Osteoarthritis and Cartilage

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Sustained release of locally delivered celecoxib provides pain relief for osteoarthritis: a proof of concept in dog patients

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SUMMARY

Objective: Drug delivery platforms that allow for gradual drug release after intra-articular administration have become of much interest as a treatment strategy for osteoarthritis (OA). The aim of this study was to investigate the safety and efficacy of an intra-articular sustained release formulation containing celecoxib (CXB), a cyclooxygenase-2 (COX-2) selective inhibitor.

Methods: Amino acid-based polyesteramide microspheres (PEAMs), a biodegradable and non-toxic platform, were loaded with CXB and employed in two *in vivo* models of arthritis: an acute inflammatory arthritis model in rats ($n = 12$), and a randomized controlled study in chronic OA dog patients ($n = 30$). In parallel, the bioactivity of sustained release of CXB was evaluated in monolayer cultures of primary dog chondrocytes under inflammatory conditions.

Results: Sustained release of CXB did not alleviate acute arthritis signs in the rat arthritis model, based on pain measurements and synovitis severity. However, in OA dog patients, sustained release of CXB improved limb function as objective parameter of pain and quality of life based on gait analysis and owner questionnaires. It also decreased pain medication dependency over a 2-month period and caused no adverse effects. Prostaglandin E_2 levels, a marker for inflammation, were lower in the synovial fluid of CXB-treated dog OA patients and in CXB-treated cultured dog chondrocytes.

Conclusion: These results show that local sustained release of CXB is less suitable to treat acute inflammation in arthritic joints, while safe and effective in treating pain in chronic OA in dogs.

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Introduction

The impact of osteoarthritis (OA) has doubled since the mid-20th century; the prevalence and burden is expected to rise in the coming decades[1]. Recent efforts identify clinical OA phenotypes[2]

but the first line treatment remains similar for all phenotypes. Inflammation in OA has gained much interest for specific therapeutic targeting with inflammatory processes as the common denominator for all clinical phenotypes.

Pro-inflammatory mediators produced by articular chondrocytes (ACs) and synovial lining cells promote OA progression and severity of the symptoms[3]. In experimental rat OA, gene expression levels of prostaglandin E synthase (*ptges*) and prostaglandin-endoperoxide synthase 2 (*ptgs2*, encoding cyclooxygenase 2), two cyclooxygenase-2 (COX-2) regulated genes, were higher in the knee cartilage than in the control healthy knees[4]. Similarly, synovial fluid (SF) Prostaglandin E_2 (PGE_2) levels increase in

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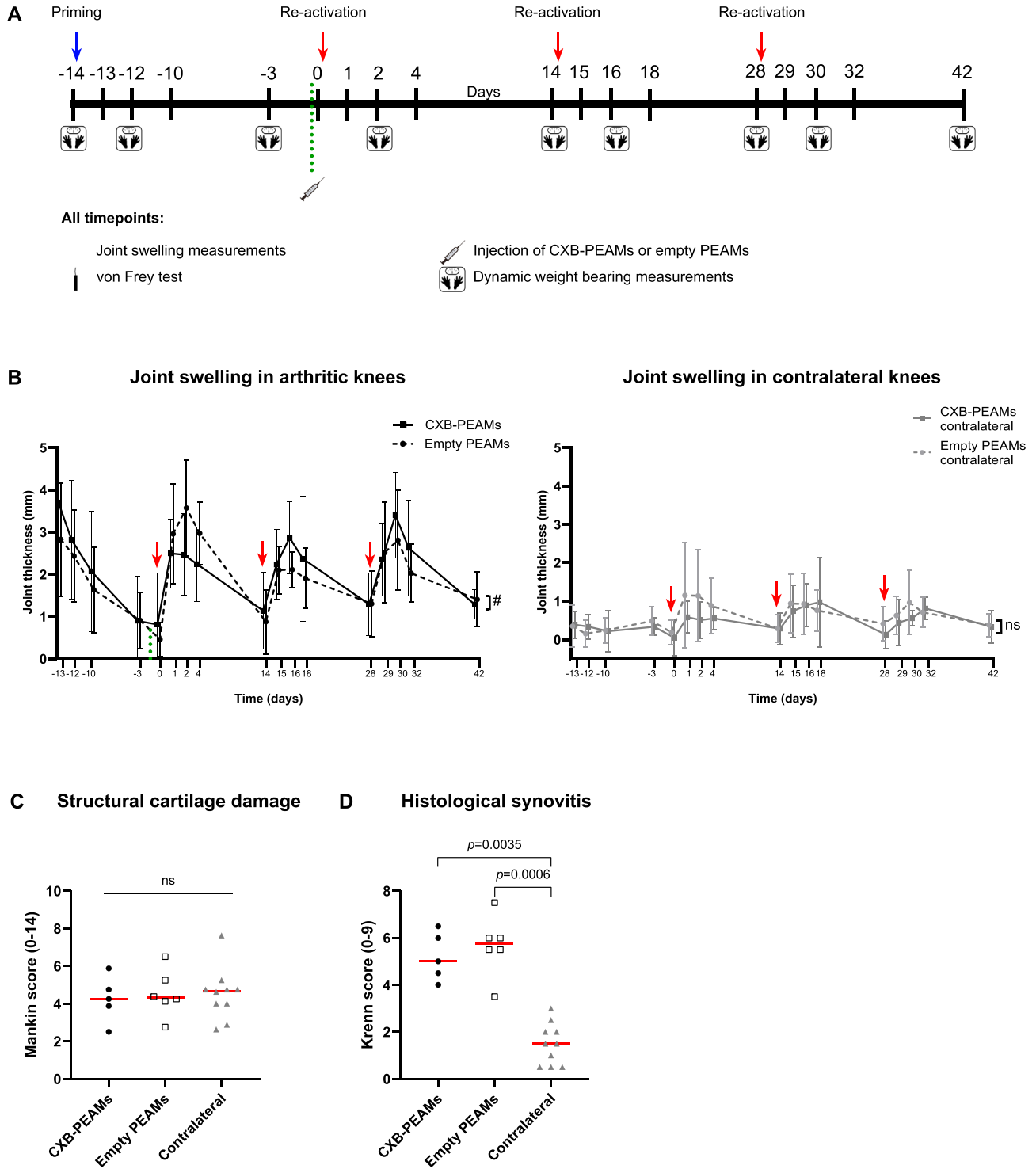*E-mail address:* m.a.tryfonidou@uu.nl (M.A. Tryfonidou).

