Enhanced Bioavailability of 6-Mercaptopurine After Rectal Administration in Rats

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INTRODUCTION

6-Mercaptopurine (6-MP) is one of the major drugs used in the maintenance therapy for children with acute lymphoblastic leukemia (ALL). Remission can now be induced in about 95% of children with ALL; however, approximately one-third of the patients relapse thereafter. Possible causes of such relapses are drug resistance and/or suboptimal drug effects as a result of poor compliance or low bioavailability of 6-MP (1).

Previous studies have reported low and highly variable bioavailability of oral 6-MP in man (1). The low and variable bioavailability of 6-MP administered orally probably results from crystal polymorphs, with extremely low 6-MP solubility (2), first-pass effects in the alimentary canal and/or liver (1,3), and genetic polymorphism (4). Kuroda *et al.* (2) have reported that 6-MP exists in three polymorphic forms. Form III was 6 to 7 times more soluble than form I, corresponding to a 1.5 times greater extent of bioavailability (EBA) than that of form I following oral administration to rabbits as an aqueous suspension (5). Three major enzymes, hypoxanthine phosphoribosyltransferase (HGPRT), xanthine oxidase (XO), and thiopurine methyltransferase (TPMT), are responsible for the metabolism of 6-MP. HGPRT converts 6-MP to pharmacologically active metabolites (thiopurine nucleotides) (3), whereas XO converts 6-MP to pharmacologically inactive metabolites (8-hydroxy-6-methylmercaptopurine, 6-thiouric acid). Zimm *et al.* (6) have demonstrated the inhibition of first-pass metabolism of 6-MP by allopurinol, which is a XO inhibitor. Pretreatment with allopurinol enhanced the bioavailability of oral 6-MP fivefold. One important catabolic route for 6-MP is thiol methylation catalyzed by TPMT. It has been reported that TPMT shows pharmacogenetic polymorphism (4).

Because of the poor bioavailability of oral 6-MP, rectal administration of 6-MP to rats was evaluated. The kinetics and the extent of systemic bioavailability of 6-MP after oral and rectal administrations have not been compared in man or in animals. In the present study, plasma levels and the area under the plasma concentration–time curve (AUC) for 6-MP were determined after intravenous (iv), oral, and rectal administrations in male Wistar rats. The EBA of 6-MP after rectal administrations was compared with that after oral dosing.

MATERIALS AND METHODS

Materials. 6-MP was obtained from Sigma Chemical Co., Ltd. (St. Louis, MO). Macrogol 1000 and 4000 were obtained from Wako Pure Chemical Industries, Ltd. (Osaka). Witepsol H-15 was obtained from Hoei Pharmaceutical Co., Ltd. (Osaka). All other chemicals were of reagent grade.

Preparation of Suppositories and Quantitation of 6-MP in a Suppository. Preparation of suppositories and quantitation of 6-MP in a suppository were performed according to a previously described method (7).

Animal Experiments. Male Wistar rats weighing 280 to 310 g were fasted overnight before the experiment. Rats were anesthetized with 20% (w/v) urethane (1 g/kg body weight, intraperitoneal). The right femoral vein was cannulated with PE-10 tubing (Clay Adams, Parsippany, NJ) to instill saline solution and to inject the drug. The left femoral artery was cannulated with PE-50 (Clay Adams) filled with heparinized saline solution (Shimizu Pharmaceutical Co., Ltd., Shizuoka) (50 IU/ml) to collect blood samples. 6-MP prepared in saline solution containing 1 *N* sodium hydroxide (final concentration, 3% alkali) was administered to rats at 10 mg/kg body weight iv or by gastric intubation (oral). For rectal administration, a suppository of 6-MP was inserted into the anus at 10 mg/kg body weight, which was fixed with a glass bead cover (7-mm diameter) to prevent the overflow of melted suppository and closed with surgical adhesive (Aron Alpha glue, Toa-Gousei Co., Tokyo). A crossover design was not conducted. Blood samples (0.2 ml) were collected from the femoral artery at 0, 5, 10, 15, 20, 30, and 45 min and at 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 hr following iv dosing and at 0, 10, 20, 30, and 45 min and at 1.0, 1.3, 1.7, 2.0, 2.5, 3.0, 4.0, 5.0, and 6.0 hr following rectal and oral administrations. Blood samples were cooled on ice, and plasma was immediately separated by centrifugation at 2000*g* for 10 min. The separated plasma was stored at −20°C until analysis. Plasma concentrations of 6-MP were determined by the HPLC method as reported previously (8).

