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Characterization of osteoarthritis in cats and meloxicam efficacy using objective chronic pain evaluation tools

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ABSTRACT

This study aimed to characterize osteoarthritis (OA)-related chronic pain and disability in experimental cats with naturally occurring OA. Peak vertical ground reaction force (PVF), accelerometer-based motor activity (MA) and the von Frey anesthesiometer-induced paw withdrawal threshold were used to define OA and to test the efficacy of meloxicam. A diagnosis of OA was based on radiographic and orthopedic examinations. Cats with OA ($n = 39$) and classified as non-OA ($n = 6$) were used to assess the reliability and sensitivity of the parameters to assess OA over 3 weeks while being administered placebo medication. A randomised parallel design study was then used to investigate the effects on OA of daily oral meloxicam treatment for 4 weeks at different dose rates (0.025 mg/kg, $n = 10$ mg/kg; 0.04 mg/kg, $n = 10$; 0.05 mg/kg, $n = 9$), compared to cats administered a placebo ($n = 10$).

The test–retest repeatability for each tool was good (intra-class correlation coefficient $\geqslant 0.6$). The PVF and the von Frey anesthesiometer-induced paw withdrawal threshold discriminated OA ($P < 0.05$). Meloxicam did not add to the PVF improvement observed in placebo-treated cats during the treatment period (adj-$P \leqslant 0.01$). The 0.025 and the 0.05 mg/kg meloxicam-treated cats experienced a higher night-time (17:00–06:58 h) MA intensity during the treatment period compared to the placebo period (adj-$P = 0.04$, and 0.02, respectively) and this effect was not observed in the placebo group. The high allodynia rate observed in the 0.04 mg/kg meloxicam-treated group may explain the lower responsiveness to the drug. The von Frey anesthesiometer-induced paw withdrawal threshold demonstrated no responsiveness to meloxicam. The results from this study indicated that daily oral meloxicam administration for 4 weeks provided pain relief according to night-time MA.

Introduction

Osteoarthritis (OA) in cats develops with aging and is responsible for causing disability and chronic pain (Hardie et al., 2002; Lascelles, 2010; Slingerland et al., 2011; Bennett et al., 2012a). While OA is very common in older cats, it is poorly diagnosed in clinical practice (Clarke et al., 2005; Lascelles, 2010). This may be related to the mismatch between an orthopedic evaluation and the severity of OA structural changes, and/or the lack of validated chronic pain assessment tools in cats (Clarke and Bennett, 2006; Lascelles et al., 2012). Unfortunately, this situation leads to an absence of approved medication for the treatment of OA-associated chronic pain in cats in North America (Lascelles and Robertson, 2010; Bennett et al., 2012b).

Assessment of chronic pain in animals is challenging. This challenge may be related to the need to consider the impact of pain on physical disability and the associated changes that occur in the central nervous system due to chronic pain. Quantification of limb impairment using the peak vertical ground reaction force (PVF) has been used to objectively evaluate OA-associated pain/disability in cats (Guillot et al., 2012). Moreover, in this species, OA induces a decrease in owner-assessed daily activity, a reluctance to jump and to walk up stairs, and other altered behaviors (Bennett and Morton, 2009; Zamprogno et al., 2010; Slingerland et al., 2011).

Accelerometer-based motor activity (MA) assessment is a complementary approach, which provides an objective quantification of functioning limitations related to OA-associated chronic pain (Lascelles et al., 2007b, 2010; Guillot et al., 2012). Moreover, several studies in humans suggest that mechanical allodynia is an essential part of the OA-associated chronic pain assessment (Hendiani et al., 2003; Imamura et al., 2008; Arendt-Nielsen et al., 2010).

It was hypothesized that PVF, MA and the von Frey anesthesiometer-induced paw withdrawal threshold would provide

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complementary assessments of OA-associated chronic pain in cats. The objectives for this study were as follows: (1) to assess the reliability and sensitivity (discriminatory ability) to OA of PVF, MA monitoring, and von Frey anesthesiometer-induced paw withdrawal threshold testing; (2) to test the dose-dependent efficacy of a non-steroidal anti-inflammatory drug (NSAID), meloxicam, in OA cats using these three objective techniques to assess chronic pain.

Materials and methods

Cat selection and experimental protocol

The Institutional Animal Care and Use Committee approved the study protocol (Rech-1482), and the Canadian Council on Animal Care Guidelines were followed regarding the care and handling of the cats.

Experimental cats were selected based on a normal physical and neurological evaluation, complete blood count, blood biochemical profile, and urine analysis. A thorough orthopedic examination was performed to detect changes in gait, posture, and the presence of joint pain. Cats with limb deformities or signs of acute disease were disqualified from the study. The cats were also screened using computed radiographs (CR) of the mediolateral and caudocranial stifle projections. The coxofemoral, carpal and tarsal joints as well as the mediolateral projection of shoulders and elbows were also screened. These CRs were performed under intramuscular sedation using medetomidine (0.02 mg/kg; Domitor 1 mg/mL, Pfizer Canada Animal Health) and morphine (0.1–0.2 mg/kg; Morphine Sulfate Injection 10 mg/mL, Sandoz). The radiographic OA severity was graded as previously described (D'Anjou et al., 2008; Guillot et al., 2012).

Of the 120 cats examined, 48 satisfied the inclusion criteria and were classed into three different OA-status groups (Fig. 1), as follows: OA-status group 0 (non-OA cats) consisted of young cats (three females and three males) with normal orthopedic examinations and no radiographic OA; OA-status group 1 consisted of 19 female and 13 male cats with an abnormal orthopedic examination and radiographic OA; OA-status-group 2 consisted of five female and five male cats with abnormal orthopedic examinations but no radiographic OA (Table 1).

