Impairment in locomotor activity as an objective measure of pain and analgesia in a rat model of osteoarthritis

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Received January 5, 2020; Accepted July 24, 2020

DOI: 10.3892/etm.2020.9294

Abstract. A major problem with current animal models of pain is their lack of face validity and their vulnerability for false positive results. The present study evaluated the efficacy of the open field locomotor system, as an objective measure of pain-related behavior and analgesic efficacy in rodents. Adult, male, Sprague-Dawley rats (180-250 g) received intra-articular injections of monoiodoacetate (MIA; 1 mg) in the left knee joint. Mechanical allodynia using von Frey filaments, the weight bearing difference test and the open field locomotor activity test were performed every other day for 21 days, following the MIA injection. The antinociceptive effects of ibuprofen (50 and 100 mg/kg) on the MIA-induced nociception were also evaluated. MIA induced a significant reduction in the paw withdrawal threshold (PWT) and a significant alteration in the weight bearing difference compared with control rats. Similarly, MIA induced a significant reduction in locomotor activity, with respect to X total counts, that represent the overall locomotor activity in the horizontal plane, and X ambulatory counts, which in turn represent small scale movements, such as scratching and grooming, and lastly, Z total counts, that represent rearing or standing. Both doses of ibuprofen resulted in a significant reversal of the MIA-induced alterations in PWT and weight bearing difference. Furthermore, the two doses of ibuprofen resulted in a significant reversal of the MIA-induced reduction in locomotor activity, with respect to X ambulatory counts, but not Z total counts. Only the higher dose of ibuprofen reversed the X total counts. The open field locomotor system may successfully be used to predict the analgesic efficacy of compounds in models of joint inflammation and osteoarthritis.

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Key words: pain, osteoarthritis, locomotion, antinociception, ibuprofen, rat

Introduction

Osteoarthritis (OA) is a chronic multifactorial condition characterized by progressive joint degeneration and subchondral bone sclerosis, which may result in the formation of bone cysts and marginal osteophytes (1). OA has been indicated to be the most recurrent health problem in the middle age (45-65 years) and elderly (>65 years) (2). Of note, chronic pain remains the primary concern and predominant clinical feature of patients with OA and is typically poorly treated using occupational therapy (3,4).

Current preclinical studies, which are largely reliant on animal models and laboratory pain testing, are widely used to develop more suitable analgesics for patients with OA (5,6). Indeed, a number of animal models have been used to investigate OA, and have effectively been found to predict the clinical efficacy of therapies, that are universally used against pain, including ibuprofen and acetaminophen (7). However, a gold standard animal model, that may aid in standardization across clinical groups, has not yet been established.

Numerous models of OA, including the monoiodoacetate (MIA) model, and behavioral tests, such as von Frey filament testing and static or dynamic analysis of weight distribution, have been used to investigate potential analgesic compounds (8). Of note, false positive results in evoked and non-evoked pain measures may also be observed owing to effects other than analgesia, such as sedation, motor side effects or drug-induced anxiety (9). The negative contradictory findings between rodents and humans have guided a number of researchers to question whether rodents should be used to model human chronic pain and examine the efficiency of analgesic compounds (10). To overcome these concerns, an incorporation of suitable additional assays, such as locomotor activity, may assist with the identification of these issues in OA models and reduce false positives (11). Indeed, a benefit of using an open field locomotor activity analysis may be the recognition of non-specific side effects, including sedation, by the computed animal activity system (12).

Therefore, using traditional methods to measure pain, such as von Frey and weight bearing, in combination with novel pain-related measures, such as the locomotor test, may potentially decrease the possibility of obtaining false positive

results and would provide an improved understanding of the translational capability of preclinical findings.

To decrease the translational gap between rodent and human pain research, the present study aimed to evaluate the rat locomotor activity in the MIA model of OA, which includes alterations that are at least partially pain-related. Using a computed animal activity system, the current study aimed to measure both horizontal (X axis) and vertical (Z axis) locomotor activity, which represent both the overall locomotor activity and repetitive small-scale movements, such as scratching and grooming. Furthermore, ibuprofen was used, as a positive control, and its effects were evaluated using well-established techniques of mechanical allodynia evaluation (von Frey and weight bearing) and the newly proposed locomotor activity system.

