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# Antinociceptive effect of clinical analgesics in a nonhuman primate model of knee osteoarthritis

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ABSTRACT

A number of potential analgesic pharmacotherapies developed in preclinical osteoarthritis animal models have failed clinical trials. A possible basis for the lack of translation of preclinical findings to clinical efficacy is the use of a preclinical species that is distinct from that of humans. The current study tested clinical analgesics in a nonhuman primate model of knee osteoarthritis. Following a medial meniscectomy, the animals developed a robust ipsilateral reduction in knee pressure threshold (hyperalgesia) and an ipsilateral reduction in weight bearing (resting pain). The serotonin-noradrenalin reuptake inhibitor duloxetine and opioid morphine increased ipsilateral pressure threshold and weight bearing. By contrast, the anticonvulsant pregabalin did not affect either pressure hyperalgesia or resting pain. The current findings in the nonhuman primate model of osteoarthritis parallel clinical findings, in that duloxetine and opioids are used in the management of osteoarthritis pain whereas pregabalin is not. The current findings also suggest the possible differentiation of pharmacotherapeutics in a nonhuman primate model, of distinguishing potential clinically useful analgesics for the management of osteoarthritic pain from those that are not.



## 1. Introduction

Osteoarthritis is a common disability of aging, characterized by decreased functioning and pain, and the worldwide incidence of osteoarthritis is expected to rise with the aging of the global population (Wittenauer et al., 2013). Acute injury and surgery to knee intraarticular tissues and athletic activity that stress the knee joints also lead to an osteoarthritic state (Roos et al., 1998; Takeda et al., 2011). Since there are currently no treatments that slow or reverse the progression of osteoarthritis, current therapeutics focus on managing pain in order to improve function.

A number of pathophysiological mechanisms, based on findings in rodent osteoarthritis models, have been proposed to mediate evoked knee joint hypersensitivity and pain at rest observed in the clinical state (Zhang et al., 2013). Injection of monosodium iodoacetate into the knee joint of a number of nonhuman animal species leads to apoptosis of chondrocytes as well as inflammation and long-lasting, robust pain-related behaviors, such as ipsilateral cutaneous hypersensitivity and ipsilateral weight bearing (Little and Smith, 2008). Surgical destabilization of the knee joint, such as by medial meniscectomy, leads to rapid pathological changes in the articular cartilage as well as long-lasting pain-related behavior (Little and Smith, 2008; Rojas-Ortega et al., 2015). Cytokines released during the initial stages of osteoarthritis in rats, mice and guinea pigs sensitize knee joint primary afferent sensory neurons and these neurons develop spontaneous activity as well (Kelly et al., 2012). Changes in the physiological properties of primary afferents in turn leads to changes in basal activity and responding of postsynaptic spinal dorsal horn neurons to cutaneous stimulation (Zhang et al., 2013). Glia are likely to be involved as they have a key role in modulating the synaptic milieu. Increased spinal dorsal horn glial activity has been observed in rats with osteoarthritic pain, which could be a consequence of increased presynaptic primary afferent activity or postsynaptic spinal neuron activity (Sagar et al., 2011). Based on preclinical rodent findings, a number of potential therapeutics for clinical osteoarthritis pain have been developed.

However, few, if any, therapeutics developed in this manner have successfully completed double-blinded, placebo-controlled clinical trials (Huggins et al., 2012; Malfait and Little, 2015; Miller et al., 2014). While a number of issues have been identified that could underlie the limited translation of preclinical findings to a clinical treatment, one issue could be the use of rodents, which are

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phylogenetically distant to humans. A consequence of this distance is that molecular target functioning in one species markedly differs from that of other species (Chen et al., 2013).

A model of knee osteoarthritis pain was created in cynomolgus macaques, a species that is phylogenetically closer to humans compared to rodents, which demonstrated pain-related behavior that was partially ameliorated by the nonsteroidal anti-inflammatory drug diclofenac (Little and Smith, 2008; Ogawa et al., in press). In the current study, the model's pharmacological isomorphism (or "predictive validity") was further elaborated with analgesics used to manage osteoarthritic pain, the serotonergic-norepinephrine reuptake inhibitor duloxetine and the opioid morphine (Geyer and Markou, 1995). As a further test of pharmacological isomorphism, the anticonvulsant pregabalin, not FDA-approved for use in the management of osteoarthritis pain, was evaluated as well.

## 2. Materials and methods

Female cynomolgus macaques (*Macaca facsicularis*; n=9; Shin Nippon Biochemical Laboratories (Kagoshima, Japan)) were approximately 8 years of age at the beginning of the study.

