EFFECT OF THE CENTRALLY ACTING ANTITUSSIVES ON ASCITES TUMOR CELLS

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Abstract—Dextromethorphan, dimemorfan, dihydrocodeine and oxymethebanol, centrally
acting antitussives, were examined for their effect on Ehrlich carcinoma cells and sarcoma-
180 cells in vitro or in vivo. The tumor cells were suspended in Hanks balanced salt
solution (pH 7.4) supplemented with 2% bovine albumin, and they were incubated with
and without 1 mM drugs at 37°C for 120 min. The incubation of the tumor cells with
dextromethorphan or dimemorfan resulted in a decrease in the proportion of the viable
cells (less than 25% after 120 min). However, no significant change was observed in
the proportion of the viable tumor cells during the incubation with and without the other
drugs (80-83% after 120 min). In addition, mice given the tumor cells i.p. were injected
intraperitoneally with drugs (20-80 mg/kg/day) once daily for 5 successive days, and
their survival time was observed. There was a slight difference in the survival time
between mice treated with and without dextromethorphan or dimemorfan. However, a
significant difference was found in the survival time between mice treated with and
without dextromethorphan when mice given Ehrlich carcinoma cells were injected with
the drug (40 mg/kg/time) twice a day for 5 days (about 18 days and 29 days). These
results indicate that dextromethorphan and dimemorfan are cytotoxic to the tumor cells
in vitro and in vivo.

Recently, we have reported that pentazocine is cytotoxic to Ehrlich ascites
carcinoma cells in vitro, but morphine has little effect on the viability of the tumor cells
in vitro (1). The pharmacological action of pentazocine is similar to that of morphine (2,
3). However, pentazocine is a non-narcotic analgesic, and morphine is narcotic. This
raises the question as to whether non-narcotic analogues of morphine might affect
the viability of tumor cells. In the present study, dextromethorphan, dimemorfan, di-
hydrocodeine and oxymethebanol (drotebanol), centrally acting antitussives, were
examined for their effect on Ehrlich carcinoma cells and sarcoma-180 cells in vitro or in vivo.
Dextromethorphan and dimemorfan are non-
narcotic antitussives, while dihydrocodeine
and oxymethebanol are narcotic (3-6). It
was found that dextromethorphan and
dimemorfan are cytotoxic to Ehrlich carcinoma
cells or sarcoma-180 cells in vitro, whereas
dihydrocodeine and oxymethebanol have
little effect on the viability of these tumor
cells in vitro.

Materials and Methods
Drugs: The drugs used were as follows:
dextromethorphan hydrobromide (Maruko Pharmaceut. Co., 5 mg/ml solution),
dimemorfan phosphate (Yamanouchi Pharmaceut. Co.), dihydrocodeine phosphate
(Takeda Chem. Indust.), and oxymethebanol (Sankyo Co., 1 mg/ml solution). Dimemorfan
phosphate and dihydrocodeine phosphate were dissolved in physiological saline (20 mM) and stored at 4°C until use.

**Tumor cell suspension:** Ehrlich carcinoma cells or sarcoma-180 cells were inoculated intraperitoneally into female mice of the ddY strain, 7–8 weeks of age. At 10 days after inoculation, the tumor cells were obtained from the mice, and they 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 (1), and they were suspended in BSS (pH 7.4) supplemented with 2% bovine albumin fraction V (Wako Pure Chem. Indust.).

**Cytotoxicity test of drugs:** The in vitro effect of drugs on Ehrlich carcinoma cells or sarcoma-180 cells was examined as described previously (1). About 20 ml of the tumor cell suspension (2×10⁶ cells/ml) in 50-ml test tubes were incubated with and without drugs at 37°C for 30 to 120 min in a shaking water bath. After incubation, the tumor cells collected by centrifugation were washed twice and suspended in BSS. The proportion of the viable tumor cells was examined by the trypan blue test (1).

**Examination of antitumor activity of drugs:** The in vivo effect of drugs on Ehrlich carcinoma cells or sarcoma-180 cells was examined as described previously (7). Female mice of the ddY strain, 7–8 weeks of age, were used in this study. Ehrlich carcinoma cells or sarcoma-180 cells were obtained from mice at 10 days after i.p. inoculation of the cells, and they were suspended in BSS. Groups of 10 mice each were inoculated intraperitoneally with Ehrlich carcinoma cells or sarcoma-180 cells (2×10⁶ viable cells/mouse). The i.p. injection of drugs into mice (20–80 mg/kg/day) was began 24 hr after inoculation of the tumor cells and was continued once daily for 5 successive days. Mice given the tumor cells and treated with physiological saline for 5 days were used as a control (untreated mice). The survival time of the animals was observed for 60 days.

In addition, mice given the tumor cells i.p. were injected intraperitoneally with various doses of drugs (10–40 mg/kg/time) twice a day for 5 successive days, and their survival time was observed thereafter.