Materials and methods

Animals. A total of 29 Adult, male, Sprague-Dawley rats (180-250 g) were used for behavioral experiments. Rats (4-6 weeks old) were purchased from Jordan University of Science and Technology (Irbid, Jordan). The rats were housed in a temperature-controlled environment (22±1˚C; 12 h light/dark cycle; relative humidity, 40-60%) at the animal house unit of The University of Jordan. Animals had free access to food and water, their bedding was altered twice a week and their health status was monitored daily, with no mortalities observed. All experiments were performed in The University of Jordan laboratories. The protocols were approved by the Ethics Committee of The University of Jordan (approval no. 19/2018/322). Behavioral experiments were performed in agreement with the Animals (Scientific Procedure) Act 1986 and the guidelines of the International Association for the Study of Pain (13).

Induction of the osteoarthritic pain model. For the induction of OA, the rats received intra-articular injections of MIA (1 mg in 50 μl saline) in the left knee joint. The selection of the MIA dose was based on previous studies, in which MIA exhibited significant effects in different assays (14,15). This procedure was performed under transient isoflurane (3%) inhalation anesthesia. In the control group, the rats received intra-articular injections of vehicle (3% Tween-20 in saline), in an equivalent amount (50 μl). After determining the baseline nociceptive responses, behavioral testing was performed every other day for 21 days, following MIA injection. At the end of each experiment (day 21), the rats were deeply anesthetized using diethyl ether inhalation and following a negative toe-pinch reflex, rats were euthanized using decapitation.

Assessment of mechanical allodynia. The responsiveness to a punctate pressure stimulus was evaluated using the von Frey filament test. The rats were individually placed in plastic cages, which grant full access to the paws using a wire mesh bottom. Behavioral acclimatization to the testing room was then permitted until cage inspection, and main grooming activities had ceased for at least 25 min. Subsequently, the 'up-down' method was used when applying the von Frey filaments (2-15 g, with logarithmically incremental stiffness; Bioseb; cat. no. BIO-VF-M) to the mid-plantar surface of the left hind paw to evaluate the withdrawal threshold. Every time, the von Frey filament was held for 6-8 sec perpendicularly to the planter aspect of the hind paw. The data are presented as paw withdrawal thresholds (PWT) measured in grams (16,17).

Assessment of weight bearing difference. To assess the postural equilibrium, the difference in weight distribution between the two hind paws was calculated in each rat, using a static weight bearing instrument (Static Weight Bearing Touch: Incapacitance test; Bioseb; cat. no. BIO-SWB-TOUCH-M). The weight was normally equally distributed between both hind paws. The level of pain was assessed by evaluating the weight bearing difference between the injured and uninjured paws. The incapacitance test consists of a plexiglass chamber, in which each rat was allowed to move freely until settled in an appropriate position without leaning on either side of the chamber. The rat then adapted to a suitable weight distribution between the hind paws depending on the degree of pain. The distribution of the weight between the hind paws was measured over a period of 10 sec and the values which applied to each sensor were indicated on the screen in the control unit. These data were subsequently used to calculate the ratio of left hind paw contribution in total weight bearing (18).

Assessment of open field locomotor activity. To evaluate the alterations in locomotor activity, a computed animal activity system (Opto-M4; Columbus Instruments International) was used (18,19). This open field system consists of a 45x25x20 cm arena with two horizontal planes of detector-emitter pairs across the width of the arena, which are positioned 5 and 10 cm above the cage floor. Each horizontal plane is monitored by 16 infrared beams, that are spaced 2.54 cm apart. The total number of beam interruptions occurring due to rat movements were calculated every 5 min using the infrared beams, and sent to a central computer. The animals were placed individually 60 min following the drug injections. The total number of beam interruptions was recorded for 20 min and stored every 5 min. This allows the system to continuously monitor the horizontal (X axis) and vertical (Z axis) activity. The horizontal activity is represented by the lower plane, while the vertical activity is represented by the upper plane. This system also calculates both the total and ambulatory counts. X total counts register a count every time an infrared beam is broken in the lower plane (horizontal counts). This represents both the overall locomotor activity and repetitive small-scale movements, such as scratching and grooming. X ambulatory counts register a count only when a new beam is broken, allowing it to measure actual locomotion (distance traveled in beams rather than centimeters or inches). Z total counts register a count every time an infrared beam is broken in the upper plane (vertical counts) and is utilized to detect rearing or standing on the hind paws.

