Characterization of the Rat Monoiodoacetate Model of Osteoarthritis: Evoked and Non-evoked Pain Response Assessments

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Background

The National Institutes of Health Helping to End Addiction Long-term℠ Initiative, or NIH HEAL Initiative℠, Preclinical Screening Platform for Pain (PSPP) program aims to accelerate the discovery and development of new non-opioid, non-addictive pain therapeutics. Toward this goal, PSPP is collaborating with PsychoGenics, Inc. to validate preclinical models and endpoints to enable screening and profiling of assets, including small molecules, biologics, natural products, and devices. Here, we describe the validation of one such effort to optimize the monoiodoacetate (MIA) model of osteoarthritis pain in the rat.

Methods

Animals: Adult male and female Sprague Dawley rats (n=10, each sex) were used in these studies. All housing and testing of the animals were in accordance with the Principles of Laboratory Animal Care and the approval of PsychoGenics Inc., Institutional Animal Care and Use Committee in AAALAC-accredited facilities.

Intraarticular injection of monoiodoacetate (MIA) into the knee joint: To examine the effects of MIA on hind paw tactile sensitivity and hind paw weight bearing, MIA (0.3 – 3 mg) was injected intraarticularly into the left hindlimb knee joint in preliminary studies. Based on the results from these studies, a dose of 1 mg of MIA was selected for subsequent studies as this dose produced the lowest variability in behavioral responses and was devoid of any observed adverse effects. For the intraarticular injection procedure, rats were briefly anesthetized using isoflurane (1-5% to effect, inhalation) and received a single intraarticular injection of MIA (50 µl) into the left hindlimb knee joint through the patellar tendon using a 26 gauge needle attached to a 1 ml syringe.

Hind paw tactile sensitivity: Hind paw tactile sensitivity was measured by applying von Frey filaments (0.4, 0.6, 1, 2, 4, 6, 8, 15 g; Semmes-Weinstein, Stoelting, Wood Dale IL, USA) to the plantar hind paw and determining the paw withdrawal threshold (PWT) using the "up-down" method (Chaplan et al. 1994 J Neurosci Methods 53:55-63). A maximum of 9 filament applications were used to determine the 50% Withdrawal Threshold, and based on Chaplan et al. (1994), animals that did not respond to any filament were assigned a threshold of 15.0 g, while animals that responded to all filaments were assigned a threshold of 0.25 g. The PWT was determined for both the left and right hind paws, and assessments were made by a blinded investigator.

Hind limb knee joint pressure sensitivity: Knee joint sensitivity to a pressure stimulus was assessed using the Rodent Pincher Device (Bioseb). Rats were lightly restrained, the device was placed on the animal knee joint, and a progressive quantified squeeze force was applied across the joint. The force that elicited a response characterized by a knee withdrawal or vocalization was designated as the knee pressure response threshold (gm).

Dynamic weight bearing: Weight bearing on the hind paws of rats was assessed using the Dynamic Weight Bearing 2.0 Instrument (Bioseb). Rats were placed in the chamber for a period of 3 minutes per test where they were able to move freely. Hind paw weight bearing, in addition to other parameters (paw surface area, time on sensor pad), were recorded in real time and stored on a computer.

Data analysis: Data were analyzed using two-way repeated measures ANOVA with Bonferroni's or Dunnett's post hoc test when appropriate. Effects with $p<0.05$ were considered to be statistically significant. Power analysis and effect size were determined using SAS/STAT, and appropriate sample size was based on a power value of 0.8 to ensure adequate power for F-tests for two-way interactions. Data are represented from individual animals and are summarized as mean±sem.

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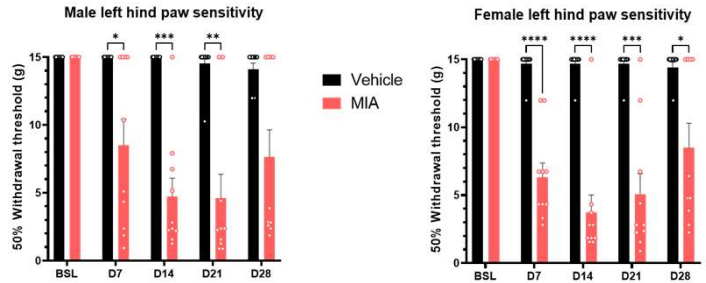
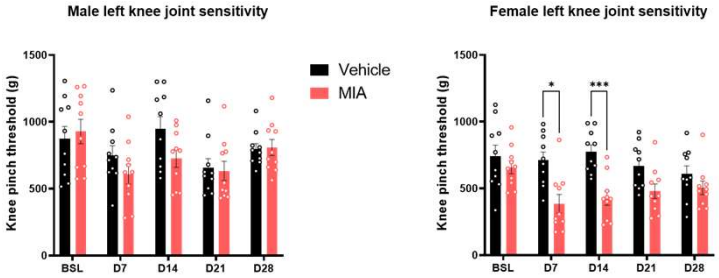
Hind paw tactile sensitivity



