Table I, the systemic available fractions[6] are set by Eq. 29 for the drug, and by Eq. 30 for its relevant metabolite.

TABLE I. *AUC*'s Derived from the Moment of Plasma Concentration *vs.* Time Curve of Drug and Its Metabolite for Different Routes of Administration

Agent	Route of administration	*AUC*[a]	Eq.
Drug	Intraperitoneal	$AUC_D^{D_{ipl}}=F_{ipl}\text{dose}^{D_{ipl}}[F_{H1}(CL_{20}+CL_{21})+(1-F_{H1})F_{H2}CL_{21}]/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(23)
		$AUC_M^{D_{ipl}}=F_{ipl}\text{dose}^{D_{ipl}}[F_{H1}CL_{12}+(1-F_{H1})F_{H2}(CL_{10}+CL_{12})]/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(24)
	Intravenous	$AUC_D^{D_{iv}}=\text{dose}^{D_{iv}}(CL_{20}+CL_{21})/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(25)
		$AUC_M^{D_{iv}}=\text{dose}^{D_{iv}}CL_{12}/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(26)
Preformed metabolite	Intravenous	$AUC_M^{M_{iv}}=\text{dose}^{M_{iv}}(CL_{10}+CL_{12})/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(27)
		$AUC_D^{M_{iv}}=\text{dose}^{M_{iv}}CL_{21}/(CL_{10}CL_{20}+CL_{10}CL_{21}+CL_{12}CL_{20})$	(28)

a) Superscript indicates the route of administration of the drug (D) and performed metabolite (M).

$$F_d=\frac{AUC_D^{D_{ipl}}/F_{ipl}\,\text{dose}^{D_{ipl}}}{AUC_D^{D_{iv}}/\text{dose}^{D_{iv}}}$$

$$=F_{H1}+(1-F_{H1})F_{H2}(AUC_D^{M_{iv}}/\text{dose}^{M_{iv}})/(AUC_D^{D_{iv}}/\text{dose}^{D_{iv}}) \quad (29)$$

$$F_m=\frac{AUC_M^{D_{ipl}}/F_{ipl}\,\text{dose}^{D_{ipl}}}{AUC_M^{D_{iv}}/\text{dose}^{D_{iv}}}$$

$$=F_{H1}+(1-F_{H1})F_{H2}(AUC_M^{M_{iv}}/\text{dose}^{M_{iv}})/(AUC_M^{D_{iv}}/\text{dose}^{D_{iv}}) \quad (30)$$

Then, the available fraction as the sum of both redox forms is given by Eq. 31.

$$F=\frac{(CL_{10}AUC_D^{D_{ipl}}+CL_{20}AUC_M^{D_{ipl}})/\text{dose}^{D_{ipl}}}{(CL_{10}AUC_D^{D_{iv}}+CL_{20}AUC_M^{D_{iv}})/\text{dose}^{D_{iv}}}$$

$$=F_{ipl}[1-(1-F_{H1})(1-F_{H2})] \quad (31)$$

Setting F_{H2} as unity,

$$F=F_{ipl} \quad (32)$$

If drug metabolism occurs in the absence of a reversible process, one can set the area $AUC_D^{M_{iv}}$ and the opposite clearance CL_{21} at zero in Eqs. 3—6 and Eqs. 23—29.

Results and Discussion

Plasma Concentrations of AH and HH after iv and ipl Administrations of Agents in Laboratory Animals Semilogarithmic plots of the mean plasma concentration *vs.* time of AH and HH after bolus iv and ipl administrations of AH or HH in rats, guinea pigs and rabbits are illustrated in Figs. 1, 2 and 3, respectively. The pharmacokinetic parameters obtained are summarized in Table II.

