# ANTITUMOR ACTIVITY OF PRUMYCIN

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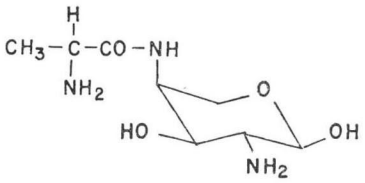
The antifungal antibiotic, prumycin, was studied for antitumor activity against several tumor systems. It was found to possess potential antitumor activity against a well-established mouse mammary adenocarcinoma in C3H/He mice. It was also active in prolongation of the lifespan of mice bearing P-388 lymphocytic leukemia. Moreover, prumycin did not depress the white blood cell counts in the mouse peripheral blood. However, severe alopecia was observed in mice treated with this agent at dosage levels near the $LD_{50}$.

Prumycin is an antifungal antibiotic isolated from the culture broth of *Streptomyces* sp. strain No. F-1028 in 1971[1,2]. The chemical structure of this compound was shown to be 4-D-alanyl-2,4-diamino-2,4-dideoxy-L-arabinose[3], as shown in Fig. 1. This antibiotic is effective against phytopathogenic fungi such as *Sclerotinia scleotiorum, Sclerotinia cinerea, Botrytis fabae* and *Botrytis cinerea* but inactive against most bacteria and yeasts[2]. Recently, the synthesis of prumycin derivatives has been achieved by HASEGAWA *et al*[4]. However, the antitumor activity of this antibiotic was not studied until the present work.

Fig. 1. Structure of prumycin.




This paper reports the antitumor activity and side effects of prumycin in various tumor systems.

## Materials and Methods

### Animals

Male ddY, $BDF_1$, $CDF_1$, and female C3H/He mice weighing about 20 g were used. These mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals. Male Donryu rats, about 150 g body weight, were obtained from Nihon Rat Co. Each experimental group comprised 5~8 animals.

### Tumor

Sarcoma 180 ascites type was obtained from the National Cancer Center (Tokyo) and has been maintained by intraperitoneal passage in ddY mice. For antitumor experiments, $5 \times 10^6$ cells were inoculated subcutaneously at the axillary region of ddY mice. Leukemia P-388 was donated by the Cancer Chemotherapy Center (Tokyo). For the antileukemia test, $BDF_1$ mice were inoculated intraperitoneally with P-388 $1 \times 10^6$ cells or L-1210 $1 \times 10^5$ cells. A spontaneous mammary adenocarcinoma, which originated from the mammary gland of C3H/He female mouse in our laboratory and was designated as KSP-1, has been passaged in solid form in the same strain. C3H/He female mice were subcutaneously inoculated with this tumor by trocar for the antitumor test. LEWIS lung carcinoma from the Cancer Chemotherapy Center (Tokyo) was inoculated by trocar into C57BL/6 mice for passage and into $BDF_1$ mice for the antitumor test. An ascites variant of fibrosarcoma originally

induced in BALB/c mice with 3-methylcholanthrene, designated Meth 1, has been passaged using the same strain of mice. For the antitumor test $CDF_1$ mice were inoculated subcutaneously with $1 \times 10^6$ tumor cells. EHRLICH ascites carcinoma, $5 \times 10^6$ cells, was inoculated intraperitoneally or intravenously into ddY mice. YOSHIDA sarcoma and AH-130 ascites hepatoma were inoculated with $5 \times 10^6$ cells subcutaneously into Donryu rats.

Chemical agents

Prumycin hydrochloride was prepared according to the method of ŌMURA *et al*[3]. Mitomycin C (Kyowa Hakko Kogyo), adriamycin (Kyowa Hakko Kogyo), chromomycin $A_3$ (Takeda Chem. Ind.), actinomycin D (Makor Chemicals), bleomycin (Nihon Kayaku) and carbazilquinone (Sankyo) were used as reference antitumor agents. These agents were dissolved in physiological saline solution and 0.2 ml was administered intraperitoneally into animals starting 24 hours after tumor inoculation unless otherwise specified. Dose and administration schedules are described in the Results.

