was suspended in 3 mM acetate buffer (pH 4.5), and the mixture was allowed to stand with stirring in the dark for 72 hr at 55°.[12] The reducing power was determined at intervals by the Somogyi-Nelson method.[20,21]

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Effect of Chondroitin Sulfate A in Combination Therapy with Mitomycin C on Sarcoma 180 Ascites Tumor

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Chondroitin sulfate A (CSA) was found to show a strong synergistic effect with mitomycin C in combination therapy of sarcoma 180 ascites tumor implanted in dd mice. Namely, 50% of mice each implanted with 1×10^6 tumor cells of ascitic form were found to survive on combination treatment with mitomycin C (0.3 mg/kg/day, 5 times) and CSA (1 and 10 mg/kg/day, 5 times). Enhancement of acid phosphatase and β-D-glucuronidase activities of the peritoneal cells in the mice given the combination therapy was evident as compared with those of mice of the control group.

Keywords——acid phosphatase; antitumor agent; chondroitin sulfate A; combination therapy; β-D-glucuronidase; peritoneal cells

Only a few papers have been published on tumor therapy by means of combinations of immunopotentiators with antitumor agents in order to minimize the side reaction and to increase the effect of the latter agents.[2] Previous studies have indicated that modified polysaccharides prepared by the introduction of fatty acid and phosphate groups, *i.e.*, palmitoyldextran phosphate[3] and stearoylmannan phosphate,[4] showed synergistic action with several antitumor agents against transplantable mouse tumors. It has recently been reported by Niitani *et al.*[5] that dextran sulfate showed a synergistic effect with mitomycin C against tumor. These findings seem to indicate that a polyanionic nature is one of the essential factors for the synergistic effect.

Here, we report that chondroitin sulfate A (CSA), a normal component of connective tissue, showed a synergistic effect with mitomycin C against sarcoma 180 ascites tumor. The mechanism was assumed to involve enhancement of the lysosomal enzyme activities in the peritoneal cells treated with CSA.

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Materials and Methods

Animal and Tumor——Male dd mice weighing 18 ± 2 g were used. The tumor used was sarcoma 180 ascites, initially supplied by the National Cancer Center Research Institute of Japan, and maintained in our laboratory in an ascites form.

Chemicals——Sodium chondroitin sulfate A (CSA) (Seikagaku Kogyo Co., Tokyo; Lot WEA 9082) and mitomycin C (Kyowa Hakko Kogyo Co., Tokyo) were freshly prepared in saline.

Assay for Antitumor Activity——Ten mice/group were used and about 1×10^6 sarcoma 180 cells were intraperitoneally implanted in the mice. CSA, at doses of 0.1, 1, and 10 mg/kg, was intraperitoneally administered 24 hr after the tumor inoculation, and 1 hr later, 0.3 mg/kg of mitomycin C was intraperitoneally administered daily for 5 consecutive days. Life span elongation was observed for up to 60 days. The test was repeated twice.

Assay for Hydrolase Activity——Three mice/group were used, and the test was repeated 3 times. Assay for lysosomal enzymes of mouse peritoneal cells was conducted as follows[6]: CSA was injected into the peritoneal cavity of the mice, and 2, 5, and 10 days later, the peritoneal cells were collected with 5 ml of PBS, washed with saline, and finally suspended in saline at 5×10^6 cells/ml.

In the assay of acid phosphatase and β-D-glucuronidase, *p*-nitrophenylphosphate disodium salt and *p*-nitrophenyl-β-D-glucuronide were used as substrates, respectively; 0.1 ml of the cell suspension, 0.1 ml of 0.1 M substrate in water, and 0.8 ml of 0.1 M acetate buffer, pH 4.5, were mixed and incubated at 37° for 30 min. Next, 1.0 ml of 0.1 N NaOH was added to the solution, and the color was estimated at 420 nm.

