Effects of combined swimming exercise and non-steroidal anti-inflammatory drugs on inflammatory nociception in rats

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Abstract. Pain is a growing health problem with an increasing prevalence, and represents a large burden worldwide. Pain control can be achieved through pharmacological and non-pharmacological (such as exercise) interventions. The prolonged use of analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs), is accompanied by numerous side effects. No previous studies have examined whether exercise may enhance the analgesic effect of NSAIDs. In the present study, the effect of ibuprofen and swimming exercise on nociception threshold were investigated using a rat model of inflammatory pain. A prophylactic swimming protocol and a treatment swimming protocol were used. In the two protocols, nociception was induced by intraplantar injection of Complete Freund's Adjuvant. The authors hypothesized that swimming exercise may enhance ibuprofen-induced antinociception. In the control group, nociception lasted for 17 days, and ibuprofen produced an antinociceptive effect at a dose of 32 mg/kg. However, swimming exercise enhanced ibuprofen-induced antinociception in the two swimming protocols. Notably, ibuprofen produced a significant increase in the nociception threshold at a dose of 10 mg/kg in the prophylactic swimming group. In addition, the duration of inflammation did not exceed 8 days under either swimming protocol. In conclusion, the combination of ibuprofen and swimming exercise was effective in controlling nociception in a rat model of inflammatory pain. Based on these observations, the combined use of exercise and ibuprofen may be a viable intervention for the control of chronic pain, and may decrease the potential for drug-induced side effects.

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Introduction

Pain is a major public health problem and an economic burden. In the United States alone, recent estimates indicate that 100 million adults suffer from pain-associated complaints (1). In Europe, severe and frequent pain is associated with reduced quality of life in the five largest European Union countries (2). In addition, the treatment of pain places a large burden on the economy and health services. Indeed, it is estimated that the annual cost of pain in the United States alone ranges between $560 and $635 billion (including the direct healthcare costs and health-associated loss of productivity); this figure is greater than the annual costs of heart disease, cancer and diabetes management combined (3).

Pain is classified into either acute or chronic types depending on its duration. One-fifth of the world's population is thought to suffer from chronic pain (4). Opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications for the management of chronic pain (5). Despite their well-documented analgesic effects, opioids and NSAIDs have a number of disadvantages if used over a long period. For example, the chronic use of opioids increases the risk of serious side effects, such as tolerance, constipation and physical dependence (6). By contrast, the chronic use of NSAIDs (including ibuprofen) is associated with an increased risk of gastrointestinal bleeding, ulcers and thrombotic events (6).

Given the limitations associated with the use of NSAIDs and opioids for the management of chronic pain, alternative strategies have been proposed, such as combining the above analgesics with non-pharmacological interventions (7,8). The use of such a multimodal approach is expected to have added benefits over the sole use of pharmacological agents. For instance, combining analgesics with non-pharmacological interventions may achieve the same therapeutic effect of NSAIDS or opioids at lower doses. This dose reduction may subsequently decrease the risk of adverse drug side effects and, in turn, reduce treatment costs. Of note, the lowest effective dose of NSAID for the shortest time period is recommended where possible (9). A number of non-pharmacological interventions have been proposed for the management of chronic pain (10); with exercise as the most recommended

intervention. The World Health Organization recognizes insufficient physical activity as a leading risk factor for the increase in chronic pain worldwide (11). In addition, a review of the literature indicated that exercise is effective in reducing chronic pain, whilst improving functional and psychological status (12). A limitation of the studies that have investigated the benefits of exercise in the management of chronic pain is the use of subjective outcome measures, such as questionnaires, as a primary method of assessment. These studies, although useful in predicting or confirming associations, fail to establish causal relationships and are inherently not well controlled. Accordingly, the use of a suitable animal model would be a supplemental approach to study the effect of exercise on chronic pain using more objective outcome measures.

Complete Freund's Adjuvant (CFA) is a solution that is widely used to study nociception in animals (13). CFA consists of heat-killed *Mycobacteria* in suspension, which induces a local inflammatory reaction at the site of injection. These injections induce peripheral tissue injury accompanied by an increased sensitivity to thermal and mechanical stimuli. The authors of the current study hypothesized that swimming exercise may produce an additive effect on NSAID-induced antinociception. To test this hypothesis, intraplantar injections of CFA were used to induce nociception in experimental rats. The effect of swimming exercise, alone or in combination with ibuprofen, on the threshold of nociception was then examined. To the best of the authors' knowledge, this is the first study to evaluate the effects of combined swimming exercise and NSAIDs in a rat model of chronic inflammatory pain.

Materials and methods

Animals. A total of 78 adult (8 weeks old) male Sprague Dawley rats (weight, 180-250 g at the time of study commencement) were used in this study. All rats were obtained from the animal housing facility at Jordan University of Science and Technology (JUST; Irbid, Jordan). Rats were housed in stainless steel wire cages, with 3 rats/cage. The temperature was controlled at 22±2˚C and the rats were exposed to 12 h light/dark cycles with lights on between 6:00 a.m. and 6:00 p.m. Tap water and standard chow were provided *ad libitum*. All experimental procedures were conducted between 9:00 a.m. and 3:00 p.m. All rats were kept for 14 days for habituation prior to the initiation of any intervention. The study protocol was approved by the National Committee of Animal Care and Use at JUST.