Pharmacological treatments. The effects of different doses of ibuprofen (50 and 100 mg/kg) on MIA-induced nociceptive behavior were assessed. Ibuprofen was diluted in 3% Tween-20 in saline. The rats were treated with ibuprofen by intraperitoneal injections at day 7 following MIA injection, when mechanical allodynia had developed. The control groups, which received MIA injections without ibuprofen treatment,

received intraperitoneal injections of vehicle (3% Tween-20 in saline) in equal amounts to the ibuprofen treatment. Blinded behavioral experiments were performed 1 h post-injection to evaluate the nociceptive behavior. The behavior was assessed based on alterations in the mechanical PWT and the ratio of the left hind paw contribution in total weight bearing.

Statistical analysis. Data regarding MIA-induced mechanical allodynia are expressed as the mean ± SEM of PWT in grams. The ratio of left hind paw contribution in total weight bearing is also presented as the mean ± SEM. Regarding the locomotor activity, data are presented as the mean ± SEM of X total counts, X ambulatory counts and Z total counts in percentage, and were calculated using the following equation: Percentage counts=counts following MIA/saline/baseline counts. These data were subsequently analyzed using two-way ANOVA with treatment and time as the main factors, allowing both between and within group comparisons, followed by a Holm-Sidak post hoc test, as appropriate. Data regarding the effects of ibuprofen on MIA-induced mechanical allodynia are presented as the mean ± SEM of percentage antinociception, and were calculated according to the following equation: Percentage antinociception=(reading following drug application-reading before drug application)/(reading before MIA injection-reading before drug application). Data regarding the recovery effects of ibuprofen on MIA-induced reduction in locomotor activity are presented as the mean ± SEM of percentage locomotor activity recovery and were calculated as follows: Percentage locomotor activity recovery=(reading following drug application-reading before drug application)/(reading before MIA injection-reading before drug application). These data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test as appropriate. Statistical analysis was performed using GraphPad Prism v6 statistical program (GraphPad Software, Inc.).

