International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Histopathological Changes in Three Variations of Wistar Rat Adjuvant-Induced Arthritis Model

Gabriela Laste[1,2,3], Izabel Cristina Custódio de Souza[1,5], Vinicius Souza dos Santos[1], Wolnei Caumo[1,2], Iraci L.S.Torres[1,2,3]

[1]Pain Pharmacology and Neuromodulation: Animal Model Laboratory, Pharmacology Department, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, CEP 90050-170, Porto Alegre, Brazil.

[2]Graduate Program in Medicine: Medical Sciences, Universidade Federal do Rio Grande do Sul, CEP 90035-003, Porto Alegre, Brazil.

[3]Animal Research Unit, Hospital de Clínicas de Porto Alegre, CEP 90035-003, Porto Alegre, Brazil.

[4]Central of Pathology, Hospital de Clínicas de Porto Alegre, CEP 90035-003, Porto Alegre, Brazil.

[5]Universidade Federal de Pelotas, CEP 96010-610, Pelotas, Brazil.

Manuscript No: IJPRS/V3/I2/00234, Received On: 24/04/2014, Accepted On: 24/05/2014

ABSTRACT

This study aims to describe histopathological changes observed in three Wistar rat variations of adjuvant-induced arthritis model inducing mono arthritis and poly arthritis. Complete Freund's adjuvant (CFA) was injected in variation I: 200 µL of emulsion injected into the right footpad; CFA-induced arthritis variation II: 100 µL of CFA injected into the right footpad and two injections of 100 µL into the tail; CFA-induced arthritis variation III: 300 µL of CFA injected into the right footpad. All animals presented joint damage, revascularization, and synovial proliferation. Analysis of histological scores on the 15th day showed that variation I had the highest scores for synovial inflammation (3.0±0) and subcutaneous inflammation (3.0±0), and variation III presented the highest scores for cartilage (2.3±1.1) and bone erosion (2.3±1.1). On the 21st day, once again variation I showed the highest scores for synovial (2.3±0.6) and subcutaneous inflammation (3.0±0.6), despite a decrease in synovial inflammation scores on the 21st day (3.0 to 2.3). Cartilage erosion was more frequent in variation II (2.0±1.1), and bone erosion was similar in all models. Our findings suggest that arthritis was successfully induced in all three variations of Wistar rat arthritis model, with variable severity in terms of histological findings and clinical manifestations. The most homogenous response was obtained with the use of an emulsion to induce arthritis, however with lesser severe manifestation.

KEYWORDS

Adjuvant-Induced Arthritis, Wistar Rats, Histopathological Abnormalities

INTRODUCTION

Rheumatoid arthritis (RA) is a condition characterized by chronic joint inflammation, progressive destruction of periarticular bone, and periarticular osteoporosis[1]. The increased secretion of pro-inflammatory mediators, such as interleukin-1 (IL-1ß) and tumor necrosis factor (TNF), maintain the chronic inflammation, the progression of cartilage and bone lesions[2]. These cytokines, interacting with growth factors, stimulate the overgrowth of

***Address for Correspondence:**
Iraci Lucena da Silva Torres
Departamento de Farmacologia - ICBS, UFRGS
Rua Sarmento Leite, 500 sala 202
90050-170 - Porto Alegre, RS, Brazil.
E-Mail Id: iracitorres@gmail.com

synovial cells to form a mass of synovial tissue with exuberant new blood vessels, called pannus, which invades the cartilage and bone via osteoclast activation and protease production[2].

Animal models of rheumatoid arthritis have been extensively used in research, to investigate the pathogenesis of inflammatory arthritis, and in the pharmaceutical industry, for to test the potential anti-arthritic agents. Important criteria to select a model include: 1) its ability to predict the efficacy of agents under investigation in humans; 2) easy handling, reproducibility of data, reasonable duration of test period; and 3) pathology and/or pathogenesis similar to human disease[3]. In the rheumatoid arthritis area, excellent models have shown good track records for predictability[3].

Complete Freund's adjuvant (CFA)-induced arthritis in rats presents similar features to rheumatoid arthritis in humans, and the model has been widely used in etiopathogenetic and investigational drug studies[4,5]. Adjuvant-induced arthritis (AIA) was initially observed by accident, when CFA was used for immunization[6]. Since then, AIA has been extensively used as a model for rheumatoid arthritis, despite some histopathological differences. Some clinical features of reactive arthritis are also seen in AIA, e.g., iridocyclitis, nodular skin lesions, genitourinary lesions, and diarrhea. Finally, the model features some aspects of rheumatoid fever, namely, ulcerative colitis and sarcoidosis[7]. AIA is a rather aggressive, monophasic, self-remitting form of arthritis[8]. As a result, the time period used to measure the effects of drugs or other agents in modulating the disease is limited[8]. Therefore, in some cases, it may be desirable to start the modulation before the disease becomes clinically apparent, e.g., between days 7 and 10 after immunization[8].