Results and Discussion

Figure 1 shows the effect of combination therapy with CSA and mitomycin C, of which the former is a well-known normal component of connective tissue. Fifty to sixty percent of the mice treated with CSA (1 and 10 mg/kg/day, × 5 times) and mitomycin C survived for more than 60 days after implantation, with noticeable tumor regression at that time, whereas CSA at a dosage of 0.1 mg/kg/day (5 times) in combination with mitomycin C proved to be ineffective. Tumor-bearing mice injected with CSA alone did not show a longer survival period than the control group.

As regards the mechanism of the above antitumor effect, it is quite reasonable to assume that the phagocytic cells of the peritoneal cavity might be initially activated by CSA, and the cells might then attack the tumor cells, rendering them more vulnerable to mitomycin C. It is well-known that macrophages play an important part in antitumor immunity, and

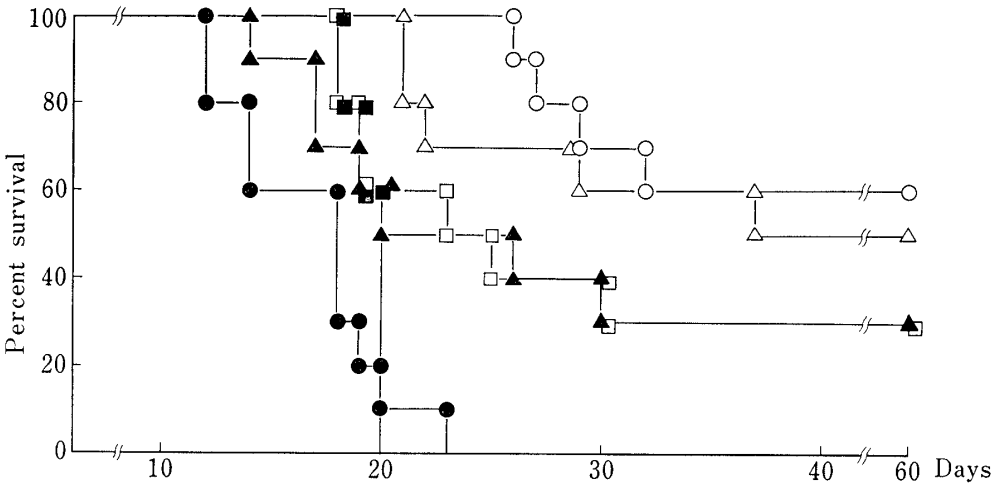

Fig. 1. Effect of Combination Treatment with Chondroitin Sulfate A and Mitomycin C on the Survival of Mice implanted with Sarcoma 180 Ascites Tumor Cells

Mice were implanted *i.p.* with 1×10^6 cells of sarcoma 180, and beginning 24 hr later, a solution of CSA in 0.9% NaCl and/or mitomycin C was injected *i.p.* for 5 successive days.
●, tumor-bearing control; □, CSA 0.1 mg/kg/day+mitomycin C 0.3 mg/kg/day × 5 times; ○, CSA 1 mg/kg/day+mitomycin C 0.3 mg/kg/day × 5 times; △, CSA 10 mg/kg/day+mitomycin C 0.3 mg/kg/day × 5 times; ▲, mitomycin C 0.3 mg/kg/day × 5 times, ■, CSA 1 mg/kg/day × 5 times.

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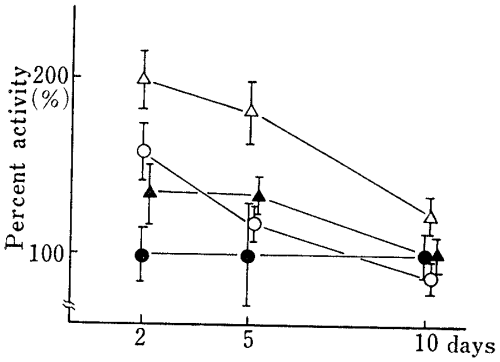

Fig. 2. Effect of Single and Combination Treatments with Chondroitin Sulfate A and Mitomycin C on the Acid Phosphatase Activity of the Peritoneal Cells in Mice

Mice were injected *i.p.* with CSA in 0.9% NaCl, and 1 hr later, mitomycin C was injected *i.p.* in the same vehicle.
●, normal control; ○, CSA 1 mg/kg; △, CSA 1 mg/kg+mitomycin C 0.3 mg/kg; ▲, mitomycin C 0.3 mg/kg.