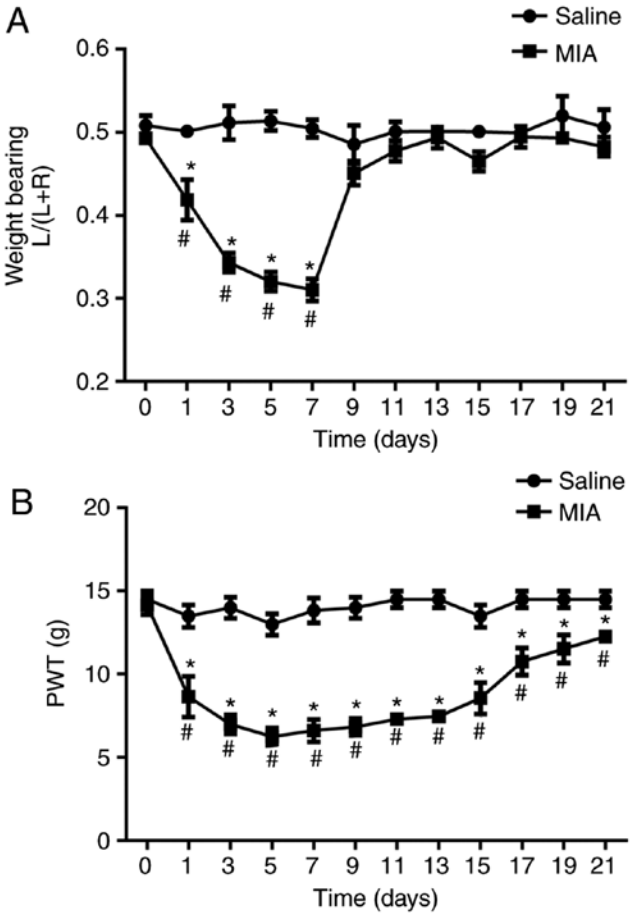

Figure 1. Effects of intra-articular injection of MIA or vehicle (3% Tween-20 in saline) on mechanical allodynia in rats. (A) Ratio of the left hind paw contribution in total weight bearing (L/L+R). (B) Mechanical PWT was measured in grams. Data are presented as the mean ± SEM of n=6 rats. Data was analyzed using two-way ANOVA with Holm Sidak post hoc test. *P<0.05 vs. day 0; #P<0.05 vs. saline group. L, amount of weight bearing in the left hind paws in grams; R, amount of weight bearing in the right hind paw in grams; MIA, monoiodoacetate; PWT, paw withdrawal threshold.

Results

Effects of MIA on nociceptive behavior. At day 1 post MIA injection, the left hind paw contribution in total weight bearing was significantly decreased compared with that in the vehicle-treated control group (0.5±0.01 vs. 0.42±0.02; P<0.05; Fig. 1A). This effect persisted for ~1 week (Fig. 1A). Furthermore, the mechanical PWT was significantly decreased compared with that in the vehicle-treated control group, at day 1 post MIA injection (13.5±0.74 vs. 8.4±0.84; P<0.05; Fig. 1B). This reduction persisted for 21 days, suggesting that the development of nociceptive behavior following MIA treatment.

Effects of MIA on open field locomotor activity. At day 1 post MIA injection, the X total and X ambulatory counts were significantly decreased compared with that in the vehicle-treated control group (100.4±16.3% vs. 37±2.9% and 104.9±10.6% vs. 31.5±5.1%, respectively; P<0.05; Fig. 2A and B). At day 5, the Z total counts were also significantly decreased compared with that in the vehicle-treated control group (102.2±11.2 vs. 54.8±8.5%; P<0.05; Fig. 2C). The reduction of the X total and X ambulatory counts persisted for ~21 days, while the reduction of Z total counts persisted from day 5 to day 13 post-MIA injection (Fig. 2A-C). These data suggested that the rats in the osteoarthritic pain model exhibited a deficit in non-evoked measures (locomotor activity), in addition to the alterations in evoked sensory sensitivity (PWT and weight bearing), which may suggest a nociceptive behavior.

Effects of ibuprofen on the osteoarthritic pain model. At 1-week post MIA injection, when the PWT and left hind paw contribution in total weight bearing were significantly decreased compared with that in the control group, different doses of ibuprofen (50 and 100 mg/kg) were injected intraperitoneally. Behavioral experiments were performed 1 h post-injection to assess the analgesic properties of the drug. Both doses significantly restored the left hind paw contribution in total weight bearing compared with that in the vehicle-treated control group (-2.2±6.6 vs. 55.59±8.7% and -2.2±6.6 vs. 99.2±3.6%, respectively; both P<0.05; Fig. 3A). With respect to mechanical allodynia, both doses of ibuprofen significantly restored the PWT compared with that in the vehicle-treated control group (-7.1±11.3 vs 45.3±10.1% and -7.1±11.3 vs. 50.4±13%, respectively; both P<0.05; Fig. 3B).

