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Effect of allopurinol on the first-pass metabolism of 6-mercaptopurine in the rat

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In a previous report [1], we investigated the characteristics of intestinal absorption of three anticancer antimetabolites, 5-fluorouracil, ftorafur and 6-mercaptopurine, by *in vitro* and *in situ* absorption techniques in rats. Most of the 6-mercaptopurine that was lost from the lumen appeared as its metabolite, 6-thiouric acid, in the lumen. A small serosal transfer of 6-mercaptopurine was confirmed using an *in vitro* method. These results indicate that the rate of intestinal first-pass metabolism of 6-mercaptopurine will be high after oral administration. Loo *et al.* [2] reported that the gastrointestinal absorption of 6-mercaptopurine is incomplete and variable in humans.

6-Mercaptopurine is normally metabolized to 6-thiouric acid by xanthine oxidase *in vivo* [3]. Allopurinol is used for the treatment of gout and other hyperuricemic states as a potent inhibitor of xanthine oxidase [4]. Elion *et al.* [5, 6] demonstrated that the catabolism of 6-mercaptopurine to 6-thiouric acid is diminished by the administration of allopurinol. This metabolic inhibition resulted in a several-fold potentiation of the antitumor activities of 6-mercaptopurine when tested against Adenocarcinoma 755 in mice. Coffey *et al.* [7] reported that daily administration of allopurinol orally to cancer patients almost completely inhibits the production of 6-thiouric acid from 6-mercaptopurine administered intravenously. Nonetheless, no effect was observable on the pharmacokinetics of 6-mercaptopurine in these patients [7].

The present study was undertaken to examine the effect of allopurinol on the first-pass metabolism of 6-mercaptopurine in rats.

Materials and methods

Materials. Allopurinol and 6-mercaptopurine were purchased from Nakarai Chemicals, Ltd. (Kyoto, Japan). 6-Thiouric acid was prepared according to the method reported by Elion *et al.* [8]. All other chemicals were of reagent grade.

Animal experiments. Male Wistar albino rats weighing 250–300 g were used throughout the study. The animals were housed in an air-conditioned room and maintained on a standard laboratory diet. The rats were fasted overnight but had free access to water. Animals were anesthetized with pentobarbital, given intraperitoneally. After complete anesthesia, 6-mercaptopurine and allopurinol dissolved in 0.1 M sodium hydroxide were administered simultaneously, orally by gastric intubation and intravenously into a femoral vein. Blood samples were removed periodically from the femoral vein. The area under the blood concentration versus the time curve (AUC) was calculated by the trapezoidal rule up to the last measurement.

Analytical methods. 6-Mercaptopurine in blood was analyzed by spectrofluorometry after modifying the method described by Maddocks [9]. A Shimadzu RF-510 spectrofluorometer (Shimadzu Co. Ltd., Kyoto, Japan) was used. Blood samples were diluted with 0.4 M NaOH and washed with ether containing 1.5% isoamyl alcohol. 6-Mercaptopurine in the aqueous layer was reacted with 0.3% phenylmercury acetate to produce 6-(phenylmercury)-mercaptopurine, which was extracted with toluene and then re-extracted with 0.1 M HCl. 6-Mercaptopurine in the acidic layer was oxidized by 2 mM potassium chromate to get fluorescence. The reaction was stopped by 0.4% sodium metabisulfite. After adding 5.0 M NaOH, relative fluorescence of the solutions was measured at 398 nm with an excitation wavelength of 288 nm.

Results and discussion

Figure 1 shows the blood concentration of 6-mercaptopurine following intravenous administration. 6-Mercaptopurine was eliminated rapidly from the blood. Following oral or intravenous coadministration of allopurinol, the blood level of 6-mercaptopurine increased. Intravenous injection of allopurinol was more effective than oral administration. Allopurinol administered intra-