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Fig. 1

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response to anterior cruciate ligament transection[5]. Additionally, PGE$_2$ levels in SF of human[6] and dog OA patients[7] correlate with pain. Therefore, long-term inhibition of COX-2 to reduce PGE$_2$ secretion may be a solution for effective relief of OA-related pain and inhibit disease progression.

Celecoxib (CXB) is a selective COX-2 inhibitor suggested to have disease-modifying effects[8,9]. Osteophyte formation and bone marrow lesions were reduced in a post-traumatic rat OA model after oral treatment with CXB[10]. However, oral non-steroidal anti-inflammatory drugs (NSAIDs) can lead to adverse effects, including hypertension (>10%) and gastro-intestinal damage (30—50%), of which 1—2% of the patients experience serious injury[11]. Moreover, systemic drug administration entails suboptimal local drug exposure, with articular levels reaching only 23—50% of those in the circulation[12], which is below the minimum inhibitory concentration of CXB. Local intra-articular drug concentrations rather than blood plasma levels determine the clinical effect, as illustrated in arthritis patients whose SF levels of orally administered anti-inflammatory drugs correlated with the magnitude of relief of clinical symptoms arthritis[13]. As such, intra-articular application is an obvious solution. In a post-traumatic rabbit OA model, intra-articularly injected CXB inhibited expression of pro-inflammatory mediators and prevented further structural cartilage damage[14]. However, rapid diffusion and clearance of drugs after direct intra-articular injection require frequent intra-articular injections that are a burden to the patient and not without risk. Extended delivery of NSAIDs from biomaterial carriers would circumvent this problem.

In vitro release of CXB from amino acid-based polyesteramide microspheres (PEAMs), a biodegradable and non-toxic platform, was demonstrated for >75 days[15]. Intra-articularly injected CXB-PEAMs were present in rat OA knee joints >12 weeks and lowered local PGE$_2$ levels[15]. Furthermore, intra-articular delivery in a pre-clinical rat OA model of PEAMs with 2—13× higher CXB loading doses than those employed in the earlier study, attenuated synovial inflammation and inhibited the progression of subchondral bone changes[16].

As PEA-mediated CXB delivery appears to be a promising avenue for the treatment of OA, the current study aims to further delineate its applicability in two *in vivo* arthritis models reflecting two main patient groups. In the first, acute inflammation and pain during severe flares play a central role, which are observed in a subset of OA patients and also in rheumatoid arthritis. The second consists of pet dogs diagnosed with clinical and radiological OA. It is estimated that >10% of the dog population is affected by spontaneous OA[17] and OA dog patients are considered a well-accepted model for human OA within the concept of 'One Medicine'[18].

Method

A detailed description of all methods can be found in the online supplementary methods.

Synthesis and characterization of PEAMs

PEAMs were synthesized and characterized according to previously published methods[19]. CXB-loading of the PEAMs resulted in 24.1 wt% CXB. The CXB release profile in phosphate buffered saline (PBS) was determined by HPLC as described previously[20]. The profile confirmed an initial burst release at day one followed by a gradual increase of CXB release in the days thereafter, reaching a cumulative release of 32% at day 14 (Fig. S1).

Induction of experimental acute arthritis in rats

The study design [Fig. 1(A)] was approved by the National Commission of animal experiments (AVD108002015282), was supervised by the local Animal Welfare Body (WP#800-15-282-01-003) and is in accordance with the ARRIVE guidelines. To study the long-lasting effects of local controlled CXB delivery, acute arthritis was induced in one knee joint (left) of twelve 8-weeks old female Sprague—Dawley rats (214 ± 12 g; Charles River laboratories, The Netherlands) as described previously[21]. Briefly, local synovitis was induced in all rats by priming the left joint (*i.e.*, experimental joint) with a peptidoglycan-polysaccharide (PGPS) at day −14 by intra-articular injection of PGPS (100P fraction with 5 mg rhamnose/mL, 25 μL PGPS of 0.17 mg/mL, Lee Laboratories). Baseline assessments were performed by IR. Thereafter, the animals were re-randomized based on the degree of pain/swelling, to ensure similar weight-bearing deficiency across treatment groups. Local synovitis was reactivated at day 0, 14, and 28 with intravenous PGPS injections (0.28 mg/mL). The treatment (60 mg/mL empty PEAMs or 80 mg/mL CXB-PEAMs (V = 25 μL, amounting to 402 μg CXB/joint) *n* = 6 per group, randomly divided) was intra-articularly administered 2.5 h prior to the reactivation at day 0 (IR), whereafter outcome measures were determined (IR/SV, both blinded for the treatment throughout the study). The administered CXB dose was the highest dose shown to be effective in the ACLT rat OA model[16].

Outcome measures to evaluate acute inflammation in rat knee joints

Knee joint swelling was determined by the average of three consecutive measurements using a digital caliper and upon subtraction of the baseline measurements prior to priming (day -14). Mechanical hypersensitivity was assessed by applying von Frey hairs to the hind paw[22]; the 50% threshold was determined using

