6) H.H. Kausch, D.G. Fesko, and N.W. Tschoegl, *J. Colloid and Interface Sci.*, **37**, 603 (1971).
7) D.N. Sutherland, *J. Colloid and Interface Sci.*, **25**, 373 (1967).
8) I. Goodarz-nia, *J. Colloid and Interface Sci.*, **52**, 29 (1975).

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Effect of Prior Administration of Aminophylline, Caffeine, or Propranolol on the Antitumor Activity of 6-Mercaptopurine or 6-Mercaptopurine Riboside

Ken-ichi Sasaki,* Shinobu Furusawa, and Giichi Takayanagi

Cancer Research Institute, Tohoku College of Pharmaceutical Sciences, 4-4-1, Komatsushima, Sendai, 983, Japan

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The effect of prior administration of aminophylline, caffeine, or propranolol on the antitumor activity of 6-mercaptopurine (6-MP) or 6-mercaptopurine riboside (6-MPR) against Ehrlich solid tumor in ddY male mice was studied. These three drugs each weakened the action of 6-MP but had less effect on the action of 6-MPR. Considering the variation in the activities of hypoxanthine–guanine phosphoribosyltransferase and xanthine oxidase, we suggest that higher doses of aminophylline and propranolol may promote the conversion from 6-MP to biologically inactive thiouric acid or hypoxanthine rather than to biologically active thioinosinic acid.

Keywords——aminophylline; caffeine; propranolol; antitumor activity; 6-mercaptopurine; 6-mercaptopurine riboside; Ehrlich solid tumor; mice; hypoxanthine–guanine phosphoribosyltransferase; xanthine oxidase

6-Mercaptopurine (6-MP) is converted *in vivo* into biologically active thioinosinic acid (TIMP) by hypoxanthine–guanine phosphoribosyltransferase (HGPRTase), or is oxidized to thiouric acid, a noncarcinostatic metabolite, by xanthine oxidase.[1] Recently Higuchi *et al.*[1a,1b] reported that the effects of 6-MP may be modified by drug-metabolizing enzymes in the mouse liver.

In the previous paper,[2] we demonstrated that butoctamide stimulated the HGPRTase activity and inhibited the xanthine oxidase activity of mouse liver, and enhanced the antitumor activity of 6-MP on Ehrlich solid tumor in mice. Thus, the combined use of such drugs with 6-MP may change the activity of drug-metabolizing enzymes in the liver, leading to changes in the antitumor activity of 6-MP.

Aminophylline, caffeine, and propranolol have an effect on cyclic AMP (c-AMP) formation or degradation in cell. Gericke and Chandra[3] reported that c-AMP inhibits the growth of transplanted NKL-lymphosarcoma in mice.

On the other hand, it has been reported by many investigators[4,5] that adenyl cyclase and phosphodiesterase activities and the content of c-AMP in tumor cells are lower than in normal cells. However, the relationship between content of c-AMP and tumor growth is still uncertain.

In this work, we examined the effects of prior administration of aminophylline, caffeine, or propranolol on the antitumor activity of 6-MP or 6-mercaptopurine riboside (6-MPR).

Materials and Methods

Animals——Male ddY mice weighing 20—22 g were used in all experiments.

Drugs——The drugs used were as follows: aminophylline (theophylline ethylenediamine, Neophyllin®, Eisai Co.), caffeine (caffeine and sodium benzoate, Fuso Yakuhin Kogyo Co., was used; doses are expressed

in terms of caffeine), propranolol hydrochloride (Sumitomo Kagaku Kogyo Co.), sodium pentobarbital (Tokyo Kasei Kogyo Co.), 6-MP (Kohjin Co.), 6-MPR (Morishita Seiyaku Co.), cyclic AMP (cyclic 3′,5′-adenosine monophosphate, Nakarai Chemical Co.), dibutyryl cyclic AMP (dibutyryl cyclic 3′,5′-adenosine monophosphate salt, Dbc-AMP, Sigma Chemical Co.). Aminophylline, caffeine, propranolol, 6-MPR, c-AMP and Dbc-AMP were each dissolved in 0.9% NaCl, while 6-MP was dissolved in a small volume of 0.1 N NaOH and diluted with 0.9% NaCl. c-AMP and Dbc-AMP were injected close to the cell-inoculation site and other drugs were injected intraperitoneally at a volum of 0.01 ml/g of mouse weight.

