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Pregabalin for the treatment of syringomyelia-associated neuropathic pain in dogs: A randomised, placebo-controlled, double-masked clinical trial

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Abstract

Pregabalin is the first-line treatment for neuropathic pain (NeP) in humans. Dogs with Chiari-like malformation and syringomyelia (CM/SM) associated with NeP could benefit from pregabalin. The aim of this study was to evaluate the efficacy of pregabalin for NeP in dogs with CM/SM. Eight dogs with symptomatic CM/SM were included in a double-masked, randomised, crossover placebo–controlled clinical trial. All dogs received anti- inflammatory drugs as base-line treatment during placebo or pregabalin phase of 14±4 days each. Analgesic efficacy was assessed with a daily numerical rating scale (NRS) recorded by dog owners (0–10, 10=worst pain) and quantitative sensory testing at baseline, placebo and pregabalin phases. Blood samples were collected to report pregabalin exposure and to assess renal function.

Daily NRS scores recorded by dog owners in the pregabalin group were lower than in the placebo group ($P=0.006$). Mechanical thresholds were higher with pregabalin compared to baseline or placebo ($P=0.037$, $P<0.001$). Cold latency at 15 °C was prolonged on the neck and humeri with pregabalin compared to baseline ($P<0.001$ for both) or placebo ($P=0.02$, $P=0.0001$). Cold latency at 0 °C was longer on pregabalin compared to baseline and placebo ($P=0.001$, $P=0.004$). There was no pregabalin accumulation between first and last dose. This study demonstrates the efficacy of pregabalin for the treatment of NeP due to CM/SM on daily pain scores recorded by dog owners. Pregabalin significantly reduced mechanical hyperalgesia, cold hyperalgesia (0 °C) and allodynia (15 °C) compared to placebo. Pregabalin was non-cumulative and well tolerated with occasional mild sedation.

Keywords: Chiari-like malformation; Neuropathic pain; Pregabalin; Quantitative sensory testing; Syringomyelia

Introduction

Chiari-like malformation and syringomyelia (CM/SM) is a disease complex described in dogs, which can cause clinical signs suggestive of central neuropathic pain (NeP; Rusbridge and Jeffery, 2008). Clinical signs such as vocalisation, avoidance and perhaps scratching behaviour in response to noxious and non-noxious stimuli have been suggested to represent hyperalgesia and allodynia respectively (Nalborczyk et al., 2017; Plessas et al., 2012; Rusbridge and Jeffery, 2008).

The assessment of effective analgesics to manage NeP in dogs has been limited and has focused on either assessment of the cardinal clinical signs of CM/SM or on quality of life (QoL) scoring (Plessas et al., 2012; Plessas et al., 2015). The use of quantitative sensory testing (QST) could help to evaluate more objectively the efficacy of treatment, may increase sensitivity to discriminate between NeP-affected vs unaffected dogs and help to identify the possible mechanisms involved (Backonja et al., 2009).

Current treatment for NeP disorders is limited by a lack of clinically tested, effective analgesics to provide sufficient respite for affected dogs and humans (Finnerup et al., 2015; Moore, 2016). In humans with NeP, pregabalin and amitriptyline are recommended as first line therapy (NICE CG 173, 2013[1]). In veterinary medicine, some data suggest that gabapentin and potentially pregabalin might improve NeP signs associated with CM/SM (Plessas et al., 2012; Plessas et al., 2015).

The pharmacokinetics of pregabalin have been investigated in healthy laboratory dogs after oral administration of a single 4 mg/kg dose (Salazar et al., 2009). The terminal half-life of approximately 7 h, suggests that two administrations per day may be appropriate, unlike gabapentin, which has a terminal half-life of 3 to 4 h and requires its administration three times a day (KuKanich, 2013). Dewey et al. (2009) evaluated long-term pregabalin at 3 to 4 mg/kg three times daily for idiopathic epilepsy as an add-on to phenobarbital and/or potassium bromide administration. Increase in sedation or ataxia levels were the only side-effects reported. There are currently, however, no published randomised controlled trials evaluating the drug exposure and efficacy of pregabalin in the treatment of canine NeP.

