EVIDENCE THAT A PHYSICAL ACTIVITY PROTOCOL CAN REDUCE SYNOVIAL LEUKOCYTE COUNT IN ARTHRITIC RATS

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Introduction: Physical activity is thought to be beneficial to arthritis, delaying disability and/or improving joint function. In vivo studies using experimental models of arthritis may provide useful information about such benefits. Objective: The aim of the present study was to evaluate the effects of the low-intensity exercise on a model of CFA-induced arthritis in rats. Methods: Articular incapacitation was measured by the paw elevation time in 1-min periods of stimulated walk. Edema was evaluated by the knee-joint diameter. Synovial exudate was sampled after 10 days for leukocyte count. The exercise protocol consisted of a 2-min period of stimulated walk in the 1st day, 10 min in the 2nd day, and 20 min from the 3rd to the 10th day; The control animals were submitted to 1-min period of stimulated walk once a day over 10 days. Corticosteroid involvement was assessed by treating the animals with aminoglutethimide. Results: The exercise protocol produced a slight but sustained reduction of disability and joint swelling associated with a large reduction in the synovial leukocyte count. Aminoglutethimide only prevented the effect on synovial leukocyte count. Conclusion: These results suggest that a low-intensity physical activity does not aggravate the symptoms of arthritic animals, in fact showing a slight improvement, and still can markedly reduce the migration of leukocytes into the synovial space.

Keywords: physical exercise; synovial fluid analysis, arthritis treatment, complete Freund's adjuvant.

INTRODUCTION

Rheumatoid arthritis (RA) is a very common autoimmune disease which affects 1-1.5% of the world population, with high prevalence in women[1,2]. Nevertheless, it can occur at any age, with more frequent beginning in adults aged 40-60 years[1]. RA I a chronic disorder, systemic and of unknown etiology, marked by inflammatory alterations in the synovial membrane (synovitis) and in the articular structures, characterized by the development of autoantibodies and by the presence of *pannus* – a granulation tissue made in the synovia by proliferation of fibroblasts and inflammatory cells which invade and destroy the structures close to the articulation, such as the articular cartilage, the subchondral bone, the tendons and the ligaments[3]. Additionally, pain, edema and articular stiffness caused by arthritis lead to incapacities which affect the activities of daily living (ADLs) as well as professional activities. Thus, an estimation based on the quality of life related to health indicates the progression of the disease and/or therapeutic efficiency[4,5]. Early diagnosis and immediate start of the treatment are important for the control of the disease activity and to prevent functional incapacities and irreversible articular damage[6].

Currently, exercises may not be recommended by rheumatologists, based on the hypothesis that they may accelerate articular damage and cause inflammation aggravation[7]. The chronic feature of RA affects the musculoskeletal, neuromuscular, integumentary and cardiopulmonary systems, with progressive independence loss, causing incapacities[8]. However, recent findings suggest that RA patients may benefit from physical activities[4], presenting pain decrease, improvement in articular function and delay of functional incapacities[9].

Physical activity is also associated with quality of life improvement among adult individuals[5]. Research conducted with RA patients is difficult to be suitably conducted due to the exhaustive tests which are repeated and by the ingestion of medication or daily habits which may interfere in the inflammatory process and response to exercise. Thus, *in vivo* studies using arthritis experimental models may provide useful information[2,10,11]. Nevertheless, a problem associated with this approach may be the stress induced to the animals, since they are made to perform tasks related to physical activity. Animals submitted to stress will probably release corticosteroids, which may reduce the clinical signs of inflammation, and hence, present benefits. The aim of this study was to pharmacologically assess this possibility in an arthritis model induced by CFA in rats submitted to a moderate physical activity task.

METHODS

Animals

The experiments were performed with Wistar male rats (275-350g) approximately 60 days old at the beginning of the experiments, which were kept in a room with controlled temperature (20 ± 1ºC), with free access to water and food and submitted to artificial light with 12-hour light/dark cycle. All experiments were conducted according to the ethical guidelines of the International Association for Pain Study[12] and this study ws previously analyzed and approved by the Ethics in Animal Use Committee of the Federal University of Santa Catarina (CEUA – UFSC), under protocol number 1,160,066/2006.

