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Clinical Trial

A dual amylin and calcitonin receptor agonist inhibits pain behavior and reduces cartilage pathology in an osteoarthritis rat model

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SUMMARY

Objectives: Pain and disability are the main clinical manifestations of osteoarthritis, for which only symptomatic therapies are available. Hence, there is a need for therapies that can simultaneously alter disease progression and provide pain relief. KBP is a dual amylin- and calcitonin-receptor agonist with antiresorptive and chondroprotective properties. In this study we investigated the effect of KBP in a rat model of osteoarthritis.
Methods: Medial meniscectomy (MNX) was performed in 39 rats, while 10 underwent sham surgery. Rats were treated with KBP and/or naproxen. Nociception was assessed by mechanical and cold allodynia, weight bearing asymmetry, and burrowing behavior. Blood samples were collected for biomarker measurements, and knees for histology. Cartilage histopathology was evaluated according to the advanced Osteoarthritis Research International (OARSI) score and KBPs *in vitro* antiresorptive effects were assessed using human osteoclasts cultured on bone.
Results: The MNX animals displayed an increased nociceptive behavior. Treatment with KBP attenuated the MNX-induced osteoarthritis-associated joint pain. The cartilage histopathology was significantly lower in rats treated with KBP than in MNX animals. Bone and cartilage degradation, assessed by CTX-I and CTX-II plasma levels, were decreased in all KBP-treated groups and KBP potently inhibited bone resorption *in vitro*.
Conclusions: Our study demonstrates the effectiveness of KBP in ameliorating osteoarthritis-associated joint pain and in protecting the articular cartilage, suggesting KBP as a potential drug candidate for osteoarthritis.

Introduction

Joint pain is the hallmark symptom of knee osteoarthritis (OA), due to cartilage degeneration and subchondral bone remodeling, and the pain is the main reason for individuals to seek medical assistance. Although the structural changes are the main contributors to OA pathogenesis, inflammation also plays an important role in disease progression[1]. OA-associated pain primarily affects the arthritic joint, but widespread sensitization has been reported for many patients[2].

No disease-modifying OA drugs (DMOADs) are currently available for patients[3]. Hence, there is an unmet clinical need in the treatment of OA patients. Targeting multiple aspects of the disease such as pain and joint swelling as well as delaying disease progression, rather than a single disease mechanism, could potentially improve the quality of life of OA patients substantially. At present, pharmacological therapies consist mainly of pain relievers with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and intra-articular therapies, such as glucocorticoids and hyaluronic acid[4]; if unresponsive to treatment, the joint may ultimately have to be

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replaced. Clinical and experimental observations have suggested that the structural integrity of the articular cartilage is linked to and dependent upon normal subchondral bone turnover[5]. Due to the abnormal biomechanical stresses in OA joints, an ideal therapeutic strategy may be targeting the regulation of both cartilage and bone cell activity, while providing analgesia.

Salmon calcitonin (sCT) is an interesting molecule for the treatment of OA[6]. It is a 32-amino acid peptide hormone and dual amylin and calcitonin-receptor agonist (DACRA) that activates both calcitonin and amylin receptors with high affinity[7] and a prolonged response[8]. sCT has potent antiresorptive effects, as it inhibits osteoclasts[9], and it prevents collagen degradation by articular chondrocytes[10]. The antiresorptive and chondroprotective effects of sCT in OA have been demonstrated both *ex vivo* and *in vivo*[11]. Studies have found that sCT treatment can provide analgesia a rat model of OA[12], protect the subchondral bone and articular cartilage[13,14], and has anti-inflammatory properties[15]. In canine OA studies, sCT counteracts the progression of joint lesions[16], indicating the importance of inhibiting subchondral bone turnover for cartilage protection. Similarly, in experimental arthritis studies, the inhibition of bone resorption protects the cartilage from erosion[10,17]. The analgesic effects of sCT have been demonstrated in patients with osteoporosis and Paget's disease[18], but the underlying analgesic mechanisms remain unclear[19].

KBP is an analogue of sCT, developed to overcome the challenges identified within clinical studies investigating sCT which failed to improve joint space narrowing and WOMAC scores in OA patients[20]. KBPs are designed to have greater potency than sCT and have shown promising results as potential treatments for type 2 diabetes and obesity in rats[21,22], but may also have potential in arthritic diseases[23]. In this study, we utilized the medial meniscectomy (MNX) model of OA, known to cause destabilization of the joint which eventually leads to rapid generation of the joint[24], to investigate the effects of KBP on the bone and cartilage pathology in OA, as well as its anti-nociceptive effects, alone or in combination with a standard of care NSAID.