The aim of this study was to evaluate the efficacy of pregabalin for treatment of canine NeP in a randomised placebo-controlled double-masked study. We hypothesised that pregabalin would demonstrate a superior effect on pain assessment outcomes compared to baseline and placebo. The primary efficacy endpoint was the 'daily pain assessment' with numerical rating scale (NRS; score from 0 [no pain] to 10 [worst pain]) recorded by dog owners and the secondary endpoint was the QST.

Materials and methods

The study was performed as a double masked, randomised, crossover placebo-controlled trial. Client-owned dogs with clinical signs compatible with NeP (at least cervical hyperaesthesia or spontaneous vocalisation) and with CM ± SM confirmed by Magnetic Resonance Imaging (MRI) scan performed within the preceding 6 months were included. The definition of CM and SM was made according to the British Veterinary Association Scheme for CM and SM[2] as grade 2 for both.

A recent MRI was necessary to ensure that the clinical signs were not related to other painful myelopathies such as intervertebral disc disease. Images followed the same guidelines.[2] A European Diplomate in Veterinary Neurology performed a neurological examination on the first visit and confirmed the diagnosis of CM/SM being the only clinically relevant diagnosis. All dogs received non-steroidal anti-inflammatory drugs (NSAIDs) for at least 2 days prior to the start of the trial.

Dogs that presented a heart murmur or heart disease could be included, but heart failure was an exclusion criterion. Dogs with chronic kidney disease in IRIS stage 3 or higher (serum creatinine >180 µmol/L, International Renal Interest Society[3]), were excluded from the study. Although previous pregabalin treatment was an exclusion criterion, previous treatment with gabapentin was accepted as long as the last dose had been given at least 5 days before starting the trial. If additional treatment for other conditions was necessary, additional drugs could be administered with the exception of those referenced in Appendix A. Dogs with dermatological conditions were excluded following a dermatological exam performed during the first visit.

Any adverse event, even unrelated with the administration of the drug or the procedures performed during the trial, was followed up, but the dog was excluded from the study. Dogs were also withdrawn if the owners were unable to comply with the daily dosing routine or were unable to attend any visit.

Study design

The study consisted of a baseline phase with NSAIDs as sole treatment and two periods during which the dogs additionally received pregabalin (5 mg/kg twice daily, orally) or placebo for 14 ± 4 days each. Animals were randomly assigned (block randomisation, Excel, Microsoft Office 2016) to one of two sequences (pregabalin-placebo or placebo-pregabalin) as displayed in Fig. 1. The baseline treatment ensured some analgesia was provided for the two-week period to avoid ethical or welfare constrains during the placebo treatment phase.

The study was carried out under the European Directive 2010/63/EU on the protection of animals used for scientific purposes (Project License PPL 70 / /8152). The study was monitored to Good Clinical Practice standards, according to VICH GL9[4] and was approved by the Royal Veterinary College Ethics and Welfare Committee on the 29th August 2013 (URN 2013 1243).

Pregabalin and placebo administration

The pregabalin formulation was the oral solution Lyrica (20 mg/mL, bottle of 473 mL Pfizer).

The placebo formulation was reverse-engineered according to Good Manufacturing Practices[5] from qualitative information available about the vehicle used in Lyrica. Dog owners and the investigator remained masked to the treatment. The administration of the first dose occurred during Visit 1 (Fig. 1). At 24 h after the first dose, the dogs continued with the same treatment at home twice daily for 14 ± 4 days until Visit 2. Dog owners recorded each dose given and the time of administration on a diary to confirm the compliance.

The treatments were switched over at Visit 2 (Fig. 1). A dispenser administered the medication in the hospital so the primary investigator remained masked to the treatment during treatment switch. The dogs that were receiving pregabalin during period 1, received their last dose of pregabalin followed by the first dose of placebo 24 h thereafter.

Alternatively, the dogs that were receiving placebo during period 1, received their first dose of pregabalin followed by another dose 24 h thereafter. After Visit 2, dogs continued with the second treatment twice daily for 14 ± 4 days, until Visit 3. The dogs were officially signed off the study after the last sample collection of Visit 3 and transferred to the care of their usual veterinarian. If efficacious, open-label pregabalin treatment could be continued by the primary veterinarian.

Efficacy assessment

Daily pain scores (NRS) recorded by dog owners

During the two periods of medication, dog owners recorded in a booklet a pain score with NRS at the end of each day of medication at home. They were assessing spontaneous vocalisations, phantom scratching episodes and exercise impairment (Plessas et al., 2012) to score the pain severity daily (from 0 [no pain] to 10 [worst pain]) after being previously instructed how to perform it.

