Knee osteoarthritis pain following medial meniscectomy in the nonhuman primate
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SUMMARY
Objective: A number of promising compounds developed for osteoarthritic pain have failed to demonstrate clinical efficacy. To enhance preclinical translational research for osteoarthritis, a model of knee osteoarthritis pain was developed in Macaca fascicularis and the effects of two distinct pharmacological classes of drugs were tested on pain-related behavior.

Design: Behavioral assessments were developed specifically for the macaque. Baseline knee pressure threshold and weight bearing were assessed prior to a unilateral medial meniscectomy (MMx). Fifteen days following MMx, macaques underwent a once daily exercise regimen for 36 days. Sixty-seven days following MMx, macaques were assigned to one of three treatment groups (n = 3/group), either non-steroidal anti-inflammatory drug (NSAID) diclofenac, NK1 receptor antagonist aprepitant or vehicle, and treated for 5 days. Animals were tested 3–4 h after p.o. dosing and testing was performed blinded. Treatment utilized a crossover design—each animal received all treatments—and a 9-day washout period was utilized between treatments.

Results: Vehicle-treated macaques consistently demonstrated decreased ipsilateral pressure threshold (“hyperalgesia”) and decreased weight bearing. While diclofenac increased weight bearing and pressure threshold, full attenuation of pain was not obtained. No significant improvement of either knee pressure or weight bearing was observed with aprepitant.

Conclusions: Unilateral MMx in the macaque evoked pain-related behaviors and knee joint pathology reminiscent of osteoarthritis. The behavioral endpoints were sensitive to NSAID treatment but not sensitive to NK1 receptor block, which parallel clinical findings. The current macaque osteoarthritis model could be used to test potential treatments for osteoarthritis pain.

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Introduction

Knee osteoarthritis is characterized by pain and diminished knee functioning. While osteoarthritis is usually associated with aging, acute injury and surgery to knee intraarticular tissues and athletic activity that stress the knee joints also lead to an osteoarthritic state. Osteoarthritis has been characterized as a disease that involves the entire joint, not only the articular cartilage but subchondral bone, synovium, muscles and other periarticular tissues as well. Since there are currently no drugs that moderate the progression of osteoarthritis, treatment primarily focuses on managing pain to improve functioning.

Knee osteoarthritis models in rodents suggest numerous potential mechanisms mediate osteoarthritic pain. For example, a number of pro-inflammatory mediators and pronociceptive neuropeptides, such as substance P, have been found to be increased within the synovium and peripheral nerves that innervate the affected knee joint. Thus, blocking inflammatory processes, such as blocking the generation of prostaglandins by the cyclooxygenase (COX) enzyme, leads to significant albeit temporary pain relief. While non-steroidal anti-inflammatory drugs (NSAIDs) that inhibit the COX enzyme relieve pain such that osteoarthritis patients may perform daily tasks, prolonged use of NSAID could lead to gastrointestinal ulceration and other complications. Thus, in addition to safer drugs, drugs that show efficacy in a wide range of osteoarthritis patients are needed.

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Despite numerous mechanistic insights obtained from preclinical studies, turning these findings into useful treatments is a significant challenge for translational research\(^5\). A part of the problem could be that rodent-based models incompletely recapitulate the human pain state\(^6\). Also, the function of pain-related targets and their relevance to pain processing appears to greatly differ between rodents and humans\(^7\). Thus, one way of reducing uncertainty regarding clinical efficacy of novel therapeutics is to develop preclinical disease models in a species that is phylogenetically close to humans\(^8\)-\(^10\).

The current study developed a nonhuman primate (NHP) model of knee osteoarthritis following a medial meniscectomy (MMx) and methods to quantify pain-related behavior. Robust knee osteoarthritis pathology has been observed in aged Macaca fascicularis and in grivet monkeys (Chlorocebus aethiops) following MMx but pain-related behaviors in NHP with osteoarthritis have yet to be quantified\(^11\)-\(^13\). An important consideration in whether or not a model mirrors the clinical state is its sensitivity, or lack of sensitivity, to clinical therapeutics (or pharmacological isomorphism)\(^14\). Diclofenac is the most commonly prescribed NSAID and is frequently used in osteoarthritis clinical trials as a comparator drug\(^15\). The substance P (or neurokinin 1; NK1) receptor appears to have a prominent role in maintaining pain in the arthritic state and NK1 receptor antagonists demonstrated significant antinociception in rodent osteoarthritis models\(^15\),\(^16\). Thus, the efficacy of aprepitant, a clinically available NK1 receptor antagonist with excellent brain penetration, on pain-related behavior was compared to that of diclofenac in the current macaque model\(^17\). The primary outcome measures were knee pressure threshold and weight bearing\(^18\),\(^19\). To minimize the number of animals used in the current study, a three-treatment period crossover design was utilized, wherein all of the animals received all three treatments (diclofenac, aprepitant and vehicle).

