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Studies on Drug Nonequivalence. X.[1] Bioavailability of 6-Mercaptopurine Polymorphs[2]

Teruyoshi Yokoyama,*,[a] Tsuneo Umeda,[a] Koji Kuroda,[a] Tsutomu Kuroda[b] and Shozo Asada[c]

Hospital Pharmacy, Kobe University School of Medicine,[a] Kusunoki-cho, Ikuta-ku, Kobe Pharmacy, Miki City Hospital,[b] Kasa, Miki and Kobe Women's College of Pharmacy,[c] Motoyamakitamachi, Higashinada-ku, Kobe

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The gastrointestinal absorption of 6-mercaptopurine (6-MP) polymorphs was studied in rabbits, and their absorption kinetics were analyzed.

Form III of 6-MP was absorbed more efficiently from the gastrointestinal tract than form I. It was found that the absorption kinetics after oral administration of 6-MP could be satisfactorily accounted for by a two-compartment model with two consecutive first-order input steps. Form III had an extent of bioavailability about 1.5 times greater than that of form I. This result may be attributed to a difference of K_1 and/or K_a between the polymorphic forms I and III.

Keywords——6-mercaptopurine polymorphism; oral administration; intravenous administration; pharmacokinetics of 6-mercaptopurine; two-compartment model; bioavailability

In the previous paper,[3] the authors reported that 6-mercaptopurine (6-MP), which has significant activity against human leukemia, exists in three polymorphic forms and that form III had a solubility about 6—7 times that of form I.

In the present investigation, the authors carried out gastrointestinal absorption studies of 6-MP polymorphs in rabbits, and subsequently analyzed the absorption kinetics of 6-MP polymorphs to assess their bioavailability.

Experimental

Preparation and Identification of 6-MP Polymorphs——6-MP polymorphs, form I and form III were prepared and identified as described in the previous paper.[3]

Animal Studies——(i) For intravenous administration, white male rabbits weighing from 2.1 to 2.5 kg were used. 6-MP was dissolved in 0.1 N NaOH and diluted with 1/15 M phosphate buffer (pH 7.3) and administered intravenously at three dose levels (6, 12.5 and 25 mg kg body weight). After administration, blood samples were withdrawn from an ear vein with a heparinized syringe at regular intervals, and were centrifuged. Plasma was separated and stored at $-5°$ until analyzed.

(ii) For oral administration, a two-way cross-over design was employed. White male rabbits weighing from 2.1 to 2.8 kg were fasted for 18 hr before drug administration. The 6-MP doses were 12.5, 25 and 50 mg/kg body weight. Each 6-MP polymorph (form I or form III) was suspended in distilled water and immediately administered into the stomach through a catheter. After administration, plasma samples were obtained by the same method as in the case of intravenous administration.

Measurement of 6-MP Concentration in Plasma——The high-pressure liquid chromatography (HPLC) method reported by Day *et al.*,[4] was applied with a slight modification as follows: 0.5 ml of plasma was placed in a test tube and 100 μl (10 μg) of internal standard (6-methylthio-2-hydroxypurine) was added. The solution was then deproteinized by addition of 1.5 ml of MeOH. After being mixed for 15 sec and standing for 15 min, the mixture was centrifuged at 3000 rpm for 10 min. One ml of supernatant was transferred to another test tube, and evaporated to dryness under a stream of nitrogen. The residue was reconstituted with 100 μl of MeOH and 10 μl of the sample was injected into the HPLC apparatus.

The apparatus used was a high-pressure liquid chromatograph (Shimadzu, model LC-3A) equipped with a variable wavelength photometric detector (Shimadzu, model SPD-2A). The conditions for analysis were as follows: column, 25 cm/4 mm i.d.; packing, Shimadzu LC-PC_8; mobile phase, 10% MeOH and 0.005 M 1-heptanesulfonic acid; flow rate, 1 ml/min; wavelength, 313 nm; sensitivity, 0.005 a.u.f.s.

Test of Significance——Student's paired t test was used.

Results

Plasma Concentration of Drug after Intravenous Administration

Figure 1 shows the time course of the plasma concentration of 6-MP after intravenous administration of 12.5 mg/kg to rabbits. The 6-MP plasma concentration declined in a biphasic manner. This profile was, therefore, consistent with the following two-exponential equation.

$$C_1 = Ie^{-\alpha t} + Je^{-\beta t} \quad (1)$$

From this equation, the distribution rate constant and the elimination rate constant were evaluated. In this case, the pharmacokinetic parameters were derived by assuming that the elimination of 6-MP occurs either from a central compartment, or a peripheral compartment. The pharmacokinetic parameters obtained are summarized in Table I.

