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Effects of one minute and ten minutes of walking activity in rats with arthritis induced by complete Freund's adjuvant on pain and edema symptoms

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ABSTRACT

This study evaluated the effects of two protocols of exercise on nociception, edema and cell migration in rats with CFA-induced arthritis. Female Wistar rats (200 – 250 g, n = 50) was monoarthritis-induced by complete Freund's adjuvant (CFA; *Mycobacterium butyricum*, 0.5 mg/mL; 50 μL) into the right knee joint (TF; n = 24) or right ankle joint (TT; n = 26). Incapacitation was measured by the paw elevation time (TEP; s) in 1-min periods of observation. The edema of the knee or ankle joints was evaluated by the variation of the articular diameter (DA, cm) and by the paw volume variation (EP, mL), respectively. Both were measured during 10 consecutive days. Two protocols of exercise were performed: (a) in the constant exercise group (TF, n = 6; TT, n = 6) performing 1 minute of daily exercise on the cylinder; (b) variable exercise group (TF, n = 6; TT, n = 7), the exercise increased by 1 minute per day. The control groups (TF, n = 12; TT, n = 13) didn´t perform the exercise. After 10 days, the animals were euthanized for total (CT; cells/mm^3) and differential leukocyte counts (mononuclear — MON, and polymorphonuclear — PMN, cells/mm^3) of the articular inflammatory exudate. The variable exercise protocol inhibited incapacitation and edema for both joints. However, cell migration decreased only in the TF. The constant exercise reduced edema in both joints, and cell migration was decreased in the TT. However, the incapacitation was not reduced. Variable exercise seemed to be more effective in reducing the inflammatory parameters than constant exercise.

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Efeitos de um minuto e dez minutos de deambulação em ratos com artrite induzida por adjuvante completo de Freund sobre os sintomas de dor e edema

Palavras-chave:
Atividade física
Artrite
Incapacitação articular
CFA

RESUMO

Este estudo avaliou o efeito de dois protocolos de exercício na nocicepção, edema e migração celular em ratos com artrite induzida por CFA. Ratos Wistar fêmeas (200 – 250 g, n = 50) foram induzidos à monoartrite por adjuvante completo de Freund (CFA, *Mycobacterium butyricum*; 0,5 mg/mL; 50 µL) na articulação do joelho direito (TF; n = 24) ou tornozelo direito (TT; n = 26). A incapacitação articular foi mensurada pelo tempo de elevação da pata (TEP; s) em 1 minuto de avaliação. O edema do joelho ou tornozelo foi avaliado pela medida do diâmetro articular (AD, cm) e pelo edema de pata (EP, mL), respectivamente. Ambos foram avaliados durante 10 dias consecutivos. Dois protocolos de exercício foram realizados: (a) exercício constante (TF, n = 6; TT, n = 6), realizando 1 minuto diário de exercício no cilindro (3 r.p.m.); (b) exercício variável (TF, n = 6; TT, n = 7), exercício com aumento de 1 minuto por dia, totalizando 10 minutos no último dia. Os grupos-controle (TF, n = 12; TT, n = 13) não realizaram exercício. Após 10 dias, os animais foram eutanasiados para contagem total (células/mm^3) e diferencial (mononucleares e polimorfos nucleares; células/mm^3) de leucócitos do tecido inflamado. O exercício variável inibiu a incapacitação e o edema em ambas as articulações. Entretanto, reduziu a migração total de leucócitos apenas na articulação TF. O exercício constante inibiu o edema nas duas articulações e reduziu a migração total de leucócitos da articulação TT. Porém, não reduziu a incapacitação. O exercício variável pareceu ser mais efetivo em reduzir os parâmetros inflamatórios em comparação com o exercício constante.

Introduction

Considering the functional limitations resulting from rheumatoid arthritis (RA), early diagnosis and prompt treatment are essential for the control of disease activity and for the disability and irreversible joint damage prevention.[1] In clinical practice, usually the patient starts treatment in the acute phase only with analgesic action and after the reduction of pain and swelling, starts the process of carefully strengthening joint protection. Only at a late stage the patients begin physical reconditioning.[1] Until recently, health professionals (physicians, physiotherapists and others) suggested that their RA patients avoid exercise and keep resting.[2] Meanwhile, it is still advisable not to perform exercises during crises.[3] However, the chronic nature of RA leads to inactivity that can cause muscle weakness, joint stiffness and limitation of joint movement.[4]

Studies published by Vlieland suggest that patients with rheumatoid arthritis can benefit from physical activity safely.[5] Shih and colleagues also argue that the practice of physical activity has shown benefits for individuals with RA, by significantly decreasing pain and improving gait and overall function.[6] Thus, physical activity seems to be associated with better quality of life among individuals with arthritis.[7] Considering the degree of physical and mental impairment, the disabling potential of RA and the improve of quality of life generated by physical activity, research has become necessary to verify the influence of exercise on the functional clinical status of patients with this disease, even in small daily doses of movement.