After 2 weeks of quarantine, the cats were housed together in two similar dedicated rooms (surface around 8 × 12 m). Cats were acclimatized for 4 weeks, which included the following: (1) twice-weekly training for PVF assessment (to move freely across the pressure sensitive mat, and to perform progressive stair exercises); (2) von Frey anesthesiometer-induced paw withdrawal threshold testing; and (3) 1 week of conditioning to wear the accelerometer device. The room environment and the cats' health were controlled and recorded daily, and the cats were weighed each week. The cats were fed according to the food manufacturer's recommendations once daily in the afternoon with a standard certified commercial cat food (Hill's Prescription Diet w/d Feline). Water was supplied ad libitum. In both rooms, the cats were allowed to move freely, with free access to toys, covers, height platforms, and one large window. Beds in quiet locations were also freely accessible.

Initially, all cats received 3 weeks of the placebo (identical to Metacam Oral Suspension without the active ingredient) orally once daily in the morning, thus providing an acclimatization period for the drug administration. During this placebo period, outcome reliability (repeatability and stability) and sensitivity (discriminatory ability) to OA were assessed. The OA cats were then divided into four treatment groups using a controlled randomization according to the PVF values obtained during the placebo period, and the OA-status-group, providing homogeneous treatment groups on these criteria. Each group then received either meloxicam (Metacam Oral Suspension 0.5 mg/mL, Boehringer Ingelheim Vetmedica) or placebo (n = 11) for 4 weeks at the following dose rates: 0.025 mg/kg (n = 11); 0.04 mg/kg (n = 10); 0.05 mg/kg (n = 10). Following the controlled randomization, each treatment group was similarly represented in both living rooms. A 6 week recovery period occurred during which no treatment was administered to test the persistence effects of the treatment. All evaluations of the OA status and the provided treatment were conducted blindly (Fig. 1).

Measurement of PVF

Peak vertical of the ground reaction force was acquired using a floor mat-based plantar force measurement system (Walkway System WE4, Tekscan) and was managed using Walkway Research software v.7.0. Prior to each kinetic measurement, equilibration and calibration of the system was performed. The cats were coaxed using positive reinforcement (treats, clicker, brushing, etc.) to trot across the walkway at a comfortable speed (0.8–1.4 m/s). Speed was computed by the software using the time and distance of a given stride. Only the four-foot strikes of the first stride were considered. Among all kinetic gait parameters generated, only the maximal loading, referred to as the PVF, was considered, as supported by a previous study (Guillot et al., 2012).

For each session, a maximum of three valid trials (the cat moved across the entire mat undisturbed, consistently, in a straight line, and at the correct speed) were obtained for each cat, with an a priori maximum of 16 consecutive trials allowed. The number of trials needed to obtain the three valid trials was recorded. The PVF was recorded before and immediately after approximately 3 min of stair exercises. Stair exercises on a 10 m long staircase consisted of running up, down, and then up again. The post-exercise measurement was performed within 60 s of the final excursion up the stairs.

Based on the PVF expressed as a percentage of bodyweight (% BW), the most affected limb of the before and after-exercise sessions was determined for each cat. The most affected limb was defined as the limb that generated the lower PVF value most frequently during a trial, among all of the analyzed trials during the placebo period (maximum of 3 × 3 = 9 trials). If an equal number of lower values was detected for each limb, the limb with the lower average PVF value was chosen. For each day of evaluation, the kinetic gait analysis outcome of each session (before/after exercise) was calculated by averaging the three valid trials of the most affected limb PVF of each cat.

Motor activity assessment

The MA was assessed using a collar-attached accelerometer-based activity sensor (ActiWatch, Minimitter/Respironics, Bio-Lynx Scientific Equipment) maintained in place from day (D) −21 to D69 (see Fig. 1). The device was set for local time and configured to create 1 count value per 2 min. The amplitude of each count was subsequently translated to a numeric value (from 0 to infinite) referring to the intensity count of MA. To exclude periods where human activity and handling interfered with the cats' activity, only 3 days per week (Friday, Saturday and Sunday), between 17:00 and 06:58 h, were considered for the analyses. This was supported by our pilot study data (Guillot et al., 2012), where MA of OA cats was more affected during night-time. Additionally, all adverse events were recorded and excluded. Data were expressed as the average total intensity counts. Thus, for each week of evaluation, the MA outcome was obtained for each cat by calculating the median of the three periods.

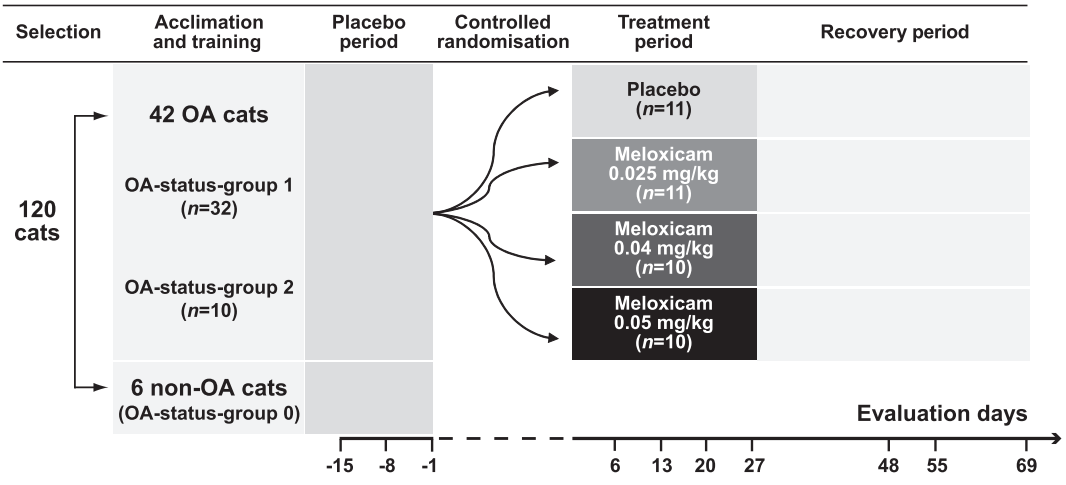

Fig. 1. Study design diagram. For the treatment effect analyses, only 10 cats per treatment group were kept, except in the 0.05 mg/kg meloxicam-treated group, which totaled nine cats, due to three withdrawals as outlined in Results section.