Materials and methods

Animals

All animal procedures were performed in accordance with guidelines from the Animal Welfare Division of the Danish Ministry of Justice under the institutional license issued to Nordic Bioscience (2015-15-0201-00469). An everyday inspection of the animals health, behavior and well-being was conducted according to the Danish National Regulations and guidelines. Female Lewis rats ($n = 49$, Envigo, DK) at 6–8 weeks-of-age, with a mean weight of 170–200 g, were housed in groups of 3–4 under controlled temperatures on a 12-h light–dark cycle with unrestricted access to water and food in standard type IV cages, enriched with standard wood chips, red-tinted huts, nesting material and sticks.

OA induction and therapy

The rats were allocated into treatment groups according to their baseline burrowing performance in order to exclude the animals that did not burrow and to result in similar burrowing average and variance. The MNX model of OA was induced as previously described[25]. Rats were anaesthetized with isoflurane prior to surgery and the right leg was shaved and disinfected. Medial collateral ligament was transected and full thickness cut through the medial meniscus was made; the meniscus was then removed. Xylocain was used as topical analgesic, and Rimadyl (1 ml/kg) and Baytril (1 ml/kg) as post-operative drugs for 3 days. Sham-operated animals underwent the same procedure with the exception that the meniscus was left intact. Treatment groups were MNX ($n = 9$); sham ($n = 10$), MNX treated with KBP (MNX + KBP, $n = 10$); MNX treated with naproxen (MNX + Napr, $n = 10$); MNX treated both naproxen and KBP (MNX + KBP + Napr, $n = 10$).

KBP peptide (Synpeptide, Shanghai, China) was dissolved in saline for subcutaneous delivery in a dose of 10 μg/kg/day, according to reports in animal models of obesity[21] and unpublished studies performed in rheumatoid arthritis animal models. Naproxen was dissolved in 1 % carboxymethyl cellulose with 0.5% Tween 80, (all from Sigma–Aldrich, St. Louis, MO, USA), and administered by oral gavage at 4 mg/kg, as previously described[26]. Both drugs were administered once daily at 12 p.m., from the day of the surgeries until termination of the study. All treatment outcome measurements were made by an observer blinded to treatment, and behavioral testing was performed once a week starting 1 week after the OA induction.

Mechanical allodynia

Mechanical allodynia was assessed by measuring the withdrawal thresholds of both hind paws in response to the application of von Frey filaments using the up-down method, as previously described[27]. After habituation to the procedure, mechanical allodynia was assessed before surgery and up to 55 days post-surgery.

Weight-bearing test

The animals were placed in an incapacitance tester (Colombus Instruments, Columbus, OH, USA), consisting of two separate platforms, to detect the weight placed on each hind leg. Three, 4-s measurements were obtained per animal. The weight-bearing distribution was assessed pre-surgery and weekly until the termination of the study. The weight-bearing ratio was calculated as the amount weight placed on the MNX leg divided by the total weight placed on both hind legs.

Cold hypersensitivity

Cold hypersensitivity was assessed via the application of acetone to the hind paws of the animals, and the responses were recorded. A score of 0 was given to the rats that did not show any reaction (stamp, flick or paw withdrawal). If a response to the acetone was noted, then another 20 s was recorded to determine the nature of the response[28]. The rats received a score of 0–4; 0 = no response, 1 = quick withdrawal, flick or stamp of the paw (total reaction time <1 s), 2 = prolonged withdrawal or repeated flicking (total reaction time 1–3 s), 3 = repeated flicking together with licking at the ventral side of the paw (total reaction time 3–10 s), 4 = prolonged licking (total reaction time >10 s). The acetone test was performed three times per paw with at least 10 min in between the tests, and the scores were combined to yield a maximum possible score of 24[29]. Cold hypersensitivity was assessed at baseline and 2, 4, 6 and 8 weeks after surgery.

Burrowing

The burrowing test was performed, as described by Rutten *et al.*[30] and the allocation into treatment groups as described by Deacon *et al.*[31]. Burrowing performance was determined by weighing the amount of gravel remaining in the tube and the test was performed once per week.