In CFA-induced arthritis models, the disease is induced by an intradermal injection of *Mycobacterium tuberculosis* suspended in mineral oil into the hind footpad or base of the tail[3,9,10]. Different doses of injected CFA have been reported in the literature with Wistar[4,11], Lewis[12-14], and Sprague Dawley[15] rats. Wide variations in the frequency and severity of lesions are also observed in different rat strains[16-19]. For example, male Lewis and Sprague Dawley rats have been reported to achieve success rates as high as 90 to 100%[3,8,20]. Conversely, some studies have shown that Wistar rats are less susceptible to AIA than other strains[8,21]. Finally, differences in the susceptibility have also been reported in relation to age: younger rats (1 to 7 days old) are usually not susceptible, whereas older animals (>9 months) tend to be relatively resistant[3,8].

Considering the variable susceptibility to CFA-induced arthritis in different rat strains demonstrated in the literature and the different doses and modes of administration adopted, the aim of this study was to describe histopathological changes observed in three variations of Wistar rat AIA model inducing mono arthritis and poly arthritis.

MATERIALS AND METHOD

Animals

Male Wistar rats weighing 300-350 g were housed in groups of five in 49x34x16-cm polypropylene home cages. All animals were maintained under a standard 12-hour light/dark cycle (lights on at 07:00 a.m. and off at 07:00 p.m.) in a temperature-controlled environment (22 ± 2°C). Animals had ad libitum access to water and chow.

All experiments and procedures were approved by the Institutional Committee for Animal Care and Use (Application No. 120220 - Graduate Research Group at Hospital de Clínicas de Porto Alegre - GPPG-HCPA), were compliant with Brazilian guidelines regulating the use of animals in research (Law No. 11,794), and adhered to the ethical and methodological standards of the Principles of Laboratory Animal Care (Laboratory guide for the care and use of animals, 8th ed., 2011).

All possible measures were taken to minimize animal suffering and external sources of pain and discomfort. In addition, the minimum

number of animals required to produce reliable scientific data were used.

Experimental Design

Rats were habituated to the maintenance room for 1 week prior to the experiment.

Subsequently, animals were randomly divided into three different groups, of 3-4 animals each, for arthritis induction: CFA-induced arthritis variation I; CFA-induced arthritis variation II; and CFA-induced arthritis variation III. Animals were evaluated 15 and 21 days after CFA injection.

Drugs and Chemicals

Complete Freund's adjuvant (CFA) was purchased from Difco, Detroit, MI, USA (H37 Ra).

CFA-Induced Arthritis Models

Briefly, inflammation was induced in the three Wistar rat variations of CFA arthritis model following isoflurane anesthesia, as follows:

CFA-induced arthritis variation I: 200 µL of emulsion (1:1; CFA and saline) injected into the right footpad[4] to induce monoarthritis;

CFA-induced arthritis variation II: 100 µL of CFA injected into the right footpad and two injections of 100 µL into the tail (one concomitantly with the injection into the footpad and the other 24 hours after)[12] to induce poly arthritis;

CFA-induced arthritis variation III: 300 µL of CFA injected into the right footpad[22] to induce mono arthritis.

Clinical Scoring

The polyarthritis severity was graded on a scale of 0–4[23]: grade 0, no disease; grade 1, slight swelling; grade 2, moderate redness and swelling; grade 3, severe redness and swelling of the whole paw; and grade 4, maximum swelling and deformity of the paw involving multiple joints.

The maximum joint score was 16, including all paws.

Histology and Histological Scoring

The animals were sacrificed (on the 15th day and on the 21st day following CFA injection), and hind paws were excised and fixed in 10% buffered formalin for 7 days. Paws were then decalcified with nitric acid 10% for 27 hours. Tissues were sectioned and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin.

A comprehensive histological scoring system, previously described[24], was used to classify specimens. Briefly, tibiotarsal joints (ankle region) were histologically evaluated and scored (0=absent, 1=mild, 2=moderate, 3=severe) for the following parameters: synovial inflammation; subcutaneous inflammation; cartilage erosion; and bone erosion.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0 (SPSS, Chicago, IL, USA). Kruskal Wallis was performed for clinical scores analysis and Fisher's Exact Test for histological scores. Significance was set at $P<0.05$.

RESULTS

Evaluation of CFA Arthritis Variations on the 15th Day

When analyzing CFA-induced arthritis variation I, of the three rats evaluated, only one presented paw edema in the four paws (Figures 1A and 1B). Conversely, in variation of CFA model II rats, all four rats showed inflammation in the four paws, in addition to skin desquamation, epilation, and red nodular swellings at the base of the tail (Figures 1D and 1E). A nodular inflammatory reaction was also observed in all variation II rats affecting the external ears and nose (Figure 1D). Among variation III rats, two developed edema in the contralateral paw and one in the four paws (Figures 1G and 1H). However, it was founded a significant difference in variation II on 14th day from Variation III on 21st day (Kruskal Wallis test/Dunn; P=0.017) (Table 1).

