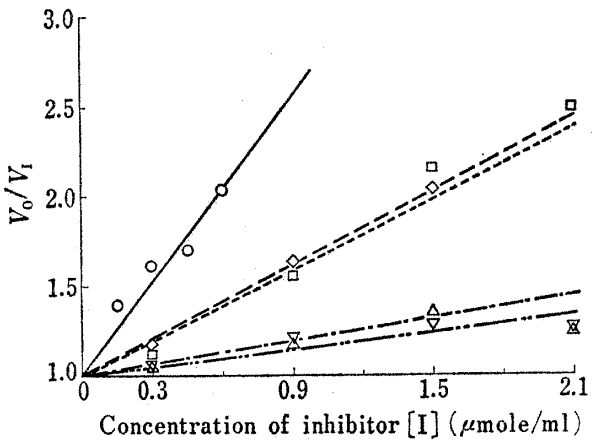

Fig. 2. Effect of Derivatives of AICA on the Activity of Guanine Deaminase

-○-: AICA -◇-: FAICA -□-: AICAR
-▽-: TAICA -△-: TAICAR

nated in the enzyme preparation.[8] But none of the above compounds was detected in the mixture after 30 min incubation. The activity of AICA was reduced by the introduction with either formyl to the amino group at position 5 or ribofuranosyl to the nitrogen at position 1.

Effects of Derivatives of 5-Amino-4-imidazolethiocarboxamide

Two mercapto derivatives having antitumor activity were tested. TAICA and TAICAR were almost inactive against guanine deaminase. 5-Formamido-4-imidazolethiocarboxamide, which had potent antitumor activity, could not be examined because of its insolubility in the incubation mixture. When the oxygen of the carboxamide group at position 4 of AICA was substituted with sulfur, the inhibitory activity was reduced.

To conclude, an inhibitor more active than AICA for guanine deaminase (3.5.4.3) was not found among some derivatives of AICA tested in this investigation, and these results suggested that the amino group at position 5, the hydrogen at position 1 and the oxygen of carboxamide group at position 4 of AICA were necessary for the inhibition of the enzyme activity.

Acknowledgement The authors are indebted to laboratory members of the Central Research Laboratories, Ajinomoto Co., Inc. for their supply of the test compounds.

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The Effects of DBM on Rat Ascites Tumors and Antibody Production

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DBM is 1,6-dibromo-1,6-dideoxy-D-mannitol (dibromomannitol, myelobromol, NSC-94100) as shown in Fig. 1. The effectiveness of DBM in chronic myeloid leukemia was first reported by Eckhardt, *et al.*[2,3] Szentkláray[4] attained complete remission in patients suffering from polycythemia vera. In experimental tumor this compound has an excellent effect against solid strain of Yoshida sarcoma on transplantation subcutaneously.[5] Several cross-resistant tests have been carried out also with various types of antitumor agents with DBM-

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2) S. Eckhardt, C. Sellei, I.P. Honváth and L. Institoris, *Cancer Chem. Rep.*, 33, 57 (1963).
3) C. Selfei and S. Ekhardt, *Rev. Franc. Etude. Clin. Biol.*, **8**, 483 (1963).
4) J. Szentkláray, *Orv. Hetil.*, **107**, 2182 (1966).
5) E. Csányi, *Arzneim.-Forsch.*, **14**, 668 (1964).

resistant Yoshida subcutaneous sarcoma.[6] However, little work has been done to study of antitumor effect using an ascites strain of Yoshida sarcoma and other rat tumors. While, this antitumor drug has no immunosuppressive activity in the kidney homotransplantations of dogs.[7]

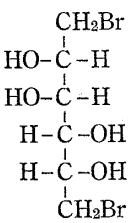

Fig. 1. DBM

The present study deals with the antitumor effect of DBM on Yoshida ascites sarcoma, nitrogen mustard (HN2)-resistant Yoshida ascites sarcoma (RA_{C-22}, YS_{C-20}) and rat ascites hepatoma (AH-13, AH-66, AH-7974). We studied also on antibody producing effect when the drug was administered intraperitoneally to mice after the infection of sheep red cells.

Table I illustrates the results of primary screening on cytotoxic effects. The cell effects were observed markedly in two strains, Yoshida sarcoma and ascites hepatoma AH-13. The maximum cytomorphological changes of the tumor cells appeared 48 hours after the intraperitoneal injection of DBM, and 72 hours after the oral administration of the drug, respectively. The effects have scarecely been revealed on the HN2-resistant Yoshida sarcomas and rat ascites hepatoma AH-66 or AH-7974 as far as administration of 1000 mg DBM per kg body weight of the rat.

