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Mechanism of the antitumor activity of 5,5′-bis(2′-tetrahydropyranyl) secalonic acid D against Meth-A

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Summary. *The mechanism of the antitumor activity of 5,5′-bis (2′-tetrahydropyranyl) secalonic acid D (PSA) was examined in Balb/c mice bearing Meth-A fibrosarcoma. IP-injected PSA showed remarkable antitumor activity against IP-implanted Meth-A tumor. Antitumor activity of PSA was not abolished by treatment with silica as an antimacrophage agent or anti-asialo GM_1 antiserum that selectively eliminates natural killer cells. Although it was significantly suppressed by treatment with antithymocyte globulin in Balb/c mice, PSA was effective against Meth-A tumors implanted in athymic Balb/c mice. PSA inhibited in vitro Meth-A proliferation as effectively as mitomycin C and was not effective against Meth-A tumor implanted SC at a site where direct contact of PSA and Meth-A cells was unlikely. These results suggest that the antitumor activity of PSA was mainly achieved by inhibiting Meth-A cell proliferation, although the host T cell-mediated immunity was partly involved in the eventual therapeutic efficacy of PSA.*

Introduction

Secalonic acid D (Fig. 1) is an antibiotic that was isolated from the culture filtrates of *Penicillium oxalicum* [3, 11]. This antibiotic belongs to a group of ergot pigments (ergochromes) produced in the sclerotoria of the fungues *Claviceps purpurea* when grown on rye, and possesses antitumor activity as well as strong antimicrobial activity toward *Bacillus subtilis,* with considerable toxicity in mice. As a part of search for a new chemically modified compound with potent antitumor activity and low toxicity, PSA was obtained by tetrahydropyranylization of the alcoholic OH-groups of the original antibiotic. PSA shows remarkable antitumor activities against Ehrlich ascitic carcinoma, sarcoma-180, murine tumors induced with Rous sarcoma virus [12], and the rat bladder carcinoma, BC-47 [6]. The antitumor activity of PSA has so far been poorly understood, although it has been reported to have both immunopotentiating and antiproliferation activities [6, 12]. In this paper, we characterize the mechanism of antitumor action of PSA in mice bearing the syngeneic fibrosarcoma, Meth-A.

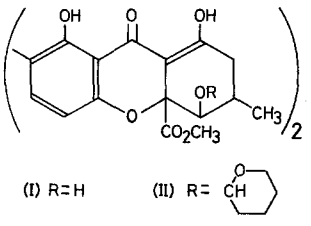

Fig. 1. Chemical structure of secalonic acid D (I) and PSA (II)

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Materials and methods

Animals and tumors. Female Balb/c mice, 7–9 weeks old and weighing about 20 g, were obtained from Charles River Japan, Inc. (Astugi, Japan). Nude mice (Balb/c genetic background) were bred in our laboratory. Meth-A has been maintained by IP passages in Balb/c mice at weekly intervals.

Chemicals. PSA and mitomycin C were obtained from Asahi Chemical Ind. Co. (Tokyo, Japan) and Kyowa Hakko Co. (Tokyo, Japan), respectively. Rabbit antithymocyte globulin (ATG) [2] and normal rabbit globulin (NRG) were purchased from Microbiological Associates (MD, USA) and Cappel Labs. Inc., (PA, USA), respectively. Anti-asialo GM_1 was obtained from Wako Pure Chemical Ind. (Osaka, Japan). Silica (particle size 0.007 μm) was obtained from Sigma Chemical Co. (MO, USA) and RPMI-1640 medium from Nissui Seiyaku Co. (Tokyo, Japan). Fetal bovine serum was purchased from Flow Labs. Inc., (MD, USA). All other reagents used were of analytical grade.

Solubilization of PSA. PSA was synthesized according to the method of Ishida [5]. Since this compound is only sparingly soluble in phosphate-buffered saline (PBS), the compound was first dissolved in dimethylsulfoxide containing 5% Tween 80, and then one volume of this solution was diluted by adding nine volumes of PBS (pH 7.8) to form an emulsion.

