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Bone cancer pain model in mice: evaluation of pain behavior, bone destruction and morphine sensitivity

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Abstract

The primary aim of the study was to correlate pain development during bone cancer growth with objectively obtained tumor-induced changes in bone morphology. Additionally morphine sensitivity of this bone pain was evaluated. Mice were injected into the femur with osteolytic NCTC2472 cells, and behaviorally followed during a 3-week period. During the observation period increasing pain behavior was observed in tumor-bearing animals. Tumor mice exhibited spontaneous and movement-evoked lifting, the latter evoked through non-noxious palpation of the tumor. Limb use during forced ambulation on a rotarod decreased to substantial non-use of the affected limb by day 23. On day 23, micro-computer tomography scans of the tumor-bearing bones were evaluated for bone destruction. Different bone parameters indicative of osteolysis or fragmentation were significantly correlated with pain behavior. In a separate group of mice the effects of different morphine doses on pain behavior were evaluated on days 17 and 21 of tumor growth. Spontaneous lifting and movement-evoked lifting were sensitive to morphine treatment, although stress-induced analgesia due to repeated restraint might minimize movement-evoked lifting in mice. Limb use during forced ambulation was only slightly ameliorated by high morphine doses.

Keywords: Bone cancer; Cancer pain; Opioid; Morphine; Mice model; Metastasis; Breakthrough pain; Osteolysis; CT-scan

1. Introduction

There is a need for drugs that effectively and safely treat cancer pain since up to 90% of patients with metastatic or advanced cancer will experience moderate to severe chronic pain (Portenoy and Lesage, 1999), while nearly half of them have inadequate or under managed pain control (De Wit et al., 2001). Severe cancer-induced bone pain usually occurs in patients with bone metastases, as frequently observed in the case of lung, breast or prostate cancer, the most common human cancers (Mundy, 2002). Because in bone cancer pain, there is a unique neurochemical reorganization of the spinal cord, analgesics effective to treat inflammatory or neuropathic pain are frequently insufficient in this pain state (Honore et al., 2000c). Major contributors to this unique pain state of malignant bone disease are osteoclastic bone resorption (Mantyh et al., 2002; Mundy, 2002; Clohisy and Mantyh, 2003) and the malignant disease itself; moreover, it involves peripheral and central sensitization of the nervous system (Clohisy and Mantyh, 2003). Additionally, bone resorption weakens the bone, so that mechanical stress can place the bone under torsion, thus exciting mechanosensitive fibers present in the periosteum. The presence of afferent nerve fibers within mineralized bone and bone marrow may explain the clinical observation that many patients report skeletal pain well in advance of any radiological evidence of bone destruction (Mach et al., 2002).

Recently, animal models were described to investigate pain due to bone cancer, in which tumor cells are injected locally into the bone (Schwei et al., 1999; Honore et al., 2000a; Wacnik et al., 2001; Medhurst et al., 2002; Walker et

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al., 2002). The present study used the technique of Schwei et al. (1999) to introduce a femoral bone cancer in mice. Animals were injected with osteolytic NCTC2472 cells, and subsequently evaluated for pain behavior under different conditions during a 3-week period. In order to correlate objectively obtained tumor-induced changes in bone morphology with pain behavior, the amount of bone destruction was evaluated through analysis of μCT scan images of the tumor bearing bones on day 23 after induction of tumor growth. In a second part of the study, the effects of morphine on the pain behavior were evaluated in animals in which the tumor had produced significant changes in bone morphology.

2. Methods

2.1. Cells

Osteolytic NCTC2472 mouse fibrosarcoma cells (American Type Culture Collection (ATCC), Rockville, MD) were maintained in NCTC 135 medium, containing 10% horse serum (Gibco) and passaged two times weekly according to ATCC recommendations.

2.2. Animals

Male C3H/HeNCrl mice (Charles River, Sulzfeld, Germany) were kept individually in ventilated cages on a 12-h day/night cycle. Food and water were given ad libitum. On day 0, animals were anaesthetized (xylazine 10 mg/kg i.p. followed by ketamine 100 mg/kg i.p.) and the left limb was shaved and disinfected with povidone-iodine followed by 70% ethanol. Osteolytic NCTC2472 cells were injected into the distal femur after opening of the knee joint, based on the technique of Schwei et al. (1999). Briefly a left knee arthrotomy was performed. Lidocaine 0.05% was applied locally on the exposed condyles to block pain development. Between the femur condyles a light depression was made using a dental bur. A 30-gauge needle was used to perforate the cortex, and tunnel the shaft of the femur. A 300-μl syringe with a 29-gauge needle was used to inject a volume of 20 μl medium containing no or 2.5×10^5 NCTC2472 cells, referring to as sham and tumor-bearing mice. Afterwards, the injection hole was sealed with dental acrylic (Paladur, Heraeus Kulzer, Wehrheim, Germany). All described tests were approved by the Institutional Ethical Committee for Animal Experiments and conform to the European Communities Council Directive (86/609/EEC).

2.3. Experiments

2.3.1. Experiment 1: pain behavior over time

To evaluate the pain behavior over time, a group of sham injected animals and a group of tumor cell injected animals were behaviorally tested during a 3-week period: the day before surgery and on days 5, 7, 9, 11, 13, 14, 15, 16, 18, 19, 20, 21 and 22 after tumor induction.