Studies performed in humans are difficult to control, because repeated tests become strenuous and drug intake or daily habits can interfere with the inflammatory process and the response to exercise. *In vivo* studies in animals, using experimental models of arthritis induction, may produce more information on this issue. The induced arthritis by Complete Freund's Adjuvant (CFA) is a suitable model, because it mimics the signs and symptoms of human RA, including histopathological changes, cellular infiltration, hypersensitivity and edema in the affected joint.[8] Thus, this study aims to evaluate the effect of daily walking activity, lasting 1 minute and 10 minutes, on the parameters of disability, joint swelling and leukocyte migration into the knee joint or ankle of rats with CFA induced arthritis.

Materials e methods

Animals

The experiments were performed using female Wistar (250-300 g) rats, aged approximately two months, allocated in standardized boxes, containing six animals per box, animals were kept at controlled temperature (20 ± 1°C) and with a dark/light cycle of 12 h using artificial lighting. The animals were fed with standard laboratory food and water was available *ad libitum*. All experiments were conducted according to the ethical guidelines of the International Association for Study of Pain (IASP) and approved by the local Ethics Committee for Animal (se (CEUA - UFSC, protocol number 1160066, and CETEA/CAV - UDESC protocol number 01/26/06).[9]

Drugs and reagents

The following substances were used: Complete Freund's Adjuvant - CFA *Mycobacterium tuberculosis* (1 mg/mL, Sigma®), Freund's Complete Adjuvant - CFA *Mycobabterium butyricum* (0.5 mg/mL, Difco®); sodium chloride isotonic solution (0.9%, Aster®), halothane gas (250 mL, 1:1 v/v, 2- 4%, diluted in hospital O2, Cristalia®), iodinated alcohol (1%, Rialcool®), aqueous solution of lauryl sulphate (2.5%, Vetec®).

Joint incapacitation test

Joint disability was measured using the right paw elevation time (PET), in seconds, with the help of the registry system proposed by Ferreira and Tonussi, which allows the evaluation of nociception declared by animals.[10] In this log system, the animals are forced to walk on a stainless steel cylinder (30 cm in width and 30 cm in diameter) rotating continuously at a constant speed of 3 rpm for 60 seconds. Metallic shoes were adjusted on the hind legs; only the right paw shoe was connected to a computer to record the total time of non-contact of the paw with the surface of the cylinder during a period of 60 seconds. The animals were accustomed to the shoes, by placing them on the animals for at least one hour before testing. The PET of naïve animals, that is, without any intra-articular treatment, is approximately 10 seconds. The increase in PET after intra-articular injection of phlogistic agents indicates development of joint disability.[10] PET was registered immediately before stimulation with CFA (baseline value) and after 24 h, during all days of the experiment.

Assessment of joint swelling

After CFA induction of arthritis, the rats were monitored daily for joint swelling. For the evaluation of tibiofemoral joint swelling (TF), the change in joint diameter (JD cm) was registered with the help of a non digital caliper (accuracy, 0.05 mm).[11] The evaluation of the tibiotarsal joint (TT) or paw edema (EP mL) was carried out with the aid of a plastic bucket filled with lauryl sulphate in water (2.5%) coupled to a precision electronic balance (Acculab, V-121). For this procedure, the animal was restrained with the help of a polyethylene cone and its paw was inserted to immediately above the tibiotarsal joint.

The displacement of the liquid column inside the bucket was registered in milliliters. Each gram of paw weight corresponds to 1 mL of liquid displaced from the bucket.[12] To ensure that the measurements were made at exactly the same articulation, daily markings were done using a waterproof pen. The edema was measured immediately before stimulation with CFA (baseline measurement) and after 24h, before the evaluation of joint incapacitation at each day of the experiment.

