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Measurement of the Fractional Ratio of Demethylation of Imipramine in Rat by Using a Co-administration Technique

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Desipramine (DMI), a metabolite of imipramine (IP), has approximately the same antidepressant activity as the mother compound. It is important to clarify the fractional ratio of metabolism from IP to DMI ($fm^{IP\text{-}DMI}$). We have developed a new technique to estimate $fm^{IP\text{-}DMI}$ by the use of a co-administration technique. After co-administration of a mixture of IP-d_4 and DMI-d_0 to rats, area under the plasma concentration–time curve (AUC) and renal excretion of DMI-d_4 and DMI-d_0 were examined. $fm^{IP\text{-}DMI}$ estimated from the ratio of AUC of DMI-d_4 to that of DMI-d_0 was almost the same as $fm^{IP\text{-}DMI}$ calculated from the ratio of the amount of excreted DMI-d_4 to that of DMI-d_0. This means that $fm^{IP\text{-}DMI}$ can be calculated by using the renal excretion data instead of AUC. The relationship between $fm^{IP\text{-}DMI}$ and IP-d_4 dose is also discussed.

Keywords——imipramine; desipramine; metabolism fractional ratio; co-administration technique; stable isotope

If a metabolite "M" derived from a drug "X" has a pharmacological activity or toxicity, the estimation of the fractional ratio of metabolism of a drug[1] ($fm^{X\text{-}M}$) is considered to be of great importance in the analyses of drug effect, toxicity and regimen. Although $fm^{X\text{-}M}$ can be theoretically estimated from the ratio of the area under the plasma concentration–time curve (AUC) of M following the administration of X or M by use of a cross-over technique, a large number of subjects is needed to get a firm conclusion, because there are large daily variations and individual differences in AUC of M. Pang *et al.*[2] estimated fm of phenacetin to acetoaminophen by the use of a double tracer technique following co-administration of ^{14}C-phenacetin and ^{3}H-acetoaminophen. However, this method is not applicable to human experiments owing to the radiation hazard and is not applicable to a drug which does not show high plasma concentration because of the limitation of maximum specific activity of a carbon-14-labeled compound (the maximum specific activity of a compound labeled with one carbon-14 atom is around 60 mCi/mmol). It is difficult to estimate fm accurately because a large error is involved in measuring radioactivity in the double tracer technique.

Imipramine (IP) has been widely used as an antidepressant drug. IP is metabolized by two main metabolic routes, demethylation and hydroxylation at the 2 position of the iminodibenzyl nucleus.[3] Desipramine (DMI), which is a metabolite derived from the former route, has approximately the same pharmacological activity as IP.[4] The matabolic route of the tricyclic antidepressant is greatly variable, depending on sex, age[5] and race[6] differences. There are large individual differences in the plasma concentrations of IP and DMI, or in the ratio of IP to DMI in plasma in the steady state.[7] Taking these findings into consideration, it is of great importance to estimate fm of IP to DMI ($fm^{IP\text{-}DMI}$). However, we have not been able to find any report dealing with the determination of $fm^{IP\text{-}DMI}$.

Co-administration techniques using a mixture of drug labeled with a stable isotope and non-labeled drug have been widely applied to bioavailability and bioequivalency tests.[8] Chart 1 shows the principle of our method. A mixture of IP-d_4 and DMI-d_0 is orally administered to

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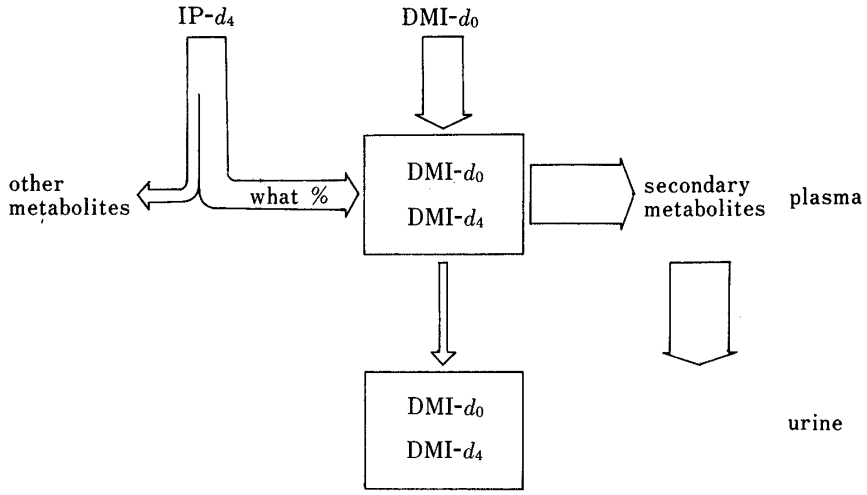