Table I. Primary Screening Data of DBM

Tumor	MTD (mg/kg)[a] *i.p.* or *p.o.*[c]	MED (mg/kg)[b] *i.p.*[c]	*p.o.*[c]
Yoshida sarcoma	>1000	50	100
RA_{C-22}	>1000	>1000	>1000
YS_{C-20}	>1000	1000	>1000
AH-13	>1000	25	50
AH-66	>1000	>1000	>1000
AH-7974	>1000	1000	>1000

a) maximum tolerated dose *b*) minimum effective dose *c*) routes of DBM administration

Table II. Prolongation Experiment of Survival Time

Tumor	Drug	Route	Dose (mg/kg)	Survivor 30 days	60 days	Survival[a] days
Yoshida sarcoma	Control[b]	ip		0/10		8
	DBM	ip	75 ×12	0/10		12
	DBM	ip	150 ×12	0/10		12
	DBM	po	75 ×12	0/10		12
	DBM	po	175 ×12	0/10		12
	MT-C	ip	0.25×12	0/10		14
	MT-C	po	0.25×12	0/10		8
AH-13	Control[b]	ip		0/10		7
	DBM	ip	75 ×12	10/10	8/10	>60
	DBM	po	75 ×12	10/10	9/10	>60
	MT-C	ip	0.25×12	8/10	5/10	42
	MT-C	po	0.25×12	2/10	2/10	7

a) Survival times when 50% of the hosts were dead. *b*) Physiological saline solution was injected.

Then we studied further on life prolongation test of Yoshida sarcoma and AH-13 bearing rats as shown in Table II. Mitomycin C (MT-C) which is a potent antitumor agent was used as a reference drug. The comparisons yielded the data shown in Table II. DBM was effective

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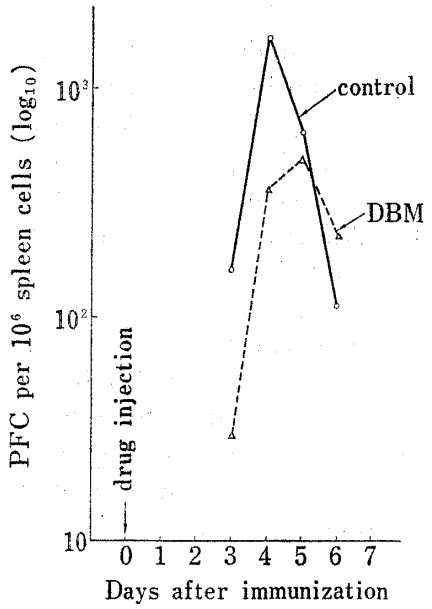

Fig. 2. Effect of DBM on the Number of Hemolitic Plaque Forming Cells in the Spleens of Immunized Mice

DBM injection was performed 1 hour after immunization.

both intraperitoneal and oral administrations to the AH-13 bearing rats, but was less effective on the Yoshida ascites tumor. In view of these results, DBM has no extensive effects against the rat ascites tumors widely. Body weight of animals given DBM, however, was not evidently decreased through these experiments. According to Csányi,[8] the LD_{50} of DBM for rats on single intraperitoneal injection is 1700 mg/kg and on oral administration is 2200 mg/kg, respectively. These data show the low toxicity of the drug to the animals.

As shown in Fig. 2, the number of antibody plaque forming cells (PFC) in the spleen of DBM treated mice was slightly low but insignificant degree compared with control mice given saline alone. It seems that cytostatic effect may develop without any severe damage of the immunological reaction of the animals. Moreover, the patients may be treated to cure with these mild effects of the drug.

Experimental

DBM was purchased from Medimpex Co., Budapest.

Procedure of Antitumor Screening——Animals used in these experiments were female Donryu rats, weighing 100—150 g, supplied from Nippon Rat Co. All animals were kept on standard diet CE-2 of CLEA Japan Co. with unlimited supply of water.

Tumor cells used were Yoshida ascites sarcoma, HN2-resistant lines of Yoshida sarcoma, RA_{C-22}, YS_{C-20} which have 100-fold resistance against HN2 *in vitro* and rat ascites hepatomas, AH-13, AH-66 and AH-7974.

The procedure was almost similar to that described by Yoshida, *et al.*[9] Three-day-old tumor cells grown in Donryu rats were used. Three days after intraperitoneal inoculation of 10^6 tumor cells, a single intraperitoneal or oral administration of the drug was given as the primary screening. For determination of life span-prolongation effects, rats were inoculated intraperitoneally with 10^6 cells of Yoshida or AH-13 tumors which had been grown for 3 days in the peritoneal cavity of rats. The drugs to be tested were given three days after the inoculation, and the additional dosages were given daily for two weeks.

Determination of Immunosuppressive Activity——Non-imbred ICR-JCL mice were obtained from Japan CLEA Co. They were 5—6 weeks of age at start of an experiment and weighed 25—34 g. The animals were kept on the CE-2 diet and water *ad libitum*.

Sheep red blood cells obtained commercially from Shiihashi Co. were washed three times in $NaHCO_3$-free Tyrode solution by serial low speed centrifugation, and resuspended in the Tyrode to a concentration of 10^9 red cells per ml.

Test and control mice were immunized by intravenous inoculation of 4×10^8 sheep red cells and 3 animals of each group were sacrificed at closely spaced intervals thereafter, on the 3rd, 4th, 5th and 6th day after immunization. DBM (475 mg/kg) were injected intraperitoneally at 1 hour after the infection of the antigen.

Determination of antibody production in the spleen was carried out according to the method of Jerne, *et al.*[10] However, a freshly prepared spleen cell suspension in Eagle's medium was treated with NH_4Cl solution because of removal of red cells. And plaque assays were performed triplicate in each of the test animals and the mean of triplicate counts was expressed as the number of hemolitic plaque forming cells per 10^6 spleen cells.

Acknowledgement The authers wish to express gratitude to Dr. Yoshio Sakurai of Cancer Institute for his relevant information and generous supplies of the tumor cells.

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