The evaluation of antitumor activity against solid tumor was based on the comparison of the tumor size of the treated group (T) with that of control group (C), T/C. In the ascites tumor or intravenously inoculated tumor, antitumor activity was evaluated by the percentage increase in lifespan over the controls. The $LD_{50}$ of prumycin was calculated from the number of survivors at 14 days after a single intraperitoneal or intravenous injection into ddY mice or Donryu rats. In order to examine the bone marrow toxicity of prumycin and mitomycin C, mice were intraperitoneally injected with these agents starting one day after a sarcoma 180 inoculation. Blood was taken from these mice and the white blood cells were counted with a Micro Cell Counter (Toa Medical Electronics) 4 or 7 days after tumor inoculation. For the histological examination C3H/He mice bearing mammary adenocarcinoma and normal ddY mice were given prumycin at 75 mg/kg and 150 mg/kg intraperitoneally. Then, tumor mass from C3H/He mice and, skin and right femur from ddY mice were removed at specified time intervals and conventional paraffin sections were prepared and stained with hematoxylin and eosin as a routine method.

## Results

### Acute Toxicity of Prumycin

The $LD_{50}$ of prumycin by a single intraperitoneal and intravenous injection were 155 and 144 mg/kg respectively in mice, and about 70 mg/kg by intraperitoneal injection in rats. Most animals which recieved a lethal dose of prumycin died within 2 days after injection, and in the surviving mice severe alopecia was observed from about 4 days after administration and continued for a few weeks (Plate 1).

### Antitumor Effects of Prumycin

Effect on sarcoma 180 solid type:

Table 1 shows the inhibitory effect of prumycin and mitomycin C on the growth of solid sarcoma 180 implanted in ddY mice. At a dose of 75 mg/kg of prumycin and 4.2 mg/kg of mitomycin C, which are approximately equal to 1/2 of $LD_{50}$, they suppressed the tumor growth to almost the same extent, that is T/C of 0.47 and 0.42, respectively. However, prumycin did not show as high antitumor activity as mitomycin C did when the dosage was increased. On the other hand, none of the mice treated with prumycin exhibited the depression of white blood cell counts when measured 4 or 7 days after tumor inoculation, whereas mice given mitomycin C showed a severe decrease in white blood cell counts even at the dose of 4.2 mg/kg, 1/2 of $LD_{50}$.

Effect on mouse mammary adenocarcinoma:

Mammary adenocarcinoma KSP-1, was implanted subcutaneously by trocar into C3H/He mice, and 9 days later the mice with tumor mass about 500 $mm^3$ in volume were chosen and given

prumycin or other agents administered intraperitoneally on days 9, 12 and 15. Prumycin was found to be highly effective against this established mammary adenocarcinoma as shown in Fig. 2. Three injections of 50 mg/kg of prumycin, about the $LD_{50}$ in total, caused more rapid regression of the tumor than mitomycin C (2.8 mg/kg × 3) did. The administration of adriamycin 5 mg/kg × 3, about

Table 1. Effect on sarcoma 180 (s.c.-i.p.)

| Compounds | Dose (mg/kg/day) | Treatment[a] schedule | Tumor volume[b] ($mm^3$) (Mean±SD) | T/C (Day 7) | Body wt.[c] change (g) | WBC[d] ($/mm^3$) |
|---|---|---|---|---|---|---|
| Control | None | — | 749±371 | — | +4.1 | 8,300* |
| | | | | | | 12,900** |
| Prumycin | 150 | Day 1 | 313±126 | 0.42 | +0.9 | 8,800* |
| | 100 | Day 1 | 353±110 | 0.47 | +0.5 | 8,800* |
| | 75 | Day 1 | 366± 99 | 0.49 | +1.3 | 8,000* |
| | 50 | Day 1 | 661±265 | 0.88 | +3.6 | N.T. |
| Mitomycin C | 5.6 | Day 1 | 179± 87 | 0.24 | +0.1 | 2,800* |
| | 4.2 | Day 1 | 313±132 | 0.42 | +3.3 | 3,600* |
| Prumycin | 20 | Days 1~6 | 286± 94 | 0.38 | −3.6 | 11,100** |
| | 10 | Days 1~6 | 457±134 | 0.61 | +0.4 | N.T. |
| | 5 | Days 1~6 | 621±164 | 0.83 | +2.3 | N.T. |

a) Sarcoma 180 ($5 \times 10^6$ cells) were inoculated subcutaneously into ddY mice on day 0.
b) Mean tumor volume on day 7.
c) Body weight change shows the difference of the body weight measured on days 1 and 7.
d) White blood cell counts on days 4 (*) and 7 (**).

