ELSEVIER

European Journal of Pharmacology 367 (1999) 13–17

ejp

Influence of antineoplastic drugs on morphine analgesia and on morphine tolerance

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Received 13 July 1998; revised 17 December 1998; accepted 22 December 1998

Abstract

The possible influence of cisplatin, methotrexate, adriamycin and vincristine on thermal pain threshold, morphine analgesia and development of morphine tolerance was investigated in mice. In the hot-plate test, the nociceptive threshold was not affected by acute or repeated administration of any of the antineoplastic drugs used. The analgesic activity of morphine was significantly reduced by pretreatment with cisplatin, intraperitoneally (i.p.) injected at the dose of 2 mg/kg. In contrast, methotrexate, subcutaneously (s.c.) injected at the dose of 1 and 5 mg/kg, adriamycin (1 and 3 mg/kg s.c.), vincristine (0.25 and 0.5 mg/kg i.p.) and a lower dose of cisplatin (1 mg/kg i.p.) had no effect. The development of tolerance to morphine analgesia was delayed by adriamycin but was not influenced by the other antineoplastic drugs used. These data show that, of the four antineoplastic agents used in this study, cisplatin may interfere in the mechanism of action of morphine, and that adriamycin may delay the development of opiate tolerance.

Keywords: Antineoplastic drug; Morphine; Pain; Analgesia; Tolerance; Methotrexate; Adriamycin; Cisplatin; Vincristine; (Mouse)

1. Introduction

Pain is a frequent and severe symptom in advanced cancer. Moreover, antineoplastic treatments also can cause pain. Thus, pain relief, usually by means of symptomatic analgesic treatment, is a fundamental and formidable task in the care of cancer patients, and opioid analgesics remain the mainstay of such treatment (World Health Organization, 1990; Busnhell and Justin, 1993; Cherny, 1996; Thurlimann and Stoutz, 1996). As a rule, cancer patients are concurrently treated with analgesics and antineoplastic drugs, yet there have been few studies concerning the possible influence of antineoplastic agents on the analgesic activity of opioid drugs and on the development of tolerance to such analgesic activity (Cohen et al., 1965). The aim of this study was to investigate this possibility.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (Charles River, Calco, Como, Italy), weighing 25–30 g, were used. They were maintained five per cage in air-conditioned colony-rooms (temperature: 22 ± 1°C; humidity: 60%) with free access to standard food pellets and tap water, on a natural light–dark cycle. They were acclimatized to our housing conditions for at least 1 week before experimental use. The experiments were performed in compliance with the standards and suggestions of the European Community for the care and the use of animals for scientific purposes (CEE Council 86/609, and Italian D.L. 27/01/92, No. 116).

2.2. Thermal pain threshold measurement

Responsiveness to nociceptive stimulation was measured by means of a conventional hot-plate apparatus (Socrel DS-35, Ugo Basile, Comerio, VA, Italy). The animals were placed one at a time on an electrically heated metal plate that was kept at the constant temperature of 50 ± 0.4°C. The latency either to forepaw licking or to

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00966-2

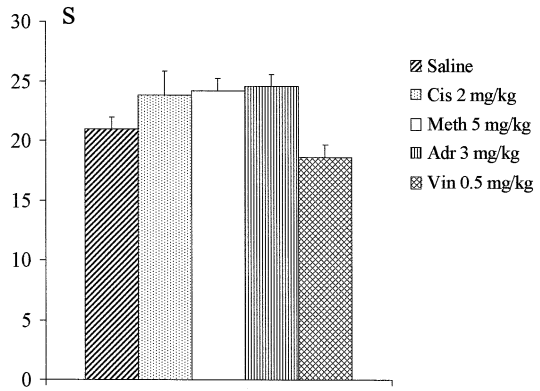

Fig. 1. Influence of cisplatin (Cis, 2 mg/kg i.p.), methotrexate (Meth, 5 mg/kg s.c.), adriamycin (Adr, 3 mg/kg s.c.) and vincristine (Vin, 0.5 mg/kg i.p.) on the thermal pain threshold measured in the hot-plate test (50 ± 0.4°C). Data are means ± S.E.M. of latency in seconds (s) from 14 animals for each group.

jumping was recorded by means of an electronic timer started and stopped by a foot switch. A cut-off time of 60 s was adopted. The analgesic effect of morphine was calculated as a percentage of the maximum possible effect (%MPE) (Woolfe and MacDonald, 1944; Eddy and Leimbach, 1953) according to the formula:

$$\frac{TL - BL}{60 - BL} \times 100$$

where TL = test latency, BL = baseline latency, 60 = cut-off time in seconds. Baseline latency was the mean of the latencies recorded in the last two out of three baseline tests, performed at 30-min intervals.