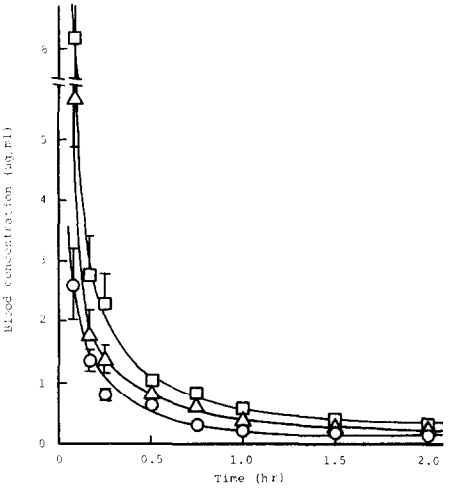

Fig. 1. Effect of allopurinol on the blood concentration of 6-mercaptopurine following intravenous administration. Each point represents the mean ±SE of at least three experiments. Key: (○) 6-mercaptopurine (2 mg/300 g) alone; (△) with allopurinol (0.6 mg/300 g) administered orally; and (□) with allopurinol (0.6 mg/300 g) administered intravenously.

venously is assumed to have inhibited hepatic xanthine oxidase. The blood concentration of 6-mercaptopurine after oral administration is presented in Fig. 2. Although the blood level of 6-mercaptopurine was low, an increased blood concentration of drug was found following the oral and intravenous coadministration of allopurinol. On the basis of these results, the AUC was calculated by the trapezoidal rule. The results are summarized in Table 1. The AUC of 6-mercaptopurine after oral administration was remarkably smaller than that following intravenous administration and was taken to be an indicator of first-pass metabolism. A significant increase in the AUC for 6-mercaptopurine was observed following the oral or intravenous coadministration of allopurinol. Especially, the

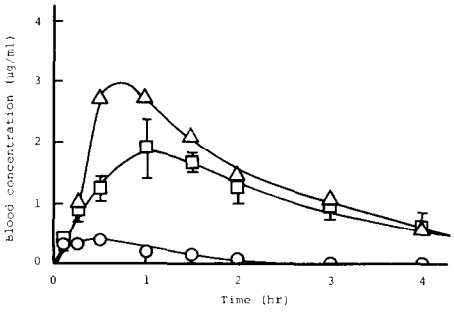

Fig. 2. Effect of allopurinol on the blood concentration of 6-mercaptopurine following oral administration. Each point represents the mean ±SE of at least three experiments except in some cases (two experiments, with allopurinol administered orally). Key: (○) 6-mercaptopurine (5 mg/300 g) alone; (△) with allopurinol (1.5 mg/300 g) administered orally; (□) with allopurinol (1.5 mg/300 g) administered intravenously.

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Table 1. Effect of allopurinol on the first-pass metabolism of 6-mercaptopurine*

Drug	AUC (min × μg/ml) p.o.	AUC (min × μg/ml) i.v.
6-Mercaptopurine alone	28.7 ± 9.6	265.2 ± 10.3
With allopurinol (i.v.)	258.9 ± 21.2	502.6 ± 33.1
With allopurinol (p.o.)	372.9	372.1 ± 14.3

* Dose of 6-mercaptopurine p.o., 5 mg/300 g; i.v., 2 mg/300 g. Dose of allopurinol 0.6 mg/300 g, i.v. administration of 6-mercaptopurine; 1.5 mg/300 g, p.o. administration of 6-mercaptopurine. In the case of the intravenous administration of 6-mercaptopurine, the AUC was corrected to a dose of 5 mg/300 g.

first-pass metabolism of 6-mercaptopurine was inhibited completely by the oral coadministration of allopurinol, suggesting the importance of the intestinal wall in the first-pass metabolism. Sackler [10] reported that xanthine oxidase is localized at the brush border area of the mucosal epithelial cells and that the oxidative activity toward hypoxanthine per gram wet weight of tissue is 100-fold greater in the intestine than in hepatic tissue. Therefore, the high concentration of allopurinol in the intestinal lumen after oral administration may have effectively inhibited the metabolism of 6-mercaptopurine in the intestinal wall. Although further work is necessary, the present results should contribute to the design of a dosage regimen of 6-mercaptopurine in humans.

In summary, a high rate of first-pass metabolism of 6-mercaptopurineafter oral administration was found. Its first-pass metabolism was inhibited markedly by the oral coadministration of allopurinol.

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