On day 23, in order to evaluate the efficacy of an opioid, the same animals were tested 1 h after an i.p. administration of 15 mg/kg morphine sulphate. Body weight of the mice was recorded throughout the experimental period to get an idea on general health status. At the end of the experiment the femur of the left hind limb was sampled and scanned by μCT.

2.3.2. Experiment 2: morphine sensitivity

To further obtain an idea of morphine sensitivity in this bone cancer pain model, an additional group of tumor cell-injected animals was behaviorally tested on days 16, 17 and 21. On days 17 and 21, these animals were retested 1 h after i.p. administration of morphine sulphate 10 or 40 mg/kg. Also here, the femur of the left hind limb was sampled and scanned by μCT at the end of the experiment.

2.4. Assessment of nociception

All tests were performed during the light phase and before each test, the animals were habituated to the laboratory room for at least 30 min. The observer was blinded to the surgical and pharmaceutical treatment of the animals. The number of animals in each group is indicated on the graphs.

To measure spontaneous lifting behavior animals were habituated for 5 min in a colorless acrylic cylinder of 20 cm diameter and thereafter observed during 4 min for spontaneous lifting behavior of the left hind limb. Every lift of the left hind limb not related to walking or grooming was considered to be one flinch, and the duration of the lift was counted until the paw again touched the walking surface. Subsequently movement-evoked lifting was evaluated. For this, the tumor site was palpated in a non-noxious way for 2 min, thereafter the animal was observed during 2 min for lifting behavior of the left hind limb. Finally limb use during forced ambulation was obtained by placing the animals on a mouse rotarod (ENV-575M®, Med Associates, Georgia, US) at a speed of 16 rounds per minute for 2 min. Limb use was scored: 4=normal; 3=limping; 2=partial non-use of left hind limb; 1=substantial non-use of left hind limb; 0=non-use of left hind limb.

2.5. Evaluation of bone destruction

On day 23, all animals were sacrificed by use of CO_2 gas. Thereafter, the left hind limb was dissected from the body and the femur, including surrounding muscular tissue, was fixed in 10% phosphate-buffered formalin for 1 week and transferred to 70% ethanol solution for further storage.

A standardized cone beam μCT scan was performed of the left limb using a high resolution (8.870 μm pixel size) in vivo X-ray μCT system for small animal imaging (Skyscan 1076®, Skyscan, Aartselaar, Belgium). After standardized

reconstruction, the data sets for each bone were resampled using Ant: 3D-creator vs. 2.2 e (Skyscan) so that the medial axis of the bone was centrally oriented for each bone. The same thresholds were used for all samples. Hereafter, two- and three-dimensional bone parameter analyses were performed on a 5-mm long femur bone segment situated proximally from the proximal end of the patellar trochlea using CTanalyzer vs. 1.02 (Skyscan).

2.6. Statistics

Throughout the manuscript data are expressed as mean±S.E.M. Animals were assigned to different treatment groups in a randomized way.

In experiment 1 pain behavior between the sham group and tumor bearing group was compared using a Mann–Whitney *U*-test for independent samples, exact two-sided *P*-values were used. In experiment 2, behavioral measurements were compared within animals before and after morphine treatment using a Wilcoxon signed rank test.

For comparison of bone parameters between the sham group and the tumor-bearing groups of experiments 1 and 2, respectively, a Mann–Whitney *U*-test was done and one-sided *P*-values were used (probability 95%, α=0.01). Correlations between bone parameter values and pain behavior were calculated within all animals of both groups by means of a Spearman rank correlation test. Significance was determined using a Monte Carlo estimate of exact one-sided *P*-values with a 99% confidence interval.

3. Results

3.1. Experiment 1: pain behavior over time

3.1.1. Spontaneous lifting behavior

On day 9 after tumor cell injection, some animals displayed spontaneous lifting behavior of the tumor-bearing limb (Fig. 1). The duration of lifting gradually increased over time reaching an average maximal level of 131±22.0 s at day 18, which was about 55±9.3% of the 4-min observation time (Fig. 1; left panel). On day 22 after tumor cell injection, this mean lifting time was 103±18.2 s, or 43±7.6% of the total observation time. Similar observations were made concerning the number of flinches that by day 22 had increased to 44±3.3 during a 4 min period (Fig. 1; right panel). In the animals of the sham group, injected with medium, only occasional lifting of the left hind limb was observed, i.e. 1.9±0.25% of the time lifting (4.7±0.6 s) and 5.1±0.5 flinches per 4 min on average over the experimental period. On day 23 animals were given 15 mg/kg morphine i.p., resulting in a significant decreased lifting behavior in tumor-bearing animals (P<0.05). Lifting was reduced to 1.6±0.64% of the observational period (3.8±1.5 s) and 9±3 flinches on average. Thus, 15 mg/kg morphine completely antagonizes pain behavior in this test.