Induction of inflammation. CFA was used to elicit an immune response at the site of injection. A volume of 100 μl CFA (containing 1 mg/ml *Mycobacterium tuberculosis*; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used to induce the inflammation. On the day of injection, the rats received light anesthesia using an isoflurane vaporizer (Ugo Basile S.R.L, Gemonio, VA, Italy). Intraplantar CFA or its vehicle (saline) was administered to the left hind paw (CFA paw), while the right hind paw (control paw) received saline.

Mechanical allodynia. Mechanical allodynia was assessed by measuring the paw withdrawal threshold. Following the acclimation period and prior to any injection, rats were habituated to a plastic apparatus placed over a wire mesh bottom that facilitates poking of the paw from below for 30 min on two consecutive days. Threshold readings were measured for three consecutive days to serve as a baseline prior to any CFA injections. Assessment was conducted using Aesthesio® von Frey filaments (Bioseb, Vitrolles, France) in a simple up-down method, as suggested previously (14). The filament sizes ranged between 0.4 and 15.0 g. The results were recorded as binary values (i.e., response or no response).

Swimming protocol. The swimming protocol described by Ozbek *et al* (15) and Wang *et al* (16) was used in the present study. In this protocol, rats were randomly assigned to one of six experimental groups (n=8 in each group): i) No CFA + ibuprofen + sham swimming; ii) 100 μl CFA + ibuprofen + sham swimming; iii) no CFA + ibuprofen + swimming treatment; iv) no CFA + ibuprofen + prophylactic swimming; v) 100 μl CFA + ibuprofen + swimming treatment; and vi) 100 μl CFA + ibuprofen + prophylactic swimming.

Rats were placed in one of two identical metal tanks measuring 60x100x60 cm (width, length and depth, respectively). The tanks were filled with tap water at 32±1˚C, and a drop of soap was added to minimize the surface tension and floating behavior. On the first day, the rats were allowed to swim for a 5-min duration session. Subsequently, the duration of the swimming exercise was gradually increased by 5 min/day until the rats were able to swim for 30 min continuously. All swimming sessions were supervised to ensure that every rat received the required exercise. In rare instances when signs of floating appeared, gentle stirring of the water was performed by the observer to create a water current and stimulate swimming. Rats receiving sham swimming (control) were introduced to shallow water (5 cm), for 30 min/day for 5 days/week. Rats in the swimming experiments were divided to two subgroups: Prophylactic and treatment. In the prophylactic swimming group, rats received the swimming exercise for 30 min continuously each day, 5 days/week for 5 weeks (2 weeks prior to CFA injection and 3 weeks after CFA injection). Rats receiving swimming treatment swam for 30 min continuously each day, 5 days/week for three weeks after CFA injection only. Following swimming, the rats were removed from the tank and gently dried with a cloth before they were returned to their cages.

Assessment of mechanical allodynia was performed daily directly prior to the swimming sessions. A summary of the general workflow is presented in Fig. 1.

Drugs. Ibuprofen was administered at four doses (vehicle, 3.2, 10 and 32 mg/kg; based on a pilot study conducted in the laboratory), and each dose was administered after at least 48 h had elapsed since the previous dose to eliminate any leftover effect. Thus, ibuprofen doses were administered in a randomized Latin-square design on days 5, 7, 9, and 11 post-CFA injection. Ibuprofen powder was obtained from the Jordanian Pharmaceutical Manufacturing Company Co. PLC (Naour, Jordan) and dissolved in a mixture of 20% ethanol, 10% cremophor oil and 70% water to prepare the required concentrations. The assessor was blinded to the ibuprofen doses throughout the experiment. On the ibuprofen test day, the von Frey readings were performed twice, immediately

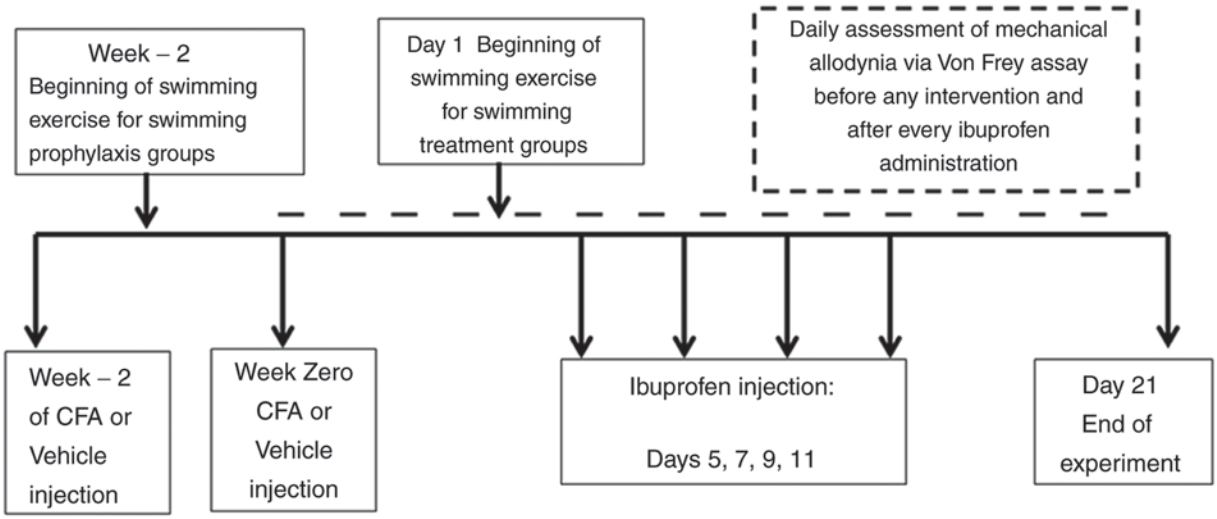

Figure 1. Timeline of the experimental design.

prior to drug administration and 60 min following ibuprofen administration.