Fig. 2-a. Comparison of the effect of prumycin, mitomycin C and adriamycin on mouse mammary adenocarcinoma.

Compounds were administered on days 9, 12 and 15.

Values in parentheses mean the body weight change from day 9 to day 18.

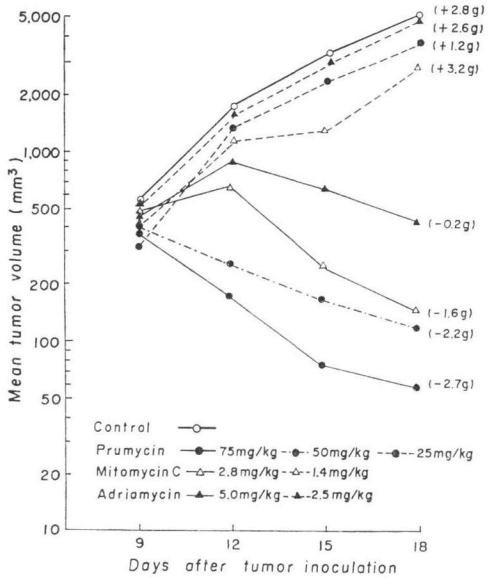



Fig. 2-b. Comparison of the effect of prumycin, carbazilquinone and chromomycin $A_3$ on mouse mammary adenocarcinoma.

Compounds were administered on days 9, 12 and 15. Values in parentheses mean the body weight change from day 9 to day 18.

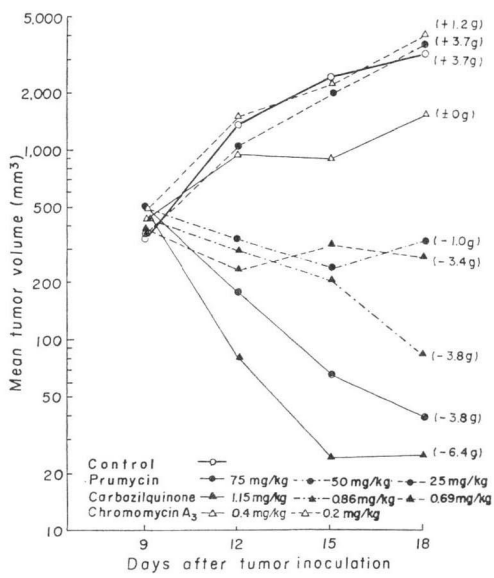


Table 2. Effect on P-388 (i.p.-i.p.)

| Compounds | Dose (mg/kg/day) | Treatment[a] schedule | Survival days (Mean±SD) | ILS[b] (%) |
|---|---|---|---|---|
| Control | None | — | 10.2±0.4 | |
| Prumycin | 200 | Day 1 | 13.4±4.5 | 31 |
| | 150 | Day 1 | 15.2±0.4 | 50 |
| | 100 | Day 1 | 13.8±1.0 | 40 |
| | 75 | Day 1 | 13.2±1.0 | 30 |
| Mitomycin C | 4.2 | Day 1 | 18.4±0.8 | 80 |
| Control | None | — | 10.8±1.0 | |
| Prumycin | 20 | Days 1~7 | 15.0±5.9 | 38 |
| | 10 | Days 1~7 | 14.2±3.2 | 31 |
| | 5 | Days 1~7 | 14.0±2.1 | 30 |
| Mitomycin C | 1 | Days 1~7 | 20.4±1.2 | 89 |

[a] Leukemia P-388 ($1\times10^6$ cells) were inoculated intraperitoneally into $CDF_1$ mice on day 0.
[b] Increased lifespan.

$LD_{50}$ in total, was less effective than prumycin and mitomycin C at the comparable dose. But prumycin failed to demonstrate substantial antitumor activity at a dose of 25mg/kg although adriamycin did at 2.5 mg/kg and mitomycin C at 1.4 mg/kg. This tumor was quite sensitive to carbazilquinone, though severe loss of body weight was observed. Chromomycin $A_3$ did not suppress the tumor size even at a dose of 0.4 mg/kg×3 which is equal to about $2LD_{50}$'s in total.

Effect on lymphocytic leukemia P-388:

The effect of prumycin on the lifespan of mice bearing P-388 is shown in Table 2. When prumycin was given at a dose of 150 mg/kg on the day after tumor inoculation, a maximum increase in lifespan (50%) was observed and no additional therapeutic benefit was obtained with a multiple injection schedule. As shown in Table 2, mitomycin C displayed stronger antitumor activity against P-388 than prumycin by both single-dose and multiple-dose treatment.

