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Antinociceptive and chondroprotective effects of prolonged β-caryophyllene treatment in the animal model of osteoarthritis: Focus on tolerance development

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ABSTRACT

Osteoarthritis (OA) is a chronic joint disease in which cartilage degeneration leads to chronic pain. The endocannabinoid system has attracted attention as an emerging drug target for OA. However, the therapeutic potential of cannabinoids is limited by psychoactive side-effects related to CB1 activation and tolerance development for analgesic effects. β-Caryophyllene (BCP) is a low-efficacy natural agonist of CB2 and a common constituent of human diet with well-established anti-inflammatory properties. The results presented herein show the anti-nociceptive and chondroprotective potential of BCP in an animal model of OA induced by intra-articular injection of monoiodoacetate (MIA). Behavioural assessment included pressure application measurement and kinetic weight bearing tests. Histological assessment of cartilage degeneration was quantified using OARSI scoring. Experiments established the dose-response effects of BCP and pharmacological mechanisms of the antinociceptive action dependent on CB2 and opioid receptors. Chronic BCP treatment was able to hamper cartilage degeneration without producing tolerance for the analgesic effects. The data presented herein show that BCP is able to produce both acute and prolonged antinociceptive and chondroprotective effects. Together with the safety profile and legal status of BCP, these results indicate a novel and promising disease-modifying strategy for treating OA.

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1. Introduction

Osteoarthritis (OA) is a chronic joint disease in which cartilage degenerates and is one of the most common disorders causing chronic pain and disability among older adults (Allen and Golightly, 2015). OA has been recognized by the World Health Organization as a "priority disease" (report WHO/EDM/PAR/2004.7) and one of the top 5 healthcare costs in Europe (Cross et al., 2014). OA pathology is multifactorial, involving the remodelling of subchondral bone, synovial inflammation and loss of articular cartilage (Goldring, 2012). Current treatment is mostly based on symptomatic care using nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, naproxen or diclofenac, which are not able to stop or slow disease progression. Moreover, NSAIDs do not always provide adequate pain relief, and their use is restricted because of serious side effects, including bleeding, ulcers, stroke, and myocardial infarction (van Walsem et al., 2015).

As current treatment options in OA are very limited, OA patients would benefit greatly from some ability to self-manage their condition. One of the most influential lifestyle factors in health and diseases is diet. Recent evidence points to the roles of healthy dietary choices in arthritis management. For example, adherence to a Mediterranean diet is associated with a lower prevalence of OA (Veronese et al., 2017), whereas an anti-inflammatory diet containing foods rich in n–3 fatty acids, fibre, antioxidants, and probiotics has a positive impact on the Disease Activity Score in rheumatoid arthritis (Vadell et al., 2020). The aforementioned studies focused on the role of nutritional constituents of food such as vitamins or fatty acids; however, bioactive compounds such as alkaloids or terpenes should also be considered.

Research conducted by Gertsch's group led to the identification of β-Caryophyllene (BCP) as a dietary agonist of cannabinoid receptor type 2 (CB2) (Gertsch et al., 2008) with anti-inflammatory and analgesic potential (Klauke et al., 2014). BCP is found in large quantities in the essential oils of many different spice and food plants, such as oregano (*Origanum vulgare* L.), cinnamon (*Cinnamomum* spp.) and black pepper

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(*Piper nigrum* L.) (Jayaprakasha et al., 2003; Jirovetz et al., 2002; Mockute et al., 2001). CB2 is widely distributed on immune cells, where it is responsible for mediating cytokine release (Pertwee, 2009). Additionally, its expression has been shown in chondrocytes, bone and synovial tissue (Dunn et al., 2016; Ofek et al., 2006; Richardson et al., 2008). CB2 activation in the spinal cord and peripheral nervous system results in well-documented analgesic effects involving peripheral endorphin release (Ibrahim et al., 2005; Starowicz and Finn, 2017). In