During the 5 days preceding the experiment all mice were familiarized with handling, weighing, and injection: once a day, they were removed from their home-cages, injected with saline, and then placed on the unheated plate of the algesimeter and allowed to explore for 60 s.

2.3. Acquisition of tolerance to the analgesic effect of morphine

Morphine tolerance was produced by daily injection of 15 mg/kg of morphine sulphate subcutaneously (s.c.) for 9 consecutive days (Trujllo and Akil, 1991). The baseline pain threshold was calculated (as described above) as the mean of the latencies recorded in the last two out of three baseline tests performed at 30-min intervals. The effect of morphine was measured 30 min after treatment on days 1, 3, 5, 7 and 9. The development of tolerance was defined as a significant reduction of the analgesic effect of morphine compared with the effect produced by the first treatment.

2.4. Drugs and treatments

The following drugs were used: morphine sulphate (Salars, Como, Italy), methotrexate (Cyanamid Italia, Catania, Italy), cisplatin (Rhone-Poulenc Italia, Milano, Italy), adriamycin hydrochloride (Pharmacia, Milano, Italy), vincristine sulphate (Eli Lilly Italia, Sesto Fiorentino, Firenze, Italy).

Mice were randomly assigned to one of the following treatments: (1) saline intraperitoneally (i.p.) plus (30 min later) saline s.c.; (2) saline i.p. plus (30 min later) morphine s.c. (15 mg/kg); (3) cisplatin i.p. (1 or 2 mg/kg) plus (30 min later) saline s.c.; (4) cisplatin i.p. (1 or 2 mg/kg) plus (30 min later) morphine s.c. (15 mg/kg); (5) methotrexate s.c. (1 or 5 mg/kg) plus (45 min later) saline s.c.; (6) methotrexate s.c. (1 or 5 mg/kg) plus (45 min later) morphine s.c. (15 mg/kg); (7) adriamycin s.c. (1 or 3 mg/kg) plus (30 min later) saline s.c.; (8) adriamycin s.c. (1 or 3 mg/kg) plus (30 min later) morphine s.c. (15 mg/kg); (9) vincristine i.p. (0.25 or 0.5 mg/kg) plus (30 min later) saline s.c.; (10) vincristine i.p. (0.25 or 0.5 mg/kg) plus (30 min later) morphine s.c. (15 mg/kg).

The doses of the antineoplastic drugs were chosen as being those that have been reported to be effective against experimental tumors in mice (Mead et al., 1961; Adamson et al., 1965; Rosenberg et al., 1969; Rosenberg and Van-