Local delivery of CXB-PEAMs in acute arthritic rat knee joints. (**A**) Experimental design acute arthritis model: rat knee joints (left) were primed with intra-articular injection of 0.17 mg/mL PGPS at day −14 (blue arrow) and re-activated with 0.28 mg/mL intravenous PGPS injections (red arrows) to stimulate severe flares. CXB-PEAMs or empty PEAMs (control) were administered as single intra-articular dose 2.5 h prior to the first re-activation at day 0 (green dotted line). The second and third reactivations were administered on day 14 and 28. Joint swelling measurements as an indication for synovitis and mechanical hypersensitivity measurements at the hind paw for pain-like behaviors were conducted at all time points, while dynamic weight bearing measurements (scale icon) were conducted at various days throughout the experiment. The animals were euthanized at endpoint on day 42 and the knee joints were histologically analyzed. (**B**) Swelling of the joint (synovitis) for *n* = 6 knees per group, *n* = 5 knees for CXB-PEAMs after 28 days calculated as the increase in knee thickness compared to baseline thickness at day −14 and presented as mean ± SD. One CXB-treated rat reached the humane endpoint which was defined as lameness in combination with severe swelling of both hind paws and no improvement after additional pain medication consisting of 5 mg/kg carprofen. This was reached at day 18 and the rat was excluded from the study. #: *P* = 0.799 (**C**) Structural cartilage damage and (**D**) synovitis were scored on coronal knee joint histological sections at day 42 (*n* = 10 contralateral; *n* = 6 empty PEAMs; *n* = 5 CXB-PEAMs). Data of individual knees are presented as dot plots including the mean (red lines). ns: not significant.

the up-down method[23]. A decrease in 50% threshold indicates increased hypersensitivity, and thus increased pain sensation. Weight-bearing and paw surface areas were analyzed using the dynamic weight-bearing (DWB) apparatus (Bioseb, module version 1.4.2.98; Boulogne, France) as described previously[21].

Upon termination of the experiment, all hind limbs were collected and processed for histological analysis as described in the supplementary methods. Five μm coronal knee joint sections were stained with Safranin-O/Fast green to evaluate cartilage degeneration using the Mankin score[24] (0: "completely healthy" to 14: "total joint destruction"). Synovitis was evaluated using the Krenn score[25] (0: "healthy" to 9: "severe synovitis") on hematoxylin and eosin-stained sections. Scoring was done independently in a random and blinded fashion (AT/IR). The average score was used for further analysis.

Efficacy of local controlled CXB delivery in OA dog patients

Release behavior and bioactivity of CXB-PEAMs were evaluated in primary dog AC culture in which inflammation was simulated by exogenous tumor necrosis factor (TNF) using a culture system described previously[26]. Briefly, healthy ACs were enzymatically isolated from cartilage from six experimental dogs from unrelated experiments (AVD108002015282). Cells were expanded till passage 2 in expansion medium (High glucose Dulbecco's modified medium (DMEM) Glutamax, Gibco, Waltham, USA) and refreshed every 3–4 days. Cells were co-cultured with chondropermissive medium (High glucose DMEM, 1% [v/v] penicillin-streptomycin (GE Healthcare, Chicago, USA), 1% [v/v] ITS+ premix (Corning; Corning, NY, USA), 0.04 mg/mL bovine serum albumin (Sigma–Aldrich), 0.1 mM ascorbic acid (Sigma–Aldrich)) in the presence of empty- or CXB-PEAMs that were placed in Transwell® baskets (pore size 0.4 μm, polycarbonate membrane, Corning). CXB-PEAMs containing $7*10^{-4}$ M CXB, from now on referred to as 10^{-4} M, were used to inhibit COX-2 activity. After 4 h, 10 ng/mL recombinant TNF (R&D Systems, Minneapolis, USA) was provided as a pro-inflammatory stimulus. The transwells with the empty-PEAMs or CXB-PEAMs were transferred to newly plated cells of each donor after 72 h. This was repeated 8 times amounting to a total culture period of 28 days where bioactivity of the released CXB was determined. Every 72 h, medium was collected and stored at −20°C.

CXB medium levels were measured using enzyme-linked immunosorbent assay (ELISA; Neogen Corporation, Lansing, USA) according to the manufacturer's instructions. PGE_2 (Cayman Chemical, Ann Arbor, USA) for $n = 3$ AC donors and C–C motif chemokine ligand 2 (CCL2; Kingfisher Biotech, Saint Paul, USA) for $n = 6$ AC donors were measured in the culture medium according to manufacturers' instructions.

Randomized controlled study in chronic OA dog patients

The study was conducted upon approval of the departmental Ethical Committee (#AVR17-06). Dogs were considered eligible for the study if they met the inclusion criteria (Table S1) and upon written owner consent. Both owners and assessing veterinarians were blinded to the treatment during the entire study period. A random list for the vials containing CXB-PEAMs ($n = 20$) or placebo ($n = 10$) was created in advance (Excel 2016, Microsoft) and dogs were thereby randomly allocated to one of the treatment arms by order of enrollment. For the arthrocentesis and intra-articular injection, dogs were sedated. SF was collected, centrifuged (5 min, 1300g), aliquoted, and stored at −20°C. Suspensions of 70 mg PEAMs/mL for 20 wt% CXB-loaded PEAMs (9.3 mg CXB/mL) and empty-PEAMs were prepared as previously reported[27]. Dosing of the treatment depended on body weight categories, *i.e.*, 0.5 mL

(15–30 kg), 1 mL (30–45 kg), and 1.5 mL (>45 kg) microsphere solution, corresponding to 4.6, 9.3, and 14 mg CXB, respectively, based on results of a preclinical study[16]. Dogs in the placebo group received the same quantity of empty-PEAMs. One- and 2-month follow-up consisted of clinical examination, force plate analysis, owner questionnaires (primary outcome), plain radiographs, SF analysis briefly described below and in detail in supplementary methods. Owners were allowed to use analgesics they were already using prior to the study and were asked to document medication(s) administered during the study ('additional pain relief medication') and note adverse reactions. Any pain medication was discontinued 4 days prior to baseline and follow-up measurements to minimize a possible (beneficial) effect of the oral medication on the subjective and objective read out.

Outcome measures for relief of clinical signs in dog OA

All dogs underwent full clinical and orthopedic examination prior to, and at the 1- and 2-month follow-up visits. Lameness was recorded on a 4-point scale. Radiographic projections of each joint were used for evaluating radiographic OA (Table S2) prior to inclusion and 2 months after treatment. Osteophyte height was measured as described previously[28].

Ground reaction forces (GRFs) were measured (9261 Kistler Instrumente)[29] during each visit with a single force plate with the dogs on a leash at a walking gait. Ten valid measurements per limb and per time point were collected, minimally eight when limited by the stamina of the dog and their owners. GRFs in the mediolateral (Fx), craniocaudal (Fy) and vertical (Fz) direction were normalized for body weight (N/kg) whereafter Symmetry indices (SIs) were calculated[30].