The primary investigator (SSM) performed the physical and neurological examinations and QST. The dogs stayed in hospital for at least 24 h at Visit 1, Visit 2, and Visit 3, during which the following parameters were recorded.

Quantitative sensory testing

The somatosensory function was then assessed with QST following the Sensory Threshold Examination Protocol (STEP) described previously (Sanchis-Mora et al., 2017). The stimuli were applied on the skin of six body areas (BA) in a randomised order in unrestrained dogs. A threshold was obtained when the animal responded according to previously used criteria (Sanchis-Mora et al., 2017) with the addition of phantom scratching. The body areas tested were lateral neck, humeri and tibias (bilaterally; Appendix BB). It was necessary to clip a 1.5 × 1.5 cm square of hair for each BA. The STEP consisted of the evaluation of the sensory modalities briefly explained below. Von Frey filaments (Bioseb) were used for tactile sensory thresholds (TST) and tactile allodynia. The 50% response technique described by Sanchis-Mora et al. 2017) was used. Mechanical stimulus was applied with an algometer (ProdPro, Topcat Metrology Ltd). Heat stimulus was applied using a handheld thermal probe (HotPro, Topcat Metrology Ltd). Both the mechanical thresholds (MT) and heat thresholds (HT) were measured in triplicate for each BA. Cold / /cool stimuli were applied using a handheld thermal

probe (NTE-2A, Physitemp Instruments) set at 0±0.2 °C and 15±0.2 °C, respectively. The latency (measured in s) between cold/cool application and the time at which the animals responded to the probe was recorded. The measurements were performed in triplicate for each BA and for each temperature.

Blood and urine samples

At each visit, 1 mL of blood was taken for plasma pregabalin concentration measurement at baseline (0 h, time of treatment administration), at 90±9 min post-dose administration (presumed time of peak plasma concentration, (Salazar et al., 2009)) and at 12 h±72 min post-dose administration (trough concentration; Fig. 1). Blood samples were taken through a preplaced peripheral catheter (Introcan IV catheter, Braun Vetcare) according to the method from Elliott et al. (2010). The samples were transferred into EDTA tubes, then centrifuged at 1000 g for 10 min (Jouan C3i-CR3I Multifunction Centrifuge, Thermo). Two aliquots of at least 150 µL of EDTA plasma were separated and stored at −80 °C within 6 h after collection.

Plasma pregabalin concentration was measured by liquid chromatography tandem mass spectrometry using a validated method (Appendix C). Plasma creatinine was measured on each visit using a calibrated portable machine (creatinine cartridges, ISTAT-1, VetScan Abaxis). A urinary sample was collected by free catch for urinary dipstick analysis (Siemens Multistick Siemens Healthcare) and urinary specific gravity.

Statistical analysis

Sample size calculation[6] estimated that nine dogs would be required to demonstrate an increase 3 N (standard deviation, SD = 1.9) in MT in the neck area with 90% power and 5% type I error rate. The mean and SD were based on previous non-published data testing in asymptomatic and symptomatic CM/SM dogs.

Baseline, pregabalin and placebo were considered as three phases for statistical comparisons. Data were analysed using a commercial statistical software (SPSS 21, IBM and R[7]). For continuous data, normality of distribution was verified by Kolmorov–Smirnov's test and by visual assessment of Q–Q plots and histograms.

Linear mixed models were used to assess effects of treatment period (baseline, pregabalin and placebo), BA and their interaction for TST, MT and HT, and dog identification was considered as a random effect.

Cold thresholds at 0 (0 se latency or >1 s latency) and 15 °C (censored at 60 s) were analysed with generalized linear mixed effects model and mixed effects Cox model, respectively, with the same fixed and random effects as above. Additionally, linear mixed effects model was used to assess the effects of pregabalin versus placebo and number of days within treatment on owner-recorded daily NRS scores.

It was not possible to test the sequence effect due to the small sample size. Post hoc comparisons were made when appropriate using the Fisher's Least Significant Difference (LSD). The mean peak and trough plasma concentrations after the first and last dose were compared with a paired t-test to

evaluate an increase in plasma concentration by the end of the period.