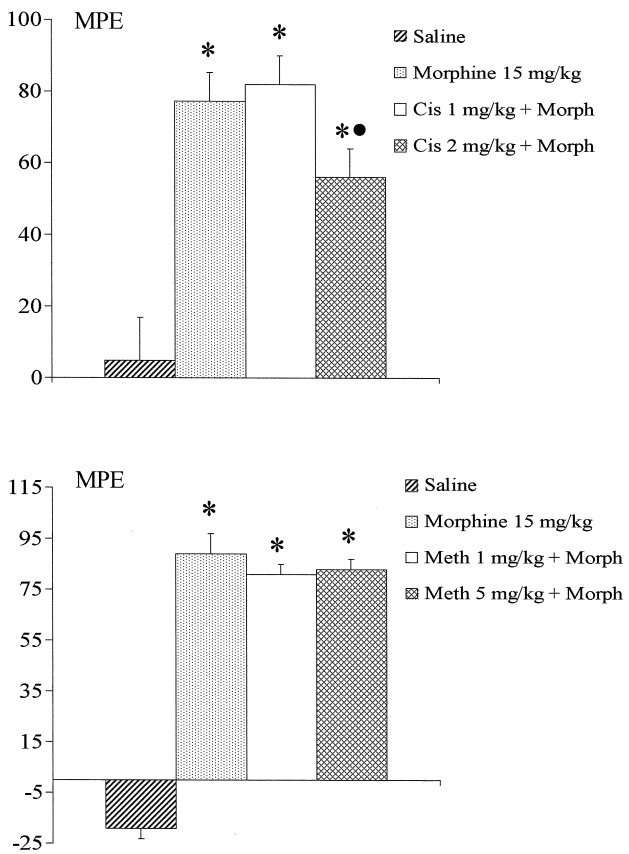

Fig. 2. Influence of cisplatin (Cis, 1 and 2 mg/kg i.p.) and methotrexate (Meth, 1 and 5 mg/kg s.c.) on the analgesic activity of morphine (15 mg/kg s.c.) measured in the hot-plate test (50 ± 0.4°C). Data are means ± S.E.M. of %MPE from 14 animals for each group. * $P < 0.05$ vs. saline group; • $P < 0.05$ vs. morphine group (ANOVA followed by Newman–Keuls multiple comparison test).

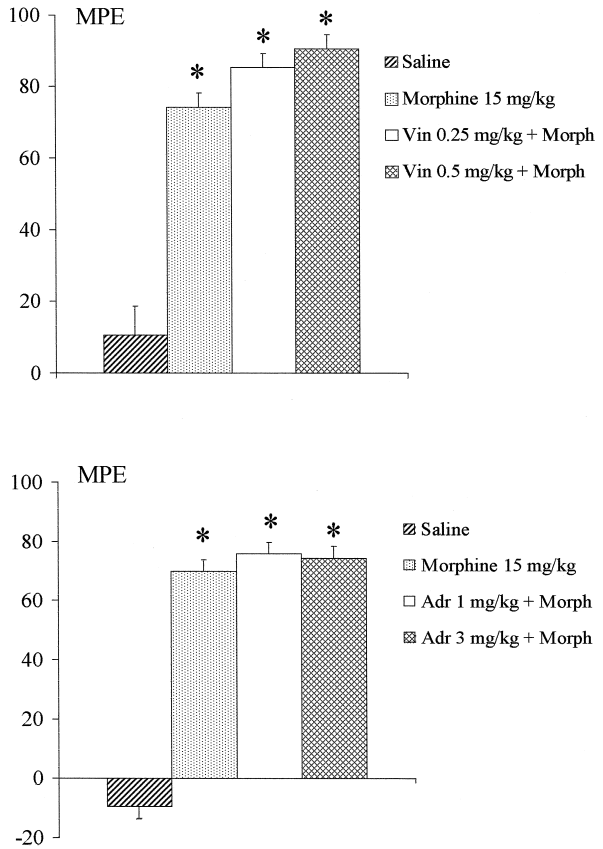

Fig. 3. Influence of vincristine (Vin, 0.25 and 0.5 mg/kg i.p.) and adriamycin (Adr, 1 and 3 mg/kg s.c.) on the analgesic activity of morphine (15 mg/kg s.c.) measured in the hot-plate test (50 ± 0.4°C). Data are means ± S.E.M. of %MPE from 14 animals for each group. * $P < 0.05$ vs. saline group (ANOVA followed by Newman–Keuls multiple comparison test).

Camp, 1970; Arena et al., 1971; Bertino et al., 1977; Nahabedian et al., 1988).

The above treatments were given either acutely (once: influence of acute administration of antineoplastic drugs on thermal pain threshold and on the analgesic activity of morphine) or once a day for 9 consecutive days (influence of the repeated administration of antineoplastic drugs on thermal pain threshold and on the development of morphine tolerance; in this case, however, only one dose of each antineoplastic drug was used, i.e., 2 mg/kg in the case of cisplatin, 1 mg/kg in the case of methotrexate, 3 mg/kg in the case of adriamycin, 0.25 mg/kg in the case of vincristine). The thermal pain threshold was measured 30 min after morphine (or saline) injection. Fourteen mice per group were used. The volume of either s.c. or i.p. injections was 1 ml/100 g b.w. Cisplatin and adriamycin were dissolved in 0.9% NaCl; methotrexate, vincristine and morphine were dissolved in distilled water.

2.5. Statistical analysis

Data were analyzed by means of analysis of variance (ANOVA), followed by Student–Newman–Keuls test.