Algesimetric test

The knee incapacity test of rats was previously described in detail by Tonussi and Ferreira[13]. In this recording system, a device assisted by a computer measures the total time in which the hind paw ipsilateral to the injected knee did not enter in contact with a stainless steel spinning cylinder (30cm diameter) in continuous rotation at steady velocity of thee rotations per minute (r.p.m.) and for a 60-second period of estimated deambulation (that is, time of paw elevation -TPE). Due to the slow rotation, the animals calmly try to remain on the top of the cylinder. Animals with no treatment usually present TPE of approximately ten seconds, while the inflammation in the knee articulation increases this value only in the affected limb.

Edema evaluation

In order to quantify the inflammatory edema, non-digital pachymeter was used to measure the mediolateral articular diameter (AD) on each day after the intra-articular injection, before the incapacity test[14]. Data are presented as the difference between the means of the AD values daily measured after the intra-articular injection of CFA and values measured immediately before the intra-articular injection of CFA (in centimeters).

General procedures

Arthritis by adjuvante was induced by an intradermic injection of 50 µL of CFA (*Mycobacterium butyricum*, Difco-USA, 0.5 mg/mL) in the base of the tail, followed by a second injection inside the right knee articulation after seven days. The injections were administered under anesthesia by halotane (2-4% in oxygen, Cristália Laboratory, Brazil). The animals were divided in two groups, control and exercise. In the control group, the animals performed one minute of daily deambulation in the cylinder according to the time of the incapacity test per se. In the exercise group, the animals performed deambulation during two minutes on the first day, tem minutes on the second day, and 20 minutes from the third to the tenth day. The incapacity test was performed always during the first minute of this activity. Both incapacity and edema were measured during ten consecutive days. The control and exercise groups were subdivided in groups treated with water (0.1 ml/100 g, oral) and aminoglutethimide (Orimeten®, 50 mg/kg, oral) and were named group water-control; water-exercise; amino-control and amino-exercise. The treatments were given one hour before the incapacity test, for ten consecutive days, according to the protocols previously described.

Sampling of the synovial liquid cells

After ten days from the evaluation of the incapacity test and edema, the animals were euthanized for leukocytes count of the synovial liquid wash. The synovial cavity of the knee articulation was open and 5 µL of synovial fluid were collected for the swab preparation. The swab was stained by May-Grunwald-Giemsa and used for leukocytes differential count (mononuclear and polymorphonuclear cells – cells/mm^3 – MON and PMN, respectively) in an optical microscope (100 x augmentation). Immediately after the collection of the pure synovial fluid, wash of the articular cavity was performed with 100 µL of physiological solution 0.9% containing anticoagulant EDTA (5%), and diluted in Turk solution (1:20) for five minutes. A sample of it was used for leukocytes total count (TC, cells/mm^3) with aid of a Neubauer camera[14].

Statistical analysis

All statistical analyses were performed using *GraphPad Prism*® 4.0 and SPSS 17.0 for Windows®. The data obtained were submitted to the Shapiro-Wilk test for verification of data normality, and to the Leven test for verification of homogeneity of variances. Comparisons of two curves or means were performed using paired and no-paired *t* test, respectively. The results are presented as mean ± standard deviation of six animals at significance level of five percent.

RESULTS

The presente exercise protocol did not aggravate incapacity and articular edema, right on the contrary, these parameters presented slight reduction in intensity. This reduction was considered statistically significant for remaining constant during the entire recording period, which provides great sensibility to the repeated measures tests used. The incapacity test evidenced that the group water-control presented TPE value higher when compared to the values in the curve of the group water-exercise ($P < 0.001$) (figure 1A). The same situation was observed in the group treated with aminoglutethimide with TPE of the group amino-control significantly higher when compared to the group amino-exercise ($P < 0.05$) (figure 1B). The incapacity curve of the group treated with water presented delayed and less intense peak when compared to the group treated with aminoglutethimide. The maximum TPE for the groups ater was found on the second day for the group water-exercise (TPE on the second day = 23.2 ± 5.8 s) and on the fourth day for the group water-control (TPE on the second day = 24.6 ± 4.8 s; TPE on the fourth day = 26.5 ± 3.7 s), which were lower than the ones found in the groups treated with aminoglutethimide hich presented higher values on the first day after stimulation by CFA (group amino-exercise: TPE on the first day = 25.9 ± 6.1 s and TPE on the second day = 16.8 ± 2.7 s; group amino-control: TPE on the first day = 28.7 ± 5.7 s and TPE on the second day = 25.2 ± 5.7 s). After having reached the maximum TPE value, incapacity in the groups treated with aminoglutethimine decreased earlier when compared to the values of the groups treated with water, and both decreased before the inflammation signs; that is to say, edema and leukocytes infiltration.