**Method**

**Animals**

Female, young adult (about 8 years of age), purpose-bred cynomolgus macaques (M. fascicularis) were purchased from Shin Nippon Biochemical Laboratories (\(n = 9\); Kagoshima, Japan) and were between 2.4 and 5.9 kg at the beginning of the study.

This study was conducted in compliance with the relevant Japanese laws and guidelines concerning the use of experimental animals and adhered to principles stated in the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Research Council, 2011. All study procedures were reviewed and approved by the Hamamatsu Pharma Research, Inc. (HPR) Animal Care and Use Committee. Macaques were housed in individual stainless steel cages in a dedicated primate unit where room temperature and humidity were continuously monitored. Although individually housed, animals maintained auditory, visual and olfactory contact with neighboring conspecifics and were provided with manipulanda (e.g., metal mirrors). Animals were fed standard NHP diet (Oriental Yeast Co., Ltd., Chiba, Japan) and water was available ad libitum. In addition, animals received supplementary fresh fruits, vegetables and treats. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

**Exercise regimen**

A pilot study showed that activity, or exercise, is needed to evoke pain-related symptoms following MMx. In this pilot study (\(n = 3\)), knee pressure and weight bearing were measured once a week for 4 weeks without an exercise routine following MMx. Neither ipsilateral weight bearing nor decreased knee pressure was observed in these animals. In a second pilot study, \(n = 6\) animals underwent a 35-day exercise routine beginning 10 days after MMx. Decreased ipsilateral knee pressure was observed 35 days following MMx and decreased ipsilateral weight bearing was observed beginning 21 days after MMx. Based on the second pilot study, the current study utilized a 5 day per week, 36 day exercise period. Interestingly, such a dependence on an exercise routine to evoke both osteoarthritis symptoms and pathology following MMx surgery has been observed in large animals as well as rats\(^20\),\(^21\).

In the current study, before MMx surgery, macaques were trained for 2 weeks, 5 days per week, to jump from cage to cage, a distance of 2 m. Macaques were trained to jump 50 times (one round trip was one jump). Exercise was halted for animals that demonstrated hesitation or fatigue and animals were rested for about 5 min before continuing exercise. Macaques underwent the exercise regimen beginning 15 days after MMx and for 5 days in between testing cycles (Fig. 1(A)).

**Training and behavioral assessment**

Prior to use in experiments, macaques were habituated to restraint in a monkey chair and to a “monkey walker,” a modified monkey chair that allows the animal to stand and freely ambulate. Before MMx, body weight, knee diameter, knee skin temperature, weight bearing and knee pressure threshold were measured. (For knee skin temperature and knee diameter measurement methods, see Supplementary Information.)

Weight bearing was measured while the animal was restrained in a monkey walker and knee pressure threshold was measured while the animal was in a monkey chair. The sequence of behavioral testing before and after MMx surgery was weight bearing followed by knee pressure threshold.

**Weight bearing**

The force (kg) exerted by the ipsilateral and contralateral legs was measured using two weight scales (Tanita Co., Tokyo, Japan), in a manner similar to that used for rodents\(^22\). The mean of three measurements of each leg at each time point was calculated and weight bearing was expressed as a percent (%) of the ipsilateral leg compared to total weight bearing of the ipsilateral and contralateral legs:

\[
\text{Weight bearing} \% = \frac{\text{weight on the ipsilateral leg} - \text{weight on the contralateral leg}}{\text{weight on the ipsilateral leg}} \times 100
\]

In uninjured macaques, weight bearing of each leg was 50%.

