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Pharmacological evaluation of opioid and non-opioid analgesics in a murine bone cancer model of pain

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Abstract

The intramedulary injection of osteosarcoma cells in the mouse femur has served as a laboratory model to study bone cancer pain. However, the efficacy of different classes of analgesics has not fully been analyzed in this model. Therefore, the acute antinociceptive properties of different classes of drugs were evaluated on post-inoculation day 15 when the degrees of spontaneous pain and mechanical hypersensitivity in the ipsilateral inoculated hind paw reached almost their maximal effects. At high doses, the opioids fentanyl, morphine, and tramadol had full efficacies for all pain parameters tested. Antagonism experiments with naloxone (10 mg/kg s.c.) or its peripheral analogue methylnaltrexone (10 mg/kg s.c.), suggest that the analgesic effects of fentanyl were predominantly mediated by centrally located μ-opiate receptors.

Acetaminophen, the non-steroidal anti-inflammatory drug indomethacin, and the COX-2-inhibitor celecoxib did not significantly improve pain behavior. The tricyclic antidepressants amitriptyline and desipramine significantly reduced spontaneous pain behavior but this only at sedative doses; the serotonin reuptake inhibitor fluoxetine had limited efficacy. Also with the anticonvulsants lamotrigine, topiramate, and gabapentin limited or no efficacies were found. In conclusion, the present study provided integrated information about the tumor-induced bone pain in mice, and clarified acute efficacies of different categories of analgesics for the spontaneous lifting, limb-use impairment, and mechanical hypersensitivity. Moreover, the finding that bone cancer-pain behaviors are attenuated by various established compounds further supports the validity of the murine bone cancer model for the study of bone cancer pain and its use for the identification of novel treatments.

Keywords: Analgesics; Bone cancer pain; Hypersensitivity; Opioid; Murine bone tumor model; Anticonvulsants; Antidepressants; NSAIDS; COX-2; Acetaminophen

1. Introduction

Cancer pain resulting from metastasis to skeletal bone is characterized by pathological symptoms, such as hyperalgesia and hypersensitivity to mechanical stimuli, as well as spontaneous pain, and is generally considered to respond relatively poorly to pharmacotherapy (Koltzenburg, 1998; Sindrup and Jensen, 1999). In order to study the neurobiological mechanisms underlying the development of bone cancer pain and to find novel and potentially more effective treatments, a number of rodent models have been developed during the last decade (Martin and Eisenach, 2001). These models are based on either a unilateral injection of tumor cells into bone skeleton or on a unilateral injection close to the bone (Schwei et al., 1999; Medhurst et al., 2002).

The murine bone cancer model appears to be one of the most frequently used preclinical models for the study of cancer pain and its treatment. The model, which is based on a unilateral femoral inoculation, shows many of the pathophysiological properties of chronic cancer pain in human subject (Honore et al., 2000). In addition, this chronic bone cancer model has been demonstrated to be sensitive to a number of compounds, which are used clinically for the symptomatic treatment of chronic cancer and neuropathic pain (El Mouedden and Meert, 2005; Luger et al., 2002).

In bone cancer models, it was reported that spontaneous pain, limb-use impairment, or hypersensitivity reactions to mechanical stimuli could be attenuated by the opioids fentanyl and morphine (El Mouedden and Meert, 2005; Luger et al.,

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2002), acetaminophen (Saito et al., 2005), the non-steroidal anti-inflammatory drug indomethacin (Saito et al., 2005), and the anticonvulsant gabapentin (Donovan-Rodrigez et al., 2005; Kuraishi et al., 2003). It should be noted, however, that these reference compounds were mostly studied singularly (i.e. in separate studies), and that their efficacy was assessed under different experimental conditions and against different modalities using different behavioral readouts. Due to this lack of standardization, the relative potency and efficacy of these compounds remains unclear, making it more difficult to appraise the potential of novel experimental compounds.

The aim of the present study was to assess the pharmacological sensitivity of the femoral bone cancer model in mice to opioids, indomethacin, the cyclooxygenase-2 inhibitor celecoxib (Chan et al., 1999), and to a number of antidepressants and anticonvulsant reference compounds which were reported to have antihyperalgesic and analgesic properties in this model or in other model(s) of neuropathic pain. Efficacy of the compounds against spontaneous paw lifting, limb-use impairment and mechanical hypersensitivity was assessed after acute administration of these drugs. In order to estimate the specificity of the antihyperalgesic and antiallodynic effect, behavioral reactivity was also tested at the contralateral non-operated hind paw. An antihyperalgesic or antiallodynic effect was considered to be specific if it could be demonstrated that it occurred at a dose that did not affect reactivity of the non-operated hind paw.

2. Materials and methods

2.1. Animals

Experiments were performed on C3H/He male mice (Charles River, Sulzfeld, Germany) weighing 25–30 g. The mice were housed, in accordance with the National Institutes of Health guidelines, in boxes of temperature- and humidity-controlled environment, and maintained on a 12 h light/dark cycle with free access to chow and water. All experiments were conducted following the ethical guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and were approved by the Local Animal Care Ethics Committee. Animals could adapt to the laboratory conditions for at least 1 week before surgery took place. Each animal was used only once.

2.2. Cell culture and implantation

NCTC clone 2472 fibrosarcoma cells, originally derived from a connective tissue tumor in a C3H mouse, were obtained from the American Type Cell Culture Collection, Rockville, MD, USA. All cells were maintained as described previously (El Mouedden and Meert, 2005).