Chart 1. Strategy for Estimation of $fm^{IP\text{-}DMI}$

rats. The concentrations of DMI-d_4 (derived from IP-d_4) and DMI-d_0 in plasma and urine are quantitated by gas chromatography-mass spectrometry-selected ion monitoring (GC-MS-SIM) using DMI-d_8 as an internal standard. DMI-d_4 and DMI-d_0 are excreted into urine in the same ratio as that in plasma. So, there is a possibility that $fm^{IP\text{-}DMI}$ might be calculated from the ratio of DMI-d_4 to DMI-d_0 in urine even if the amount of DMI excreted into urine is very small.

This paper presents a comparison of $fm^{IP\text{-}DMI}$ estimated from AUC data and from renal excretion data in rats, which show a similar metabiolic pattern to humans, prior to a human experiment.

Experimental

Instruments and Conditions——SIM profiles of DMI were measured on a Shimadzu GCMS-QP 1000 GC-MS equipped with a capillary column interface (Shimadzu SPL-G9) in the chemical ionization mode under the same conditions as described previously.[9]

Labeled and Non-labeled Compounds——IP[1,3,7,9-d_4] (IP-d_4)·HCl and DMI[1,2,3,4,6,7,8,9-d_8] (DMI-d_8)·HCl were synthesized in our laboratory.[10] DMI-d_0·HCl was kindly supplied by Japan Ciba-Geigy Co. Ltd.

Administered Solutions——Administered Solution Ia: A mixture of 25 mg of IP-d_4·HCl and 25 mg of DMI-d_0·HCl was dissolved in 5.0 ml of H_2O.

Administered Solution Ib: A mixture of 40 mg of IP-d_4·HCl and 10 mg of DMI-d_0·HCl was dissolved in 2.0 ml of H_2O.

Administered Solutions IIa, IIb, IIc and IId: Mixtures of 5.0 mg of DMI-d_0·HCl and 1.25 (IIa), 5.0 (IIb), 25.0 (IIc) or 50.0 mg (IId) of IP-d_4·HCl were dissolved in 2.5 ml of H_2O, respectively.

Animal Experiment I——Three male Wistar rats weighing about 180 g were used as one group. Rats were starved for 12 h before and for 6 h after drug administration. Water was available *ad libitum*. Administered solution Ia or Ib (0.5 ml each) was orally administered to the rats. After administration, 1 ml of blood was taken from the jugular vein at 1, 2, 4, 8, 12, 24, 36, 48 and 72 h later. Plasma was obtained by centrifugation (3000 rpm × 10 min). Urine was collected during the 0—24, 24—48 and 48—72 h periods, and the total volume made up to 25 ml with H_2O in each case. The plasma and urine samples were stored at −20°C until analysis.

Animal Experiment II——Three male Wistar rats weighing about 175 g were used as one group. Administered solutions IIa, IIb, IIc or IId (0.5 ml each) were orally administered to the rats. After administration, urine was collected during the 0—24, 24—48, and 48—72 h periods.

Quantitation of DMI——After adding 20.1 ng of DMI-d_8 as an internal standard and 3.8 ml of H_2O to 200 μl of plasma, DMI-d_4 and DMI-d_0 in plasma were determined according to the method described previously.[9] After adding 80.4 to 201.0 ng of DMI-d_8 to 1 to 4 ml of urine, DMI-d_4 and DMI-d_0 in urine were determined according to the method mentioned above.