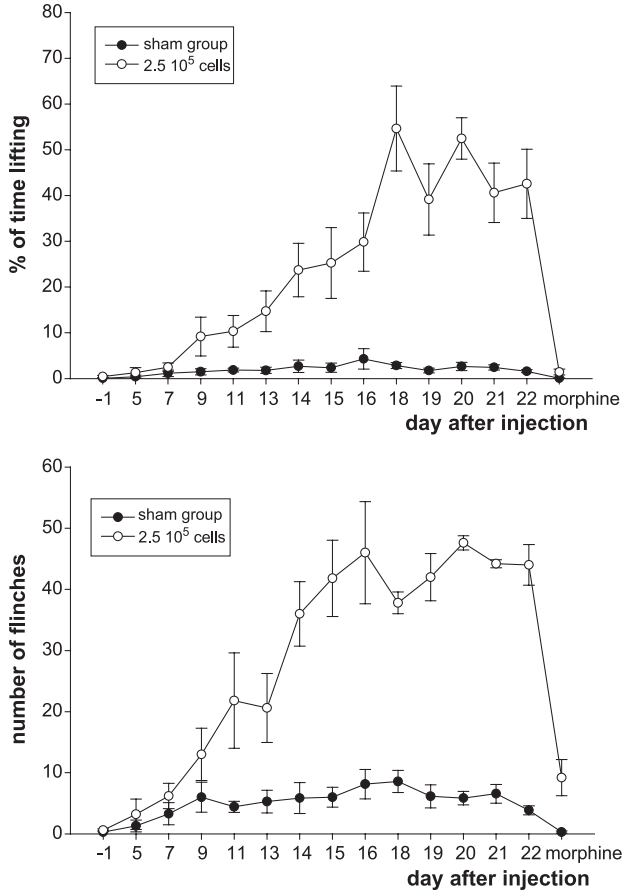

Fig. 1. Spontaneous lifting behavior over time of the tumor-bearing left hind limb in mice after injection of 20 µl medium containing either no cells (n=7) or 2.5×10^5 NCTC2472 cells (n=5). Lifting behavior, % of time lifting as well as number of flinches, increased with time after injection. On day 23, animals were given 15 mg/kg morphine i.p. resulting in a strong and significant decreased lifting behavior in tumor-bearing animals (one-sided P<0.05).

3.1.2. Movement-evoked lifting

On day 7 after tumor cell injection the first animals demonstrated palpation-induced lifting behavior of the tumor-bearing limb (Fig. 2). This lifting behavior thereafter increased with time reaching an average maximal level of 54±5.6 s, i.e., 45±4.7% of the 2-min observation time, on day 22 after tumor cell injection. Also the number of flinches increased to an average of 20±2.3 in a 2 min period. The animals of the sham group, injected with medium, demonstrated no substantial lifting behavior. In this group occasional lifting of the left hind limb was observed, namely 2.1±0.4% of the time lifting (2.5±0.5 s) and 1.8±0.3 flinches per 2 min on average over the experimental period. As compared to spontaneous lifting, palpation-induced lifts were less numerous though one lift had a duration that was on average three times longer than a spontaneous lift. Therefore, the mean duration of the lifting behavior was higher after palpation than when occurring spontaneously. This palpation-induced lifting can be regarded as a sign of movement-evoked pain sensation. On day 23, animals were given 15 mg/

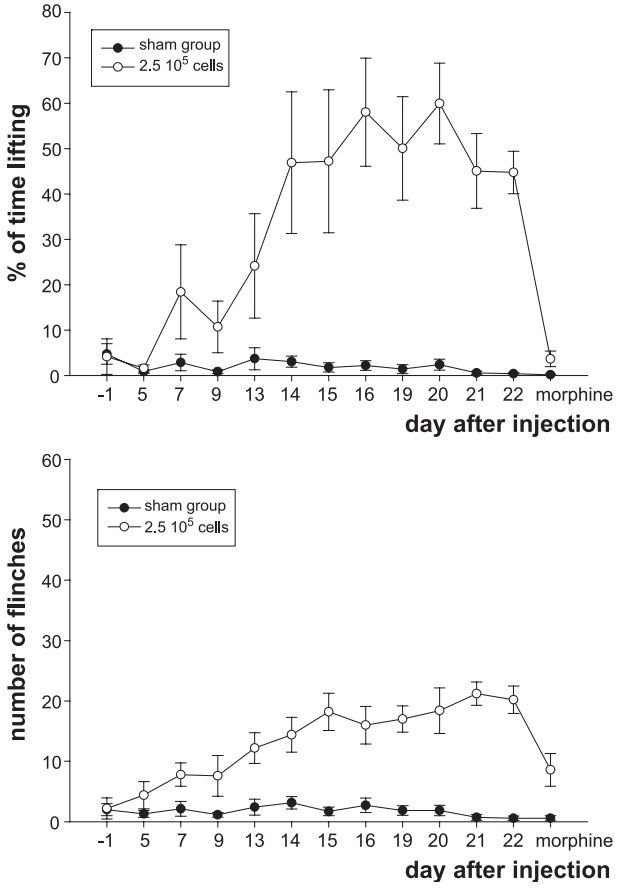

Fig. 2. Movement-evoked lifting behavior over time after 2 min of non-noxious palpation of the tumor-bearing left hind limb in mice. Lifting behavior, % of time lifting and number of flinches, was measured during a 3-week period after injection of 20 μl medium containing either no cells (n=7) or 2.5×10^5 NCTC2472 cells (n=5). Lifting behavior increased with time after injection of tumor cells. The animals of the sham group demonstrated no substantial lifting behavior. On day 23, animals were given 15 mg/kg morphine i.p., resulting in a strongly decreased lifting behavior in tumor-bearing animals (one-sided P<0.05).

kg morphine i.p., resulting in a significant decreased lifting behavior in the tumor-bearing animals. The mean duration of lifting behavior in tumor-bearing animals was now 4.4±2.0 s, or 3.7±1.7% of the 2-min period, divided over a mean of 8.6±2.7 flinches (P<0.05), indicating that 15 mg/kg morphine also antagonizes movement-evoked pain behavior.