For tumor cell inoculation, mice were placed in an enclosed chamber and anesthetized with a combination isoflurane/air (1.5%, 0.5 L/min) in preparation for surgery. When the animal did not respond anymore to a paw pinch, it was removed from the chamber and fitted with a facemask that delivered a combination of isoflurane/air (1.5%, 0.5 L/min) continuously throughout the procedure. The left knee of the mice was bent and placed facing the experimenter; it was shaved and disinfected with povidone–iodine followed by ethanol 70%. A minimal skin incision was made and the patellar ligaments were cut, exposing the condyles of the distal femur. A 23-gauge needle was inserted at the level of the intercondylar notch and the intramedullary canal of the femur to create a cavity for injection of the cells. Approximately 2.5×10^5 cells in a volume of 20 μl of medium were injected unilaterally into the intramedulary cavity of the femur using a syringe. To prevent leakage of cells outside the bone, the injection site was sealed with dental acrylic (Paladur, Heraeus Kulzer, GmbH, Wehrheim, Germany); the surgical procedure was ended by stitching the skin of the knee.

Sham control animals were subjected to operation procedure with the exception that the medium was injected without any cell loading but housed in the same room and conditions in which the testing procedure was performed to try to minimize any stress associated with novel environmental cues.

2.3. Pain behavior assessments

2.3.1. Spontaneous lifting

Spontaneous lifting of the hind paws was measured as described by El Mouedden and Meert (2005). Spontaneous lifting was performed at room temperature and before each test animals were habituated to the laboratory room for at least 30 min. For testing itself, animals were placed and habituated in a transparent acrylic cylinder of 20 cm diameter put on the surface of a glass plate. The animals were observed for 4 min for spontaneous lifting behavior of the left hind paw. Data were expressed as % withdrawal time over total session time. 0% was the normal value observed in most non-operated and sham-operated animals.

2.3.2. Limb-use impairment on rotarod

Limb-use impairment on the rotarod was measured as described by El Mouedden and Meert (2005). After spontaneous lifting assessment, animals were immediately placed on a mouse rotarod (ENV-575M®, Med Associates Inc., GA, USA) at a speed of 16 rpm for 2 min and limb-use during the forced ambulation was scored according to the following criterion: 4 = normal; 3 = limping; 2 = partial non-use of left hind paw; 1 = substantial non-use of left hind paw; 0 = non-use of left hind paw.

The sedative effects of drugs were also examined by determining animals' ability to support their own body weights when forced to run on rotarod at a speed of 16 rpm. Animals that fell from the rotarod within 1 min were considered as positive for sedation/motor impairment.

2.3.3. Assessment of mechanical hypersensitivity

Mechanical hypersensitivity was tested in mice as described by Hofmann et al. (2003) and Vermeirsch et al. (2004). In a quiet room, mice were placed in acrylic cages with wire grid floors 30 min before the start of testing. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer (electronic anesthesiometer, Senselab® Somedic, Horby, Sweden) which was applied manually to the mid-plantar hind paw with a gradual increase in pressure. The end point was

characterized by the removal of the paw followed by clear flinching movements. After the paw withdrawal, the intensity of the pressure was automatically recorded. The value for the response was obtained by averaging three measurements. The animals were tested before and after treatments.

The drug effects are expressed as withdrawal threshold ratio (%) and calculated according to the following formula:

$$\text{Withdrawal threshold ratio}(\%) = \frac{\text{Left paw postdrug threshold} - \text{Left paw predrug threshold}}{\text{Right paw predrug threshold} - \text{Left paw predrug threshold}} \times 100$$

Withdrawal threshold ratio is 100% when the left paw threshold has increased to normal values.

2.4. Pharmacological testing for intrinsic paw lifting and limb-use on the rotarod

Pharmacological testing was performed at random at 15 days post-surgery in animals with confirmed pain behavior. Baseline spontaneous and mechanical hypersensitivity was checked on the day before pharmacological testing, in order to ascertain behavioral pathology. Baseline values were considered to be valid if the Von Frey test values for the left operated and right non-operated hind paw were around 3 and 8 g, respectively. Animals fulfilling this criterion were selected for pharmacological testing on the next day. Each treatment group consisted of 6 mice (receiving either saline or one dose of a compound).

Mice were treated subcutaneously by injection of drugs in the neck in front of the shoulder blades. Sham animals were treated subcutaneously with saline. Injection of saline, administered by the same route as the other drugs, did not significantly affect the nociceptive threshold either in sham-operated or in tumor-beraing mice. The tested compounds included the opioids fentanyl (0.02, 0.04, 0.08, 0.16, and 0.64 mg/kg, s.c. $t=60$ min), morphine (2.5, 5, 10, 20 and 40 mg/kg, s.c. $t=60$ min), tramadol (6.25, 12.5, 25, 50, and 100 mg/kg, s.c. $t=60$ min), and loperamide (2,5, 5, and 10 mg/kg, s.c. $t=60$ min); the tricyclic antidepressants amitriptyline (2.5, 5, 10, 20, and 40 mg/kg, s.c., $t=60$ min) and desipramine (5, 10, 20, 40, and 80 mg/kg, s.c., $t=60$ min); the SSRI fluoxetine (5, 10, 20, 40, and 80 mg/kg, s.c., $t=60$ min); the anticonvulsants gabapentin (12.5, 25, 50, 100, and 200 mg/kg, s.c., $t=60$ min), topiramate (12.5, 25, 50, 100, and 200 mg/kg, s.c., $t=60$ min), lamotrigine (2.5, 5, 10, 20 and 40 mg/kg, s.c.); the antipyretic acetaminophen (paracetamol) (12.5, 25, 50, 100, and 300 mg/kg, s.c., $t=60$ min); the COX-2-inhibitor celecoxib (2.5, 5, 10, 20 and 40 mg/kg, s.c., $t=60$ min) and the NSAID indomethacin (5, 10, and 30 mg/kg, s.c.). All drugs or vehicle solutions were tested 1 h after treatment. Because no differences were observed between the various vehicles and saline, the control data were pooled and referred to as the saline group.

