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Studies on Drug Nonequivalence. XI.[1] Pharmacokinetics of 6-Mercaptopurine Polymorphs in Rabbits

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6-Mercaptopurine (6-MP) polymorphs were administered to rabbits as intravenous injection, oral aqueous solution, oral hard capsule and effervescent capsule. When the form III powder was given in hard capsules, the dissolution from the capsule was very slow and the plasma levels after oral administration were also lower than those obtained with form I. On the other hand, the form III effervescent capsule prepared to enhance the dissolution gave an extent of bioavailability (EBA) about 1.5 times greater than that of form I.

The time course data after oral administration of each dosage form were analyzed by means of the compartment model method and the statistical moment method. It appears that the difference of EBA after oral administration as the capsule dosage forms was chiefly attributable to the difference of apparent absorption rate.

These results could be explained in terms of the differences of the mean disintegration and dissolution time (MDDT) obtained by means of the moment method.

Keywords——6-mercaptopurine polymorphism; pharmacokinetics of 6-mercaptopurine; two compartment model; statistical moment method; the mean residence time (MRT); the mean disintegration and dissolution time (MDDT)

In the previous papers,[1,2] we reported that 6-mercaptopurine (6-MP) polymorphic form III had a solubility about 6—7 times greater than that of form I, and we also suggested that form III had a greater bioavailability than form I following oral administration to rabbits as an aqueous suspension. However, we were unable to elucidate the absorption kinetics of 6-MP polymorphs in detail.

In the present study, we assessed the pharmacokinetics of 6-MP polymorphs after administration of different dosage forms, *e.g.*, intravenous injection, oral aqueous solution, oral hard capsule and effervescent capsule. The evaluation of pharmacokinetic parameters were carried out by means of the compartment model and the statistical moment analysis method.

Experimental

Preparation and Identification of 6-MP Polymorphs——6-MP polymorphic forms I and III were prepared and identified as described in the previous paper.[2]

Preparation of Capsule Dosage Forms——For the hard capsule forms, each polymorph was manually filled in hard gelatin capsules (JP #4 capsule). Each capsule contained 64.3—68 mg of 6-MP. The effervescent capsule forms were prepared by packing each polymorph, corn starch and "Barosu effervescent granula"[3] (2: 3: 2) into hard gelatin capsules (JP #1 capsule). Each capsule contained 246—250 mg of these mixtures.

Dissolution of 6-MP from Capsule Forms——Dissolution of 6-MP from capsule forms was tested according to the modified method of the XIXth U.S.P. with stirring at 25 rpm at 30°C. The test solution was 1000 ml of the first medium of the IXth JP (pH 1.2). Dissolved drug was assayed spectrophotometrically at 325 nm.

Animal Studies——Animal experiments were carried out as described in the previous paper.[1] 6-MP doses were 25 mg/kg body weight in every case. For oral administration, the gastric emptying rate of rabbits was controlled according to the method of Maeda *et al.*[4]

a) Intravenous Injection: 72.5 mg of 6-MP was dissolved in 1 ml of 0.1 N NaOH and diluted to 2 ml with 1/15 M phosphate buffer (pH 7.3), and this solution was intravenously administered in 1 min.

b) Aqueous Solution: 64 mg of 6-MP was dissolved in 1 ml of 0.1 N NaOH and diluted to 20 ml with 1/15 M phosphate buffer (pH 7.3), and this solution was orally administered into the stomach through a catheter.

c) Capsule: Capsules were orally administered and 20 ml of water was given immediately.

Measurements of 6-MP Concentration in Plasma——The high-pressure liquid chromatography method was used, as described in the previous paper.[1] In this study, a column packed with Nucleosil-C_{18} (10 μm) was used to enhance the reproducibility at low plasma concentration levels.

Computer Analysis——The curve-fitting program used was the MULTI program written in BASIC[5] for an NEC N4700 minicomputer system. The least-squares algorithm used was the simplex method at the preliminary fitting, and the converged values were futher analyzed by the modified Marquardt method.