After iv administration of AH, the disappearance of AH from the systemic circulation is at least biphasic, as shown in Figs. 1A, 2A and 3A, and a major characteristic of the reductive metabolite HH generated from AH in each

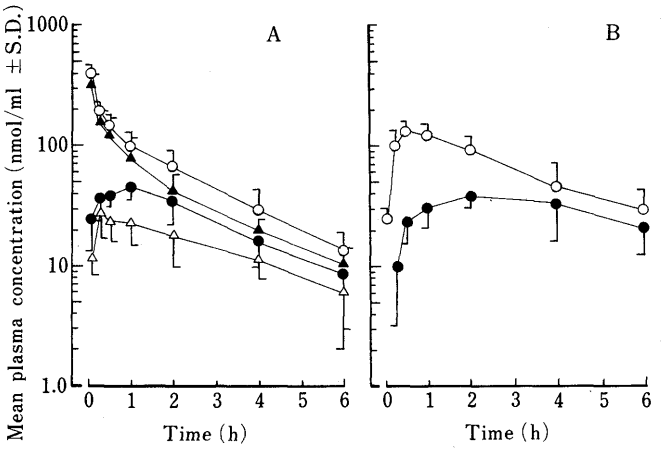

Fig. 1. Mean Plasma Concentration *vs.* Time Courses of Each Redox Form after Intravenous and Intraperitoneal Administrations of Acetohexamide AH or Hydroxyhexamide HH in Rats

A: ○, AH following iv dosing of 0.046 mmol/kg of AH ($n=5$); ●, metabolite HH generated from AH; ▲, HH following iv dosing of 0.046 mmol/kg of HH ($n=5$); △, metabolite AH generated from HH. B: ○, AH following ipl dosing of 0.046 mmol/kg of AH ($n=4$); ●, metabolite HH generated from AH.

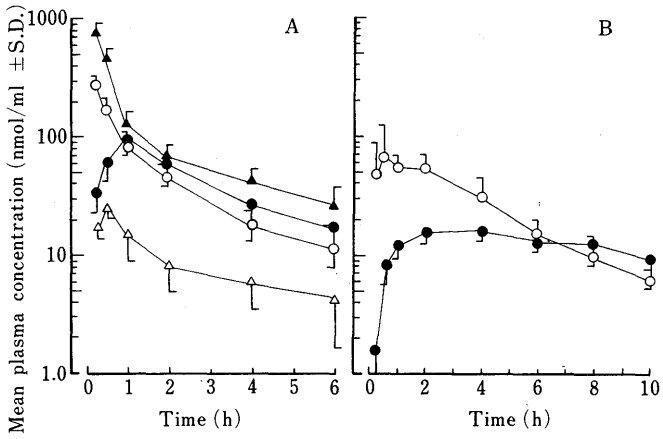

Fig. 2. Mean Plasma Concentration *vs.* Time Courses of Each Redox Form after Intravenous and Intraperitoneal Administrations of Acetohexamide AH or Hydroxyhexamide HH in Guinea Pigs

A: ○, AH following iv dosing of 0.046 mmol/kg of AH ($n=3$); ●, metabolite HH generated from AH; ▲, HH following iv dosing of 0.046 mmol/kg of HH ($n=3$); △, metabolite AH generated from HH. B: ○, AH following ipl dosing of 0.139 mmol/kg of AH ($n=3$); ●, metabolite HH generated from AH.

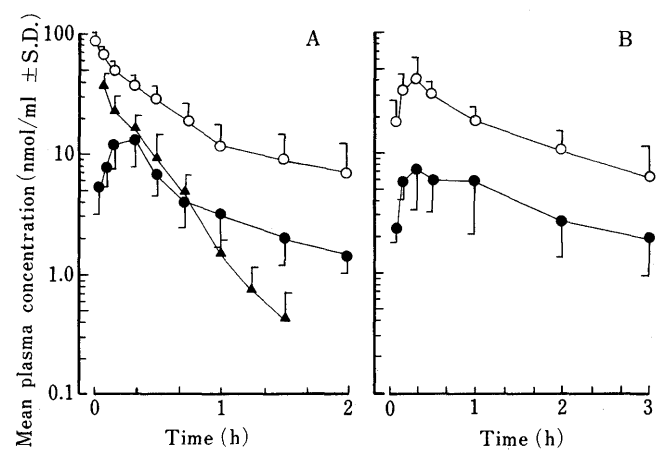

Fig. 3. Mean Plasma Concentration *vs.* Time Courses of Acetohexamide AH and Hydroxyhexamide HH after Intravenous and Intraperitoneal Administrations of AH or Preformed HH in Rabbits

A: ○, AH following iv dosing of 0.041 mmol/kg of AH ($n=4$); ●, metabolite HH generated from AH; ▲, HH following iv dosing of 0.041 mmol/kg of HH ($n=3$). B: ○, AH following ipl dosing of 0.041 mmol/kg of AH ($n=2$); ●, metabolite HH generated from AH.