Data Analysis——The elimination rate constant (k_{el}) of DMI-d_0 was determined by least-squares linear regression of the logarithmic plasma concentration–time profiles at the final four points. AUC and the mean

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residence time (MRT) in the systemic circulation were calculated by means of the trapezoidal rule using the following equations:

$$\mathrm{AUC}=\int_0^T C_p(t)\mathrm{d}t \tag{1}$$

$$\mathrm{MRT}=\int_0^T tC_p(t)\mathrm{d}t \Big/ \int_0^T C_p(t)\mathrm{d}t \tag{2}$$

where T is final sampling time, and C_p (t) is plasma concentration at the time t.[11]

Estimation of $fm^{IP\text{-}DMI}$[12]——AUC of DMI-d_4 following the administration of IP-d_4 (AUC[DMI-d_4]) may be related to $fm^{IP\text{-}DMI}$ as follows:

$$\mathrm{AUC[DMI}\text{-}d_4]=fm^{IP\text{-}DMI}\, F_{(DMI,IP)}\, \mathrm{dose[IP}\text{-}d_4]/CL^{DMI} \tag{3}$$

in which $F_{(DMI,IP)}$ is defined as the apparent hepatic availability of DMI that is immediately derived from IP and that is also immediately further eliminated, and CL^{DMI} is the systemic clearance of DMI. Equation 3 holds true for all administration routes of IP, because the entire dose of IP is completely absorbed and the liver is the only drug-elimination organ for IP and DMI.[13]

Because the entire dose of DMI-d_0 is absorbed into the portal blood as the intact form,[13] AUC of DMI-d_0 (AUC [DMI-d_0]) following its oral administration is given by:

$$\mathrm{AUC[DMI}\text{-}d_0]=F_{(DMI)}\, \mathrm{dose[DMI}\text{-}d_0]/CL^{DMI} \tag{4}$$

where $F_{(DMI)}$ is the hepatic availability of DMI. Comparison of Eqs. 3 and 4 shows that:

$$fm^{IP\text{-}DMI}=\frac{\mathrm{AUC[DMI}\text{-}d_4]/\mathrm{dose[IP}\text{-}d_4]}{\mathrm{AUC[DMI}\text{-}d_0]/\mathrm{dose[DMI}\text{-}d_0]}\times\frac{F_{(DMI)}}{F_{(DMI,IP)}} \tag{5}$$

If the eliminations of IP and DMI may be described in terms of an operationally well-mixed liver, the first-pass effect of DMI would be the same as the sequential first-pass effect of the immediately formed DMI from IP, that is, $F_{(DMI)}=F_{(DMI,IP)}$. Thus, Eq. 5 reduces to:

$$fm^{IP\text{-}DMI}=\frac{\mathrm{AUC[DMI}\text{-}d_4]/\mathrm{dose[IP}\text{-}d_4]}{\mathrm{AUC[DMI}\text{-}d_0]/\mathrm{dose[DMI}\text{-}d_0]} \tag{6}$$

Although renal clearance of DMI (CL_R^{DMI}) is insignificant, the cumulative amount of DMI-d_0 (A_e[DMI-d_0]) or DMI-d_4 (A_e[DMI-d_4]) in urine following the oral administration of DMI-d_0 or IP-d_4 is given by:

$$A_e[\mathrm{DMI}\text{-}d_0]=CL_R^{DMI}\,\mathrm{AUC[DMI}\text{-}d_0] \tag{7}$$

$$A_e[\mathrm{DMI}\text{-}d_4]=CL_R^{DMI}\,\mathrm{AUC[DMI}\text{-}d_4] \tag{8}$$

Dividing Eq. 8 by Eq. 7 gives:

$$A_e[\mathrm{DMI}\text{-}d_4]/A_e[\mathrm{DMI}\text{-}d_0]=\mathrm{AUC[DMI}\text{-}d_4]/\mathrm{AUC[DMI}\text{-}d_0] \tag{9}$$

Substituting Eq. 9 in Eq. 6 gives:

$$fm^{IP\text{-}DMI}=\frac{A_e[\mathrm{DMI}\text{-}d_4]/\mathrm{dose[IP}\text{-}d_4]}{A_e[\mathrm{DMI}\text{-}d_0]/\mathrm{dose[DMI}\text{-}d_0]} \tag{10}$$