Biochemical analysis of rat plasma

Blood samples were collected from overnight-fasted rats 2 h after the morning dosing on day 3 and 31 post-surgery. Baseline samples were obtained in the morning from overnight-fasted rats without prior dosing of the compounds. The plasma levels of CTX-I and CTX-II were determined through the use of commercially available kits (IDS, The Boldons, UK) according to the manufacturer's instructions.

Isolation of knees

The ipsilateral joint was collected at termination, 8 weeks after surgery, fixated in 10% formalin for 1 week and decalcified in 15% EDTA for 5 weeks. The knees were infiltrated with paraffin using Tissue-Tek VIP 5 Jr. (Sakura Finetek, Alphen aan den Rijn, The Netherlands), embedded in paraffin, and cut into 5 μm thick coronal sections using a HM 360 microtome (Microm International GmbH, Walldorf, DE). The sections were deparaffinized and rehydrated according to standard procedures prior to all stainings.

Cartilage joint pathology

Sections were stained with Safranin O (SafO)/Fast green, dehydrated, and mounted in Pertex to assess cartilage degradation, using the Osteoarthritis Research International (OARSI) advanced grading methodology[32]. The OARSI score system consists of six OA grades, with subcriteria for a more detailed examination. If the subcriteria are met then 0.5 is added to the grade, giving a total possible range of 0–6.5. OA stages (0–4) are used to identify the horizontal extent of cartilage damage, independently from the grade that was given. The reported score is the combination of grade and stage: score = grade × stage. Scoring was performed by two blinded observers on the medial area of the tibial plateau. Several slides per animal were scored and averaged, and the inter-observer mean was reported.

Resorption assay

Mature human osteoclasts derived from $CD14^+$ monocytes were generated from peripheral blood, as previously described[33], of anonymized blood donors obtained from a blood bank. For the resorption experiment, cortical bone (CB) slices from one bovine knee were used. CB slices were washed with PBS and placed in 96-well culture plates. The osteoclasts were lifted by trypsinization, scraped off, counted and seeded into the wells at a density of 50,000 cells/CB slice in 200 μl medium. Two CB slices without osteoclasts were cultured in parallel for background measurements. Plates were incubated 1 day at 37°C and 5% CO_2 followed by a medium change supplemented with resorption inhibitors: 300 nM diphyllin (V-ATPase inhibitor, Sigma–Aldrich), 10 nM KBP, 1 nM KBP, 0.1 nM KBP, 0.01 nM KBP, or vehicle (medium). Media was collected after 6 h for measurement of CTX-I release, to assess the osteoclast-mediated bone resorption. CTX-I, a biomarker detecting C-terminal type I collagen fragments, was measured using the CrossLaps for Culture ELISA (IDS, The Boldons, UK).

Statistical analyses

Data derived from body weight, pain assessments and plasma biomarkers were statistically analyzed by repeated measures two-way analysis of variance (ANOVA), with the assumption that the data were normally distributed, with Dunnett's multiple comparisons test comparing all groups to the MNX group. The cartilage scoring data was analyzed by Kruskal–Wallis test with Dunn's multiple comparison test comparing all groups to the MNX group. Data derived from the osteoclast cultures were analyzed using one-way ANOVA with Dunnett's multiple test for comparison against the vehicle. Body weight was expressed as percent change from baseline. Data from the acetone tests were normalized by subtracting baseline (BL) values from the experimental values for each rat. von Frey data were normalized and shown as percent change from baseline. Plasma biomarker data were normalized as fold increase compared to baseline. All data are presented in the text as mean (95% confidence interval), except the cartilage score which is presented as mean rank differences, and graphically as mean (S.E.M.). A two-tailed $P < 0.05$ was taken to indicate statistical significance. All plots were generated in GraphPad Prism 7.01 (Graph Pad Software, La Jolla, CA, USA).

Results

KBP reduces body weight

The health status of the animals was monitored throughout the study, with frequent body weight measurements (Fig. 1) and observation. No observable decrease in body weight was evident within the MNX group compared to sham (−2.1 (−5.9 to 1.8) *g*, $P = 0.5$). Animals treated with KBP monotherapy (7.6 (3.7–11.4) *g*, $P = 0.001$) and combination therapy with naproxen (8.7 (4.8–12.5) *g*, $P = 0.001$) had a substantially decreased body weight compared to the MNX group, as has been reported previously[21]. The general health of the animals was good, with no signs of impaired locomotion or distress.