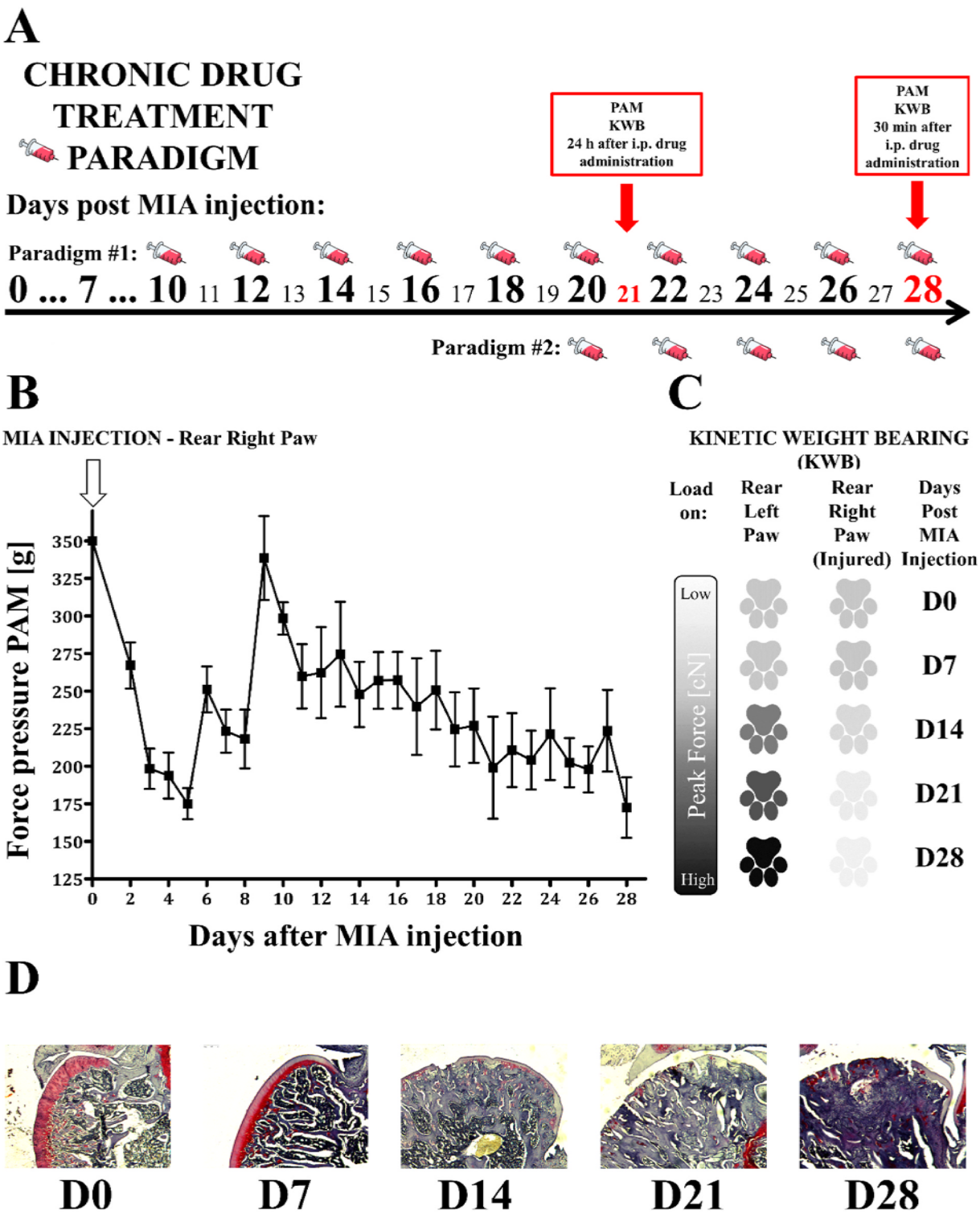

Fig. 1. Schematic representation of pharmacological chronic treatment paradigms. Drugs were administered i.p. every other day, starting on D10 or D20. The behavioural assessment was performed on D21 and D28 (Fig. 1A). Development of pain phenotype in the animal model of osteoarthritis was established in our previous studies (Fig. 1B and C; N = 8), for PAM details please see Malek et al., 2015, while for KWB details please see Bryk et al. (2021). Pictures below present samples of histologically stained cartilage undergoing degeneration following i.a. administration of MIA. Detailed histological data regarding MIA model of OA in available in publication by Bryk et al. (2020) (D0 group N = 4; D7 group N = 4; D14 group N = 3; D21 group N = 3; D28 group N = 6). Abbreviations: PAM – Pressure Application Measurement; KWB – Kinetic Weight Bearing; RL – Rear Left Paw; RR – Rear Right Paw (injured).

contrast to CB1 agonists, such as Δ^9-tetrahydrocannabinol, CB2 agonists do not produce psychoactive or rewarding side effects (Morales et al., 2016). Preclinical research has revealed a significant role of CB2 receptors in mediating the susceptibility to OA, as deletion of the CB2 receptor leads to more severe cartilage degeneration in a surgical model of OA (Sophocleous et al., 2015), while the overexpression of the CB2 receptor, attenuates joint pain manifestations in a mouse monoiodoacetate (MIA) model of OA (La Porta et al., 2013). Moreover, chronic treatment with the CB2-selective agonist HU308 reduces the severity of OA in the whole joint following surgical induction of OA (Sophocleous et al., 2015), possibly due to the reduction of the proteoglycan production by chondrocytes.

Previously, our group have revealed promising disease-modifying properties of synthetic CB2 agonists and their molecular underpinnings in OA but the analgesic effects of CB2 agonists declined with a prolonged use (Mlost et al., 2021). There are a few advantages of BCP over synthetic selective CB2 agonists. First, BCP is a naturally occurring compound that is very common in our diet, and it has been approved as a food additive, taste enhancer, and flavouring agent by the U.S. Food and Drug Administration and European agencies (Chicca et al., 2014). Second, BCP is a low-efficacy CB2 agonist, in contrast to high-efficacy synthetic CB2 agonists; therefore, it may be less likely to induce receptor desensitization/downregulation or consequential tolerance for analgesic effects. Additionally, BCP, as a natural compound, is most likely a polypharmacological agent (Francomano et al., 2019), in contrast to novel highly selective CB2 agonists. Targeting multiple biological sites with low efficacy may result in synergistic anti-inflammatory and analgesic effects; for example, BCP may simultaneously activate CB2 and peroxisome proliferator-activated receptors (PPAR) (Wu et al., 2014), which are involved in immunoregulation and inflammation control.