Table 1
Age, radiographic and orthopedic features of the selected cats at the start of the study by OA-status group.

Feature	OA-status group		
	0	1	2
Mean age [SD] (year)	2.8 [1.4]	8.0 [2.4]	8.9 [1.4]
Median radiographic scores (range)			
Forelimbs	0 (0–0)	1 (0–9)	0 (0–0)
Hind limbs	0 (0–0)	3 (0–11)	0 (0–0)
Median radiographic OA-affected joint number (range)			
Forelimbs	0 (0–0)	1 (0–4)[a]	0 (0–0)
Hind limbs	0 (0–0)	2 (0–4)[b]	0 (0–0)
Presence of gait alteration (%)	0	43.7	40
Presence of posture alteration (%)			
Forelimbs	0	9.4	10
Hind limbs	0	37.5	60.0
Presence of subjective pain as noted by a blinded observer (%)			
Forelimbs	0	9.3	22.3
Hind limbs	0	100	100

[a] The affect joints in 32 cats were shoulder (12/32 = 37.5%), elbow (9/32 = 28.1%), and carpal (4/32 = 12.5%) joints.
[b] The affect joints in 32 cats were coxofemoral (21/32 = 65.6%), tarsal (12/32 = 37.5%), and stifle (11/32 = 34.4%) joints.

Punctate tactile allodynia quantification

Secondary punctate allodynia responses were evaluated using gradually increased pressure applied on the plantar paw surface with a mechanical von Frey polypropylene probe (Rigid Tip 0.7 mm^2 of surface 28 G, IITC Life Science) fitted on a hand-held force transducer and paw withdrawal threshold monitoring anesthesiometer. The tip was placed perpendicularly to the plantar surface of each paw (without manipulating the limb) in turn in a pre-defined order while the cat was standing up partially restrained in a meshed cage specifically designed for this evaluation. The stimulus was stopped as soon as the paw was withdrawn or the cat showed pain behaviors, such as vocalisation, agitation, or avoidance. For each animal, the peak of force in grams was recorded, and duplicate measurements of each paw were obtained with a 60 s interval between both stimuli. The data under 2 g were discarded, and a maximal cut-off value of 200 g was applied. For each evaluation day, the von Frey anesthesiometer-induced paw withdrawal threshold outcome was obtained by averaging all of the available threshold values ($n = 8$) for each cat.

In addition to these parameters, an allodynia threshold was set at 40 g for the front paws and 50 g for the hind paws according to the data distribution. These thresholds were determined based on the first quartile values of the OA cats placebo period data (lowest round value close to the mean of the 12 calculated first quartile values for the fore and hind paws respectively), and importantly, no non-OA cat presented such low values, or lower, twice for a duplicate. A cat was considered to be allodynic if at least one of its paws presented duplicate values under the fixed threshold.

Statistical method

All analyses were conducted two-sided with an α threshold of 0.05 using a statistical software program (SAS system, version 9.2). Outcome normality was verified using the Shapiro–Wilk test.

During the placebo period, outcome repeatability was assessed by computing the intra-class correlation coefficient (ICC), a measure of the proportion of variance that is attributable to objects of measurement. It is accepted that ICCs > 0.6 suggest satisfactory, and >0.8 excellent stability (Faries and Yalcin, 2007). Mixed model analyses for repeated measures allowing to model the covariance structure (Littell et al., 2006) provided information about outcome stability over time (day effect assessment) and the outcome ability to discriminate over an OA cat's status (OA-status-group effect estimates; whole model details are provided in Table 2). A Tukey–Kramer adjustment was used to obtain adjusted *P*-values (adj-*P*) for multiple comparisons.

Mixed model analyses for repeated measures were also conducted to test the effect of treatments on the different outcomes (whole model details are provided in Table 2). To consider multiple comparisons, adjusted *P*-values were computed using the Bonferroni correction (original *P*-value multiplied by the number of comparisons of interest, i.e., 3).

Results

Departure from the protocol and health follow up

Punctate tactile allodynia was not evaluated for one cat in the OA-status-group 0 because of constant non-reliable behavior throughout the placebo period. During the treatment period, three OA cats (all of which originated from OA-status-group 1) were withdrawn from the study. One 0.05 mg/kg meloxicam-treated cat was withdrawn due to the occurrence of vestibular syndrome, one 0.025 mg/kg meloxicam-treated cat was withdrawn because it had chronic diarrhea, which required repeated isolation, and one placebo-treated cat was withdrawn due to the development of an aversion to being handled. Finally, there were 10 cats per treatment group, except in the 0.05 mg/kg meloxicam-treated group, which had nine cats.

No clinical side effects related to meloxicam administration were observed in the 31 meloxicam-treated cats, no significant change in complete blood count, blood biochemistry or urine analysis was observed, and particularly, there were no individual increases in liver or kidney parameters to values outside of the normal range. However, data were discarded during the last week of MA because the sensor stopped recording in the majority of cats (75%) between D62 and D68 due to low batteries.