TABLE II. Pharmacokinetic Parameters[a] Calculated by the Moment Method after Administration of Acetohexamide (AH) and Hydroxyhexamide (HH) in Laboratory Animals

Animal species	Administration site		Intravenous (iv)		Intraperitoneal (ipl)		
	Compound (dose, mmol/kg) (experimental No.)						
Rat	AH	λ_D	(h^{-1})	0.377±0.142	AH	λ_D	0.361±0.068
	(0.046)	$AUC_D^{D_{iv}}$	(μmol·h/ml)	0.499±0.222	(0.046)	$AUC_D^{D_{ipl}}$	0.452±0.115
	(n=5)	$MRT_D^{D_{iv}}$	(h)	2.58±1.51	(n=4)	$MRT_D^{D_{ipl}}$	2.93±0.81
		$CL_{app,D}$[b]	(l/h·kg)	0.104±0.036			
		λ_M	(h^{-1})	0.318±0.065		λ_M	0.291±0.048
		$AUC_M^{D_{iv}}$	(μmol·h/ml)	0.218±0.045		$AUC_M^{D_{ipl}}$	0.233±0.063
		$MRT_M^{D_{iv}}$	(h)	3.36±0.87		$MRT_M^{D_{ipl}}$	4.59±0.45
	HH	λ_M	(h^{-1})	0.436±0.275			
	(0.046)	$AUC_M^{M_{iv}}$	(μmol·h/ml)	0.335±0.107			
	(n=5)	$MRT_M^{M_{iv}}$	(h)	2.23±1.04			
		$CL_{app,M}$	(l/h·kg)	0.150±0.054			
		λ_D	(h^{-1})	0.263±0.051			
		$AUC_D^{M_{iv}}$	(μmol·h/ml)	0.121±0.050			
		$MRT_D^{M_{iv}}$	(h)	3.91±0.81			
Guinea pig	AH	λ_D	(h^{-1})	0.222±0.011	AH	λ_D	0.221±0.021
	(0.046)	$AUC_D^{D_{iv}}$	(μmol·h/ml)	0.373±0.120	(0.139)	$AUC_D^{D_{ipl}}$	0.300±0.049
	(n=3)	$MRT_D^{D_{iv}}$	(h)	2.81±0.33	(n=3)	$MRT_D^{D_{ipl}}$	4.11±0.68
		$CL_{app,D}$	(l/h·kg)	0.120±0.013			
		λ_M	(h^{-1})	0.226±0.013		λ_M	0.123±0.042
		$AUC_M^{D_{iv}}$	(μmol·h/ml)	0.351±0.018		$AUC_M^{D_{ipl}}$	0.297±0.086
		$MRT_M^{D_{iv}}$	(h)	4.06±0.27		$MRT_M^{D_{ipl}}$	17.4±7.1
	HH	λ_M	(h^{-1})	0.220±0.001			
	(0.046)	$AUC_M^{M_{iv}}$	(μmol·h/ml)	0.845±0.064			
	(n=3)	$MRT_M^{M_{iv}}$	(h)	2.80±0.29			
		$CL_{app,M}$	(l/h·kg)	0.055±0.008			
		λ_D	(h^{-1})	0.150±0.002			
		$AUC_D^{M_{iv}}$	(μmol·h/ml)	0.084±0.006			
		$MRT_D^{M_{iv}}$	(h)	5.92±0.27			
Rabbit	AH	λ_D	(h^{-1})	0.649±0.019	AH	λ_D	0.801±0.006
	(0.041)	$AUC_D^{D_{iv}}$	(μmol·h/ml)	0.063±0.022	(0.041)	$AUC_D^{D_{ipl}}$	0.058±0.003
	(n=4)	$MRT_D^{D_{iv}}$	(h)	1.19±0.20	(n=2)	$MRT_D^{D_{ipl}}$	1.35±0.004
		$CL_{app,D}$	(l/h·kg)	0.699±0.205			
		$X_{ren,D}$[c]	(μmol/kg)	1.47±0.30	(3.6%)		
		λ_M	(h^{-1})	0.973±0.705		λ_M	0.525±0.109
		$AUC_M^{D_{iv}}$	(μmol·h/ml)	0.0126±0.0062		$AUC_M^{D_{ipl}}$	0.0131±0.0089
		$MRT_M^{D_{iv}}$	(h)	1.21±0.59		$MRT_M^{D_{ipl}}$	2.05±0.49
		$X_{ren,M}$	(μmol/kg)	3.48±0.95	(8.5%)		
	HH	λ_M	(h^{-1})	2.65±0.92			
	(0.041)	$AUC_M^{M_{iv}}$	(μmol·h/ml)	0.0142±0.0018			
	(n=3)	$MRT_M^{M_{iv}}$	(h)	0.353±0.004			
		$CL_{app,M}$	(l/h·kg)	2.92±0.38			
		$X_{ren,M}$	(μmol/kg)	6.09±2.93	(14.9%)		