Statistical analysis. The primary dependent measure to assess mechanical allodynia was the paw withdrawal threshold. Results are expressed as the mean ± standard error of the mean. Analysis was performed using repeated measures two-way analysis of variance followed by Bonferroni's post hoc test. All statistical analyses and tests were performed using GraphPad Prism software version 6.07 (GraphPad Software, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

The percentage change in antinociception was calculated using the following equation: Change (%) = [(threshold after ibuprofen-threshold before ibuprofen)/(baseline threshold-threshold before ibuprofen)] x100.

Results

Effect of ibuprofen on CFA-induced mechanical allodynia. Fig. 2 illustrates the effect of ibuprofen on CFA-induced mechanical allodynia. The CFA vehicle did not produce any significant difference in nociception threshold between the two paws [$F(1, 14)=0.2628$; $P=0.6162$; Fig. 2A]. In the CFA group, the threshold was significantly lower in the CFA paw compared with the control paw from day 4 until 20 days following CFA injection [$F(1, 14)=54.56$; $P<0.0001$; Fig. 2B]. However, ibuprofen (32 mg/kg) partially reversed CFA-induced allodynia [$F(1, 28)=21.74$; $P<0.0001$], without producing any significant effect in the CFA vehicle group [$F(1, 28)=2.853$; $P=0.1023$; Fig. 2C and D].

Effect of swimming exercise and ibuprofen on the CFA vehicle groups. Fig. 3 illustrates the effect of prophylactic swimming and swimming treatment on the paw withdrawal threshold in the absence and presence of ibuprofen. No significant difference in the nociception threshold between the two paws in the groups receiving prophylactic swimming [$F(1, 14)=0.00001421$; $P=0.9970$] or swimming treatment [$F(1, 14)=0.003911$; $P=0.9510$; Fig. 3A and B] was observed. In addition, ibuprofen did not produce a significant change in threshold in either group, regardless of ibuprofen dose (Fig. 3C and D).

Effect of swimming exercise and ibuprofen on CFA-induced mechanical allodynia. Fig. 4 illustrates the effects of ibuprofen in combination with prophylactic swimming or swimming treatment on CFA-induced mechanical allodynia. In the prophylactic swimming group, ibuprofen administration produced a dose-dependent increase in nociception threshold, which was significant at 10 and 32 mg/kg (from 3.22 to 6.7 g and from 3.83 to 11 g, respectively) compared with pre-ibuprofen administration [$F(1, 56)=42.16$; $P<0.0001$]. In addition, ibuprofen produced a significant increase in the nociception threshold at 32 mg/kg in the swimming treatment group [$F(1, 56)=14.90$; $P=0.0003$].

Effect of swimming exercise and ibuprofen on CFA-induced mechanical allodynia. Fig. 5A illustrates the effect of prophylactic swimming and swimming treatment on paw withdrawal threshold in the presence of ibuprofen. Prophylactic swimming produced a significantly higher threshold when compared with the sham swimming group on days 2-4 and 15-20 following CFA injection. Likewise, swimming treatment produced a significant increase in threshold compared with sham swimming, which was significant on days 1-3 and 14-20 following CFA injection. Statistical analysis revealed a significant effect of swimming exercise on mechanical allodynia in prophylactic swimming and swimming treatment groups when compared with the sham swimming group [$F(2, 21)=14.54$; $P=0.0001$]. Fig. 5 also demonstrates the effect of different swimming exercise protocols on CFA-induced mechanical allodynia in the CFA paw following the administration of ibuprofen. The nociception threshold following administration of 10 mg/kg ibuprofen was 3.53 g in the sham swimming group, 7 g in the swimming treatment group, and 6.77 g in the prophylactic swimming group. The thresholds in the latter two groups were significantly higher compared with that in the sham swimming group ($F=29.33$; $P<0.0001$; Fig. 5B). Similarly, the thresholds in the swimming treatment and prophylactic swimming