Pharmacokinetic Analysis. Plasma concentration (C) vs time (t) data of 6-MP after iv administration were fitted to a biexponential equation ($C = Ae^{-\alpha t} + Be^{-\beta t}$), where A, B, α, and β are hybrid parameters (9). The AUC values after iv, oral, and rectal administrations were calculated by the trapezoidal rule from the observed values and extrapolated to time infinity. The data were analyzed according to the nonlinear least-squares regression analysis by the computing program MULTI (10). The apparent EBA after oral and rectal administrations was estimated by comparing each AUC value with that obtained after an equivalent iv dosing, respectively. Maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained di-

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rectly from the observed values. The data are shown as mean ± SD. Significant differences in the AUC values were estimated by the analysis of variance (ANOVA in STAX Institute, Tokyo).

RESULTS AND DISCUSSION

Content Uniformity of the Suppositories. The content of 6-MP in the suppositories prepared with macrogol and Witepsol bases was 5.02 ± 0.13 and 5.04 ± 0.25 mg/g suppository, respectively.

Pharmacokinetics. Plasma concentration–time curves for 6-MP after bolus iv, oral, and rectal administrations are shown in Fig. 1. The plasma concentrations of 6-MP after iv administration was found to decline biexponentially with time. Estimated pharmacokinetic parameters for 6-MP after iv administration were as follows: A = 7740 ± 1870 ng/ml; B = 340 ± 190 ng/ml; α = 0.138 ± 0.016 min^{-1}; β = 0.035 ± 0.005 min^{-1}; and elimination half-life $t_{1/2\beta}$ = 20.1 ± 5.2 min. The AUC value and the mean EBA of 6-MP after various administration routes are summarized in Table I.

Oral administration of 6-MP (solution) to rats showed relatively fast absorption of 6-MP from the digestive tract (Fig. 1); however, as suspected, plasma levels of 6-MP were very low compared to iv administration. Consequently, the AUC value was very small (Table I). The mean C_{max} of 6-MP after oral administration was 106.0 ± 58.9 ng/ml (range, 15.4 to 235.1), with a mean t_{max} of 1.1 ± 0.6 hr (range, 0.5 to 1.7). The EBA was only 21%.

Previously, we have studied whether the pharmacokinetics of 6-MP would show a dose dependency following three single oral doses (50, 87.5, and 175 mg/m^2) in eight children with acute leukemia in remission (11). Although the mean (±SD) values for t_{max} and $t_{1/2\beta}$ were fairly comparable among the three doses examined, 1.1 ± 0.2 and 1.2 ± 0.2 hr, respectively, the mean values for C_{max} and AUC increased disproportionately (88 ± 123 to 326 ± 194 to 653 ± 344 ng/ml for C_{max} and 147 ± 180 to 451 ± 177 to 1291 ± 415 ng · hr/ml for AUC, respectively), when the doses increased from 50 to 87.5 and then to 175 mg/m^2. Consequently, the mean apparent oral clearance decreased disproportionately with the increasing doses (32.7 ± 47.6 to 3.6 ± 2.0 to 2.5 ± 0.8 L/min/m^2). We hypothesized that this presystemic elimination of 6-MP in a nonlinear fashion might exist in the saturation of the first-pass metabolism and that first-pass elimination of oral 6-MP might bring about low plasma concentrations, low bioavailability, and large interindividual variations. Therefore, in order to avoid the first-pass metabolism of oral 6-MP in the alimentary canal and/or liver, we have conducted rectal administrations of 6-MP to rats as a preexamination for a clinical application.

Two kinds of suppository bases, Witepsol H-15 (oleaginous base) and macrogol (aqueous soluble base), were used for the preparation of 6-MP suppositories. When the Witepsol suppository was applied to rats, the absorption rate of 6-MP was the slowest among the three preparations [solution (oral) and two kinds of suppositories] (Fig. 1). The C_{max} was not statistically different from that after oral administration ($P > 0.05$); however, the t_{max} was delayed by a factor of 2 compared to oral administration ($P < 0.05$). The observed data were as follows: C_{max} of 52.5 ± 30.1 ng/ml (range, 19.5 to 92.9), with a t_{max} of 2.6 ± 0.7 hr (range, 1.3 to 4.0). The AUC value was almost twice the oral value, and the EBA was approximately 43%. When the macrogol suppository was applied to rats, 6-MP was absorbed rapidly from the lower rectal mucosa, its C_{max} and t_{max} were 179.9 ± 95.2 ng/ml (range, 86.5 to 292.3) and 1.3 ± 0.5 hr (range, 0.8 to 2.0), respectively. These values were not significantly different from those after oral administration ($P > 0.05$). The mean AUC value after iv administration was significantly

