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Antitumor Effect of Polysaccharides and Its Relationship to Biogenetic Amines in Tumor Tissues under Vitamin B₆ Deficient Feeding

From the result of screening test by the host-mediated antitumor bioassay¹⁾ we (F.F. and T.I.) found many antitumor polysaccharides, especially from *Basidiomycetes* and reported on the antitumor activities against transplanted sarcoma 180 and the chemical characterization.²⁾ In the studies on the mechanisms of the antitumor polysaccharides T. Okuda, *et al.* found some correlation between the host-mediated antitumor activity and complement C3 inactivation.³⁾

Furthermore it was found by skin reaction using guinea pig that in sarcoma 180 bearing mice when treated with the antitumor polysaccharides, more C3a was formed than in untreated mice or in mice treated with polysaccharides having no antitumor activity.³⁾ It was suggested that histamine release *in vivo* may be stimulated by administration of the antitumor polysaccharides.

On the other hand, we (T.O., M.S. and I.T.) reported that a vitamin B₆ antagonist, 3-aminopyridoxol (3-NH₂-POL) more highly inhibited the growth of *Saccharomyces carlsbergensis* 4228 (ATCC 9080) than 4-deoxyprydoxol (4-DOP) did.⁴⁾ The 50% inhibition index of 3-NH₂-POL was 35, while that of 4-DOP was 80 or 90. Vitamin B₆ is a coenzyme to form histamine from histidine and serotonin from 5-hydroxytryptophan.

In this communication we report on the host-mediated antitumor effect and biogenetic amine value in the tumor when treated with antitumor polysaccharide and vitamin B₆ antagonists under feeding on vitamin B₆ deficient diet. An antitumor polysaccharide used in this study was EA₃, which was isolated from *Flammulina velutipes* and shown to be a β (1 \rightarrow 3)-glucan.⁵⁾ Vitamin B₆ deficient diet ingredients are as follows: vitamin free test casein 18.0%, sucrose 73.8%, corn oil 4.0%, McCollum salt 4.0% and choline chloride 0.2%, and vitamin supplements per 100 g are vitamin A 1200 IU, vitamin D 120 IU, thiamine-HCl 1.0 mg, riboflavine 1.0 mg, niacin 4.0 mg, pantothenic acid 6.0 mg, iso-inositol 13.0 mg, PA-BA 20.0 mg, biotin 20 mcg and folic acid 20 mcg. Complete diet is a diet added with pyridoxine-HCl 1.0 mg to the above. The mice were fed for 6 weeks since 2 weeks prior to the tumor implantation.

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- 2) a) T. Ikekawa, M. Nakanishi, N. Uehara, G. Chihara, and F. Fukuoka, *Gann*, **59**, 155 (1968); b) T. Ikekawa, N. Uehara, Y. Maeda, M. Nakanishi, and F. Fukuoka, *Cancer Res.*, **29**, 734 (1969); c) G. Chihara, J. Hamuro, Y. Maeda, Y. Arai, and F. Fukuoka, *ibid.*, **30**, 2776 (1970); d) Y. Yoshioka, T. Ikekawa, M. Noda, and F. Fukuoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1175 (1972).
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The ascites tumor of sarcoma 180 (about 6×10^6 cells) was transplanted subcutaneously into the right groin of ICR mouse. The polysaccharide and vitamin B₆ antagonists were administered by intraperitoneal injection 24 hours after tumor implantation and administration of the latter was continued as a rule every other day through 4 weeks, and that of the former for 10 days. At the end of 4 weeks after transplantation the animals were sacrificed and the tumors were weighed, and inhibition ratios were calculated comparing with tumor weight of the control mice. The concentration of the amines extracted from the homogenized tumor tissues were determined by fluorometric method.⁶⁾

