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Estimation of the Rates of Available Fraction for Some 4-Substituted Acetophenone Derivatives in the Rat: Reversible Drug-Metabolite Pharmacokinetics

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4-Acetamidoacetophenone (Ia), 4-acetylbenzenesulfonamide (IIa) and acetoexamide (IIIa) and the respective reduced compounds, 4-substituted α -hydroxyethylphenyl derivatives, are in a reversible drug-metabolite relationship in rats. The pharmacokinetic profiles of these agents were studied after intraportal (pv) administration in comparison with those after intravenous (iv) administration using an interconversion model. Fundamental clearances, CL_{10} , CL_{12} , CL_{20} and CL_{21} , were calculated using four AUC (area under the plasma concentration-time curve) values obtained after iv administration of the drug and preformed metabolite. The hepatic available fraction of parent drug, F_{H1} and sequential hepatic available fraction of its metabolite, F_{H2} were estimated by the following equations.

$$F_d = (AUC_{D^{pv}}/\text{dose}^{D^{pv}})/(AUC_{D^{iv}}/\text{dose}^{D^{iv}}) = F_{H1} + (1 - F_{H1})F_{H2}CL_{21}/(CL_{20} + CL_{21})$$

$$F_m = (AUC_{M^{pv}}/\text{dose}^{D^{pv}})/(AUC_{M^{iv}}/\text{dose}^{D^{iv}}) = F_{H1} + (1 - F_{H1})F_{H2}(CL_{10} + CL_{12})/CL_{12}$$

Keywords—metabolite pharmacokinetics; 4-substituted acetophenone; reversible metabolism; AUC ; fundamental clearance; portal vein administration; hepatic available fraction; interconversion model

It is important to evaluate the disposition and bioavailability of a drug generated by metabolism after the administration of a prodrug. If the species of interest undergoes reversible biotransformation, the usual concept of drug disposition is not entirely adequate.

Compounds such as sulindac,¹⁾ canrenone²⁾ and various corticosteroids and sex steroids^{3,4)} have metabolites that can revert in part to the parent drug. A complicating factor in pharmacokinetic investigation of these compounds is that reversible metabolism negates the traditional meanings of area under the plasma concentration-time curve (AUC), clearance, distribution volume and bioavailability when these pharmacokinetic parameters are calculated by classical methods.

Previously,⁵⁾ the authors reported that some 4-substituted α -hydroxyethylphenyl derivatives and the respective oxidized ketones including acetoexamide, an oral antidiabetic agent, were in a reversible drug-metabolite relationship in rats.

The purpose of the present paper is to present comprehensive equations which characterize the pharmacokinetics of a reversible drug-metabolite system using 4-acetamidoacetophenone (Ia), 4-acetylbenzenesulfonamide (IIa) and acetoexamide (IIIa), and their preformed reductive metabolites, 4-($\pm$)- α -hydroxyethylphenylacetamide (Ib), 4-($\pm$)- α -hydroxyethylphenylsulfonamide (IIb) and ($\pm$)-hydroxyhexamide (IIIb).

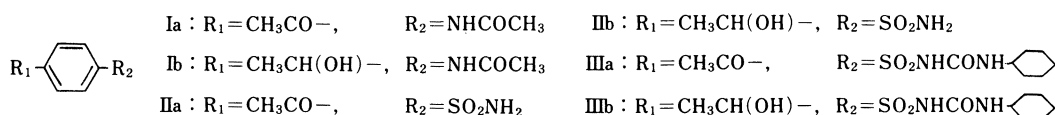


Chart 1

Experimental

Materials—Compound IIIa was of pharmaceutical grade, and IIa, IIb, IIIb and tolcyclamide (CY) were synthesized in this laboratory.⁵⁾ Compound Ia, 4-toluenesulfonamide (4-TS) and phenacetin (PH) were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Compound Ib was synthesized by treating Ia (10 mmol) in MeOH (30 ml) with NaBH₄ (15 mmol) in the same manner as described in the previous paper.⁵⁾ mp 68–71 °C (recrystallized from ether–petroleum ether). Other reagents were of special reagent grade.

