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Evaluation of pain behavior and bone destruction in two arthritic models in guinea pig and rat

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Abstract

The primary aim of the study was to describe and correlate pain behavior and changes in bone morphology in animal models of arthritis both in rats and guinea pigs. Either complete Freund's adjuvant (CFA) or mono-iodoacetate (MIA) solution was injected into the left knee joint to obtain a model for rheumatoid arthritis and osteoarthritis, respectively. Subsequently, animals were behaviorally tested during a period of 12 days after CFA injection and at least 19 days after MIA injection. During these observation periods increasing pain behavior was observed, characterized by decreased von Frey mechanical thresholds and weight bearing on the affected limb. In Hargreaves' paw flick test slightly increased thermal hypersensitivity was observed in some instances in guinea pigs. In rats there was also decreased limb-use during forced ambulation. To evaluate bone destruction μ-computed tomography scans of the arthritic knee were taken on the last experimental day. Different bone parameters indicative of osteolysis and decreased trabecular connectivity were significantly correlated with the observed pain behavior. Detailed description of morphological changes in arthritic joints better characterizes the different animal models and might add to the knowledge on the working mechanisms of analgesic compounds that have an influence on bone structures in arthritis.

Keywords: Arthritis; Complete Freund's adjuvant; Iodoacetate; Animal model; Osteolysis; CT scan; Bone; Pain

1. Introduction

Arthritic pain is a common and often debilitating chronic pain condition characteristic of a variety of arthritic diseases. In preclinical research on arthritic pain a frequently used rat model is obtained after intravenous injection of complete Freund's adjuvant (CFA) to induce polyarthritis. Unfortunately these animals cannot be kept for a longer period as severe systemic changes that affect behavior, physiology and biochemistry will develop and provoke ethical concerns (Butler et al., 1992). Other investigators injected CFA into the plantar skin of the hind paw (Stein et al., 1988) or intradermally at the base of the tail (Awouters et al., 1976), thus inducing polyarthritis of the toe and ankle joints. Also in these models systemic illness will develop if animals have to be kept for chronic studies. Therefore Butler et al. (1992) injected CFA into a single joint, namely the tibio-tarsal joint, thus inducing a predictable monoarthritis rat model, that can be used for a period up to 6 weeks without development of systemic CFA-induced changes. Bone morphologic changes in this rat model were briefly documented using radiographs of the joints (Butler et al., 1992). In CFA monoarthritis neutrophils and other inflammatory cells play an important role, which is characteristic of rheumatoid arthritis (RA). A different kind of arthritic pathology in the rat is observed after injection of sodium mono-iodoacetate (MIA) into a joint. MIA induces a chronic arthritic pathology via the inhibition of glycolysis. It thereby targets the avascular cartilage and causes chondrocyte death, thus providing a basis for studies on the mechanisms of pain as in osteoarthritis (OA) (Fernihough et al., 2004, 2005; Guzman et al., 2003).

The aim of our study was to describe in detail changes in bone morphology after induction of arthritis and to evaluate pain behavior. To cover two important types of arthritis, RA and OA respectively, as well CFA- as MIA-induced arthritis in the knee joint was evaluated. Development of the models was done in parallel in two animal species to ensure the possibility of evaluating new pharmacological pain targets for arthritis in

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different species. After CFA injection into the knee joint, animals were evaluated for pain behavior during a 12-day period, after MIA injection during a 19-day period for guinea pigs or 33-day period for rats. Pain manifested as a change in weight bearing, von Frey threshold and limb-use. At the end of the experiment the degree of bone destruction was measured through analysis of μ-computed tomography (μCT) scan images of the arthritic knees. In both rat and guinea pig correlation coefficients were estimated for correlations between arthritis-induced changes in bone morphology and pain behavior.