a) $AUC=\int_0^\infty C\,dt+C_T/\lambda$, and $MRT=(\int_0^\infty tC\,dt+TC_T/\lambda+C_T/\lambda^2)/AUC$, where λ is the terminal elimination slope. *b*) Plasma clearance: $CL_{app}={}_{iv}\text{dose}/AUC$. *c*) X_{ren} indicates the cumulative amounts of AH (D) or HH (M) excreted into urine during 48 h after dosing of AH or HH. Percent of drug dose excreted into urine is parenthesized.

species is the nearly parallel decline in the terminal AH concentration *vs.* time curves. The same characteristic is observed in the terminal curves of AH and its metabolite HH following ipl dosing of AH (Figs. 1B, 2B and 3B). The metabolite HH generated from AH in guinea pigs yields a higher plasma concentration than that of the parent drug with passage of time. A similar effect was recognized after oral administration of AH to humans.[9]

After iv administration of preformed metabolite HH, on the other hand, the disappearance of HH in plasma is biphasic with a similar decline to that of AH in two species, but not the rabbit. In rabbits (Fig. 3A), its plasma concentration following iv dosing of HH decreases rapidly with a monophasic decline despite the fact that the metabolite HH generated following iv dosing of AH shows a similar terminal slope to that of parent drug AH. Besides, no appearance of oxidized reversible metabolite AH being recognized, HH disappearance proceeds in the absence of the reversible metabolic process in rabbits. This conclusion is supported by the fact that the oxidized metabolite AH is not detectable in urine excreted following iv dosing of HH in rabbits.

The MRT of a drug is a hybrid parameter which is dependent on both CL_{app} and $V_{ss,app}$, and yet represents the time for 63.2% of an iv dose to be eliminated.[4b] The rate limitation in metabolite kinetics involving no reversible metabolic process depends on the inter-relationship between the clearance and volume terms for both drug and metabolite. This can be appreciated by considering the ratio of MRT_M to MRT_D by

TABLE III. Comparison of Parameters[a] Characterizing the Disposition of Acetohexamide (AH) and Hydroxyhexamide (HH) in Laboratory Animals

Parameter	Rat		Guinea pig		Rabbit	
	AH	HH	AH	HH	AH	HH
CL_{10} (l/h·kg)	0.0431		0.0795		0.0732	
CL_{12} (l/h·kg)	0.0712		0.0565		0.577	
CL_{20} (l/h·kg)	0.124		0.0465		2.89	
CL_{21} (l/h·kg)	0.0395		0.0135		—	
Apparent clearance CL_{app} (l/h·kg)	0.0922	0.137	0.123	0.0544	0.650	2.89
Real clearance CL_{real} (l/h·kg)	0.114	0.163	0.136	0.0601	0.650	2.89
Apparent distribution volume at steady-state $V_{ss,app}$ (l/kg)	0.238	0.306	0.347	0.152	0.773	1.019
Real distribution volume at steady-state $V_{ss,real}$ (l/kg)	0.211	0.229	0.317	0.139	0.773	1.019
Mean residence time MRT (h)	2.58	2.23	2.81	2.80	1.19	0.353
Sojourn time $\bar{S}$ (h)	1.84	1.40	2.33	2.32	—	—

a) See text for definition of symbols.

$$\frac{MRT_M^{Miv}}{MRT_D^{Div}}=\frac{V_{ss,app,M}CL_{app,D}}{CL_{app,M}V_{ss,app,D}} \quad (33)$$

Substituting each MRT for rabbits given in Table II into Eq. 33, the MRT ratio is obtained as 0.297, less than unity. Therefore, the formation rate of the metabolite is the limiting factor in the kinetics in rabbits.

