RAPID COMMUNICATION

Effects of inhaled cigarette smoke on the myo-articular system of female rats with collagen-induced arthritis

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INTRODUCTION

Rheumatoid arthritis (RA) imposes substantial costs on society, due to both the costs of treatment and the increase in work-related deficiencies; it also accelerates mortality.[1] Studies have shown that cigarettes are a factor in the development of RA and also in the worsening of an already present condition, potentially modifying the gravity of RA.[2,3] However, the importance of tobacco on the overall effects of RA remains unknown.[2] It was shown that the prevalence of RA in Caucasian women with a genetic predisposition and who smoked was greater than in those with a genetic predisposition but did not smoke.[4]

In addition, RA, either isolated from or associated with smoking, leads to a compromised musculoskeletal system. If this condition is not controlled and is combined with a sedentary lifestyle, a compromised musculoskeletal system can cause a series of complications that culminate in a decrease in functionality and quality of life that is associated with increased rates of morbidity and mortality relative to the healthy population.[5]

Thus, the objective of this study was to analyze the effects of cigarette smoke on the myo-articular system of rats with induced arthritis.

METHODS

Animals and Experimental Groups

This study was approved by the Ethics Committee of FEPAR (protocol n° 5597/9) and was performed using 24 twelve-week-old Wistar rats (256.21 $\pm$ 17.42 g) that had been maintained in a controlled environment.[6]

The rats were divided into the following 4 groups (n = 6): 1) CONT – control group; 2) RA – rheumatoid arthritis-induced group; 3) CI – cigarette group; and 4) RACI – rheumatoid arthritis and cigarette group.

Collagen-induced arthritis and cigarette smoke inhalation

RA was induced by the subcutaneous injection of 10 μg of a solution of type II human collagen + aluminum hydroxide (which acts as an adjuvant).[7] The induction of RA was evaluated clinically by the observation of edema and erythema in the tibia-fibula (knee) and talocrural (ankle) joints.[8,9,10] The measurement of edema in the tibia-fibula joint in the flexed position and in the talocrural joint in the neutral position was performed using a digital caliper (*Vonder*) one week after injection of the solution and on the day the animals were euthanized.[7,11] The rats were monitored every day starting day 7 after immunization. Because the peak time of arthritis onset is approximately 30 days, the experiment was maintained for 28 days.[10]

The animals were exposed to cigarette smoke in a 10-L box.[10,11] The cigarette smoke was collected using a 60-ml syringe that was attached to a lit commercial brand cigarette that contained the following ingredients: 4 mg tar, 0.4 mg nicotine and 5 mg carbon monoxide.[12] The aspirated smoke was then injected into the box, which contained 3 animals.[13] One cigarette was used per rat.[10,11,14] The animals were exposed three times a day for 10 minutes (every 6 hours, beginning at 7:00 a.m. and with a 12-hour interval between treatments) for a consecutive period of 28 days.[10,11,13,14]

All of the animals were anesthetized on day 29, and the soleus muscle was dissected and removed. While under the effect of the anesthetic, the animals were euthanized by intra-cardiac puncture.[4]

Muscle fiber cross-sectional area (MFCSA) procedures

After dissection, each muscle was weighed separately using an analytical balance (*Instrument Company, Huntingdon, England*).[15] The muscles were then straightened over a flat surface (Styrofoam), and the muscle length was measured using a digital electronic caliper (*Vonder*) and magnifying glass (*Olympus*). The soleus muscle was divided longitudinally into two equal parts using a scalpel (*Feather*).[15]

The fragment removed from the medial half of the belly of each soleus muscle was cross-sectioned (10 μm) and stained with Harris hematoxylin and eosin (H & E) for a histo-morphometric analysis of the MFCSA.[15]

Photomicrographs of the histological cuts were made using a light microscope (*Axyophot*, *Carl Zeiss*, *Oberkochen*, *Germany*) and captured using a video-image system (*Applied Spectral Imaging*, *Migdal Ha'emek*, Israel) and the *Case Data Manager Expo program* (*Applied Spectral Imaging*, *Migdal Ha'emek*, Israel, version 4.0) in the Postgraduate Cell Biology Department of the Federal University of Paraná, Brazil.[15] The cross-sectional areas of 100 fibers selected at random from each muscle were measured with the *UTHSCSA ImageTool* 3.0 software (developed at the *University of Texas Health Science Center at San Antonio*, Texas and available at http://ddsdx.uthscsa.edu/dig/itdesc.html).[15,16]