**Knee pressure threshold**

The current method used to assess joint responsiveness to pressure in macaques was based in part on the four-point Ritchie index\(^10\). An observer assigns a score based on the patient’s facial response to pressure applied to a joint. For example, a score of 2 is assigned if the patient “complained of pain and winced” and a score of 3 is assigned if the “patient complained of pain, winced and withdrew the joint.” Facial expression is used clinically to assess pain states in patients that are unable to verbally respond (e.g., infants and patients with dementia)\(^23\).

Pressure thresholds (kg) of the knee joint were measured with a hand-held pressure meter (Matsumiya Medical Co., Ltd., Tokyo, Japan). The tip of the meter (9 mm diameter) was slowly pushed against the medial joint space and medial condyle of the femur. The amount of force needed to evoke a response, a pain-related facial...
expression (e.g., flinching of the facial muscles around the eyes and/or contraction of the skin at the back-top of the head), was recorded as the pressure threshold. If no response was observed, the cut-off value (3 kg) was assigned. The mean of three measurements from each leg at each time point was calculated and the pressure threshold was expressed as a percent (%), of the ipsilateral knee vs the contralateral, uninjured knee:

\[
\% \text{ of contralateral knee} = \frac{\text{pressure threshold of the ipsilateral knee}}{\text{pressure threshold of the contralateral knee}} \times 100.
\]

**MMx surgery**

Macaques were anesthetized with 25 mg/kg ketamine (i.m.), supplemented as needed with 25 mg/kg (i.m.) pentobarbital. The surgical site was prepped with povidone-iodine and a MMx of the right knee was performed using aseptic technique. The skin just above the medial collateral ligament was incised and the anterior horn of the medial meniscus was detached from the tibia. The posterior horn of the medial meniscus was visualized and loosened from the tibia. Then, the entire medial meniscus was removed from the articular capsule. The incision was closed in layers and the animals were allowed to recover from anesthesia before being returned to their home cages. To prevent post-operative infection, animals were treated with cefazolin (25 mg/kg, i.m., twice daily for 5 days). To alleviate acute post-operative pain, animals were treated with buprenorphine (0.01 mg/kg, i.m., 2 times per day for 3–7 days). Animals were allowed unrestricted access to food and water.

In a separate group of macaques \((n = 3)\), a sham surgery was performed following baseline behavioral assessments. Under anesthesia, the skin was incised as per MMx surgery. The wound was closed and animals were allowed to recover from anesthesia. Post-operative care was given as previously described.

**Drug administration and test cycles**

Baseline behavioral tests were performed 67 days following MMx ("Day 0"; Fig. 1(A)). Stable pain-related behaviors were attained 49 days after MMx and randomization to treatment groups \((n = 3/\text{group})\) was performed 67 days after MMx [Fig. 1(B)]. Animals were allocated such that there were no significant differences between groups in terms of mean knee pressure threshold and weight bearing as measured on Day 0. Macaques were dosed once per day for 5 days with either diclofenac (10 mg/kg, p.o., Sigma Chemical Co., St. Louis, MO, USA), aprepitant (10 mg/kg, p.o., Ono Pharmaceutical Co., Osaka, Japan) or 1 ml/kg of vehicle (0.5% (w/v) methyl cellulose solution in water). Macaques were tested after each daily dosing, 3 h after diclofenac dosing and 4 h after aprepitant dosing. Vehicle-treated macaques were tested 3 h after dosing. The dose and testing time points were determined from pharmacokinetic data obtained from the literature and extrapolated from package inserts for each drug.

A previous study in a rat osteoarthritis model indicated that repeated dosing (e.g., celecoxib) may be necessary in order to observe antinociception in the weight bearing test. To balance the need for repeated dosing and at the same time reduce the risk of
gastrointestinal lesioning following repeated oral diclofenac treatment, animals in the current study were dosed for 5 days.

Between Test Cycles, macaques underwent a concurrent 9-day washout period and a 5-day exercise period. Macaques underwent three test cycles—each animal received all three treatments [Fig. 1B].

Testing was performed in a blinded manner. Staff performing the tests were given a testing schedule and were unaware of the treatment and when the animals were dosed.

Knee joint histology

Three macaques were euthanized with an overdose of pentobarbital 130 days post-MMx. The ipsilateral and contralateral femoral–tibial joints were removed and stored in 10% buffered formalin overnight for overnight shipping to a histological laboratory for processing (KAC, Kyoto, Japan). Knee joints were decalcified and embedded in paraffin according to a modified method based on Little et al.26 The femoral–tibial joints were coronally sectioned and alternating 3 μm sections were stained with safranin O and counterstained with Fast Green and hematoxylin based on a previously described method27. Pathology of the tibia and femur was classified according to the "histologic-histochemical grading system" (Mankin score)28 by an observer unaware of whether the tissue came from the ipsilateral or contralateral knee joint.