Table 1: Clinical scores evaluation

	Variation I		Variation II		Variation III	
Variable	**15th day**	**21st day**	**15th day**	**21st day**	**15th day**	**21st day**
Total clinical scores	4 [4;-]	4 [4;4]	12 [12;12]*	9 [6;12]	7 [7;-]	6.5 [3;-]

Kruskal Wallis test followed by Dunn. Data are expressed as median [quartiles].

* Different from Variation III on 21st day (P=0.017).

Table 2: Histological scores (0=absent to 3 = severe) of right ankles 15 and 21 days after arthritis induction with CFA injection

	Variation I		Variation II		Variation III		*P*
Variable	**15th day n (%)**	**21st day n (%)**	**15th day n (%)**	**21st day n (%)**	**15th day n (%)**	**21st day n (%)**	
Synovial inflammation 0 1 2 3	- - - 3 (100%)	- - 2 (66.7%) 1 (33.3%)	- 1 (25%) 1 (25%) 2 (50%)	- 1 (25%) 2 (50%) 1 (25%)	- - 1 (33.3%) 2 (66.7%)	- 1 (33.3%) 1 (33.3%) 1 (33.3%)	0.857
Subcutaneous inflammation 0 1 2 3	- - - 3 (100%)	- - - 3 (100%)	- - 4 (100%) -	- - 1 (25%) 3 (75%)	- - 1 (33.3%) 2 (66.7%)	- - - 3 (100%)	0.021*
Cartilage erosion 0 1 2 3	- 1 (33.3%) 1 (33.3%) 1 (33.3%)	1 (33.3%) - 1 (33.3%) 1 (33.3%)	1 (25%) 2 (50%) 1 (25%) -	- 2 (50%) - 2 (50%)	- 1 (33.3%) - 2 (66.7%)	1 (33.3%) - 1 (33.3%) 1 (33.3%)	0.867
Bone erosion 0 1 2 3	1 (33.3%) - 2 (66.7) -	1 (33.3%) - 1 (33.3%) 1 (33.3%)	3 (75%) - 1 (25%) -	1 (25%) 1 (25%) - 2 (50%)	- 1 (33.3%) - 2 (66.7%)	1 (33.3%) - 1 (33.3%) 1 (33.3%)	0.549

CFA = complete Freund's adjuvant.

* significant difference between groups (Fisher's Exact test)

Histological analyses showed periarticular panniculitis affecting the tibiotarsal joints of the induced paw in all three models assessed (Figure 2). Inflammatory infiltrates contained giant cells, plasmocytes, lymphocytes, and especially macrophages and neutrophils. There were no cases of subcutaneous tissue necrosis, and edema was similar in all animals. All animals presented joint damage, revascularization, and synovial proliferation. However, variations I and III presented higher levels of bone erosion when compared with variation I. Variation I showed the highest histological scores for synovial inflammation (3.0±0) and subcutaneous inflammation (3.0±0). Variation III presented the highest scores for cartilage erosion (2.3±1.1) and bone erosion (2.3±1.1) (Table 2 and Figure 2).

However, it was found statistically difference between groups for subcutaneous inflammation (Table 2; P=0.021; Fisher's Exact test).

Evaluation of CFA Arthritis Variations on the 21st Day

After 21 days of CFA injection, all rats in variation I group developed edema in one paw only (Figure 1C). In model II rats, systemic arthritis affected the four paws in two rats and the contralateral paw in the other two (Figure 1F). As also observed on the 15th day after CFA injection, variation II animals presented red nodular swellings, desquamation, and epilation at the base of their tail, in addition to a nodular inflammatory reaction affecting the external ears and nose. All rats in the variation III group presented edema in one paw (Figure 1I).