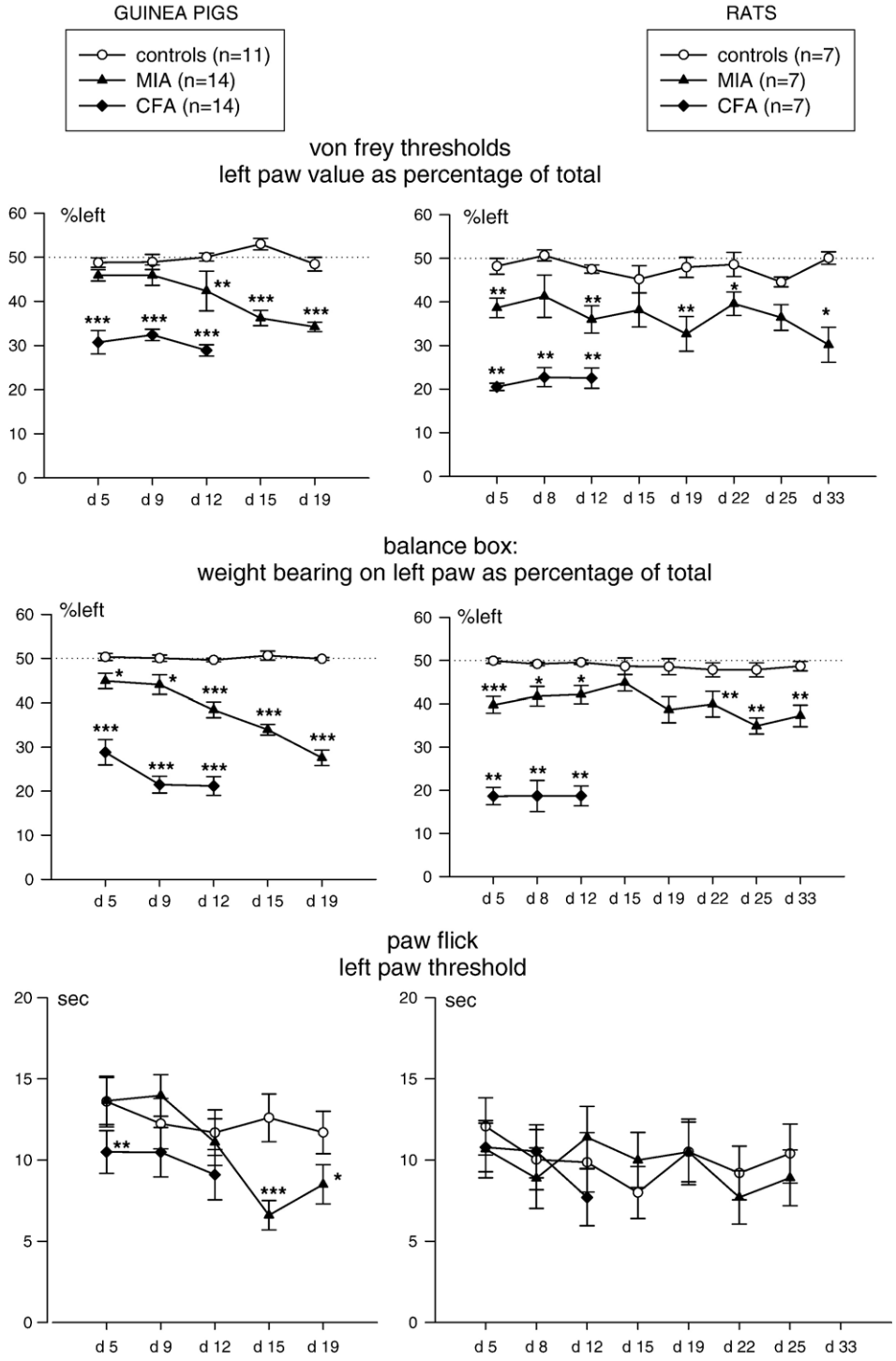

Fig. 1. Pain behavior in either MIA- or CFA-induced monoarthritis in the left knee joint of guinea pigs (left graphs) and rats (right graphs), mean values±s.e.m. Results from animals with arthritis were compared to control animals (MWU test exact 2-sided P values, *$P<0.05$, **$P<0.01$, ***$P<0.001$). For the von Frey test and balance box, the ratio of the left paw value to the sum of values of both paws is given. For the paw flick test the left paw threshold is given.

2. Methods

2.1. Animals

Sprague Dawley rats (Harlan, Eystrup, Germany) and Dunkin Hartley Guinea pigs (Charles River, Kissleg, Germany), both male and weighing 300–320 g, were maintained individually in ventilated cages on a 12 h light/dark cycle at a temperature of 22±1 °C. During housing water and food were available ad libitum. All tests were performed according to guidelines of the Institutional Ethical Committee for Animal Experiments, those for animal research according to IASP, and the guidelines as described to maintain the AAALAC accreditation of our lab.

2.2. Induction of arthritis

On day 0 animals were anaesthetized with isoflurane administered in a mixed N_2O/O_2 gas and the left knee was shaved and disinfected with povidone–iodine followed by 70% ethanol. Following the technique of Butler et al. (1992) monoarthritis was induced by intracapsular injection into the left knee joint. Either 50 µl of complete Freund's adjuvant (5 mg killed *Mycobacterium butyricum* in 1 ml of paraffin oil) or 50 µl sodium mono-iodoacetate (40 mg/ml) (Sigma-Aldrich NV, Bornem, Belgium) was injected through the patellar ligament, using a 300 µl syringe with a 29G needle. Control animals were injected into the left knee joint with 50 µl saline. The number of animals per experimental group is given in the figures.

2.3. Assessment of pain behavior

The animals were behaviorally tested every 3 to 4 days after induction of arthritis with the observer blinded to the type of injection the animals had received. 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 tests performed were respectively, an automated von Frey test for mechanical hypersensitivity (Meert and Vermeirsch, 2005), measurement of weight bearing on both hind limbs and a Hargreaves' paw flick test for heat hypersensitivity. In rats additionally limb-use during forced ambulation on a rotarod was evaluated.

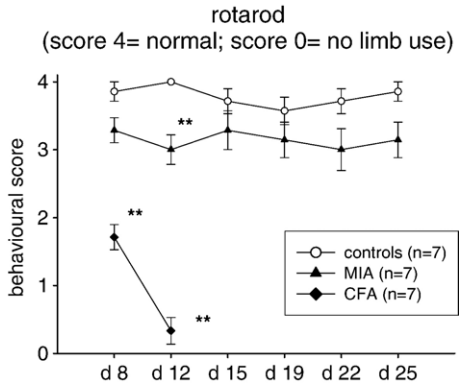

Fig. 2. Limping behavior in either MIA- or CFA-induced monoarthritis in the left knee joint of rats, mean values±s.e.m. Results from animals with arthritis were compared to control animals (exact 2-sided *P* values, $^{**}P<0.01$).

