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Relationship between Antitumor Effect of Polysaccharides and Biogenic Amines in Tumor Tissues

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Relationship between the host-mediated antitumor effect and biogenic amines was investigated. Histamine and serotonin values in solid tumor of sarcoma 180 were periodically determined by fluorometric method. When treated with the antitumor active polysaccharide (EA_5), significantly high histamine values were detected at the end of 2 weeks after tumor transplantation. Although some increase of the histamine value was also found at 2 and 3 weeks by treatment with the antitumor inactive polysaccharide (EA_{505}) and/or cyclic adenosine monophosphate it was not significant in comparison with the remarkable increase in the antitumor active polysaccharide (EA_5) treatment. Any remarkable increase of serotonin values in tumor tissues was not found.

In our previous paper we reported that polysaccharides from *Flammulina velutipes* (Curt. ex Fr.) Sing., an edible mushroom, showed remarkably high antitumor activity by the host-mediated antitumor bioassay using solid tumor of sarcoma 180, but not so high activity by the total packed cell volume method using ascites tumor of sarcoma 180.[2—4]

In order to elucidate the mechanism of the host-mediated antitumor effect of the polysaccharides from biochemical approach, relation between the effect and the biogenic amines in tumor tissues was investigated as previously reported.[5] It was found that there was some correlation between serotonin values in tumor tissues and the antitumor effect when experimental animals were treated with an antitumor active polysaccharide and vitamin B_6 antagonists under vitamin B_6 deficient feeding. But no clear correlation between the antitumor effect and histamine values in the tumor tissues was found at the end of four weeks after transplantation.[5] In this study we deal with time course of biogenic amine values in the tumor tissues, and some correlation between the antitumor effect and periodic biogenic amine values is found.

Experimental

Materials——Antitumor polysaccharides used in this study, EA_5 and EA_{505} were isolated from *F. velutipes*, and in the antitumor bioassay against solid tumor of sarcoma 180 inhibition ratio of EA_5 was 84% at a dose of 1 mg/kg/day in 10 days administration, and complete regression was observed in 4 out of 10 mice, but that of EA_{505} was only 18% and no complete regression was observed at the same dose.[2,4,6] Therefore, the former was used as an antitumor active polysaccharide and the latter as an antitumor inactive polysaccharide.

Antitumor Bioassay——Seven-day-old ascites tumor of sarcoma 180 (about 8×10^6 cells) were subcutaneously transplanted in the right groin of ICR mice, and beginning 24 hrs after transplantation the polysac-

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charide samples were administered daily at a dose of 1 mg/kg/day except one week's group. In one week's group the samples were administered until the mice were sacrificed. Cyclic AMP was intraperitoneally daily administered at a dose of 13.6 mg/kg/day until the mice were sacrificed in each group. At the end of each week after tumor transplantation mice of each group were sacrificed and the solid tumors were dissected out. Inhibition ratios were determined by comparing the tumor weight of the treated mice with that of the untreated mice.

Determination of Biogenic Amines[5,7]——a) Histamine: About 1 g of the tumor tissue was homogenized and histamine was extracted with 7 ml of 0.4 N perchloric acid aq., and it was extracted with 10 ml of *n*-BuOH after adding 0.5 ml of 5 N NaOH aq. and excess NaCl. To 5 ml of the *n*-BuOH solution 4 ml of 0.1 N HCl aq. and 15 ml of *n*-heptane was added and shaken vigorously, and then the amine was extracted to a water layer. To 2 ml aliquot of the amine solution 0.4 ml of 1 N NaOH aq. and 0.1 ml of *ortho*-phthalaldehyde (OPT) reagent were added. After adding 0.2 ml of 3 N HCl aq. the optical density of the fluorescence at 450 mμ resulting from activation at 360 mμ was measured.

b) Serotonin: The tumor tissue (*ca.* 1 g) was homogenized with 4 ml of 0.1 N HCl aq. and the extract was adjusted pH 10 with Na_2CO_3 aq. After adding 5 ml of borate buffer, 10 ml of *n*-BuOH and excess NaCl, it was shaken vigorously.