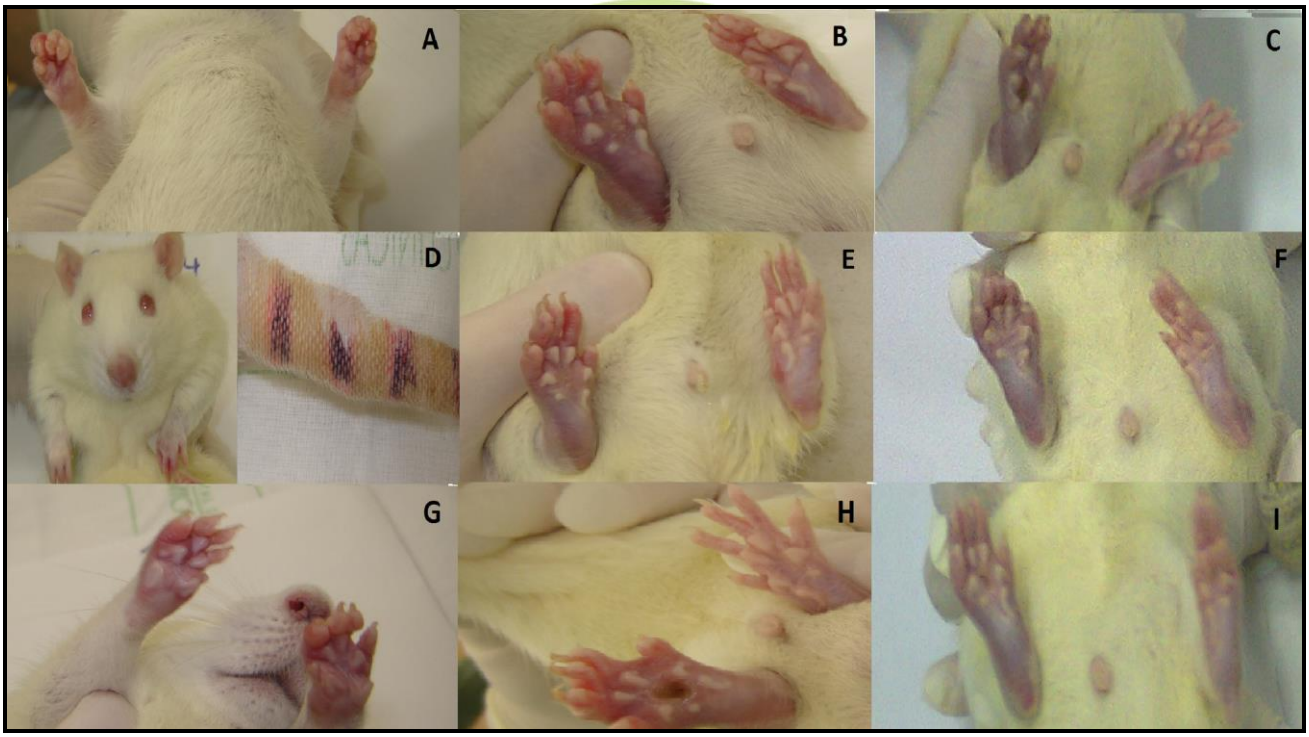

Figure 1: Wistar rats showing edema after induction of arthritis with CFA in three different variations

Figures **A**, **B**, and **C** show variation of CFA-induced arthritis I: **A**) edema in forepaws 15 days after induction; **B**) edema in hind paw at 15 days; **C**) edema in hind paw 21 days after induction. Figures **D**, **E**, and **F** show variation of CFA-induced arthritis II: **D**) edema in forepaws 15 days after induction, with desquamation, epilation, and red nodular swellings at the base of the tail, and nodular inflammatory reaction affecting external ears and nose; **E**) edema in hind paw at 15 days; **F**) edema in hind paw at 21 days. Figures **G**, **H** and **I** show variation of CFA-induced arthritis III: **G**) edema in forepaws 15 days after induction; **H**) edema in hind paw at 15 days; **I**) edema in hind paw 21 days after induction.