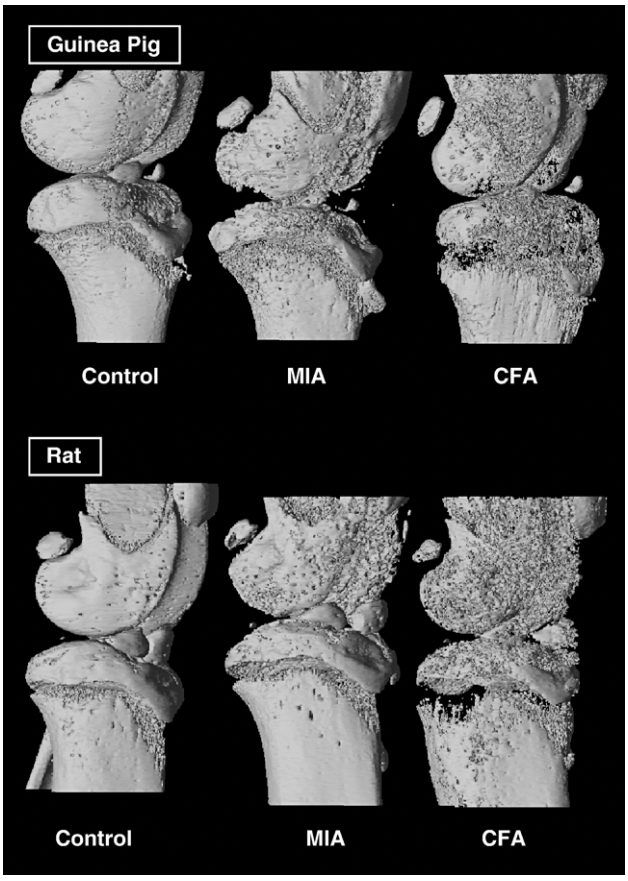

Fig. 3. Visualization of the knee joints of guinea pigs and rats. Compare the smooth surface of a control knee joint (left), with the bone erosions observed in a knee after MIA injection (middle) and after CFA (right). In the arthritic joints osteolytic defects are obvious. In the rat CFA-injected knee some extracortical bone formation is visible on the left side of the tibia.

Von Frey thresholds for measuring mechanical hypersensitivity were obtained 1 h after habituation to the test cage. Gradually increased pressure was applied with a mechanical von Frey probe (1.0 mm of diameter, Senselab® Somedic, Hörby, Sweden) perpendicularly into the mid-plantar surface of the paw. The stimulus was continued until the hind paw was withdrawn or elevated such that the force leveled off. The peak of force in grams was recorded. For each animal 3 measurements of each hind paw were taken with a 60 s interval between stimuli. The percentage is given of the left paw von Frey threshold to the sum of the left and right paw threshold.

For measuring weight bearing, the animals were placed in a Plexiglas cage with each hind paw on a small electronic balance (balance box, Somedic, Hörby, Sweden) and once the animal was settled, 3 measurements of 5 s were done, generating the weight in gram that each individual hindpaw could bear. The weight bearing on the left hind limb is given as percentage of total weight bearing on both hind limbs.

In the Hargreaves' paw flick test for heat hypersensitivity a radiant heat produced by a light source (Plantar test®, IITC Inc. Life Science, Woodland Hills, CA, USA) was administered to

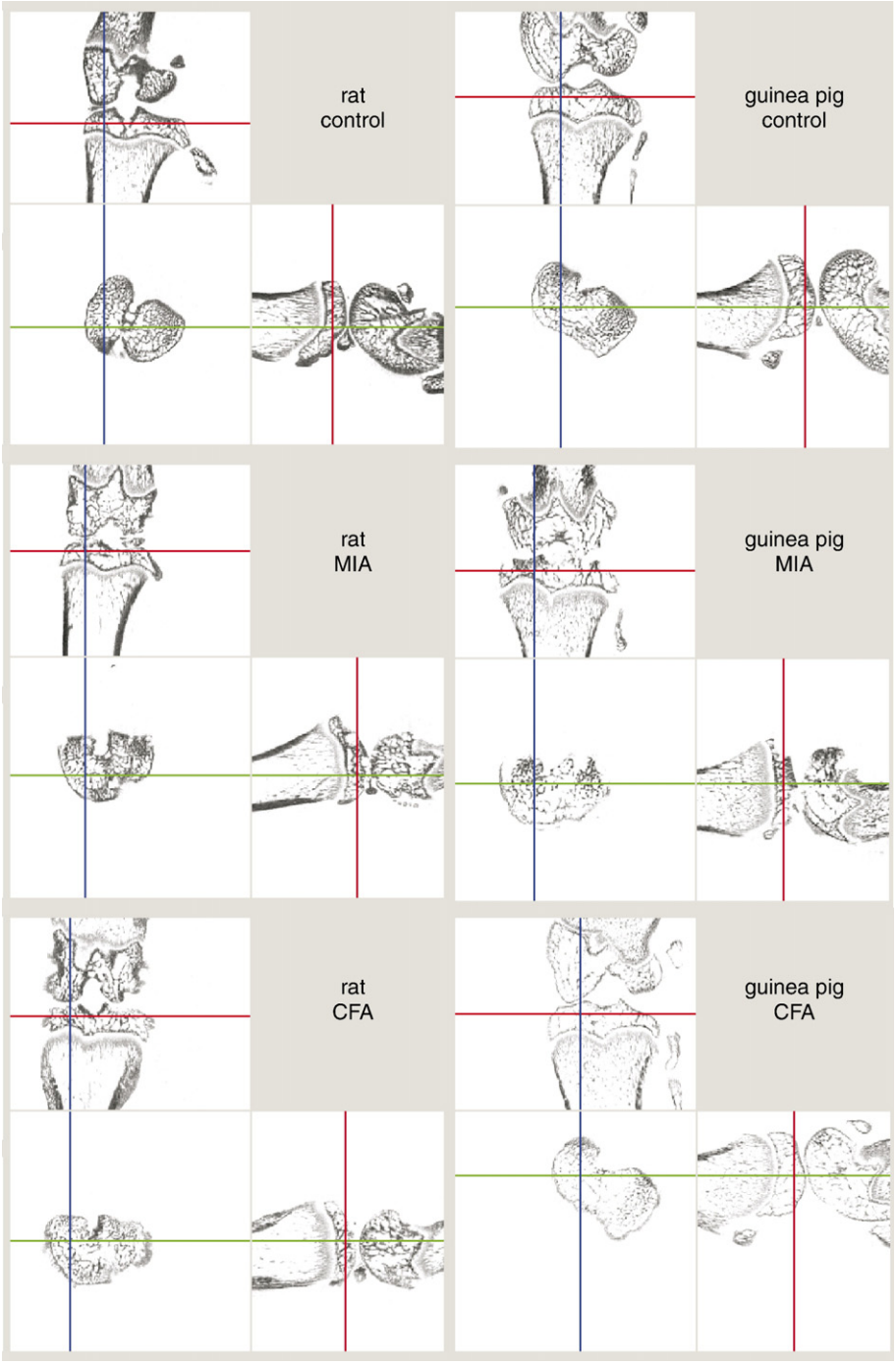

Fig. 4. Saggital, transverse and axial section through a control knee joint (upper images), MIA- (middle images) and CFA-induced arthritic (lower images) knee joint of a rat (left) or guinea pig (right). In the arthritic joint there is a loss of trabecular structures, and thinning of compact bone. In the CFA-injected rat limb this induces a periosteal reaction with irregular calcifications, thus extending the bone perimeter.

the plantar surface of the hind paw, with an intensity such that no tissue damage occurs at the duration of the cut-off latency of 20 s. The heat stimulus was given until the animal withdrew its paw or until the cut-off time was reached. Withdrawal was defined as lifting, licking or flinching of the paw. For each animal 3 measurements of each hind paw were taken with a 5 min interval between measurements.