2.5. Pharmacological testing of mechanical hypersensitivity

In separate groups of animals additional testing was performed to evaluate the effects of the various compounds on mechanical hypersensitivity. To do so only the highest doses

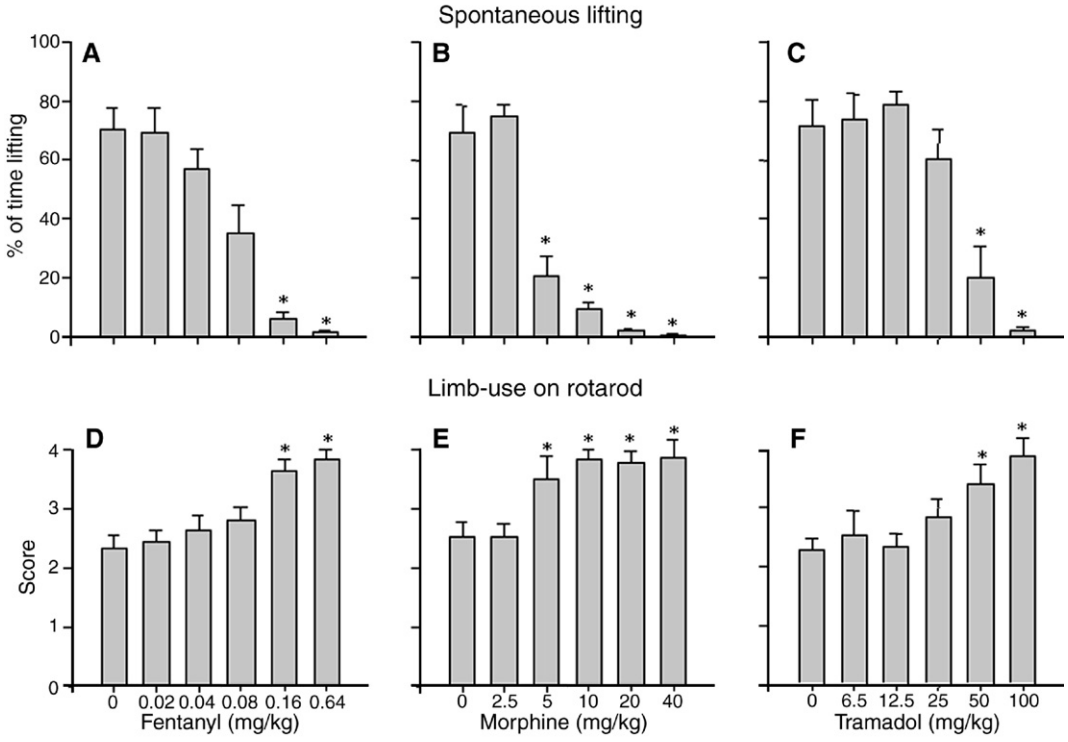

Fig. 1. Effects of fentanyl (A and D), morphine (B and E), and tramadol (C and F) on spontaneous lifting and limb-use impairment on the rotarod in the cancer tumor-induced bone pain model in mice. Fentanyl, morphine, and tramadol were administered subcutaneously 60 min before the measurement of pain parameters. The results are expressed as mean ± S.E.M values of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the saline-treated mice.

were tested. Also here animals were prescreened and drug testing occurred 1 h after subcutaneous administration on day 15. The doses used are presented in the Results section.

2.6. Effects of opioid antagonists on the antinociceptive effects of fentanyl in the bone cancer model

To evaluate whether the general opioid antagonist naloxone and the peripheral antagonist methylnaltrexone can reverse the effects of fentanyl, several additional groups of animals were treated subcutaneously with 0.16 mg/kg fentanyl, 10 mg/kg naloxone or 10 mg/kg methylnaltrexone alone or in combination (Kogel et al., 2005). The doses of the opioid-antagonists were selected on the basis of existing data in mice.

All these animals were tested on spontaneous paw lifting, limb-use on the rotarod, and mechanical hypersensitivity using the automated Von Frey test.

2.7. Statistics

Data were analyzed by a one-way analysis of variance (ANOVA), followed, where appropriate, by Mann–Whitney *U*-testing. Results were considered statistically significant at $P<0.05$ (two-tailed). Data are presented as mean±SEM.

3. Results

The osteosarcoma tumor inoculation into left femur of mice produced pronounced bone destruction and pain-related behavior which peaked 2 weeks after surgery in >90% of the operated animals (El Mouedden and Meert, 2005). At day 15, all tumor-inoculated mice used for testing were confirmed with hypersensitivity as assessed by spontaneous lifting and mechanical hypersensitivity. The selected mice ($n=6$/group) were randomly distributed between different groups of treatment with saline or a drug solution. All treatment groups had comparable baseline values before drug treatment (Mann–Whitney *U* test, exact 2-sided $P>0.05$).

3.1. Spontaneous lifting and limb-use impairment on the rotarod

Compared to the saline-treated tumor-bearing mice, the subcutaneous administration of fentanyl (0.02–0.64 mg/kg), morphine (2.5–40 mg/kg), and tramadol (5–100 mg/kg) produced a dose-dependent attenuation of spontaneous lifting ($P<0.001$) (Fig. 1 A, B, and C) and improved limb-use on the forced ambulatory rotarod ($P<0.001$) (Fig. 1 D, E, and F). Administration of significantly higher doses of fentanyl (≥0.16 mg/kg), morphine (≥5 mg/kg) and tramadol (≥50 mg/kg) reduced almost all pain parameters to normal values observed in sham operated animals (score on rotarod=4; paw lifting <5 s). At these doses no sedation was present. Animals even started to become hyperactive with fentanyl and morphine.