Results

Plasma Concentration of Drug after Intravenous Administration

Closed circles in Fig. 1 show the time course of the plasma concentration of 6-MP after intravenous administration. The curve fitting of time course data was evaluated by using Akaike's information criterion (AIC).[6] To determine the number of exponential terms, mono and biexponential equations are fitted to the plasma concentration data by means of the least-squares method. It was found that the biexponential fitting gave a smaller AIC value than the monoexponential.[6] Therefore, the curve fitting analysis of the time course data was carried out by applying a two compartment open model.

From the biexponential equation, the distribution rate constants k_{12}, k_{21} and elimination rate constant k_{10} were evaluated in the manner reported previously.[1] The parameters obtained are shown in Table I.

Plasma Concentration of Drug after Oral Administration as an Aqueous Solution

Open circles in Fig. 1 show the time course of plasma concentration of the drug after oral administration as an aqueous solution.

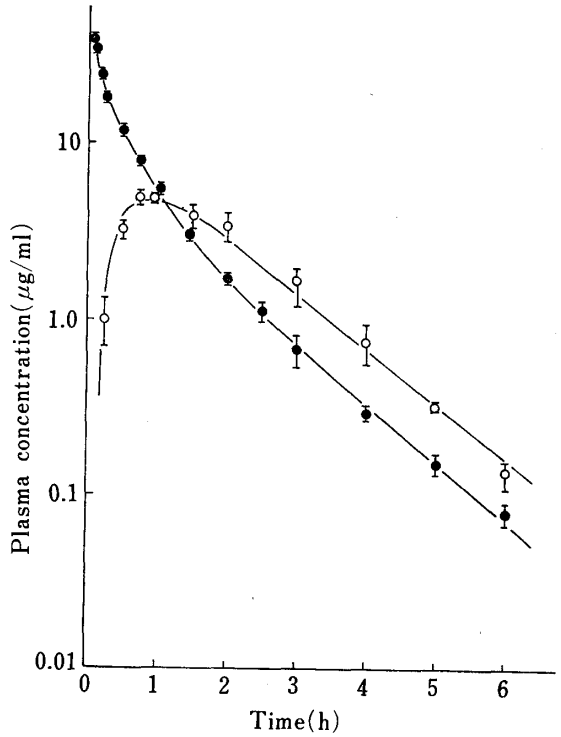

Fig. 1. Plasma Concentrations of 6-MP following Intravenous and Oral Administration

●: *i.v.* ○: aqueous solution.
Each point represents the average ± S.E.

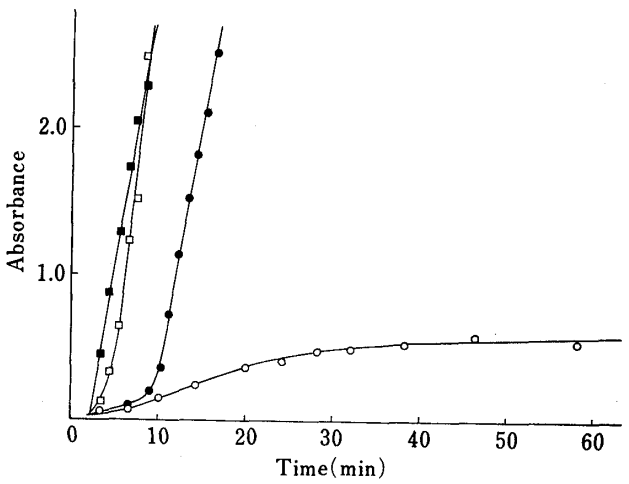

Fig. 2. Dissolution of 6-MP Capsules in 0.1 N HCl Solution

●: form I hard capsule.
○: form III hard capsule.
■: form I effervescent capsule.
□: form III effervescent capsule.

The apparent absorption process was complate at one and a half houres after administration and subsequently the apparent elimination was similar to the β phase for intravenous injection. The plasma concentration-time curve after oral administration as a solution was analyzed by applying a two compartment model with first-order absorption.

The pharmacokinetic parameters were estimated by simultaneous calculation of the equation for both the intravenous and solution dosage forms using the MULTI program,[5] since the simultaneous fitting yielded acceptable parameters. Good agreement was obtained between the predicted value and observed plasma concentration. The rate constant of absorption, k_a was also obtained by the method of Loo–Riegelman.[7] The calculated concentration curve and parameters predicted are shown in Fig. 1 and Table I, respectively.