Finally in rats limb-use during forced ambulation was evaluated by placing the animals on a rotarod (ENV-575®, Med Associates Inc., Georgia, US) at a speed of 16 rounds per minute. 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.

2.4. Evaluation of bone destruction

On the last experimental day all animals were sacrificed by use of CO_2 (day 12 for CFA-induced arthritis, day 19 or 33 for

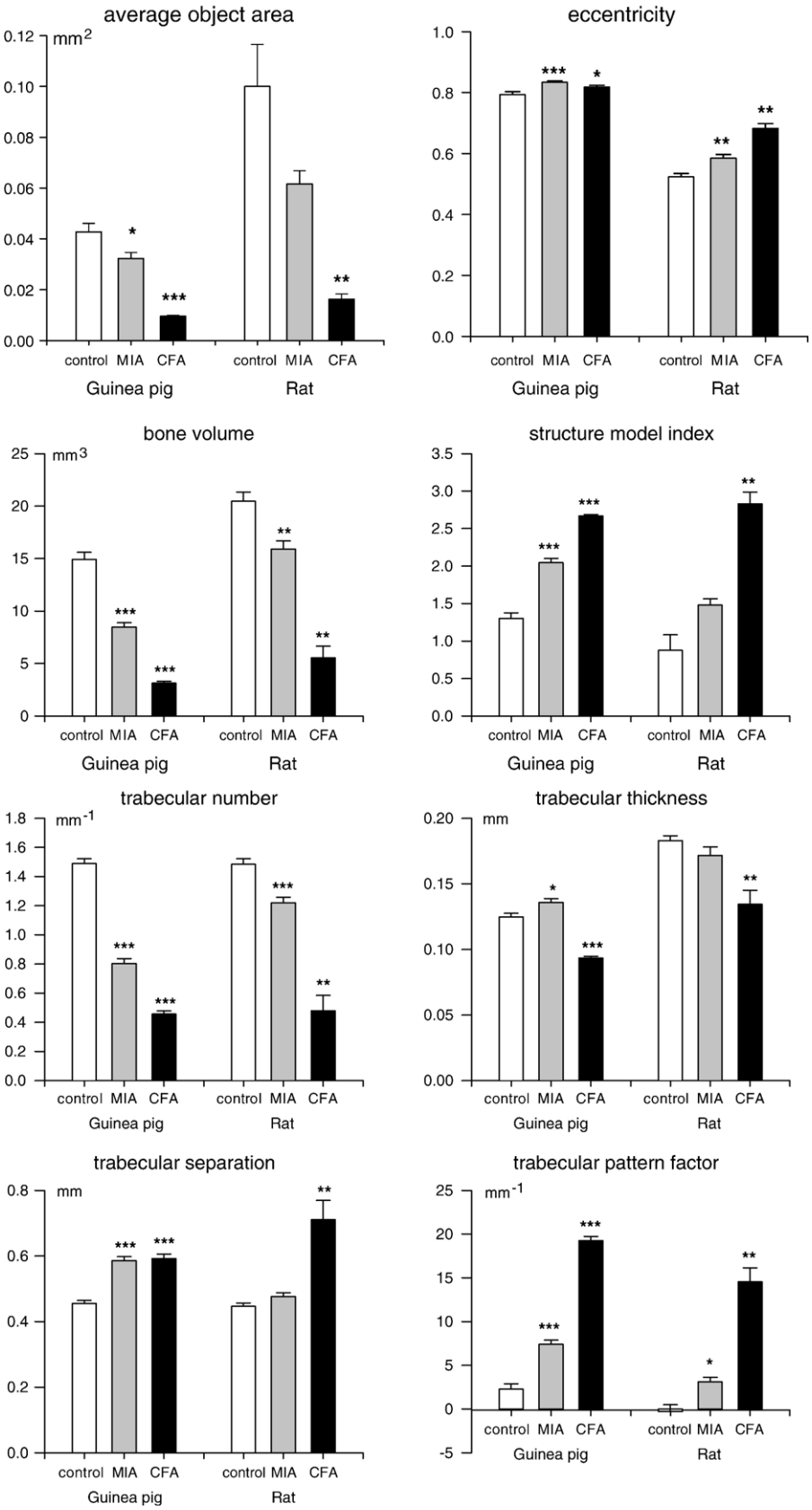

Fig. 5. Bone specific parameters in MIA- or CFA-induced monoarthritis in the left knee joint of guinea pigs and rats. The proximal 1.5 mm of the tibial bone was analyzed and data of control animals (white bars) were compared to data of animals with MIA- (gray bars) or CFA-induced (black bars) arthritis, mean values ± s.e.m. (exact 2-sided P values, *$P<0.05$, **$P<0.01$, ***$P<0.001$).

MIA-induced arthritis in guinea pigs or rats, respectively). Thereafter the left hind paw, including surrounding muscular tissue, was dissected from the body.