Synovial fluid leukocyte count

After euthanasia, the joint capsule was exposed to collect 5 mL of synovial fluid to prepare synovial fluid smears on glass slides. The smear of synovial fluid from each animal was stained with May-Grünwald stain and Giemsa, which was used to obtain the differential leukocyte count (MON, mononuclear cells, PMN, polymorphonuclear) using an optical microscope (100 × magnification). Data was expressed as MON/mm^3 and PMN/mm^3. Immediately after collection of pure synovial fluid, the joint cavity was washed with 100 mL of 0.9% saline containing EDTA (5%) and then it was diluted in Turk solution (1:20) for 5 minutes. This fluid was used for total leukocyte count (TC; cells/mm^3), with the aid of a Neubauer chamber and an optical microscope (40× magnification).[11] The synovial fluid collection for leukocyte count was performed only on the 10th day of the experiment.

Experimental procedures

Arthritis induction was performed by two injections of CFA (*Mycobacterium butyricum*, 0.5 mg/mL, 50 µL). The first injection, administered intradermally (id), was given at the base of the tail. The second injection, administered intra-articularly (i.art), was given at the femorotibial (TF, n = 24) or tibiotarsal (TT, n = 26) joint of the animals, 21 days after the first injection of CFA. For both injections, the animals were anesthetized with halothane gas (3%).

The animals were subdivided into the following groups: experimental (E1 or E10), groups of constant and variable exercise respectively, and controls (C1 and C10) of constant and variable groups, respectively.

The animals in the experimental group underwent two exercise protocols: A) constant exercise, in which the animals performed 1 minute of daily walking activity for 10 consecutive days, and B) variable exercise in which the animals performed the ambulation exercise with gradual increase of 1 minute per day, to the amount of ten minutes in the 10th (consecutive) day. Both protocols were performed in a stainless steel cylinder at 3 rpm of continuous speed.

The animals in the control groups (C1 and C10) did not excercise. With the exception of the 1st, 5th and 10th days, when they were evaluated for joint incapacitation. However, these animals were handled daily only for edema assessment . Also, they were placed daily in the cylinder (without movement), with the objective of exposing them to the apparatus, which can be a source of stress for these animals.

The data for edema (JD or EP) was expressed as the difference between the baseline measurement and the measures taken each day during the 10 days of evaluation. The data for disabling joint (PET) were expressed as they were measured; this way, the "day zero" corresponds to the baseline measurement and the subsequent days correspond to the ten days of exercise treatment. At the end of the experiments, on the 10th day, the analysis of synovial fluid leukocyte count (MON, PMN and TC) was performed.

Statistical analysis

The results obtained were analysed with the Shapiro-Wilk test to verify the normality of the data. Later the Student t test was applied to detect differences between experimental groups (E1 and E10) and their respective controls (C1 and C10) at a significance level of 5%. Statistical analysis was performed using SPSS for Windows® v.20.0.

Results

Constant exercise

According to the results, constant exercise caused a decrease in PET during the experiment for TF joint, but no significant differences were observed compared to the control group. For TT joint, there was only a trend towards the reduction of PET on the 10th day compared to the control group (Fig. 1A and A').

For the joint swelling assessment, it was found that walking for 1 minute produced a significant reduction of JD in TF joint (p <0.001) and of EP in TT joint (p < 0.001) against controls during all the experimental time (Fig. 1B and B').

In leukocyte migration, constant exercise produced no significant change in the TF joint compared to control group. However, in TT joint a significant reduction in leukocyte migration was noted, both for TC, as for PMN and MON, in the E1 group compared to the C1 group (Fig. 1C and C').

Variable exercise

The results revealed that variable exercise caused a reduction in PET during the experiment for TF joint, but with a significant difference compared to the control group in the 10th day of evaluation (p < 0.01). But in TT joint we observed a significant decrease in PET control group (p < 0.01) from the fifth day of evaluation on (Fig. 2A and A').

The variable exercise protocol also promoted a significant reduction of JD in TF joint (p < 0.001) and of EP in TT joint (p < 0.001), when compared to the control group during the whole time of the experiment (Fig. 2B and B').

As for the leukocyte migration, the variable exercise protocol significantly reduced the concentration of TC ($p \leq 0.01$), PMN ($p \leq 0.01$) and MON ($p \leq 0.05$) in TF joint compared to the control group. On the other hand, in TT joint a significant change in the leukocyte migration due to the variable exercise was not observed. There could only be seen a trend toward reduction of mononuclear cells (Fig. 2 C and C').