3.1.3. Limb use on rotarod

To evaluate ambulation-induced pain behavior, animals had to run on a rotarod for 2 min and limb use was scored. At day 7 after tumor cell injection, limb use was clearly affected in tumor-bearing animals compared to sham-operated animals (Fig. 3). This limb use thereafter decreased with time and on day 22 after tumor cell injection animals showed partial to substantial non-use of the tumor-bearing limb. The animals of the sham group, injected with medium, showed no problems when running on the rotarod. On day 23, animals were given 15 mg/kg morphine i.p., resulting in the tumor cell-injected animals in a slightly increased limb

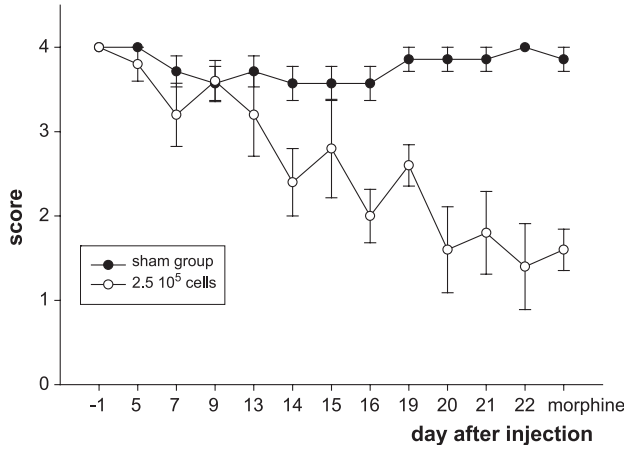

Fig. 3. Use of the tumor-bearing limb over time during forced ambulation on the rotarod turning at 16 rounds per minute. A decreased limb use is observed in tumor-bearing animals over a 3-week period after injection of 20 μl medium containing 2.5×10^5 NCTC2472 cells (n=5). The animals of the sham group (n=7), injected with medium, demonstrated normal limb use. On day 23, animals were given 15 mg/kg morphine i.p. resulting in a slightly, but not significant, ameliorated limb use in tumor-bearing animals.

use during forced ambulation, from substantial to partial non-use of the tumor-bearing limb.

3.1.4. Body weight

With regard to body weight no significant changes were observed. In the sham-operated group of mice body weight showed an initial decrease after surgery but then again increased to become higher than the initial weight from day 14 onwards. In the group of tumor-bearing animals, the mean body weight showed a tendency to decrease during the 23-day period after tumor cell inoculation, never regaining the initial body weight (Fig. 4).

3.2. Experiment 2: morphine sensitivity

Morphine sensitivity of the bone tumor-related pain behavior was tested in a separate group of tumor-bearing

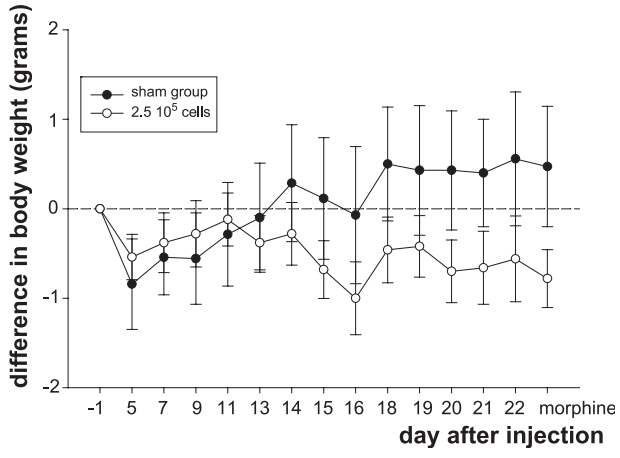

Fig. 4. Mean change in body weight ± S.E.M. of the different groups of animals during the 23-day period after surgery.

animals. Mice were randomly distributed between three groups of six animals receiving a treatment on days 17 and 21 with either vehicle, 10 or 40 mg/kg morphine. No significant differences in behavioral testing before drug treatment were observed between these groups (Mann–Whitney *U*-test, exact two-sided *P*-value). Animals were tested just before and 1 h after i.p. administration of vehicle, 10 or 40 mg/kg morphine (Fig. 5). As compared to lifting behavior before treatment, vehicle-treated animals showed no amelioration of pain behavior. In contrast morphine-treatment induced a significant ($P<0.05$) decrease in duration of spontaneous lifting on days 17 and 21, while the decrease in number of flinches was only significant for the 40-mg/kg treatment. After 10 mg/kg morphine treatment, mean duration of lifting and number of flinches per 4 min decreased from 13.9% and 21.0 to 3.4% and 11.8 on day 17, and from 16.7% and 30 to 10.2% and 16.5 on day 21, respectively. In animals treated with 40 mg/kg morphine mean duration of lifting and number of flinches per 4 min decreased from 8.7% and 18.5 to 0.7% and 4.0 on day 17, and from 27.6% and 41.8 to 6.5% and 12.0 on day 21, respectively.