The aim of the present study was to elucidate the analgesic and chondroprotective effects of BCP. Drugs were administered with various treatment schedules, including those appropriate for acute dose response studies and two chronic treatment paradigms (Fig. 1); starting from day 10 after MIA injection (D10); or starting from day 20 after MIA injection (D20). Doses were selected based on literature with starting dose of 10 mg/kg (Al Mansouri et al., 2014; Alberti et al., 2017; Javed et al., 2016; Varga et al., 2018). For comparison, 1 g of black pepper allows for extraction of 22 mg of essential oil that can contain 63% of BCP, yielding 14 mg of BCP in 1 g of black pepper (Orav et al., 2004). The average daily intake of spices (including black pepper, cloves, oregano and cinnamon, all of which containing substantial amounts of BCP) in USA is 4 g, however in India, the average amount of spices used per dish is 10 g and up to 27 g in Thailand (Bhathal et al., 2020). Assuming consumption of 4 g of black pepper containing 14 mg of BCP, we could presume daily intake of 54 mg BCP per average 60 kg person, giving a dose around 1 mg/kg "A simple practice guide for dose conversion between animals and human" (Nair and Jacob, 2016) suggests equivalent dose for rat as human dose multiplied by 6.2, based on body surface area, while this do not include physiological differences that should further increase the dose. We hypothesize that physiologically relevant doses of BCP possess antinociceptive and disease-modifying potential surpassing the effects of synthetic CB2 full agonists. Behavioural effects were assessed with pressure application measurement (PAM) and a kinetic weight bearing (KWB) instrument allowing for non-invasive gait analysis of freely moving animals at D21 and D28 after MIA injection. We have also established a mechanism of BCP action through its coadministration with distinct receptor antagonists.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Hamburg, Germany) around 55th postnatal day, initially weighing 225–250 g were used for all experiments. The animals were housed as five rats per cage under a standard 12-h/12-h light/dark cycle with food and water available ad libitum. Animals were housed in conventional cages on aspen wood bedding without environmental enrichment. All experiments were approved by the Local Bioethics Committee of the Institute of Pharmacology (Cracow, Poland, approval number 1130/2014 and 125/2018). All pharmacological experiments (including treatment and behavioural assays) were performed in the morning hours (08:00–12:00). Tissue dissection was performed at the end of the experiments. Care was taken to implement the "3 Rs" rule (replacement, reduction and refinement) to reduce the number of animals used and their suffering during the experiments. Total number of 92 animals was used in the present study.

2.2. Drugs and reagents

BCP was kindly provided by Prof. Jurg Gertsch (Bern, Switzerland). AM630, Naloxone and GW6471 were obtained from Tocris Bioscience (Bristol, UK). MIA, dimethyl sulfoxide (DMSO) and Kolliphor EL were obtained from Sigma-Aldrich (Poznan, Poland). GW833972A was obtained from Carbosynth (Compton, United Kingdom). BCP alone or in combination with antagonists was dissolved in a vehicle solution containing 5% DMSO, 5% Kolliphor® EL and 5% ethanol in 0.9% saline. MIA was dissolved in 0.9% saline. Total administration volume for i.p. administration was 2 ml/kg.

2.3. OA induction

Animals were deeply anaesthetized with 5% isoflurane in 100% O_2 (3.5 L/min) until the flexor withdrawal reflex was abolished. The skin overlying the rear right knee joint was shaved and swabbed with 100% ethanol. A 27-gauge needle was introduced into the joint cavity through the patellar ligament, and 1 mg of MIA, which is an irreversible GADPH inhibitor, diluted in 50 μL of 0.9% saline was injected into the joint (intra-articular, i.a.) to induce OA-like lesions. MIA inhibits chondrocyte glycolysis and produces cartilage degeneration and subchondral bone alterations. The MIA model reproduces osteoarthritis-like histological lesions and functional impairment similar to that observed in human disease (Guingamp et al., 1997). Sham-treated animals received i.a. administration of 50 μL of 0.9% saline into the right rear knee joint. The age and weight of the animals were selected to allow comfortable access for i.a. injection, whereas only male rats were selected for the experiment to minimize variability related to the estrous cycle throughout the course of the chronic treatment paradigm. The rats were sacrificed 28 days after MIA injection (D28) as a humane end-point as the cartilage was no longer able to further degenerate, and sufficient time was available to study the effects of the prolonged pharmacological treatment. MIA model of osteoarthritis have been chosen due to progressive degeneration of cartilage and subchondral bone, allowing us to study disease-modifying properties of tested drugs. 1 mg of MIA was selected based on our previous findings, which revealed full cartilage degeneration in the given dose (Bryk et al., 2020) and development of pain phenotype that was indistinguishable from the higher dose of 3 mg MIA (Bryk et al., 2021).