A standardized cone beam μCT scan of the left limb was performed using a medium resolution (17.75 μm pixel size) X-ray μCT-system for small animal imaging (Skyscan 1076®, Skyscan, Aartselaar, Belgium). After reconstruction using standardized parameters and thresholds for all samples, two and three dimensional bone parameter analysis was performed on a 1.5 mm long bone segment situated distally from the proximal end of the tibial plateau using CTanalyzer version 1.3.2.2 (Skyscan, Aartselaar, Belgium). Calculation methods of bone parameters are described on the Skyscan website (www.skyscan.be) and by Chappard et al. (2001).

2.5. Statistics

Throughout the manuscript data are expressed as mean±s.e.m. Animals were assigned to different experimental groups in a randomized order. Between the control group and arthritis group pain behavior and bone parameters, respectively, were compared using a Mann–Whitney *U* test for independent samples (exact 2-sided *P* values).

Correlations between bone parameter values and pain behavior were calculated within all animals of both arthritis groups by means of a Spearman rank correlation test. Data of the last experimental day were used to calculate these correlation coefficient estimates. Significance was determined using a Monte Carlo estimate of exact *P* values with a 99% confidence interval.

3. Results

Body weight of the animals was monitored throughout the experiment (data not shown). Body weights of the different experimental groups were comparable, although the body weight increase over time in MIA- and CFA-injected guinea pigs, respectively, tended to be slightly smaller as compared to control animals. Similar observations were made in the rat experiments, additionally the CFA-injected rats had a decreased body weight on day 5 to 92%±2.2% of that on day 1, thereafter it increased comparably to control animals.

3.1. Evaluation of pain behavior

Pain behavior due to monoarthritis in the left knee joint of guinea pigs and rats (Fig. 1) was characterized by clearly decreased von Frey thresholds in the arthritic left paw, this was compensated by slightly increased thresholds in the right paw (results right paw not shown). Similar observations were made for weight bearing, namely a decrease for the left paw compensated by an increase for the right paw as compared to control animals. It must be emphasized that the decreased von Frey thresholds were due to a decreased counter-pressure of the affected limb and not to hypersensitivity for mechanical stimuli. In the paw flick test heat sensitivity was increased in the arthritic left paw in guinea pigs, while in rats no clear change was observed. This increased sensitivity in guinea pigs was observed especially in CFA-injected animals, and in a later stage it was also observed in MIA-injected animals. In rats limb-use during forced ambulation was decreased to substantial non-use of the affected limb in CFA-arthritic animals and limping behavior in MIA animals (Fig. 2).

3.2. Evaluation of bone destruction

To evaluate bone destruction μCT scans of knee joints were taken on the last day of the experiment (Figs. 3 and 4). There

Table 1
Correlation coefficient for correlation between several bone parameters and different tests for pain behavior in 2 models of arthritis in the guinea pig and rat

	Guinea pig			Rat			
	VF	BB	PF	VF	BB	PF	RR
CFA							
Average object area	0.79***	0.72***	0.32	0.93***	0.79**	0.20	0.79**
Mean eccentricity	−0.42*	−0.31	−0.28	−0.58*	−0.74**	−0.65*	−0.85***
Bone volume	0.73***	0.83***	0.39	0.90***	0.75**	0.21	0.81***
Trabecular number	0.67***	0.81***	0.34	0.86***	0.78**	0.30	0.81***
Trabecular thickness	0.76***	0.80***	0.35	0.83***	0.68*	0.17	0.74**
Trabecular separation	−0.61**	−0.84***	−0.27	−0.67*	−0.65*	−0.37	−0.85***
Trabecular pattern factor	−0.76***	−0.77***	−0.29	−0.91***	−0.79**	−0.30	−0.81***
Structure model index	−0.79***	−0.68***	−0.27	−0.87***	−0.76**	−0.31	−0.83***
MIA							
Average object area	0.54**	0.32	0.21	0.36	0.54*	0.55*	−0.17
Mean eccentricity	−0.57**	−0.58**	−0.02	−0.35	−0.58*	−0.55*	−0.22
Bone volume	0.79***	0.69***	0.35	0.50*	0.78**	0.60*	0.20
Trabecular number	0.72***	0.69***	0.42*	0.50	0.76**	0.30	0.25
Trabecular thickness	−0.24	−0.49*	−0.33	0.22	0.45	0.72**	−0.28
Trabecular separation	−0.68***	−0.71***	−0.53**	−0.08	−0.20	−0.13	−0.01
Trabecular pattern factor	−0.72***	−0.59**	−0.34	−0.42	−0.65*	−0.54*	−0.01
Structure model index	−0.76***	−0.66***	−0.36	−0.41	−0.59*	−0.46	0.06

Correlation coefficient estimates for correlations between bone parameters and pain behavioral measurements (VF: von Frey thresholds, BB: balance box, PF: Hargreaves' paw flick test, RR: rotarod performance) (*$P<0.05$; **$P<0.01$; ***$P<0.001$).