Additionally, articular edema was also reduced by exercise. Significant decrease ($p < 0.05$) of articular diameter (figure 1C) was produced by the exercise protocol in the group treated with water

from the first to the third day. In the group treated with aminoglutethimide, this difference was even more significant, being observed during the entire test ($p < 0.001$) (figure 1D).

Similarly to incapacity, articular diameter (AD) in the group treated with aminoglutethimide presented earlier and wider peak than the group treated with water. The AD in the groups treated with aminoglutethimide was higher on the first and second days (group amino-exercise: AD first day = 0.41 ± 0.05 and AD on the second day = 0.38 ± 0.06; group amino-control: AD first day = 0.48 ± 0.05 and AD second day = 0.43 ± 0.03) while the groups treated with water presented higher peaks on the second and third days (group water-control: AD second day = 0.41 ± 0.03; group water-exercise: AD third day 0.37 ± 0.03).

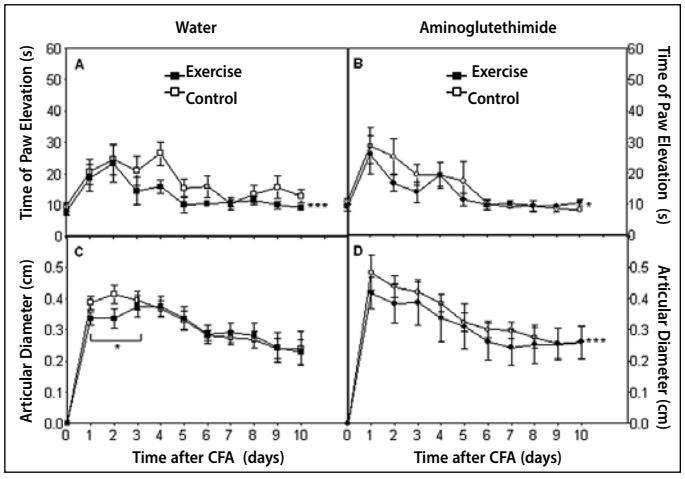

Figure 1. Exercise effect on pain and edema after pre-treatment with corticosteroid inhibitor. Animals which perform exercise and the control received aminoglutethimide (50mg/kg, o.a.) or distilled water (0.1ml/ 100g, o.a.) for tem consecutive days, one hour before the incapacity test. * and *** indicate statistic differences of the control group for $P < 0.05$ and $P < 0.001$, respectively (paired Student's *t* test). All results were expressed as mean ± standard deviation of six rats per group.

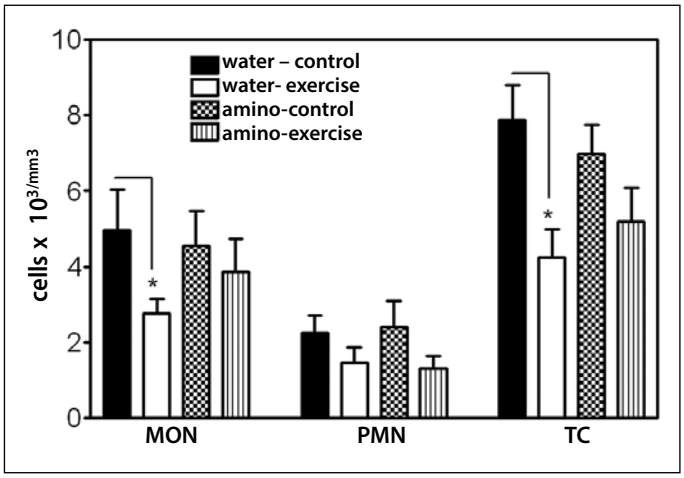

Figure 2. Exercise effect in the leukocytes infiltration after pre-treatment with corticosteroid inhibitor. Animals which performed exercise and control ones received aminoglutethimide (50mg/kg, o.a.) or distilled water (0.1ml/ 100g, o.a.) for ten consecutive days. Total synovial leukocytes (TC) were counted on the tenth day. PMN and MON represent the polymorphonuclear and mononuclear cells, respectively. * indicates statistical difference of th control group for $P < 0.05$ (paired Student's *t* test). All results were expressed as mean ± standard deviation of six rats per group