Animal Experiments—A heparin-treated polyethylene cannula was surgically introduced into the jugular vein of anesthetized male Wistar rats (Shizuoka Laboratory Animal Center, Hamamatsu, Japan) weighing 250–380 g, to obtain blood samples. The animals (3–4 animals/group) were given agents intravenously (iv) or through the portal vein (pv) as follows: Ia and Ib (0.05 mmol/kg), IIa and IIb (0.1 mmol/kg), and IIIa and IIIb (0.031 mmol/kg). After dosing, blood samples were drawn at appropriate time intervals and then centrifuged to separate the plasma.

Sample Analysis—A sample of 200 μ l of plasma was shaken vigorously for 10 min with 2.0 ml of 0.1 M phosphate buffer (pH 5.0) and 5.0 ml of extractive solvent containing the internal standard (Table I), and then centrifuged for 20 min at 3000 rpm. A 4.0 ml aliquot of the separated organic phase was evaporated *in vacuo* at 40 °C, the residue was dissolved in 100 μ l of the mobile phase, and 20 μ l of this solution was injected into a high-performance liquid chromatography (HPLC) column. HPLC was carried out using a Shimadzu LC-6A apparatus equipped with a variable-wavelength photometric detector (Shimadzu SPD-6A) and reversed-phase column. Analytical conditions are summarized in Table I.

TABLE I. Chromatographic Conditions at Room Temperature

Sample	Ia and Ib	IIa and IIb	IIIa and IIIb
Extraction solvent	C ₂ H ₄ Cl ₂	C ₆ H ₆ :AcOEt (1:1)	C ₆ H ₆ :AcOEt (1:1)
Internal standard	PH	4-TS	CY
(in extraction solvent)	(0.5 μ g/5 ml)	(2.5 μ g/5 ml)	(10 μ g/5 ml)
Column	5 μ m Shim-Pack CLC-ODS (0.6 i.d. $\times$ 15 cm)	Cosmosil 5 C ₁₈ (0.46 i.d. $\times$ 15 cm)	Cosmosil 5 C ₁₈ (0.46 i.d. $\times$ 15 cm)
Mobile phase	MeOH–H ₂ O (6:4)	MeCN–0.1% AcOH (3:7)	MeCN–0.2% AcOH (1:1)
Flow rate (ml/min)	1.0	0.6	1.2
UV (wavelength, nm)	256	235	240
AUFS (abs)	0.04	0.064	0.064

UV, ultraviolet. AUFS, absorbance unit full scale.

Retention times were as follows: 4.0 min for Ia, 3.5 min for Ib, 3.8 min for IIa, 5.5 min for IIb, 3.6 min for IIIa and 5.2 min for IIIb. It was confirmed that the retention time of each compound formed as a metabolite coincided with that of the same compound used as a parent drug.

Pharmacokinetic Calculation—The AUC was calculated by applying the trapezoidal rule with extrapolation to infinity (Eq. 1). The terminal elimination rate constant (λ) was determined by least-squares linear regression of the logarithm of plasma concentration profiles.

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

Theoretical

An interconversion model which may be appropriate for the reversible drug–metabolite analysis is illustrated in Fig. 1.⁶⁾ This model assumes that both the parent drug and its relevant metabolite have linear and stationary dispositions and that elimination and interconversion processes of the drug and metabolite are restricted to their central compartments.