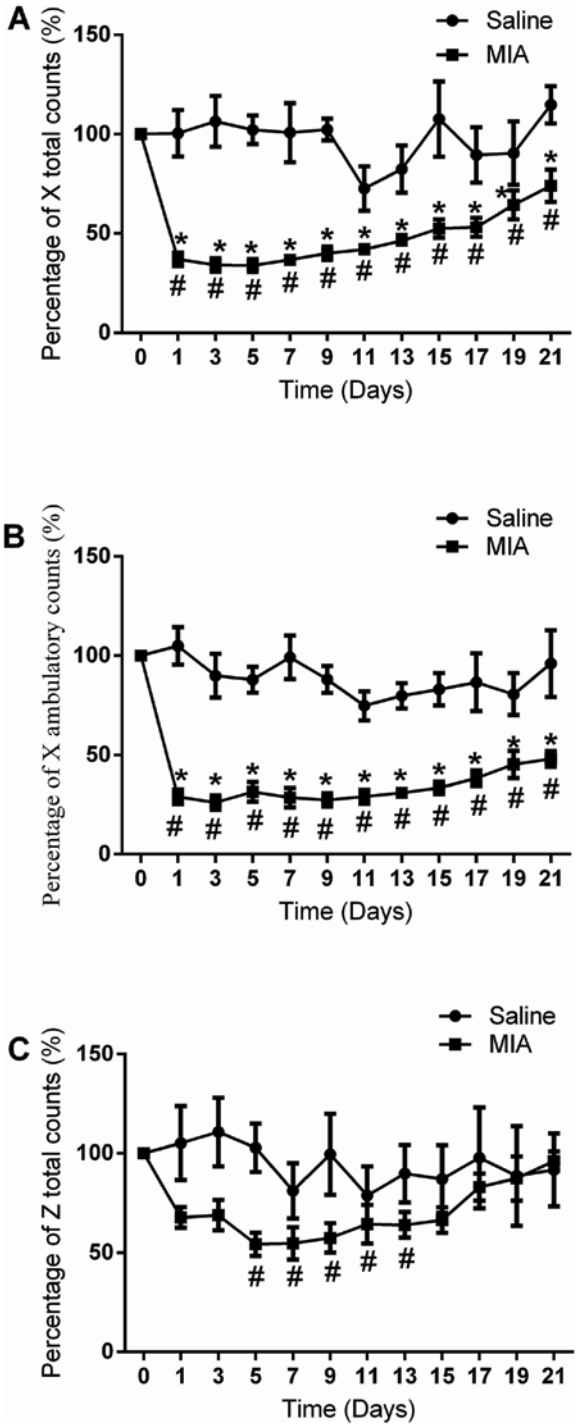

Figure 2. Effects of intra-articular injection of MIA or vehicle (3% Tween-20 in saline) on locomotor activity in rats. (A) X total counts. (B) X ambulatory counts. (C) Z total counts. A significant reduction was observed in X total and ambulatory counts, while the reduction of Z total counts persisted from day 5 to day 13 in MIA-treated compared with that in the vehicle-treated rats. Data are presented as the mean ± SEM of n=6 rats. Data was analyzed using two-way ANOVA with Holm Sidak post hoc test. *P<0.05 vs. day 0; #P<0.05 vs. saline group. MIA, monoiodoacetate.