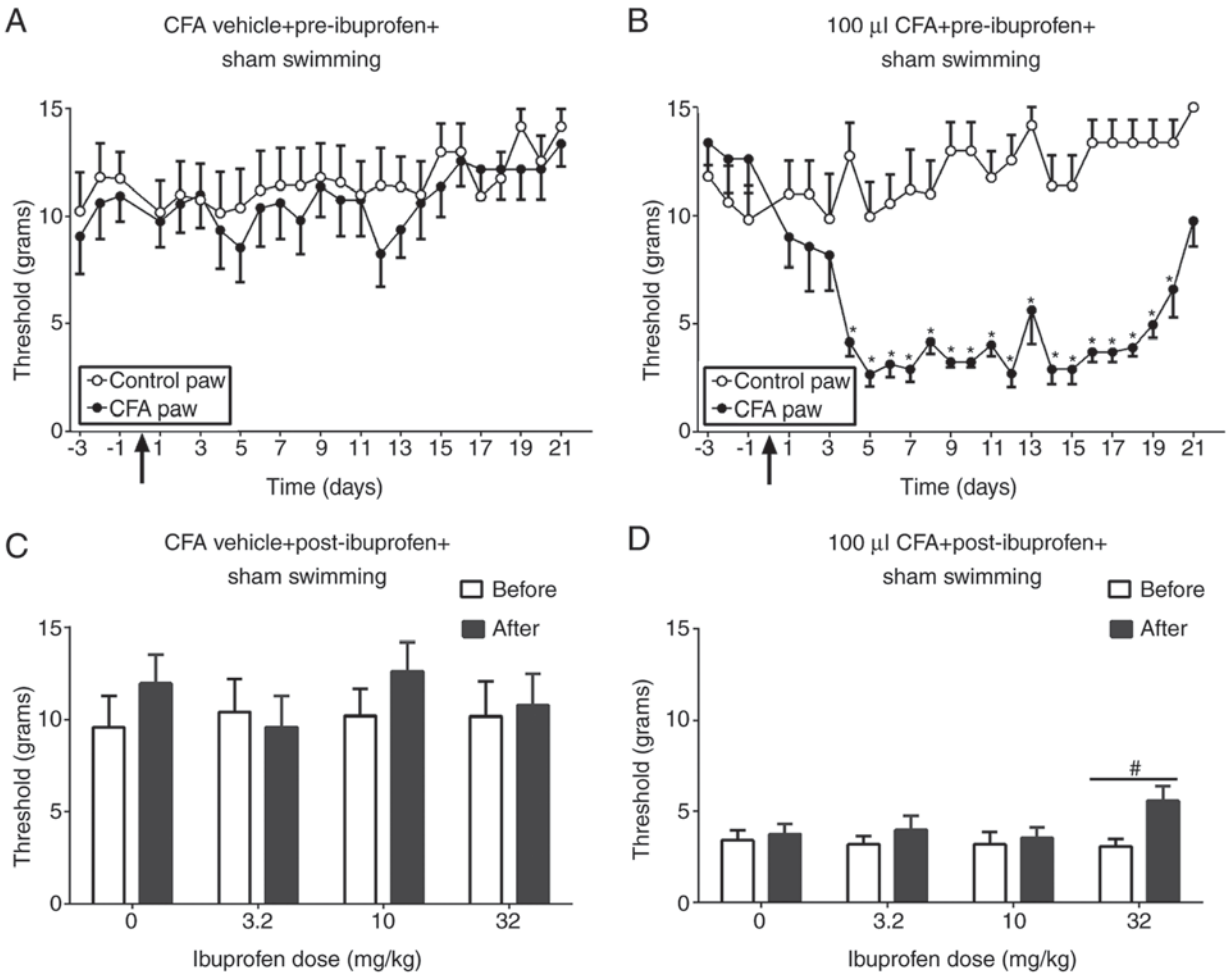

Figure 2. Effect of ibuprofen on CFA-induced mechanical allodynia. Panels (A) and (B) demonstrate the results of daily mechanical allodynia prior to any injection on each day. *P<0.05 vs. the control paw. Arrows indicate the time of CFA injection. Panels (C) and (D) demonstrate the effect of ibuprofen on CFA-induced mechanical allodynia in the CFA-injected paw. Ibuprofen doses or vehicle were injected on days 5, 7, 9, and 11 in a Latin-square design. #P<0.05 indicates a significant within-dose effect. Each data point represents the mean ± standard error of the mean (n=8 rats). CFA, Complete Freund's Adjuvant.

groups were significantly higher compared with the threshold in the sham swimming group following the administration of 32 mg/kg of ibuprofen (F=12.37; P<0.0001; Fig. 5B); these were 5.58 g in the sham swimming group, 10.57 g in the swimming treatment group, and 11 g in the prophylactic swimming group.

When the change in threshold was converted into a percentage change in antinociception, 32 mg/kg ibuprofen only produced only a 23.11% change in the sham swimming group. In comparison with the sham swimming group, the percentage change was significantly higher in the swimming treatment and prophylactic swimming groups, such that it was increased to 68.12 and 91%, respectively (F=9.189; P=0.0002; Fig. 5C). Only the prophylactic swimming group produced a significantly greater change in percentage antinociception following the administration of 10 mg/kg ibuprofen (43.9%) when compared with the sham swimming group, which was only 4.30% (F=20.22; P<0.0001; Fig. 5C).

Discussion

The present study examined the effect of swimming treatment and prophylactic swimming in combination with ibuprofen on CFA-induced nociception in rats. There were three principal findings: First, swimming treatment and prophylactic swimming shortened the duration of CFA-induced nociception when compared with the non-swimming group. Second, the two types of swimming enhanced the efficacy of ibuprofen-induced antinociception. Third, prophylactic swimming increased the potency of ibuprofen to produce antinociception. Collectively, these results indicated that exercise may enhance the antinociceptive effect of ibuprofen in inflammatory conditions

CFA is commonly used in rodents to induce nociception for up to 4 weeks (17,18). The results of the current study are consistent with the well-established profile of CFA-induced inflammation reported in previous literature (19). Ibuprofen is widely used in the clinic for the management of different types of inflammatory pain, including osteoarthritis and rheumatoid arthritis (20). In the present study, ibuprofen produced significant antinociception effects in CFA-induced mechanical allodynia at a dose of 32 mg/kg without shortening the duration of CFA-induced mechanical allodynia. This result was consistent with previous studies that examined the dose-associated effects of ibuprofen in CFA-induced mechanical allodynia (20,21). An additional study reported that the median effective dose of ibuprofen alone was 22 mg/kg, with no significant antinociception observed below 17.8 mg/kg (22).