Results

Descriptive demographics, clinical data and neurological exam

Nine Cavalier King Charles Spaniels (four males and five females) were recruited for the study from February 2016 to August 2016 (Fig. 2). The median age was 6 years old (range, 1.1–9 years) and the median bodyweight was 9.6 kg (range, 6.6–13.8 kg). All dogs presented with cervical hyperaesthesia on palpation and five dogs showed scratching behaviour, either phantom scratching or making contact with the skin without evidence of skin/ear disease.

The baseline NSAID was meloxicam 0.05 mg/kg once daily for seven dogs and carprofen 2 mg/kg once daily for two dogs. Two dogs were previously treated with gabapentin at 10 mg/kg twice daily. Gabapentin administration was stopped between 6 and 10 days before starting the trial. These two dogs were randomly allocated to placebo phase during the first period.

Six dogs had a heart murmur. One had cardiac investigation due to bradyarrhythmia. The electrocardiogram revealed benign second-degree auriculo-ventricular block, Mobitz type II and echocardiography revealed a trivial mitral valve regurgitation and mild tricuspid regurgitation. None of the dogs received additional medication except one dog (ocular treatment with fusidic acid twice daily).

Clinical history and neurological examination results are summarised in Appendix D. Urine analysis and plasma creatinine values were within normal limits for all dogs.

One dog dropped out of the study at day 7 of the first period due to an ailment unrelated to the study (Fig. 2). This dog developed haemorrhagic diarrhoea at the same time as other dogs in the household. Nevertheless, this dog was subsequently excluded as the treatment with NSAIDs may have been contraindicated. This dog was receiving placebo during the first period and its data were excluded from the statistical analysis. Sedation was reported in two other dogs. No other side effects were reported.

Dog owner daily pain assessment

The daily pain scores (NRS) recorded by dog owners was significantly lower during pregabalin treatment phase (mean 3.17 ± SD 2.3 units) compared to the placebo phase (mean 4.24 ± SD 2.4 units, $P = 0.006$, Fig. 3). Number of days within treatment did not significantly affect daily NRS scores recorded by dog owners ($P = 0.470$).

Quantitative sensory testing

For each of the QST modalities, there was main effect of body area ($P < 0.001$) and treatment phase effect for TST ($P = 0.002$), MT ($P = 0.001$), CL_0 ($P = 0.001$) and CL_{15} ($P = 0.001$), but not for HT ($P = 0.094$). However, the interaction between BA and treatment was not statistically significant in any of the QST modalities ($P > 0.05$) except CL_{15} ($P = 0.005$; see below).

On post hoc comparison, baseline and pregabalin TST were significantly higher than for the placebo ($P=0.001$ and $P=0.005$ respectively). Pregabalin TST were not significantly different from baseline $P=0.567$. MT for pregabalin were significantly higher than baseline and placebo ($P=0.037$ and $P<0.001$, respectively) overall (represented as mean ± SD in Fig. 4), whereas placebo and baseline were not different ($P=0.097$).

Under pregabalin treatment, CL_0, was significantly longer from baseline ($P=0.001$) and placebo ($P=0.004$). Placebo and baseline phases were not different from each other ($P=0.686$; Fig. 5). For CL_{15}, the interaction between treatment phase and BA was statistically significant ($P<0.005$). Neck and humeri showed longer latencies on pregabalin compared to baseline ($P<0.001$ for both) or placebo ($P=0.02$, $P=0.0001$, respectively; Fig. 6). Latencies were longer during baseline than placebo for humeri ($P=0.001$) but not the neck ($P=0.167$).

Pregabalin plasma concentration

Peak plasma concentration at first dose (mean 4128 ng/mL, range 2420–5538 ng/mL) and at last dose (mean 4669 ng/mL, range 3704–6023 ng/mL) were not significantly different ($P=0.153$). Trough plasma concentration (12 h after peak) at first dose (mean 1047 ng/mL, range 541–1844 ng/mL) and at last dose (mean 1075 ng/mL, range 413–2381 ng/mL) were not significantly different ($P=0.769$). Pregabalin was undetectable during the placebo phase.

Discussion

This is the first randomised controlled trial reporting the efficacy of pregabalin in dogs with NeP. Daily NRS scores recorded by dog owners were significantly lower during pregabalin treatment phase compared to placebo. Pregabalin also improved mechanical hyperalgesia and cold allodynia as defined by Allchorne et al. (2005), compared to placebo and baseline.