Dissolution from Capsules

It is well known that differences in dissolution from capsules results in differences in the subsequent blood levels. Therefore, the *in vitro* dissolution from each dosage form was examined before animal absorption experiments.

As shown in Fig. 2, the dissolution from the hard capsules of form III was slower than that of form I. Furthermore, after 90 minutes, the hard capsule of form III maintained its capsular appearance. This phenomenon was attributed to a high aggregation of form III, just as in the case of chloramphenicol capsules described by Aguiar *et al.*[8]

On the other hand, in the case of the effervescent capsules prepared to avoid the effect of aggregation, no difference in dissolution between forms I and III could be seen.

Plasma Concentration of Drug after Oral Administration as a Capsule Dosage Form

The plasma levels found after oral administration as a capsule dosage form are shown in Figs. 3 and 4. Although the polymorphic form III had a higher solubility, its plasma concentration after oral administration as a hard capsule form was lower than that of form I. These results were attributed to the marked aggregation of form III powder.

On the other hand, when each polymorphic form was administered as an effervescent capsule form, the plasma concentration of form III was larger than that of form I, and the

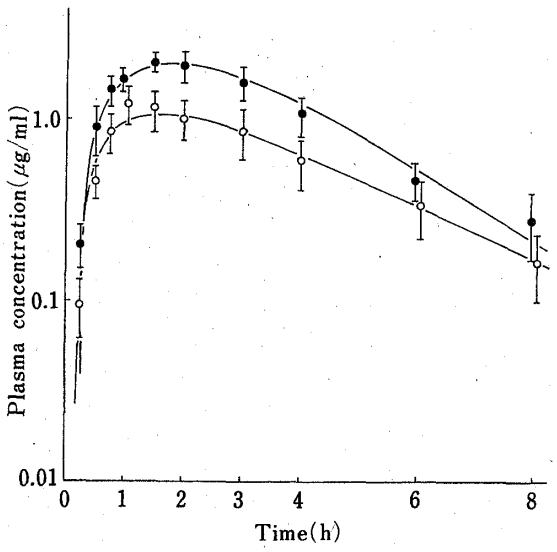

Fig. 3. Plasma Concentrations of 6-MP Polymorphs after Oral Administration as Hard Capsules

●: form I. ○: form III.
Each point represents the average ± S.E.

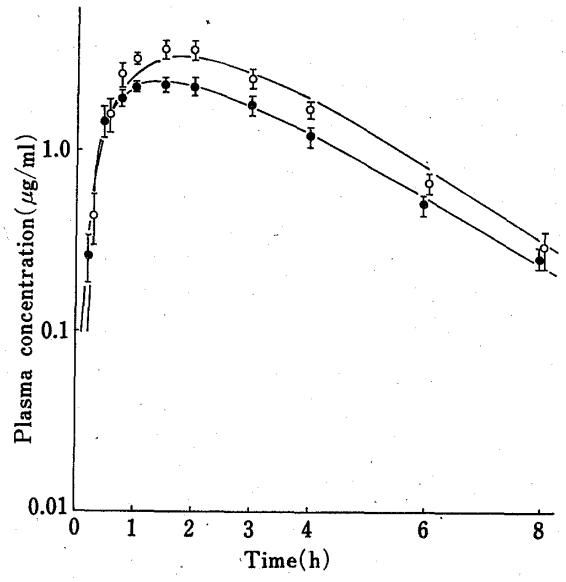

Fig. 4. Plasma Concentration of 6-MP Polymorphs after Oral Administration as Effervescent Hard Capsules

●: form I. ○: form III.
Each point represents the average ± S.E.

extent of bioavailability (EBA) of form III was about 1.5 times greater than that of form I, as shown in Fig. 4 and Table I.

The analysis of plasma concentration time course was carried out according to the two compartment open model with first-order absorption. The computer analysis was done by simultaneous fitting of the equation for both the intravenous and the capsule dosage form. Each calculated concentration curve fitted the observed plasma data well.

The k_a value calculated by the MULTI program was in agreement with the value obtained by the Loo–Riegelman method.