Total leukocyte migration to the synovial fluid was also significnatly decreased by the exercise protocol in the group treated with water ($P < 0.05$) (group water-exercise: CT/mm^3 = 4,241 ± 750; MON/mm^3 = 2,768 ± 393; PMN/mm^3 = 1,474 ± 396. Group water-control: CT/mm^3 = 7,875 ± 929; MON/mm^3 = 4,975 ± 1,063; PMN/mm^3 = 2,254 ± 455); however, differently from incapacity and edema, the treatment by aminoglutethimide inhibited, at least partially, this exercise effect (group amino-exercise: CT/mm^3 = 5,192 ± 893; MON/mm^3 = 3,871 ± 854, PMN/mm^3 = 1,321 ± 310. Group amino--control: CT/mm^3 = 6,992 ± 770; MON/mm^3 = 4,567 ± 919;PMN/mm^3 = 2,410 ± 677) (figure 2).

DISCUSSION

The present study has shown that controlled physical activity may effectively reduce parameters such as pain, edema and cellular migration in rats submitted to arthritis induced by CFA. Among these effects, reduction in cellular migration to the synovial space was undoubtfully the most relevant and it is also of major clinical importance, since it may determine reduction of articular destruction associated with this degenerative disease. Experimental arthritis is associated with dramatic alterations in the properties of the response of articular afferents[15], which is believed to increase pain which follows arthritis. Moreover, pain associated with the articulation disease is induced or aggravated during movement and by local mechanical stimulation in the articulation affected[16]. In fact, Butler *et al.* (1991) studied the swimming effects (in water at 37°C, three weekly times) in the pain behavior in an arthritis model induced by CFA and reported lower pain threshold in response to pressure to the aw in exercised animals, despite absence of aggravation of the articular stiffness and mobility[17]. In the present study, although the animals present previous adaptation in the incapacity induced by inflammation, consistent difference can be observed between exercised and non-exercised animals, which suggests antinociceptive effect.

Other important characteristics observed were reduction of articular diameter and the number of leukocytes in the synovial liquid. Reduction of articular diameter reflects in reduction of inflammatory edema in periarticular tissues; besides that, it has been seen that this parameter is also reduced by anti-inflammatory agents like aspirin and glucocorticoids[18,19]. Thus, the reduction observed here suggests that there is an important positive effect of exercise in this model of articular inflammation. Additionally, the leukocytes infiltration is the most relevant event during arthritis, since it is involved in the tissue destruction and in all its long-term consequences. Pharmacological strategies, including anti-inflammatory agents, are hence guided to the inhibition of leukocytes migration[20] as an effort to limit progression of the disease, since anti-inflammatory drugs may only produce symptomatic relief without preventing progression of the disease. Therefore, the noticeable inhibition of synovial leukocytes by the exercise protocol was of great importance. When united, these inhibiting effects in the periarticular edema and synovial leukocytes suggest that a similar exercise protocol may also contribute to the conventional anti-inflammatory therapeutics in clinics.

Pre-treatment with aminoglutethimide (a mitochondrial inhibitor P450 scc) was conducted in order to suppress increase of endogenous corticosteroids during the exercise protocol [21]. The results

showed that remarkable differences relative to leukocytes migration observed between the group treated with aminoglutethimide and the group control suggest that this effect is due to the corticosteroid increase during the deambulation protocol. Increase of corticosteroids and other markers after exercise protocols has been presented by other studies[22,23], indicating that forced exercise protocols induce a stress response in rats. Such corticosteroids increase is supposedly responsible for the decrease of synovial leukocytes and the consequent antiedematogenic and anti-hyperalgesic effects due to their known anti-inflammatory activity[24]. In fact, previous studies have shown decrease in the expression of the inflammation induced by COX-2[25] and decrease of the IL-1b plasma levels[26], after forced exercise protocols. Both findings have been consistent with increase in the corticosteroids levels and the consequent suppressive effect in the expression of pro-inflammatory genes. However, in the present study, the anti- hyperalgesic and antiedematogenic effects induced by the exercise protocol were resistant to the aminoglutetihmide treatment, suggesting that improvement of articular function produced by the exercise protocol was independent from corticosteroid increase and also efficient in happening even with the presence of leukocytes migration.

CONCLUSION

This study suggests that low-intensity physical activity does not aggravate the symptoms of the arthritic animals, in fact, they present light improvement. Exercise may even remarkably reduce leukocytes migration to the synovial space.

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