X_1 and X_2 are the amounts of drug in compartment #1 and of metabolite in compartment #2, respectively. The drug (D) may be administered intravenously (iv) into compartment #1 of distribution volume V_1 to give a concentration C_D . Alternatively, the drug may be

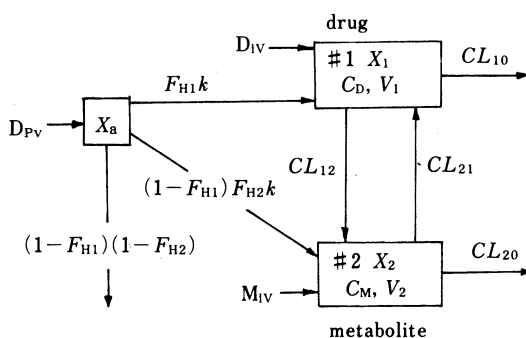


Fig. 1. Simple Interconversion Model with First-Order Input Step

administered through the portal vein (pv), where X_a is the amount of drug at the administered site, and k is the first-order rate constant describing the drug transfer. The metabolite (M) occupies a distribution volume V_2 at compartment #2 to give a concentration C_M . CL_{12} and CL_{21} are the clearance rates for the metabolic interconversion, and CL_{10} and CL_{20} are the clearance rates for the irreversible loss of drug and metabolite, respectively. If drug metabolism occurs in the absence of a reversible process, one can set the opposite clearance (CL_{21}) to be zero.

The fundamental clearances, CL_{10} , CL_{12} , CL_{20} and CL_{21} of the system⁷⁾ can be obtained using four AUC values produced after intravenous administration of doses of the drug and preformed metabolite as follows:

$$CL_{10} = \frac{AUC_M^{Miv} \text{dose}^{Div} - AUC_D^{Div} \text{dose}^{Miv}}{AUC_D^{Div} AUC_M^{Miv} - AUC_M^{Div} AUC_D^{Miv}} \quad (2)$$

$$CL_{12} = \frac{AUC_M^{Div} \text{dose}^{Miv}}{AUC_D^{Div} AUC_M^{Miv} - AUC_M^{Div} AUC_D^{Miv}} \quad (3)$$

$$CL_{20} = \frac{AUC_D^{Div} \text{dose}^{Miv} - AUC_M^{Miv} \text{dose}^{Div}}{AUC_D^{Div} AUC_M^{Miv} - AUC_M^{Div} AUC_D^{Miv}} \quad (4)$$

$$CL_{21} = \frac{AUC_D^{Miv} \text{dose}^{Div}}{AUC_D^{Div} AUC_M^{Miv} - AUC_M^{Div} AUC_D^{Miv}} \quad (5)$$

After an intraportal dose of drug (D_{pv}), a certain fraction of X_a enters compartment #1 intact (F_{H1}) escaping hepatic metabolism. A part of the remaining fraction $(1 - F_{H1})$ of X_a undergoes first-pass metabolism, entering the systemic circulation (compartment #2) as the metabolite. The F_{H2} is the fraction of the generated metabolite which enters compartment #2 escaping sequential hepatic biotransformation. In this system, first-order linear differential equations describing the rates of change across the compartment for the drug and metabolite can be written:

$$dX_a/dt = -[F_{H1} + (1 - F_{H1})F_{H2} + (1 - F_{H1})(1 - F_{H2})]kX_a = -kX_a \quad (6)$$

$$dX_1/dt = F_{H1}kX_a - (CL_{10} + CL_{12})X_1/V_1 + CL_{21}X_2/V_2 \quad (7)$$

$$dX_2/dt = (1 - F_{H1})F_{H2}kX_a + CL_{12}X_1/V_1 - (CL_{20} + CL_{21})X_2/V_2 \quad (8)$$

The Laplace transforms⁸⁾ of the plasma concentration–time equation of the drug $\tilde{C}_D(s)$ and its metabolite $\tilde{C}_M(s)$ after intraportal administration of the drug are given by Eqs. 9 and 10, respectively.