Discussion

In the present study we observed that exercise, especially the variable modality, had an effect in nociception reduction at the two joints evaluated. Additionally, both exercise protocols, constant and variable, demonstrated an effect on edema reduction at these two joints. The constant exercise decreased the amount of leukocytes only in TT joint. The variable exercise reduced the number of leukocytes in TF joint, showing only a statistical trend for TT joint, regardian a decrease of mononuclear cells. This shows that exercise has some effect in reducing the inflammatory exudate.

Several studies in the literature[6,7,13-18,22] show that exercise in patients with arthritis decreases their pain, regulates blood pressure, increases muscle and bone strength, increases lean mass and decreases fat mass, improving psychological well-being, reducing the risk of depression and improving moods.[13] According to the U.S. Department of Health and Human Services, these benefits occur with-

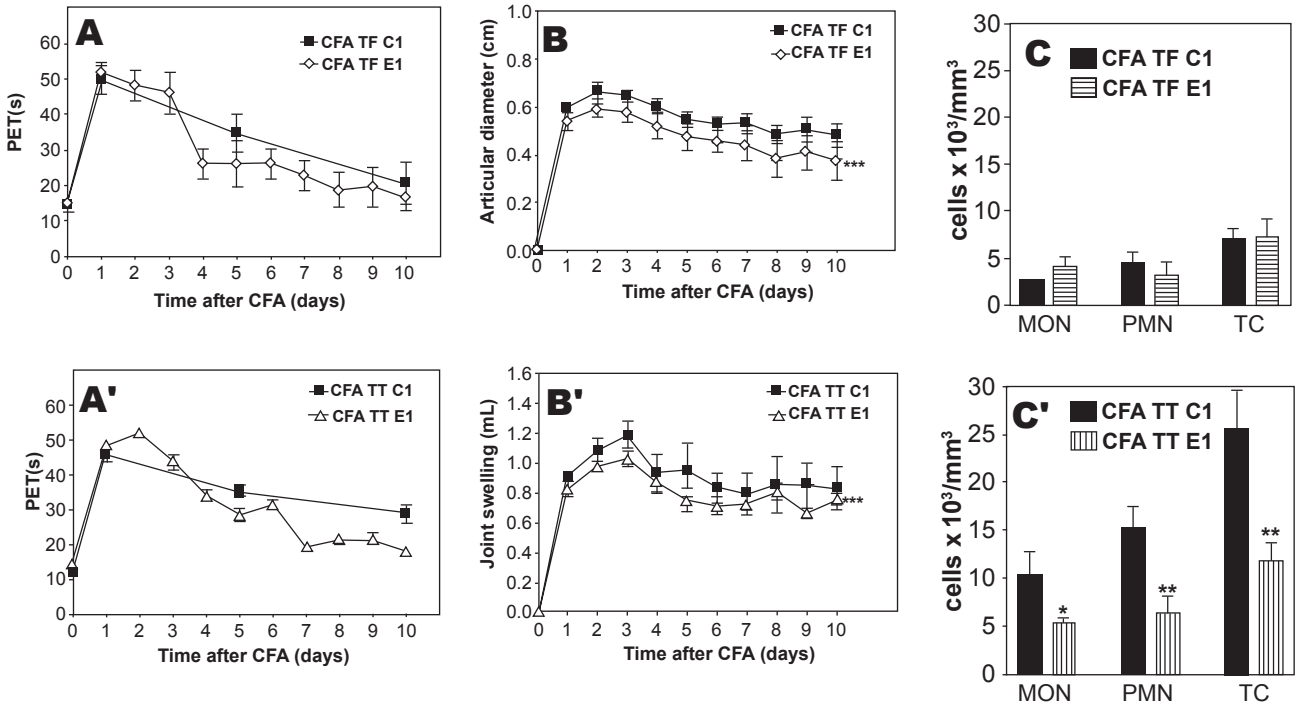

Fig. 1 – Effect of constant exercise on joint disability (A and A'), on swelling (B, B') and on leukocyte migration of (MON, mononuclear cells, PMN, polymorphonuclear, and TC, total cells) (C and C') of tibiofemoral (above, CFA TF E1) and tibiotarsal (below, CFA TT E1) joints of female rats with CFA-induced (0.5 mg/mL, *Mycobabterium butyricum*, 50 µL) arthritis. The control groups (CFA TF C1 and CFA TT C1) did not carry out the exercise. Both groups received intra-articular CFA (n = 25). * $p \leq 0.05$, Student's t test for independent samples.