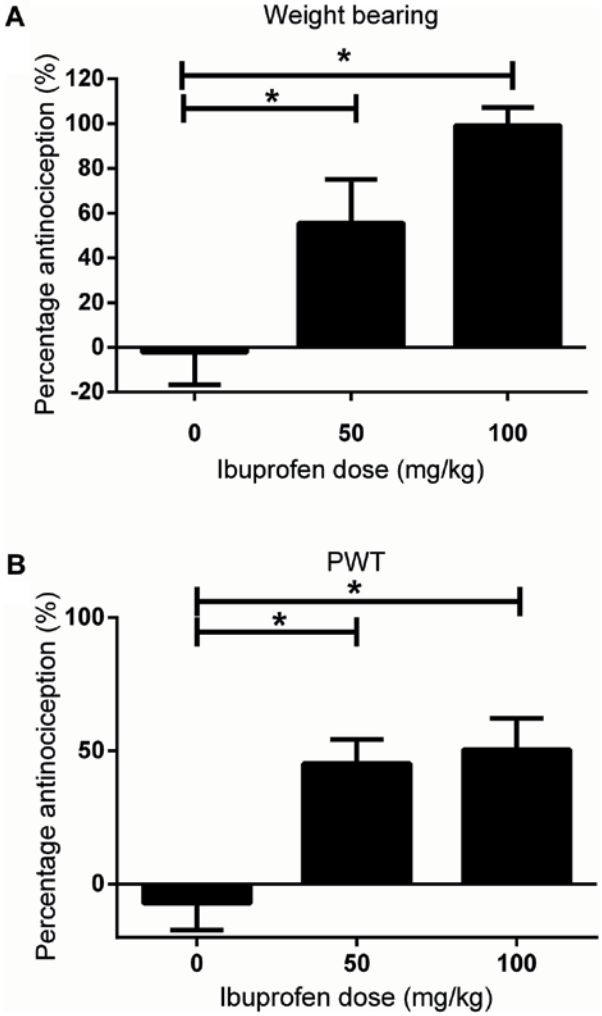

Figure 3. Effects of ibuprofen (50 and 100 mg/kg) or vehicle (3% Tween-20 in saline) on MIA-induced mechanical allodynia in rats. The rats received an intraperitoneal injection of ibuprofen or vehicle at day 7, following MIA injection. Alterations in (A) the left hind paw contribution in total weight bearing and (B) PWT, 1 h post drug injection. Data are presented as the mean ± SEM of percentage antinociception. n=5 rats for the control group and n=6 rats for each of the treated groups (50 and 100 mg/kg). Data was analyzed using one way ANOVA followed by Bonferroni post hoc test. *P<0.05. MIA, monoiodoacetate; PWT, paw withdrawal threshold.

Effects of ibuprofen on open field locomotor activity. To determine if the inhibitory effects of MIA injections on locomotor activity influenced the interpretation of the analgesic effects of ibuprofen, additional experiments were performed.

At 1 week post MIA injection, when the locomotor activity was significantly reduced compared with that in the control group, different doses of ibuprofen (50 and 100 mg/kg) were injected intraperitoneally. At 1 h post-injection, the analgesic properties of the drug were assessed. With respect to the X total counts, only the 100 mg/kg dose significantly restored the counts compared with that in the vehicle-treated control group (22.4±5.2 vs. 5.5±4.6%; P<0.05; Fig. 4A). Both doses (50 and 100 mg/kg) significantly restored the X ambulatory counts compared with that in the vehicle-treated control group (17.4±3 vs. -2.1±3.7% and 28.9±6.1 vs. -2.1±3.7%, respectively; both P<0.05; Fig. 4B). These data suggested that the antinociceptive behavior, that was observed following drug injection, may attribute to the analgesic effects of ibuprofen rather than the reduction in locomotor activity, and also nullified any sedative effect of the drug influencing the nociceptive behavior of the rats. On the other hand, neither dose of ibuprofen restored the Z total counts compared with that in the vehicle-treated control group (40.7±4.5 and 43.8±21.5, respectively, vs. -7.5±12.5%; Fig. 4C).