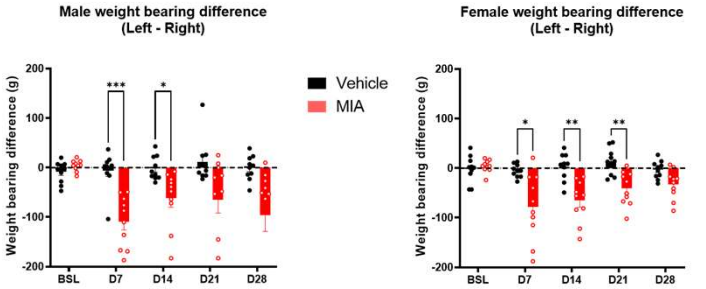
Ipsilateral hind paw tactile sensitivity to von Frey filament stimulation in male and female rats over time following intraarticular injection of MIA (3 mg/50 µl) or vehicle (saline; 50 µl). Baseline paw withdrawal thresholds were recorded prior to intraarticular injections which occurred on Day 0 (BSL). Data represented as mean±sem; n=10/group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Bonferroni's test. Paw withdrawal threshold on the contralateral paw was not affected by MIA injection into the left hindlimb knee joint (data not shown).

Hind limb knee joint pressure sensitivity



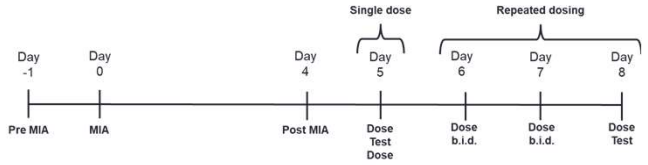
Ipsilateral hind limb knee joint pinch sensitivity to stimulation using a forceps pinch instrument in male and female rats over time following intraarticular injection of MIA (3 mg/50 µl) or vehicle (saline; 50 µl). Baseline knee joint pinch response thresholds were recorded prior to intraarticular injections which occurred on Day 0 (D0). Data represented as mean±sem; n=10/group; *p<0.05, ***p<0.001, Bonferroni's test.

Dynamic weight bearing

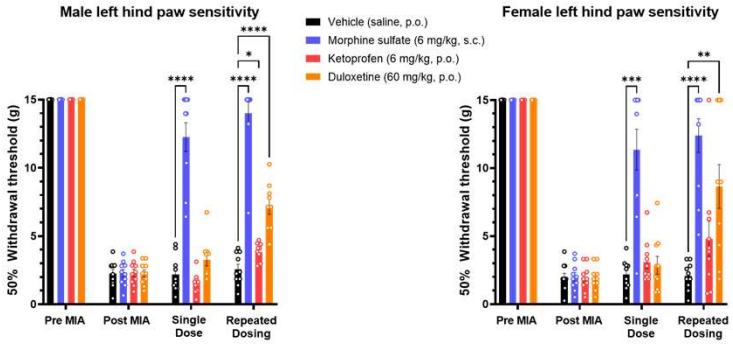


Dynamic weight bearing deficits represented as difference in weight bearing (Left – Right) in male and female rats over time following intraarticular injection of MIA (3 mg/50 µl) or vehicle (saline; 50 µl). Data represented as mean±sem; n=10/group; * p<0.05, **p<0.01, ***p<0.001, Bonferroni's test

Testing timeline

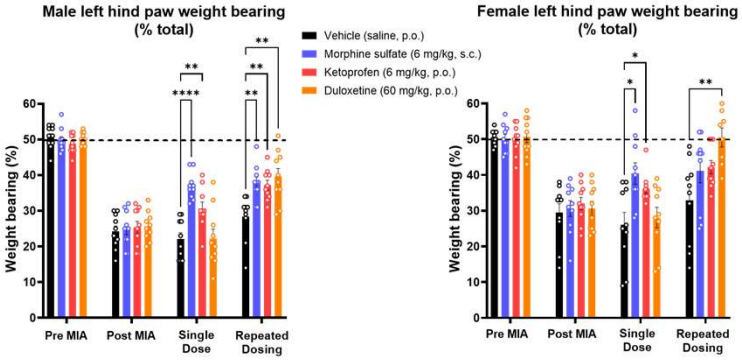


Validation of tactile sensitivity



Ipsilateral hind paw tactile hypersensitivity to von Frey filament stimulation in male and female rats following intraarticular injection of MIA (1 mg/50 µl) and effects of reference analgesic compounds at 1 hour post-dosing following a single dose (Day 5) and repeated dosing (Day 8). Data represented as mean±sem; n=10/group; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Dunnett's test.

Validation of dynamic weight bearing



Ipsilateral hind paw dynamic weight bearing deficits in male and female rats following intraarticular injection of MIA (1 mg/50 µl) and effects of reference analgesic compounds at 1 hour post-dosing following a single dose (Day 5) and repeated dosing (Day 8). Data represented as mean±sem; n=10/group; *p<0.05, **p<0.01, ****p<0.0001, Dunnett's test

Conclusion

The results from these validation studies demonstrate reproducible hind paw tactile hypersensitivity and weight bearing deficits in male and female rats following intraarticular injection of MIA (1 mg/50 µl) into the knee joint. Knee joint pressure hypersensitivity was less robust and reproducible in male and female rats. Repeated dosing of the reference analgesic compounds ketoprofen and duloxetine produced greater efficacy in reversing hind paw tactile hypersensitivity and weight bearing deficits compared to a single dose. These results demonstrate that single and repeated treatment paradigms may be used to identify and differentiate novel therapeutics for osteoarthritis pain.