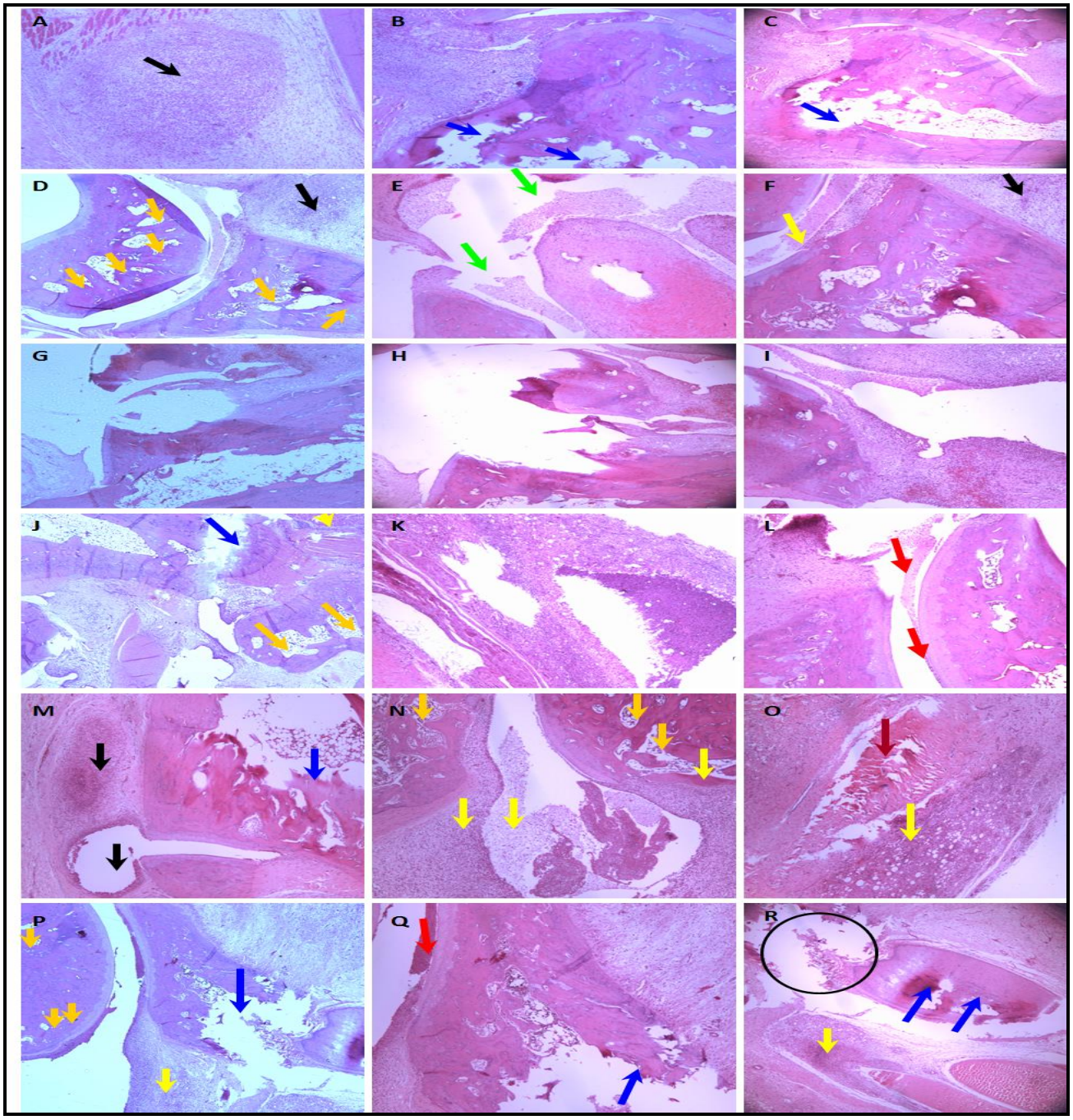

Figure 2: Hematoxylin and eosin staining of sections from the right hind paws of rat variations groups.

Black arrow = synovial inflammation; blue arrow = bone erosion; orange arrow = revascularization; green arrow = synovial tissue destruction; yellow arrow = inflammatory infiltrates invading the bone; red arrow = articular synovial proliferation; brown arrow = muscle destruction. The circle shows partial destruction of bone and synovial tissues. **A**, **B**, and **C**: variation of CFA-induced arthritis I at 15 days. **D**, **E**, and **F:** CFA variation of CFA-induced arthritis I at 21 days; **G**, **H**, and **I**: variation of CFA-induced arthritis II at 15 days, showing almost complete destruction of articular tissue and fully inflamed synovial tissues; **J**, **K**, and **L**: variation of CFA-induced arthritis II at 21 days; **M**, **N**, and **O**: variation of CFA-induced arthritis III at 15 days; **P**, **Q**, and **R**: variation of CFA-induced arthritis III at 21 days. Figures presented at 50x.

Inflammation patterns were similar on the 21st day to those observed on the 15th day. Again, variation I rats showed the highest histological scores for synovial (2.3±0.6) and subcutaneous inflammation (3.0±0.6) (Table 2 and Figure 2).

Notwithstanding, synovial inflammation showed a reduction on the 21st day when compared with the 15th day (3.0 to 2.3). Cartilage erosion was more frequent in variation II rats (2.0±1.1). Bone erosion scores were similar in all groups.

Overall, synovial inflammation scores decreased with time (i.e., from day 15 to 21) in all groups, whereas subcutaneous inflammation showed a slight increase in variations II and III. Cartilage erosion scores increased in variation II and reduced in the other two. Bone erosion increased in variations I and II, but not in variation III. However, again it was found statistically difference between variations for subcutaneous inflammation (Table 2; P=0.021; Fisher's Exact test).

DISCUSSION

Our study histologically analyzed three variations of CFA-induced arthritis model at two different time points and showed that arthritis was successfully induced in all male Wistar rat variations groups, although some histopathological and clinical differences were found. The lowest degree of variability was observed in variation I, in which arthritis was induced using a prepared emulsion comprising CFA and saline. This result can be related that most animals developed mono arthritis.