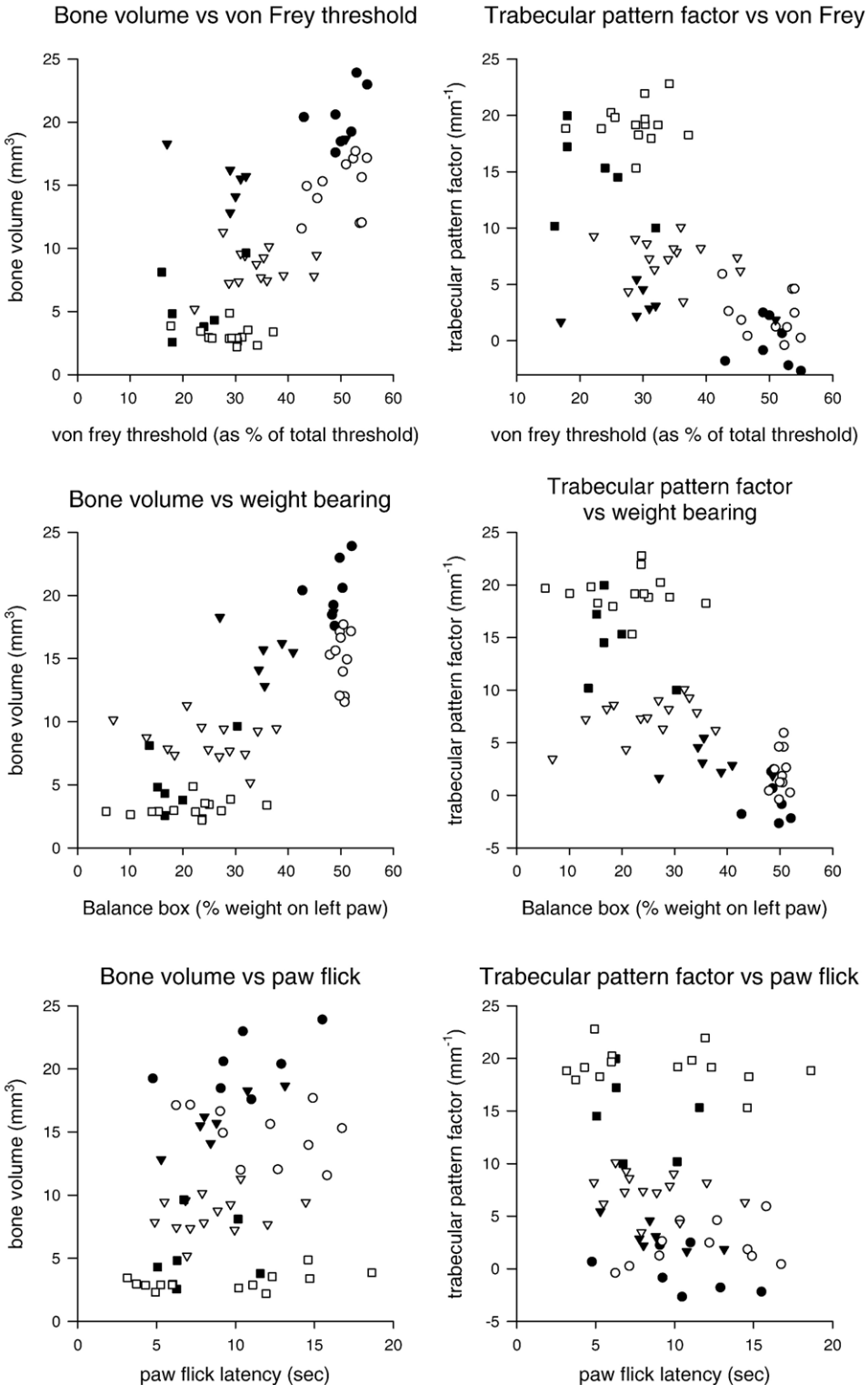

Fig. 6. Scatter plots of pain behavior versus bone volume (a parameter for osteolysis (left graphs)) or trabecular pattern factor (a parameter for fragmentation (right graphs)). Data are plotted for control groups (circles), MIA-injected (triangles) and CFA-injected animals (squares), as well rats (black) as guinea pigs (white).

was extensive destruction of the distal end of the femur and proximal end of the tibia with several osteolytic lesions. In rats with CFA arthritis some disorganized calcifications were present extracortically and seemed to be associated with the periosteum. This was not observed in MIA-injected rat knees nor in either model in guinea pigs. In control knees no bone destruction or extracortical calcifications were seen.

Several bone parameters were obtained after computer analysis of the scanned images. The bone parameters that were evaluated were bone volume, trabecular number, thickness

and separation, and structure model index, trabecular pattern factor, eccentricity and average object area (Fig. 5). These latter three parameters need some explanation. Structure model index indicates the relative prevalence of rods and plates in a three dimensional structure such as trabecular bone (Hildebrand and Ruegsegger, 1997). It is of importance in osteoporosis of trabecular bone which is characterized by a transition from plate-like to rod-like architecture. Trabecular pattern factor is an index of connectivity of trabecular bone defined by Hahn et al. (1992), its increase means a more disconnected trabecular structure. Eccentricity is an elliptic parameter indicating departure from circular shape by lengthening, thus increased eccentricity indicates a change of shape of the bone circumference, becoming less circular. Average object area is a useful indicator of structural connectivity, fragmentation due to bone resorption results in small disconnected objects, thus decreasing the average object area. Changes in bone parameters were significant if compared with the control group. After a two dimensional slice by slice analysis in arthritic animals a decreased average area of bone fragments indicates fragmentation of trabecular bone due to bone loss. There was an increased mean eccentricity signaling departure from circular shape by lengthening, probably due to a periosteal reaction which changed the shape of the bones. In arthritic animals the three dimensional analysis indicated a decreased bone volume and trabecular number and thickness, and increased trabecular separation because of osteolysis. The increased trabecular pattern factor indicates a more disconnected trabecular structure, while the increased structure model index is a sign of transformation of plate like trabecular bone into rods due to bone breakdown. Generally strongest changes in bone parameters were observed for CFA-injected joints, while MIA-induced changes were less pronounced. Similar observations were made in both animal species for most parameters.

Correlation coefficients between bone parameters and pain behavior were estimated (Table 1). Within the same model differences were observed between rat and guinea pig especially for the MIA model. Between models bone parameters and pain behavior were similarly correlated in the guinea pig, but in the rat clear differences were observed between MIA- and CFA-induced arthritis.