In this study, the daily NRS assessment recorded by dog owners appeared to be a solid parameter. Longitudinal data do not rely on a single time point as for QST evaluation. Daily scoring therefore nullifies the bias of assessment at isolated points in time in the context of fluctuating clinical signs (Colloca et al., 2016). Similarly, in human clinical trials, pregabalin efficacy in peripheral neuropathy has been evaluated with success using patients' daily pain scores (Jenkins et al., 2012). We cannot exclude that other degenerated clinical problems, even if not demonstrated on MRI scans, could have also improved with pregabalin, and therefore improved dogs' mobility. The sample size calculation was based on MT data (single measurement), as there was not preliminary data on daily NRS pain assessment (repeated measures at home). Further significant changes could have been evidenced if we had more dogs included, especially when testing for interactions. For example, to demonstrate that NRS was significantly different between placebo and pregabalin treatments on a single day, post hoc sample size calculation recommended 13 dogs. We only demonstrated a difference of pregabalin over placebo for the overall duration of treatment with the current sample size.

Based on the response to pregabalin on the affected areas (neck and humeri) this study suggests that CM/SM dogs presenting clinical signs of NeP had mechanical hyperalgesia and cold hyperalgesia and allodynia. Pregabalin and gabapentin are efficacious if there is central sensitisation and nerve

damage is present (Patel and Dickenson, 2016). Visual inspection of the plotted data suggested a large improvement, especially for CL_{15} in both, humeri and neck. Reduction of cold allodynia at 15 °C with pregabalin was the clearest result, consistent with previous findings in rats with NeP (Lau et al., 2013). Pregabalin has been shown to decrease hyperalgesia and allodynia for cold, brush and mechanical testing in small cohort of humans with NeP (Attal et al., 1998; Dirks et al., 2002). Although there was a treatment effect on CL_0, there were no differences on the interaction with BA.

HT thresholds showed no differences between treatment phases and baseline, suggesting HT may not be a sensitive test for CM/SM dogs. Another study in dogs with spinal cord injury found a lack of thermal sensation compared to control dogs (Gorney et al., 2016). Human patients with SM showed impairment of thermal sensation, however, those presenting evoked and spontaneous pain had different QST profiles (Hatem et al., 2010). This may be also occurring in dogs with CM/SM.

There is no reported therapeutic window for the analgesic effect of pregabalin in people or in dogs with NeP, just a therapeutic range for seizure control in man (Arroyo et al., 2004; Berry and Millington, 2005). The mean steady-state trough concentration in the current study (1075 ng/mL) was sufficient to produce the analgesic effect. In elderly people, a reduced dose is indicated for patients with impaired renal function[8]). However, there is no data about pharmacokinetics of pregabalin in dogs with renal impairment. No pregabalin accumulation was seen in this study, supporting the safety of pregabalin in non-azotaemic dogs.

The main limitation of this study is the small sample size. The small number of dogs recruited in approximately 7 months could be because the incentive to dog owners (free treatment after completing the study) was modest compared to the limiting high cost of the MRI. Additionally, dogs already on gabapentinoids could not participate unless the treatment was withdrawn, and this was not always possible or ethical in severely affected dogs. Another limitation of this study was that the sedation observed in two dogs was not objectively quantified nor assessed for all dogs. Sedation can alter sensory thresholds slowing the reaction time (Gustorff et al., 2001). However, if sedation would have affected sensory thresholds, a generalized altered sensory threshold should have been found for all stimuli applied, not just in one region with specific stimuli types.

Finally, a control group with a healthy cohort of Cavalier King Charles Spaniels would have been needed to compare baseline thresholds. The high prevalence of this disease (Thofner et al., 2015) and financial constraints of imaging in dogs with no clinical signs made this not possible.

Conclusions

This is the first double-masked crossover placebo control clinical trial in dogs with NeP to investigate the efficacy of pregabalin on daily pain scores recorded by dog owners. Pregabalin also significantly reduced mechanical hyperalgesia and cold hyperalgesia and allodynia compared to placebo. Pregabalin was well tolerated with mild sedation in few cases. A larger sample is needed to confirm effect of pregabalin on other outcome variables.