Antitumor Activity——The antitumor activity was estimated by weighing the tumor mass of drug-treated and saline-treated mice on the 15th day after the inoculation of Ehrlich ascites carcinoma (1×10^6 cells). Aminophylline, caffeine, or propranolol was injected *i.p.* once a day for 7 days, and cells were inoculated into the left thigh at the time of the final injection of these drugs. c-AMP and Dbc-AMP were injected close to the cell-inoculation site once a day for 7 days starting 24 hr after the cell inoculation. 6-MP or 6-MPR in combination with aminophylline, caffeine, or propranolol was injected once *i.p.* 24 hr after the cell inoculation. In combination with c-AMP, 6-MP was injected *i.p.* at the time of the injection of c-AMP.

Sleeping Time——Sleeping time was defined as the time between the loss and the recovery of righting reflex after the intraperitoneal injection of 50 mg/kg of sodium pentobarbital.

Enzyme Assay——For the assay of enzyme activities in the liver, mice were fasted for 12 hr before sacrifice. Xanthine oxidase activity in the liver homogenate was measured by the method of Roussos.[6] HGPRTase activity was assayed in $30000\times g$ supernatant of 10% liver homogenate prepared in 1 mM Tris buffer (pH 7.5) according to the method of Atkinson and Murray.[7]

Statistical Evaluation——Student's *t* test was used to determine the statistical significance of differences.

Results and Discussion

As shown in Table I, the mean sleeping time upon intraperitoneal administration of sodium pentobarbital (50 mg/kg) was 47.1 min. Prior administration of aminophylline (20 or 50 mg/kg/day × 7), caffeine (10 or 25 mg/kg/day × 7), or propranolol (2 or 10 mg/kg/day × 7) significantly shortened the sleeping time induced by pentobarbital. It is surmised that these drugs may change the activity of hepatic drug-metabolizing enzymes and thus cause a decrease in the activity of pentobarbital.

Table I. Effects of Aminophylline, Caffeine, and Propranolol on the Antitumor Activity of 6-MP or 6-MPR and on the Sleeping Time induced by Pentobarbital

Pretreatment (*i.p.*)	mg/kg/day	Antitumor activity[a] (Inhibitory %)		Sleeping time[b] mean ±S.E. (min)
		6-MP (80 mg/kg) mean±S.E.	6-MPR (300 mg/kg) mean±S.E.	
Saline	—	42.0±2.8	38.7±4.9	47.1±1.7
Aminophylline	20×7	27.5±3.0	40.0±2.9	35.0±5.4*
Aminophylline	50×7	24.4±1.0**	26.4±2.3	25.5±4.5*
Caffeine	10×7	39.2±4.8	32.5±3.2	25.3±5.3**
Caffeine	25×7	25.5±2.5	30.8±1.7	30.2±3.6**
Propranolol	2×7	15.6±0.9**	24.0±4.3	20.9±4.9**
Propranolol	10×7	18.7±1.6*	34.1±4.3	20.6±3.9**

Groups of 10 mice were used. *a*) The antitumor activity was estimated from the weight of the solid tumor compared with that of the saline-treated control on the 15th day after Ehrlich ascites carcinoma (1×10^6 cells) inoculation. Aminophylline, caffeine, propranolol, or saline (as a control) was injected *i.p.* once a day for 7 days, and cells were inoculated into the left thigh at the time of the final injections of these drugs. 6-MP, or 6-MPR was injected once *i.p.* 24 hr after the cell inoculation. b) Time between the loss and recovery of the righting reflex after 50 mg/kg, *i.p.*, of pentobarbital. Significant differences from the corresponding control group are indicated as *($p<0.05$) and ** ($p<0.01$).