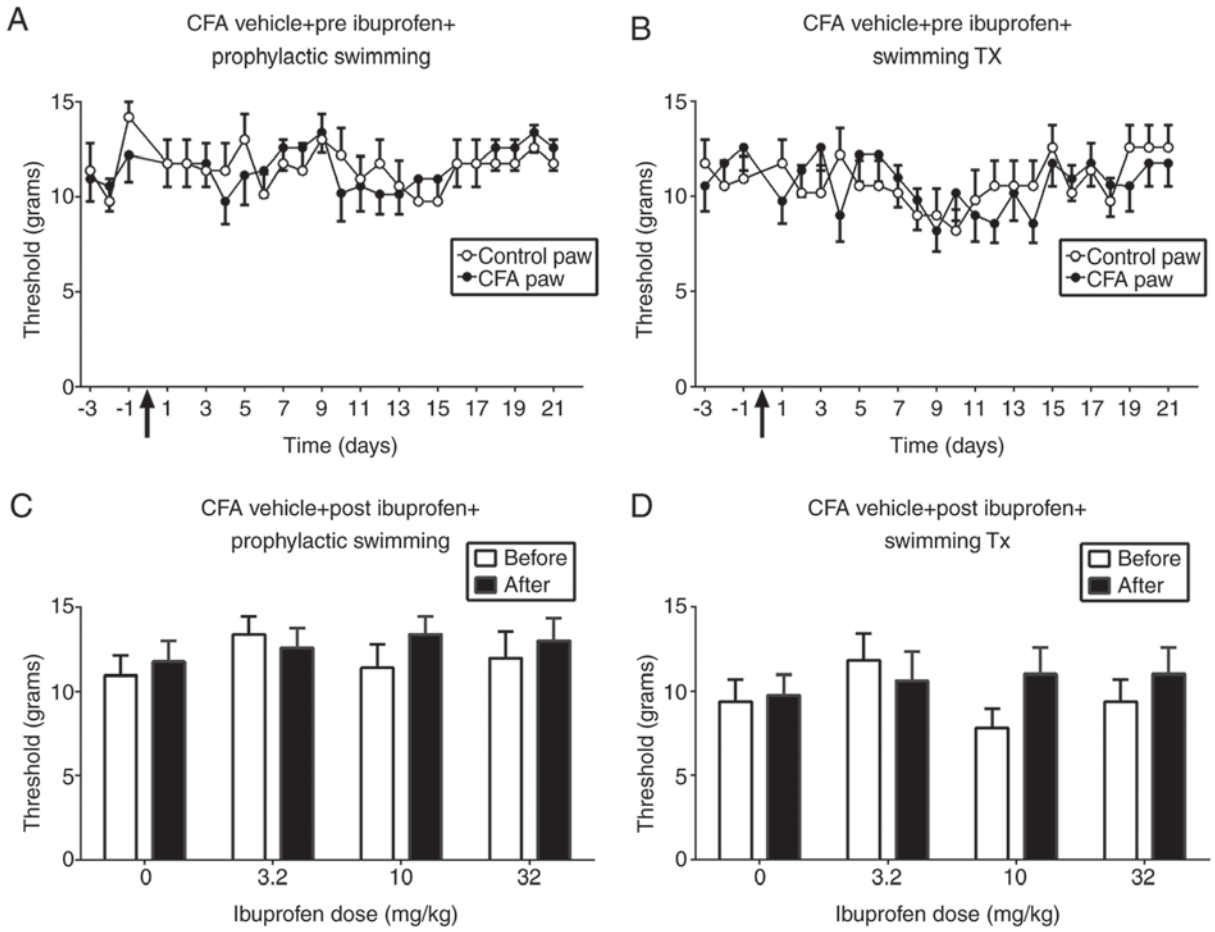

Figure 3. Effect of swimming exercise and ibuprofen on the nociception threshold. Panels (A) and (B) demonstrate the results of the daily paw withdrawal threshold prior to any injection on each day in the prophylactic swimming and swimming treatment groups, respectively. Arrows indicate the day of CFA vehicle injection. Panels (C) and (D) demonstrate the effect of ibuprofen on the CFA vehicle-injected paw withdrawal threshold together with prophylactic swimming or swimming treatment, respectively. Ibuprofen doses or vehicle were injected on days 5, 7, 9, and 11 in a Latin-square design. Each data point represents the mean ± standard error of the mean (n=8 rats). CFA, Complete Freund's Adjuvant.

The present study demonstrated that swimming exercise decreased the duration of CFA-induced mechanical allodynia. The duration of mechanical allodynia produced by CFA was 15 days in the control group (between days 4 and 19), while in the swimming groups the duration of mechanical allodynia was decreased to only 10 days. Similar results were reported previously in a post-ischemic inflammatory model (23,24). Additional studies have reported that exercise facilitates the recovery of brain and spinal cord injuries in a relatively short time frame, with better physical and cognitive outcomes (25,26). The current study adds to a growing body of evidence indicating that swimming exercise shortens the recovery period of inflammatory conditions, including osteoarthritis.