For movement-evoked lifting, in morphine-treated groups as well as in the vehicle-treated group, a decrease in lifting behavior was observed on both time points. This decrease was most significant in the animals that received a 40-mg/kg morphine treatment ($P<0.05$). After 10 mg/kg morphine treatment, the mean duration of lifting and

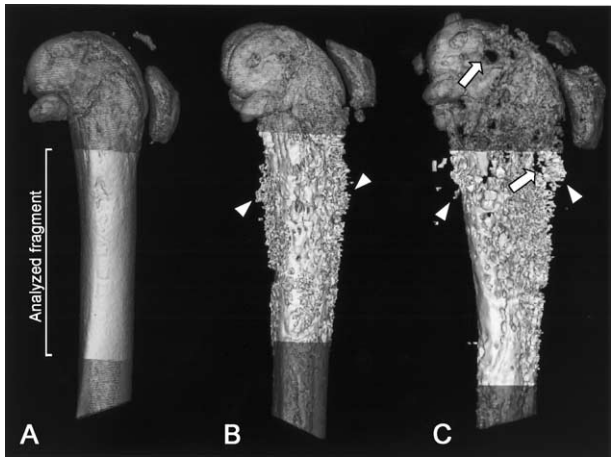

Fig. 6. μCT scan images of bones sampled on day 23 after tumor injection. A sham-injected femur (A) and tumor cell-injected femoral bones (B and C) of experiments 1 and 2, respectively. The highlighted area is the 5-mm-long bone fragment that was used for bone parameter analysis. In the sham-injected femur, no bone destruction or new bone formation was seen. As can be observed, there was extensive destruction of tumor-bearing femoral bones with several osteolytic lesions all along the distal femoral cortex (arrows). Also, some disorganized new bone formation was present (arrowheads).