KBP protects cartilage structure

Articular joint pathology was assessed in the isolated surgery knees. The induction of OA pathology as a result of the MNX can be clearly seen in the cartilage in the medial tibia plateau [Fig. 2(A)]. When compared to the sham group, the MNX animals had significant OA pathology (31.2, $P < 0.001$) as judged by the OARSI score [Fig. 2(B)]. MNX animals treated with KBP were found to have a significantly lower OARSI score compared to MNX (17.9, $P = 0.02$), indicating a positive effect upon cartilage health. Animals treated with the combination treatment had a lower OARSI score than the MNX group, but this was not statistically significant (9.3, $P = 0.66$). Interestingly, the combination therapy did not have increased efficacy compared to the KBP monotherapy. Furthermore, naproxen monotherapy appeared to have no effect upon cartilage integrity compared to the MNX group (2.8, $P > 0.9$).

KBP reduces nociceptive behavior

To test the anti-nociceptive potential of KBP, we analyzed the mechanical allodynia, cold hypersensitivity, burrowing behavior, and weight-bearing of the MNX rats. OA pain-like behavior was fully developed 9 days post- MNX (−57.52 (−76.26 to −38.78), $P = 0.001$ for mechanical allodynia and 3.21 (1.02–5.39), $P = 0.002$ for cold hypersensitivity), and persisted through day 51 (Fig. 3(A) and (B), see un-normalized data Supplementary Fig. 1). Interestingly, KBP monotherapy could increase paw withdrawal thresholds significantly compared to the MNX group (−32.35 (−51.1 to −13.61), $P = 0.001$, Fig. 3(A)). Naproxen monotherapy and combination therapy resulted in significantly increased paw withdrawal thresholds [Fig. 3(A)] compared to the MNX treated animals (−58.06 (−76.81 to −39.32), $P = 0.001$ and −63.29 (−82.03 to −44.54), $P = 0.001$ respectively). Consistent with the anti-nociceptive effect of KBP found in mechanical allodynia, cold hypersensitivity was greatly reduced in the KBP-treated group

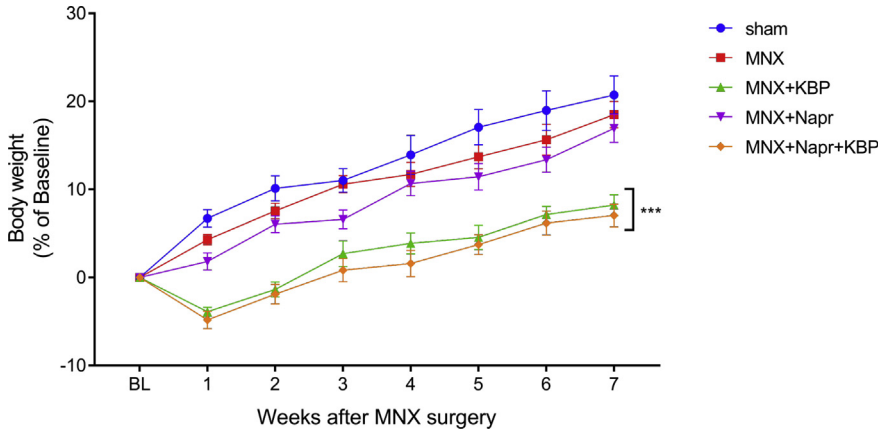

Fig. 1. Body weight monitoring. Change in body weight presented as % of initial body weight. Error bars indicate the SEM, $n = 10$/group except $n = 9$ in MNX group. Statistical significance compared to the MNX is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