As regards antitumor activity, the inhibitory effect of a single administration of 6-MP or 6-MPR against Ehrlich solid tumor was found to be dose-dependent (Fig. 1). The effect of 6-MP (80 mg/kg) or 6-MPR (300 mg/kg) on the tumor was examined alone and in combination with aminophylline, caffeine, or propranolol (Table I). The mean tumor weight ±S.E. of the control group on the 15 th day after the cell inoculation was 6.43±0.36 g. A single dose of 6-MP at 80 mg/kg or of 6-MPR at 300 mg/kg showed an inhibitory effect on tumor

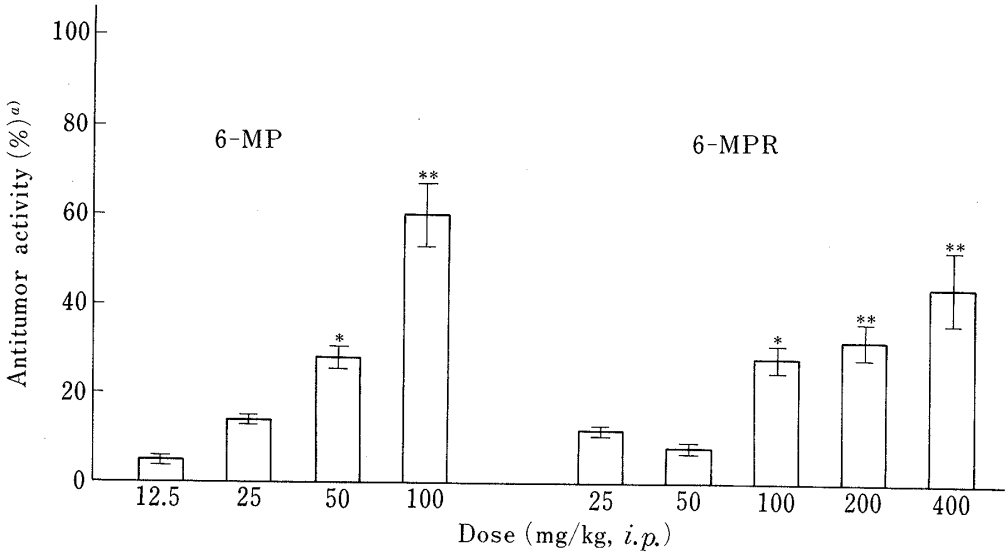

Fig. 1. Dose–dependent Inhibition of Tumor Growth by 6-MP and by 6-MPR

a) See Table I
Significant differences from the corresponding control (saline-treated) group are indicated as * ($p<0.05$) and ** ($p<0.01$).

growth, the tumor weight being 3.71±0.25 g or 3.94±0.50 g, respectively. After pretreatment with aminophylline, caffeine, or propranolol for 7 days, the inhibitory effect of a single injection of 6-MP or 6-MPR on the tumor growth decreased. However, the effects of drug pretreatment on the activity of 6-MPR were weaker than on that of 6-MP. Thus, the antitumor effect of 6-MP or 6-MPR was influenced by pretreatment with these drugs. In mice treated with these drugs for 3 days prior to the administration of 6-MP, the antitumor activity of 6-MP was weakened similarly.

As regards drug-metabolizing enzymes in the liver, it was observed that enzyme activities were changed considerably by pretreatment with any one of aminophylline, caffeine, and pro-

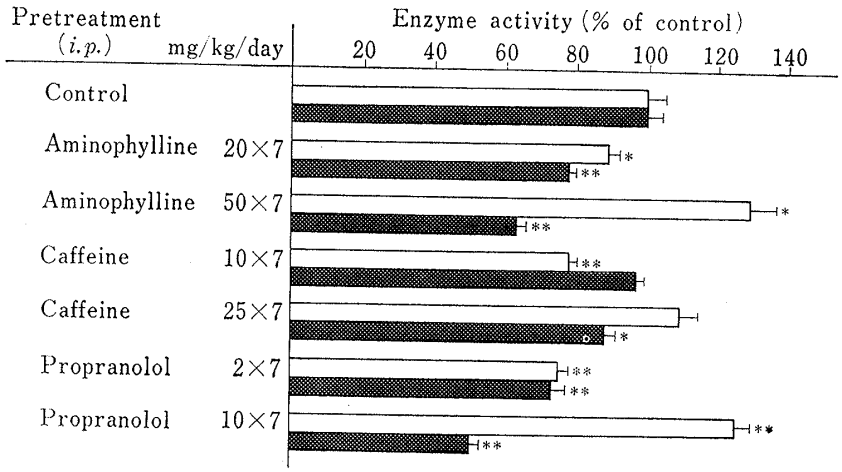

Fig. 2. Effects of Aminophylline, Caffeine, and Propranolol on Xanthine Oxidase and HGPRTase Activities in Mouse Liver