In CFA-induced arthritis variation I, only one rat developed polyarthritis, the others had monoarthritis. In variation III, most rats had monoarthritis, although two developed polyarthritis. All variation II rats developed polyarthritis, however the intensity of response varied among animals. It should be noted that the low dose of CFA used in variation I did not cause polyarthritis and showed a more homogeneous response. It must be noted that CFA injections in the tail (variation II) are associated with polyarthritis induction[25,26], as we observed. And the CFA injection in the footpad is associated with mono arthritis[27,28] and/or arthritis in the two footpads[29,30]. Despite these differences, we observed that all animals injected with CFA developed arthritis due to intense subcutaneous changes associated with granulomatous inflammation, (e.g., as in panniculitis), as previously reported[31].

In humans with arthritis, the synovial fluid is enriched predominantly with neutrophils; other structures also present include macrophages, T lymphocytes, dendritic cells, and activated fibroblasts. The primary site of irreversible tissue damage is the junction between the synovial membrane lining the joint capsule and the cartilage/bone; a mass rich in macrophages often termed the pannus. The cells of the pannus migrate over the underlying cartilage and into the subchondral bone, causing subsequent erosion of these tissues[32,33]. These activated macrophages, lymphocytes, and fibroblasts, as well as their products, can also stimulate angiogenesis, which may explain the increased vascularity found in the synovium of patients with rheumatoid arthritis[33]. In our sample, both bone/cartilage erosion and angiogenesis were observed, although the pannus was not present.

Corroborating this study, previous authors had reported that successful immunization would lead to the development of macroscopically visible inflammation of ankles, wrists, and/or interphalangeal joints, starting within 9 to 17 days post-immunization[3,8]. Disease severity usually increases over a period of 2 weeks and then diminishes[8]. In our study, on the 21st day after inflammation induction, a slight decline in histological scores was observed in all three variations. This corroborates previous studies that demonstrated that the inflammation induced by CFA had a peak in the 14th post injection, and it decreased during the days after[3,10,11]. Terrier et al., assessing severe CFA arthritis in Lewis rats, found that infiltrates were more intense and the (sub) synovial tissue more edematous in areas of focal necrosis[34]. At late disease stages, the pannus (membrane from granulation tissue, which is chronic and progressive and produces joint erosion) extends over the surface of articular cartilage, resulting

in fibrous ankylosis[34]. We did not observe these rheumatoid arthritis characteristic events in any of our three variations.

In another model of arthritis, the rats were immunized using homologous or heterologous type II collagen. The resulting polyarthritis shows marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption, and periosteal proliferation, in addition to moderate to marked synovitis and periarticular inflammation[35]. Lesions caused by type II collagen arthritis are more similar to those seen in human arthritis than AIA lesions, in that there is a more extensive pannus associated with cartilage destruction. Notwithstanding, AIA has been used much more extensively for pharmaceutical testing, and therefore more data are available for comparison with humans[3]. Also, Brand et al. have shown that collagen arthritis is elicited in genetically susceptible strains of mice by immunization with type II collagen emulsified in CFA, which restricts the range of animals used for induction[36].

In our study, male Wistar rats were used. In the study by Dimitrijevic et al., half of the Wistar rats assessed were resistant to AIA, and both Wistar and Lewis rats developed moderate adjuvant arthritis[21]. However, illness characteristics differed between the two strains in respect to disease duration and body weight loss, suggesting a lower susceptibility of Wistar rats to AIA[21]. In Lewis rats, a high susceptibility to inflammatory diseases has been reported[37], probably related to a deficient secretion of corticotropin-releasing hormone in the hypothalamus in response to inflammation[38]. In fact, HPA axis hyporesponsiveness has been used to explain the behavioral and immunological vulnerability of compromised animals[31].

In conclusion, our CFA-induced arthritis models showed some similarities with arthritis in humans[39]. In other words, the three Wistar rat CFA-induced arthritis models developed arthritis of variable clinical and histological severity. The most homogenous inflammatory response was obtained with the use of an emulsion to induce arthritis (variation I), with a minimally severe manifestation when compared to the other arthritis models tested here and in other studies.

ACKNOWLEDGEMENT

This study was supported by the following Brazilian funding agencies: National Council for Scientific and Technological Development - CNPq (Dr. I.L.S. Torres, Dr. W. Caumo); Coordination for the Improvement of Higher Education Personnel - CAPES (G. Laste, I.C.C. de Souza); Graduate Research Group (GPPG) - Hospital de Clínicas de Porto Alegre - HCPA (Dr I.L.S. Torres – Grant no. 120220).); FAPERGS/PRONEM (Dr. I.L.S. Torres, grant no. 11/2050).

REFERENCES

1. Schett, G., Redlich, K., Hayer, S., Zwerina J., Bolon B., Dunstan C., Görtz B., Schulz A., Bergmeister H., Kollias G., Steiner G., & Smolen J. S. (2003). Osteoprotegerin protects against generalized bone loss in tumor necrosis factor-transgenic mice. *Arthritis & Rheumatism 48*, 2042–2051.