Acetohexamide-Hydroxyhexamide Disposition in Laboratory Animals Pharmacokinetic parameters for each redox form in laboratory animals calculated from traditional mammillary moment analysis and interconversion moment analysis are presented in Table III.

The irreversible clearance process of metabolite CL_{20} is larger than that of AH (CL_{10}) at 0.124 l/h·kg for rats and 2.89 l/h·kg for rabbits, as reflected in their apparent plasma clearances. In guinea pigs, CL_{20} is conversely smaller than CL_{10} at 0.0465 l/h·kg. The metabolic process CL_{12} operating on HH is greater than the back conversion process CL_{21} in the rat and guinea pig.

Apparent systematic clearance represents the total nonreversible eliminating activity, which is always less than the total metabolic turnover of the interconversion system. Therfore, the apparent clearance of each redox form is smaller than the real clearance by about 10—20% in rats and guinea pigs.

Comparing the volumes of distribution obtained from mammillary analysis with the real volumes of distribution for interconverting compounds in rats and guinea pigs, the real V_{ss} is smaller than the apparent V_{ss}.

Sojourn time is defined in the interconversion system to represent the average length of time that a molecule of a compound exists in the system before being either irreversible removed or converted to its metabolic pair. The metabolic turnover of the parent or metabolite is well reflected by the respective sojourn times. It has been reported[5] that the residence time of the compound is given by adding these sojourn times together after weighting them by the fraction of the dose undergoing N cycles through the whole interconversion system.[10] Consequently, MRT's are larger than the respective sojourn times of the redox form in rats and guinea pigs, as shown in Table III. In guinea pigs, the sojourn times, $\bar{S}_D$ and $\bar{S}_M$, of the redox forms are in good accordance at 2.3 h.

Estimation of the Available Fraction The available fractions calculated mathematically using Eqs. 29—32 are presented in Table IV. The F values are almost unity in rats and rabbits, and 0.273 in guinea pigs. On the assumption that the sequential first-pass effect of metabolite HH may be small enough to be negligible in these experimental animals, F_{ipl} is equal to F. Then, the available fraction F_{H1} of AH and sequential available fraction F_{H2} of its metabolite HH are obtained by solving Eqs. 29 and 30 in rats and guinea pigs, and by solving Eqs. 30 and 31 in rabbits, simultaneously.

TABLE IV. Available Fractions Estimated by Using the AUC's and Clearance Values

Available fraction[a]	Rat	Guinea pig	Rabbit
F	0.997	0.273	1.0
F_{ipl} $(=F)$[b]	0.997	0.273	1.0
F_{H1}	0.88	0.98	0.69
F_{H2}	0.95	1.0	0.99
F_d	0.91	0.98	0.92
F_m	1.07	1.03	1.04
$F_{H2}\ \frac{(AUC_M^{Miv}/dose^{Miv})}{(AUC_M^{Div}/dose^{Div})}$	1.45	2.41	1.14

a) See text for definition of symbols. b) Determined by considering the sequential first-pass effect of metabolite HH to be negligible.

The F_{H2} values are nearly unity in all species in accordance with the assumption mentioned above. On the other hand, F_{H1} values are appreciably less than unity at 0.88 for rats, 0.98 for guinea pigs and 0.69 for rabbits.

It has been reported[11] that AH reduction to HH is catalyzed by ketone reductase, and AH reducing activity is observed in the cytosolic fractions of liver and kidney in rats and rabbits, and particularly of heart in rabbits. These facts imply that the appearance of the reduced metabolite HH in the plasma is caused not only by first-pass metabolism but also by biotransformation in the systemic circulation following ipl dosing of AH.