$$\tilde{C}_D^{D_{pv}}(s) = \frac{\text{dose}^{D_{pv}}}{V_1} \frac{k \left[F_{H1} \left(s + \frac{CL_{20} + CL_{21}}{V_2} \right) + (1 - F_{H1}) F_{H2} \frac{CL_{21}}{V_2} \right]}{(s+k) \left[\left(s + \frac{CL_{10} + CL_{12}}{V_1} \right) \left(s + \frac{CL_{20} + CL_{21}}{V_2} \right) - \frac{CL_{12} CL_{21}}{V_1 V_2} \right]} \quad (9)$$

$$\tilde{C}_M^{D_{pv}}(s) = \frac{\text{dose}^{D_{pv}}}{V_2} \frac{k \left[F_{H1} \frac{CL_{12}}{V_1} + (1 - F_{H1}) F_{H2} \left(s + \frac{CL_{10} + CL_{12}}{V_1} \right) \right]}{(s+k) \left[\left(s + \frac{CL_{10} + CL_{12}}{V_1} \right) \left(s + \frac{CL_{20} + CL_{21}}{V_2} \right) - \frac{CL_{12} CL_{21}}{V_1 V_2} \right]} \quad (10)$$

where s is the Laplace parameter. AUC values are given as $AUC = \lim_{s \rightarrow 0} \tilde{C}(s)$.⁹⁾ Therefore,

$$AUC_D^{D_{pv}} = \frac{\text{dose}^{D_{pv}} [F_{H1}(CL_{20} + CL_{21}) + (1 - F_{H1}) F_{H2} CL_{21}]}{CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20}} \quad (11)$$

$$AUC_M^{D_{pv}} = \frac{\text{dose}^{D_{pv}} [F_{H1} CL_{12} + (1 - F_{H1}) F_{H2} (CL_{10} + CL_{12})]}{CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20}} \quad (12)$$

The general solutions for the AUC 's under different administration routes of drug and preformed metabolite are summarized in Table II (Eqs. 11–16).

TABLE II. Areas under the Curve of Drug and Its Metabolite after Intravenous and Portal Vein Administrations

Compound	Route of administration	AUC^a	Eq.
Drug	Intraportal	$AUC_D^{D_{pv}} = \text{dose}^{D_{pv}} [F_{H1}(CL_{20} + CL_{21}) + (1 - F_{H1}) F_{H2} CL_{21}] / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(11)
		$AUC_M^{D_{pv}} = \text{dose}^{D_{pv}} [F_{H1} CL_{12} + (1 - F_{H1}) F_{H2} (CL_{10} + CL_{12})] / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(12)
	Intravenous	$AUC_D^{D_{iv}} = \text{dose}^{D_{iv}} (CL_{20} + CL_{21}) / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(13)
		$AUC_M^{D_{iv}} = \text{dose}^{D_{iv}} CL_{12} / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(14)
Preformed metabolite	Intravenous	$AUC_M^{M_{iv}} = \text{dose}^{M_{iv}} (CL_{10} + CL_{12}) / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(15)
		$AUC_D^{M_{iv}} = \text{dose}^{M_{iv}} CL_{21} / (CL_{10} CL_{20} + CL_{10} CL_{21} + CL_{12} CL_{20})$	(16)

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

The rates of systemic available fraction may be defined by Eq. 17, dividing Eq. 11 by Eq. 13, for the drug, and by Eq. 18, dividing Eq. 12 by Eq. 14, for the generated metabolite:

$$F_d = \frac{AUC_D^{D_{pv}} / \text{dose}^{D_{pv}}}{AUC_D^{D_{iv}} / \text{dose}^{D_{iv}}} = F_{H1} + (1 - F_{H1}) F_{H2} CL_{21} / (CL_{20} + CL_{21})$$

$$= 1 - (1 - F_{H1}) [1 - F_{H2} CL_{21} / (CL_{20} + CL_{21})] \quad (17)$$

$$F_m = \frac{AUC_M^{D_{pv}} / \text{dose}^{D_{pv}}}{AUC_M^{D_{iv}} / \text{dose}^{D_{iv}}} = F_{H1} + (1 - F_{H1}) F_{H2} (CL_{10} + CL_{12}) / CL_{12}$$

$$= 1 + (1 - F_{H1}) [F_{H2} (CL_{10} + CL_{12}) / CL_{12} - 1] \quad (18)$$

The relationship described in Eq. 18 may be applicable to a drug-metabolite system in the absence of reversible metabolism.¹⁰⁾