**Results**

**Cytotoxicity of drugs:** First, the proportion of viable Ehrlich carcinoma cells was examined after incubation for 30 to 120 min in the presence or absence of drugs. When Ehrlich carcinoma cells were incubated with 1 mM dextromethorphan or dimemorfan, the proportion of the viable cells was reduced markedly within 120 min (about 0.4% for dextromethorphan and 22% for dimemorfan after 120 min, P<0.01) (Fig. 1 and Table 1). Whereas, no significant change was observed in the proportion of the viable tumor cells during the 120-min incubation with 1 mM...
dihydrocodeine or oxymethohebanol (81–83% after 120 min). The proportion of viable Ehrlich carcinoma cells after incubation for 120 min without drugs was 80–82%.

A decrease in the proportion of the viable cells was also observed in sarcoma-180 cells incubated with 1 mM dextromethorphan or dimemorfan. The proportion of the viable tumor cells after incubation for 120 min with and without 1 mM of these drugs was less than 24% and about 80%, respectively (P<0.01) (Table 2). The proportion of viable sarcoma-180 cells was 80–84% after incubation for 120 min with 1 mM dihydrocodeine or oxymethohebanol.

**Antitumor activity of drugs:** Dextromethorphan and dimemorfan were cytotoxic to Ehrlich carcinoma cells or sarcoma-180 cells in vitro, as described above. The in vivo effect of these drugs was then examined against Ehrlich ascites carcinoma or ascites sarcoma-180 in mice.

Tables 3 and 4 show the survival time in groups of mice that had been given Ehrlich carcinoma cells or sarcoma-180 cells and injected with drugs once daily for 5 days. In groups of mice given Ehrlich carcinoma cells, the survival time of mice treated with dextromethorphan or dimemorfan was 100–140% of that of the untreated animals. Similarly, the survival time of mice given sarcoma-180 cells and treated with these drugs was 90–140% of that of the corresponding untreated animals. On the other

### Table 1. Proportion of viable Ehrlich carcinoma cells treated with various concentrations of antitussives

| Antitussives       | Drug concentrations (mM) |  |  |
|--------------------|--------------------------|---|---|
|                    |                          | 1 | 0.1 | 0.01 | 0 |
| Dextromethorphan   | 0.4±0.1  
| Dimemorfan         | 22.1±1.6*  
| Dihydrocodeine     | 81.4±1.3*  
| Oxymethohebanol    | 82.5±0.9*  |

Ehrlich carcinoma cells were suspended in Hanks balanced salt solution (pH 7.4) supplemented with 2% bovine albumin (2×10^6 cells/ml), and they were incubated with and without drugs at 37°C for 120 min. After incubation, the proportion of the viable tumor cells was examined by the trypan blue test. Each value represents the mean±S.E. of 6 experiments. *Significantly different from the corresponding value of tumor cells incubated alone (P<0.01).

### Table 2. Proportion of viable sarcoma-180 cells treated with various concentrations of antitussives

| Antitussives       | Drug concentrations (mM) |  |  |
|--------------------|--------------------------|---|---|
|                    |                          | 1 | 0.1 | 0.01 | 0 |
| Dextromethorphan   | 23.8±2.9*  
| Dimemorfan         | 15.7±4.1*  
| Dihydrocodeine     | 79.5±2.0  
| Oxymethohebanol    | 81.6±1.3  |

Sarcoma-180 cells were suspended in Hanks balanced salt solution (pH 7.4) supplemented with 2% bovine albumin (2×10^6 cells/ml), and they were incubated with and without drugs at 37°C for 120 min. After incubation, the proportion of the viable tumor cells was examined by the trypan blue test. Each value represents the mean±S.E. of 6 experiments. *Significantly different from the corresponding value of tumor cells incubated alone (P<0.01).
hand, the survival time of mice treated with dihydrocodeine was less than 100% of that of the untreated animals, irrespective of the tumor cells examined. In the following experiments, mice receiving the tumor cells were injected with

| Antitussives       | Doses of drugs (mg/kg/day) | Survival time of mice |
|--------------------|-----------------------------|-----------------------|
|                    | Days                        | %                     |
| Dextromethorphan   | 80  | 25.3±2.3*                      | 138.0                 |
|                    | 60  | 21.9±2.4                       | 117.7                 |
|                    | 40  | 18.5±2.2                       | 99.5                  |
|                    | 20  | 17.8±1.7                       | 95.7                  |
|                    | 0   | 18.6±1.1                       | 100.0                 |
| Dimemorfan         | 80  | 2.5±0.2                        | 13.0                  |
|                    | 60  | 18.5±2.0                       | 96.4                  |
|                    | 40  | 26.6±1.5*                      | 138.5                 |
|                    | 20  | 19.2±2.2                       | 100.0                 |
|                    | 0   | 19.2±1.5                       | 100.0                 |
| Dihydrocodeine     | 80  | 18.6±2.0                       | 97.3                  |
|                    | 60  | 18.5±1.5                       | 96.8                  |
|                    | 40  | 18.3±1.3                       | 95.8                  |
|                    | 20  | 18.0±1.2                       | 94.2                  |
|                    | 0   | 19.1±1.8                       | 100.0                 |