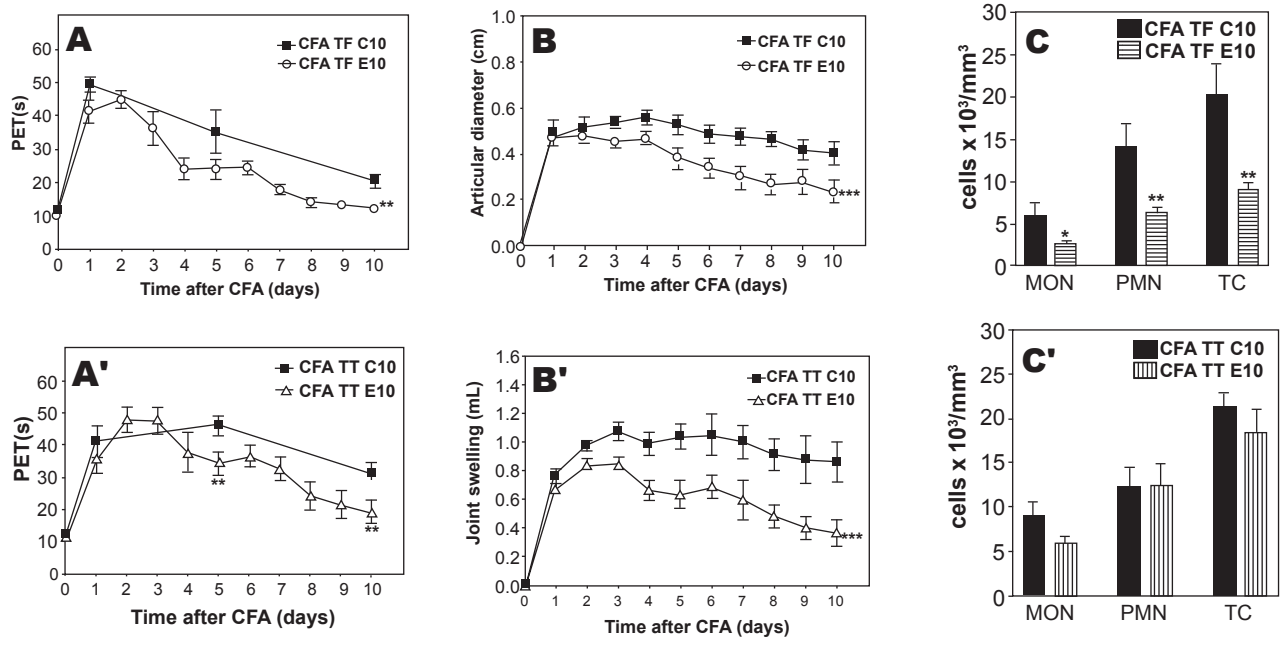

Fig. 2 – Effect of constant exercise on joint disability (A and A'), on swelling (B, B') and on leukocyte migration (MON, mononuclear cells, PMN, polymorphonuclear, and TC, total cells) (C and C') of tibiofemoral (above, CFA TF E10) and tibiotarsal (below, CFA TT E10) joints of female rats with CFA-induced (0.5 mg/mL, *Mycobabterium butyricum*, 50 µL) arthritis. The control groups (CFA TF C10 and CFA TT C10) did not carry out the exercise. Both groups received intra-articular CFA (n = 26). * $p \leq 0.05$, Student's t test for independent samples.

out adverse effects on immune function or in the disease state.[13]

More specifically in relation to the analgesic effects of exercise, other studies have shown that physical activity decreases pain in patients with RA.[2,6,7,14-18] Astrand, in his studies, suggested that physical activity is a therapeutic modality for pain relief in patients with RA, bringing benefits in mobility of periarticular structures, including joint capsules, tendons and muscles.[18] Ekdhal et al. suggest that this hypoalgesic effect is due to the release of β-endorphins caused by exercise.[19] However, in our experiment we did not verify the possibility of endorphins release.

On the other hand, studies of Raja et al. argue that mobilization within the limits of range of motion in an inflamed joint can lead to sensitization of primary afferent nociceptors, and even a slight movement of the joint can cause pain.[20] Schaible and Grubb agree with this theory, assuming that in the disease associated with joint pain, this feeling is induced or aggravated during movement.[21] Taking into account that the joint incapacitation test evaluates spontaneous pain during mobilization, the results obtained in this study regarding variable exercise effects contradict these theories, showing that nociception was reduced in the animals that took part in the exercise. Even in relation to the constant exercise, where no statistical significance was perceived, the curve showed a tendency to decrease nociception.