The rate of available fraction as the sum of the drug and its relevant metabolite is given by Eq. 19.^{6b)}

$$F = \frac{(CL_{10}AUC_D^{D_{pv}} + CL_{20}AUC_M^{D_{pv}})/\text{dose}^{D_{pv}}}{(CL_{10}AUC_D^{D_{iv}} + CL_{20}AUC_M^{D_{iv}})/\text{dose}^{D_{iv}}} = 1 - (1 - F_{H1})(1 - F_{H2}) \quad (19)$$

The fraction, $CL_{21}/(CL_{20} + CL_{21})$ for the first-time conversion of metabolite to parent drug is a fundamental parameter of the interconversion system,⁷⁾ and may be given by Eq. 20, obtained by dividing Eq. 16 by Eq. 13.

$$f_d = \frac{(AUC_D^{M_{iv}}/\text{dose}^{M_{iv}})}{(AUC_D^{D_{iv}}/\text{dose}^{D_{iv}})} = CL_{21}/(CL_{20} + CL_{21}) \quad (20)$$

Similarly, $CL_{12}/(CL_{10} + CL_{12})$ for the first-time conversion fraction of drug to metabolite may be given by Eq. 21, obtained by dividing Eq. 14 by Eq. 15.

$$f_m = \frac{(AUC_M^{D_{iv}}/\text{dose}^{D_{iv}})}{(AUC_M^{M_{iv}}/\text{dose}^{M_{iv}})} = CL_{12}/(CL_{10} + CL_{12}) \quad (21)$$

The hepatic available fraction, F_{H1} and sequential hepatic available fraction, F_{H2} are obtained by solving the simultaneous Eqs. 17 and 18.

Results and Discussion

Plasma Concentrations of Drugs and Their Metabolites after Intravenous and Intraportal Administration

Figure 2 shows semilogarithmic plots of the mean plasma concentration vs. time after administration of the agents in rats. That is: the three figures (IA, IIA and IIIA) on the left side indicate, respectively, the plots for drugs and their reversible metabolites after intravenous administrations at 0.05 mmol/kg dose level of Ia, 0.1 mmol/kg dose level of IIA and 0.031 mmol/kg dose level of IIIA, the central three figures (IB, IIB and IIIB) indicate those after intravenous administrations at 0.05 mmol/kg dose level of Ib, 0.1 mmol/kg dose level of IIB and 0.031 mmol/kg dose level of IIIB, and the three figures (IC, IIC and IIIC) on the right side of Fig. 2 indicate those after administrations through the portal vein at the 0.05 mmol/kg dose

TABLE III. Terminal Slopes and Areas Obtained by Administrations of Drug and Preformed Metabolite in Rats^{a)}