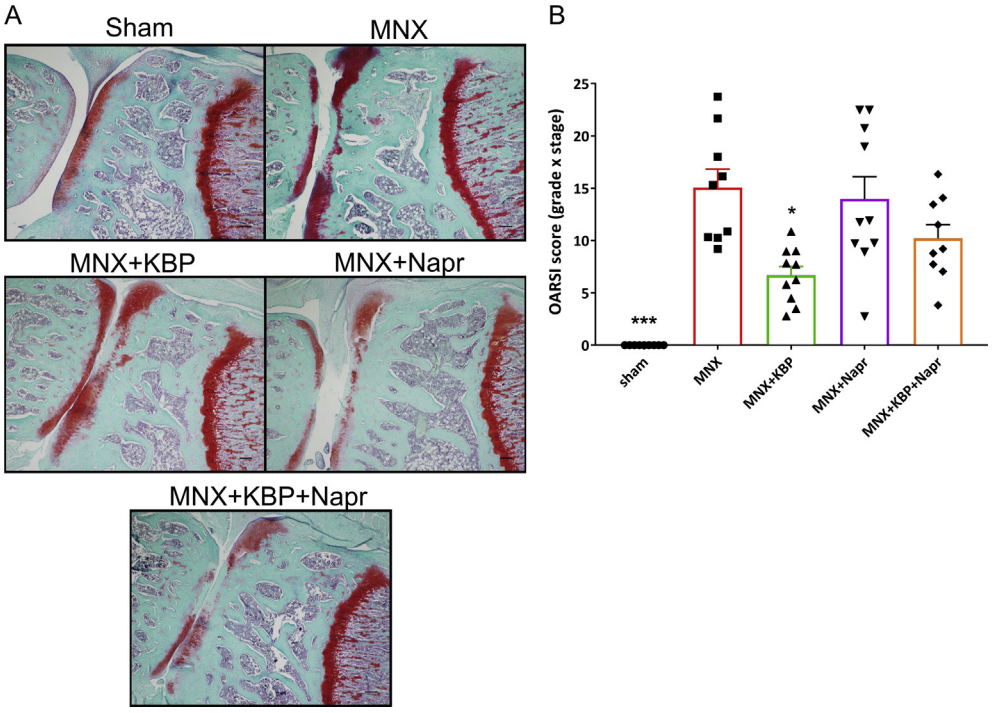

Fig. 2. Effect of KBP on cartilage pathology 8 weeks after surgery. Representative micrographs of the medial tibia plateau stained with SafO + Fast green staining (A) to assess cartilage damage according to the Osteoarthritis Research International (OARSI) advanced grading methology (B). Scale bars in A indicate 200 μm. Statistical significance compared to the MNX in B is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. $n = 9$ for the MNX, sham and MNX + KBP + Napr, and $n = 10$ for the MNX + KBP and MNX + Napr groups.

compared to the MNX group (2.2, (0.003–4.37), $P = 0.05$, Fig. 3(B)). Combination therapy was also found to significantly reduce cold hypersensitivity compared to the MNX group (2.2, (0.02–4.39), $P = 0.05$), whilst naproxen was not found to be significant different (0.93 (−1.26 to 3.12), $P = 0.66$).

We further assessed the burrowing behavior and the MNX rats showed the same vigorous burrowing performance as the sham (−150.9 (−534.8 to 232.9), $P = 0.72$), which decreased over time [Fig. 3(C)]. Additionally, no significant difference was observed between groups. The burrowing data should be cautiously interpreted as the test has low sensitivity. Furthermore, we observed an overall decrease in burrowing within the sham group, and therefore the sensitivity of the test decreased over time. OA induction by MNX did not induce any weight-bearing asymmetry, no difference between sham and MNX was found (−0.01 (−0.04 to 0.02), $P = 0.77$) Fig. 3(D)). Hence the effects of the therapies on weight-bearing could not be established.

KBP suppresses bone and cartilage turnover

The effects of KBP on bone resorption and cartilage degradation were investigated by measuring CTX-I and CTX-II. The MNX group was not found to be significant different compared to the sham for CTX-I (0.15 (−0.05 to 0.35), $P = 0.22$) and CTX-II plasma levels (0.13 (−0.19 to 0.46), $P = 0.76$) [Fig. 4(A) and (B)]. Nonetheless, KBP monotherapy had a significant reduction in the plasma levels of CTX-I (0.67, (0.47–0.87), $P < 0.001$)) and CTX-II (0.73, (0.39–1.06), $P < 0.001$) compared to the MNX. The combination therapy resulted