2.4. Treatment paradigms

Drugs were administered i.p. in three treatment regimens: for acute and two chronic schemes. Time points for acute drug testing were selected based on previous studies (Malek et al., 2015), which showed development of a severe pain phenotype and cartilage destruction after the 21st day (D21) following OA induction, which is why acute treatment was performed on D21 to establish dose-response effects. Acute antinociceptive effects of BCP were measured either in time-course with PAM or 30 min after i.p. drug administration with KWB. Two chronic treatment paradigms were used: #1) a long-term treatment starting on the 10th Day (D10) and administered every other day (for a total of 10

drug injections) aiming at establishing the disease-modifying potential of preventive treatment when the cartilage is not yet fully degenerated, and #2) a short-term treatment starting on day 20 (D20) and continued every other second day (total of 5 drug injections) to reflect the clinical situation in which the patient seeks medical help when the pain becomes significant. Vehicles were administered starting on either D10 or D20. A schematic representation of the pharmacological treatment paradigms is shown in Fig. 1. In chronic treatment paradigm, at D21 drugs were administered 24 h prior to behavioural testing, whereas at D28 drugs were administered 30 min prior to behavioural testing. Animals were immediately returned into their home cages after treatment. The experimenters performing the behavioural tests were blinded to the treatments, and the rats were randomly assigned to each treatment group.

2.5. The pressure application measurement

The PAM device (Ugo Basile, Italy) was used for the assessment of joint hyperalgesia. A quantifiable force was applied for direct stimulation of the joint, and the automatic readout of the response was recorded. The animals were held lightly, and the operator placed a thumb with a mounted force transducer unit on one side of the animal's knee joint and a forefinger on the other. A gradually increasing squeeze force was applied across the joint at a rate of approximately 30 g/s with a maximum test duration of 15 s or applied 500 g force. Using calibrated instrumentation, the force in grams applied was displayed on a digital screen and recorded. The test end-point was determined when the animal withdrew its limb or showed any behavioural signs of discomfort or distress, such as freezing of whisker movement, wriggling or vocalizing. The peak gram force (gf) applied immediately before the limb base unit recorded withdrawal was designated as the limb withdrawal threshold (LWT), and the mean LWTs were calculated. The baseline measurements were obtained 30 min before i.p. drug administration, whereas acute drug effects were assessed from 30 to 240 min following treatment. To compare the acute nociceptive effects in the dose-response experiment, each LWT was calculated as the maximum possible effect - %MPE = [(test LWT–baseline LWT/maximum possible LWT – baseline LWT) x 100], which allowed us to minimize individual differences as the results were normalised to baseline measurements, i.e. increase in %MPE was proportional to baseline pain threshold of the animal allowing us to more precisely estimate acute antinociceptive effects of the given dose and make comparison.

2.6. Kinetic weight bearing

To characterize pain behaviour in the MIA model, we used kinetic weight bearing (KWB), a novel instrument developed by Bioseb (France). Sensors placed on the ground measure weight borne by each individual paw during the walking sequence of a freely moving animal, while a built-in camera detects body shape and the centre of gravity of the animal, which is then used for further analysis. The rats were trained for a week to move through a corridor (50 × 130 cm) before the actual experiment. Measurements were made on D21 and D28 following MIA administration, either 24 h or 30 min after drug administration, depending on treatment paradigm (details in figure caption). Data collection was terminated when 5 validated runs were obtained or after 5 min of acquisition. All collected runs for each animal were then averaged for further statistical analysis. If the animal did not run during this time window, it was excluded from further analysis; thus, the number of samples from KWB may vary. All the recorded data were then validated and refined by a blinded observer, who carefully examined video-recordings and verified that animal was not stopping during the run or that detected signal was ascribed to proper paw.

2.7. Histological assessment

Histological analysis was performed on sagittal sections of the medial femoral condyle. Femoral condyle has been chosen based on our XMT results which showed no changes in tibia morphology (Mlost et al., 2021), however medial part was chosen based on results by Sophocleous et al. (2015) (Sophocleous et al., 2015), which revealed higher OARSI scoring in medial compartment of knee joints in Cnr2 / mice. Samples were decalcified in 10% formic acid for 10 days and then processed through increasing concentrations of sucrose, embedded in Leica OTC Tissue Freezing Medium and frozen in liquid nitrogen. A Leica CM1860 cryostat was used to cut 8–12 μm coronal sections through the entire cartilage sample at 45 μm intervals, yielding 10–13 different levels of sample. The sections were then stained with safranin-O and haematoxylin according to standard techniques. Histological evaluation of the severity of the osteoarthritis was performed by an observer (AS) blinded to the pharmacological treatment according to the Osteoarthritis Research Society International (OARSI) scoring system.

2.8. Statistical analysis

The analysis was performed using Prism V.5 (GraphPad Software). Data was first examined for gaussian distribution by Shapiro-Wilk normality test and the equality of variances by Brown–Forsythe test. All results were normally distributed and had equal variances. Changes in limb withdrawal threshold throughout the time course were analysed using two-way analyses of variance with a Bonferroni post hoc test limited to the comparison of the treatment groups vs the vehicle group. The kinetic weight bearing data were analysed using a one-way analysis of variance with Bonferroni multiple comparison test for rear paws in the respective treatment groups. Histological data were analysed using a one-way analysis of variance with Dunnett's post hoc test for comparison of the treatment effects compared to that of the vehicle group. The number of animals used in specific experiments is denoted under the graphs. The data were considered significant only when P 0.05. All data analyses were performed under blinded conditions.