Effect on methylcholanthrene induced fibrosarcoma:

Methylcholanthrene induced fibrosarcoma, $5\times10^6$ cells, was inoculated subcutaneously into $CDF_1$ mice. Inhibitory effect of prumycin, mitomycin C and bleomycin by single intraperitoneal administration at 24 hours after tumor inoculation was determined by measuring the tumor size on days 7 and 18. As shown in Table 3, the tumor growth was strongly inhibited by bleomycin at a dose of 80 mg/kg and 40 mg/kg while the $LD_{50}$ was determined to be about 160 mg/kg in our experiment, but both doses also caused severe loss of body weight. On the other hand, prumycin showed somewhat weak antitumor effect at a dose of around 100 mg/kg (2/3 of $LD_{50}$) compared with mitomycin C at a dose of 4.2 mg/kg (1/2 of $LD_{50}$). The tumor growth was suppressed during the first week after implantation by treatment with prumycin on day 1, but later the tumor resumed it's growth, resulting in an almost identical tumor size between the treated mice and the control mice. Mitomycin C and bleomycin suppressed tumor growth for a longer period of time after administration.

Effect on LEWIS lung carcinoma:

LEWIS lung carcinoma was implanted subcutaneously into $BDF_1$ mice and drug treatment was started on the 7th day after implantation when the tumor was about 100 $mm^3$ in volume. Prumycin or other agents were given on days 7, 10 and 13, and the tumor volume was measured on days 16 and

Table 3. Effect on methylcholanthrene-induced sarcoma (s.c.-i.p.)

| Compounds | Dose[a] (mg/kg/day) | on Day 7 | | | on Day 14 | | |
|---|---|---|---|---|---|---|---|
| | | Tumor volume ($mm^3$) (Mean±SD) | T/C | Body wt.[b] change (g) | Tumor volume ($mm^3$) (Mean±SD) | T/C | Body wt.[b] change (g) |
| Control | None | 131.7±34.9 | — | +0.4 | 811±208 | — | +1.0 |
| Prumycin | 150 | 37.6±25.8 | 0.29 | +0.3 | 464±216 | 0.57 | +1.3 |
| | 100 | 46.4±23.5 | 0.35 | +0.8 | 573±145 | 0.71 | +1.2 |
| | 75 | 56.0±14.7 | 0.43 | +0.2 | 682±166 | 0.84 | +0.8 |
| Mitomycin C | 5.6 | 12.9±12.0 | 0.10 | +0.4 | 106± 26 | 0.13 | +0.6 |
| | 4.2 | 31.3±19.9 | 0.24 | +0.4 | 254±241 | 0.31 | +1.0 |
| Bleomycin | 80 | 10.0± 8.4 | 0.08 | −4.6 | 120± 17 | 0.15 | −2.7 |
| | 40 | 33.9±19.1 | 0.26 | −3.0 | 246±100 | 0.30 | −0.6 |

One million tumor cells were inoculated subcutaneously into $CDF_1$ mice on day 0.

[a] Test compounds were administered intraperitoneally on day 1.

[b] Body weight change shows the difference of the body weight measured on days 1, and 7 or 14.

Table 4. Effect on LEWIS lung carcinoma

| Compounds | Dose[a] (mg/kg/day) | on Day 16 | | | on Day 20 | | |
|---|---|---|---|---|---|---|---|
| | | Tumor volume ($mm^3$) (Mean±SD) | T/C | Body wt.[b] change (g) | Tumor volume ($mm^3$) (Mean±SD) | T/C | Body wt.[b] change (g) |
| Control | None | 2,190±498 | — | +0.2 | 4,241±1,428 | — | +4.0 |
| Prumycin | 75 | 729±355 | 0.33 | −3.5 | 2,241±1,903 | 0.53 | +0.8 |
| | 50 | 1,313±249 | 0.60 | −0.5 | 2,770±1,053 | 0.65 | +4.3 |
| | 25 | 1,793±235 | 0.82 | +0.9 | 3,269± 490 | 0.77 | +5.3 |
| Mitomycin C | 2.8 | 1,647±362 | 0.75 | −1.5 | 1,798± 368 | 0.42 | −0.6 |
| | 1.4 | 2,040±232 | 0.93 | +0.5 | 2,821± 425 | 0.67 | +3.7 |
| Adriamycin | 5.0 | 1,646±483 | 0.75 | −1.7 | 1,938± 666 | 0.46 | −0.1 |
| Bleomycin | 20 | 1,197±242 | 0.55 | 0 | 1,623± 408 | 0.38 | +2.8 |
| | 10 | 1,911±279 | 0.87 | −0.5 | 2,281± 614 | 0.54 | +3.2 |

[a] Test compounds were administered intraperitoneally on days 7, 10 and 13.