□: xanthine oxidase*a*); ■: HGPRTase*b*)
Groups of 8 mice were used. Pretreatment with drugs was carried out as described in Table I. Measurements of enzymes were performed 24 hr after the final injection of drugs. Each value is the mean activity and horizontal bars represent the standard errors of the means. Significant differences from the corresponding control group are indicated as * ($p<0.05$) and ** ($p<0.01$).

a) Activity was expressed as % of control uric acid formed. The control value was 210.3 ±10.0 nmol/min/g of liver.

b) Activity was expressed as % of control guanosine-5′-monophosphate formed. The control value was 317.4±12.9 nmol/min/g of liver.

pranolol. In particular, upon administration of higher doses of aminophylline and propranolol, the HGPRTase activity decreased and the xanthine oxidase activity increased to some extent as compared with the control values (Fig. 2).

It is known that the content of c-AMP is decreased by administration of propranolol (an inhibitor of adrenergic β-receptors) and is increased by aminophylline or caffeine (an inhibitor of phosphodiesterase). As regards the relationship between tumor growth and content of c-AMP in cells, Gericke and Chandra[3] reported an inhibitory effect of c-AMP on tumor growth.

In the present study, the effects of c-AMP and Dbc–AMP on the growth of Ehrlich solid tumor were examined. c-AMP and Dbc–AMP were injected close to the cell-inoculation site according to the method of Gericke and Chandra[3] once a day for 7 days starting 24 hr after the cell inoculation. However, no inhibitory effect was observed upon administration of c-AMP (50 or 150 mg/kg, *i.p.*) or Dbc–AMP (5, 25 or 125 mg/kg, *i.p.*). In contrast, administration (once a day for 7 days) of 20 mg/kg, *i.p.*, of 6-MP inhibited tumor growth to about 60%. The inhibitory effect of c-AMP (50 mg/kg/day$\times$7, into cell-inoculation site) combined with 6-MP (20 mg/kg/day$\times$7, *i.p.*) was similar to that of 6-MP (20 mg/kg/day$\times$7, *i.p.*) alone. These experimental results are not in accord with the report on the antitumor activity of c-AMP by Gericke and Chandra.[3]

The major experimental results can be summarized as follows. (1) The antitumor activity of 6-MP on Ehrlich solid tumor in mice was decreased by a high dose of aminophylline or propranolol. (2) Pentobarbital-induced sleeping time (measured to monitor the activity of hepatic drug-metabolizing enzymes) was shortened by pretreatment with either of the above drugs. (3) On pretreatment with a high dose of aminophylline or propranolol, HGPRTase activity was decreased and xanthine oxidase activity was increased significantly.

Thus, it seems likely that prior administration of higher doses of aminophylline and propranolol promote the conversion from 6-MP to biologically inactive thiouric acid or hypoxanthine rather than to biologically active TIMP.

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References and Notes

1) *a*) T. Higuchi, T. Nakamura, and H. Uchino, *Cancer Res.*, **37**, 3668 (1977); *b*) T. Higuchi, T. Nakamura, and H. Uchino, *Biochem. Pharmacol.*, **27**, 2507 (1978); *c*) G.B. Elion, S. Callahan, H. Nathan, S. Bieber, R.W. Roundles, and G.H. Hitchings, *Biochem. Pharmacol.*, **12**, 85 (1962).
2) K. Sasaki, S. Furusawa, and G. Takayanagi, *Nippon Yakurigaku Zasshi*, **77**, 131 (1981).
3) D. Gericke and P. Chandra, *Hoppe-Seyler's Z. Physiol. Chem.*, **350**, 1469 (1969).
4) R.R. Burk, *Nature*, **219**, 1272 (1968).
5) M. d'Armiento, G.S. Johnson, and I. Pastan, *Proc. Nat. Acad. Sci. U.S.A.*, **69**, 459 (1972).
6) G.G. Roussos, "Methods in Enzymology," Vol. 12, ed. by L. Grossman and K. Moldane, Academic Press, New York, 1968, p. 5.
7) M.R. Atkinson and A.W. Murray, *Biochem. J.*, **94**, 64 (1965).