To assess treatment outcome on the behavior and function of their dog, owners filled in a questionnaire including some questions from the Canine Brief Pain Inventory (CBPI)[31] supplemented with questions relating to mobility; owners were asked to focus on their dog's behavior of the last 7 days. To make the scores more intuitive for Dutch owners, scales were flipped (*i.e.*, 1 = lowest; 10 = excellent). Synovial PGE_2 and CCL2 levels were measured by ELISA according to the manufacturers' instructions (1:10 diluted) as described before.

Statistical analysis

GraphPad 9.0.0 was used for statistical evaluation. Normality of the data was checked by assessing the Q–Q plots, histograms, and Shapiro–Wilks tests. All tests were subjected to Benjamini-Hochberg *post-hoc* correction for multiple testing and P-values <0.05 were considered significant.

For the rat study, differences in joint swelling, 50% threshold, and DWB parameters during the priming (day −14 to day 0) and the three reactivation periods (*i.e.*, day 0 (day 1–13), 14 (day 15–27) and 28 (day 29–42)) between treatment groups, were determined with mixed model analysis with "time" and "treatment" as fixed effects and "rat" as random effects. Differences in the area under the curve (AUCs) per period between the treatment groups were evaluated by Welch's *t*-tests. Differences in histological scores (*e.g.*, Krenn and Mankin score) were evaluated with non-parametric Kruskall–Wallis tests.

For the *in vitro* analysis, differences in PGE_2 and CCL2 secretion by the ACs in response to empty- and CXB-PEAMs, were evaluated with a two-way ANOVA (factors: treatment; donor).

For the dog study, the sum score of all questions and δ-sum score (sum score at 1 or 2 months − sum score at baseline) were computed. The sum score was analyzed as repeated measures in a Friedman test to determine the treatment effect over time within

the group. To determine efficacy, *i.e.*, difference between treatment arms, the δ-sum score at each time point was subjected to Kruskal−Wallis tests correcting therefore the baseline. Force plate outcomes were analyzed with linear mixed-effects models of non-transformed [peak propulsive force (PPF)] and log transformed data [peak vertical force (PVF), vertical impulse (VI)] with fixed ("time") and random ("dog") effects to study differences within the group, while efficacy was evaluated with Brown-Forsythe ANOVA to evaluate differences between groups at the same timepoint.
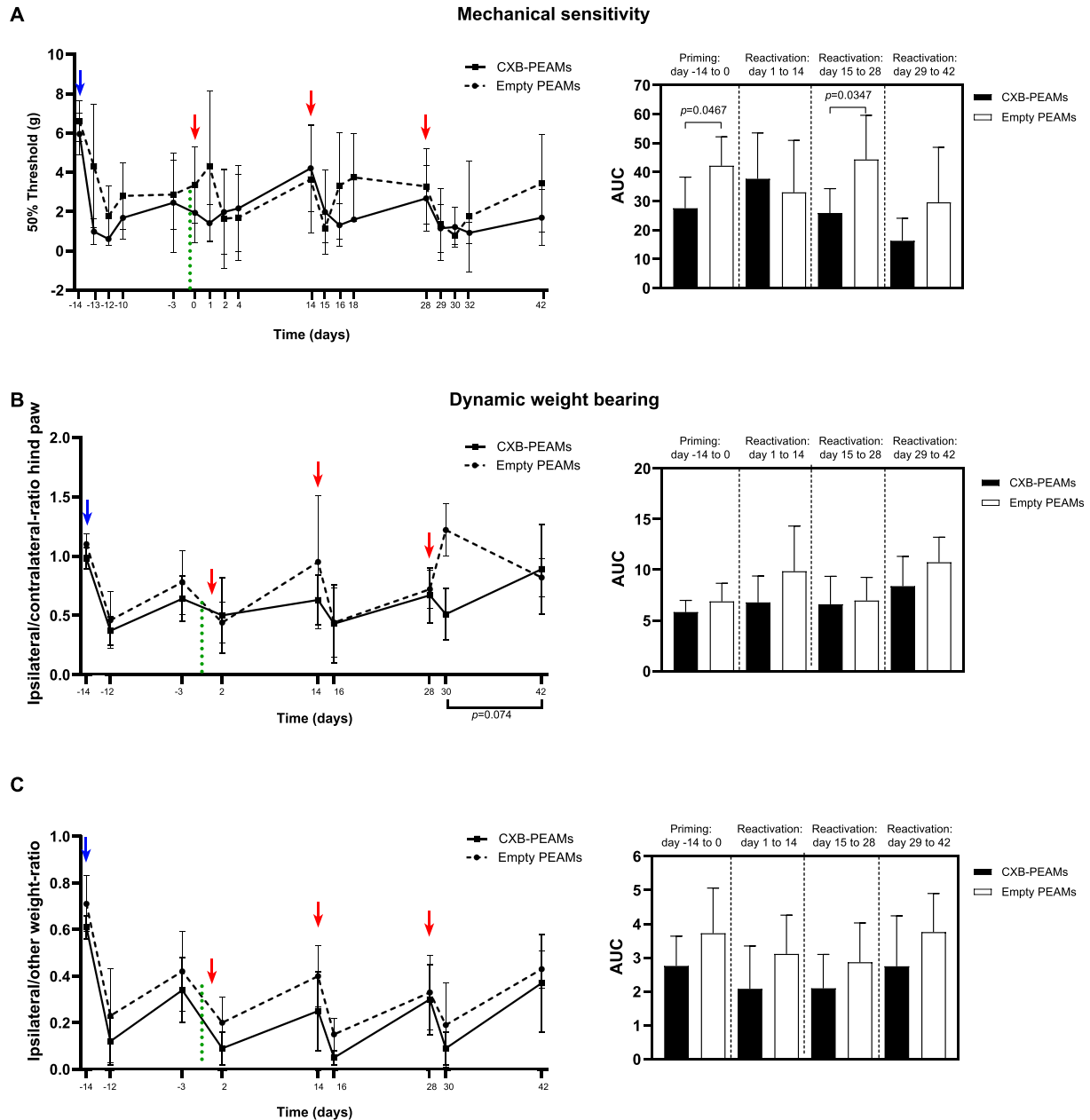