Conflict of interest statement

This study was supported, in part, by an RVC internal grant and Transpharmation Ltd. None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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Footnotes

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[8]See: Package leaflet: Information for the user Lyrica 20 mg/ml mg/mL oral solution Pregabalin https://www.medicines.org.uk/emc/files/pil.4120.pdf (Accessed 27 June 2019).

Figure legends

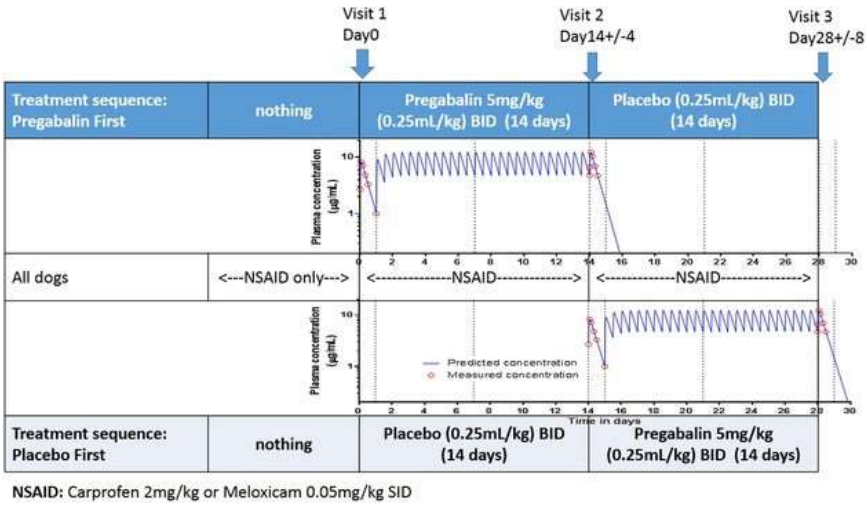

Fig. 1 Group allocation during the two period crossover study and timeline of the visits. Representation of Visit 1, Visit 2 (treatment switch) and Visit 3 for dogs receiving pregabalin or placebo first. The blue line is the average plasma concentration-time curve simulated from the pharmacokinetics from Salazar et al. 2009. The red dots are the times at which plasma samples were obtained for measurement of pregabalin in the current study.

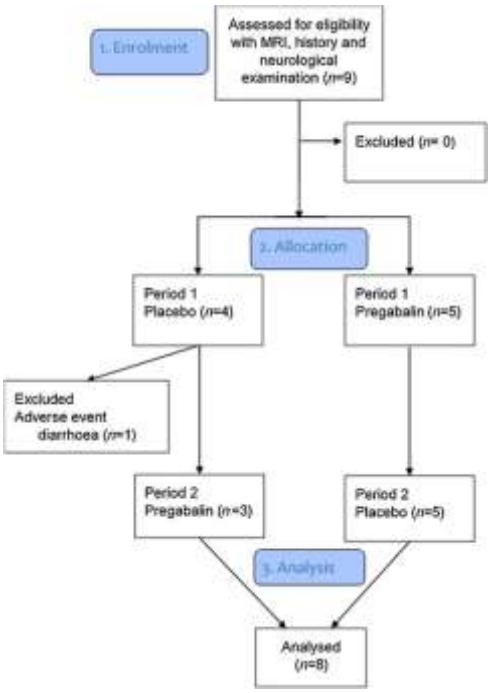

Fig. 2 Dog allocation diagram. The two periods consisted on a 2-week treatment with non-anti-inflammatory drug and placebo or pregabalin; then they switched treatment for another 2-week period. One dog was excluded on the first week of the Period 1 because she developed haemorrhagic diarrhoea non-related to treatment.

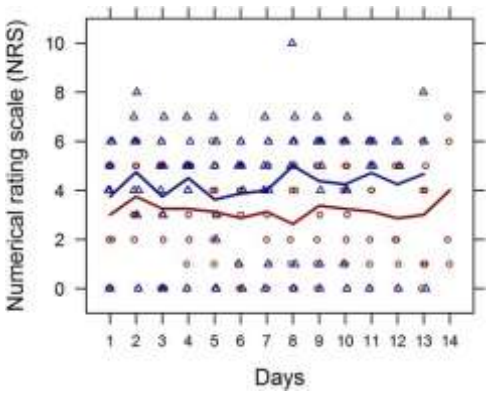

Fig. 3 Mean and individual daily pain scores recorded by dog owners using numerical rating scale (NRS; from 0 [no pain] to 10 [worst pain]) based on the level of spontaneous yelping, scratching episodes and exercise impairment. Red triangles represent pregabalin phase daily scores with the red line representing the mean and blue triangles represent placebo phase daily scores with the blue line representing the mean value.