To 5 ml of the *n*-BuOH solution, 20 ml of *n*-heptane and 1.5 ml of 0.1 N HCl aq. were added and shaken for *ca.* 5 min. To 0.2 ml of aliquot of the serotonin extract 1.2 ml of OPT reagent was added and heated on a boiling water bath for 15 min. After cooling the optical density of the fluorescence at 470 mμ resulting from activation 360 mμ was measured.

Taking the biogenic amine values of the control mice as 100, the numerical values of the treated mice were calculated.

Results and Discussion

In the antitumor bioassay to test the host-mediated action of the polysaccharides inhibition ratio was generally determined at the end of 5 weeks after tumor transplantation. Inhibition ratio of EA_5 showed 84% at a dose of 1 mg/kg at the end of 5 weeks after transplantation, but that of EA_{505} was only 18% at the same dose.[2,4,6] Inhibition ratios of EA_5 and EA_{505} were 69 and 3%, respectively, at the end of 4 weeks after transplantation as shown in Table I.

Table I. Antitumor Effect and Biogenic Amine Value

Experimental group	Antitumor effect Inhibition ratio 4 weeks	Amine value Histamine 1	2	3	4 weeks	Serotonin 2 weeks
EA_5	69%	139	768	289	179	115
EA_{505}	3	60	234	209	88	158
EA_{505} + cyclic AMP	5	216	302	269	68	180
Cyclic AMP	−18	71	389	189	86	189
Control	—	100	100	100	100	100

Table I also shows the amine values in the tumor tissues at the end of each week. When treated with EA_5, the histamine value was more than seven times of that of the control at the end of 2 weeks, and after 3 weeks it decreased gradually. No significant difference was found in serotonin values of each experimental group at the end of 2 weeks and it was not detectable by fluorometric method at other weeks.

It was reported that biogenic amines, especially histamine, enhanced cyclic AMP level *in vivo*,[8] and moreover, cyclic AMP enhanced antibody formation.[9] Considering these results we tested the antitumor effect of the inactive polysaccharide (EA_{505}) in combination with

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cyclic AMP although cyclic AMP itself is inactive. However, no synergic antitumor effect was found between them. When treated with the antitumor active polysaccharide (EA_5), significantly high histamine values were detected at the end of 2 weeks after transplantation. Although some increase of the histamine value was also found at 2 and 3 weeks by treatment with the antitumor inactive polysaccharide (EA_{505}) and/or cyclic AMP, it was not significant in comparison with the remarkable increase in the antitumor active polysaccharide (EA_5) treatment.

Similarity of the antitumor activity between serotonin and the active polysaccharide was discussed and it was noted that the serotonin content increased in case of treatment with an active polysaccharide.[10] However, our experimental result showed that the histamine content in the solid tumor markedly increased, particularly at the end of 2 weeks, but the serotonin content did not increase even when treated with the active polysaccharide. Though it might be thought as if serotonin is related to mechanism of the host-mediated action from similarity of the antitumor effect of serotonin and polysaccharides, a conclusion may be proper that serotonin was not practically concerned with mechanism of the so-called "host-mediated" antitumor effect.

When treated with polysaccharides, the host-mediated tumor inhibitory effect is generally obscure at the end of 2 weeks, but when treated with the active polysaccharide the histamine content showed significant increase at that time in comparison with the control or the inactive polysaccharide as described above. This result showed that some essential action of the host-mediated antitumor effect by the active polysaccharide initiated before revelation of the apparent effect of tumor inhibition. This does not conflict with the reports on X-ray irradiation studies and other works.[11–13] In addition, this result indicated something in common with the fact that the local lymphoid reaction was found at an early period by treatment with the active polysaccharides after implantation.[14]

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