In the present study, a non-weight bearing exercise (NWB) (i.e., swimming) was selected, rather than weight-bearing (WB) exercise (e.g., walking and running). WB exercises generate force activity that exerts load on skeletal regions. On the other hand, NWB exercises are performed while the bodyweight of the individual is supported artificially (i.e., without the person supporting his/her own weight), and thus have the advantage of being performed without any added load from the bodyweight itself (27). The choice of either type of exercise depends on the specific considerations of the patient. For example, engaging individuals with joint pain in WB exercises has the potential to exacerbate disease symptoms. This is attributed to the excessive loading of the joint, which can increase swelling, inflammation and pain (28). Age and risk of falling are also significant considerations that should be considered when selecting the type of exercise. Falling is the primary cause of fatal and nonfatal injuries in individuals aged >65 years (29) and 54% of falls occur during exercise, with 15% while walking (30). Water-based exercise increases energy expenditure with decreased impact loading on the joints or injured tissues. The resistance characteristics of water supports bodyweight during exercise, and thus positive outcomes will be achieved while avoiding the joint load and increased falling risk associated with WB exercise (31). In one prospective randomized study, water gymnastics was effective in reducing the intensity of back pain during pregnancy (32).

For the aforementioned reasons, swimming, as an NWB exercise, was selected to examine its effect on nociception in combination with ibuprofen in the present study. Swimming exercise leads to a significant reduction in acetic acid-induced nociception in mice (33). In addition, repeated sessions of swimming exercise was observed to significantly reduce mechanical allodynia in a mouse model of chronic neuropathic pain (34). The swimming protocol used in these previous studies is

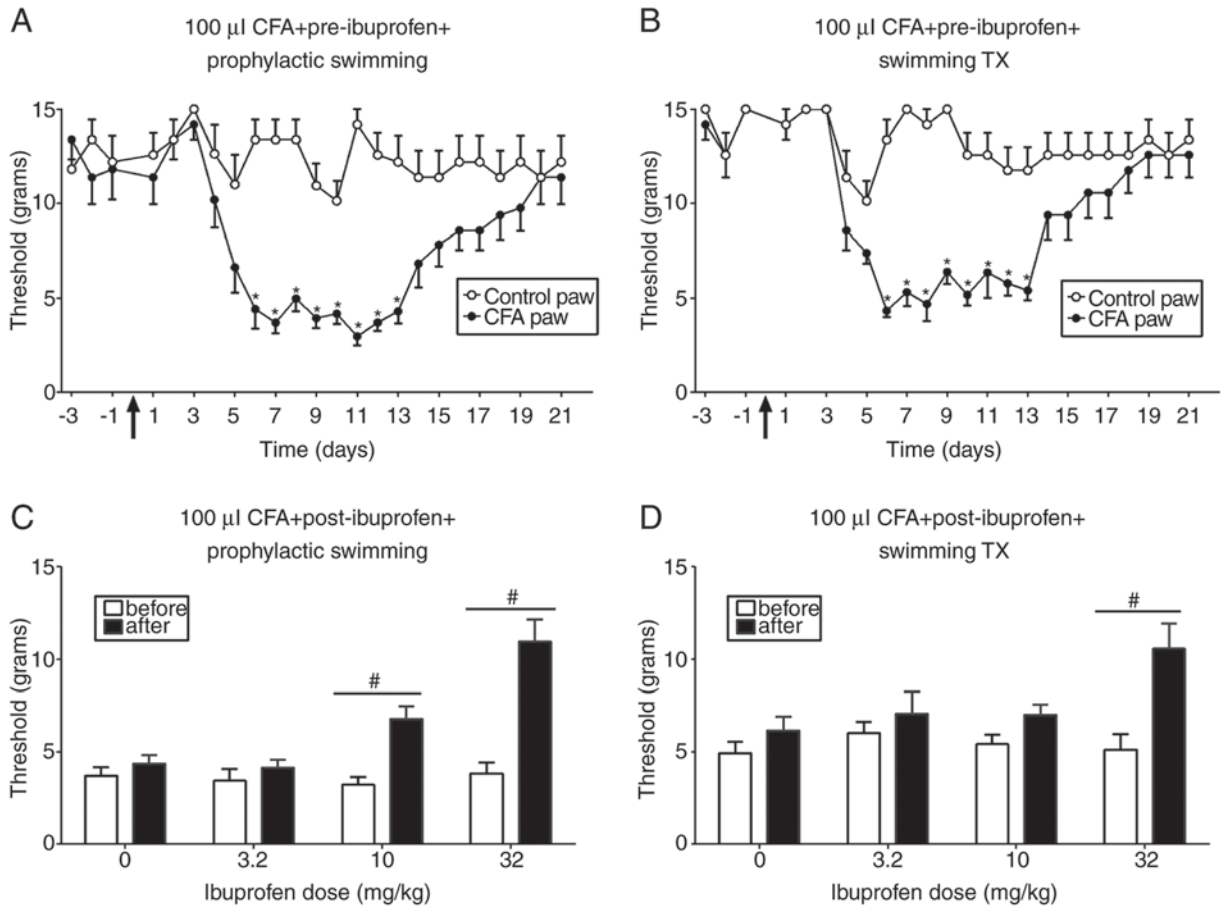

Figure 4. Effect of swimming exercise and ibuprofen on CFA-induced mechanical allodynia. Panels (A) and (B) illustrate the effect of ibuprofen on CFA-induced mechanical allodynia in the prophylactic swimming and swimming treatment groups, respectively. The arrows indicate the time of injection. *P<0.05 vs. Control paw. Panels (C) and (D) demonstrate the effect of ibuprofen and swimming on CFA-induced mechanical allodynia in the CFA-injected paw. Ibuprofen doses or vehicle were injected on days 7, 9, 11, and 13 in a Latin-square design. #P<0.05 indicates a significant within-dose effect. Each data point represents the mean ± standard error of the mean (n=8 rats). CFA, Complete Freund's Adjuvant.