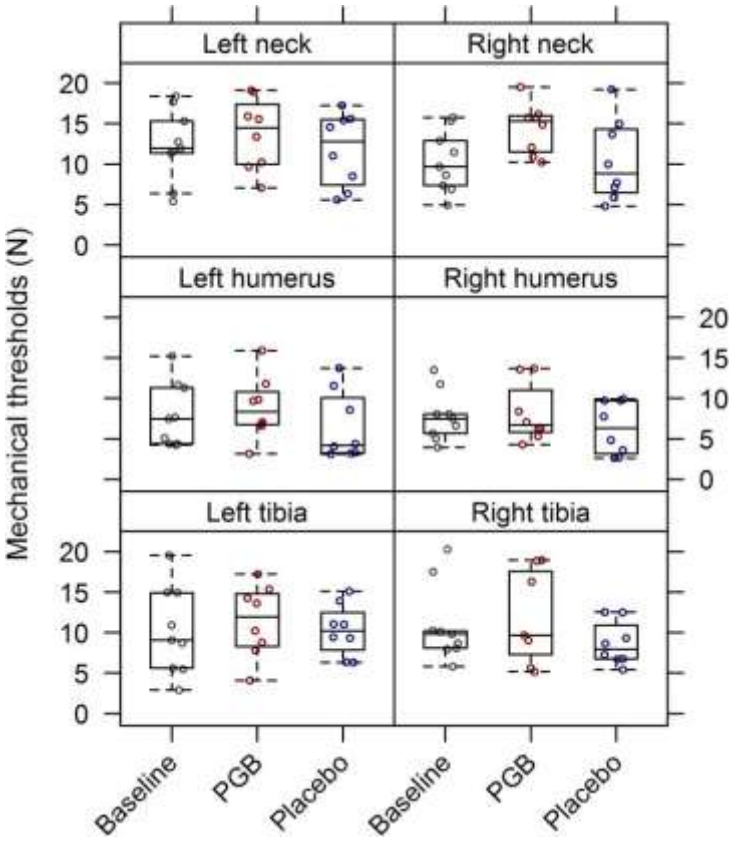

Fig. 4 Box plot of the mechanical thresholds in Newton (N) obtained at baseline, placebo and pregabalin treatments in the different body areas. There was a significant effect of treatment group ($P=0.001$) but the interaction treatment group and body area was not significant. MT during pregabalin were significantly higher than baseline and placebo ($P=0.037$ and $P<0.001$, respectively). Placebo MT were not significantly different from baseline ($P=0.097$). PGB: pregabalin.