Fig. 2

CXB-PEAMs do not alleviate pain in acute arthritis in rats. Knees were primed at day −14 (blue arrow) with an intra-articular injection of PGPS and flares were evoked with intravenous injections of PGPS at 0, 14, and 28 days (red arrows). Single intra-articular injections CXB-loaded PEAMs (solid lines) or empty PEAMs (placebo; dotted lines) were given 2.5 h prior to re-activation on day 0 (green dotted line). (**A**) Mechanical sensitivity of the hind paws and (**B**−**C**) dynamic weight bearing was measured at various time points throughout the experiment for *n* = 6 rats per group (*n* = 5 rats for CXB-PEAMs after 42 days), presented as mean ± SD and corresponding area under the curve (AUC) are provided for the priming and the three re-activation periods upon intra-venous injection of PGPS. Only *P*-values <0.05 are annotated in this figure. An increase in mechanical sensitivity indicates that local CXB release did not alleviate arthritis-induced pain.

Visual lameness scores were analyzed via contingency tables and Fisher's exact tests for lame vs non lame dogs at each time point between treatment arms. SF biomarkers were evaluated with non-parametric Wilcoxon signed-rank tests for differences within groups between time points. To determine efficacy, in the subset of paired SF data, the change in PGE_2 (δPGE_2) and CCL2 ($\delta CCL2$) was computed and subjected to statistical analysis using the unpaired t test for δPGE_2 and the Mann Whitney test for $\delta CCL2$.

To evaluate dependency on additional pain relief medication, a log rank test was run to determine differences in the survival distribution ("analgesics free period") between the treatment- and placebo group. To assist interpretation, the mean difference (MD) with the 95% confidence interval (CI) is provided as MD [lower CI; upper CI] for the continuous clinical data.

Results

Effect of local delivery of CXB on acute arthritis signs in rat knee joints

Treatment with CXB-PEAMs did not significantly reduce joint swelling compared to empty-PEAMs during the three reactivation periods (day 1–42) ($P = 0.799$) although a slight reduction was apparent in the first period [Fig. 1(B)]. The degree of structural cartilage degeneration was not changed by PGPS priming compared to control, nor was it affected by any treatment [Fig. 1(C)]. Synovitis was significantly increased in both the joints treated with empty-PEAMs and joints treated with CXB-PEAMs compared to the contralateral control knees, supporting that the PGPS model induced synovial changes in the primed knees at day 42 [Fig. 1(D)]. Local application of CXB-PEAMs did not ameliorate synovitis compared to empty-PEAMs during the entire experiment.

Local delivery of CXB does not alleviate pain-like behaviors in rats with arthritic knee joints

During the priming period (day-14 to day 0), rats developed mechanical hypersensitivity. Despite randomization, mechanical sensitivity of the hind paw during the priming period was higher in rats allocated to CXB-PEAMs, based on the significantly smaller (AUC) of the 50% threshold, compared to those allocated to receive empty-PEAMs at day 0. Each intravenous injection of PGPS increased mechanical sensitivity after each reactivation. Only after the second reactivation, mechanical sensitivity in CXB-treated rats was further increased compared to controls, as seen by a decrease of 50% threshold based on the AUC for the three reactivation periods following intravenous administration of PGPS at day 0, day 14, and 28 [Fig. 2(A)]. All DWB parameters were similar between groups throughout the entire experiment, though the ipsilateral/contralateral-ratio tended to be lower during the 3rd reactivation period for the CXB-treated rats ($P = 0.074$), indicating that the rats unloaded the experimental limb [Fig. 2(B) and (C)].

Sustained release of CXB from PEAMs alleviates pain in clinical dog OA

The capacity of PEAM-mediated CXB release to suppress inflammation was evaluated in dog ACs stimulated by TNF. CXB medium levels at day 4 and 14 confirmed sustained release from the PEAMs [Fig. S3(A)]. Culturing without PEAMs or with empty-PEAMs did not affect PGE_2 secretion by dog ACs, but this was significantly decreased after exposure to CXB-PEAMs [Fig. S3(B)]. Culturing ACs with empty-PEAMs increased CCL2 production which was prevented by CXB-PEAM- [$P = 0.003$; Fig. S3(C)].

Baseline demographics and read outs of the thirty included dogs are provided in Table I and supplementary data (Fig. S4, Tables S3 and S4). There were no major adverse effects during the study period. Peri-articular joint swelling 1–3 days after placebo administration occurred in 2 dogs, which resolved after a few days without additional treatment.

The sum score of the owner's questionnaire was considered the primary patient-oriented read-out. CXB-PEAMs-treated dogs improved in the sum score questionnaire at 1 month ($P = 0.0035$; rank sum difference −18) and 2 months ($P = 0.0150$; rank sum difference −15) after treatment [Fig. 3(A)], while the placebo group

Parameter	CXB-PEAMs	Empty PEAMs (placebo)
Joint distribution	hip ($n = 5$), knee ($n = 6$), Elbow ($n = 9$)	hip ($n = 1$), knee ($n = 5$), elbow ($n = 5$)
Age (median (range))	5 (9 mo–13 yrs)	7.5 (2 yrs–10 yrs)
Body weight (mean (SD))	32 (12)	34 (10)
Gender	3 M, 7 MN, 2 F, 9 FN	3 M, 2 MN, 5 FN
Duration of NSAID use in months (mean (SD))	12 (12)	15 (17)
Multimodal therapy (i.e., medications in addition to NSAID)	Tramadol ($n = 3$), Tramadol + phenylbutazone ($n = 1$)	–
Previous surgery to the affected joint	8/20 (40%)	5/10 (50%)
Sumscore questionnaire (median (range))	46 (32–81)	49 (20–88)
Visual lameness score (median (range))	1 (1–3)	2 (1–3)
SI − PVF (mean (SD))	16.3 (20.6)	20.3 (25.8)
SI − VI (mean (SD))	18.3 (23.5)	27.5 (29.9)
SI − PPF (mean (SD))	28.8 (24.7)	41.8 (35.8)
Radiographic OA score	3 (1–3)	3 (2–3)
Osteophyte score	2 (1–3)	2 (1–3)

Mo, months; yrs, years; M, male; MN, male neutered; F, female; FN, female neutered; SI: symmetry index; PVF, peak vertical force; VI, vertical impulse; PPF, peak propulsive force; SD: standard deviation.

