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Effect of Chondroitin Sulfate on the Growth of Solid Ehrlich Ascites Tumor under the Influences of Other Interstitial Components

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SUMMARY

The influences of collagen, L-hydroxyproline, fibrinogen, egg albumin, calf serum, and muramidase on tumor growth were studied in relation to the growth-promoting activity of chondroitin sulfate using solid hypotetraploid Ehrlich ascites tumor.

One ml of the solutions of substances tested, chondroitin sulfate, and the mixtures of the substance and chondroitin sulfate were given, respectively, s.c. into the back of SMA and ddN mice, followed immediately by the injection of Ehrlich tumor. Average tumor weights in experimental groups were compared with those in control groups, which were given isotonic saline on Day 8 after tumor inoculation.

The effect of chondroitin sulfate, which stimulated the growth of tumor, was markedly inhibited by egg albumin, calf serum, and muramidase. It was, however, only weakly inhibited by a collagen preparation, L-hydroxyproline, or fibrinogen, though they tended to inhibit the tumor growth.

It may be considered that inhibitory effect of these substances on the growth-promoting activity of chondroitin sulfate was due to the decrease of the negative electric charge of chondroitin sulfate.

INTRODUCTION

In a previous paper, this author (20, 21) demonstrated the promoting effect of chondroitin sulfate on the growth of solid Ehrlich ascites tumor, which tended to be accelerated as the amounts or concentrations of chondroitin sulfate were increased. He also indicated that chondroitin sulfate counteracted the inhibitory effect of hydrocortisone acetate on the tumor growth (22). The exact mechanism, however, of the action of chondroitin sulfate on the tumor growth could not be deduced from these results, though it was conceivable that chondroitin sulfate helped tumor growth by protecting the surface of tumor cells and promoting the exchange of their metabolite.

In order to elucidate the mechanism of the growth-promoting activity of chondroitin sulfate, relations between the effects of chondroitin sulfate and other interstitial components on the tumor growth were investigated. Effects of collagen, L-hydroxyproline, fibrinogen, egg albumin, calf serum, and muramidase were examined by measuring tumor weights by the same procedures as described in the previous paper (20, 21, 24).

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MATERIALS AND METHODS

Animals used throughout this experiment were male ddN mice 60–70 days old, obtained from Nihon Clea Co. Ltd., Tokyo, and male SMA mice [described in Staats' Listing (19)] 70–100 days old, obtained from the Centre Supplying Laboratory Animals in Nagoya University School of Medicine. They were fed with a standard pellet (CA-1, Nihon Clea Co. Ltd., Tokyo) and given drinking water *ad libitum*.

Tumor cells used in this study were Ehrlich hypotetraploid stock (Kaziwara 4n) (12) maintained in adult male SMA and ddN mice through serial i.p. transplantation at 7- or 8-day intervals in this laboratory.

One ml of the substances tested was injected s.c. into the back of each mouse, immediately followed by injection of 0.1 ml of the Ehrlich tumor ascitic fluid, containing 8×10^6 cells, into the same site. In the control, isotonic saline was injected before the tumor inoculation. These animals were killed on the 8th day after tumor inoculation, and the solid tumor which developed s.c. was excised and weighed. The results of the experiments were evaluated on the basis of the average weight of tumor tissues in the experimental groups in comparison with the control groups.

The substances tested were prepared in the following ways: Chondroitin sulfate C (average molecular weight about 50,000), obtained from Kaken Yakukako Co. Ltd., Tokyo, was dissolved in isotonic saline. Collagen (from bovine achilles tendon) was obtained from Tokyo Chemical Industry Ltd., Tokyo. Collagen substrate was prepared according to Ehrmann and Gey's procedure (4): 2 gm of collagen were dissolved in 100 ml of 0.3% acetic acid solution and then centrifuged at 3,000 rpm for 30 min; the supernatant was reconstituted by dialysis against distilled water.

Fibrinogen (Bovine Fibrinogen, Fraction I, Daiichi Pure Chemicals Co., Ltd., Tokyo), L-hydroxyproline (Katayama Chem. Co. Ltd., Osaka), albumin (egg albumin, Maruzen & Co., Nagoya), and muramidase (*N*-acetylmuramide glycanonhydrolase) (Neuzym, Eizai Co. Ltd., Osaka) were dissolved in isotonic saline. Serum (calf serum, Chiba Serum Research Institute, Chiba Prefecture) was used without any preliminary treatment.

The mixture of chondroitin sulfate and each of the materials was prepared as follows: 200 mg of chondroitin sulfate C were dissolved in each 10 ml of the collagen substrate and the calf serum and also in the same quantity of 2% solutions of L-hydroxyproline, albumin, fibrinogen, and muramidase.