Outcome reliability and sensitivity to OA during the placebo period

The ICC (95% confidence interval) of the most affected limb PVF expressed as % BW before and after exercise was 0.49 (0.32–0.66) and 0.60 (0.40–0.70), respectively. Moreover, mixed model analyses showed no significant change over time (days) of the most affected limb PVF before and after exercise ($P = 0.84$ and 0.40 respectively), using BW ($P < 0.01$), velocity ($P < 0.05$) and the maximum number of trials ($P < 0.01$) as covariates. The ICC of the MA was 0.87 (0.80–0.92), and no change over time was detected ($P = 0.33$). Finally, the ICC of the von Frey anesthesiometer-induced paw withdrawal threshold was 0.78 (0.67–0.87), and no change over time was detected ($P < 0.40$).

The most affected limb PVF analyses before and after exercise showed a significant OA-status-group effect ($P < 0.01$ and $P = 0.02$, respectively; Fig. 2A), with group comparison showing that the cats in OA-status-group 1 were more affected. For the OA-status-group 1 cats ($n = 29$), all most affected limbs (pre and post exercise session) were hind limbs with a median (range) radiographic score of 2 (0–6), 27 (=93.1%) of which presented radiographic OA. Affected joints were coxofemoral (18/27 = 66.7%), tarsal (8/27 = 29.6%), and stifle (6/27 = 22.2%) joints. For the OA-status-group 0 and 2 cats, all most affected limbs were also hind limbs and, according to their selection criteria, with no radiographic OA. The MA intensity demonstrated no OA-status-group or interaction of day with OA-status-group effects

Table 2
Details of the mixed model analyses.

Models[a,b]	Outcomes	Outcome transformation[c]	Fixed effects	Covariance structures	Covariates[d]
Outcome variability assessment and OA status discrimination	PVF	Log-transformed	OA-status-group, day, and their interaction	Compound symmetry with a heterogeneous selection group	BW, velocity and maximum number of trials
	Motor activity	Log-transformed		Toeplitz with a heterogeneous selection group	–
	Von Frey anesthesiometer-induced paw withdrawal threshold	–		Type 1 autoregressive	–
Treatment effect assessment	PVF	Log-transformed	Treatment, period, and their interaction	Compound symmetry	BW, velocity and maximum number of trials
	Motor activity	Log-transformed		Toeplitz with a heterogeneous treatment group	–
	Von Frey anesthesiometer-induced paw withdrawal threshold	Square root transformed		Spatial power	–

[a] All models used a mixed model method for repeated measures and provided fixed effect estimates by restricted likelihood modeling. The homogeneity of variance was assessed using the absolute values of the residuals of the mixed model, and the best structure of the covariance model was assessed using a graphical method (plots of covariance vs. lag in time between pairs of observation compared to different covariance models), as well as using information criteria that measure the relative fit of competing covariance models. Also, residuals of the models were thoroughly studied to assess the model's validity (Littell et al., 2006).
[b] All models used day as the repeated factor.
[c] Outcome transformations were recommended following residual analysis results.
[d] Covariates of the models were thoroughly assessed and only significant ones were kept in each final model.

($P > 0.14$; Fig. 2B). Finally, the von Frey anesthesiometer-induced paw withdrawal threshold analyses indicated a significant OA-status-group effect ($P = 0.02$; Fig. 2C). Particularly for OA-status-group 1 cats, the von Frey anesthesiometer-induced paw withdrawal threshold was lower than that of the OA-status-group 0 cats (adj-$P = 0.02$).

Effect of meloxicam

The before-exercise PVF data were not considered to be adequate to test for the effects of meloxicam because this outcome was not sufficiently reliable and sensitive to OA, compared to the after-exercise PVF data. Descriptive statistics for the different outcomes over the evaluation days are provided in Table 3 (most affected limb PVF after exercise and essential covariates: velocity and maximum number of trials), Table 4 (MA) and Table 5 (von Frey anesthesiometer-induced paw withdrawal threshold).

The most affected limb PVF after-exercise analyses indicated an overall period effect ($P < 0.01$), but showed neither a significant treatment effect nor an interaction of treatment with period effect ($P > 0.30$). After excluding the OA-status group 2 cats, complementary analyses of the most affected limb PVF after exercise showed an overall period effect ($P < 0.001$) and no significant treatment effect ($P = 0.78$). However, there was a significant interaction of treatment with period effect ($P < 0.04$). In particular, there was a significant increase in PVF for the 0.025 and 0.05 mg/kg meloxicam-treated cats during the treatment compared to the placebo period (adj-$P < 0.01$). The placebo-treated cats also experienced a significant increase in PVF between the placebo and the treatment period (adj-$P = 0.01$) in these complementary analyses (Fig. 3).

The MA analyses revealed an overall period effect ($P < 0.01$) and no significant treatment effect ($P = 0.35$). However, there was a significant interaction of treatment with period effect ($P < 0.01$). There was an increase in MA intensity of meloxicam, but not of placebo-treated cats between the placebo and the treatment periods (Fig. 4). The increase in MA intensity was significant for the 0.025 and the 0.05 mg/kg meloxicam-treated cats (adj-$P = 0.04$, and 0.02, respectively). Only in the group receiving the lowest dose of meloxicam was their activity significantly lower during the recovery compared to the treatment period (adj-$P < 0.01$).

Von Frey anesthesiometer-induced paw withdrawal threshold analyses did not indicate a significant period effect, a treatment effect, or an interaction of treatment with period effect ($P > 0.66$). Interestingly, the 0.04 mg/kg meloxicam-treated cats presented with a higher rate of allodynia during the treatment period (Table 6). No treatment affected either the von Frey anesthesiometer-induced paw withdrawal threshold (Table 5) or the rate of allodynia (Table 6).