Compound (Dose, mmol/kg)	Administered site			
	Intravenous (iv)		Intraportal (pv)	
	Terminal slope (h ⁻¹)	AUC ^{b)} (μmol·h/ml)	Terminal slope (h ⁻¹)	AUC (μmol·h/ml)
Ia	λ _D 0.435 ± 0.120	AUC _D ^{D_{iv}} 0.201 ± 0.002	λ _D 0.433 ± 0.093	AUC _D ^{D_{pv}} 0.151 ± 0.047
(0.05)	λ _M 0.325 ± 0.086	AUC _M ^{D_{iv}} 0.084 ± 0.020	λ _M 0.236 ± 0.055	AUC _M ^{D_{pv}} 0.074 ± 0.020
Ib	λ _M 0.411 ± 0.012	AUC _M ^{M_{iv}} 0.153 ± 0.005		
(0.05)	λ _D 0.354 ± 0.022	AUC _D ^{M_{iv}} 0.069 ± 0.012		
IIa	λ _D 0.457 ± 0.048	AUC _D ^{D_{iv}} 0.092 ± 0.004	λ _D 0.555 ± 0.258	AUC _D ^{D_{pv}} 0.074 ± 0.033
(0.1)	λ _M 0.303 ± 0.047	AUC _M ^{D_{iv}} 0.176 ± 0.022	λ _M 0.453 ± 0.357	AUC _M ^{D_{pv}} 0.203 ± 0.081
IIB	λ _M 0.450 ± 0.057	AUC _M ^{M_{iv}} 0.316 ± 0.016		
(0.1)	λ _D 0.382 ± 0.026	AUC _D ^{M_{iv}} 0.030 ± 0.001		
IIIa	λ _D 0.286 ± 0.042	AUC _D ^{D_{iv}} 0.382 ± 0.112	λ _D 0.354 ± 0.147	AUC _D ^{D_{pv}} 0.307 ± 0.028
(0.031)	λ _M 0.340 ± 0.149	AUC _M ^{D_{iv}} 0.148 ± 0.099	λ _M 0.257 ± 0.040	AUC _M ^{D_{pv}} 0.157 ± 0.041
IIIB	λ _M 0.366 ± 0.046	AUC _M ^{M_{iv}} 0.203 ± 0.040		
(0.031)	λ _D 0.275 ± 0.266	AUC _D ^{M_{iv}} 0.068 ± 0.054		

a) Each value is the average ± S.D. of 3–4 experiments. b) Calculated by use of the trapezoidal rule.

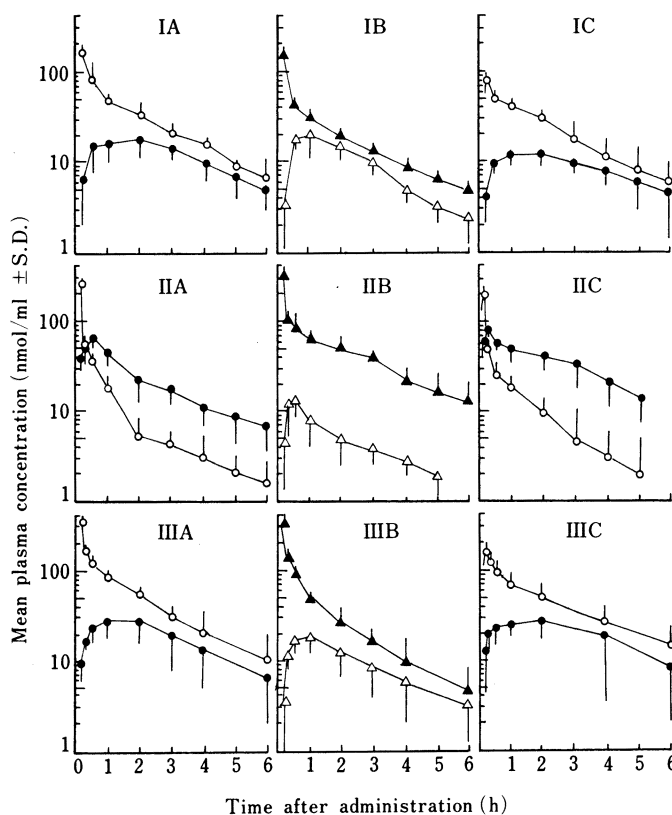


Fig. 2. Mean Plasma Concentration vs. Time Courses Produced after Administration of Drug and Preformed Metabolite in Rats

Points are given as the average $\pm$ S.D. of 3–4 experiments.