TABLE I. Pharmacokinetic Parameters[a] Calculated by the Compartment Model Method after Administration of 25 mg/kg to Rabbits

	Intravenous administration	Solution	Hard capsule		Effervescent capsule	
			Form I	Form III	Form I	Form III
n	9	4	9	9	7	8
B.W. (kg)	2.91±0.01	2.56±0.06	2.57±0.05	2.72±0.05	2.86±0.07	2.84±0.01
AUC (μg·h/ml)	19.15±2.78	11.50±3.30	9.00±2.87	5.53±3.19	10.12±1.90	14.39±3.88
EBA (%)	100	60.07	46.98	28.90	52.84	75.15
P (μg/ml)	31.59±2.82	−18.44±1.55	−0.125±0.92	−1.40±0.45	−3.38±0.91	1.54±2.16
α (h^{-1})	2.91±0.33	2.83±0.39	2.93±0.36	3.14±0.42	3.02±0.29	2.65±0.47
Q (μg/ml)	7.48±1.08	−6.34±59.59	−13.93±4.92	−2.71±1.09	−9.69±3.40	−24.99±9.45
β (h^{-1})	0.77±0.03	0.804±0.04	0.784±0.03	0.789±0.04	0.778±0.03	0.815±0.05
R (μg/ml)		18.36±58.36	13.27±4.42	3.24±0.82	11.06±2.88	22.85±8.29
k_a (h^{-1})		0.743±0.29[b]	0.500±0.04[c]	0.364±0.04[c]	0.474±0.03[c]	0.519±0.04[c]
		(1.10±0.23)[d]	(0.470±0.12)[d]	(0.449±0.12)[d]	(0.492±0.07)[d]	(0.552±0.16)[d]
k_{21} (h^{-1})[e]	1.18	1.19	1.20	1.30	1.08	1.13
k_{10} (h^{-1})	1.90	1.90	1.90	1.90	2.16	1.90
k_{12} (h^{-1})	0.536	0.636	0.608	0.727	0.550	0.426

$C_p = Pe^{-\alpha t} + Qe^{-\beta t}$ for $i.v.$ administration and $C_p = Pe^{-\alpha t} + Qe^{-\beta t} + Re^{-k_a t}$ for oral administration. ($\alpha > \beta$)

a) Parameters were computed by means of the MULTI program. Each value is a mean±S.D. *b*) "True"absorption rate constant. *c*) Apparent absorption rate constant. *d*) Calculated by the Loo-Riegelman method.[7]

e) $k_{21} = \frac{\alpha\beta[\mathrm{AUC}]V_1}{F \cdot D}$, V_1 is the value obtained on $i.v.$ administration.

Pharmacokinetic Analysis of 6-MP Concentration Time Course by the Statistical Moment Method

The application of statistical moment analysis to pharmacokinetic was recently described by Riegelman *et al.*[9] and Yamaoka *et al.*[10] This method is model-independent.

The mean residence time (MRT) and the mean absorption time (MAT) were defined as follows,

$$\mathrm{MRT} = \frac{\int_0^\infty t\,C_p\,dt}{\int_0^\infty C_p\,dt} \tag{1}$$

$$\mathrm{MAT} = \mathrm{MRT_{po}} - \mathrm{MRT_{iv}} \tag{2}$$

MRT values were evaluated from the time course data of plasma concentration by use of a linear trapezoidal equation (from zero to eight h) and extrapolation to infinite time. The mean *in vivo* disintegration and dissolution time (MDDT) was defined as follows:

$$\mathrm{MDDT_{capsule}} = \mathrm{MAT_{capsule}} - \mathrm{MAT_{solution}} \tag{3}$$

Fig. 5 shows the meaning of MRT, MAT and MDDT. The result of pharmacokinetic analysis of 6-MP by means of the moment method are summarized in Table II.

The MDDT of all capsule dosage forms of 6-MP is larger than the $\mathrm{MAT_{solution}}$, and it appears that the disintegration and dissolution of 6-MP is the rate-determining step.