Results and Discussion

We defined $fm^{IP\text{-}DMI[AUC]}$ and $fm^{IP\text{-}DMI[RE]}$ as $fm^{IP\text{-}DMI}$ estimated from the AUC data (Eq. 6) and from the renal excretion data (Eq. 10), respectively. As $fm^{IP\text{-}DMI}$ should be discussed in relation to the drug effect, $fm^{IP\text{-}DMI[AUC]}$ should be mainly considered. However, $fm^{IP\text{-}DMI[RE]}$ may be more useful from the viewpoint of the burden for a patient and the number of samples, if $fm^{IP\text{-}DMI[RE]}$ is coincident with $fm^{IP\text{-}DMI[AUC]}$. The sensitivity of GC-CI-MS-SIM for DMI was 50 pg (injected amount) and the maximum plasma concentration of DMI in rats was about 500 ng/ml when 5 mg of IP was orally administered.[9] So, it was deduced that the AUC of DMI in rats can be accurately determined, if 1 ml of blood is taken after co-administration of the mixtures of 2.5 or 10 mg of IP-d_4 and 2.5 mg of DMI-d_0.

The plasma concentration curves of DMI-d_4 and DMI-d_0 in rats following the co-administration of IP-d_4 and DMI-d_0 are shown in Fig. 1. The maximum plasma con-

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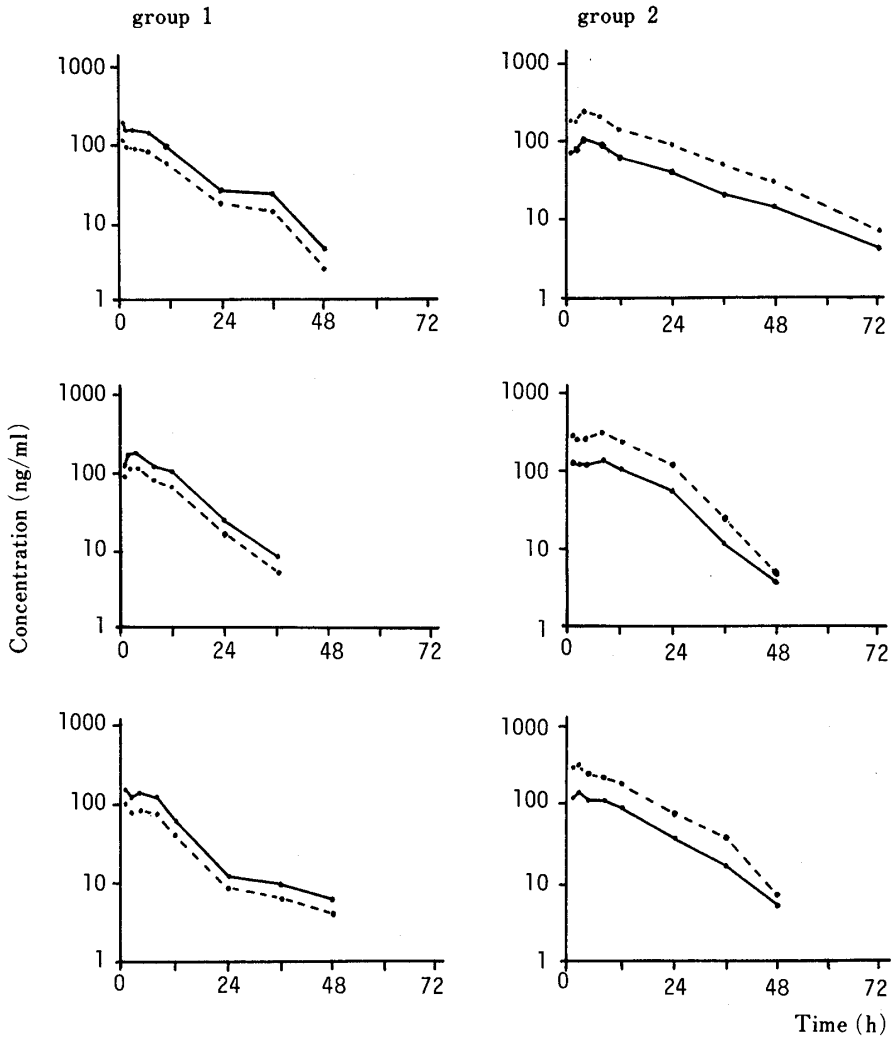

Fig. 1. Plasma Concentration Curves of DMI-d_4 and DMI-d_0 Following the Oral Administration of a Mixture of IP-d_4 and DMI-d_0

Group 1 received IP-d_4 (2.5 mg) and DMI-d_0 (2.5 mg), and group 2 received IP-d_4 (10.0 mg) and DMI-d_0 (2.5 mg). ----, DMI-d_4; ——, DMI-d_0.