The AUC_M ratio of metabolite F_m cited above in Eq. 30 can be rearranged to Eq. 34.

$$F_m=\frac{AUC_M^{Dipl}/F_{ipl}\,dose^{Dipl}}{AUC_M^{Div}/dose^{Div}}$$

$$=1+(1-F_{H1})[F_{H2}(AUC_M^{Miv}/dose^{Miv})/(AUC_M^{Div}/dose^{Div})-1] \quad (34)$$

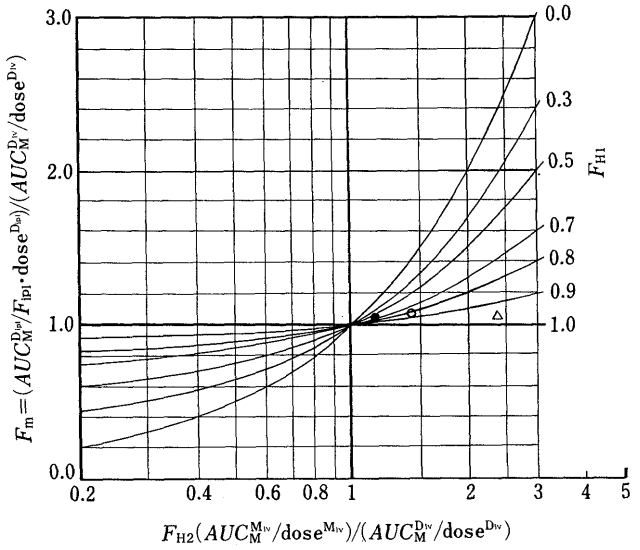

Fig. 4. Relationship among the Ratios of AUC obtained from Intravenous and Intraperitoneal Drug Administrations and the Available Fractions of Drug Dose

Equation (34) in the text was used to draw the curves. ○, rat; △, guinea pig; ●, rabbit.

If first-pass elimination of drug is negligible ($F_{H1}=1$) or the term $[F_{H2}(AUC_M^{M_{iv}}/dose^{M_{iv}})/(AUC_M^{D_{iv}}/dose^{D_{iv}})-1]$ in Eq. 34 is zero, the AUC_M ratio F_m becomes unity. On the contrary, when this term takes a positive value, the F_m value is larger than unity. Furthermore, the F_m value becomes less than unity for a negative value of this term.

Figure 4 illustrates the relationship between the ratios of AUC at various values of F_{H1} and F_{H2}. The experimental results in all species are plotted in Fig. 4.

Conclusion

An interconversion model which accounts for both systemic and first-pass formation of the metabolite HH generated from AH was employed in order to assess the contribution of the active metabolite following drug administration in three laboratory species.

1) Biotransformation of each redox form is reversible in rats and guinea pigs. However, the reductive biotransformation of AH to HH is irreversible and its rate is limited in rabbits.

2) In consequence of a first-pass elimination after ipl administration of AH, the F_{H1} values are 0.88 for rats and 0.69 for rabbits. Both F_{H1} and F_{H2} being close to unity in guinea pigs, the appearance of the reduced metabolite HH in the plasma seems to originate mainly from biotransformation in the systemic circulation.

3) The AUC for a metabolite is a useful parameter to help to evaluate the relative importance of the particular metabolite.

When the first-pass elimination of the drug and sequential first-pass elimination of its metabolite are appreciable, the magnitude of the AUC_M ratio of metabolite F_m is dependent upon not only the F_{H1} of the drug but also the F_{H2} of the generated metabolite, as is clear from Eq. 34. The illustration in Fig. 4 may be useful intuitively to evaluate the extent of availability of a metabolite generated following oral or ipl administration of prodrug.

References and Notes

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10) $$MRT_D^{D_{iv}}=\bar{S}_D+\sum_{N=1}^{\infty}\left[\left(\frac{CL_{12}CL_{21}}{(CL_{10}+CL_{12})(CL_{20}+CL_{21})}\right)^N(\bar{S}_D+\bar{S}_M)\right]$$
$$MRT_M^{M_{iv}}=\bar{S}_M+\sum_{N=1}^{\infty}\left[\left(\frac{CL_{12}CL_{21}}{(CL_{10}+CL_{12})(CL_{20}+CL_{21})}\right)^N(\bar{S}_D+\bar{S}_M)\right]$$
MRT is produced by adding the sojourn time of the interconversion system, where
$$\left(\frac{CL_{12}CL_{21}}{(CL_{10}+CL_{12})(CL_{20}+CL_{21})}\right)^N$$
is used as the weighting function.[5]
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