Within guinea pigs (Table 1, left part; Fig. 6) the calculated bone characteristic parameters were poorly correlated with results of Hargreaves' test for thermal hyperalgesia, yet they were significantly correlated with von Frey and balance box measurements in both models. Highly significant positive correlations ($P<0.001$) were found between bone volume and trabecular number on the one hand and von Frey and weight bearing on the other. A highly significant negative correlation ($P<0.001$) was found between structure model index, trabecular separation and pattern factor, versus both behavioral measurements. Moreover von Frey and weight bearing showed significantly positive correlations with average object area and trabecular thickness in the CFA-arthritis model, while they were negatively correlated with eccentricity in the MIA model. Within rats (Table 1, right part; Fig. 6) the calculated bone-characteristic parameters were never significantly correlated with results of Hargreaves' test for thermal hyperalgesia. In the CFA model for RA most bone parameters had highly significant correlations with von Frey and limb-use, and moderately significant correlations with weight bearing results. Yet in the rat MIA model for OA only slightly significant correlations between bone parameters and weight bearing were found. Some of these findings are presented in Fig. 6. In this figure the lack of correlation between paw flick latency and two of the bone parameters, bone volume and trabecular pattern factor, is clearly represented. Further it is easy noticed how bone volume is positively correlated with von Frey thresholds and weight bearing, while the opposite is true for trabecular pattern factor. It can also be seen how results of the two animal species nicely overlap, or at least follow the same trend.

In summary, positive correlations were found between the pain-induced decrease in behavioral measurements and bone specific parameters that decrease in case of fragmentation of bone and due to osteolysis, namely average object area on the one hand and the latter are bone volume, trabecular number and thickness. Negative correlations were found between behavioral measurements that decrease because of pain and specific bone parameters that increase in case of bone destruction. These increased parameters are trabecular separation indicating osteolysis, trabecular pattern factor indicating loss of trabecular connectivity, structure model index meaning the transformation of plates into rod like structures, and finally eccentricity indicating the shape of the bone becoming less circular. In the MIA model in the guinea pig one exception is found as the trabecular thickness has the tendency to be negatively correlated with the behavioral measurements, yet these correlations were not or borderline significant.

4. Discussion

There are some important criteria in selection of a model for drug testing such as (a) capacity to predict efficacy in humans, (b) similar pathology and/or pathogenesis to that of human disease and (c) ease of use, reproducibility, reasonable duration of test period (Bendele et al., 1999). Yet in case of arthritis no induced rodent model can reproduce accurately the full complexity of the human disease because of important differences in animal models as compared to human. Namely, in animal models as the disease progresses much more rapidly it is characterized primarily by an acute inflammatory response (Bendele et al., 1999). Also mechanical factors differ considerably between a small quadruped and man (Fernihough et al., 2004), while important differences in therapeutic responses exist between different ages of animals, and between spontaneous and surgical models (Ameye and Young, 2006). The present study aimed to better characterize and quantify in an objective way the morphological changes that occur in the bone after induction of OA or RA in the knee joint of laboratory animals using MIA or CFA, respectively, and correlate these changes to pain behavior. In order to evaluate the effectiveness of novel antinociceptive agents and to study the pathogenesis of the disease it is desirable to have an animal model that consistently reproduces pain associated with arthritis.

The MIA model was validated as the first pain model of OA, the latter describing a range of diseases that result in a common joint pathology (Fernihough et al., 2004). The MIA model rapidly reproduces the clinical and pathologic features of OA in a small animal species via a minimally non-invasive method. There are a number of reasons why this model can be a useful tool to study the pain associated with OA (Pomonis et al., 2005). The pathological changes induced by MIA share many similarities with the pathologies associated with human OA (Fernihough et al., 2004; Guingamp et al., 1997; Guzman et al., 2003), including significant loss of cartilage and perturbations of the subchondral bone. This chronic degeneration of the joint is associated with chronic pain behavior. The rapid induction of the disease state allows timely evaluation of pain modifying compounds, while the severity of the disease can easily be manipulated by altering the concentration of MIA (Pomonis et al., 2005). Moreover consistent pain behavior in the MIA model can be modulated by clinically relevant analgesics such as morphine, paracetamol, gabapentin and non-steroidal anti-inflammatory drugs (Bendele et al., 1999; Fernihough et al., 2004). Therefore this model provides a means of evaluating drugs with novel mechanisms of action and may be more predictable for clinical efficacy that other chronic or acute OA models (Fernihough et al., 2004).

OA is regarded as a degenerative condition (Bendele et al., 1999), morphologically characterized by focal areas of destruction of cartilage, formation of bone cysts, sclerosis of subchondral bone, and presence of osteophytes at the joint margin (Bendele et al., 1999; Tessier et al., 2003), as well as variable synovitis, capsular thickening and narrowing of the joint space (Kidd, 2006). In animal models OA-induced changes in the joint morphology were investigated in several studies. In rat knees injected with MIA Bendele et al. (1999) by means of histological analysis observed chondrocyte degeneration and necrosis, and focal fragmentation of bone trabeculae with fibrosis and increased osteoclast activity. Pomonis et al. (2005) using radiographs observed osteolysis and swelling of the joints, mirrored by reductions in bone mineral content and bone mineral density. In a spontaneous OA model in guinea pigs disease progression and changes in the joint cartilage have been described using 3D-magnetic resonance imaging (Tessier et al., 2003). In the present study objective and detailed quantification of bone morphological changes indicated that MIA-induced skeletal damage was mainly characterized by osteolysis, partly resulting in loss of trabecular connectivity.