It is noteworthy that in this study a monoarthritis was created, i.e., the induction of arthritis in only one paw; during the walking activity in the joint incapacitation test, the animals remained with three "pain-free" paws for support. In this case, it is not possible to say that these animals did not feel pain in the other paws. In humans, this would not be possible, because of the bipedal support and the symmetrical characteristic of RA. These considerations complicate the linking of these findings to humans; however, it is believed that, even with these differences, the performance of physical activity contributes effectively to a clinical improvement in RA, in view of the large number of publications, including papers in humans, advocating this practice.[6,7,13-18,22]

Although rehabilitation programs consider therapeutic exercises for the treatment of RA symptoms, as far as we know there is no research carried on the reduction of edema and leukocyte migration promoted by exercise.[22] On the contrary, some authors, like Marques and Kondor, claim that the increase in edema in patients with osteoarthritis is indicative of excessive exercise.[23] According to Kavuncu and Evcik, walking can increase the intra-articular pressure in patients with knee inflammation and swelling, and that this activity should be performed only at remission of the disease.[22]

Animal studies developed by Butler et al. showed that after six injections of 0.05 mL of CFA containing 300 mg of *Mycobacterium butyricum* in a TT joint of rats submitted to a protocol of progressive swimming (increasing from 5 to 15 minutes) with frequency of three times a week for 4 weeks, no change was perceived with respect to joint swelling and mobility. However, regarding the pain threshold in response to the paw pressure test, a decrease was observed in the experimental *versus* control group.[24]

These studies contradict the results presented in this paper. However, the type of physical activity, the intensity of exercise performed or even the form of assessment chosen may have contributed to these differences. The evaluation of pain

threshold used by Butler et al. is not a model that evaluates the spontaneous pain caused by movement.[24]

Lana et al. studied the effects of high and low intensity exercise on the acute inflammatory response to induced arthritis in Wistar rats.[25] The exercises consisted of walking on a treadmill; the low-intensity activity consisted of 60 minutes of treadmill/day at a speed of 5 m/min for 12 weeks, and the high-intensity activity consisted of a progressive training that, at the end of the experiment, reached a cumulative time of 75 min and a speed of 25 m/ min.

The inflammatory response was induced by carrageenan (0.5%, 0.1 mL, TT) and the inflammatory edema volume was measured by plethysmography 24 hours after induction. Compared to untrained animals, an increase in the volume of acute inflammatory edema in animals that underwent low intensity activity was observed; however, the response was more evident in animals that underwent high-intensity exercise.

The authors admit that exercise is a form of stressful stimulus that could promote changes in homeostasis, with the reorganization of responses mainly in the neuroendocrine system, probably generating an increase of endogenous glucocorticoids concentration in serum. However, this was not observed with high-intensity exercise, which, according to the authors, gives evidence of the influence of intensity, frequency and duration of exercise on edema.[25] It is noteworthy that this study evaluated a lasting activity in an acute inflammatory response, which makes difficult the comparison against the findings obtained in the present study.

Indeed, the type of physical activity and the exercise intensity can interfere with the results obtained in RA patients. Rall and Roubenoff suggest that during the active phase of the disease, exercises with no weight-lifting are suitable and that in those patients with controlled disease, activities with load may be prescribed.[26] In fact, very vigorous exercise is not recommended for patients with active disease.[15] The Expert Arthritis and Physical Activity Panel at a meeting held in 2003 by specialists recommend that people with arthritis should safely perform 30 minutes of physical activity of moderate intensity at least three times a week.[27]

Finally, the proposal of this experiment was to demonstrate that a small amount of exercise performed from the beginning of the disease does not affect the joint and also can benefit in nociception and in the parameters of joint inflammation, such as edema and leukocyte migration. However, this study was performed in animals, which makes it difficult to relate to humans. A continuation of the study is suggested in humans, to evaluate different types of physical activity, or even different walking times, with frequent evaluation of pain and edema in patients with rheumatoid arthritis.

Conclusion

In conclusion, the walking activity, especially the variable modality, decreased nociception, edema and leukocyte migration in animals with CFA-induced arthritis. These data show that exercises can be performed shortly after the establishment of the diagnosis of RA, without risk of joint injury; the exercise is useful as adjunctive treatment of the disease and has great importance for the prognosis, as well as for improving the quality of life of patients.

Conflicts of interest

The authors declare no conflicts of interest.

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