[b] Body weight change shows the difference of the body weight measured on days 7, and 16 or 20.

20. As shown in Table 4, prumycin at a dose of 75 mg/kg/day caused marked inhibition of the tumor growth and loss of body weight on day 16, shortly after the last injection. However, rapid tumor growth and recovery of the body weight were observed on day 20. On the contrary, although none of the reference agents such as mitomycin C at the dosage used in this experiment showed a suppressive effect against the tumor on day 16, they gave suppression of tumor growth on day 20.

Effect on YOSHIDA sarcoma:

In order to compare the antitumor activity of prumycin against YOSHIDA sarcoma with other compounds, prumycin, mitomycin C, adriamycin and carbazilquinone were intraperitoneally administered at a dosage level of 1/2~1/6 of the $LD_{50}$ by the single or intermittent treatment schedule (days 1, 3, 5) and the mean tumor weight of the treated group was compared to that of the control group on day 8. As reported[5], YOSHIDA sarcoma showed marked sensitivity to mitomycin C even at a low dose such as 0.64 mg/kg (1/5 of $LD_{50}$), but it was found to be resistant to prumycin at all dosages tested.

Table 5. Effect on YOSHIDA sarcoma (s.c.-i.p.)

| Compounds | $LD_{50}$ (mg/kg) | Dose (mg/kg/day) | Treatment[a] schedule | Tumor weight[b] (g) (Mean±SD) | T/C (day 8) | Body wt.[c] change (g) |
|---|---|---|---|---|---|---|
| Control | | None | — | 2.89±0.46 | — | +31.6 |
| Prumycin | 67.5 | 33.8 | Days 1, 4, 7 | 2.55±0.96 | 0.88 | − 3.8 |
| | | 22.5 | Days 1, 4, 7 | 2.09±0.73 | 0.72 | +17.2 |
| | | 16.9 | Days 1, 4, 7 | 3.17±0.76 | 1.10 | +11.0 |
| | | 50 | Day 1 | 2.76±1.14 | 0.96 | +10.9 |
| Mitomycin C | 3.2 | 1.07 | Days 1, 4, 7 | 0.04±0.07 | 0.01 | + 3.5 |
| | | 0.8 | Days 1, 4, 7 | 0.05±0.06 | 0.02 | + 2.3 |
| | | 0.64 | Days 1, 4, 7 | 0.17±0.12 | 0.06 | +13.4 |
| | | 1.6 | Day 1 | 0.19±0.44 | 0.07 | − 4.0 |
| Adriamycin | 16.0 | 5.3 | Days 1, 4, 7 | 0.85±1.05 | 0.29 | − 4.7 |
| | | 4.0 | Days 1, 4, 7 | 0.86±0.64 | 0.30 | − 8.5 |
| | | 3.2 | Days 1, 4, 7 | 1.07±0.72 | 0.37 | − 0.9 |

a) YOSHIDA sarcoma ($5\times10^6$ cells) were inoculated subcutaneously into Donryu rats on day 0.
b) Mean tumor weight on day 8.
c) Body weight change shows the difference of the body weight measured on days 1 and 8.

Table 6. Effect on AH-130 (s.c.-i.p.)