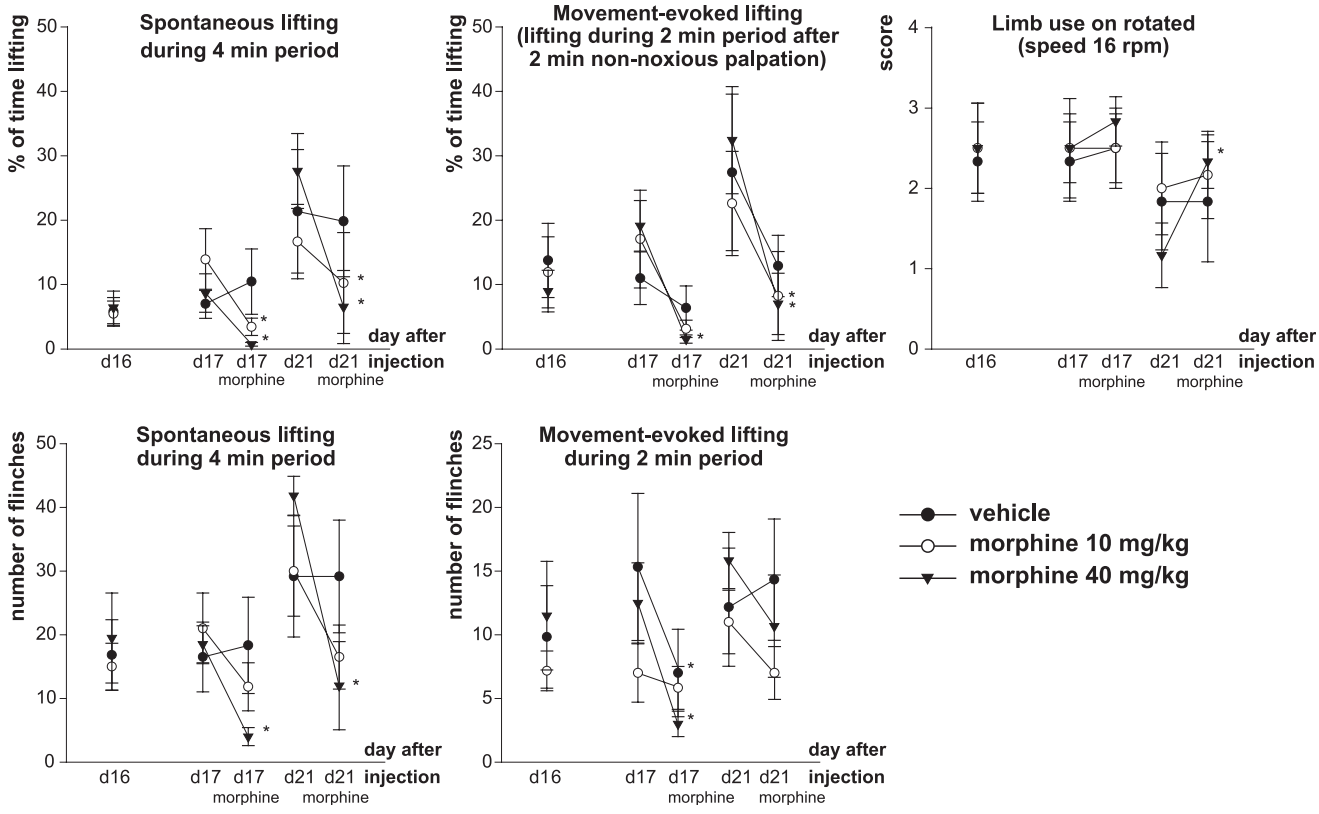

Fig. 5. To evaluate morphine sensitivity of bone cancer pain, animals were tested just before and 1 h after i.p. administration of vehicle or 10 or 40 mg/kg morphine ($n=6$ per treatment condition). As compared to lifting behavior before treatment, vehicle-injected animals showed no change in pain behavior, while morphine treatment showed a dose-dependent decrease in bone-cancer induced spontaneous lifting. Movement-evoked lifting decreased in all groups when measured two times with 1-h interval. Limb use during forced ambulation on the rotarod was only slightly better after 40 mg/kg morphine administration. *$P<0.05$ (Wilcoxon, one-sided *P*-value).

number of flinches per 2 min decreased from 17.1% and 7.0 to 3.1% and 5.8 on day 17, and from 22.6% and 11.0 to 8.2% and 7.0 on day 21, respectively. In animals treated with 40 mg/kg morphine, mean duration of lifting and number of flinches per 2 min decreased from 19.1% and 12.5 to 1.5% and 3.0 on day 17, and from 32.4% and 15.8 to 7.0% and 10.7 on day 21, respectively.

Limb use during forced ambulation on the rotarod was only slightly better after high dose morphine treatment on day 21 after tumor cell injection ($P<0.05$).