Table I

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Baseline demographics for dogs with osteoarthritis (OA) treated with celecoxib-loaded polyesteramide microspheres (CXB-PEAMs) or empty PEAMs (placebo)

did not ($P > 0.999$; rank sum difference 0). The significantly higher δ-sum score at both 1 ($P = 0.0127$; rank sum difference 16.6) and 2 months ($P = 0.0284$; rank sum difference 14.7) in the CXB-PEAM-treated group compared to placebo indicated efficacy of the CXB-PEAMs treatment [Fig. 3(B), Table S5].

Additional pain relief medication was deemed necessary for 9/10 dogs allocated to the placebo group vs 3/20 dogs that received CXB-PEAMs. The survival distribution ("additional analgesia free period") was significantly higher in the treatment vs placebo group [$\chi^2 = 25.75$, $P < 0.0001$, Fig. 3(C)] with a median medication-free

Fig. 3

Osteoarthritis and Cartilage
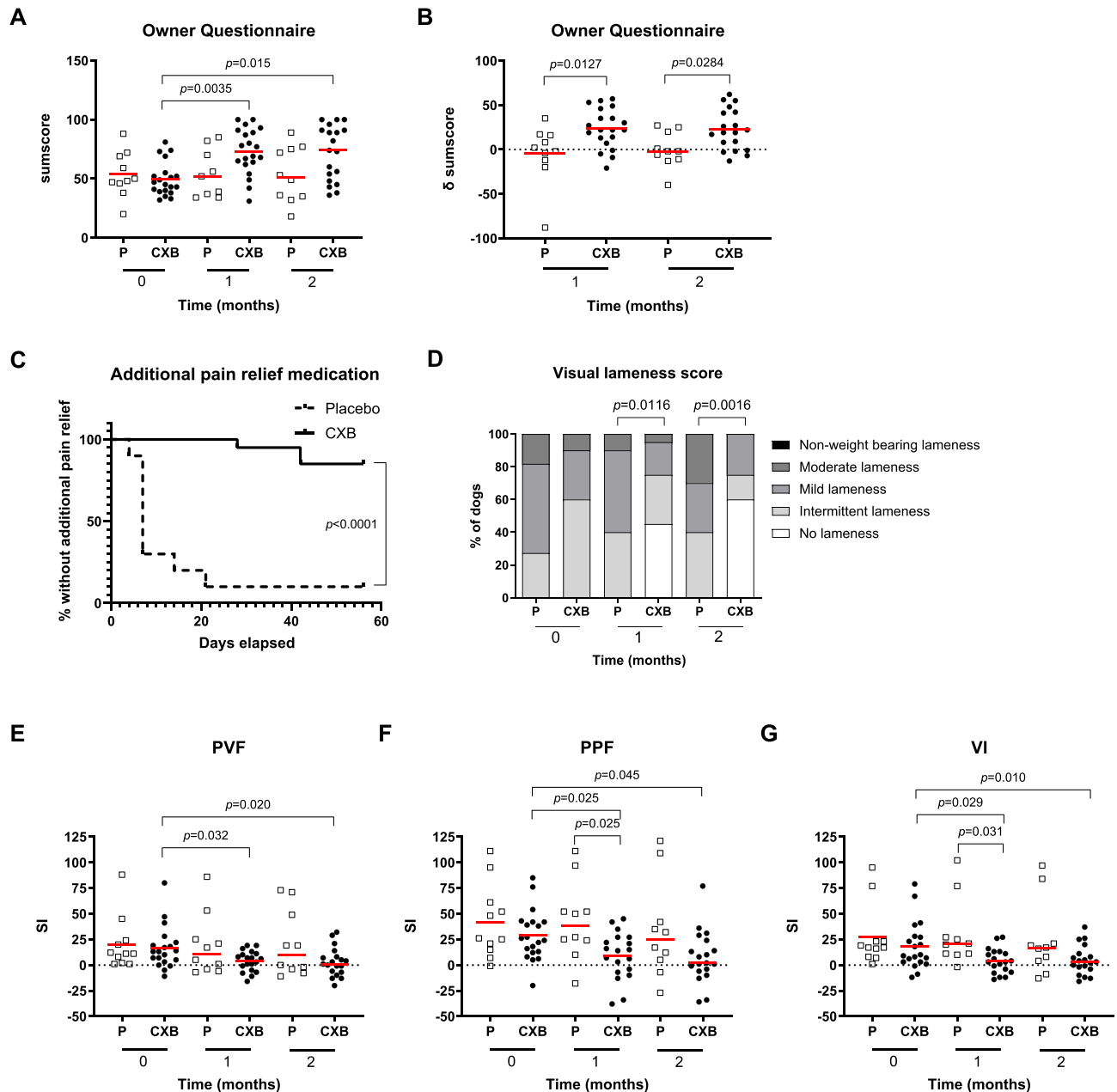
Sustained release of CXB from PEAMs alleviates pain in OA dog patients. Randomized controlled clinical trial of client-owned dogs suffering from naturally occurring OA. (**A**) Owner questionnaire sum score and (**B**) differences in sum score (δ) (sum core at 1 or 2 months − sum score at baseline) provided by the owners of the dogs treated with placebo ($n = 10$) or CXB-PEAMs ($n = 20$) at the 1- and 2-month follow-up visits. Means indicated by red bars. (**C**) Kaplan−Meier curve depicting the additional pain relief medication during the study period and (**D**) Proportions of the visual lameness scores before and after treatment with placebo or CXB-PEAMs. Symmetry indices (SI) of (**E**) the peak vertical force (PVF), (**F**) Peak Propulsive Force (PPF), and (**G**) the Vertical Impulse (VI) of placebo treated or CXB-PEAMs treated patients 1 and 2 months after administration. Only *P*-values <0.05 are annotated in this figure.

period being undefined for CXB and 7 days for placebo. Radiographic OA severity and osteophyte height did not change during the follow-up period (Table S4; $P > 0.15$).