| Compounds | $LD_{50}$ (mg/kg) | Dose (mg/kg/day) | Treatment[a] schedule | Tumor weight[b] (g) (Mean±SD) | T/C (day 10) | Body wt.[c] change (g) |
|---|---|---|---|---|---|---|
| Control | | None | — | 4.91±1.62 | — | +28.0 |
| Prumycin | 67.5 | 22.5 | Days 1, 4, 7 | 2.19±0.82 | 0.45 | +13.1 |
| | | 16.9 | Days 1, 4, 7 | 2.50±1.68 | 0.51 | +22.3 |
| | | 13.5 | Days 1, 4, 7 | 2.13±1.65 | 0.58 | +23.8 |
| Mitomycin C | 3.2 | 1.07 | Days 1, 4, 7 | 0 | 0 | +10.6 |
| | | 0.8 | Days 1, 4, 7 | 0.63±1.36 | 0.13 | +18.8 |
| | | 0.63 | Days 1, 4, 7 | 0.16±0.19 | 0.03 | +18.1 |
| Adriamycin | 16.0 | 5.3 | Days 1, 4, 7 | 0.34±0.17 | 0.07 | −41.7 |
| | | 4.0 | Days 1, 4, 7 | 1.51±2.25 | 0.31 | −10.2 |
| Chromomycin $A_3$ | 0.51 | 0.1 | Days 1, 4, 7 | 2.32±1.75 | 0.47 | −10.4 |
| | | 0.075 | Days 1, 4, 7 | 3.17±1.99 | 0.65 | − 5.0 |

a) AH-130 ascites hepatoma ($5\times10^6$ cells) were inoculated subcutaneously into Donryu rats on day 0.
b) Mean tumor weight on day 10.
c) Body weight change shows the difference of the body weight measured on days 1 and 10.

(Table 5)

Effect on AH-130:

The antitumor activity against AH-130 solid form was examined in the same manner used for YOSHIDA sarcoma. As shown in Table 6, prumycin showed a weak antitumor effect, that is 0.45 of T/C value at a dose of 22.5 mg/kg (1/3 of $LD_{50}$), and was less active than mitomycin C which completely inhibited tumor growth at a dose of 3.2 mg/kg (1/3 of $LD_{50}$). Chromomycin $A_3$ was toxic and almost ineffective at the dosage used against this tumor.

Histological examination:

Prumycin at a dose of 75 mg/kg caused severe alopecia in ddY mice 7 days after the injection, and histological examination showed atrophy of skin and hair follicles as shown in Plate 1-b. However,

Plate 1-a. Histology of skin from control mice.

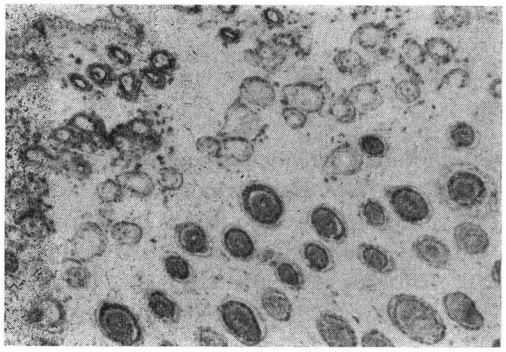


Plate 1-b. Histology of skin from mice treated with prumycin at 75 mg/kg, 7 days after the intraperitoneal injection.

Note the marked decrease in hair follicles.

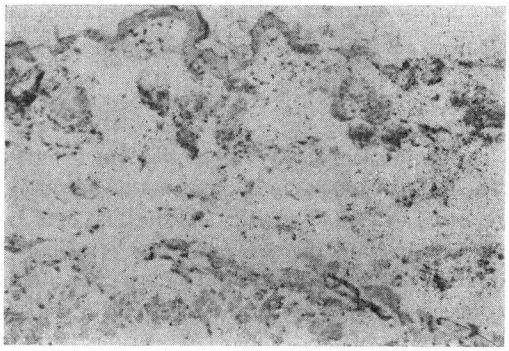


Plate 2-a. Histology of bone marrow from control mice.

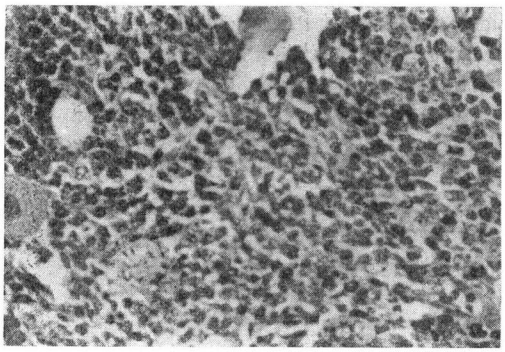


Plate 2-b. Histology of bone marrow from mice treated with prumycin at 150 mg/kg, 4 days after the intraperitoneal injection.

None remarkable change was observed.