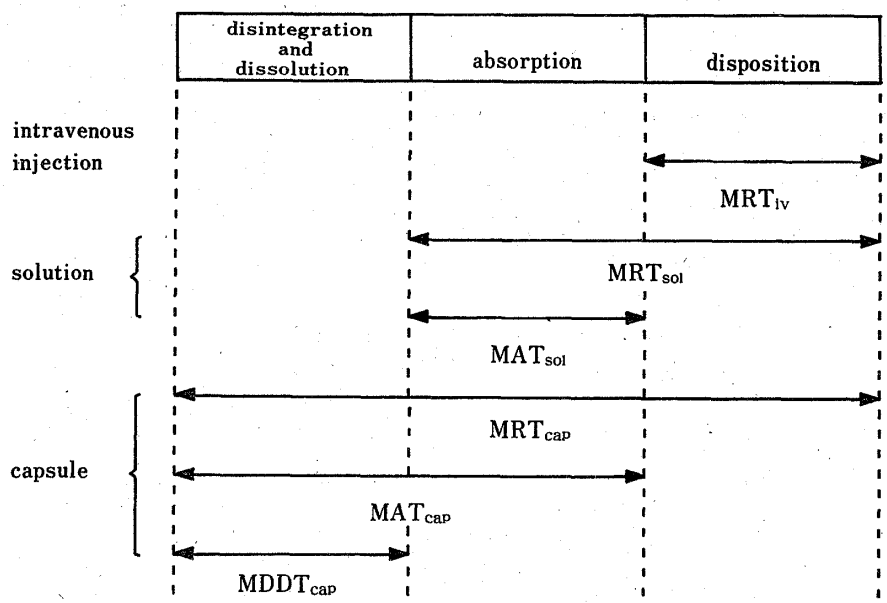

Fig. 5. Illustration ot fhe meaning of MRT, MAT and MDDT

TABLE II. Pharmacokinetic Parameters Calculated by the Moment Method after Administration of 25 mg/kg to Rabbits

	Intravenous administration	Solution	Hard capsule		Effervescent capsule	
			Form I	Form III	Form I	Form III
AUC (μg·h/ml)	19.15±2.78	11.50±3.30	9.00±2.87	5.53±3.49	10.12±1.90	14.39±3.88
EBA (%)	100	60.07	46.98	28.90	52.84	75.15
MRT (h)	0.840±0.08	1.86±0.20	3.48±1.39	4.33±1.18	3.38±0.48	3.06±0.35
MAT (h)		1.02	2.64	3.49	2.54	2.22
MDDT (h)			1.62	2.46	1.51	1.20

Each value is a mean ± S.D.

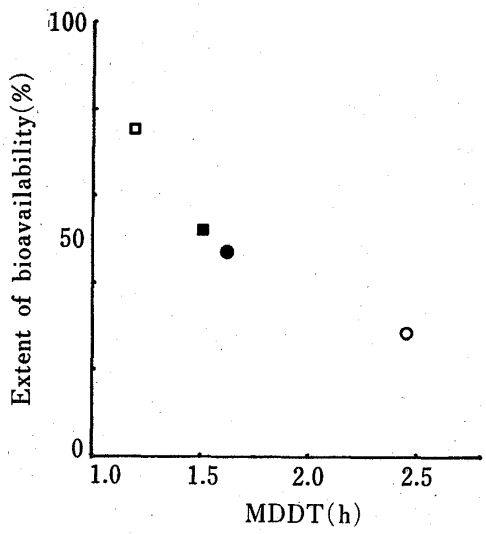

Fig. 6. Relationships between EBA and MDDT

●: form I hard capsule.
○: form III hard capsule.
■: form I effervescent capsule.
□: form III effervescent capsule.

Discussion

The purpose of this study was to clarify the differences in bioavailability of polymorphs and/or dosage forms of 6-MP pharmacokinetically.

Pharmacokinetic parameters for 6-MP after administration of each dosage form are summarized in Table I. From these results, it was considered that the absorption kinetics after oral administration of 6-MP could be described by a two compartment model with first-order absorption.

From the values of the rate constant k_a, which represents the apparent absorption of the drug, it appears that EBA increased with the apparent absorption rate, as shown in Table I. From these results, it can be assumed that the differences in bioavailability were mainly due to the differences in apparent absorption rate in the initial period.

On the other hand, the differences in bioavailability of 6-MP polymorphs and/or dosage forms were more clearly explained by the statistical moment analysis. From the values of MDDT obtained after administration of capsule dosage forms, it appears that the smaller the value of EBA is, the larger the value of MDDT is, as shown in Fig. 6.

Consequently, it was concluded that the differences in bioavailability after administration of capsule forms could be explained in terms of the differences in MDDT.

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References and Notes

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