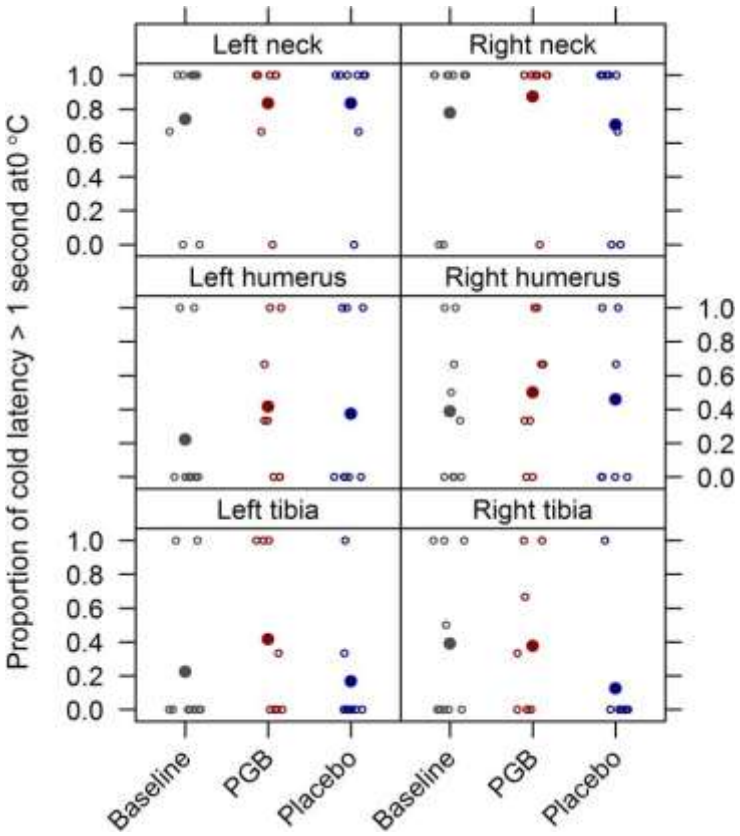

Fig. 5 Cold latency at 0 °C in s obtained at baseline, placebo and pregabalin treatments in the different body areas. Right and left sides are display separately. Individual values are represented by the opened dots and solid dots are the mean proportion across all dogs. More dogs tolerated Cold (0 °C) latency for >1 s with pregabalin compared to baseline (P = 0.001) and placebo (P = 0.004). There were no significant differences between placebo and baseline (P = 0.686), or the interaction between treatment phase and body area (P = 0.074). PGB: pregabalin.

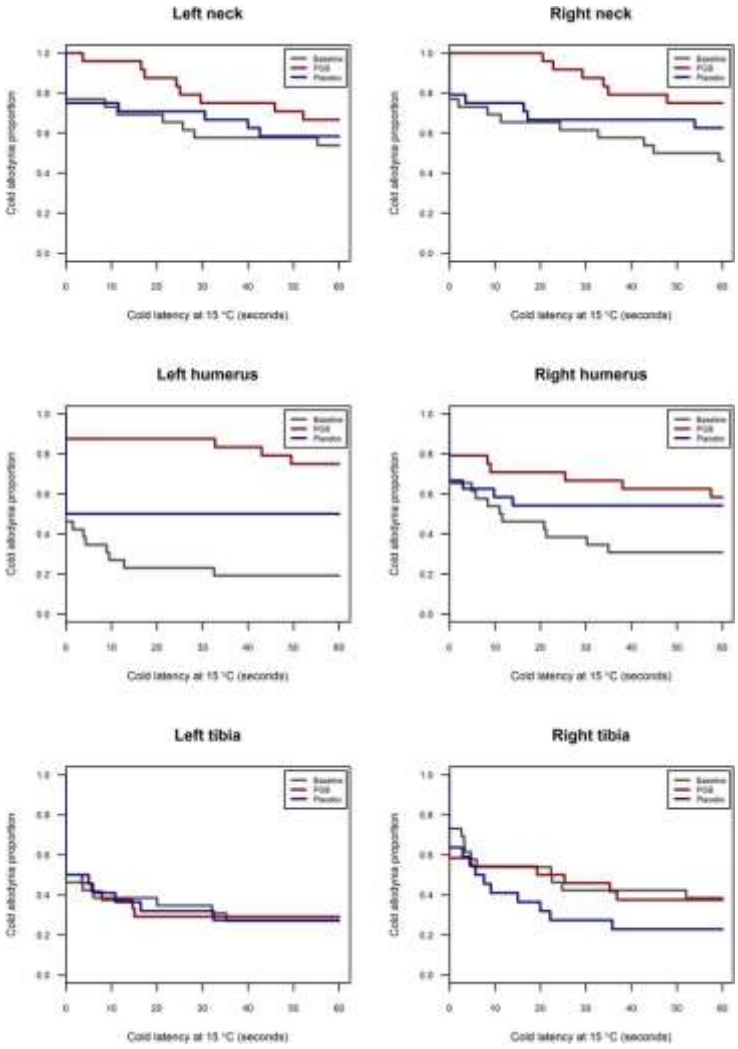

Fig. 6 Cold latency at 15 °C thresholds (measured in s) obtained at baseline, placebo and pregabalin treatments in the different body areas. Right and left sides are display separately. Neck and humeri showed longer latencies on pregabalin compared to baseline grouping both side together (right and left; $P<0.001$ for both) or placebo ($P=0.02$, $P=0.0001$, respectively; Fig. 6). Baseline and placebo were also different of each other on humeri ($P=0.001$), however not on the neck ($P=0.167$). Cold latency at 15 °C on the tibias were not significantly different between treatment phases. PGB: pregabalin.