IA: $\circ$, iv administration of 0.05 mmol/kg dose of Ia; $\bullet$, metabolite, Ib generated from Ia.
 IB: $\blacktriangle$, iv administration of 0.05 mmol/kg dose of Ib; $\triangle$, metabolite, Ia generated from Ib.
 IC: $\circ$, pv administration of 0.05 mmol/kg dose of Ia; $\bullet$, metabolite, Ib generated from Ia.
 IIA: $\circ$, iv administration of 0.1 mmol/kg dose of IIa; $\bullet$, metabolite, IIb generated from IIa.
 IIB: $\blacktriangle$, iv administration of 0.1 mmol/kg dose of IIb; $\triangle$, metabolite, IIa generated from IIb.
 IIC: $\circ$, pv administration of 0.1 mmol/kg dose of IIa; $\bullet$, metabolite, IIb generated from IIa.

IIIA: $\circ$, iv administration of 0.031 mmol/kg dose of IIIa; $\bullet$, metabolite, IIb generated from IIIa.
 IIIB: $\blacktriangle$, iv administration of 0.031 mmol/kg dose of IIb; $\triangle$, metabolite, IIIa generated from IIb.
 IIIC: $\circ$, pv administration of 0.031 mmol/kg dose of IIIa; $\bullet$, metabolite, IIb generated from IIIa.

TABLE IV. Fundamental Clearances^{a)} (l/h per kg)

Paired agent	Ia–Ib	IIa–IIb	IIIA–IIIB
CL_{10}	0.138	0.588	0.025
CL_{12}	0.168	0.740	0.068
CL_{20}	0.264	0.261	0.144
CL_{21}	0.138	0.126	0.031

a) Calculated from Eqs. 2–5.

level of Ia, 0.1 mmol/kg dose level of IIa and 0.031 mmol/kg dose level of IIIa. It is noteworthy that the respective plasma concentration–time curves of these agents decline at least in a biphasic manner. The terminal slopes and AUC's obtained are summarized in Table III.

TABLE V. Rates of Available Fraction Estimated from the Pharmacokinetic Parameters, *AUC* and Fundamental Clearance

Agent	Ia	IIa	IIIa
F_d	0.75	0.80	0.80
F_m	0.88	1.15	1.06
F_{H1}	0.72	0.73	0.77
F_{H2}	0.32	0.87	0.92
$F^a)$	0.81	0.96	0.98

a) The rate of available fraction as the sum of drug and its metabolite.

Fundamental Clearances

The fundamental clearances calculated from Eqs. 2, 3, 4 and 5 mentioned above are summarized in Table IV. For the paired agents, Ia–Ib, the irreversible clearance process for the parent drug, CL_{10} , and the back conversion process, CL_{21} , are similar with values of 0.138 l/h per kg. The metabolic process, CL_{12} , operating on Ib is larger than CL_{21} , and the irreversible process, CL_{20} is 1.5 times larger than CL_{12} with a value of 0.265 l/h per kg. An analogous situation may occur in the paired agents, IIIa–IIIb. In contrast, it is noticeable that the irreversible process, CL_{10} is 2 times larger than CL_{20} for the metabolite, with a value of 0.588 l/h per kg, in the case of the paired agents IIa–IIb.

Assessment of the Rate of Available Fraction

The comparison of *AUC*'s obtained after administration of the agents by different routes is a common method to assess bioavailability on the assumption that plasma clearances remain unchanged between treatments. The results of mathematical solution for the rates of available fraction using Eqs. 17–19 are presented in Table V.

When the term $[F_{H2}(CL_{10} + CL_{12})/CL_{12} - 1]$ in Eq. 18 mentioned above takes a positive value, F_m is larger than unity. The agents IIa and IIIa fall into this category (Table V). The F values for IIa and IIIa seem to be nearer to unity. Nevertheless, the sequential available fractions, F_{H2} are appreciably less than unity at 0.87 for IIa and 0.92 for IIIa.

In the case of Ia, the F_{H1} , F_{H2} and F values are 0.72, 0.32 and 0.81, respectively, and the value of the term $[F_{H2}(CL_{10} + CL_{12})/CL_{12} - 1]$ is negative.

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