consistent with the swimming protocol employed in the current study. This high-intensity extended swimming exercise protocol is clinically relevant, as it is the protocol recommended by the American Heart Association and the American College of Sports Medicine (35). The association recommended that all adults between 18 and 65 years of age engage in 30 min of moderately intense physical activity each day for at least 5 days/week. These recommendations were the result of cumulative evidence that revealed the health benefits of regular exercise and an active lifestyle (25). Prophylactic swimming, in particular, was reported to ameliorate diabetes-induced muscular atrophy (36), prevent bone mass loss (37), and prevent diabetes and hypertension-associated inflammatory consequences (38,39). In the present study, prophylactic swimming exercise was efficacious in preventing inflammatory pain.

The two swimming protocols employed in the present study, increased the efficacy of the antinociceptive effect of ibuprofen. Ibuprofen produced a significant increase in the nociception threshold at 10 and 32 mg/kg in the swimming treatment and prophylactic swimming groups when compared with the control group. A recent study demonstrated that combined indomethacin (another NSAID) and exercise training produced more rapid recovery from the inflammatory process associated with ischemic infarcts compared with exercise training alone (24). Although swimming exercise may produce fatigue (40), the authors of the present study considered that the observed increase in nociception threshold from combined ibuprofen and swimming treatment was primarily due to direct antinociceptive effects of ibuprofen rather than a fatigue-induced effect for three reasons: First, the percentage change in antinociception did not reach (or exceed) 100% in any group, although the equation used would allow for the detection of such an effect; second, daily swimming sessions were conducted following the administration of ibuprofen and von Frey testing to eliminate any fatigue or swimming-induced stress; finally, no change in swimming behavior was noted by the assessor in any of the treatment groups. These results are therefore consistent with the primary target of pain control, which is to relieve pain while restoring normal function and the ability to perform daily activities (41).

The results of the present study demonstrate that prophylactic swimming produces improved outcomes with respect to ibuprofen-induced antinociception when compared with swimming treatment plus ibuprofen treatment. For example, the percentage change in antinociception of 32 mg/kg ibuprofen was higher in the prophylactic swimming group when compared with the swimming treatment group. In addition, prophylactic swimming increased the potency of ibuprofen to