There were significantly less lame CXB-PEAMs-treated dogs at 1 and 2 months compared to placebo ($P = 0.012$ and $P = 0.0016$) with dogs receiving placebo having an infinite relative risk to be dependent on oral medication. Kinetic gait parameters improved significantly at 1 and 2 months after intra-articular injection with CXB-PEAMs. Specifically, PVF, VI, and PPF SIs improved at 1- and 2-months post injection of CXB-PEAMs [Fig. 3(E)–(G); Table S6], whereas no statistically significant differences were found between time-points within the placebo group. Only at 1-mo follow-up, PPF and VI SIs were significantly better in CXB-PEAM-treated dogs compared to placebo ($P = 0.025$; -33.8 [-59.9; -7.69] and $P = 0.0031$; -0.35 [-0.55; -0.15], respectively).

Effects of CXB-PEAMs on joint inflammation in dogs with OA

In joints injected with CXB-PEAMs, SF PGE_2 levels were significantly lower than at baseline [Fig. 4(A); $P = 0.025$; -474 [-1820; 24]]. CCL2 levels had significantly increased compared to baseline in the placebo-treated, but not in the CXB-PEAMs-treated joints [Fig. 4(B); $P = 0.031$; 562 [0–4486]]. The δPGE_2 tended to be lower in the CXB-PEAMs-treated joints compared to placebo ($P = 0.0768$; -599 [-1270; 72]), while $\delta CCL2$ did not differ ($P = 0.8369$; 59 [-4065; 2606]).

Discussion

Drug delivery platforms that gradually release drugs after intra-articular administration have become of much interest as a treatment strategy for OA[32]. The present study shows that controlled release of CXB from PEAMs is not suitable for treating acute arthritis but was safe and effective to treat pain related to chronic OA in dogs for at least 2 months after injection.

It has become more evident that also inflammation contributes to pain in OA[33,34]. We therefore evaluated the efficacy of CXB-PEAMs in the PGPS rat model, a model characterized by severe flares of inflammation. Despite the total dose of ~400 µg CXB-PEAMs shown to be effective in inhibiting inflammation in OA[16], which theoretically would result into at least 20% being released within the first week of injection and hence exceeds the reported IC50 for COX-2[35] by 2000–8000 times this did not reduce joint swelling nor lowered inflammation-induced mechanical hypersensitivity in the paws over the course of 42 days. Noteworthy, *in vitro* release profiles are not predictive for the *in vivo* situation, where many factors are at play that influence release of the drug and clearance from the joint[32]. There may be several reasons why CXB-PEAMs were unable to treat pain caused by the severe inflammatory state of the joint, including (a) dosing of CXB was too low for this type of arthritis and hence the release of the CXB-PEAMS did not match the needs of a fulminantly inflamed joint; (b) rapid degradation of the PEAMs due to increased levels of protease activity typically present in heavily inflamed joints like in rheumatoid arthritis[36] limiting the duration of the therapeutic effect of the released drug, and (c) the generally limited potency of NSAIDs in fulminant inflammation. PGE_2 may not be the main driver in the PGPS arthritis model, where Th2-dependent monocyte infiltration is dominating[37]. Employing a similar drug delivery system releasing the glucocorticoid triamcinolone acetonide we demonstrated major improvements on joint inflammation in the same rat arthritis model[21]. Corticosteroids exert stronger inhibitory effects on leukocytes (*e.g.*, Th2-lymphocytes) compared to NSAIDs[38]. Furthermore, the mode of action between glucocorticoids and NSAIDS is rather different. Glucocorticoids can affect inflammatory responses pre-and post-transcriptionally, for example, inhibiting transcription factors that induce cytokines or affect cytokine signaling in recipient cells[39,40], while COX-2 inhibitors target specific production of prostaglandins[41]. Pathways downstream of PGE_2 may be modulated by NSAIDs, while glucocorticoids have a broader action which may have translated to the differences seen in pain inhibition in our previous study. These observations emphasize the importance of patient stratification to determine the target population, or OA phenotype, that will respond and benefit most from the treatment.

We utilized the dog patient serving as target species and as a natural disease model for human patients[18] to evaluate safety and efficacy of the CXB-PEAMs platform for OA pain management. *In vitro*, the presence of CXB-PEAMs lowered the secretion of PGE_2 and CCL2 by ACs, indicating an anti-inflammatory effect. The present study did not specifically study biocompatibility in the OA joint. The increased secretion of CCL2 by ACs upon exposure to empty-PEAMs may relate to the increased phagocytic capacity of OA ACs and the adoption of a pro-inflammatory phenotype[42]. Whether the synovial lining macrophages in OA joints become activated upon exposure to the PEAM degradation products, as known for other materials[43], remains to be determined. It was reported that microspheres are entrapped in the synovium and surrounded by mononuclear inflammatory and giant cells; CD68$^+$ macrophages were detected in the synovial lining of OA rat knees that received empty-PEAMs[15]. Nonetheless, it remains to be determined whether the presence of macrophages is biomaterial-driven cell infiltration or relates to the OA joint environment wherein macrophages have a well-described role[44].

In the population of patient dogs suffering from OA, significant improvements in owner-perceived pain and lameness were evident in the CXB-PEAMs but not the placebo treatment, indicating a minimal caregiver placebo effect in this veterinary clinical trial. This seemingly contradicts Conzemius and Evans[45] reporting ~40% caregiver and veterinarian placebo effects in an FDA-approved dog study. Noteworthy, the latter was only based on global and subjective evaluation of lameness. Several new owner questionnaires, inspired from tools applied to human patients, have been developed and adopted for veterinary use. Recent large randomized veterinary clinical studies in the OA field employing the CBPI, reported placebo effects ranging between 16%[46] to 28%[47]. Importantly, these beneficial analgesic effects of the CXB-PEAMs were corroborated by objective measures. Dogs treated with CXB-PEAMs did not depend on oral pain medication compared to placebo over time and showed a decrease in asymmetrical limb loading during the follow-up period, indicating improvement in gait due to pain relief.