Discussion

Despite the importance of reliability testing to validate outcome measures, to our knowledge this study is the first to report on the reliability of kinetic gait analysis, accelerometer-based MA assessment and von Frey anesthesiometer-induced paw withdrawal threshold testing in cats. According to the obtained ICC, the PVF expressed as % BW test–retest reliability after exercise was better than that before exercise but overall was moderately good. The stability of the PVF values over time, after correction for BW, velocity, and the maximum number of trials, indicates that covariates are needed to provide a reliable outcome measure. Investigating and considering such covariates could avoid the difficulty encountered when comparing study results (Lascelles et al., 2007a; Guillot et al., 2012).

The cats used the lower end (around 5 PSI) of the working range of the mat (0–125 PSI), requiring a specific calibration to ensure accuracy in the measurement. System error was minimized by optimizing the recording of the floor mat-based plantar force measurement system. An equilibration profile adapted for companion animals was used, which compensates for the variation in individual sensel output, and the system was calibrated with a custom-made device of similar weight and contact area to those of the feline subjects. Although the accuracy of the calibration method may be subject to questioning, the repeatability and stability of the data were good to excellent, and hopefully the future will see the emergence of a standardized approach and a mat adapted for cat sensitivity. The MA intensity and the von Frey anesthesiometer-induced paw withdrawal threshold ICC were very good, and these outcomes were stable over time, demonstrating their good reliability in cats.

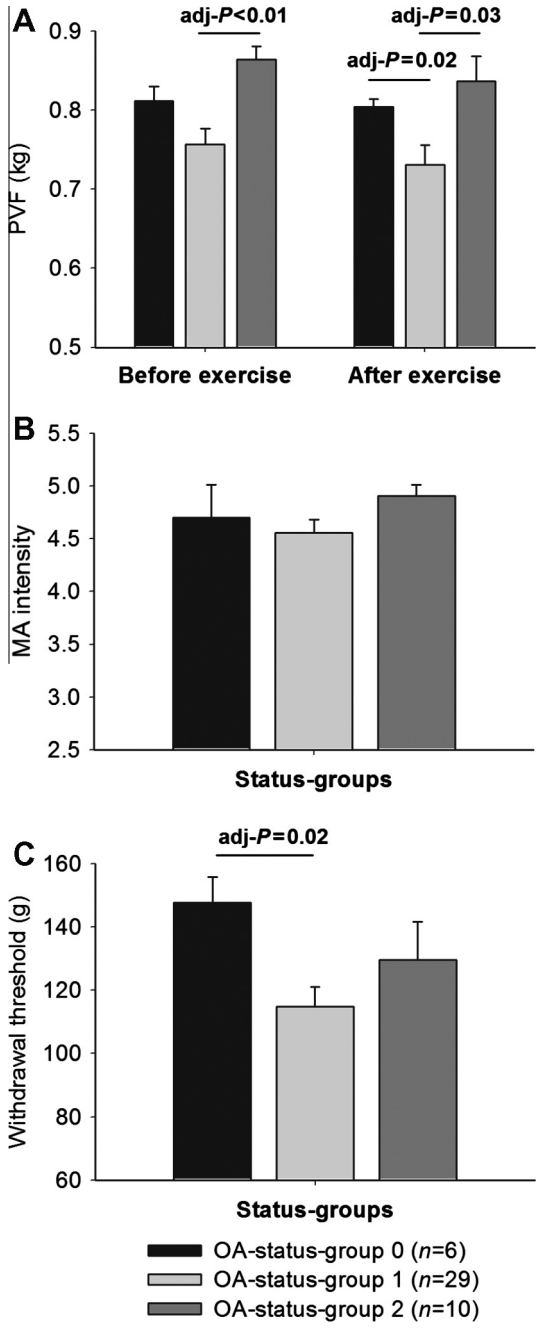

Fig. 2. Least square means of the different outcomes by OA-status-group during the placebo period. (A) Least square means of the log-transformed most affected limb PVF and SEM by OA-status group before- and after-exercise gait sessions. Before exercise, the PVF of the OA-status-group 1 cats was lower than that of the OA-status group 2 cats (adjusted *P*-value [adj-*P*] < 0.01) and similar to that of the OA-status group 0 cats (adj-*P* = 0.12). In contrast, the PVF of the OA-status-groups 2 and 0 cats were similar (adj-*P* = 0.12). After exercise, the PVF of the OA-status group 1 cats was lower than that of the OA-status groups 0 and 2 cats (adj-*P* = 0.02, and 0.03, respectively). The PVF of the OA-status groups 2 and 0 cats was similar (adj-*P* = 0.58); (B) least square means of the log-transformed motor activity and SEM by OA-status group; (C) least square means of the von Frey anesthesiometer-induced paw withdrawal threshold and SEM by OA-status group. The von Frey anesthesiometer-induced paw withdrawal threshold of the OA-status group 1 cats was lower than that of the OA-status group 0 cats (adj-*P* = 0.02), but similar to that of the OA-status group 2 cats (adj-*P* = 0.54). In contrast, the von Frey anesthesiometer-induced paw withdrawal threshold of the OA-status groups 2 and 0 cats was similar (adj-*P* = 0.44).

Significant differences between the OA-status-groups during the placebo period of the after exercise-PVF demonstrate that this outcome, in contrast to the before-exercise PVF, is able to discriminate between the functional state of non-OA and OA cats. This result suggested that the after-exercise PVF objectively quantifies chronic pain-related disability associated with OA in cats, as previously reported in dogs (Moreau et al., 2011; Rialland et al., 2012) and humans (Henriksen et al., 2010). The most affected limb PVF method was employed as a proxy for the whole cat impairment because of the gait interdependency of limbs affected, or not, by OA. Limb pressures of a given cat are biologically and statistically dependent, requiring a specific approach to be analyzed. We determined that using the most affected limb PVF method was more sensitive and easier to interpret than the use of a four dependent limbs statistical model (data not shown). The vast majority (93.1%) of cats in the OA-status group 1 presented an association of radiographic OA score to the most affected limb PVF. We relate the absence of unanimity to the recognized poor sensitivity of radiographic quantification of structural OA (Lascelles et al., 2012).