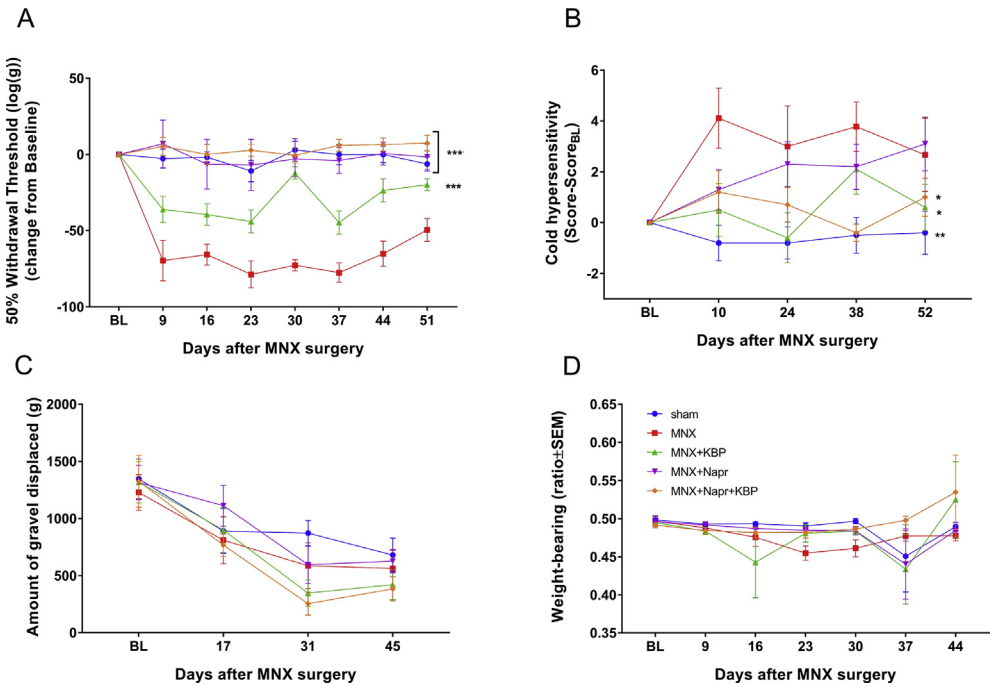

Fig. 3. Effect of KBP on pain behavior in the MNX model. Pain-like behaviors were assessed using paw withdrawal thresholds (A), cold hypersensitivity (B), burrowing behavior (C), and weight bearing asymmetry (D). Data in A are presented as the percent change from baseline and data in B are presented as the mean change from baseline. Error bars indicate the SEM, $n = 10$/group except $n = 9$ in MNX group. Statistical significance compared to the MNX is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

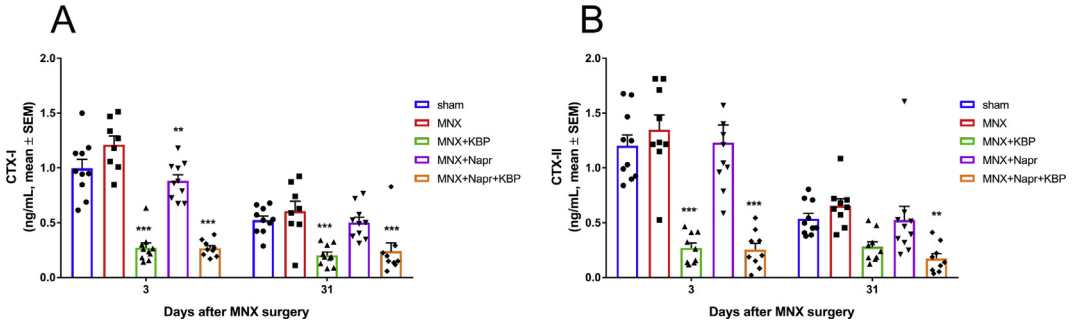

Fig. 4. KBP inhibits bone resorption and cartilage degradation *in vivo*. Bone resorption and cartilage degradation was assessed by CTX-I (A) and CTX-II (B), respectively. For CTX-I; MNX $n = 9$; sham $n = 10$; CIA + KBP $n = 10$; CIA + Napr $n = 10$; MNX + KBP + Napr $n = 9$. For CTX-II; MNX $n = 10$; sham $n = 10$; CIA + KBP $n = 9$; CIA + Napr $n = 10$; MNX + KBP + Napr $n = 9$. Data are presented as the mean fraction of each rat's baseline measurement. Error bars indicate the SEM. Statistical significance compared to the MNX is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

in a significant decrease of both CTX-I (0.65 (0.45–0.86), $P < 0.001$) and CTX-II (0.79 (0.45–1.12), $P < 0.001$) compared to the MNX, most likely due to the KBP effect. Naproxen monotherapy significantly lowered plasma CTX-I levels (0.22 (0.02–0.42), $P = 0.03$) compared to the MNX group, albeit the reduction was small and only was observed at the early time point. On the other hand, it did not show a statistically significant effect on CTX-II (0.12 (−0.2 to 0.45), $P = 0.79$).