3. Results

3.1. Acute antinociceptive effect of BCP in dose-response studies on day 21

Based on a literature search, three doses of BCP (10, 25 and 50 mg/kg) were used for the dose response experiments (Al Mansouri et al., 2014; Alberti et al., 2017; Javed et al., 2016; Varga et al., 2018). When testing knee hypersensitivity with PAM, two-way ANOVA test revealed significant effect of treatment on withdrawal threshold F (3, 96) = 15, 29; p 0,001. Post-hoc analysis revealed an increase in the paw withdrawal threshold 30 min after BCP treatment at doses of 25 mg/kg (p = 0,0028) and 50 mg/kg (p = 0,0014) in comparison to vehicle (Fig. 2A). The effects of 50 mg/kg of BCP persisted for 60 min post. i.p. drug administration (p = 0,0284), whereas the effects of 25 mg/kg BCP diminished at subsequent time points (Fig. 2A). No difference was observed between the dose of 10 mg/kg and the vehicle at any of the time points (Fig. 2A). For KWB, ANOVA did not reveal significant changes between rear left and rear right (injured) paw in all treatment groups (F(7, 26) = 2184; P = 0,0695) but we observed a significant discrepancy in peak force administered to the left and right hind paws 1 h following vehicle treatment in post-hoc analysis (p = 0,0085) (Fig. 2B), suggesting that weight bearing was restored 1 h after BCP treatment at all tested doses (Fig. 2B). However, significant effect of treatment on weight bearing was observed when BCP was combined with antagonists (F(9, 40) = 5919; P 0,0001). Weight bearing was impaired when BCP was co-administered with the CB2 antagonist AM630 (p = 0,0019) or the opioid receptor antagonist naloxone (p = 0, 0019) (Fig. 2C). On the other hand, the PPARα antagonist GW6471 did

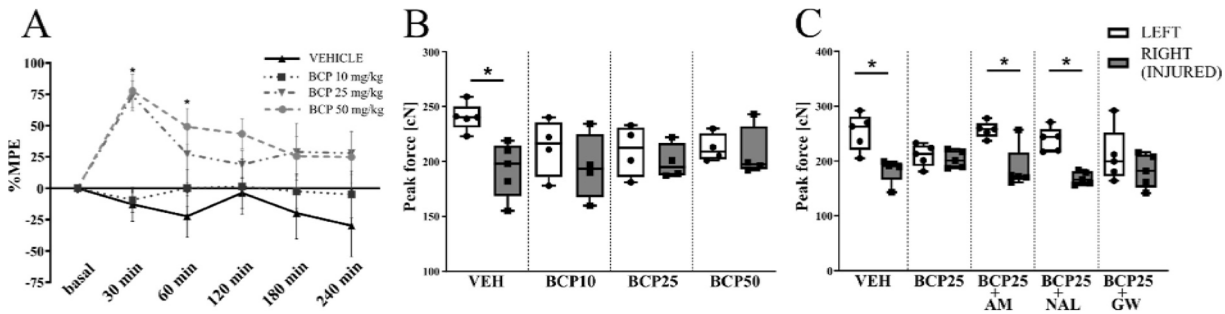

Fig. 2. Behavioural assessment of acute antinociceptive potential of BCP and mechanism of action. Dose-response relationship for the antinociceptive effects of BCP on knee joint hypersensitivity according to the pressure application measurement (PAM) test (Fig. 2A) and in kinetic weight bearing (KWB) (Fig. 2B). Fig. 2C presents the effects of BCP cotreatment (25 mg/kg) with either the specific CB2 antagonist AM630 (AM, 3 mg/kg), the non-specific opioid receptor antagonist naloxone (NAL, 1 mg/kg) or the PPARα antagonist GW6471 (GW, 1 mg/kg). The PAM results are presented as the means of the maximum possible effect percentage ± SEM from a group in which N = 5 (Fig. 2A). The KWB results are presented as individual datapoints. For dose-response assessment in KWB, VEH groups N = 5; BCP 10 mg/kg, 25 mg/kg and 50 mg/kg groups N = 4 (Fig. 2B), whereas for antagonist assessment in KWB, for all groups N = 5 (Fig. 2C). Statistical analysis was performed using two-way analysis of variance followed by Bonferroni post hoc test. Values of P 0.05 were considered significant. In Fig. 2A, - * denotes a significant difference between the VEH and pharmacological treatment groups at the same time point, whereas in Fig. 2B–C, - * denotes a significant difference at P 0.05 between paws within each treatment group.

not affect BCP action (p = 0,6630) (Fig. 2C).

