EFFECT OF PENTAZOCINE ON EHRlich ASCITES TUMOR CELLS

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Abstract—The effect of pentazocine, a non-narcotic analgesic, on Ehrlich ascites tumor cells was examined in vitro and in vivo. To test the in vitro effect of pentazocine, Ehrlich tumor cells suspended in Hanks balanced salt solution (BSS, pH 7.4) supplemented with 2% bovine albumin were incubated with various concentrations of the drug (0.10–1.0 mM) at 37°C for 120 min. After incubation, the tumor cells in BSS were inoculated subcutaneously into the right flank of mice (10⁶ cells/mouse). All mice given the tumor cells incubated alone developed solid tumor. However, no tumor growth was observed in groups of mice given the tumor cells pretreated with 0.3 or 1.0 mM pentazocine. The in vivo effect of pentazocine was then examined against Ehrlich ascites carcinoma in mice. Mice inoculated intraperitoneally with Ehrlich tumor cells in BSS (2×10⁶ cells/mouse) were given various doses of pentazocine (20–80 mg/kg/day) intraperitoneally once a day for 5 successive days. The average survival time in a group of mice given the tumor cells alone was about 19 days, and the survival time was about 29 days in a group of animals, treated with pentazocine in a dose of 80 mg/kg/day (p<0.01).

Pentazocine is a potent non-narcotic analgesic. This drug exerts an action on various mammalian cells (1, 2). As there is little information on the effect of pentazocine on tumor cells, the effects of this drug on Ehrlich ascites tumor cells were studied both in vitro and in vivo.

MATERIALS AND METHODS

Tumor cell suspension: Female ddY strain mice, 7–8 weeks of age, were used. Ehrlich tumor cells were obtained from mice at 10 days after i.p. inoculation of the cells, and were suspended in Hanks balanced salt solution (BSS, pH 7.4) containing penicillin G (100 IU/ml) and streptomycin (100 μg/ml). The tumor cells used were prepared by velocity sedimentation as described previously (3). Briefly, Ehrlich tumor cells suspended in 40 ml of BSS (about 1.5×10⁷ cells/ml) were gently loaded over the sucrose solution consisting of 2 layers (150 ml of 1.0% sucrose in BSS and 300 ml of 1.5% sucrose in BSS without phenol red) in a 500-ml flask through a needle connected with a 100-ml separatory funnel by tubing. After standing at 4°C for 90 min, the tumor cells sedimented to the bottom were collected and washed twice with BSS. These cells were then suspended in BSS (pH 7.4) supplemented with 2% bovine albumin fraction V (Wako Pure Chem. Indust.).

Cytotoxicity test of pentazocine: The tumor cell suspension (approx. 2×10⁶ cells/ml) in 100-ml flasks was incubated with various concentrations of pentazocine...
(Sankyo Co., 0.01–1.0 mM) at 37°C for 30 to 120 min in a shaking water bath. These cells were also incubated in the absence of the drug. After incubation, the tumor cells collected by centrifugation were washed twice and suspended in BSS (2×10^6 cells/ml). The tumor cell suspension was then injected subcutaneously into the right flank of mice (10^6 cells/mouse), and the tumor growth in mice was observed for 6 weeks (3, 4). In addition, the number of viable Ehrlich tumor cells was examined by the trypan blue test after incubation in the presence or absence of pentazocine, and the proportion of the viable tumor cells was calculated from the number of viable and dead cells (5).

**Examination of antitumor activity of pentazocine:** The antitumor activity of pentazocine was examined against Ehrlich ascites carcinoma in mice (6). Ehrlich tumor cells suspended in BSS were inoculated intraperitoneally into groups of 10 mice each (2×10^6 viable cells/mouse). The mice were then given various doses of pentazocine in physiological saline (20–100 mg/kg body weight, i.p. dailyx5), and the survival time was examined. A control group of animals was inoculated with the same number of tumor cells, followed by the injection of saline alone.

| Incubation time of tumor cells with pentazocine (min) | Number of mice bearing with solid tumor at 6 weeks after inoculation |
|-------------------------------------------------------|---------------------------------------------------------------------|
|                                                       | Concentrations of pentazocine (mM)                                  |
|                                                       | 1.0        | 0.3       | 0.1       | 0.01      | 0         |
| 30                                                    | 7/10       | 9/10      | 10/10     | 10/10     | 10/10     |
| 60                                                    | 0/10       | 6/10      | 9/10      | 10/10     | 10/10     |
| 120                                                   | 0/10       | 0/10      | 7/10      | 10/10     | 10/10     |

*Ehrlich tumor cells were suspended in Hanks balanced salt solution (pH 7.4) supplemented with 2% bovine albumin (2×10^6 cells/ml), and were incubated with various concentrations of pentazocine (0.01–1.0 mM) at 37°C for 30, 60 and 120 min. After incubation, the tumor cell suspension was given s.c. into the right flank of mice (10^6 cells/mouse), and the tumor growth in animals was observed for 6 weeks.

**RESULTS**

**Cytotoxicity of pentazocine:** First, the tumor growth in mice was examined after s.c. inoculation with various number of Ehrlich tumor cells (Table 1). All mice given 10^6 tumor cells per mouse developed solid tumor, whereas 7 out of 20 mice given 5×10^5 tumor cells did not. Tumor growth did not occur in a group of mice inoculated with 10^5 tumor cells.