RESULTS

Solid Ehrlich tumors were successfully produced by s.c. injection of ascitic tumor cells in all cases.

Table 1 indicates effects of the substances tested, the mixtures of each substance and chondroitin sulfate, and 2% chondroitin sulfate solution on the tumor growth. In the cases of the mixtures of chondroitin sulfate and egg albumin, calf serum, and muramidase, no significant differences were noted in the tumor weight between the mixture-treated groups and the respective control groups. In the collagen, hydroxyproline, and fibrinogen groups, however, the addition of chondroitin sulfate accelerated the tumor growth to some extent; the average tumor weights were greater in the mixture groups than in the control groups, the difference between them being statistically significant ($P < 0.001$ in each case). A significant increase of average tumor weights in 2% chondroitin sulfate-treated groups compared with those in control groups was consistently observed, as shown in previous papers (20, 21). The growth-promoting activity of chondroitin sulfate was inhibited by each of the substances tested; the difference of tumor weight between each mixture-treated group and 2% chondroitin sulfate-treated group was statistically significant. The differences were highly significant in the cases of the mixture-treated groups with egg albumin, calf serum, and muramidase, while it was considerably less significant in the cases where collagen, hydroxyproline, or fibrinogen respectively were mixed with chondroitin sulfate. These results show that the promoting activity of chondroitin sulfate on the tumor growth was intensely inhibited by egg albumin, calf serum, and muramidase, but that the inhibitory effect of a collagen preparation, hydroxyproline, or fibrinogen was weak.

Collagen preparation (undiluted) and 2% and 0.2% fibrinogen solution inhibited the growth of tumor to some degree, but no stimulatory effect on the tumor growth was observed in any other substances tested, as shown in Table 1 and Chart 1.

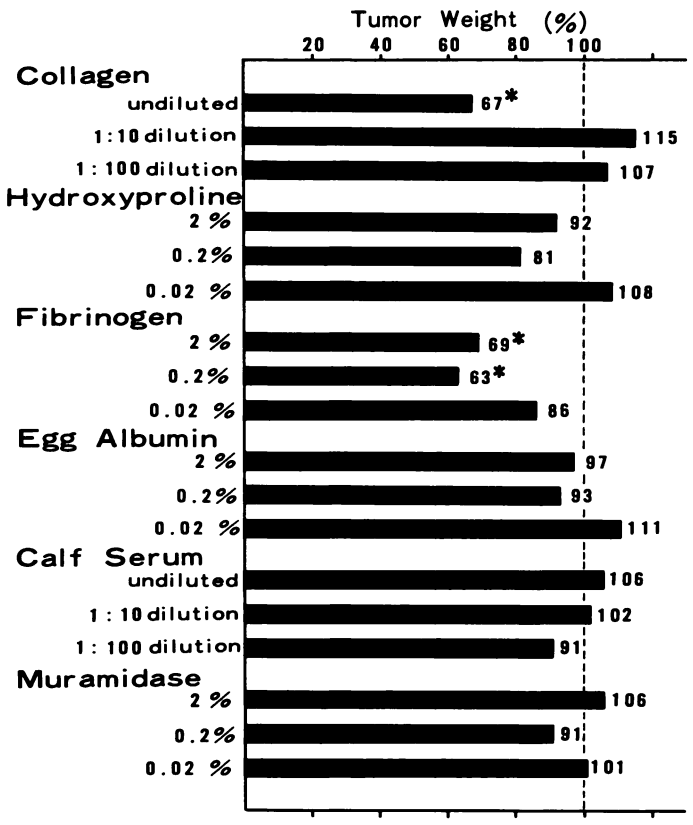

Chart 1. Effect of different concentrations of each substance tested on the growth of solid Ehrlich ascites tumor on the 8th day after inoculation. The bars indicate % of tumor size against control tumor size in each experiment. From 9 to 10 mice were used in each group. *Dashed line* indicates the tumor size of control groups (100%). The asterisk shows that the difference between average tumor weight in experimental group and that in control is significant.