In vitro tumor growth inhibition of PSA. Meth-A cells were collected from the peritoneal cavity of a tumor-bearing mouse and washed three times with RPMI-1640 medium by centrifugation. The cells were resuspended in RPMI-1640 containing 10% heat-inactivated fetal bovine serum and finally adjusted to a cell number of 2×10^4 cells/ml in each tube. After culturing the cells in the presence of different concentrations of PSA for 48 h at 37° C in a CO_2 incubator, the cell number was obtained with a Coulter Counter (Model Z_{BI}, Coulter Electronic Ind., FA, USA), and the IC_{50} concentration was calculated according to the footnote to Table 1.

Antitumor activity of PSA. Meth-A cells were injected IP into nude mice (10^6 cells/mouse) or either IP (2×10^5 cells/mouse)

Table 1. 50% inhibitory concentration (IC_{50})[a] of PSA and mitomycin C

Agent	IC_{50} (μg/ml)
PSA	0.17 ± 0.02[a]
Mitomycin C	0.23 ± 0.08

[a] The IC_{50} is the concentration required to inhibit the growth of cells by 50%. Values shown are mean ± standard deviation of three experiments for PSA and of two experiments for mitomycin C

or SC (10^6 cells/mouse) into Balb/c mice. PSA was injected IP at a dose of 8 mg/kg daily on 7 consecutive days from day 1 after the tumor implantation. The antitumor activity was evaluated in terms of the mean survival in the treatment group as a percentage of that in control mice.

Antisera and silica injection. ATG and NRG were injected IP at a dose of 2.5 mg/0.25 ml per mouse on days −1, 1, 3, and 5. Anti-asialo GM_1 antibody was injected IP at a dose of 20 μl/head on days 1, 3, and 5. Silica was suspended in physiological saline and injected IP in a single dose of 25 mg/kg on day 1.

Results

Antitumor activity of PSA by implantation site of tumor

Antitumor activity of PSA depends on the implantation site of the tumor. As shown in Fig. 2, antitumor activity was observed when Meth-A was implanted IP. However, no antitumor activity was observed when Meth-A was implanted SC. These results suggest that direct contact between tumor cells and PSA may be required for it to exhibit antitumor activity.

In vitro growth inhibition of PSA

Table 1 shows the growth-inhibitory activity of PSA against Meth-A in comparison with that of mitomycin C. The 50% inhibition concentration (IC_{50}) of PSA against Meth-A is almost the same as that of mitomycin C. This indicates that PSA possesses strong direct growth-inhibitory action against Meth-A.

Effects of ATG, NGR, anti-asialo GM_1 antibody and silica on antitumor activity of PSA

ATG, anti-asialo GM_1 antibody or silica was injected IP. As shown in Table 2, the antitumor activity of PSA was significantly suppressed by treatment with ATG. Anti-asialo GM_1 antibody and silica did not affect its antitumor effect. These results suggest that the antitumor activity of PSA may occur only partly, if at all, by way of the host-mediated immune response.

Antitumor activity of PSA in nude mice

Antitumor activity of PSA was investigated in nude mice with congenital depression of T-cell function. As shown in Fig. 3, antitumor activity was observed in the nude mice, although it was not so effective as in the original Balb/c mice. This is consistent with the hypothesis that the antitumor activity of PSA was achieved by the direct interaction of PSA with Meth-A cells in the nude mice.

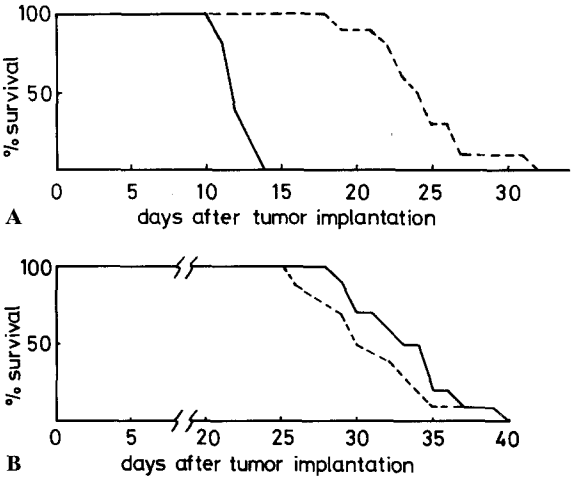

Fig. 2A and B. Effects of PSA on survival in Balb/c mice bearing Meth-A. **A** Meth-A cells (2×10^5 cells/mouse) were implanted IP. Mean survival (days) ± standard deviation (number of mice per group) in the control group (———) was 12.4 ± 1.0 (n = 11) and that in the treated group (– – –) was 24.7 ± 3.5 (n = 10). $P < 0.05$ (Mann-Whitney test). **B** Meth-A cells (10^6 cells/mouse) were implanted SC. Mean survival (days) ± standard deviation in the control group (———) was 33.6 ± 3.5 (n = 10) and that in the treated group (– – –) was 31.8 ± 4.1 (n = 10)