As shown in Table I, dose dependency was not observed. The value of biological half-life ($t_1/_{2(\beta)}$) was calculated by averaging the results at three dose levels (6, 12.5 and 25 mg/kg), and was 45.8 min.

On the other hand, Loo *et al.*[5] reported a $t_{1/2}$ value of 47 min for this drug after a single intravenous administration to patients.

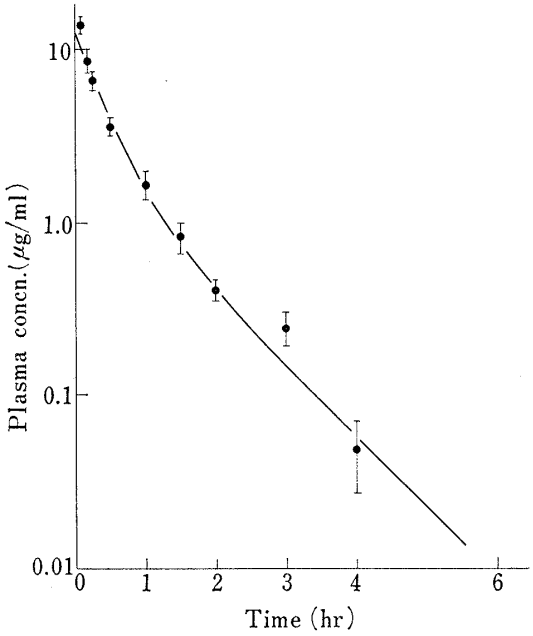


Fig. 1. Plasma Concentrations of 6-MP after Intravenous Administration of 12.5 mg/kg

Points are means ± S.E. of three experiments. The continuous line is the computed plasma concentration of 6-MP.

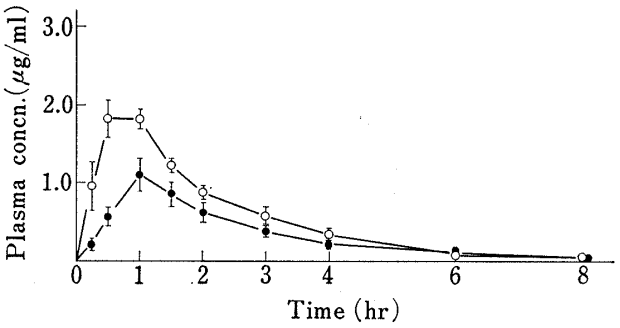


Fig. 2. Plasma Concentrations of 6-MP Polymorphs after Oral Administration of 12.5 mg/kg suspended in Distilled Water

—●—: Form I, —○—: Form III
Points are means ± S.E. of seven experiments.

Plasma Concentration of Drug after Oral Administration

The mean plasma concentration-time curves after oral administration of 6-MP polymorphs are shown in Figs. 2, 3 and 4. The mean plasma levels at intervals (0.25, 0.5, 1 and 1.5 hr) and the area under the plasma concentration-time curve (AUC) after administration of 12.5 mg/kg of form III were significantly higher than those after the same dose of form I ($p<0.05$). In the case of administration of 25 mg/kg, it was again found that form III produced a higher peak in the plasma than form I ($p<0.05$). These results indicate that form III was more efficiently absorbed than form I.

Table I. Pharmacokinetic Parameters[a] after Intravenous Administration of 6-MP

Dose mg/kg		6	12.5	25
n		3	3	3
Body weight kg		2.36±0.13	2.17±0.03	2.56±0.18
I μg/ml		6.09±1.17	10.70±1.2	23.1±1.4
α hr^{-1}		3.95±0.08	3.39±0.09	3.35±0.06
J μg/ml		1.24±0.20	3.10±0.85	5.25±0.89
β hr^{-1}		0.850±0.096	0.929±0.048	0.945±0.017
$C_0=I+J$ μg/ml		7.33	13.82	28.33
[AUC] μg·hr/ml		3.013±0.209	6.413±1.048	12.41±0.53
[AUC]/kg·dose		0.211±0.003	0.237±0.038	0.195±0.017
Model I_a	K_{12} hr^{-1}	0.964±0.128	0.690±0.025	0.610±0.015
	K_{10} hr^{-1}	2.43±0.25	2.17±0.06	2.30±0.13
	K_{21}[b] hr^{-1}	1.40±0.18	1.46±0.13	1.39±0.09
	V_1[c] ml/kg	844.3±99.3	947.9±147	883.2±20.9
	V_2[d] ml/kg	1186±289	1156±50.0	1183±24.8
Model I_b	K_{12}[e] hr^{-1}	3.39±0.15	2.86±0.05	2.91±0.10
	K_{20} hr^{-1}	0.995±0.102	1.11±0.07	1.09±0.04
	K_{21} hr^{-1}	0.409±0.080	0.354±0.034	0.295±0.030
	V_1[f] ml/kg	844.9±99.6	948.2±147	886.1±22.8
	V_2[g] ml/kg	2051±137	1943±493	1862±159