The effects of loperamide, a peripheral μ-opioid agonist, were also examined in this model of tumor-induced bone pain ($n=6$ per treatment group). Administration of loperamide (2.5–

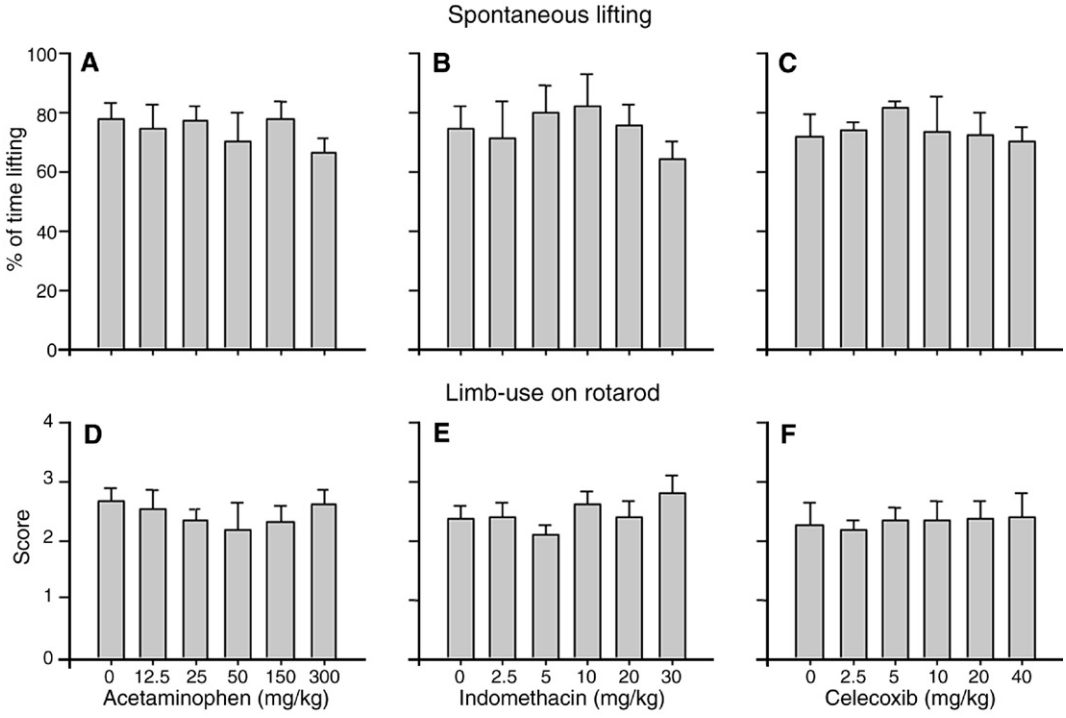

Fig. 2. Effects of acetaminophen (A and D), indomethacin (B and E), and celecoxib (C and F) on spontaneous lifting and limb-use impairment in the tumor-induced pain in mice. Acetaminophen, indomethacin, and celecoxib were administered subcutaneously 60 min before the measurement of pain parameters. The results were expressed as the mean±S.E.M. of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the saline-treated mice.

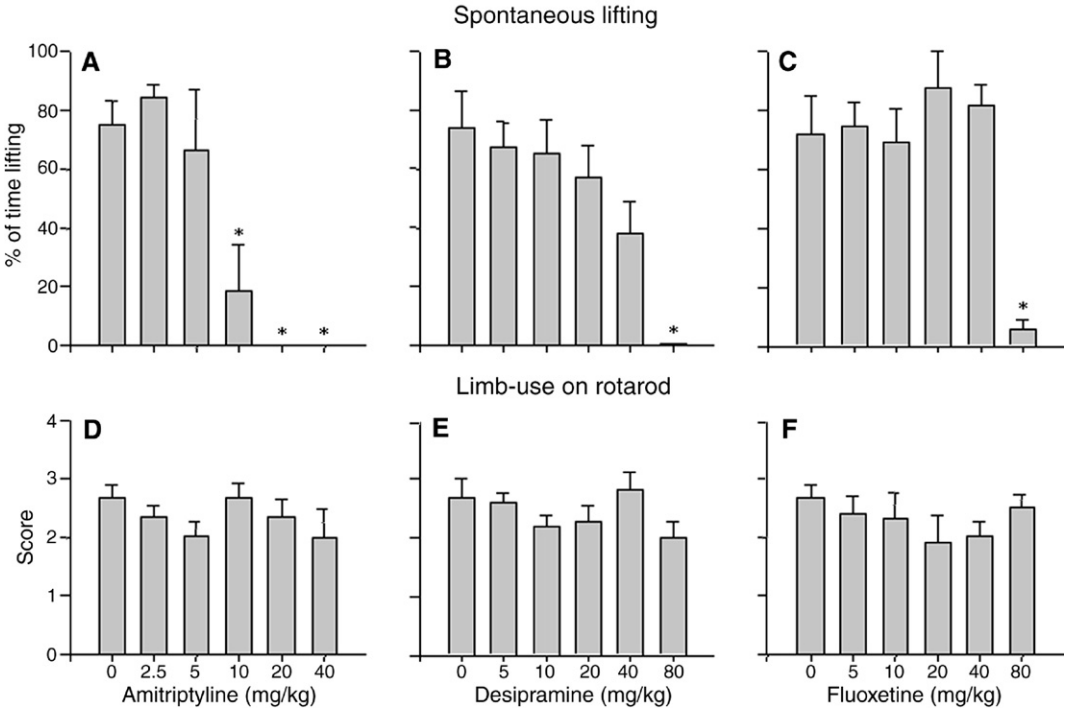

Fig. 3. Effects of amitriptyline (A and D), desipramine (B and E), and fluoxetine (C and F) on spontaneous lifting and limb-use impairment in the tumor-induced bone pain in mice. Amitriptyline, desipramine, and fluoxetine were administered subcutaneously 60 min before the measurement of pain parameters. The results were expressed as mean ± S.E.M. values of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the saline-treated mice.