Our radiographic OA scoring method involves estimation of osteophytes/entesophytes (0–3), subchondral sclerosis (0–3) and effusion (0–1). It was found to have lower sensitivity to detect structural OA than that of magnetic resonance imaging (MRI) in cats (Guillot et al., 2012). For one cat in particular, despite the absence of lesions detected on radiographs, several OA lesions were detected on the MR images (Guillot et al., 2012). Therefore, since PVF is a reflection of the overall condition of the cat and is influenced by both biomechanical alteration and pain perception, it seemed logical to include all cats presenting clinical signs of OA. The controlled exercise may have increased the joint pain during use or induced muscular fatigue in impaired limbs, or both. Moreover, the lower PVF values of the OA-status group 1, compared to the OA-status group 2, suggested that the cats with OA radiographic signs presented with more disability than those with only some OA clinical signs. Hence, PVF and radiographic evidence of OA should be considered as an interesting stratification factor of pain-related disability in future studies investigating OA in cats.

The OA-status group 1 cats presented a lower MA than the cats of the other status groups during the placebo period. But these differences were not significant, which could be related to a lack of power due to the high intra-group variability. This variability may be explained by the behavior-dependent nature of the MA, which makes inter-group comparison difficult (Lascelles et al., 2010; Milgram, 2011). This highlights the need to use each cat as its own control in MA analyses.

The significantly lower von Frey anesthesiometer-induced paw withdrawal threshold of the OA-status group 1 compared to the non-OA cats suggests that central sensitization (induced by continuous and intense nociceptive input from the OA joint) is an underlying mechanism of pain in OA cats, as is already thought in humans (Arendt-Nielsen et al., 2010; Mease et al., 2011; Woolf, 2011). To our knowledge, this is the first report of involvement of allodynia related to OA in cats.

Despite the increase in PVF in the meloxicam-treated cats, our study failed to demonstrate a significant effect of meloxicam against placebo on this parameter. We hypothesized that chronic pain associated with OA induces a decrease in the use of the affected limb, leading to muscle atrophy and limb impairment over time. However, this loss of function related to chronic pain may have been progressively restored with the weekly exercise performed by all cats, masking improvement attributable to the meloxicam treatment. This exercise-related improvement is suggested particularly by the PVF values in the placebo-treated cat group. The increase in PVF was maintained up until the last time-point of the recovery period.

The inclusion of OA-status-group 2 animals may have limited our ability to show an improvement in PVF due to meloxicam because these animals were not impaired based on this parameter

Table 3
Mean and standard deviation (SD) of the most affected limb PVF, velocity and maximum number of trials (MT) of the after-exercise session by treatment group over days.

Treatment (from D0 to D27)	Day																			
	−15		−8		−1		6		13		20		27		48		55		69	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PVF (% BW)																				
Placebo	44.9	8.4	43.9	6.9	51.3	6.4	48.2	5.1	50.0	6.7	49.6	7.5	49.4	5.0	50.6	6.9	51.5	5.2	53.0	7.9
Meloxicam 0.025 mg/kg	44.7	6.7	46.5	8.2	47.1	9.1	49.1	5.3	49.1	8.7	49.0	8.5	50.6	8.2	52.1	8.0	50.4	9.0	51.4	5.7
Meloxicam 0.04 mg/kg	45.4	6.2	44.5	8.4	44.9	5.7	43.5	5.0	46.7	4.5	43.9	5.2	45.2	4.6	47.9	6.3	47.5	8.1	49.5	6.4
Meloxicam 0.05 mg/kg	41.2	8.7	44.8	5.0	44.9	4.5	47.1	6.3	47.0	5.2	46.4	4.8	47.0	4.1	50.3	5.2	48.4	6.5	47.3	4.6
Velocity (m/s)																				
Placebo	1.27	0.14	1.26	0.28	1.36	0.11	1.30	0.19	1.26	0.19	1.30	0.16	1.31	0.26	1.31	17.1	1.37	0.10	1.35	0.88
Meloxicam 0.025 mg/kg	1.39	0.33	1.29	0.19	1.31	0.13	1.37	0.16	1.37	0.14	1.38	0.14	1.41	0.18	1.37	18.7	1.35	0.17	1.38	0.19
Meloxicam 0.04 mg/kg	1.28	0.26	1.53	0.23	1.39	0.21	1.36	0.15	1.40	0.12	1.37	0.12	1.32	0.23	1.33	19.6	1.42	0.18	1.42	0.20
Meloxicam 0.05 mg/kg	1.25	0.22	1.34	0.15	1.31	0.80	1.31	0.16	1.25	0.21	1.29	0.22	1.34	0.20	1.31	26.4	1.35	0.21	1.35	0.15
MT																				
Placebo	8.6	4.2	11.4	4.2	7.6	3.2	9.1	3.8	8.2	4.0	9.1	3.9	8.9	4.3	8.6	4.4	8.1	4.4	7.2	3.2
Meloxicam 0.025 mg/kg	11.6	4.5	10.4	4.4	11.6	4.4	12.6	3.8	10.0	4.2	8.1	2.8	8.4	3.0	8.2	2.6	7.8	3.0	9.2	3.9
Meloxicam 0.04 mg/kg	9.5	3.5	9.7	4.0	8.7	2.9	11.5	3.1	9.2	2.7	9.3	3.1	7.8	2.7	8.0	2.9	8.2	2.9	6.2	1.1
Meloxicam 0.05 mg/kg	11.0	4.8	6.8	2.6	9.0	4.4	7.7	3.6	9.9	4.6	8.3	3.5	8.1	4.3	7.6	3.4	6.8	1.2	7.8	3.6

Grey shaded columns are evaluation days of the treatment period.