a) Data are means ± S.E.
b) $K_{21}=(I\beta+J\alpha)/C_0$
c) $V_1=\text{Dose}/K_{10}[\text{AUC}]$
d) $V_2=\dfrac{K_{12}V_1[\text{AUC}_1]}{K_{21}[\text{AUC}_2]}$, where $[\text{AUC}_2]$ was estimated from C_2 calculated by the Loo–Riegelman method.[19]
e) $K_{12}=(I\alpha+J\beta)/C_0$
f) $V_1=\text{Dose}\ (K_{21}+K_{20})/\alpha\beta[\text{AUC}]$
g) $V_2=K_{12}V_1/(K_{21}+K_{20})$

On the other hand, as shown in Fig. 4, it was found that the administration of 50 mg/kg of form I resulted in a plateau level of the drug in the plasma, These results may be attributed to an overdose of 6-MP. Consequently, it was considered that 6-MP was dissolved gradually in the gastrointestinal tract because of its extremely low solubility.

The extent of bioavailability (EBA) was calculated according to the conventional method.[6] As shown in Table II, form III had an EBA about 1.5 times that of form I.

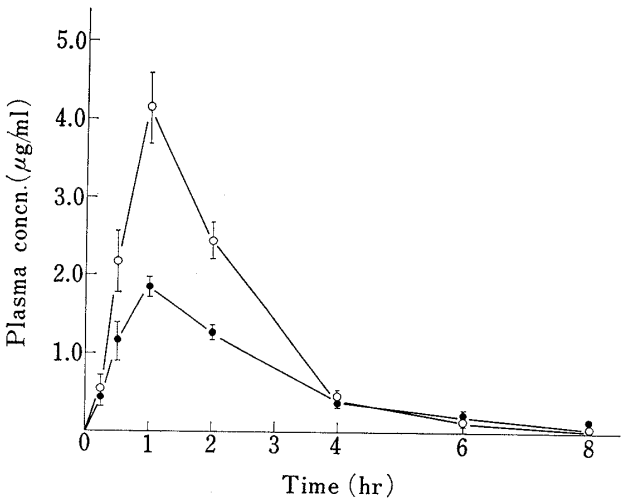


Fig. 3. Plasma Concentrations of 6-MP Polymorphs after Oral Administration of 25 mg/kg Suspended in Distilled Water

—●—: Form I, —○—: Form III
Points are means ± S.E. of five experiments.

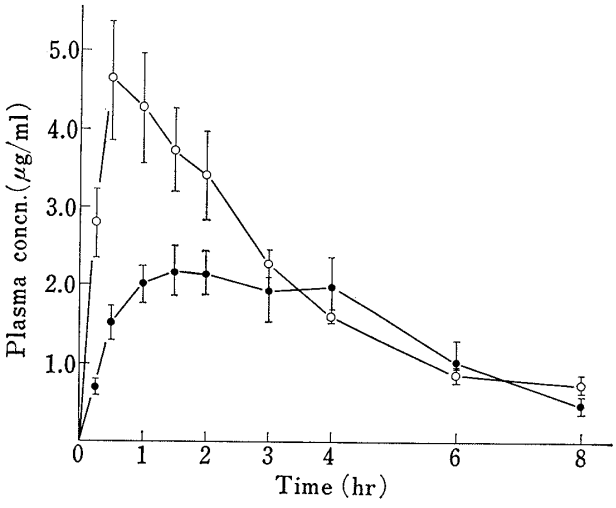


Fig. 4. Plasma Concentrations of 6-MP Polymorphs after Oral Administration of 50 mg/kg suspended in Distilled Water

—●—: Form I, —○—: Form III
Points are means ± S.E. of five experiments.

Table II. Extent of Bioavailability (EBA) of 6-MP Polymorphs after Oral Administration

Dose mg/Kg	Form I		Form III	
	12.5	25	12.5	25
[AUC][a] μg·hr/ml	3.10±0.27	5.28±0.39	4.23±0.37[b]	8.84±0.49[c]
EBA %	48.3	42.5	65.9	71.2
Bioavailability ratio	100	100	136.4	167.5
Bioavailability (f)[d] %	24.2	21.3		

a) Calcd. by use of the trapezoidal rule.
b) Significantly different from from I ($p<0.05$).
c) Significantly different from form I ($p<0.01$).
d) Bioavalability (f) was calculated by using model Ib.