3. Results

3.1. Influence of antineoplastic drugs on thermal pain threshold

Responsiveness to thermal nociceptive stimulation was not affected either by acute or by repeated administration of any of the antineoplastic drugs used. Fig. 1 shows the effect of a single administration of the highest dose of the antineoplastic drugs. Figs. 4 and 5 show the effect of the repeated (once a day for 9 consecutive days) administration of the antineoplastic drugs at a dose which did not cause death.

3.2. Influence of antineoplastic drugs on the analgesic activity of morphine

The highest dose (2 mg/kg i.p.) of cisplatin significantly reduced the analgesic activity of morphine (Fig. 2), whereas methotrexate, adriamycin and vincristine had no significant effect (Figs. 2 and 3).

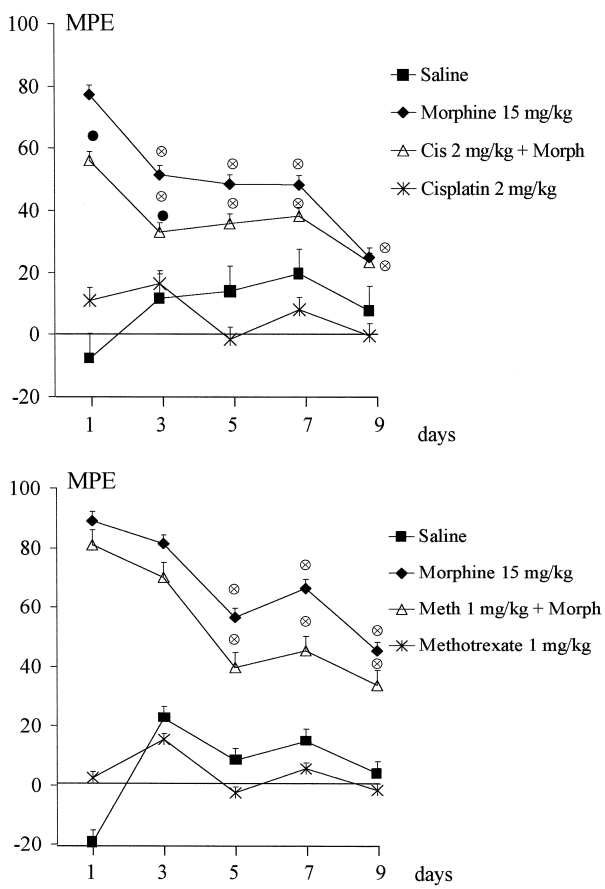

Fig. 4. Influence of cisplatin (Cis, 2 mg/kg i.p.) and methotrexate (Meth, 1 mg/kg s.c.) on the development of tolerance to the analgesic activity of morphine (15 mg/kg s.c.) measured in the hot-plate test (50 ± 0.4°C). Data are means ± S.E.M. of %MPE from 14 animals for each group. $^{\otimes}P < 0.05$ vs. data obtained the first day of treatment; $^{\cdot}P < 0.05$ vs. the corresponding values of morphine group (ANOVA followed by Newman–Keuls multiple comparison test).