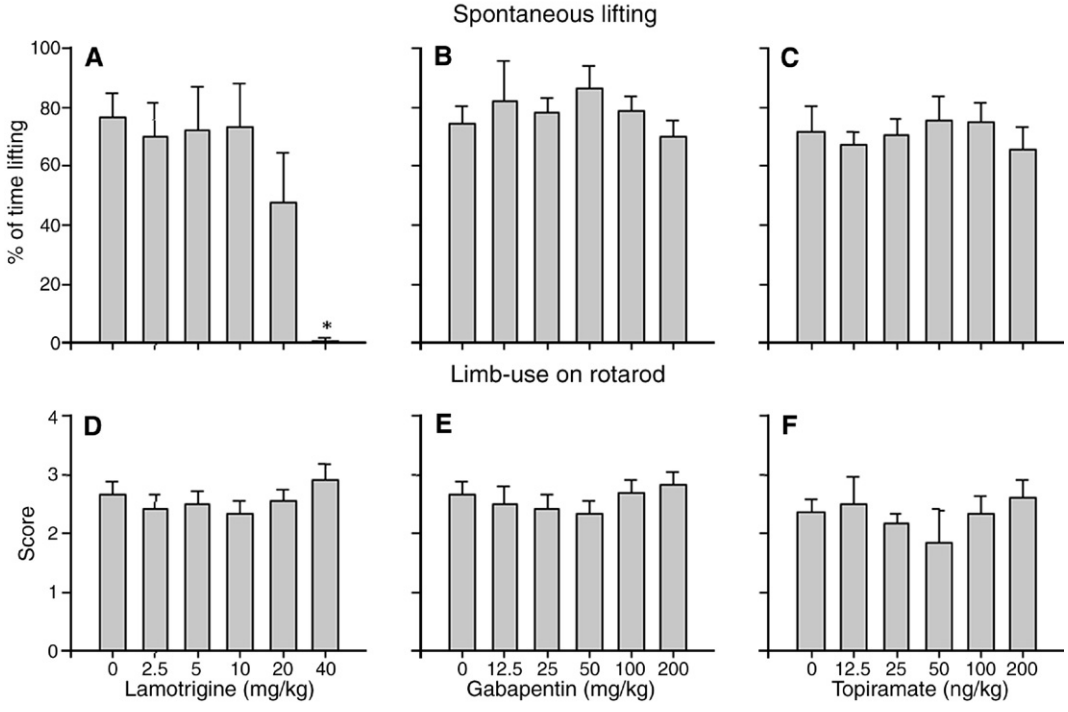

Fig. 4. Effects of lamotrigine (A and D), gabapentin (B and E), and topiramate (C and F) on spontaneous lifting and limb-use impairment in the tumor-induced bone pain in mice. Lamotrigine, gabapentin, and topiramate were administered subcutaneously 60 min before the measurement of pain parameters. The results were expressed as mean ± S.E.M. values of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the saline-treated mice.

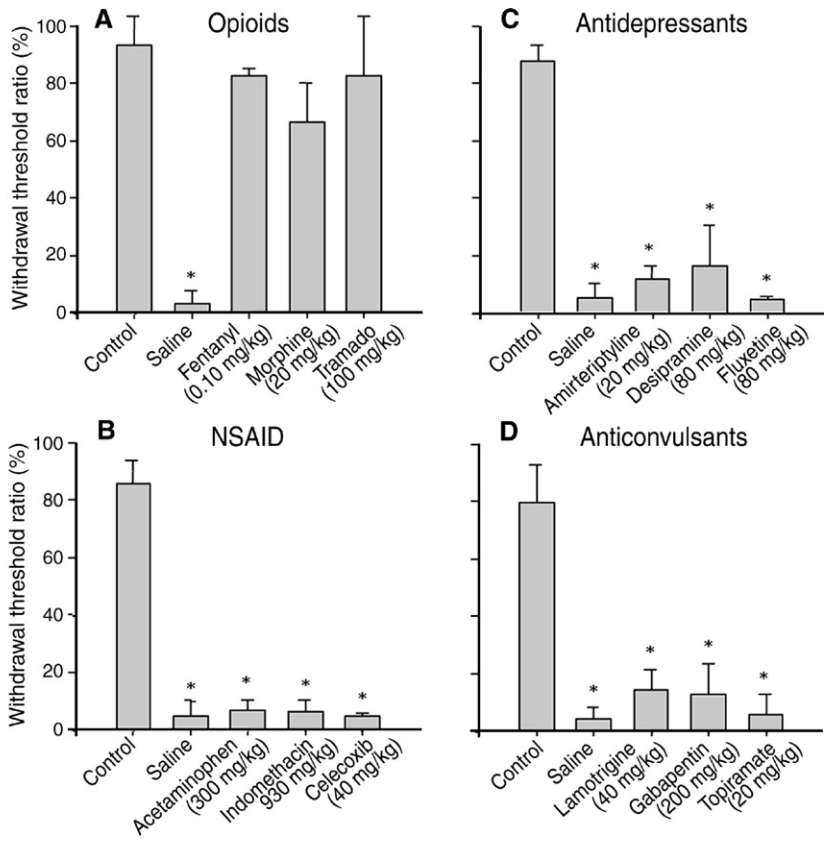

Fig. 5. Effects of opioid agonists (A), acetaminophen, nonsteroidal anti-inflammatory and COX-2-inhibitory drugs(B), antidepressants (C), and anticonvulsants (D) on mechanical hypersensitivity in the tumor-induced bone pain in mice. Compounds were injected subcutaneously 60 min before the measurement of pain parameters. The results were expressed as the mean±S.E.M. of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the sham control mice.