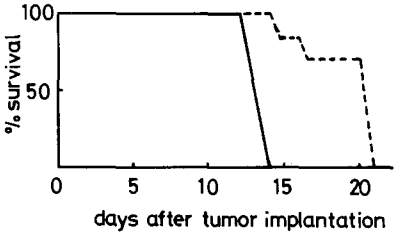

Fig. 3. Effects of PSA on duration of survival (days) in nude mice bearing Meth-A. Mean survival (days) ± standard deviation in the control group (———) was 13.5 ± 0.6 (n = 4) and that in the treated group (– – –) was 19.3 ± 2.7 (n = 6). $P < 0.05$ (Mann-Whitney test)

Table 2. Changes in antitumor activity of PSA following treatment with antisera and silica

Group no.	PSA	Treatment	No. of mice	Mean survival (days) (mean ± $\sigma n-1$)	(T/C) × 100 (%)
1.	−	−	11	11.9 ± 1.1[a]	100
2.	+	−	10	24.0 ± 3.6[a, b, c]	202
3.	+	ATG	9	19.1 ± 2.3[b]	161
4.	+	NRG	9	23.4 ± 2.1	197
5.	+	Silica	9	23.9 ± 4.5	201
6.	+	Anti-aGM_1	9	20.7 ± 2.7[c]	174

Meth-A cells (2×10^5 cells/mouse) were implanted IP
[a, b], $P < 0.05$ (Mann−Whitney)
[c] $0.1 > P > 0.05$ (Mann−Whitney)

Discussion

When PSA was injected IP it had antitumor activity against the Meth-A tumor implanted IP, but not against the tumor implanted SC (Fig. 2A, B). PSA possesses in vitro growth-inhibitory activity against Meth-A (Table 1). Therefore, PSA must reach the implanted site of the Meth-A tumor to exhibit

antitumor activity. Since PSA is only sparingly soluble in aqueous media, it may not distribute systemically in the body when PSA is injected IP. This may explain the difference in its antitumor activity according as whether the Meth-A tumor was implanted IP or SC.

Meth-A cells were found to be resistant to lysis by natural killer (NK) cells, which are known as asialo-GM_1-positive cells [4, 7, 9, 13]. Although the antitumor activity of PSA was partially suppressed in anti-asialo GM_1 antibody-treated mice, NK cells may not be responsible for the antitumor activity of PSA, because Meth-A is resistant to killing by NK cells. Antitumor activity of PSA was more markedly suppressed in the ATG-treated mice than in the NRG-treated mice (Table 2). Although PSA enhanced anti-SRBC antibody production in mice [12], we have no evidence showing that PSA activates T-cell antitumor immunity. When it is taken into account that chemotherapeutic agents in general exert less antitumor potency in an immunosuppressed host than in an immunologically competent host [1, 8, 10], abrogation of the antitumor activity of PSA by ATG does not neccessarily indicate that the antitumor activity of PSA was achieved by activation of T-cell immunity. Furthermore, the finding that PSA was therapeutically effective in nude mice indicates that PSA was effective without potentiation of T-cell immunity. These results, as well as the finding that PSA was as potent as mitomycin C in suppressing in vitro proliferation of Meth-A cells, indicates that the antitumor activity of PSA was associated with its direct interaction with Meth-A cells, suppressing their proliferation. However, it is not yet clear how much the host antitumor immunity was involved in the PSA-induced therapeutic effect in Meth-A-bearing mice. In fact, PSA is reported to augment the host humoral immunity [12]. This should be taken into consideration for further analysis of PSA antitumor activity, and is especially important for the examination of other tumors, since the antitumor immunological effector systems may be different from that for the Meth-A tumor.

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