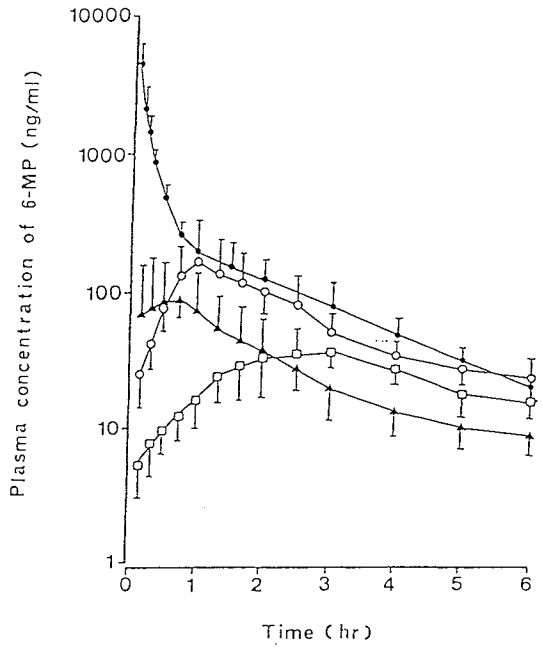

Fig. 1. Mean plasma concentration–time curves of 6-mercaptopurine following bolus intravenous (●), oral (▲), and rectal [macrogol base (○); Witepsol base (□)] administrations in rats at 10 mg/kg body weight (n = 5). The vertical lines show the SD.

Table I. The Area Under the Plasma Concentration–Time Curve (AUC) and Apparent Availability of 6-Mercaptopurine Following Intravenous, Oral, or Rectal Administration in Male Wistar Rats (Dose, 10 mg/kg; Mean ± SD; n = 5)

Route of administration	AUC (ng · hr/ml)[a]	Apparent systemic availability (%)[b]
Intravenous	1061.2 ± 276.4	100
	$P < 0.01$*	
Oral	225.3 ± 130.8	21.2
	$P < 0.01$	
Suppository	$P < 0.01$	
Macrogol type	910.0 ± 78.9	85.8
	$P < 0.01$	
Witepsol type	458.0 ± 189.8	43.2

[a] Calculated by the trapezoidal rule from the observed data and extrapolated to time infinity. Each value represents the mean ± SD (n = 5).

[b] Expressed as the percentage of the mean AUC value after iv dosing.

* Significantly different from oral or iv administration ($P < 0.01$) according to ANOVA.

different from those values calculated after both oral administration ($P < 0.01$) and rectal administration of Witepsol suppository ($P < 0.01$). However, the AUC value after macrogol suppository administration increased dramatically, to 86% not significantly different from that after iv administration ($P > 0.05$) (Table I). Therefore, the administration of 6-MP with macrogol suppository could avoid the first-pass effect of this drug in the alimentary canal and/or liver.

The t_{max} values were different (approximately, 1.3 hr) between the two kinds of suppository bases used (Fig. 1). The differences of t_{max} and C_{max} following Witepsol and macrogol suppository administrations may result from the low solubility of 6-MP (180 μg/ml in water, at 25°C) (2). Our preliminary dissolution test for 6-MP in Witepsol and macrogol suppositories showed that 6-MP in macrogol suppositories released faster than in Witepsol suppositories (7). Since 6-MP in the macrogol suppository has been solubilized already with macrogol, 6-MP would be easily absorbed from the rectal mucosa after suppository melting in rat rectum, resulting in a large C_{max} and a small t_{max} of 6-MP. In contrast, in the Witepsol suppository, it would take a long time for minute particles of 6-MP to solubilize in the rectal fluid. Hence, Witepsol suppositories show low systemic availability, although liver first-pass metabolism is avoided. The drug appears to be continuously absorbed until it is excreted in the feces as evidenced by the fact that the elimination phase following this formulation was not parallel with the elimination-phase iv following dose administration. Thus, poor systemic availability with Witepsol is due to incomplete absorption because of its slow dissolution. Absorption of 6-MP is prolonged from the Witepsol dosage form and suggests that it may be of use as a sustained release formulation.

The selection of suppository bases is important for the release and dissolution of the drug, because it affects drug bioavailability. Our results show that macrogol was the superior for the preparation of 6-MP suppositories. Rectal administration of 6-MP with macrogol suppositories may be more effective for the treatment of children with ALL, especially for patients suffering from nausea and vomiting.

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