10 mg/kg s.c.) failed to reduce significantly spontaneous paw lifting expressed as % withdrawal time over total session time (saline: 78.03±4.19; 2.5 mg/kg loperamide: 81.20±5.64; 5 mg/kg loperamide: 76.84±9.72; 10 mg/kg loperamide: 71.90±6.53) as well as to improve limb-use scores on rotarod test (saline: 1.84±0.37; 2.5 mg/kg loperamide: 2±0.26; 5 mg/kg loperamide: 1.97±0.35; 10 mg/kg loperamide: 2.16±0.40).

Acetaminophen (12.5–300 mg/kg), indomethacin (2.5–30 mg/kg), and celecoxib (2.5–40 mg/kg.) were inactive on both spontaneous paw lifting (Fig. 2. A, B, and C and limb-use on

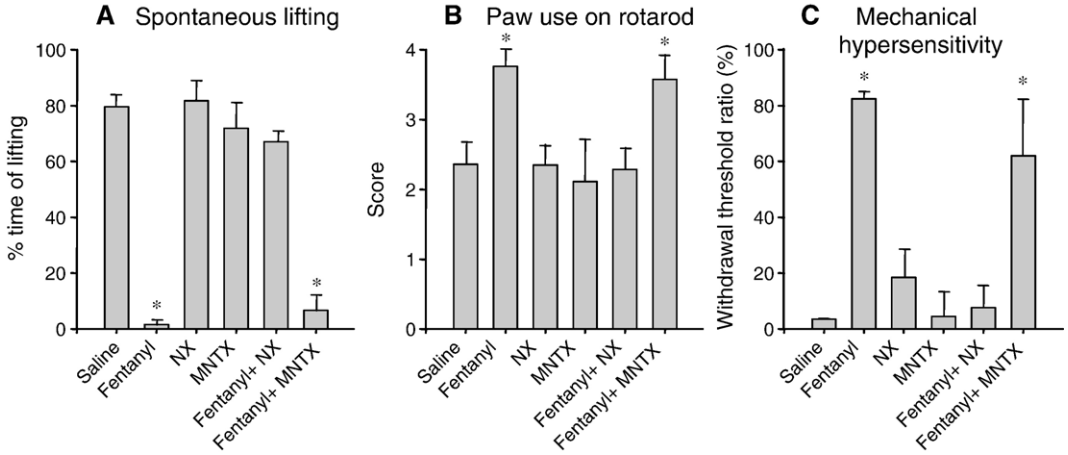

Fig. 6. Antagonism experiments with naloxone (NX; 10 mg/kg s.c.) or methylnaltrexone (MNTX; 10 mg/kg s.c.) on fentanyl (0.16 mg/kg)-induced prevention of osteosarcoma-induced spontaneous lifting, limb-use impairment, and mechanical hypersensitivity. The results were expressed as the mean±S.E.M. of six mice per group. Data were analyzed by one-way analysis of variance (ANOVA), followed by the Mann–Whitney *U* test. * $P<0.05$ significantly different from the sham control mice.

the rotarod (Fig. 2 D, E, and F). Amitriptyline and desipramine failed to improve limb-use on the forced ambulation on rotarod (Fig. 3 D and E). These drugs, however, significantly and fully reduced spontaneous paw lifting (Fig. 3 A and B) but this only at sedative doses (≥10 mg/kg amitriptyline, 80 mg/kg desipramine). Fluoxetine reduced spontaneous lifting at 80 mg/kg (Fig. 3 C), the highest dose tested without affecting rotarod limb-use (Fig. 3 F).

Of the anticonvulsant tested drugs,, lamotrigine completely reduced spontaneous paw lifting at 40 mg/kg (Fig. 4 A) without affecting limb-use on the rotarod (Fig. 4 D). However, gabapentin (12.5 to 200 mg/kg) and topiramate (12.5–200 mg/kg) were completely inactive on both spontaneous lifting (Fig. 4 B and C) and limb-use on rotarod (Fig. 4 E and F).

3.2. Mechanical hypersensitivity

The intra-femoral inoculation of osteosarcoma cells resulted in a pronounced mechanical hypersensitivity assessed by the Von Frey test ($P<0.001$) 15 days after tumor inoculation. All saline-treated tumor-bearing mice had less than 10% of control values. The administration of fentanyl (0.16 mg/kg.), morphine (20 mg/kg.), and tramadol (100 mg/kg.) reversed completely the mechanical hypersensitivity to sham control values ($P>0.05$) (Fig. 5 A). However, loperamide (10 mg/kg s. c.) was inactive on reducing mechanical withdrawal threshold (saline: 4.6±2.4; loperamide: 6.54.0±3.7; $P>0,05$).