KBP decreased CTX-I release on cortical bone

To assess the anti-resorptive potential of the KBP, bone resorption by human osteoclasts on CB slices was assessed. The V-ATPase inhibitor diphyllin was used as a positive control of osteoclast inhibition in bone resorption cultures[34]. As shown in Fig. 5, the release of the bone resorption biomarker CTX-I was significantly reduced by both diphyllin (41.68 (24.6–58.77), $P = 0.001$) and the KBP following 6 h after treatment compared to vehicle. For KBP 10 nM (19.73, (2.64–36.81), $P = 0.02$); KBP 1Nm (31.09, (14.01–48.17), $P = 0.002$); KBP 0.1Nm (32.58, (14.67–50.5), $P = 0.002$); KBP 0.01 nm (37.75, (20.67–54.84), $P = 0.001$).

Discussion

At present, no DMOADs are available to patients[3], hence there is an urgent medical need for the development of pharmaceutical agents that can both preserve bone and cartilage integrity as well as provide relief from OA-associated pain. The lack of clinical translatability of sCT was mainly due to the lower levels of exposure or due to the oral administration which will be hopefully overcomed by an injectable formulation[20]. Our study describes KBP, a novel DACRA with an increased potency than sCT, in the medial MNX rat model of OA. KBP was found to have both chondroprotective and anti-nociceptive effects in this model. KBP counteracted MNX-induced cartilage erosion and reduced plasma levels of the bone and cartilage degradation biomarkers CTX-I and CTX-II, the

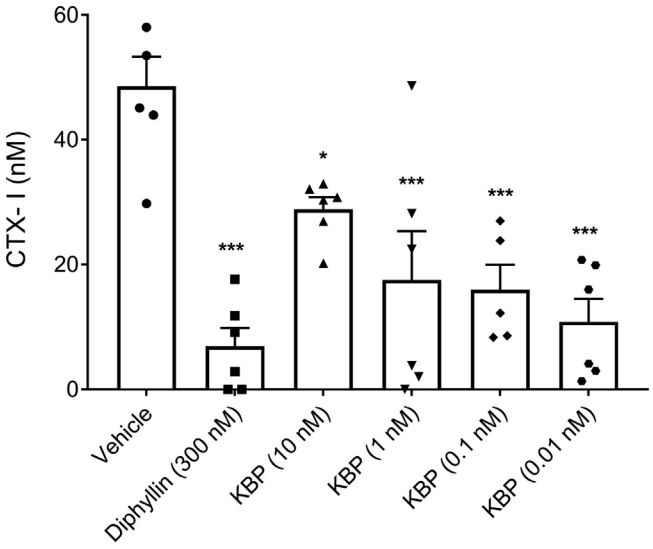

Fig. 5. KBP inhibits osteoclastic bone resorption *in vitro* inhibition. Resorption of type I collagen was assessed by measuring CTX-I. Data are presented as the mean ± SEM. Statistical significance between treated conditions and the vehicle is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

antiresorptive properties of KBP were further verified *in vitro*. OA-associated pain behavior was reduced by KBP according to two different pain behavioral models, the von Frey and acetone tests. The data described in this study suggest that DACRAs such as KBP preserve joint structure and reduce bone pain by inhibiting bone resorption and cartilage degradation, and therefore holds potential as a DMOAD with beneficial effects on both joint structure and pain.

Animal experimental arthritis studies of sCT have previously demonstrated reductions of cartilage and bone pathology[6]. In the present study, the administration of KBP significantly reduced the cartilage pathology, as indicated by the OARSI score. While the combination therapy also appeared to reduce the OARSI score, this was not statistically significant and did not provide added effects on top of the KBP which comes in contrast with the protective effect found in the joints of an inflammatory rat model of collagen induced-arthritis[23]. Our results indicate that treatment with KBP reduced the MNX-induced cartilage erosion and had a protective effect on joint health. Naproxen had little to no effect maybe due to the fact that the structural damage in this model was not inflammatory driven or it might have been implicated in a larger attenuation of pain perception than KBP, thus animals did not protect their joints[35]. Further experimentation of more dosing regimens of naproxen, preferably lower, should be tested as it might show its chondroprotective effects, especially combined with KBP.