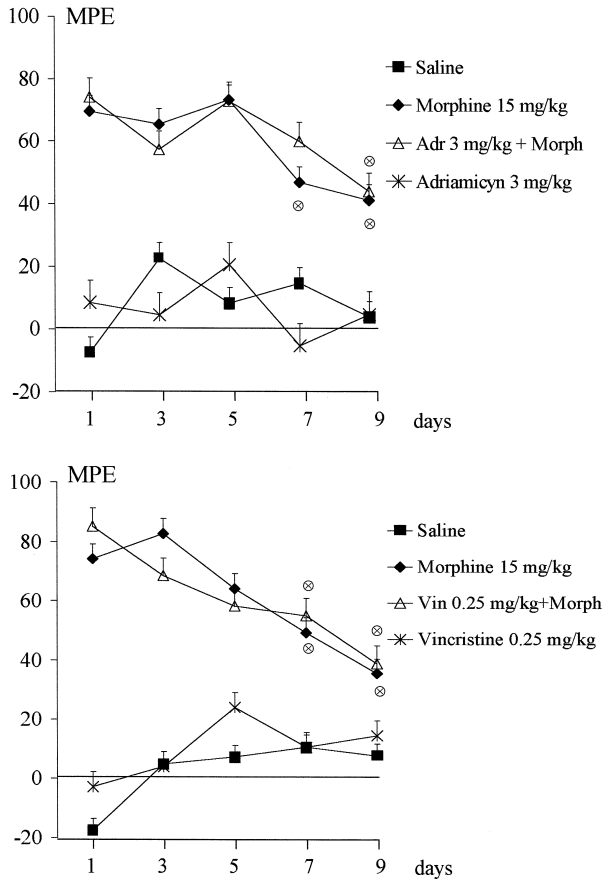

Fig. 5. Influence of adriamycin (Adr, 3 mg/kg s.c.) and vincristine (Vin, 0.25 mg/kg i.p.) on the development of tolerance to the analgesic activity of morphine (15 mg/kg s.c.) measured in the hot-plate test (50 ± 0.4°C). Data are means ± S.E.M. of %MPE from 14 animals for each group. $^{\otimes}P < 0.05$ vs. data obtained the first day of treatment (ANOVA followed by Newman–Keuls multiple comparison test).

3.3. Influence of antineoplastic drugs on the development of tolerance to the analgesic effect of morphine

The repeated administration of adriamycin (3 mg/kg s.c. once daily) delayed the development of tolerance to the analgesic effect of morphine: after seven daily treatments, the effect of morphine in these animals was still not significantly different from the effect produced by the first treatment (Fig. 5).In contrast, cisplatin, methotrexate and vincristine had no significant effect on the development of tolerance to morphine (Figs. 4 and 5).

4. Discussion

The aim of our present research (i.e., to study the possible influence of antineoplastic drugs on the analgesic activity of morphine and on the development of morphine tolerance) is of great relevance in view of the large number of cancer patients concurrently and chronically treated with both antineoplastic drugs and opioid analgesics. We chose four drugs which are widely used in cancer chemotherapy and which are representative of the main classes of antineoplastic compounds. Cisplatin, adriamycin and methotrexate interfere with the function and synthesis of DNA and RNA, and vincristine interferes with the synthesis of microtubules. Albeit indirectly, the former group of drugs eventually inhibits protein synthesis, and the latter drug hampers the mechanisms of axonal transport. So, it seemed justified to surmise that they might influence morphine analgesia and the development of morphine tolerance either by inhibiting the turnover of opiate receptors and the enzymes involved in morphine biotransformation or by affecting neuronal function.

The present results show that, in mice, only cisplatin (out of the four representative antineoplastic drugs: methotrexate, adriamycin, vincristine, and cisplatin) significantly reduced the analgesic activity of morphine, an effect which was observed already following the first treatment. Adriamycin delayed the development of tolerance to the analgesic effect of morphine, while the other drugs had no influence. Finally, none of the antineoplastic agents significantly modified, per se, the thermal pain threshold, either following a single administration or after nine daily treatments.

The sites of action of opioids—as far as analgesic activity is concerned—are in the central nervous system, both at the spinal and supra-spinal levels (Dickenson, 1991). It is possible that one major reason for the lack of influence of vincristine, adriamycin and methotrexate on the analgesic activity of morphine is the inability of these drugs to cross the blood–brain barrier (Elwood, 1989; Chabner et al., 1996). Cisplatin, which in fact interfered with the analgesic activity of morphine, can penetrate into the central nervous system (Chabner et al., 1996). Cisplatin can also enter cells by passive diffusion and within the cell it is hydrolyzed and activated. In this form it can affect the structure and permeability of the cell membrane (Eastman, 1983, 1985; Reed et al., 1986). So, its influence on the analgesic activity of morphine may be due to modification of opioid receptors, reducing their affinity, or to modification of ion channels—particularly K^+ and Ca^{2+} channels—with subsequent interference in the post-receptor steps of the mechanism of action of morphine.

As far as the development of tolerance to the analgesic activity of morphine is concerned, our present results show that it is delayed only by adriamycin, whereas cisplatin, methotrexate and vincristine have no significant influence. Besides its ability to intercalate between adjacent guanine–cytosine base pairs of DNA (Sobell, 1973), adriamycin induces the production of superoxide free radicals (Myers et al., 1977), binds to the cell membrane and alters its function (Tritton et al., 1978), and interacts with topoisomerase II (Hsiang et al., 1988). Some of these mechanisms may interfere with the compensatory modifications in target neurons that are responsible for the development of morphine tolerance.

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