The administration of 300 mg/kg acetaminophen, 30 mg/kg indomethacin or 40 mg/kg celecoxib was ineffective on the on the mechanical hypersensitivity (Fig. 5 B). Also with amitriptyline (20 mg/kg.), desipramine (80 mg/kg.), and fluoxetine (80 mg/kg.), lamotrigine (40 mg/kg.), gabapentin (200 mg/kg s.c.), and topiramate (200 mg/kg.) no activity was observed (Fig. 5 C and D).

3.3. Effects of opioid antagonists on the fentanyl-induced activity in the bone cancer model

To determine if the activity of the opioids were mediated by central or peripheral μ-opioid receptors, we examined the effects of 10 mg/kg naloxone and 10 mg/kg methylnaltrexone, on the 0.16 mg/kg fentanyl-induced antinociception in this bone cancer model (Fig. 6). Naloxone and methylnaltrexone did not have any intrinsic effects on spontaneous paw lifting, on limb-use in the rotarod test or on the mechanical hypersensitivity. Naloxone completely reversed the fentanyl activity in all 3 tests while methylnaltrexone was inactive (Fig. 6 A to C). These data suggest that the central, but not the peripheral, μ-opioid receptor plays an important role in fentanyl-induced alleviation of spontaneous and mechanical hypersensitivity.

4. Discussion

The present study assessed the pharmacological sensitivity of the chronic bone cancer pain model under standardized experimental conditions for a whole series of analgesics used in both acute and chronic pain. As reported previously (El Mouedden and Meert, 2005; Vermeirsch et al., 2004), femoral inoculation of mice with NCTC 2472 fibrosarcoma cells resulted in increased spontaneous paw lifting behavior, deterioration of limb-use on the rotarod and mechanical hypersensitivity of the affected hind paw developed within one week post-surgery. After 2 weeks all these behavioral measures for nociceptive behaviors stabilized which allows drug testing in standardized conditions.

In the present series of experiments it was demonstrated that μ-opioid agonists, fentanyl and morphine as well as the mixed μ-agonist with 5-HT/NE-reuptake properties tramadol, reduced bone cancer-induced spontaneous paw lifting, the limb-use impairment on the rotarod as well as the mechanical hypersensitivity. The analgesic efficacy of fentanyl, morphine, and tramadol was demonstrated in many different models of acute and chronic neuropathic pain (Meert and Vermeicsh, 2005). However, the efficacy of opioids is lower in tumor-bearing mice than in inflammatory and neuropathic pain models (Meert and Vermeirsch, 2005; Luger et al., 2002). Significant effects were found with doses on the order of 1–3 mg/kg morphine, 0.01–0.03 mg/kg fentanyl, and 5–10 mg/kg tramadol for inflammatory and neuropathic model. In our model of bone cancer pain, high doses of these opioids (fentanyl: ≥0.16 mg/kg, morphine: ≥5 mg/kg, and tramadol: ≥50 mg/kg) were needed to fully reverse all three tested nocicpetive pain measurements to sham control levels. At these doses no sedation or motor impairment was present as assessed by rotarod performance (Meert and Vermeirsch, 2005; Luger et al., 2002). This suggests that the antinociception observed at these doses was not the result of a significant loss of motor function. Moreover, comparable efficacies have been reported in the literature for various opioids using systemic and topical administration and different behavioral readouts in bone cancer models of mice (Brainin-Mattos et al., 2006; El Mouedden and Meert, 2005; Vermeirsch et al, 2004; Luger et al, 2002; Sasamura et al., 2002).

In order to evaluate whether the effects of opioids were peripheral or centrally mediated, antagonism studies of fentanyl were performed using naloxone and the peripheral opioid-antagonist methylnaltrexone. Both antagonists did not show any intrinsic activity on bone pain parameters used here. Naloxone, but not methylnaltrexone, did reverse the antinociceptive properties of fentanyl. As such these results point to the importance of central components of the μ-opioid agonist-induced analgesic properties in cancer pain. In addition, loperamide (2.5–10 mg/kg s.c.), a peripheral μ-opioid agonist, was inactive on tumor-induced bone pain behaviors. These results question the observations that peripheral opioids like loperamide are sufficient for producing analgesic effects in bone cancer model (Menendez et al., 2003, 2005) or that peripheral restricted opiate antagonists can reverse the analgesic efficacy of opioids (Menendez et al., 2003; Baamonde et al., 2005). Furthermore, the fact that a central spinal and/or brain analgesic component is a principle part of the treatment of bone cancer pain with opioids, is fully supported by clinical practice using classical opioid analgesics and not peripheral restricted opioids to treat (bone) cancer pain. At our knowledge there is no clinical

data comparing the peripheral versus the central acting opioids in patients with cancer pain. Reasons that can explain why certain peripheral opioids and opioid antagonists show efficacy in various readouts of murine bone cancer model have to be found in the way how the opioids are given (topically versus systematically; acute versus chronic), the behavioral readouts used to evaluate the nociceptive behavior, and the level of behavioral inhibition considered to be a significant effect.

Besides opioids, the acute antinociceptive properties of various analgesics used in the treatment of acute and chronic pain were evaluated. The antipyretic acetaminophen, the NSAID indomethacin and the COX-2-inhibitor celecoxib tested at doses up to 300, 30 and 40 mg/kg respectively, were without any activity. Here again and comparing to the literature, some comparable and conflicting results were reported. So Saito et al. (2005) reported that the COX-1-inhibitor SC560 and the COX-2-inhibitor celecoxib were inactive against bone-cancer induced mechanical changes in the Von Frey test while they reported acetaminophen and indomethacin to be active after oral administration. However, the activity of acetaminophen could be potentiated by adding an opioid, illustrating a non-optimal activity of this class of agents. Sabino et al. (2002) reported that a dose of 100 mg/kg intraperitonealy of the COX-2-inhibitor NS398 attenuated both ongoing and movement-evoked bone cancer pain; an effect remaining present after repeated administration of the COX-2-inhibitor in chow during the development of the bone tumor (Fox et al., 2004; Vit et al., 2006). Taken together these data indicate that anti-inflammatory agents can have some impact on various aspects of bone cancer pain but that high doses are needed.