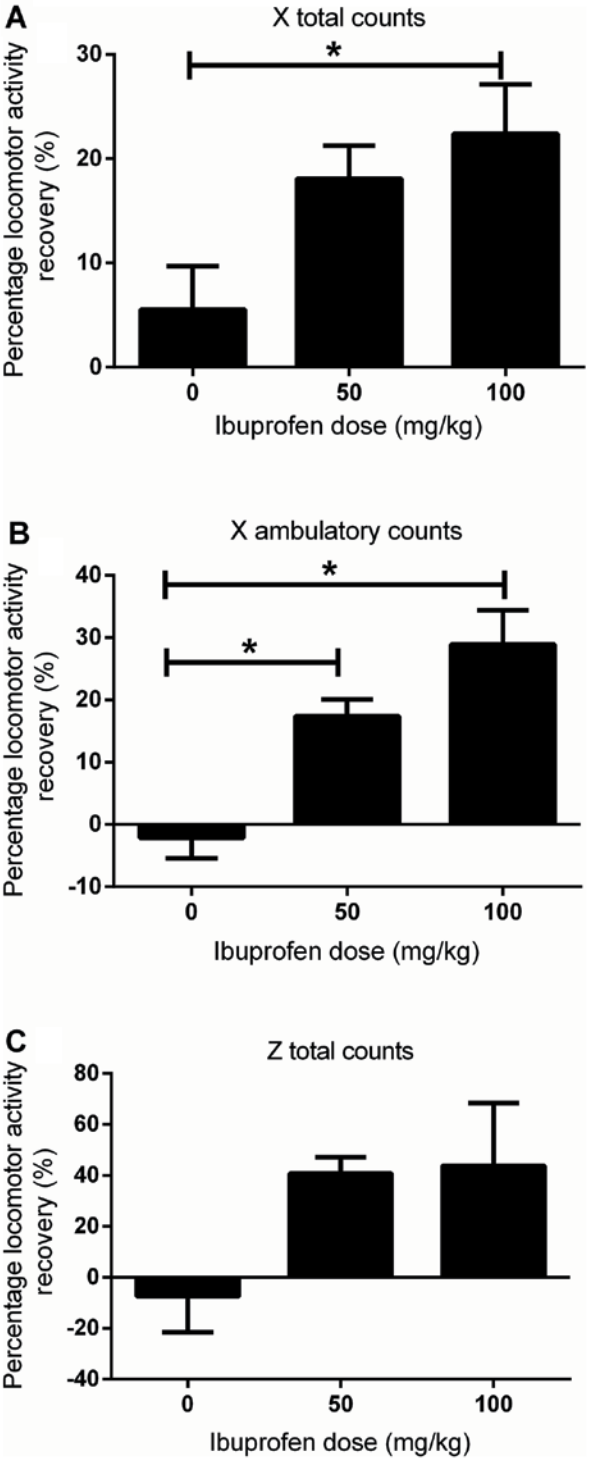

Figure 4. Effects of ibuprofen (50 and 100 mg/kg) or vehicle (3% Tween-20 in saline) on MIA-induced alterations in locomotor activity. The rats received an intraperitoneal injection of ibuprofen or vehicle at day 7, following MIA injection. (A) X total counts. (B) X ambulatory counts. (C) Z total counts. Data are presented as the mean ± SEM of percentage locomotor activity recovery. n=5 rats for the control group and n=6 rats for each of the treated groups (50 and 100 mg/kg). Data was analyzed using one-way ANOVA followed by Bonferroni post hoc test. *P<0.05. MIA, monoiodoacetate.

Discussion

The results of the present study indicated that MIA-treated rats exhibited a significant decrease in the locomotor activity compared with that in the untreated control group. These data suggested that a decline in animal activity may correspond to the inflammatory stage of the animals. This may reflect the association that has been observed between clinical OA and locomotion in rodent models or may indicate that locomotor activity was altered based on the inflammatory stage of the animals (20).

The MIA model was selected to evaluate the activity of analgesic drugs, as it has been found to induce reproducible behavioral alterations (21). Therefore, the MIA model may be used to examine the efficacy of analgesic drugs, with novel mechanisms of action for potential use in OA (22). Indeed, the MIA-induced OA model has been regularly used to evaluate the pain behavior and drug efficacy to resolve the pain in animals (5,6), and may be more indicative of the drug efficacy compared with that in other pain models that are used to test osteoarthritis drugs such as the naturally occurring models (such as elderly hamsters) and genetically modified models (23).

The present study revealed that the decrease in locomotion, which was characterized by the reduction in X total and X ambulatory counts, was associated with the decrease in the PWT, as revealed by the von Frey test. Notably, the reduced PWT remained longer compared with that in the decrease in the weight bearing of the ipsilateral paw (21 days vs. 7 days, respectively). In contrast to this finding, a previous study reported that MIA, at a dose of 1 or 3 mg, induced a significant reduction in the weight bearing of the ipsilateral hind limb from days 3-28 compared with control rats (14). The difference in the duration between the reduced PWT and the weight bearing may be attributed to various potential mechanisms, such as the chondrocyte degeneration, that has been observed at days 1-7 post MIA injection (24) or a potential transient inflammation that has been indicated at days 1-4 post MIA injection (25). The finding that both von Frey and weight bearing tests are not always consistent may suggest the presence of different pain mechanisms. The weight bearing test has been found to be more effective in measuring spontaneous pain (non-evoked painful behaviors), that is often associated with joint degeneration or inflammation arising from peripheral sensitization (15), while the von Frey test has been revealed to be more effective in identifying the evoked reflexive responses and eventually evaluating both peripheral and central sensitization (26). Similarly, the MIA-induced reduction in locomotor activity (X total counts and X ambulatory counts) lasted for the duration of the experiment, indicating that locomotion impairment in the horizontal direction was dependent on both peripheral and central sensitization. On the other hand, the MIA-induced reduction of Z total counts, which represented the vertical movement such as rearing, lasted for 13 days compared with 21 days in the case of X counts, indicating that locomotion impairment in the vertical direction was similar to the deficit in weight bearing and was primarily dependent on peripheral sensitization. On the contrary, a previous study has demonstrated that MIA injection produced prolonged impairment (21 days) in the locomotor activity both in horizontal and vertical directions (20). This discrepancy may be attributed to the higher dose of MIA used in the previous study (3 mg/kg) compared with that used in the preset study (1 mg/kg).