3.2. Chronic antinociceptive effects of BCP

We observed significant asymmetry in the peak force parameter between the left and right hind paws in vehicle-treated OA rats D21 (p = 0,0072) and D28 (P = 0,0008) following MIA administration (Fig. 3A–B). BCP treatment starting on D10 (paradigm #1) restored peak force asymmetry by D21 at both a subthreshold dose of 10 mg/kg (p > 0,9999) and a regular dose of 25 mg/kg (p = 0,5381) (Fig. 3A). The effects of both the 10 and 25 mg/kg doses of BCP persisted to D28, suggesting no tolerance development for the analgesic effects for both of the effective doses (p > 0,9999 and p = 0,1084, respectively) (Fig. 3B) in the long-term paradigm #1. In short-term treatment paradigm #2, BCP administration on D20 (25 mg/kg) elicited a prolonged analgesic effect as asymmetry was restored by D21 (p = 0,4471; assessed 24 h after a single BCP treatment, Fig. 3A). Similarly, no tolerance for the analgesic effect was observed after 25 mg of BCP in long paradigm #2, as there was no significant discrepancy in peak force between hind paws on D28 (p > 0,9999) (Fig. 3B). Chronic coadministration of either AM630 or Naloxone in paradigm #2 blocked the analgesic action of BCP on both D21 (p = 0,0165 and p = 0,0003, respectively) and D28 (p = 0,0147 and p = 0,0463, respectively) (Fig. 3A–B).

3.3. Chondroprotective effects of BCP

ANOVA analysis revealed significant effect of treatment upon OARSI scoring (F(5, 12) = 3,33; P = 0,0407). Histological evaluation of knee joint samples revealed marked cartilage deterioration in the vehicle group (Fig. 4A), resulting in a mean OARSI score of approximately 20 (Fig. 4G). A significant decrease in OARSI scores was observed following BCP treatment #1 starting on D10 (Fig. 4G) at both tested doses – 10 mg/kg (Figs. 4B) and 25 mg/kg (Fig. 4C) – compared with score in the vehicle group (p = 0,0105 and p = 0,0465, respectively). In shorter paradigm #2, 25 mg/kg BCP administered starting on D20 failed to improve OARSI scoring by D28 (p = 0,1151) (Fig. 4D and G), similarly we did not observe improvement of OARSI scoring with combinatorial treatment with BCP 25 mg/kg with 3 mg/kg of AM630 from D20 (p = 0,3107) (Fig. 4E and G). Moreover, GW833972A, which is β-arrestin biased full agonist of CB2 receptor, failed to significantly improve OARSI scoring when administered from D10 (p = 0,3605).

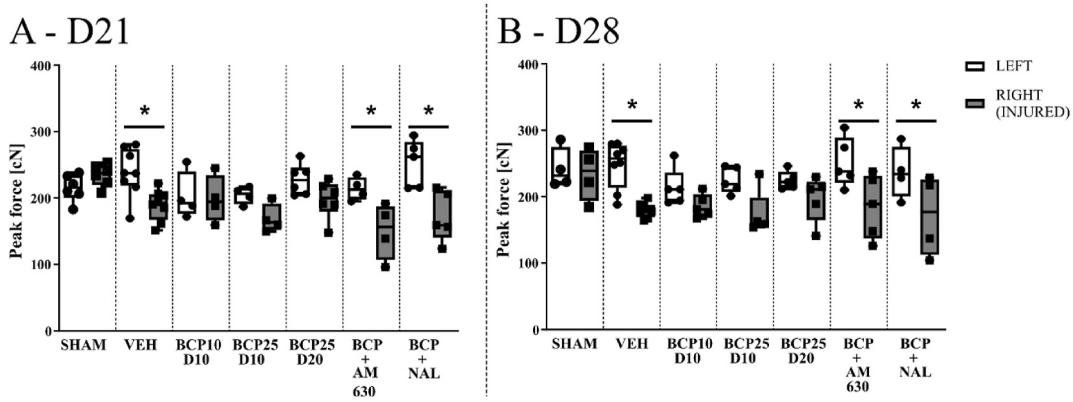

Fig. 3. Gait analysis in osteoarthritic rats in a chronic treatment schedule. BCP was administered i.p. every other day in two treatment paradigms – #1) longer, starting on D10 (at dose 10 mg/kg (BCP10 D10) or 25 mg/kg (BCP25 D10) and #2) shorter, starting on D20 (at dose 25 mg/kg (BCP25 D20). BCP at dose 25 mg/kg was coadministered from D20 with antagonists; AM630 at dose 3 mg/kg (BCP + AM) or naloxone at dose 1 mg/kg (BCP + NAL). Experiments were performed for 24 h (Fig. 3A - D21) or 30 min (Fig. 3B - D28) post drug i.p. administration. Individual datapoints are presented on scatterplot. At D21, for SHAM group N = 6; VEH group N = 8; BCP10 D10 group N = 4; BCP25 D10 group N = 4; BCP25 D20 group N = 6; BCP + AM630 group N = 4; BCP + NAL group N = 5 (Fig. 3A). At D28, for SHAM group N = 4; VEH group N = 8; BCP10 D10 group N = 5; BCP25 D10 group N = 5; BCP25 D20 group N = 5; BCP + AM630 group N = 5; BCP + NAL group N = 4 (Fig. 3B). Statistical analysis was performed using one-way analysis of variance followed by the Bonferroni post hoc test for the respective left and right rear paws. * denotes significance with P 0.05 between paws within each treatment group.