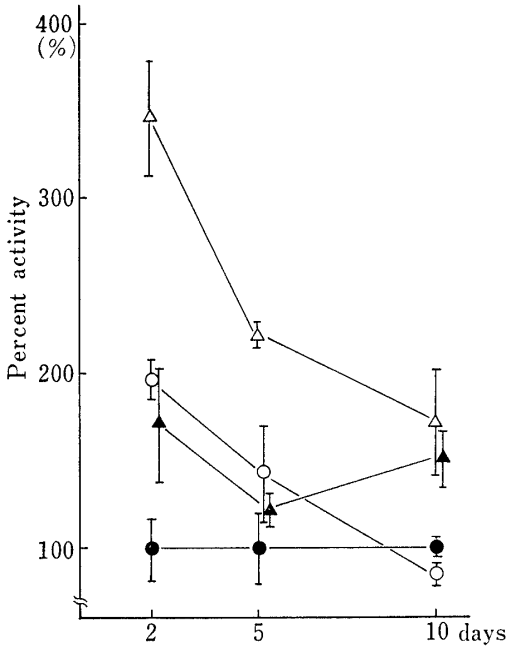

Fig. 3. Effect of Single and Combination Treatments with Chondroitin Sulfate A and Mitomycin C on the β-Glucuronidase Activity of the Peritoneal Cells in Mice

Mice were injected *i.p.* with a solution of CSA in 0.9% NaCl, and 1 hr later, mitomycin C was injected *i.p.* in the same vehicle.
●, normal control; ○, CSA 1 mg/kg; △, CSA 1 mg/kg+mitomycin C 0.3 mg/kg; ▲, mitomycin C 0.3 mg/kg.

that hydrolases such as acid phosphatase and β-glucuronidase located in the lysosomes of macrophages appear to assist the manifestation of antitumor acitvity. Therefore, in this paper, kinetic studies on these enzyme activities of the peritoneal cells were also conducted. Figures 2 and 3 show that the acid phosphatase and β-glucuronidase activities of the peritoneal cells in the mice treated with CSA and mitomycin C were increased as compared with those of mice which received CSA or mitomycin C alone. The peritoneal cells from the mice administered CSA and mitomycin C showed the most marked enhancement of both acid phosphatase and β-glucuronidase activities at 2 days; the activity of acid phosphatase was twice that in peritoneal cells from control mice, and the β-glucuronidase activity of the cells from mice given CSA and mitomycin C was 3.5 times that of the control mice. On the other hand, the peritoneal cells of mice treated with CSA or with mitomycin C alone also showed activities statistically different from those of the control group although lower than those of the group treated with CSA and mitomycin C. Five to ten days after administration of CSA and mitomycin C, the enzyme activities of the peritoneal cells gradually decreased, but remained significantly higher than those from other groups. However, in peritoneal cells obtained from the mice treated with mitomycin C alone, the hydrolase activity was not significantly different from that of the control mice, indicating that these cells did not suffer from the cytoxicity of mitomycin C at the dose used in the present study. Several polysaccharide sulfates have been shown to activate lysosomal acid hydrolase activities.[6,7] Niitani *et al.*[5] reported that dextran sulfate had an antitumor effect when injected with mitomycin C, and the effect was assumed to depend on the increased release of lysosomal enzymes. Thus, it can reasonably be concluded that polyanions which have been shown to activate lysosomal enzymes may be useful in cancer therapy as relatively non-toxic synergistic agents with mitomycin C.

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