centrations of DMI-d_0 and DMI-d_4 were observed between 1 and 8 h after administration. AUC, MRT, k_{el} and $fm^{IP\text{-}DMI[AUC]}$ calculated from the plasma concentration curves of DMI-d_4 and DMI-d_0 are shown in Table I. There were large differences in AUC, K_{el} and MRT in rats. On the other hand, little difference was observed in the ratio of AUC of DMI-d_4 to that of DMI-d_0. This AUC of DMI-d_4 was smaller than the reported AUC of DMI determined after oral administration of IP alone to rats.[14] The reason for this difference is unclear. AUC of DMI-d_4 increased in proportion to the administered dose of IP-d_4. However, the $fm^{IP\text{-}DMI[AUC]}$ values of the two groups, calculated from Eq. 6, were almost coincident.

The renal excretions of DMI-d_4 and DMI-d_0 and $fm^{IP\text{-}DMI[RE]}$ are shown in Table II. The total amounts of DMI-d_4 or DMI-d_0 excreted into urine were less than 1% of administered IP-d_4 or DMI-d_0 and renal excretion was completed within 48 h after administration. Although $fm^{IP\text{-}DMI[RE]}$ was slightly higher than $fm^{IP\text{-}DMI[AUC]}$, they are in reasonable agreement. From these results, it is apparent that $fm^{IP\text{-}DMI}$ can also be calculated from renal excretion data of DMI-d_4 and DMI-d_0.

Both $fm^{IP\text{-}DMI[AUC]}$ and $fm^{IP\text{-}DMI[RE]}$ in group 2 were slightly lower than in group 1. This means that there were dose-dependent changes in $fm^{IP\text{-}DMI}$. As the evaluation of $fm^{IP\text{-}DMI}$ is

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TABLE I. Pharmacokinetic Parameters of DMI-d_4 and DMI-d_0 in Rats Following the Oral Administration of a Mixture of IP-d_4 and DMI-d_0

	AUC (ng·h/ml)[a]		AUC ratio[b]	MRT (h)[a]	k_{el} (h^{-1})	$fm^{IP\text{-}DMI[AUC]}$[c]
	d_0	d_4		d_0	d_0	
Group 1[d]						
1	2804	1714	0.611	12.9	0.078	0.638
2	2577	1676	0.650	10.4	0.098	0.678
3	2133	1368	0.641	11.8	0.060	0.669
Mean	2505	1586	0.634	11.7	0.079	0.662
±S.D.	341	191	0.020	1.3	0.019	0.021
Group 2[e]						
1	2373	5349	2.254	23.3	0.045	0.588
2	2836	6263	2.208	14.0	0.059	0.576
3	2610	5415	2.075	14.4	0.072	0.542
Mean	2606	5676	2.179	17.2	0.072	0.569
±S.D.	232	510	0.093	5.3	0.025	0.024

a) AUC and MRT were calculated from data at time 0 to 72 h. When DMI was not detected in plasma at 48 or 72 h, its concentration at these times was set as 0. *b*) AUC ratio = AUC of DMI-d_4/AUC of DMI-d_0. *c*) $fm^{IP\text{-}DMI[AUC]} = \dfrac{\text{AUC of DMI-}d_4\text{/dose of IP-}d_4\text{ (mol)}}{\text{AUC of DMI-}d_0\text{/dose of DMI-}d_0\text{ (mol)}}$. *d*) Administered dose: IP-d_4 (2.5 mg) and DMI-d_0 (2.5 mg). *e*) Administered dose: IP-d_4 (10.0 mg) and DMI-d_0 (2.5 mg).

TABLE II. Renal Excretion of DMI-d_4 and DMI-d_0 in Rats Following the Oral Administration of a Mixture of IP-d_4 and DMI-d_0