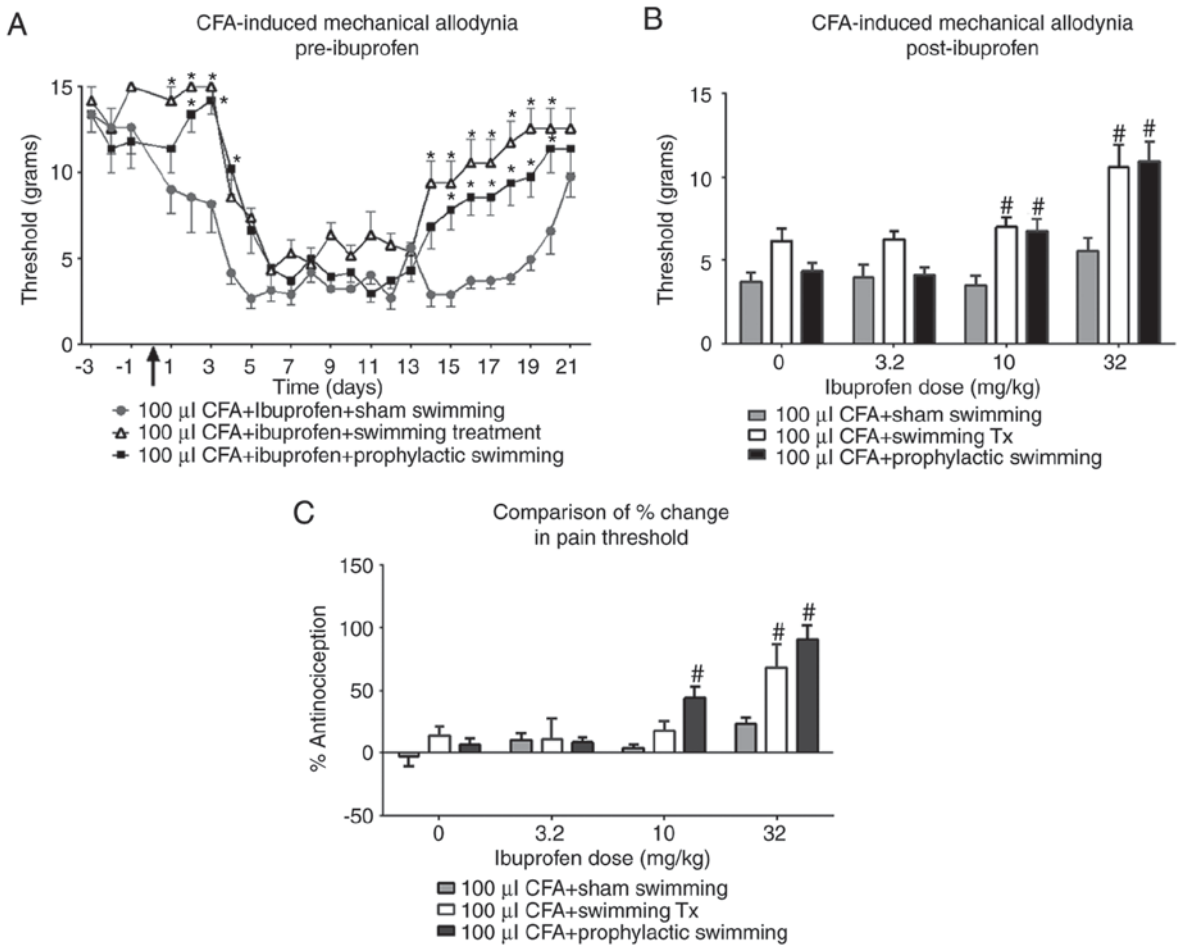

Figure 5. Effect of swimming exercise and ibuprofen on CFA-induced mechanical allodynia. (A) Effect of swimming exercise on CFA-induced mechanical allodynia. The arrow indicates the time of injection. *P<0.05 vs. the sham swimming group. (B) The absolute effect of ibuprofen in the different treatment conditions. (C) Percentage change in antinociception following ibuprofen administration under the different treatment conditions. #P<0.05 vs. sham swimming at the same dose. Each data point represents the mean ± standard error of the mean (n=8 rats). CFA, Complete Freund's Adjuvant.

produce antinociception, such that the lower doses (10 mg/kg) produced a significant increase in the nociception threshold. The present results thus support the recommendation of continuous physical activity as a strategy to decrease pain in patients with chronic arthritis (42). Growing evidence has recently changed the common misconception that exercise may be harmful to patients suffering from pain (43). It has also been reported that people with an active lifestyle have a decreased risk of developing inflammatory diseases that are associated with chronic pain (12). Furthermore, it is recommended that individuals with no active injury should be encouraged to exercise regularly as a type of prophylactic pain management strategy (44). Finally, the results of the current study are consistent with a previous study demonstrating that physically active subjects reported a 1.5-4-fold lower prevalence of daily analgesic use upon developing chronic pain conditions (45).

The mechanism by which exercise enhances the antinociceptive effect of ibuprofen was not investigated in the present study. However, earlier studies have demonstrated that repeated exercise in rats increases the cerebrospinal fluid and plasma concentrations of endogenous opioids with long-lasting antinociception (46). Endorphins are produced from the pituitary gland and the hypothalamus following pain, excitement and exercise; they lead to analgesia and a sense of well-being by activating μ-opioid receptors (47-49). Increased production of endogenous opioids results in antinociceptive effects in animals and humans (50). These results were further confirmed when exercise-induced antinociception was reversed by naltrexone; an opioid receptor antagonist (51). Furthermore, combining opioids and NSAIDs produced synergistic antinociceptive effects in many preclinical nociception models, such as a post-operative rat pain model (52) and neuropathic pain model (53), as well as in clinical studies (54).

One limitation of the present study was the use of a subjective nociception assessment method (von Frey). To overcome this subjectivity, the assessor was blinded to the administered ibuprofen doses. The administration of CFA was not able to be blinded due to the obvious signs of inflammation (i.e., redness and swelling). A second limitation is the lack of data regarding the molecular assessment of inflammatory biomarkers. The assessment of inflammatory biomarkers is important, as they may determine the mechanism by which exercise is able to enhance ibuprofen-induced antinociception. The biomarkers usually assessed in similar studies include interleukin (IL)-6, IL-1β, tumor necrosis factor-α, endogenous endorphins, C-reactive protein and numerous others (55). Given the positive effects of swimming exercise on nociception demonstrated in the current study, it is hypothesized that

swimming may also produce a decrease in the concentration of inflammatory biomarkers. As such, future studies that will measure inflammatory biomarker levels in an animal model of inflammatory pain with and without exercise is warranted. Further studies may include the evaluation of NSAIDs other than ibuprofen, as well as assessing the effects of swimming sessions of different durations, to examine the possibility of constructing a dose-response relationship for swimming exercise intervention.

In conclusion, the results of the present study demonstrated that combined ibuprofen and swimming exercise may be effective in controlling nociception in a rat pain model. The ability of exercise to enhance ibuprofen-induced antinociception suggests that this combination may be recommended as an effective intervention to help control chronic pain. In addition, prophylactic swimming may be combined with ibuprofen administration to enhance its potency, and thus decrease its side effects.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article. However, detailed datasets and individual data points are available from the corresponding author on reasonable request.

Authors' contributions

AA was responsible for designing the study and writing the manuscript. ZK was responsible for conducting the experiments and writing. SK was involved in the conception of the study and data interpretation. MaA performed the statistical analysis and writing. MoA was responsible for designing the study and writing the manuscript.

Ethics approval and consent to participate

The study protocol was approved by the National Committee of Animal Care and Use at Jordan University of Science and Technology and complied with the National Research Council Guide for the Care and Use of Laboratory Animals.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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