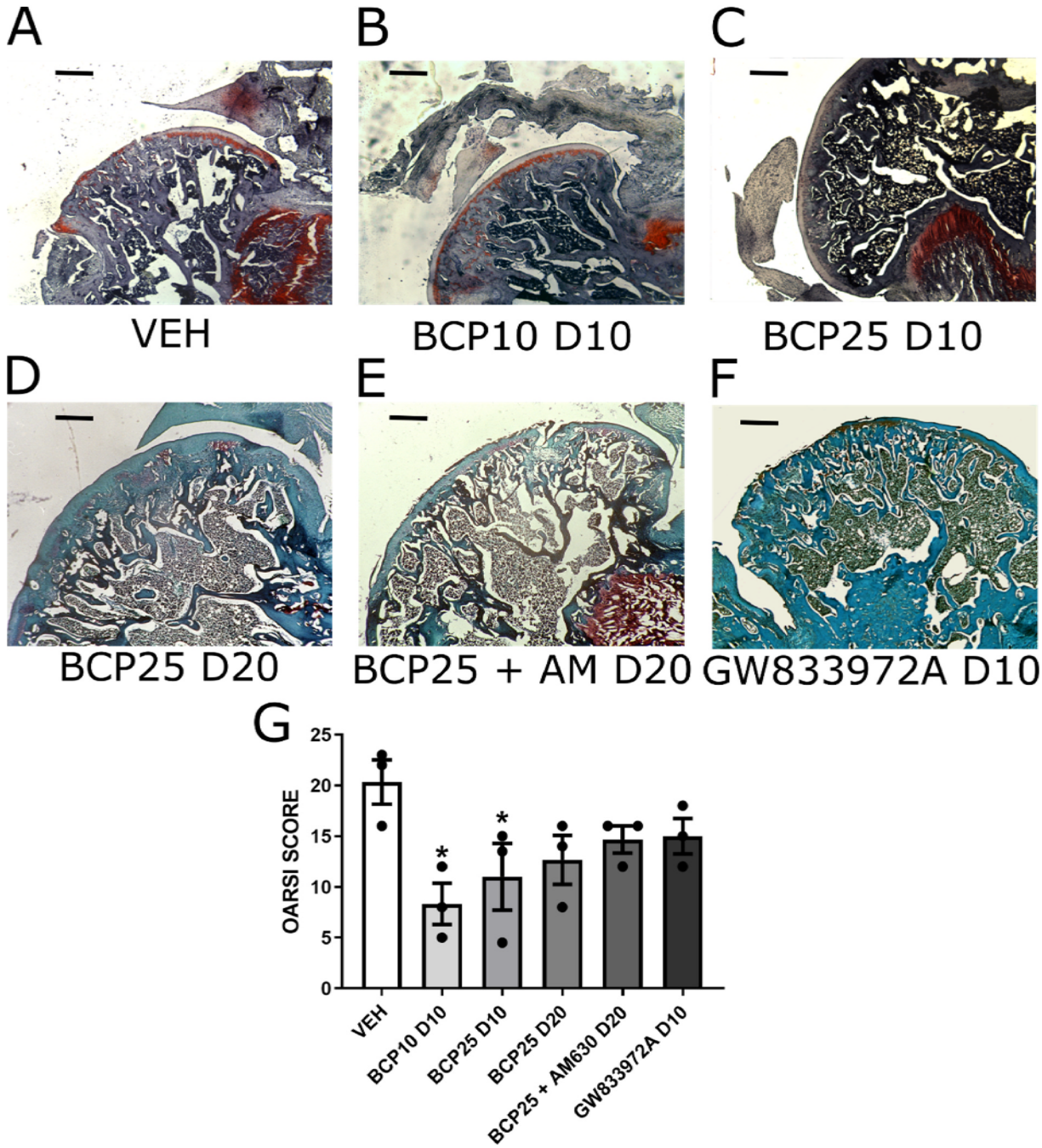

Fig. 4. Histological evaluation of rat knee cartilage following chronic pharmacological treatment with CB2 ligands. Drugs were administered i.p. every other day starting either on D10 or D20. Samples were collected on D28. Each panel shows a representative sample from: A) the vehicle group; B) the group treated with 10 mg/kg of BCP starting on D10; C) the group treated with 25 mg/kg of BCP starting on D10; D) the group treated with 25 mg/kg of BCP starting D20; E) the group treated with 25 mg/kg of BCP and 3 mg/kg of AM630 starting on D20; G) Scatterplot representation of the OARSI scores from abovementioned groups are presented in bars as the means ± SEM from a group of N = 3 for all groups (VEH N = 3; BCP 10 D10 N = 3; BCP25 D10 N = 3; BCP25 + AM630 D20 N = 3; GW833972A N = 3). Black scale bar represents 500 μm. Statistical analysis was performed using one-way analysis of variance followed by Dunnett's post hoc test. * denotes significance with P 0.05 vs. vehicle group.

4. Discussion

Up to date, several studies have already shown the antinociceptive, anti-inflammatory and even anti-arthritic properties of BCP (Alberti et al., 2017; Ghelardini et al., 2001; Irrera et al., 2019; Klauke et al., 2014; Segat et al., 2017) and essential oils containing BCP in large concentrations (Machado et al., 2018). The data presented herein show that BCP in physiologically relevant and dietary available doses is able to produce both acute and prolonged antinociceptive effects and decrease cartilage degeneration in the MIA model of osteoarthritis. BCP doses lower than 25 mg/kg, were ineffective in eliciting acute antinociception; however, in the KWB test, even 10 mg/kg of BCP was able to restore impaired weight bearing, which is possibly mediated by the anti-inflammatory action. In chronic treatment paradigm, both low and moderate doses of BCP were able to restore impaired weight bearing throughout the course of the experiment and hamper cartilage degeneration.