Statistical analysis

Behavioral evaluation prior to drug treatment

The effect of MMx on knee pressure threshold and weight bearing up to 61 days post-MMx, before assignment to treatment groups, was analyzed using a one-way repeated measures analysis of variance (ANOVA) followed by Dunnett’s test for multiple comparisons. Statistical analysis was performed using GraphPad Prism 4.02 (GraphPad Software, San Diego, CA). P < 0.05 was taken as statistically significant.

Crossover drug treatment

Drug testing utilized a 3-period, 3-treatment crossover design. A repeated measures ANOVA was performed which included these factors: treatment (vehicle, diclofenac, aprepitant), period (Test Cycle 1, Test Cycle 2, Test Cycle 3), group (three groups: carry-over) and time (6 time points in each Test Cycle: 1st day of drug testing, 35 days following MMx (P < 0.01 vs pre-MMx, Dunnett’s test). Decreased ipsilateral weight bearing persisted for at least 61 days post-MMx, before the first day of drug testing.

Knee joint histopathology

A representative light photomicrograph of the contralateral and ipsilateral medial femoral–tibial joints from a macaque 130 days after MMx is shown in Fig. 2. Fig. 2(A) shows the normal thickness and smooth articular cartilage surface of the contralateral knee joint. While safranin O staining is somewhat diminished in the superficial cartilage of the femur and tibia, safranin O staining can be observed throughout the rest the articular cartilage. Based on these findings, the Mankin scores for the femur and tibia were 1 and 0, respectively. By contrast, in the ipsilateral knee joint, full depth cartilage erosion (to the tidemark) can be observed and the subchondral bone appears to be thickened [Fig. 2(B)]. Where present, the surface of the articular cartilage is rough and uneven and proteoglycan is missing up to the mid zone of the cartilage. Upon higher magnification of existing cartilage, chondrocytes clusters ("cloning") can be observed [Fig. 2(C)]. Based on these findings, the Mankin scores for both the femur and tibia are 8.

Pain-related behaviors following MMx

Knee pressure threshold

Prior to MMx, knee pressure threshold was 100.3 ± 2.1%, indicating that the thresholds of the right and left knee were similar [Fig. 4(A)]. A statistically significant decline in ipsilateral pressure threshold ("hypalgesia") was observed beginning 28 days following MMx (P < 0.01 vs pre-MMx, Dunnett’s test). Pressure hyperalgesia persisted for at least 61 days post-MMx, before the first day of drug testing.

Weight bearing

Macaques demonstrated normal weight bearing before MMx (50.1 ± 2.2%; Fig. 4(B)). A statistically significant decline in ipsilateral weight bearing was observed beginning 35 days following MMx (P < 0.01 vs pre-MMx, Dunnett’s test). Decreased ipsilateral weight bearing persisted for at least 61 days post-MMx, before the first day of drug testing.

Crossover drug treatments on MMx-induced osteoarthritis

Knee pressure threshold

Prior to the first treatment administration (Day 0; Fig. 5), the overall mean ipsilateral knee pressure threshold was significantly decreased compared to the pre-MMx threshold (P < 0.01, one-way repeated measures ANOVA, Dunnett’s test). Mean baseline pressure thresholds were also significantly decreased on Day 14 and Day 28 (P < 0.01 vs pre-MMx threshold, one-way repeated measures ANOVA, Dunnett’s test).

A significant treatment × time interaction was detected (F(10, 80) = 14.94, P < 0.0001; Fig. 5A). Diclofenac significantly increased pressure thresholds over time in each Test Cycle. By contrast, daily treatment with either aprepitant or vehicle did not alter knee pressure thresholds [Fig. 5A, B].