Pharmacokinetic Analysis of Plasma Concentration after Oral Administration

The authors carried out an analysis of the absorption kinetics of 6-MP polymorphs by postulating the models shown in Fig. 5. From the results of intravenous administration, a two-compartment model such as model Ia or model Ib was assumed. Bioavailability (f) was calculated by applying the hepatic-portal compartment model.[7] As shown in Table II, in the case of model Ib, the bioavailability (f) obtained was smaller than the extent of bioavailability described previously. Therefore, it appears that the model Ib is not suitable as a two-compartment model after intravenous administration.

An analysis of the absorption kinetics after oral administration was carried out by applying model IIa and model III[8] shown in Fig. 5. It was found that the plasma concentration data after oral administration of 6-MP fitted model III. In the case of model IIa, the values of K_{12}, K_{10} and K_{21} were 0.18 hr^{-1}, 0.662 hr^{-1} and 0.65 hr^{-1}, respectively, and did not fit the values obtained after intravenous administration.

Table III. Pharmacokinetic Parameters[a] after Oral Administration of 6-MP Polymorphs

Dose mg/kg	Form I		Form III	
	12.5	25	12.5	25
n	7	5	7	5
Body weight Kg	2.56±0.14	2.15±0.06	2.86±0.06	2.08±0.03
P μg/ml	−47.8±4.8	−51.2±1.6	−33.7±0.36	−103±3.5
α hr^{-1}	3.31±0.27	3.09±0.23	4.29±0.36	3.04±0.27
Q μg/ml	5.09±1.87	7.68±1.42	5.63±1.91	19.1±4.1
β hr^{-1}	1.23±0.17	1.09±0.11	1.39±0.24	1.53±0.25
R μg/ml	43.9±3.7	44.8±0.66	25.1±1.3	88.5±8.7
K_1 hr^{-1}	3.87±0.42	3.78±0.35	5.22±0.64	4.56±0.56
S μg/ml	1.10±0.31	0.96±0.31	1.86±0.30	6.90±1.35
K_a hr^{-1}	0.391±0.08 (0.442)[b]	0.289±0.06 (0.387)[b]	0.559±0.11 (0.523)[b]	0.766±0.10 (0.579)[b]
$[AUC]_{0\to\alpha}$[c] μg·hr/ml	3.10±0.27	5.28±0.39	4.23±0.37	8.84±0.49
$(C_1)_0=FD/V_1$[d] μg/ml	6.72	12.13	9.17	20.32
K_{21}[e] hr^{-1}	2.105±0.44	1.660±0.20	2.456±0.57	1.645±0.21
K_{10} hr^{-1}	2.232±0.00	2.296±0.00	2.167±0.00	2.299±0.00
K_{12} hr^{-1}	0.477±0.08	0.514±0.07	0.840±0.25	0.295±0.07

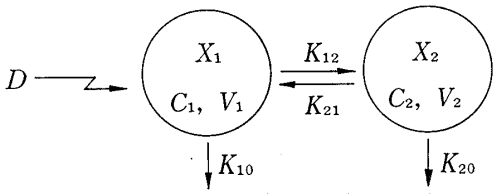
a) Parameters were computed by means of the OPTDAV-4 program using model III in Fig. 5. Each value is a mean ± S.E.
b) Calcd. by the Loo-Riegelman method.[10]
c) Calcd. by use of the trapezoidal rule.
d) V_1 is the valeu obtained on *i.v.* administration.
e) $K_{21}=\alpha\beta[AUC]/(C_1)$

Each coefficient and the rate constants were estimated by the stripping procedure, and computer fitting was then performed using these parameters.[9] The absorption rate constant, K_a was also estimated according to the method of Loo–Riegelman.[10]

Table III summarizes the computed pharmacokinetic parameters for forms I and III of 6-MP based on model III. The absorption kinetics after oral administration of 6-MP could be accounted for by four exponential terms. In addition, the absorption rate constant corresponded to the therminal slope of the plasma concentration-time curve. Therefore, oral administration of 6-MP resulted in the phenomenon called 'flip-flop,' and 6-MP had an elimination rate faster than the absorption rate.