These data are important in two contexts. First, we observed

significant therapeutic effects in the long-term treatment paradigm for the dose that was not fully effective following acute administration. Second, even a higher dose of BCP did not produce tolerance for the analgesic effects during the prolonged treatment schedule, whereas in comparison, fully effective doses of other high-efficacy CB2 agonists failed to maintain analgesia throughout the course of the experiments in both treatment regimens (Mlost et al., 2021). The antinociceptive effects of 25 mg/kg BCP were blocked by the CB2 antagonist AM630 and the opioid receptor antagonist naloxone but not by the PPARα antagonist GW6471, which suggests direct antinociceptive mechanism of action. Previous studies have already described CB2-mediated β-endorphin release from keratinocytes produces antinociception (Ibrahim et al., 2005), however the exact place of endorphin release in the present study requires further investigation.

In addition to antinociceptive effects of BCP, we have also revealed its disease-modifying properties but only when BCP was administered from D10 but not from D20, which implies the importance of preventive treatment. Interestingly, we did not observe chondroprotective effects when the selective CB2 agonist GW833972A was used starting on D10. The discrepancy in BCP and GW833972A action may be explained by 1) differences in selectivity where BCP is a non-selective natural compound and GW833972A is most likely targeting CB2 exclusively, while the chondroprotective effects of BCP could be mediated through PPARα activation as well (Shirinsky and Shirinsky, 2014; Zhou et al., 2019); 2) BCP is a low-efficacy agonist that is less likely to promote CB2 desensitization/downregulation, whereas GW833972A is not only a highly efficacious agonist but also preferentially interacts with the β-arrestin pathway, which promotes CB2 desensitization (Dhopeshwarkar and Mackie, 2016) and abrogates the therapeutic potential of CB2 agonists in OA (Mlost et al., 2021). Moreover, our previous studies have revealed the molecular underpinnings for the improvement of subchondral bone morphology and reversal of MIA-related disturbances in expression of inflammatory mediators and extracellular matrix components in the cartilage by the CB2 agonists (Mlost et al., 2021). Together these results imply that 1) prolonged treatment from very early stages of OA is necessary to hamper disease progression; 2) CB2 agonists are able to both counteract the OA pain and cartilage degeneration; 3) due to complex mechanisms underlying OA pathogenesis, natural, low-efficacy and polypharmacological compounds such as BCP are better treatment strategies for OA than selective and highly efficacious compounds.

It is also important to acknowledge the limitations of the study. First of all, sample size of the presented results is relatively small. However, obtained results were homogenous, which made it possible to obtain statistically significant conclusions. Moreover, we were able to replicate the antinociceptive effects of BCP in three independent experiments, first in the dose-response study (Fig. 2A–B), then in the antagonist study (Fig. 2C) and finally at distinct timepoints after chronic treatment (Fig. 3). In addition, results presented herein are both in line and complementary with our previous studies with synthetic, specific CB2 agonists in the same research paradigm (Mlost et al., 2021). In addition, to strong evidence of antinociceptive effects of BCP (Aly et al., 2020; Katsuyama et al., 2013; Klauke et al., 2014; Segat et al., 2017) and the well documented role of CB2 in OA pathophysiology (La Porta et al., 2013; Mlost et al., 2021; Sophocleous et al., 2015), we believe that presented findings are solid and reliable. On the other hand, it should be noted that presented evidence was obtained with chemically-induced animal model of OA. Even though, MIA model of OA is acclaimed for reproducible pharmacological studies upon the antinociceptive effects of drugs, mechanical and biochemical disturbances underlying human OA are different from MIA-induced lesions. Thus, human studies with BCP are needed to support its clinical effectiveness as nutraceutical agent with disease-modifying properties.

In conclusion, our results present superior benefits of prolonged treatment with subthreshold doses of low-efficacy and non-selective natural compound BCP over short-term treatments with higher doses of BCP or even the same or short-term treatment strategy with high-efficacy CB2 agonists (Mlost et al., 2021). Therefore, it is important to a) acknowledge the importance of successful diagnosis at the early stages of the disease and the possible beneficial effects of non-invasive interventions, such as dietary changes or supplements, and b) consider the advantages of low-efficacy agonists over high-efficacy agonists for chronic pain treatment. Taken together and considering an abundance of literature, BCP availability, safety profile and legal status our study indicate that it is a promising analgesic and disease-modifying add-on strategy for treating OA.

Author contributions

Conceptualization, J.M., P.K., and K.S.; investigation, J.M., M.K., P.K.; writing—original draft preparation, J.M.; writing—review and editing, K.S. and P.K.; visualization, J.M.; supervision, K.S.; project administration, K.S.; funding acquisition, K.S. All authors have read and agreed to the published version of the manuscript.";

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Institutional review board statement

All experiments were approved by the Local Bioethics Committee of the Institute of Pharmacology (Cracow, Poland, approval number 1130/2014 and 125/2018).

Data availability statement

The data presented in this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare no conflict of interest.

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