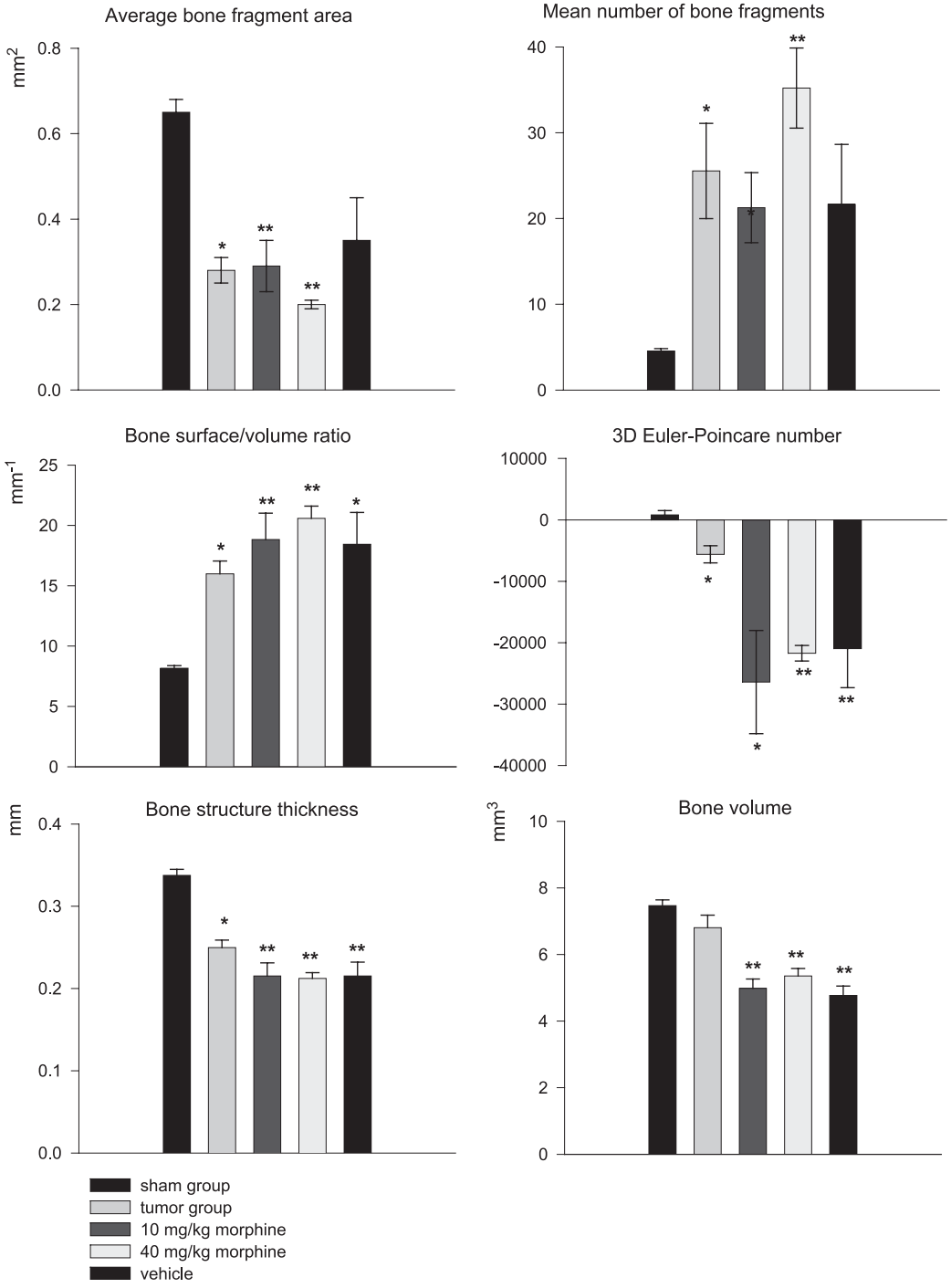

Fig. 7. Bone characteristic parameters in different treatment groups. Parameters between tumor-bearing groups within an experiment were never significantly different. As compared to the sham-operated group, groups of tumor-bearing animals were significantly different. In 2-dimensional slice by slice analysis, the increased mean number and decreased average area of bone fragments in tumor-bearing animals indicated fragmentation of bone. In 3-dimensional analysis, bone surface/volume ratio and Euler–Poincare number indicated fragmentation in tumor-bearing animals, while decreased bone fragment thickness and bone volume were due to tumor-induced osteolysis. *$P<0.01$, **$P<0.001$ (Mann–Whitney U-test one-sided P-value).

3.3. Evaluation of bone destruction

To evaluate bone destruction, μCT scans of complete femoral bones were taken on day 23 (Fig. 6). As observed, there was extensive destruction of tumor-bearing femoral bones with several osteolytic lesions all along the distal femoral cortex. Also, some disorganized new bone formation was present extracortically. In sham-injected femurs (Fig. 6A), no bone destruction or extracortical new-bone formation was seen. Several bone parameters were obtained after computer analysis from the scans (Fig. 7). In experiment 2, differences in bone parameter values between the tumor bearing groups were not significant, thus bone lesions were of the same degree in vehicle and low or high morphine dose treated mice. As compared to the sham-operated group, significant differences were observed with groups of tumor-bearing animals. In a two-dimensional slice by slice analysis, the mean number and the average area of bone fragments were higher and lower respectively in tumor-bearing animals, indicating bone fragmentation. In a three-dimensional analysis, the total bone surface to volume ratio and the Euler–Poincare number indicated fragmentation in the presence of tumor, while decreased bone structure thickness and bone volume were due to tumor-induced osteolysis.

In individual animals, pain behaviors were correlated with the calculated bone characteristic parameters. Increased spontaneous lifting behavior was highly correlated with an increased mean number and decreased average area of bone fragments and with an increased bone surface/volume ratio (R=0.68, 0.66 and 0.55, respectively; P<0.001), and to a lesser degree with a decreased Euler–Poincare number and bone fragment thickness (R=0.51 and 0.47, respectively; P<0.01). Palpation-induced lifting was highly correlated with increased mean number and decreased average area of bone fragments and increased bone surface/volume ratio (R=0.66, 0.65 and 0.56, respectively; P<0.001), and to a lesser extent with a decreased Euler–Poincare number and bone fragment thickness (R=0.54 and 0.47, respectively; P<0.01) and a decreased bone volume (0.30, P<0.05). A decrease in limb use was highly correlated with a decreased average bone fragment area, Euler–Poincare number and bone fragment thickness, and an increased mean number of bone fragments and bone surface/volume ratio (R=0.71, 0.75, 0.74, 0.65 and 0.75, respectively; P<0.001) and to a lesser extent with decreased bone volume (R=0.52; P<0.01).

4. Discussion

Recently, models used to investigate cancer-induced bone pain have been described (Schwei et al., 1999; Honore et al., 2000a; Wacnik et al., 2001; Medhurst et al., 2002; Walker et al., 2002). They allow research on the basic mechanisms underlying the development of this pain, with its cellular pathways, neurochemical changes, pharmacology and behavioral testing. To perform behavioral tests, the pain-inducing stimulus is preferentially localized in a specific and easily accessible body part. As such, these models differ from previous bone metastasis models (Lelekakis et al., 1999; Libouban et al., 2001; Rosol et al., 2003; Vanderkerken et al., 2003) in that the tumor cells are delivered locally into one specific bone, and not given systemically allowing them to metastasize at different sites of the skeleton. The models of cancer-induced bone pain compare well with aspects of human bone cancer (Cain et al., 2001). Correlations between the extent of bone destruction, painful behaviors and neurochemical readouts of pain pathways characterized the distal femora sarcoma model as a valid experimental model for bone cancer pain (Clohisy and Mantyh, 2003).