2. Klimiuk P. A., Yang H., Goronzy J. J., & Weyand C.M. (1999). Production of cytokines and metalloproteinases in rheumatoid synovitis is T cell dependent. *Clinical Immunology*, 65–78.

3. Bendele A. M. (2001). Animal models of rheumatoid arthritis. *The Journal of Musculoskeletal and Neuronal Interactions 1*, 377-385.

4. Bernardi A., Zilberstein A. C. C. V., Jäger E., Campos M. M., Morrone F. B., Calixto J. B., Pohlmann A. R., Guterres S.S., & Battastini A. M. (2009). Effects of indomethacin-loaded nanocapsules in experimental models of inflammation in rats. *British Journal of Pharmacology*, 158, 1104–1111.

5. Joe B., Griffiths M. M., Remmers E. F., & Wilder R. L. (1999). Animal models of rheumatoid arthritis and related

inflammation. *Current Rheumatology Reports*, 1,139–148.

6. Pearson C. M. (1956). Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proceedings of the Society for Experimental. *Biology and Medicine*, 91, 95-100.
7. Pearson C. M., Waksman B. H., & Sharp J. T. (1961). Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. *The Journal of Experimental Medicine*, 113,485-510.
8. John Wiley & Sons. Animal Models for Autoimmune and Inflammatory Disease. *Current Protocols in Immunology*, 1996.
9. Van Eden W., & Waksman B. H. (2003). Immune regulation in adjuvant-induced arthritis: possible implications for innovative therapeutic strategies in arthritis. *Arthritis & Rheumatism*, 48, 1788–1796.
10. Waksman B. H. (2002). Immune regulation in adjuvant disease and other arthritis models: relevance to pathogenesis of chronic arthritis. *Scandinavian Journal of Immunology*, 56, 12–34.
11. Liu M., Mao W., Guan H., Li L., Wei B., & Li P. (2011). Effects of taurochenodeoxycholic acid on adjuvant arthritis in rats. *International Immunopharmacology*, 11, 2150–2158.
12. Helyes Z., Szabo A., Nemeth J., Jakab B., Pinter E., Banvolgyi A., Kereskai L., Kéri G., & Szolcsányi J. (2004). Antiinflammatory and analgesic effects of somatostatin released from capsaicin-sensitive sensory nerve terminals in a freund's adjuvant–induced chronic arthritis model in the rat. *Arthritis & Rheumatism*, 50, 1677–1685.
13. Lorton D., Lubahn C., Klein N., Schaller J., & Bellinger D. L. (1999). Dual role for noradrenergic innervation of lymphoid tissue and arthritic joints in adjuvant-induced arthritis. *Brain, Behavior, and Immunity*, 13, 315–334.
14. Zuurmond A. M., Koudijs A., van El B., Doornbos R. P., van Manen-Vernooij B. C. T., Bastiaans J. H., Penninks A. H., van Bilsen J. H., Cnubben N. H., & Degroot J. (2011). Integration of efficacy, pharmacokinetic and safety assessment of interleukin-1 receptor antagonist in a preclinical model of arthritis. *Regulatory Toxicology and Pharmacology*, 59, 461–470.
15. Li J., Lua Y., Xiaoc C., Lua C., Niua X., & Hea X. (2011). Comparison of toxic reaction of Tripterygium wilfordii multiglycoside in normal and adjuvant arthritic rats. *Journal of Ethnopharmacology*, 135, 270–277.
16. Battisto J. R., Smith R. N., Beckman K., Sternlicht M., & Welles W. L. (1982). Susceptibility to adjuvant arthritis in DA and F344 rats. *Arthritis & Rheumatism*, 25, 1194-1199.
17. Bersani-Amado C. A., Duarte A .J., Tanji M. M., Cianga M., & Jancar S. (1990). Comparative study of adjuvant-induced arthritis in susceptible and resistant strains of rats. III. Analysis of lymphocyte subpopulations. *The Journal of Rheumatology*, 17,153-158.
18. Glenn E. M., & Gray J. (1965). Adjuvant-induced polyarthritis in rats: biologic and histologic background. *American Journal of Veterinary Research*, 26, 1180-1194.
19. Griffiths M. M. (1988). Immunogenetics of collagen-induced arthritis in rats. *International Reviews of Immunology*, 4, 1-15.
20. Pearson C. M, & Wood F. D. (1963). Studies of Arthritis and Other Lesions Induced in Rats by the Injection of Mycobacterial Adjuvant. *American Journal of Pathology*, 42, 73–95.
21. Dimitrijevic M., Laban O., Djuric V. J., Stanojevic S., Miletic T., Jovanovic V. K., Todorović C., & Radulović J. (2001). Behavior and severity of adjuvant arthritis in four rat strains. *Brain, Behavior, and Immunity*, 15, 255–265.