Table 2 shows the tumor incidence in groups of mice inoculated subcutaneously with Ehrlich tumor cells that had been incubated with various concentrations of pentazocine for 30 to 120 min. All mice given the tumor cells incubated alone...
developed solid tumor. Yet, no tumor growth was observed in mice given the tumor cells pretreated with 1.0 mM pentazocine for 60 and 120 min. Similarly, mice given the tumor cells pretreated with 0.3 mM pentazocine for 120 min had no tumor growth. The tumor growth did occur in 7 out of 10 mice given the tumor cells pretreated with 0.1 mM pentazocine for 120 min, and all animals given the tumor cells, which were pretreated with 0.01 mM pentazocine for 120 min, developed solid tumor.

The proportion of viable Ehrlich tumor cells was examined after incubation in the presence or absence of pentazocine (Figure 1). When Ehrlich tumor cells were incubated with 1.0 mM pentazocine, the proportion of the viable cells was reduced markedly within 120 min (less than 1%). The proportion of viable Ehrlich tumor cells was also decreased significantly during the incubation with 0.3 mM pentazocine (about 19% after 120 min). Only a slight change was noted in the proportion of the viable tumor cells during the 120-min incubation with 0.01 mM pentazocine (about 80%). The proportion of viable Ehrlich tumor cells before and after incubation without the drug was about 85% (80–90%) and 82% (78–85%), respectively.

Antitumor activity of pentazocine: The in vivo effect of pentazocine was examined against Ehrlich ascites carcinoma in mice. Ehrlich tumor cells were inoculated intraperitoneally into mice, and the animals were given various doses of pentazocine (20–100 mg/kg/day, i.p.) for 5 successive days. The control group of mice given the tumor cells alone died from ascites carcinoma in less than 25 days (mean survival time: about 19 days) (Table 3). The average survival time in groups of mice treated with pentazocine at doses less than 40 mg/kg/day

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Table 3. Antitumor effect of pentazocine against Ehrlich ascites carcinoma in mice

| Dose of pentazocine* (mg/kg/day) | Average survival time** (days) | (%) |
|----------------------------------|--------------------------------|-----|
| 80                               | 28.9±2.1                       | 155.4*** |
| 60                               | 23.4±1.8                       | 125.8  |
| 40                               | 17.9±1.9                       | 98.2   |
| 20                               | 18.8±2.2                       | 101.1  |
| 0                                | 18.6±1.3                       | 100.0  |

*Groups of 10 mice each were inoculated intraperitoneally with Ehrlich tumor cells (2×10^6 cells/mouse), and the animals were given i.p. various doses of pentazocine for 5 successive days thereafter. **Each value represents mean±SE of 10 mice, except one group. 2 out of 10 mice treated with pentazocine at a dose of 80 mg/kg/day died within 5 days. ***Statistically significant (p<0.01).
was similar to that in the control group of animals (18–19 days). The average survival time was about 29 days in a group of mice treated with pentazocine in a dose of 80 mg/kg/day: The average survival time in this group of mice was about 150% of that in the control group of animals (p<0.01). Almost all mice inoculated with Ehrlich tumor cells and given pentazocine at a dose of 100 mg/kg/day i.p. died within 5 days (1). Thus, the in vivo effect of pentazocine on Ehrlich tumor cells could not be tested at doses of 100 mg/kg/day or more.

**DISCUSSION**

The treatment of Ehrlich ascites tumor cells with pentazocine in vitro resulted in a decrease of the viable cells, and the tumor cells treated with the drug in vitro lost their transplantability in mice. Therefore, it is evident that pentazocine is cytotoxic to Ehrlich ascites tumor cells in vitro. The present results also indicate that pentazocine shows a weak antitumor activity against Ehrlich ascites carcinoma in mice.

It has been reported that the pharmacological action of pentazocine is similar to that of morphine, codeine or pethidine (1, 2). The results of in vitro tests with morphine, dihydrocodeine or pethidine indicated that there was no significant difference in the proportion of the viable cells between Ehrlich tumor cells incubated with and without 1.0 mM of these drugs at 37°C for 120 min (the proportion of the viable cells incubated with and without drugs: 80–85% and 82%). In addition, the growth of solid tumor was observed in all mice inoculated subcutaneously with the tumor cells pretreated with 1.0 mM of morphine, dihydrocodeine or pethidine at 37°C for 120 min. These results suggest that morphine, dihydrocodeine and pethidine have little effect on the viability of Ehrlich ascites tumor cells in vitro. Therefore, it may be concluded that pentazocine markedly differs from morphine, dihydrocodeine or pethidine in the in vitro effect on Ehrlich ascites tumor cells. Morphine, dihydrocodeine and pethidine are narcotics, while pentazocine is non-narcotic (1, 2).

The mechanism responsible for the cytotoxicity of pentazocine on Ehrlich ascites tumor cells is largely obscure. However, preliminary experiments indicated that the incubation of Ehrlich tumor cells with 1.0 mM pentazocine at 37°C resulted in a significant decrease of triglycerides and cholesterol esters in the tumor cells. The contents of triglycerides and cholesterol esters in the tumor cells treated with the drug for 30, 60 and 120 min were about 63%, 50% and 44% of those in the untreated tumor cells, respectively (the amounts of triglycerides and cholesterol esters in the untreated tumor cells: about 180 and 55 mg/10¹⁰ cells). The fatty acid pattern of neutral lipids and phospholipids from the pentazocine-treated tumor cells also differed from that from the untreated tumor cells. Thus, it is possible that pentazocine induces a change in the lipid composition of Ehrlich ascites tumor cells, resulting in the tumor cell damage (7–9). Antitumor drugs, such as alkylating agents and antimetabolites, are involved in the nucleic acid metabolism of tumor cells, and the tumor cell division is inhibited (10, 11). The pharmacological action of pentazocine on tumor cells, therefore, may differ from that of these antitumor drugs.

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