To further understand what could have contributed to clinical improvement in the dog RCT, SF PGE_2 and CCL2 levels were determined. The reduction in PGE_2 levels, but not in CCL2, suggests that relief of clinical signs was likely mediated by COX-2 inhibition in the OA dog population studied, and not via the CCL2/CCR2 signaling axis as others have reported[48,49]. Despite the strong reduction in pain outcomes in the dog population, CXB-PEAMs did not cause structural changes within the 2-month follow up period. Here, osteophytes were not affected, which contrasts with the effects seen previously in an OA rat model, where this, along with other structural changes, was reduced[16]. However, such experimental models are based on the induction of disease, hence osteophyte formation was most likely prevented rather than reverted. Furthermore, in the clinical dog study, the follow-up period might have been too: an osteophyte size increase rate of only 1 mm/3 years has been reported in the dog (*i.e.*, ~0.028 mm/2 months)[50]. The use of more advanced and sensitive imaging modalities such as CT or MRI might provide more information.
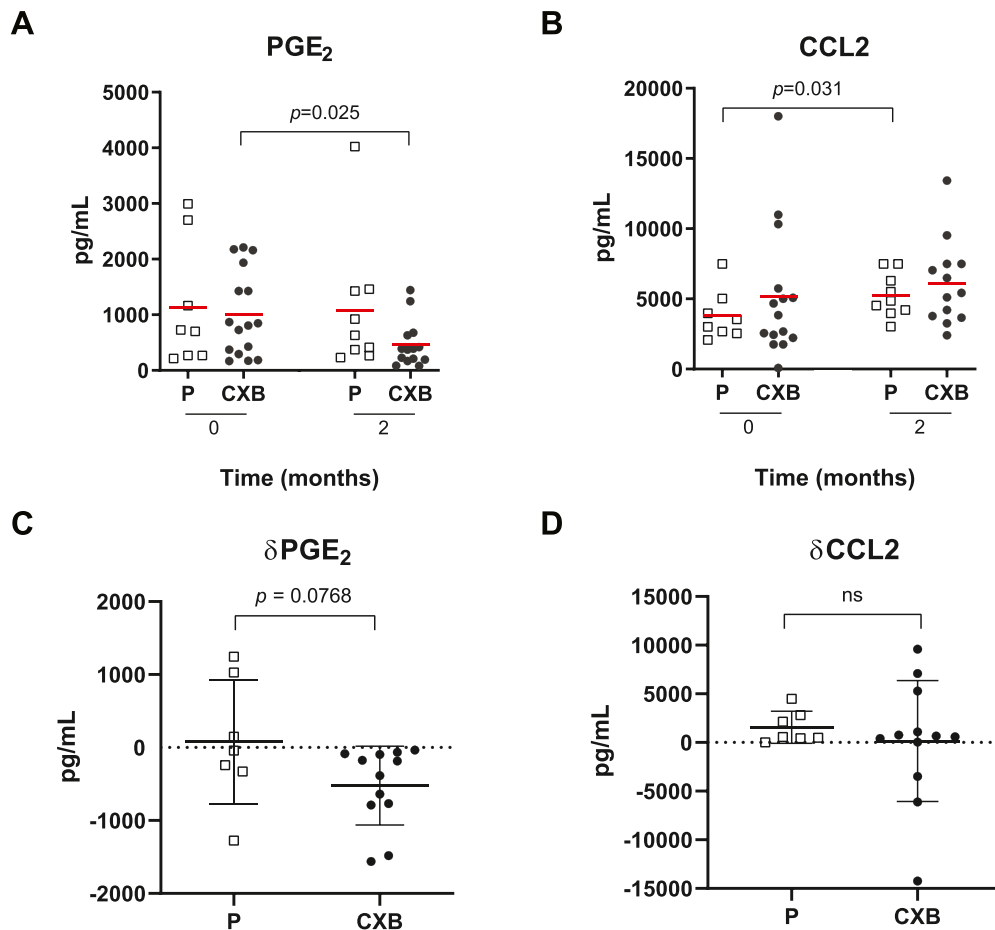
Fig. 4

Osteoarthritis and Cartilage

Pain relief after local controlled release of CXB is associated with inhibition of Prostaglandin E$_2$ secretion. (**A**) Prostaglandin E$_2$ (PGE$_2$) and (**B**) CCL2 levels in the synovial fluid of dog OA patients treated with placebo (P; empty PEAMs) or CXB-PEAMs at baseline post treatment. Data of individual dogs are presented as dot plots including the mean. There were $n = 8$ placebo and $n = 16$ CXB-treated, and $n = 9$ placebo and $n = 14$ CXB-treated synovial samples at baseline and at 2 months follow up available. Of those, $n = 7$ placebo and $n = 12$ CXB-treated paired samples were used to compute and analyze the change (δ) in synovial fluid levels over time (**C**) δPGE$_2$ and (**D**) δCCL2. Only *P*-values <0.05 and trending differences are annotated in this figure.

In conclusion, local sustained release of CXB with CXB-PEAMs is safe and effective to treat chronic OA pain in dogs suffering from natural disease, but was unsuitable to treat acute inflammation in arthritic joints with flares in rats. In OA dog patients, limb function and quality of life were improved, and additional pain medication dependency over a 2-month period was decreased without any substantial adverse effects. Follow-up studies with a larger sample size, longer follow-up time, and predefined rescue analgesia protocols are needed to investigate whether prolonged local exposure to CXB results in less osteophyte formation in dog patients with early OA. Considering the promising data in the current study, this CXB delivery platform could offer a suitable strategy for long-term OA pain management and further advance this concept towards first-in-man strategies.

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Conflict of interest

The authors from DSM Biomedical have proprietary and commercial interest in the materials discussed in this article. None of the authors has any other financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Supplementary data

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