MIA-induced pain behavior was characterized by a decrease in counter-pressure and weight bearing, increased heat sensitivity in guinea pigs and decreased use of the affected limb in rats. A decrease in weight bearing after MIA injection into the rat knee joint has been described (McDougall et al., 2006; Pomonis et al., 2005). Yet, mechanical hypersensitivity was not observed by Pomonis et al. (2005) using the paw pressure test, nor by McDougall et al. (2006) using von Frey hairs to measure secondary hyperalgesia. In contrast Fernihough et al. (2004) observed mechanical hypersensitivity and allodynia after MIA injection into the rat knee joint. They considered this as being referred pain and a more robust pain signal than the change in weight bearing. It must be emphasized that in our opinion the decreased von Frey thresholds that were observed were due to a decreased counter-pressure of the affected limb and not to mechanical hypersensitivity. The limited heat hypersensitivity that was observed might indicate that secondary hyperalgesia, which is reported in some human patients suffering from OA (Bajaj et al., 2001; Kosek and Ordeberg, 2000), was not consistently present. In man OA is the most common of articular disorders and is frequently cited as the leading cause of persistent musculoskeletal pain (Kidd, 2006). OA is a disease with a heterogeneous character, more a syndrome with many complex etiologies rather than a single disease entity. The relationship between symptoms and underlying pathology in the human turns out to be far from straightforward (Dieppe and Lohmander, 2005; Kidd, 2006; Pomonis et al., 2005). Radiographic evidence of joint damage predisposes to joint pain, but the severity of joint damage on the radiograph bears little relation to the severity of the pain experienced by patients (Creamer et al., 1996; Dieppe and Lohmander, 2005). Even up to 40% of the people with severe radiographic changes are symptom free (Davis et al., 1992). This might partly explain why bone parameters in the OA animal model were less correlated with pain behavior than in the RA model. Since OA remains a frustrating and difficult condition to treat (Chard and Dieppe, 2002) current therapies for OA are directed towards controlling symptoms maintaining function and reducing further joint damage (Fajardo and Di Cesare, 2005). Both acetaminophen and non-steroidal anti-inflammatory drugs can be given (Ge et al., 2006). In the past few decades OA therapy is evolving from symptomatic treatment to possible disease modifying solutions that need more research before being fully realized (Fajardo and Di Cesare, 2005). The present study supports the suggestion of Pomonis et al. (2005) that when using the MIA-induced OA model to study the efficacy of potential antinociceptive agents, this will be most clinically relevant when done at later time points. The reason for this is the late onset of the pain behavior.

CFA injection in laboratory animals is a well established in vivo model of RA, recapitulating many features of this pathology in humans, and with a proven track record of predictability (Bendele et al., 1999). When CFA is injected into a joint it will induce inflammation with infiltration of T-lymphocytes and macrophages (Butler et al., 1992). This will progress with paw swelling and massive infiltration of leukocytes into the synovium, and a strong and significant decrease in bone volume accompanied by severe osteolytic lesions (Romas et al., 2002). In the present study CFA-induced alterations in bone morphological parameters were observed and objectively quantified, as well in the rat as in the guinea pig. These alterations indicated osteolysis and disconnection of trabeculae probably due to bone resorption, thus weakening the strength of the bone. Butler et al. (1992) examined radiographs of rats with CFA-induced arthritis finding joint destruction and extensive bone proliferations largely confined to the region of the affected tibio-tarsal joint. Bendele et al. (1999) also described marked bone resorption and periosteal bone proliferation in rat CFA-induced polyarthritis models. In the human,

skeletal complications of RA consist of focal bone erosions and periarticular osteoporosis at sites of active inflammation and generalized bone loss (Krane, 1993; Romas et al., 2002). A remarkable observation on three-dimensional representations of the damaged rat knee in the present study is the sheet of bony proliferations formed around the bone in the joint proximity. It reflects the periosteal reaction to the joint damage caused by CFA. It has been described that in response to joint inflammation rodents have a tendency to have marked bone resorption and bone formation, especially periosteal (Bendele et al., 1999). In the guinea pig this was not observed although pain behavior and bone parameter analysis were comparable between both species. Pain in the CFA-induced arthritis model manifested as a decrease in counter-pressure and weight bearing, a decreased use of the affected limb in rats and an increased heat sensitivity in guinea pigs. Vikman et al. (2003) described thermal hyperalgesia in the CFA-injected ankle joint of rats, which was not significant in the present study. This difference in thermal hypersensitivity is probably due to the fact that Vikman et al. (2003) studied the ankle joint that, as compared to the knee joint, is more closely located to the plantar side of the paw where the heat stimulus is given. Active agents in this CFA model include corticosteroids, methotrexate and non-steroidal anti-inflammatory drugs, all drugs that are used in clinical settings (Bendele et al., 1999). Still, although non-steroidal anti-inflammatory drugs supplemented with steroid hormones remain the mainstay of arthritis treatment, they are well known to have limits to their capacity to provide relief of arthritic pain and are also limited by their side effects (Price et al., 1996). Another major unsolved problem in RA in humans is represented by bone loss, in which osteoclasts are key mediators. This bone destruction is driven by overproduction of TNF-α, a potent osteoclastogenic cytokine with a pivotal role in the pathogenesis of RA (Mulherin et al., 1996; Romas et al., 2002). Therefore osteoclasts might represent a neglected target of RA therapy that has thus far focused on anti-inflammatory treatment (Romas et al., 2002).

The present study aimed to better characterize pain behavior versus bone loss in two arthritis models, a MIA-induced OA model and a CFA-induced RA model. This was done in 2 different animal species, rat and guinea pig. As well the skeletal damage as the pain behavior were comparable in both species and for both models. Changes were observed in bone morphological parameters that were more pronounced in the CFA-induced RA model than in the MIA-induced OA model. This was reflected in the pain behavior being less severe in the latter. Pain behavior was expressed as decreased von Frey thresholds and weight bearing, slightly increased thermal sensitivity and decreased limb-use. Alterations in bone parameters, obtained after detailed analysis of high-resolution μ-CT scan images of the arthritic knees, were representative of bone loss, and fragmentation and disconnection of bone trabeculae. Highly significant correlations were obtained between pain behavior and joint destruction. Detailed description of the morphological changes that occur in the joint during the development of different kinds of arthritic pathologies better characterizes the respective animal models. It can be an important tool for evaluating the efficacy of treatment strategies in preventing or curing bone damage. Moreover it can be used to distinguish between compounds that are analgesic in the presence of severe bone destruction and compounds having analgesic properties because of preventing or curing bone damage. The severity of morphological changes in the joint illustrates that decreasing bone destruction might be an important way to diminish pain behavior in arthritis patients, as has already been shown in other models (Harada et al., 2004). This study is a continuation of our research on the relation between bone destruction and pain behavior in different animal models for pain research that involve skeletal changes. In a previous study the relation between pain behavior and bone destruction during bone cancer development in mice was investigated (Vermeirsch et al., 2004).

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