No knee pressure threshold period effect was observed (period × time, F(10, 80) = 1.69, P = 0.0980). Therefore, diclofenac efficacy was observed regardless of Test Cycle. No carry over effect was observed on knee pressure thresholds (effect of group, F(2,6) = 0.51, P = 0.6220). Therefore, no residual treatment effect on knee pressure threshold was observed on the subsequent Test Cycle.
Weight bearing

Prior to treatment administration (Day 0; Fig. 6), overall mean ipsilateral weight bearing was significantly decreased (35.9 (33.0–38.9)%; P < 0.01, one-way repeated measures ANOVA, Dunnett’s test) compared to pre-MMx weight bearing. Mean baseline ipsilateral weight bearing was also significantly decreased on Day 14 and Day 28 (P < 0.01 vs pre-MMx threshold, one-way repeated measures ANOVA, Dunnett’s test).

A significant treatment × time interaction was detected (F(10, 80) = 6.00, P < 0.0001; Fig. 6(A)). Diclofenac increased weight bearing over time in each Test Cycle. By contrast, daily treatment with either aprepitant or vehicle did not alter weight bearing [Fig. 6(A), (B)].

No period effect was observed on weight bearing (period × time, F(10, 80) = 0.69, P = 0.7348). Therefore, diclofenac efficacy was observed regardless of Test Cycle. No carry over effect was observed on weight bearing (effect of group, F(2,6) = 1.24, P = 0.3540). Therefore, no residual treatment effect on weight bearing was observed on the subsequent Test Cycle.

Discussion

Unilateral MMx in the macaque led to ipsilateral pressure hyperalgesia and decreased weight bearing, or pain on standing, and these pain-related symptoms were sensitive to diclofenac treatment. Diclofenac efficacy was observed as early as one or 2 days of treatment and persisted as long as treatment continued. In addition, ipsilateral knee joint histopathology was characterized by loss of proteoglycan from the articular cartilage, cartilage erosion and exposure of the subchondral bone and chondrocyte clusters within the remaining cartilage. Thus, the presence of persistent pain and pathology observed in the current model is highly consistent with clinical osteoarthritis. Interestingly, repeated treatment with a NK1 receptor antagonist did not significantly alter osteoarthritis pain-related symptoms, which accords with findings from a clinical trial. This finding suggests that the macaque model could be used to preclinically verify whether or not a particular mechanism has a prominent role in clinical osteoarthritis knee pain. Based on the current findings, NK1 receptors are not likely to have a prominent role in knee osteoarthritis pain.
Preclinical nonhuman animal models are crucial in developing mechanism-based therapeutics. In the case of osteoarthritic pain, encouraging results obtained in rodent models of arthritic pain have not always successfully translated to useful treatments. While there are a number of possibilities as to why potential therapeutics fail clinical testing, the utilization of rodents as the primary species in which to model clinical osteoarthritis has infrequently come under scrutiny. Rodents as a species do have advantages for use in preclinical research, such as low genetic heterogeneity, transgenic applications and the possibility to develop and apply simple behavioral endpoints, but at the same time, rodents do have significant limitations. For example, meaningful cross-species differences in receptor function and structure have been observed, which could lead to an overestimation of the significance of a particular molecular target characterized in a rodent pain model. The differential biomechanics and anatomical substrate of a small quadruped’s knee compared to the human knee also presents challenges in terms of understanding disease progression.

Thus, to enhance translatability of preclinical findings, one solution would be to use NHP, a species that is phylogenetically closer to humans than rodents, to model knee osteoarthritis.

The current macaque osteoarthritis model parallels the clinical state. Firstly, histopathology consistent with osteoarthritis was observed. Loss of proteoglycan, full-depth articular cartilage erosion and chondrocyte hyperplasia, indicating cellular attempts at repair, were observed in the ipsilateral knee joint. Also, all macaques developed significant ipsilateral weight bearing and primary hyperalgesia. Finally, pain-related symptoms were temporarily ameliorated with diclofenac, consistent with clinically observed efficacy. With respect to the efficacy of diclofenac in the macaques, it should be noted that a rough estimate of the maximum possible effect is about 40% for pressure threshold and 70% for weight bearing. Thus, while effective, diclofenac did not completely reverse these deficits. Perhaps an extended treatment period would lead to greater efficacy. Such an extended treatment period is possible, given the persistent nature of the pain-related behaviors in the current model.