Model I Bolus intravenous administration

$C_1 = Ie^{-\alpha t} + Je^{-\beta t} \quad (\alpha > \beta)$



$[AUC_1]_I = \dfrac{D\,(K_{21}+K_{20})}{V_1\,\alpha\beta}$

$[AUC_2]_I = \dfrac{D\;K_{12}}{V_2\alpha\beta}$

model I_a $(K_{20}=0)$

$\alpha + \beta = K_{12} + K_{21} + K_{10}$

$\alpha\beta = K_{21}K_{10}$

$K_{21} = (I\beta+J\alpha)/(I+J)$

model I_b $(K_{10}=0)$

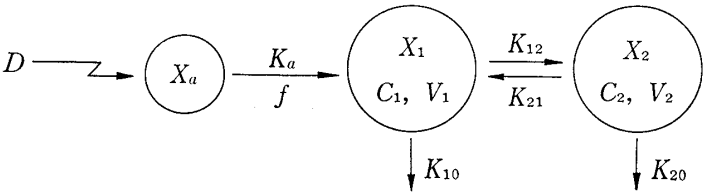
$\alpha + \beta = K_{12} + K_{21} + K_{20}$

$\alpha\beta = K_{12}K_{20}$

$K_{12} = (I\alpha+J\beta)/(I+J)$

Model II Two-compartment model with first-order absorption

$C_1 = He^{-\alpha t} + Ie^{-\beta t} + Je^{-K_a t} \quad (\alpha > \beta)$



model II_a $(K_{20}=0)$

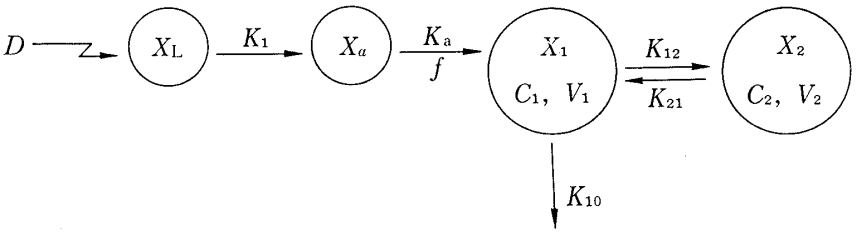
$K_{21} = \dfrac{K_a\beta H + K_a\alpha I + \alpha\beta J}{(C_1)_o K_a}$

model II_b $(K_{10}=0)$

$K_{12} = (\alpha+\beta) - \dfrac{K_a\beta H + K_a\alpha I + \alpha\beta J}{K_a(C_1)_o}$

Model III Two-compartment model with two consecutive first-order input steps

$C_1 = Pe^{-\alpha t} + Qe^{-\beta t} + Re^{-K_1 t} + Se^{-K_a t}$



$K_{21} = \alpha\beta[AUC_1]/(C_1)_o = \dfrac{K_1K_a(P\beta+Q\alpha)+\alpha\beta(RK_a+SK_1)}{K_1K_a(C_1)_o}$

Fig. 5. Pharmacokinetic Compartment Models used for 6-MP

D, dose administered; f, fraction of drug absorbed; X_L, amount of drug in dissolution site; X_a, amount of absorption site; X_1, X_2, amounts of drug in central and peripheral compartments; C_1, C_2, drug concentrations in central and peripheral compartments; V_1, V_2, volumes of central and peripheral compartments; K_1, dissolution rate constant; K_a, absorption rate constant; K_{12}, K_{21}, distribution rate constants; K_{10}, K_{20}, elimination rate constants.

Discussion

Since each polymorphic form is considered to be distributed, metabolized and excreted in a similar manner after absorption into the body, it seems reasonable that the distribution rate constant and the elimination rate constant after the administration of form I and form III are approximately equal, as shown in Table III.

On the other hand, it is well known that the gastrointestinal absorption of a poorly water soluble drug is rate-limited by the process of dissolution, and the apparent absorption rate may be decreased in such a case. In the present studies ,it can be assumed that the difference in bioavailability between the polymorphic forms I and III is due mainly to a difference in the dissolution rate. As shown in Table III, however, difference of both dissolution rate constant, K_1 and absorption rate contant, K_a were observed between forms I and III. Accordingly, the difference of mean plasma levels found in this study may be explained by the difference of K_1 and/or K_a. Further studies are in progress with a capsule dosage form.

The results of this study can be summarized as follows.

1) The polymorphic form III of 6-MP was absorbed more efficiently from the gastrointestinal tract than form I.

2) It was found that the absorption kinetics after oral administration of 6-MP could be satisfatorily accounted for by a two-compartment model with two consecutive first -order input steps, and that the elimination rate constant was several times larger than the absorption rate constant after oral administration of 6-MP.

3) Differences of K_1 and K_a were observed between forms I and III.

4) Form III had an extent of bioavailability about 1.5 times that of form I. This may be attributed to the difference of K_1 and/or K_a.

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References and Notes

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