	A_e[DMI-d_0] (μg)[a]	A_e[DMI-d_4] (μg)[a]	Renal excretion ratio[b]	$fm^{IP\text{-}DMI[RE]}$[c]
Group 1[d]				
1	13.91	9.08	0.653	0.682
2	18.14	11.25	0.620	0.647
3	12.55	8.29	0.661	0.690
Mean	14.87	9.54	0.645	0.673
±S.D.	2.92	1.53	0.022	0.023
Group 2[d]				
1	11.03	26.24	2.379	0.621
2	7.25	18.59	2.564	0.669
3	20.85	47.89	2.297	0.599
Mean	13.04	30.91	2.413	0.630
±S.D.	7.02	15.20	0.137	0.036

a) Cumulative amount of DMI-d_0 or DMI-d_4 excreted into urine within 72 h. *b*) Renal excretion ratio = A_e[DMI-d_4]/A_e[DMI-d_0]. *c*) $fm^{IP\text{-}DMI[RE]} = \dfrac{A_e\text{[DMI-}d_4\text{]/dose of IP-}d_4\text{ (mol)}}{A_e\text{[DMI-}d_0\text{]/dose of DMI-}d_0\text{ (mol)}}$. *d*) See Table I.

based on the assumption that the system behaves linearly with dose, $fm^{IP\text{-}DMI}$ evaluated under non-linear conditions does not reflect the actual fractional ratio of metabolism of IP to DMI. The relationship between $fm^{IP\text{-}DMI}$ and administered dose of IP was examined. Since $fm^{IP\text{-}DMI[RE]}$ was fairly well coincident with $fm^{IP\text{-}DMI[AUC]}$, $fm^{IP\text{-}DMI}$ was estimated from renal data of DMI-d_0 and DMI-d_4 excreted within 72 h after co-administration of a mixture of 1 mg of DMI-d_0 and various doses of IP-d_4 (0.25—10 mg). The results obtained are shown in Fig. 2. Although a slight lower $fm^{IP\text{-}DMI[RE]}$ was obtained in the 10 mg of IP-d_4 group, the $fm^{IP\text{-}DMI[RE]}$ was consistent among the other three groups. This means that no dose-dependent changes in

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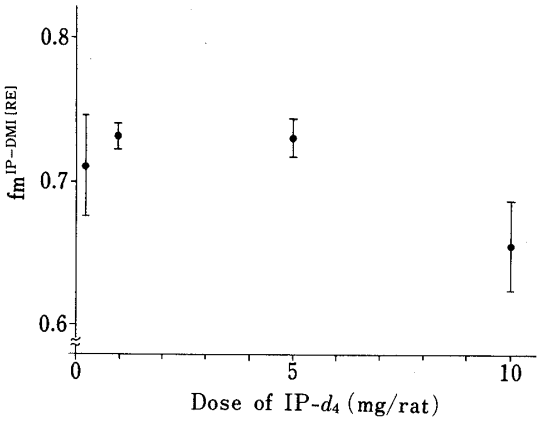

Fig. 2. Relationship between Dose of IP and $fm^{IP\text{-}DMI[RE]}$

$fm^{IP\text{-}DMI}$ occur within this administered dose range. In rats, about 70% of administered IP was metabolized through the demethylation process. This $fm^{IP\text{-}DMI}$ was slightly higher than that obtained in group 1 in animal experiment I. To compare $fm^{IP\text{-}DMI[AUC]}$ with $fm^{IP\text{-}DMI[RE]}$, blood and urine were collected from the same rats in experiment I. On the other hand, only urine was collected in experiment II. This difference of experimental conditions might account for the difference of $fm^{IP\text{-}DMI[RE]}$.

In the human co-administration experiment to estimate $fm^{IP\text{-}DMI}$, the administered dose of IP is scheduled to be 25 mg per human, an amount which is the lowest limit of the first treatment dose for depressed patients. $fm^{IP\text{-}DMI}$ was almost constant within the dose range of 0.25 to 5 mg of IP in rats. These administered doses in rats (body weight; 200 g) correspond to 75—1500 mg in human (body weight; 60 kg). Therefore, the saturation of the enzymes which metabolize IP and DMI might not occur in humans at the dose of 25 mg. So, it is expected that the co-administration technique established here for the estimation of $fm^{IP\text{-}DMI}$ in rats can be applied to humans.

$fm^{IP\text{-}DMI}$ is defined as the ratio of the intrinsic clearance for the formation of DMI to the total hepatic intrinsic clearance for the elimination of IP.[1] The evaluation of $fm^{IP\text{-}DMI}$ in human, thus, would elucidate the relationship between individual variations of plasma concentration of IP and DMI, and hepatic drug metabolizing enzyme activity of IP to DMI. Furthermore, $fm^{IP\text{-}DMI}$ would be an important parameter for deciding the IP dosing regimen in clinical treatment.

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