As shown in Table I, tumor inhibition ratio in vitamin B₆ deficient diet was about 20% higher than that of the control, and the ratio when treated with 3-NH₂-POL under vitamin B₆ deficient diet feeding was 50% higher, and when treated with 4-DOP, 87% higher than that of the control without complete regression. Under vitamin B₆ deficient diet the inhibition ratios were 10 or 20% higher than those of EA₃ under complete diet feeding. When

TABLE I. Antitumor Effect

Experimental group	Dose mg/kg × day	Inhibition ratio %	Complete regression
(A) Complete diet (control)			0/8
(B) V.B ₆ defi. diet		23	0/8
(C) V.B ₆ defi. diet 3-NH ₂ -POL	43 × 14	50	0/8
(D) V.B ₆ defi. diet 4-DOP	43 × 12	87	0/8
(E) V.B ₆ defi. diet (a) EA ₃	5 × 10	96	1/8
(b) EA ₃	1 × 10	96	2/8
(F) V.B ₆ defi. diet (a) 3-NH ₂ -POL EA ₃	43 × 13 5 × 10	98	3/8
(b) 3-NH ₂ -POL EA ₃	43 × 14 1 × 10	99	4/7
(G) V.B ₆ defi. diet 4-DOP EA ₃	43 × 14 1 × 10	95	0/7
(H) Complete diet (a) EA ₃	5 × 10	87	0/8
(b) EA ₃	1 × 10	77	1/7

TABLE II. Histamine and Serotonin Values in Tumor Tissue

Experimental group ^{a)}	Histamine	Serotonin	Experimental group ^{a)}	Histamine	Serotonin
(A)	100	100	(F) (a)	186	256
(B)	61	81	(b)	309	1180
(C)	81	91	(G)	68	328
(D)	— ^{b)}	55	(H) (a)	351	236
(E) (a)	152	180	(b)	— ^{b)}	328
(b)	41	414			

^{a)} The sample and dose are same to Table I.

^{b)} Accurate values were not obtained because of contamination of other amines in this experiment.

6) ^{a)} W. Lovenberg and K. Engelman, "Method of Biochemical Analysis," ed. by D. Glick, *Suppl. Vol.*, Interscience Publishers, New York, 1971, p. 1; ^{b)} P.A. Shore, *ibid.*, 1971, p. 89.

treated with EA₃ and the antagonist together, the inhibitory effect was remarkably high, when 3-NH₂-POL was used as an antagonist, complete tumor regression increased as shown in Table I.

Table II showed the histamine and serotonin values of tumor tissues at the end of 4 weeks after transplantation. The histamine and serotonin values decreased under vitamin B₆ deficient diet without EA₃ administration, and this tendency was an expected result. When treated with EA₃ in dosage of 5 mg/kg/day, the histamine value increased remarkably and the same tendency was found when treated with EA₃ and the antagonists together. However, this tendency was not observed in dosage of 1 mg/kg/day of EA₃. No clear correlation between antitumor effect and histamine value in the tumor tissues was found at 4 weeks after transplantation.

The constant tendency was not obtained in the histamine value at different dosage of EA₃ administration, but the serotonin value highly increased at both dosages of 5 mg/kg/day and 1 mg/kg/day of EA₃ in comparison with that of the control.

Thus it may be considerable that there is some correlation between the host-mediated antitumor effect and the serotonin value in tumor tissue. It may also be of interest that serotonin itself has some inhibitory effect on the growth of the solid tumor of sarcoma 180.⁷⁾

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11-O-Galactosyl-nogiragenin, a Prosapogenol of *Metanartheceium luteo-viride* MAXIM. obtained by Soil Bacterial Hydrolysis

In search of the genuine sapogenols of *Metanartheceium luteo-viride* MAXIM. (Japanese name: nogiran), whose steroidal sapogenols were extensively investigated by Takeda, *et al.*,¹⁾ we have been examining the usefulness of soil bacterial hydrolysis method²⁾ for the glycosides

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- 2) a) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; b) I. Yosioka, T. Sugawara, K. Yoshikawa, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 2450 (1972), and the preceding papers of the series cited therein.