Mice were inoculated i.p. with Ehrlich carcinoma cells (2×10⁶ viable cells/mouse) and injected i.p. with various doses of drugs once daily for 5 successive days thereafter. Each value represents the mean±S.E. of 10 mice. *Significantly different from the corresponding value of mice given the tumor cells alone (P<0.05).

| Antitussives       | Doses of drugs (mg/kg/day) | Survival time of mice |
|--------------------|-----------------------------|-----------------------|
|                    | Days                        | %                     |
| Dextromethorphan   | 80  | 14.3±0.7                       | 103.6                 |
|                    | 60  | 11.8±0.8                       | 88.7                  |
|                    | 40  | 18.7±2.4*                      | 136.5                 |
|                    | 20  | 12.9±1.2                       | 94.2                  |
|                    | 0   | 13.7±0.8                       | 100.0                 |
| Dimemorfan         | 60  | 18.1±2.0*                      | 132.1                 |
|                    | 40  | 15.1±0.8                       | 110.2                 |
|                    | 20  | 11.7±0.6                       | 85.4                  |
|                    | 0   | 13.7±0.9                       | 100.0                 |
| Dihydrocodeine     | 80  | 11.4±0.7                       | 84.4                  |
|                    | 60  | 12.2±0.8                       | 90.4                  |
|                    | 40  | 11.5±1.1                       | 85.2                  |
|                    | 20  | 10.9±0.7                       | 80.7                  |
|                    | 0   | 13.5±0.9                       | 100.0                 |

Mice were inoculated i.p. with sarcoma-180 cells (2×10⁶ viable cells/mouse) and injected i.p. with various doses of drugs once a day for 5 successive days thereafter. Each value represents the mean±S.E. of 10 mice. *Different from the corresponding value of mice given the tumor cells alone (P<0.1).
dextromethorphan or dimemorfan twice a day for 5 days, and their survival time was observed. In groups of mice given Ehrlich carcinoma cells, a marked difference was found in the survival time between the untreated mice and a group of animals treated with dextromethorphan in a dose of 80 mg/kg/day (Table 5). The survival time in this group of mice treated with dextromethorphan was about 160% of that of the untreated animals (about 29 days and 18 days, P<0.01). In groups of mice given sarcoma-180 cells, however, the survival time of mice treated with dextromethorphan was less than 140% of that of the untreated animals. The survival time of mice treated with dimemorfan was similar to that of the untreated animals, irrespective of the tumor cells used.

**Discussion**

The present results indicate that dextromethorphan and dimemorfan are cytotoxic to Ehrlich carcinoma cells or sarcoma-180 cells in vitro, whereas dihydrocodeine and oxymethabanol have little effect on the viability of these tumor cells in vitro. In addition, dextromethorphan and dimemorfan exhibited a weak antitumor activity against Ehrlich ascites carcinoma or ascites sarcoma-180 in mice, while dihydrocodeine showed no effect on the growth of these tumors in mice. Therefore, it may be concluded that dextromethorphan and dimemorfan markedly differ from dihydrocodeine or oxymethabanol in the effect on Ehrlich carcinoma cells and sarcoma-180 cells.

All the drugs examined are known to be centrally acting antitussives. However, dextromethorphan and dimemorfan are non-narcotic antitussives, while dihydrocodeine and oxymethabanol are narcotic (3, 6). Chemically, dextromethorphan and dimemorfan are the d-isomers of morphinan, but dihydrocodeine and oxymethabanol belong to the I-morphinans (3, 6, 8). Thus, the difference in the effect on tumor cells among the drugs examined may be related to the difference in the pharmacological properties and chemical structure of the drugs.

The mechanism responsible for the cytotoxicity of dextromethorphan and dimemorfan on tumor cells is as yet largely obscure. However, the present results strongly suggest that certain analogues of morphine exert their action on tumor cells both in vitro or in vivo. Histological studies on tumor cells indicated that degenerative changes such
as cell swelling and vesiculation of cytoplasm were observed in Ehrlich carcinoma cells and sarcoma-180 cells after incubation with 1 mM dextromethorphan or dimemorfan at 37°C for 120 min. The incubation of the tumor cells with 1 mM of these drugs for 180 min resulted in tumor cell lysis. In contrast, no significant change was observed in the structure of the tumor cells treated with and without 1 mM dihydrocodeine or oxymethébanol for 120 to 180 min at 37°C. Thus, dextromethorphan and dimemorfan appear to be lytic to Ehrlich carcinoma cells or sarcoma-180 cells in vitro.

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