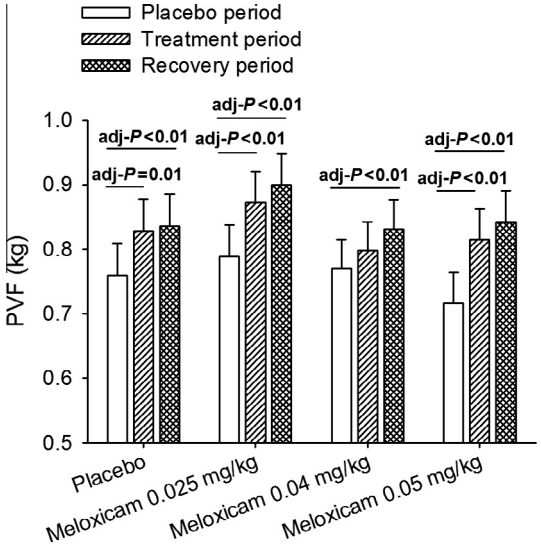

Fig. 3. Least square means and SEM of the log-transformed PVF by period and treatment after excluding OA-status group 2 cats. The placebo, 0.025 and 0.05 mg/kg meloxicam-treated cats presented a significantly higher PVF during the treatment compared to the placebo period (Least square means difference ± SEM = 0.07 ± 0.02, adjusted *P*-value [adj-*P*] = 0.01; Least square means difference ± SEM = 0.08 ± 0.02, adj-*P* < 0.01; and 0.10 ± 0.02, adj-*P* < 0.01, respectively). The treatment-placebo period difference observed in the 0.05 mg/kg meloxicam group was close to significance compared to the placebo group. Moreover, the placebo and all of the meloxicam-treated cats demonstrated a significantly higher PVF during the recovery period compared to the placebo period (adj-$P \leqslant 0.01$): Least square means difference ± SEM = 0.08 ± 0.02, 0.10 ± 0.02; 0.06 ± 0.02; 0.12 ± 0.02 for the placebo-, the 0.025, 0.04 and 0.05 mg/kg meloxicam-treated cats, respectively (n = 7, 7, 8, and 7, respectively). The pooled meloxicam groups were close to demonstrating a significant difference between the treatment and the recovery period, but the placebo group was not.

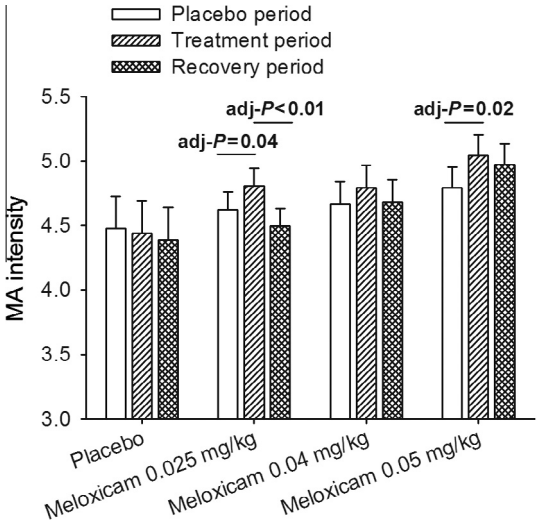

Fig. 4. Least square means and SEM of the log-transformed MA intensity by period and treatment. The placebo-treated cats presented similar MA intensity across the periods ($P > 0.5$). The 0.025 and 0.05 mg/kg meloxicam-treated cats presented a significantly higher MA intensity during the treatment compared to the placebo period (least square means difference ± SEM = 0.18 ± 0.06, adjusted *P*-value [adj-*P*] = 0.04; and 0.25 ± 0.07, adj-*P* = 0.02, respectively), but the 0.04 mg/kg meloxicam-treated cats presented a MA intensity that tended to be higher during the treatment compared to the placebo period (least square means difference ± SEM = 0.13 ± 0.05; adj-*P* = 0.08). Moreover, the 0.025 mg/kg meloxicam-treated cats presented a significantly lower MA intensity during the recovery compared to the treatment period (least square means difference ± SEM = 0.19 ± 0.05; adj-*P* < 0.01).

(Fig. 2). This hypothesis is supported by our complementary analyses excluding the OA-status group 2 animals. These analyses indicated a significant improvement in PVF in the placebo and 0.025 and 0.05 mg/kg meloxicam-treated cats, which persisted during the recovery period. Moreover, a greater relative improvement during the treatment period was observed in the meloxicam-treated cats (+10.1%, and +14.2% in the 0.025, and 0.05 mg/kg meloxicam-treated cats, respectively), compared to +8.9% in the placebo-treated cats. It is therefore possible that the improvement in limb function, as reflected by the PVF, could be related to the weekly exercise. However, improvements in limb function appeared greater with meloxicam treatment (particularly when focusing on OA-status group 1 cats). The inability to demonstrate an improvement in PVF in the 0.04 mg/kg meloxicam-treated cats may be explained by the higher rate of allodynia observed in this group (Table 6).

The observed improvement in MA intensity at night (17:00–06:58 h) following meloxicam administration suggested that there

Table 4
Mean and standard deviation (SD) of the motor activity intensity by treatment group over days.