To assess the effects of KBP specifically on cartilage degradation, we measured CTX-II which previously has been associated to the degree of cartilage degradation[36] and demonstrated a significant reduction within all groups that received KBP even though no significant elevation of CTX-II was observed in the MNX group. Similarly, KBP strongly inhibited CTX-I, but the MNX failed to induce a statistically significant increase. However the beneficial effects on bone structure of KBP combined with naproxen have been previously shown in an inflammatory arthritis model[23]. All of these reductions in CTX-I and CTX-II resulted in CTX-I and CTX-II levels that were substantially lower than the sham group, suggesting that the treatments' effects on the biomarkers were predominantly a result of inhibition of endogenous bone and cartilage turnover, and not directly in the target joint. Furthermore, any increases in the circulating biomarkers due to MNX surgery, combined with the localized nature of the lesion, are eventually obscured by the maturation of the rats[37]. *In vitro*, we demonstrated that KBP inhibits osteoclast-mediated resorption of type I collagen corresponding well to previous studies of sCT on bone resorption[38], although the dose-response indicated that at the higher doses receptor desensitization could occur, as previously seen in osteoclasts[39].

In our study, MNX induced clear pain-like behaviors according to the cold hypersensitivity and mechanical allodynia assessments, similarly to previous reports regarding MNX-induced pain[40]. As expected, naproxen monotherapy, as well as combination treatment, resulted in potent anti-nociceptive effects. The outcomes of naproxen treatment in pain assessment tests were similar to those of the sham animals, highlighting the anti-nociceptive action of NSAIDs[41]. Interestingly, KBP monotherapy reduced both mechanical allodynia and cold hypersensitivity. The anti-nociceptive effects provided by KBP mirrors a study which found that eel CT reduced cold hypersensitivity and mechanical allodynia[42]. sCT has been demonstrated to have analgesic in a clinical setting[43], which further supports that KBP may have anti-nociceptive and analgesic effects. However, while both mechanical allodynia and cold hypersensitivity tests gave clear results regarding MNX-induced pain, the burrowing test was unable to detect any effects of the MNX. Similarly, the unilateral monoiodoacetate (MIA) injection model does not result in significant effects on burrowing[44], but bilateral MIA injection does, suggesting that the burrowing test is not sensitive enough for single-joint arthritis in rats. Additionally, medial MNX does not seem to induce any significant differences in weight-bearing asymmetry.

It would be of great interest to replicate the findings of this study in MNX obese rats, as obesity is a major risk factor of OA[45] and KBP has beneficial effects upon reduction of fat mass[21]. The weight loss seen with KBP correlates with the decrease of weight observed in animal studies with sCT[46]. Future experimentation should consider testing different doses of KBP, as well as lower doses of naproxen, as this may provide more information regarding the dose-dependency and the combination of the therapies. It would be of great interest to replicate the study using male rats and see the effect of KBP on joint structure and nociception, as we know that DACRAs work well on metabolic studies using male animals[21,22]. Additionally, in order to reflect the clinical setting any potential DMOAD should be investigated within an intervention study. Despite these shortcomings, our study has generated valid and robust results through the use of multiple methods that corroborate each other. The advanced OARSI histopathology score uses quantitative and qualitative measures to evaluate cartilage pathology, and the results were highly reproducible and reliable with the use of two independent blinded observers. Furthermore, the use of multiple pain assessment techniques provide different information regarding the anti-nociceptive effects of the therapies, and clearly demonstrate the importance of combining appropriate experimental arthritis models with suitable pain assessment techniques to detect the effects of novel treatments.

In conclusion, we found that KBP, a novel DACRA, has chondroprotective and anti-nociceptive properties in a surgically induced model of OA. KBP is a promising DMOAD candidate and may overcome some of the difficulties found in treating patients with OA.

Author contributions

AK and AD performed experiments and analyzed the data. AK, HL, MAK, KVA, CST and KH contributed to the conception, study design and interpretation of the data. AK drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest

AK, MAK, KVA, CST, and KH are employees of Nordic Bioscience A/S which is a company involved in the discovery and development of biochemical biomarkers and novel therapeutic peptides, including KBPs. HL is a former employee of Nordic Bioscience A/S. KH, KVA and MAK hold patents on KBPs. MAK and KH hold stocks in Nordic Bioscience A/S. AD reports no conflicts of interest.

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Supplementary data

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