Connective tissue cross-sectional area (CTCSA) measurements

The connective tissue area of one cross-section of each soleus muscle was measured using the whole slice area (100%) of the H & E slide (10× objective). Then, all of the muscle fiber cross-sectional areas were marked and excluded using ImageTool, leaving only the connective tissue, i.e., the perimysium and endomysium. The connective tissue area was then expressed as a percentage of the whole slice area.[15]

Statistical analysis

The Shapiro-Wilks test and Levene's test were used to assess the normal and homogeneous distributions, respectively. The initial and final body weights were compared within each group using paired t-tests.[15] All of the variables analyzed (body weight, volumes of the tibia-fibula and talocrural joints, muscle weight, muscle length and cross-sectional area of the muscle fibers and connective tissue) were compared among the experimental groups (intergroup) using ANOVA, along with the *post-hoc Tukey* test for the parametric data and the *Newman-Keuls* method for the non-parametric data. Levels of $p<0.05$ were considered significant.[15]

RESULTS

Body weight

There were increases in the final body weights of the CONT, RA and RACI groups compared to the initial weights of each group ($p = 0.0006$, $p = 0.04$ and $p = 0.0019$, respectively). No significant differences in weight and muscle length were determined.

A decrease in MFCSA was observed in the RA group compared to the CONT group ($732 \pm 314\ \mu m^2$ *vs.* $1246 \pm 323\ \mu m^2$, $p = 0.02$, Figure 2C). In addition, more pronounced atrophy was observed in the RA group compared to the CI group ($732 \pm 314\ \mu m^2$ vs. $1259 \pm 411\ \mu m^2$, $p = 0.03$; ANOVA). No differences were demonstrated in the areas of connective tissue.

No statistically significant alterations were observed in the volumes of the right knee and ankle joints.

DISCUSSION

The induction of arthritis and its association with cigarettes did not prevent the weight gain that occurs during normal development in rats.[17]

In another study that examined nine-week-old female Lewis rats, there was only a 2–6% increase in the final body weight at the end of 28 days of intervention, indicating that RA caused a smaller weight gain in these animals, whereas the control group gained the expected amount of weight.[7] These results corroborate those of the present study, in which a weight gain of 2–6% was observed in the RA induced animals with or without exposure to cigarette smoke. However, while the control and RA groups increased the final body weight 6% and 5% respectively, the RACI gained only 3%. This result emphasizes the prejudicial effect of the association of RA and nicotine, as was also demonstrated in a study involving adult smokers.[18]

Conversely, the body weights of the CI group did not change, indicating that cigarettes alone prevented normal weight gain. Certain studies have shown that rats lost weight when subjected to cigarette smoke, whereas other studies have shown that these animals did not lose weight but experienced a smaller weight gain compared to the controls.[19,20]

Nevertheless, even though the RA group gained a smaller amount of body weight, no differences were observed in the muscle mass or length. Thus, it appeared that the smaller gain in body weight was not due to a loss of muscle weight.

However, it was observed that the induction of RA produced a decrease in the MFCSA and has also been described by other authors.[21]

Despite the atrophy observed in this study, no significant differences in connective tissue area were detected. This finding could be explained by the fact that structural alterations in the connective tissue are indications of a more severe stage of arthritis, both in humans and in rats.[5,8,22]

Increased knee and ankle joint volumes are indicative of the inflammation of RA and are frequently used to measure the activity of the disease.[7] Although we did not detect differences in joint volumes, another study detected a considerable increase in volume 14, 21 and 28 days after the induction of arthritis.[7] Yet another study examined DA and Lewis rats (8–12 weeks old) and demonstrated that erythema and edema were first seen in the hind limbs 10 to 12 days after the induction of RA and these signs frequently spread to the front paws of the animals.[23] In another study, the joint volume of male Sprague-Dawley rats (6–10 weeks old) began to increase 13 days after RA induction, persisted for 4–5 weeks, and then disappeared completely in some animals, leading to deformities in the paws in a more severe stage of the disease.[16] Thus, it is possible that the absence of this clinical sign in this study was due to the use of Wistar rats and to the method of arthritis induction. Some authors have recommended providing a similar booster injection seven days after the first immunization to guarantee the induction of arthritis.[7]

The use of other measurements to detect the induction of RA is recommended for future studies. Observations of the commencement and evolution of RA in rats might be clearer when using other measurements.

CONCLUSIONS

Smoking prevented normal weight gain and induced musculoskeletal atrophy in arthritic rats.

ACKNOWLEDGEMENTS

The authors are grateful for the technical support provided by Elisangela Poppin in preparing the histological slides and for the logistics support

provided by Rosinei Valle; both of these individuals are from the Faculdade Evangélica do Paraná, Brazil. Some technical support for the methodologies assessed was provided by Professor Maria Lucia da Silva Germano Jorge from the Faculdade Evangelica do Parana.

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