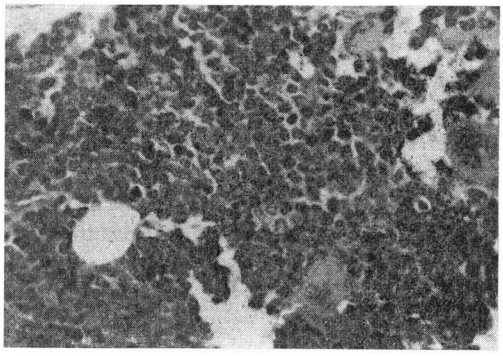


Plate 3-a. Histology of mouse mammary adenocarcinoma, KSP-1, in control mice, 11 days after implantation.

Poorly differentiated adenocarcinoma forming solid cell nests.

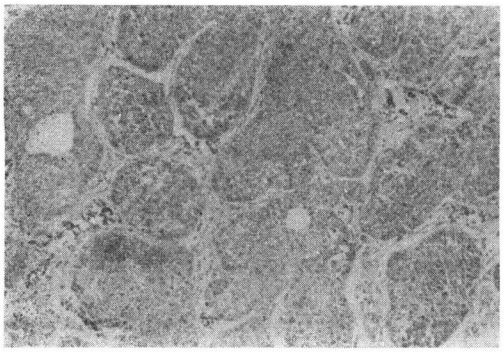
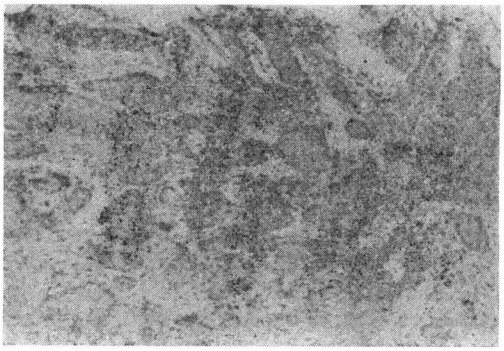


Plate 3-b. Histology of mouse mammary adenocarcinoma, KSP-1, 2 days after the intraperitoneal injection of prumycin at 75 mg/kg on day 9.

Note irregularly disrupted tumor cell nests and cords showing variable degree of degeneration and necrotic carcinoma tissue with nuclear debris in center of the tumor cell nests.



no remarkable changes were observed in bone marrow and other principal organs 4, 7, 14 and 30 days after the administration of prumycin at a dose of 150 mg/kg. (Plate 2-b and unpublished observation). When prumycin was intraperitoneally administered into C3H/He mice 9 days after implantation of mammary adenocarcinoma, tumor necrosis and atrophy of tumor cell nests were noted at a dose of 75 mg/kg 3 days after treatment. (Plate 3-b)

## Discussion

The present study was carried out to investigate the antitumor activity and toxicity of prumycin. The $LD_{50}$ of prumycin by intravenous injection was 145 mg/kg in mice, and this value was close to that reported by ŌMURA *et al.*[3] At the dosage of 1/2 of $LD_{50}$, prumycin showed no remarkable histological changes in bone marrow and other principal organs, but caused severe alopecia and atrophy of skin and hair follicles was noticed 7 days after administration. This side effect might be related to the high concentration of the antibiotic in skin, as reproted for bleomycin[6,7]. Therefore, a study of the distribution of this antibiotic in various tissues, especially in skin and bone marrow will be important for understanding the mechanism of the side effect with this agent.

Prumycin showed moderate antitumor effect against sarcoma 180, P-388 and AH-130 but was completely inactive against YOSHIDA sarcoma, L-1210 and EHRLICH ascites carcinoma (unpublished data), so the antitumor activity of prumycin was thought to be far less than that of reference agents. However, the antibiotic was found to be highly effective against mouse mammary adenocarcinoma, KSP-1, even if the treatment was begun a week after inoculation when the tumor mass had become as large as 500 $mm^3$ in volume. It may be acceptable to think that a wide antitumor spectrum is not necessarily required for an agent to be a candidate for clinical evaluation if it possess remarkable activity against a certain type of cancer and no severe treatment-limiting toxicity. In this connection, further studies will be designed to examine the effect of prumycin against human mammary tumor in athymic nude mice.

It has been reported that prumycin inhibits protein synthesis in *Botrytis cinerea*[8] but on the other hand, most antitumor antibiotics exert their cytocidal activity mainly by affecting nucleic acids. Therefore, it will be of interest to investigate the mechanism of action of this antibiotic against tumor cells.

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