Interestingly, the current behavioral findings contrast with rodent osteoarthritis models in terms of symptoms. A few previous...
studies have demonstrated a lack of ipsilateral weight bearing following MMx in rodents\cite{2,24}. In a rodent study that did show significant ipsilateral weight bearing following MMx, a single treatment of NSAID improved weight bearing\cite{42}. Differences in rat strains and assessment methods could explain the between-study variability in the expression of weight bearing. It is also possible that the rodents were relatively inactive in studies that showed a lack of significant ipsilateral weight bearing. Active rodents, such as those under an exercise regimen, show advanced stages of osteoarthritis\cite{21}. Indeed, high-level activities that stress the knee joints are a significant risk factor for osteoarthritis\cite{5}. Alternatively, it is possible that ipsilateral weight bearing is infrequently observed in rodents due to genetics or innate behavior as a prey species\cite{10}.

Further testing of the current macaque model with analgesics used in managing osteoarthritis pain, such as duloxetine, will be important in establishing the model’s pharmacological isomorphism, that is, its predictive validity\cite{13}. It will be equally important, however, to evaluate drugs that failed to show efficacy in clinical osteoarthritis pain\cite{29,31}. Given the anatomical and genetic distance between NHP and rodents, it is predicted that there will be a differentiation between these species in the efficacy of a number of drugs.

The lack of antinociception of NK₁ receptor block on osteoarthritic pain in the macaque, either after a single or repeated treatment, also contrasts with rodent findings. A number of studies demonstrated robust changes in the expression of NK₁ receptors and substance P in peripheral nerves that innervate the knee joint of arthritic rodents, suggesting a significant involvement of peripheral NK₁ receptors in osteoarthritic pain\cite{32,43}. In addition, NK₁ receptor antagonism is antinociceptive in rodent arthritic models\cite{16}. However, a clinical trial found no efficacy of NK₁ receptor antagonist lanepitant on osteoarthritis pain whereas significant efficacy was observed with the active comparator, the NSAID naproxen\cite{30}. The dose of aprepitant used in the current study (10 mg/kg) is likely to be sufficient to occupy most of the NK₁ receptors in the central nervous system (CNS). In humans, a dose of 165 mg occupies >99% of brain NK₁ receptors 4 h after oral dosing\cite{17,46}. Converting the human dose to a “macaque equivalent dose” gives 8.5 mg/kg\cite{47}. Furthermore, the human terminal half-life of aprepitant is 9–13 h. Thus, the daily peripheral and central levels of aprepitant increase gradually over the 5-day treatment period\cite{48}. The current findings in the macaque and the clinical findings indicate NK₁ receptors are not crucial in maintaining osteoarthritic pain.

A general issue in evaluating preclinical models is the choice of behavioral endpoints\cite{12}. While no overall effect of lanepitant was obtained in clinical osteoarthritic pain, knee pressure hyperalgesia and weight bearing pain were not specifically measured. In the interest of translation, future clinical studies could employ behavioral endpoints used in preclinical studies to establish their relevance to the clinical setting. While osteoarthritic patients do show changes in somatosensation as demonstrated by quantitative sensory testing, a major complaint is pain upon movement, as demonstrated by “gait disturbance”\cite{39}. A number of preclinical studies have attempted to quantify gait disturbance in arthritic quadrupeds\cite{30,50}. A method to identify gait disturbance in macaques, and its sensitivity to clinical analgesics, is currently ongoing.

**Fig. 5.** Effect of drug treatments over time on knee pressure threshold in NHP following a MMx. Pressure was applied to the knee joint and the response threshold was recorded when the animal displayed a pain-related behavior. Threshold of the ipsilateral knee was compared to that of the contralateral knee and converted into a percent (%). Baseline pressure thresholds were measured on Days 0, 14 and 28 and macaques were dosed and tested over the subsequent 5-day period. Data from individual animals are shown from all three Test Cycles (total n = 9). A. Increasing pressure thresholds over time with diclofenac treatment. By contrast, neither aprepitant nor vehicle increased knee pressure thresholds. *P < 0.05, **P < 0.01 vs pre-treatment baseline (“Pre”). B. Significant increases of pressure thresholds following diclofenac treatment compared to that of vehicle. †P < 0.01 vs vehicle. Data are expressed as mean ± S.D.
MMx in macaques leads to robust pain-related behaviors as well as osteoarthritic pathology. The current data suggests a differential pharmacological responsiveness compared to that of rodents. Thus, not only is the current model potentially useful in verifying the relevance of pain targets to human osteoarthritis pain but also in elaborating mechanisms that may be missing in rodents due to their phylogenetic distance from humans.