22. Ganjua L., Padwada Y., Singha R., Karana D., Chanda S., Chopra K. M., Bhatnagar P., Kashyap R., & Sawhney R. C. (2005). Anti-inflammatory activity of Seabuckthorn (Hippophae rhamnoides) leaves. *International Immunopharmacology*, 5, 1675–1684.

23. Kim K. S., Choi Y. H., Kim K. H., Lee Y. C., Kim C. H., Moon S. H., Kang S. G., & Park Y. G. (2004). Protective and antiarthritic effects of deer antler aqua-acupuncture (DAA), inhibiting dihydroorotate dehydrogenase, on phosphate ions-mediated chondrocyte apoptosis and rat collagen-induced arthritis. *Int Immunopharmacol*, 4, 963–73.

24. Brenner M., Meng H., Yarlett N., Joe B., Griffiths M. M., Remmers E. F., Wilder R.L., & Gulko P. S. (2005). The non-MHC quantitative trait locus Cia10 contains a major arthritis gene and regulates disease severity, pannus formation and joint damage. *Arthritis & Rheumatism*, 52, 322–332.

25. Lorton D., Lubahn C., Klein N., Schaller J., & Bellinger D. L. (1999). Dual Role for Noradrenergic Innervation of Lymphoid Tissue and Arthritic Joints in Adjuvant-Induced Arthritis. *Brain, Behavior, and Immunity*, 13, 315–334.

26. Woods J. M., Katschke K. J., Volin M. V., Ruth J. H., Woodruff D. C., Amin M. A., Connors M. A., Kurata H., Arai K., Haines G. K., Kumar P., & Koch A. E. (2001). IL-4 Adenoviral Gene Therapy Reduces Inflammation, Proinflammatory Cytokines, Vascularization, and Bony Destruction in Rat Adjuvant-Induced Arthritis. *J Immunol*, 166, 1214-1222.

27. Mythilypriyaa R., Shanthib P., & Sachdanandam P. (2008). Efficacy of Siddha formulation Kalpaamruthaa in ameliorating joint destruction in rheumatoid arthritis in rats. *Chemico-Biological Interactions*, 176, 243–251.

28. Cheppudira B. P. (2006). Characterization of hind paw licking and lifting to noxious radiant heat in the rat with and without chronic inflammation. *Journal of Neuroscience Methods*, 155, 122–125.

29. Caparroz-Assef S. M, Bersani-Amado C. A, Kelmer-Bracht A. M., Bracht A., & Ishii-Iwamoto E. L. (2007). The metabolic changes caused by dexamethasone in the adjuvant-induced arthritic rat. *Mol Cell Biochem*, 302, 87–98.

30. Talwara S., Nandakumara K., Nayaka P. G., Bansala P., Mudgala J., Mora V., Raoa C. M., & Lobo R. (2011). Anti-inflammatory activity of Terminalia paniculata bark extract against acute and chronic inflammation in rats. *Journal of Ethnopharmacology*, 134, 323–328.

31. Eberhard B. A., & Ilowite N. T. (2002). Panniculitis and lipodystrophy. *Current Opinion Rheumatology*, 14, 566–570.

32. Allard S. A., Muirden K. D., Camplejohn K. L., & Maini R. N. (1987). Chondrocyte-derived cells and matrix at the rheumatoid cartilage-pannus junction identified with monoclonal antibodies. *Rheumatology International*, 7, 153–59.

33. Choy E. H. S., Panayi G. S. P. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. Mechanisms of Disease. *The New England Journal of Medicine*, 344, 907-16.

34. Terrier F., Hricak H. L., Revel D., Alpers C. E., Reinold C. E., Levine J. (1985). Magnetic resonance imaging and spectroscopy of the periarticular inflammatory soft tissue changes in experimental arthritis of the rat. *Investigative Radiology*, 20, 813-823.

35. Terato K., Hashida R., Miyamoto K., Morimoto T., Kata Y., & Kobayashi S. (1982). Histological, immunological and biochemical studies on type II collagen arthritis in rats. *Biomedical Research*, 3, 495-523.

36. Brand D. D., Latham K. A., & Rosloniec E. F. (2007). Collagen-induced arthritis. *Nature Protocols,* 2, 1269-1275.

37. Van De Langerijt A. G., Van Lent P. L., Hermus A. R., Sweep C. G., Cools A. R., & Van Den Berg W. B. (1994). Susceptibility to adjuvant arthritis: relative importance of adrenal activity and bacterial flora. *Clininical & Experimental Immunology,* 97, 33–38.

38. Gomez F., De Kloet E. R., & Armario A. (1998). Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. *American Journal of Physiology,* 274, 420–427.

39. Sayah A., & English III J. C. (2005). Rheumatoid arthritis: A review of the cutaneous manifestations. *American Academy of Dermatology,* 53, 191-209.