In the present study, pain behavior was correlated with detailed and objectively obtained parameters describing tumor-induced bone destruction. To characterize pain development over time, mice were behaviorally evaluated after injection of NCTC2472 cells into the distal femur. A group of sham-injected animals was included to rule out the influence of invasive surgery on pain behavior. The model of Schwei et al. (1999) has the disadvantage that during cancer induction knee arthrotomy is performed, meaning tumor-unrelated soft tissue damage. Yet, this joint damage heals very fast and, in accordance with results of Schwei et al. (1999), produced no pain symptoms in the described behavioral tests. This is supported by the findings that the body weight in sham-operated animals increased over the course of the experiment, while in tumor-bearing animals a declining trend was observed. To some extent, different factors can induce this decrease in body weight: pain-induced general sickness and loss of appetite, and as the tumor is located in one of the hind limbs, reduced activity of animals implying decreased feeding behavior. Finally, there is the presence of the bone tumor itself. Severe weight loss accompanies various advanced cancers and has often been observed in experimental animals with bone metastases. This cachexia is thought to be induced by soluble factors produced by hematopoietic and cancer cells, however, its precise mechanism remains poorly understood (Iguchi et al., 2001). In tumor-bearing animals, spontaneous and palpation-induced lifting behavior developed 9 days after injection of cells of the osteolytic fibrosarcoma cell line NCTC2472 that activates osteoclasts and promotes bone destruction (Clohisy et al., 1995, 1996). As in previous studies, where this model was tested on a more limited number of time points, both pain behaviors increased over time (Schwei et al., 1999; Honore et al., 2000a,b,c) and were sensitive to morphine (Luger et al., 2002). Yet, data of experiment 2 showed that palpation-induced lifting behavior also decreased after vehicle treatment, probably caused by stress-induced analgesia (SIA) due to repeated restraint when palpating the tumor. Although care was taken to minimize the stress evoked by minimizing the restraining

method, with only the left hind limb being gently fixed, the possibility of SIA cannot be ruled out. SIA describes a phenomenon whereby exposure to acute stress results in a profound but temporary pain inhibition allowing for an effective "fight-or-flight" reaction (Bodnar et al., 1980). In rodents, environmental stressors including physical attacks and exposure to new situations, and especially in mice also human handling, can produce analgesic states via activation of endogenous pain inhibition mechanisms (Mogil et al., 2001). Therefore, more time should be left between obtaining data before and after compound administration for palpation-induced lifting behavior. As expected (Honore et al., 2000b; Luger et al., 2001, 2002), limb use during forced ambulation on a rotarod clearly decreased in tumor-bearing animals. Morphine was rather unsuccessful in ameliorating limb use; only after 40 mg/kg morphine treatment significant, but slightly ameliorated, limb use was observed. This adds to the observations by Luger et al. (2002), where on day 17 after tumor cell injection in this model doses up to 10 mg/kg morphine gave no change in limb use in the sarcoma mice, but at 30 mg/kg, limb use improved as compared to pre-treatment values. The low potency of morphine to improve rotarod performance may be due to the possibility that mice with femoral tumors experience a kind of ambulatory breakthrough pain during forced ambulation, explaining why only very high morphine doses were effective in strongly advanced disease states. In humans, classical analgesics frequently fail for treating cancer-induced bone pain because of breakthrough pains, occurring spontaneously or more commonly during weight bearing or ambulation (Portenoy and Lesage, 1999). In humans, the severity and frequency of breakthrough pain is positively correlated with the extent of bone destruction and ongoing osteoclast activity (Mercadante and Arcuri, 1998). It is one of the major problems in dealing with bone cancer pain, since opioids can be used to control ongoing pain but need to be increased to very high doses for a sufficient block of breakthrough pain, with increased risk of unwanted side effects (Portenoy and Lesage, 1999).

After documenting the pain behavior, bone destruction was measured in the same animals in an objective and detailed way using μCT scan technology. As the primary aim of cancer pain research is obtaining analgesia, independent of the prevention of metastasis or bone destruction, the analgesic efficacy of compounds for treating bone cancer pain should be evaluated in presence of severe bone destruction. It is known that reducing bone destruction, even in advanced stages, reduces pain behaviors, for instance, after inhibition of osteoclast activity using osteoprotegerin (Honore et al., 2000b; Honore and Mantyh, 2000; Luger et al., 2001). In sham-operated animals, no evidence of bone destruction was present, whereas in animals with femoral tumors extensive bone lesions were found, consisting of osteolysis and new bone formation. Cortical perforations were numerous and bone trabeculae were frequently destroyed in femoral tumors. This extensive bone breakdown and penetration of the tumor through the periosteum is in agreement with previous findings in this model (Schwei et al., 1999). Image analysis was used to quantify bone loss, fragmentation and cancer-induced new-bone formation. These detailed bone morphology data were now compared with pain-related behavioral measurements showing some significant correlations. Especially parameters assessing the degree of fragmentation of the bone, such as mean number and average area of bone fragments, and bone surface to volume ratio, were highly correlated with pain behavior. Decreased limb use was highly correlated with alterations in bone strength and normal bone architecture, which is not surprising since during ambulation, the femur will account for a large fraction of the weight-bearing of the animal. Previous studies, in which bone destruction was assessed in a more limited way by assigning scores to radiographs of tumor bearing bones, described the relationship between bone destruction and the degree of pain behavior (Schwei et al., 1999; Honore et al., 2000b). In the present study, the use of standardized high resolution cone beam μCT scanning is a more objective evaluation method to measure the degree of bone destruction, obtaining several parameters that characterize different features of the bone. Correlating pain behavior with cancer-induced bone destruction over time in subsequent experiments will increase our knowledge of the mechanisms that play a role in bone cancer pain. Moreover, relating analgesic efficacy of compounds to bone destruction is an interesting tool to evaluate their potency for treatment of bone cancer pain.

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