**Author contributions**
All authors contributed to the conception and design, analysis and interpretation of data. SO and YA were involved in collection and assembly of data and statistical analysis. SO, AH and HT were involved in drafting of the manuscript. All authors read and approved the manuscript for publication. All authors take responsibility for the integrity of the work.

**Competing interest statement**
All authors are employees of Hamamatsu Pharma Research, Inc.

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**Supplementary data**
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.joca.2016.02.006.

**References**


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**Fig. 6.** Effect of drug treatments on weight bearing in NHP following a MMx. Amount of force exerted by the legs was measured. Weight bearing of the ipsilateral limb was compared to the contralateral limb and converted to a percent (%). Baseline weight bearing was measured on Days 0, 14 and 28 and macaques were dosed and tested over the subsequent 5 day period. Data from individual animals are shown from all three test cycles (total n = 9).

A. Increasing weight bearing over time with diclofenac treatment. By contrast, neither aprepitant nor vehicle increased weight bearing. **P < 0.01 vs pre-treatment baseline (“Pre”).**

B. Significant increases of weight bearing following diclofenac treatment compared to that of vehicle. †† P < 0.01 vs vehicle. Data are expressed as mean ± S.D.
Supplementary Information

Method

*Knee diameter*

While the macaque was in a monkey chair, knee diameter (mm) was measured using a calibrated digital caliper (Mitsutoyo Co., Kanagawa, Japan). One measurement from each knee was taken at each time point. The extent of ipsilateral (right) knee swelling following MMx was reported as a percent (%) of the diameter of the ipsilateral knee vs. the contralateral uninjured (left) knee:

\[
\text{% of contralateral knee} = \frac{\text{ipsilateral knee diameter}}{\text{contralateral knee diameter}} \times 100
\]

*Knee skin temperature*

The skin temperature of both knees (°C) were measured using a contactless thermometer (HuBDIC-Global Co., Hyogo, Japan)\(^1,2\). Three measurements were performed about 2 to 3 cm away from the skin and the mean difference was calculated. The mean difference (± S.D.) between the ipsilateral and contralateral knee skin temperature was reported.

The effect of MMx on body weight, knee diameter and knee skin temperature over time was analyzed using a one-way repeated measure ANOVA followed by Dunnett’s test.
Results

General observations following medial meniscectomy

The macaques maintained weight during the experimental period (Supplementary Fig. 1A). The mean (± S.D.) weight of the macaques the day before MMx was 4.2 ± 1.2 kg and the weight of the macaques on the last day of testing (100 days after MMx) was 4.5 ± 1.1 kg. Mean weight increases were observed 72 (*P < 0.05), 95 (**P < 0.01) and 100 days (P < 0.01) after MMx (vs. pre-MMx, Dunnett’s test).

Prior to MMx, the right knee diameter was similar to the left knee diameter (99.7 ± 1.3%) (Supplementary Fig. 1B). Fifteen days following MMx, the ipsilateral knee diameter increased 12.9% (mean (95% confidence intervals, CI), 112.6 (107.0-118.2)%; (**P < 0.01 pre-MMx vs. 15 days after MMx, Dunnett’s test) and remained at about this level for the duration of the study.

Prior to MMx, the difference between the right (ipsilateral) and left (contralateral) knee skin temperature was 0.4 ± 0.6 °C (Supplementary Fig. 1C). Fifteen days following MMx, the knee skin temperature difference increased 2.0 °C (2.4 (1.6-3.1) °C; P < 0.01 pre-MMx vs. 15 days, Dunnett’s test) and knee skin temperatures were also significantly elevated 21, 28 (**P < 0.01 pre-MMx vs. 21, 28 days, Dunnett’s test) and 35 days after MMx (*P < 0.05 pre-MMx vs. 35 days, Dunnett’s test). Knee skin temperature differences at the
beginning of the drug treatment Test Cycles, on days 67 (0.6 (0.3-0.9) °C), 81 (0.4-1.2) °C) and 95 (0.3 (-0.1-0.7) °C) were not significantly different from that of pre-MMx (P > 0.05, Dunnett’s test).

References

Supplementary Figure 1.

Body weight, knee diameter and knee skin temperature following medial meniscectomy over time.

A.

B.

C.