Table 1

	Substance *A* alone			Mixture of substance *A* and chondroitin sulfate				
Substance *A*	No. of mice	% increase or decrease over control (saline) group[a]	P[a]	No. of mice	% increase or decrease over control (saline) group[a]	P[a]	% increase or decrease over 2% chondroitin sulfate group[b]	P[b]
Collagen (undiluted)	40	−20	$0.01 < P < 0.02$	40	+54	$P < 0.001$	−17	$0.02 < P < 0.05$
2% hydroxyproline	30	− 7	$0.3 < P < 0.4$	30	+34	$P < 0.001$	−25	$0.001 < P < 0.005$
2% fibrinogen	27	−30	$0.001 < P < 0.005$	28	+46	$P < 0.001$	−20	$0.01 < P < 0.02$
2% egg albumin	20	+13	$0.1 < P < 0.2$	20	+11	$0.2 < P < 0.3$	−32	$P < 0.001$
Calf serum (undiluted)	30	+21	$0.1 < P < 0.2$	29	+15	$0.2 < P < 0.3$	−42	$P < 0.001$
2% muramidase	27	− 1	$0.9 < P$	28	− 3	$0.7 < P < 0.8$	−46	$P < 0.001$
2% chondroitin sulfate	175	+83	$P < 0.001$					

Effect of collagen, L-hydroxyproline, fibrinogen, egg albumin, calf serum, and muramidase on growth-promoting activity of chondroitin sulfate on solid Ehrlich ascites tumor on the 8th day after inoculation. P[a], Statistical significance of difference in average tumor weight between experimental group and control (saline) group; P[b], Statistical significance of difference in average tumor weight between mixture-group and 2% chondroitin sulfate group. The evaluation was based on the Student *t* test.

[a] % increase or decrease of average tumor weight over control values, which are the average tumor weights in animals receiving isotonic saline.

[b] % increase or decrease of average tumor weight in mixture groups, which were given mixture solutions of chondroitin sulfate and substances tested, over those in the chondroitin sulfate group which was given 2% chondroitin sulfate alone.

DISCUSSION

Present data indicate that egg albumin, calf serum, and muramidase inhibited the promoting activity of chondroitin sulfate on the tumor growth, but it was counteracted to a certain extent by collagen, hydroxyproline, or fibrinogen. Our results also show that no stimulatory effect of these substances tested on the growth of tumor was observed except in the cases of undiluted collagen preparation and 2% and 0.2% fibrinogen, though there has been divergence of opinions on the influences of these substances (2, 5–8, 10, 13, 18).

The interactions between acid mucopolysaccharides and the substances tested in this experiment were biochemically investigated by many workers. Meyer (15) described that by local acidification in the immediate neighborhood of the fibroblasts, the precursor of collagen was denatured by the polysaccharides, the latter acting as anionic detergents rolling up the peptide chains along the acidic groups of the fibrous polysaccharide molecules. It was detected that low concentrations of heparin retarded, whereas chondroitin sulfate A and C and keratosulfate accelerated, the collagen fibril formation, and chondroitin sulfate B and hyaluronic acid had no effect (9, 25). Mathews (14) found by free solution electrophoresis at pH 7.0 that hyaluronate and chondroitin sulfate of mol. wt. 50,000 gave complexes with soluble collagen, whereas heparin and chondroitin sulfate of mol. wt. 15,000–18,000 did not and showed a schematic model for the interaction of collagen and chondroitin sulfate-protein macromolecules. It was demonstrated that acid mucopolysaccharides formed a complex by fibrinogen, albumin, and blood protein (1, 3, 11); and with regard to the interaction in aqueous solutions between the cationic protein muramidase and chondroitin sulfate, Schubert *et al.* (17) demonstrated a series of salt-like compounds whose composition was expressed by the equivalence ratio which is equivalent of polyanion per equivalent of muramidase. Mathews (14) showed that the formation and stabilization of the complex will depend largely on the effect of ionic strength, the kind of charged groups, the number and distribution of interacting groups, the size and conformation of the macromolecules, and effects of micro-ions in competitive binding and on electrostatic shielding.

Ozzello *et al.* (16) reported the growth-promoting activity of acid mucopolysaccharides *in vitro* on a strain of human mammary carcinoma cells, and they ascribed this action to the negative electric charge and the viscosity of acid mucopolysaccharides.

Previous papers by this author (20–24) have shown that chondroitin sulfate has some promoting action on the tumor growth which tends to be accelerated as the concentration of chondroitin sulfate is increased. It is conceivable that chondroitin sulfate helps tumor growth by protecting the surface of tumor cells and promoting the exchange of their metabolites through its polyanionic function. In the present study some of the cationic protein tended to counteract the promoting activity of chondroitin sulfate on the tumor growth. It may be considered that the inhibitory effect of these proteins on the chondroitin sulfate activity was due to the decrease of negative electric charge of chondroitin sulfate.

The present experiments suggest that new connective tissue which produces acid mucopolysaccharides is favorable to the growth of cancer cells, but those connective tissues in which collagen, hyaline, and deposition of protein are formed are not such a good environment for it.

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