To examine the predictive validity of the open field test for the evaluation of pain and analgesia, the effects of ibuprofen treatment on locomotion were detected in the MIA-treated rats. The results indicated that the MIA-treated rats exhibited a significant recovery of locomotor activity in the X ambulatory counts, following ibuprofen administration, in a dose-dependent manner, while only the higher dose of ibuprofen (100 mg/kg) induced a significant effect on the X total counts. Neither dose of ibuprofen (50 and 100 mg/kg) significantly reversed the reduced locomotion, with respect to the Z total counts; however, the locomotion recoveries in the presence of ibuprofen were higher compared with that in the control animals. This may be attributed to using only 5-6 rats in each experimental protocol in the present study. It can be suggested that ibuprofen may also exhibit a beneficial effect in restoring locomotion in vertical movement, such as rearing. By contrast, Bryden *et al* (27) indicated that clinically used analgesics, such as ibuprofen and morphine, did not exhibit any reversal effects on MIA-induced locomotion deficit, and additionally reported that the burrowing deficit was a more sensitive method of the analgesic effect of a drug than deficits in locomotion. The discrepancy between the results of the present study and those of the aforementioned study may be attributed to the application of a unilateral injection of MIA and a higher dose of ibuprofen in the current study vs. a bilateral injection of MIA and a lower dose of ibuprofen (30 mg/kg) in the aforementioned study (25).

The locomotion test is one of the most known primary behavioral tests and is a common method to evaluate locomotion and potentially other behaviors, such as pain, in rodents (28). The results of the current study are in agreement with those of other studies that have used different pain models and different assays of locomotion. For example, Complete Freund's adjuvant (CFA) has been found to decrease the voluntary wheel running in male and female rats (29-32). Furthermore, rotarod performance was decreased in MIA-treated rats (33) and CFA-treated mice compared with that in the control groups (34).

Measuring sedation and motor dysfunction may limit the use of certain potential antinociceptive compounds, such as cannabinoids, for pain relief (35). Indeed, the false positive effects of measuring a pain-stimulated response (von Frey test) and a pain-suppressed behavior (locomotor test) in animal models are likely to be reduced in clinical trials.

In conclusion, the results of the present study indicated that MIA-induced OA reduced motor activity, and ibuprofen significantly restored the locomotion impairment in the horizontal direction, but not in the vertical direction, suggesting that impairment of locomotion in the horizontal direction was a more sensitive method of the analgesic drug effects. Evaluation of locomotion may aid in the differentiation between the valid analgesic effects and the drug-induced motor impairment or sedative effects. This simple non-invasive quantitative and qualitative method may also aid in developing novel therapeutic strategies to treat OA in humans, and may successfully be used to predict the analgesic efficacy of compounds in models of joint inflammation and OA.

Acknowledgements

Not applicable.

Funding

The present study was funded by the Deanship of Scientific Research at The University of Jordan (grant no. 1748).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

MA, AA and MH conceived the study and acquired funding. MH and AA developed the methodology and validated the data. MA, HK and MH analyzed the data. KES, MH and MA performed the weight bearing testing and analyzed its results. AMA and MH performed the open field testing and analyzed its results. MA and MH drafted the manuscript. MA, MH and AA critically revised and edited the manuscript. KES and SAA critically revised the manuscript and designed the experiments. MH and AMA performed the von Fry testing and its analysis. KES and MA supervised the study. All authors have made substantial contributions to the conception and design of the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The protocols were approved by the Ethics Committee of The University of Jordan (approval no. 19/2018/322).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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