All study procedures were reviewed and approved by the Institutional Animal Care and Use Committee. The study adhered to principles stated in the *Guide for the Care and Use of Laboratory Animals, Eighth Edition* (National Research Council, 2011). Macaques were individually housed in stainless steel cages in a dedicated primate unit where room temperature and humidity were continuously monitored. Lighting was maintained on a 12 h light/dark cycle. Although individually housed, macaques maintained auditory, visual and olfactory contact with neighboring conspecifics and were provide with enrichment devices. Macaques were fed standard nonhuman primate diet (Oriental Yeast Co., Ltd., Chiba, Japan) and water was available ad libitum. In addition, macaques received supplementary fresh fruits, vegetables and treats. Animal care staff as well as study staff provided positive interaction with the macaques.

### 2.1. 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. Body weight, weigh bearing and knee pressure threshold were recorded before medial meniscectomy.

Macaques underwent an exercise routine. Macaques were trained to jump a distance of 2 m from the top of one cage to another for two weeks, five days per week. Macaques were trained to jump 50 times (a 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 exercise beginning 15 days after medial meniscectomy during a 36 day period, 5 days per week. Following this period, exercise frequency was every other week, 5 days per week.

#### 2.1.1. Weight bearing

While macaques were restrained in a monkey walker, the force (kg) exerted by the right and left legs was measured using two weight scales (Tanita Co., Tokyo, Japan). The mean of three measurements was calculated and ipsilateral weight bearing was expressed as a percent (%) of the ipsilateral, right leg compared to total weight bearing:

$$\%\ \text{ipsilateral weight bearing} = \text{ipsilateral weight bearing} / (\text{ipsilateral weight bearing} + \text{contralateral weight bearing}) * 100$$

Prior to medial meniscectomy, weight bearing of either the ipsilateral or contralateral leg was about 1.6 kg.

Because ipsilateral weight bearing is a function of total body weight, it is possible that weigh bearing may change over time with changes in body weight. The mean ($\pm$S.D.) body weight of macaques with a medial meniscectomy on the first day of drug testing, prior to dosing, was $4.4 \pm 1.0$ kg. On the last day of drug testing, prior to dosing with the final drug, the body weight was $4.4 \pm 1.1$ kg; during the entire study period, there was no significant change in body weight.

#### 2.1.2. Knee pressure threshold

A hand-held pressure meter (Matsumiya Medical Department Industry Mill, Ltd., Tokyo, Japan) was used to measure knee pressure threshold. The tip of the meter (9 mm diameter) was slowly pushed against the medial joint space and medial condyle of the femur. A cut-off of 3 kg was used. 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 threshold. If no response was observed, the cut-off value was assigned. The mean of three measurements was calculated and the pressure threshold was expressed as a percent (%) of the ipsilateral, right knee compared to the contralateral, uninjured left knee:

$$\%\ \text{ipsilateral pressure threshold} = \text{pressure threshold of the ipsilateral knee} / \text{pressure threshold of the contralateral knee} * 100$$

In uninjured macaques, the knee pressure threshold reached 3 kg.

The sequence of behavioral testing before and after surgery was weight bearing followed by knee pressure threshold.

### 2.2. Medial meniscectomy surgery

Macaques were anesthetized with ketamine (25 mg/kg, i.m.), supplemented with pentobarbital (25 mg/kg, i.m.) as needed. The hair was clipped from the surgical site and the skin was prepped with povidone-iodine. The knee joint was bent at a 90° angle and, using aseptic technique, a 2–3 cm skin incision was made just above the medial collateral ligament. The medial collateral ligament was bisected and the anterior horn of the medial meniscus was freed from the tibial plateau using a radio knife (ellman-Japan, Osaka, Japan) (Bove et al., 2006; Lutfi, 1975). The posterior horn of the medial meniscus was then removed using a radio knife. The skin incision was closed in layers and macaques were allowed to recover from anesthesia before being returned to their home cages. Following surgery, macaques were allowed unrestricted access to food and water. To manage post-operative pain, buprenorphine (0.01 mg/kg, i.m.) was administered 2 times per day for 3–7 days and to prevent post-operative infection, cefazolin (25 mg/kg, i.m.) was administered twice daily for 5 days.

### 2.3. Drug administration and evaluation

Drugs were tested beginning 131 days after medial meniscectomy. A previous study demonstrated significant pain-related behaviors beginning 28 days following medial meniscectomy, which persisted for at least 100 days following medial meniscectomy (Ogawa et al., in press). Prior to drug