Treatment (from D0 to D27)	Day																	
	−15		−8		−1		6		13		20		27		48		55	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo	110	74.7	117	82.2	117	74.9	136	94.7	119	90.1	104	75.4	113	76.4	108	77.0	103	64.0
Meloxicam 0.025 mg/kg	101	38.6	113	47.4	126	57.5	159	58.3	148	85.8	121	49.4	132	75.0	117	60.9	117	68.7
Meloxicam 0.04 mg/kg	119	53.8	123	67.7	132	70.1	137	64.6	150	87.9	133	66.0	143	82.0	107	39.9	126	59.4
Meloxicam 0.05 mg/kg	145	65.4	147	61.0	149	79.6	194	62.5	169	66.5	142	49.9	164	54.7	140	53.0	151	55.8

The mean (SD) values presented at each day in the above table correspond to the mean-per-group of the median in motor activity intensities of the three different periods collected over three consecutive days of the week, corresponding to the day indicated as such in the table. Grey shaded columns are evaluation days of the treatment period.

Table 5
Mean and standard deviation (SD) of the von Frey anesthesiometer-induced paw withdrawal threshold (g) by treatment group over days.

Treatment (from D0 to D27)	Day																	
	−15		−8		−1		6		13		20		27		48		55		69	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Placebo	116	36	110	33	111	41	110	39	113	43	110	39	110	43	112	31	108	38	97	31
Meloxicam 0.025 mg/kg	124	47	128	47	132	45	129	46	126	45	117	45	116	42	109	44	110	42	104	32
Meloxicam 0.04 mg/kg	113	35	109	47	115	43	119	36	100	53	102	39	113	46	118	38	106	45	112	40
Meloxicam 0.05 mg/kg	126	40	115	44	125	42	121	35	128	38	116	37	124	32	124	37	110	51	120	40

Grey shaded columns are evaluation days of the treatment period.

Table 6
Percentage of allodynic cats by treatment group over periods.

Treatment (from D0 to D27)	Period (%)		
	Placebo	Treatment	Recovery
Placebo	27	27.5	37
Meloxicam 0.025 mg/kg	30	27.5	30
Meloxicam 0.04 mg/kg	27	42.5	33
Meloxicam 0.05 mg/kg	18.5	17	30

is pain relief related to meloxicam use in OA cats. It therefore appeared reasonable to focus on the night-time period, based on previous results, including the study by Lascelles et al. (2010) who used a randomized, controlled, blinded, parallel group design and reported that activity significantly increased in the test diet group between 06:00 and 12:00 h. Furthermore, a pilot study in our own laboratory (Guillot et al., 2012) showed that the MA of normal cats was greater than that of OA cats during the night-time. Finally, it appeared logical with regard to the experimental conditions of this study to limit the MA intensity analysis to periods of time without any interference with the cats' activity. The hypothesis was, therefore, that such MA intensity analysis would reflect the treatment effect, being the only between-group element of difference. Whether the observed increase in activity during this limited period would represent a whole day beneficial effect is unclear, and will need further investigation.

The improvement in MA is in accordance with the results of a preliminary study using meloxicam in cats with OA-related pain (Lascelles et al., 2007b). In the present study, during the treatment period compared to the placebo period, there was a significant increase in the MA intensity by +3.7% for the 0.025 mg/kg meloxicam-treated group and +5.2% for the 0.05 mg/kg meloxicam-treated group, which suggested a dose-related response. Additionally, only the cats receiving the highest dose maintained an increased MA intensity during the recovery period. There was only a +2.9% increase in MA intensity in the 0.04 mg/kg meloxicam-treated group, which was close to being significant. The high rate of allodynic cats observed in this group may have also interfered with the MA improvement related to meloxicam. It may have also contributed to the higher intra-group variability encountered in this group (larger estimated SEM).

The von Frey anesthesiometer-induced paw withdrawal thresholds remained stable over time in all of the treatment groups, which is in accordance with the presumed inefficacy of NSAIDs on allodynia. It may be interesting in the future to detect the presence of allodynia in OA patients since it could influence the response to treatment, and particularly the lack of response to NSAIDs. In contrast, some drugs that target central sensitization (e.g. ionic channels or NMDA-receptor blockers, and serotonin/adrenaline reuptake inhibitors) have been suggested to be efficacious in relieving OA-associated pain in humans (Mease et al., 2011; Woolf, 2011) and dogs (Malek et al., 2012). Using a multimodal approach, such drugs should be further tested as a complementary treatment with meloxicam in OA-associated chronic pain in cats.

The true status of the OA-status group 2 remains unclear since it was based on a subjective evaluation. We decided to include the OA-status-group 2 cats because radiographs are not always able to detect OA in cats (Freire et al., 2011; Guillot et al., 2012). Based on the advanced age of these cats (their mean age was similar to the OA-status-group 1 cats, see Table 1), epidemiological studies suggested a high risk of OA, despite the absence of radiographic signs (Freire et al., 2011; Bennett et al., 2012a). The OA-status-group 1 cats presented lower PVF values than those of the OA-status-group 2 cats during the placebo period, suggesting a normal limb function of the latter. However, for both OA-status-groups, there was a positive treatment response in the MA intensity in the meloxicam-treated cats, and no response in the placebo-treated cats as indicated by an exploratory analysis of individual responses (data not shown). These results suggested that the OA-status group 2 cats may represent early onset OA cats. To avoid biases that could be associated with the introduction of the different OA-status groups, we controlled the randomization for treatment distribution in OA-groups.

Conclusions

In OA cats, PVF, MA intensity and the von Frey anesthesiometer-induced paw withdrawal threshold are reliable chronic pain evaluation tools. Each of them reflects a distinct component of the chronic pain syndrome, and as such they may be considered as complementary in cat OA pain assessment. Daily oral meloxicam administration of 0.025 and 0.05 mg/kg for 4 weeks significantly improved night-time (17:00–06:58 h) physical activity in cats suffering from OA, which suggested that meloxicam provides clinically relevant pain relief. However, meloxicam had no effect on PVF or allodynia components, the latter being generally expected from NSAIDs.

Conflict of interest statement

Dr. Mark Heit is a regular employee of Boehringer Ingelheim Vetmedica, who supervised the study for this sponsor. None of the other authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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