The antidepressants amitriptyline and desipramine and the SSRI fluoxetine reduced spontaneous paw lifting at doses starting from 10, 80 and 80 mg/kg onwards. However, within the same dose range these compounds did not affect the limb-use on the rotarod nor did they affect the mechanical thresholds. The tricyclic antidepressants have been extensively studied, and there is compelling evidence for their analgesic properties in a variety of chronic non-malignant conditions (Onghena and Van Houdenhove, 1992; Watson, 2000; Collins et al., 2000). The use of tricyclic antidepressants as analgesics in medically ill or elderly patients may be limited by the frequent occurrence of side effects (Preskorn et al., 1982; Glassman and Bigger, 1981). The present work demonstrated that the tricyclic anti-depressants amitriptyline and desipramine induced a full recovery of spontaneous lifting only at sedative doses, although it was ineffective for limb-use impairment and mechanical hypersensitivity. At our knowledge there are no reports concerning the analgesic efficacy after acute administration of antidepressants in the tumor-induced bone pain in mice. There are limited data supporting the analgesic efficacy of the selective serotonin reuptake inhibitor antidepressant drugs. This evidence is far less than that which supports the efficacy of tricyclic drugs (Dworkin et al., 2003). However, no studies have been done on cancer pain in mice. Given the established benefit of the antidepressants in patients with diverse types of neuropathic pain, the strongest indication for their use as an adjuvant analgesic in the cancer population occurs in the patient with neuropathic pain whose response to opioids has been inadequate. Despite the rationale for their use was still understood, antidepressants drugs, especially tricyclics have been widely used in the treatment of chronic pain. Because of numerous side effects, the selective serotonin reuptake inhibitors with their favorable side effect profile are preferred nowadays. Tricyclics as well as SSRIs antidepressants possess a number of possible modes of mechanisms of action, and probably the activation of the endogenous opioid receptor mechanisms (Duman et al., 2004; Schreiber et al., 1999; Anjaneyulu and Chopra, 2006), potentiation of the analgesic effect mediated by serotonergic and/or noradrenergic pathways (Pancrazio et al., 1998; Deffois et al., 1996), or interaction with adenosine receptors (Sawynok et al., 1999) are required to activate the endogeneous pain-inhibiting system.

Of the anticonvulsants, gabapentin and topiramate also revealed no antinociceptive properties at doses up to 200 mg/kg, while lamotrigine only reduced spontaneous paw lifting at 40 mg/kg. The highest dose of lamotrigine (80 mg/kg s. c.) tested resulted in a pronounced motor deficit and the animals were unable to perform the rotarod test.

The analgesic efficacy of anticonvulsants was demonstrated in many different models of acute and chronic neuropathic pain and significant effects were found with doses on the order of 3–30 mg/kg lamotrigine, 30–100 mg/kg gabapentin, and 10–50 mg/kg topiramate (Fox et al., 2003; Shannon et al., 2005; Vissers et al., 2006). The mechanism of action of anticonvulsants in pain still remains unclear. Lamotrigine has an action on voltage-gated cation channels (Lees and Leach, 1993), thus stabilising the presynaptic neural membrane and preventing the release of the excitatory neurotransmitters (Braga et al., 2002). Gabapentin acts directly on neurons, probably through its ability to bind to the α/δ_2 subunit of voltage dependent calcium channels (Gee et al., 1996; Taylor et al., 1998). Topiramate possess several mechanisms of action that may be beneficial in pain. It influences the activity of voltage-sensitive sodium and calcium channels, modulates GABA transmission and may influence glutamatergic transmission through interaction with ionotropic non-NMDA receptors (Nitu et al., 2003).

The anticonvulsant drugs are now often used to treat cancer-related neuropathic pain (Caraceni et al., 1999). The here presented negative results with the anticonvulsants contradict results of Saito et al. (2005) showing that gabapentin attenuates mechanical hypersensitivity measured with manual Von Frey filaments in cancer-induced pain in mice. However, it has recently been reported that gabapentin is only weakly active against mechanical hypersensitivity measured with the paw pressure test even at flaccidity-inducing doses in the tumor-induced bone pain in mice (Kuraishi et al., 2003). It is conceivable that different pain mechanisms underlie the tumor-induced bone model and the acute inflammatory and neuropathic pain models in which anticonvulsant are more active (Gustafsson et al. 2003; Laughlin et al., 2002; Shannon et al., 2005; Vissers et al., 2006).

In conclusion, the present study further confirmed the full efficacy of opioid receptor agonists in reducing mechanical hypersensitivity, spontaneous lifting, and limb-use impairment in the tumor-induced bone pain in mice. Overall, the present

results demonstrate that opioids can prevent spontaneous pain behavior and mechanical hypersensitivity, by acting through a central mechanism of action. With other analgesics more limited activities were seen. As such the present study provided integrated information about the time course of pain and other disease development parameters in the tumor-induced bone pain in mice, and clarified acute efficacies of different categories of analgesics in reducing the hypersensitivity and spontaneous lifting by the evaluation on post-inoculation day 15. However, also chronic treatment schedules should be evaluated in strict centralized conditions to fully characterize the present bone cancer pain model.

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