Animal models of ovariectomy in rats demonstrate characteristics similar to early stage osteoarthritis

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Short Report

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Abstract

Purpose

The aim of our study is to analyze the model of ovariectomy (OVX) in rats reproduced histological changes of osteoarthritis (OA).

Methods

For the development of the research, 12 Wistar rats were used, divided into 2 equal groups: Control Group - C (n = 6) and Osteoarthritis Group - OA (n = 6). After the 6-month experimental period, all rats were sacrificed and, subsequently, the entire knee joint complex was removed without disarticulation. For the histological evaluation of the tissue, the recommendations of the International Society for Research in OA (OARSI) were used. For data processing, each evaluation was statistically treated in both groups, comparing data from group C with the group OA. Results: Through the histological evaluation of OARSI, the evolution of OA in various tissues of the joint was evaluated. Although the OA group showed noticeable differences from group C, they were not as significant. Thus, only statistically significant favors were presented in the loss of the cartilaginous matrix (OA and C, p = 0.51), considering that the changes in the loss of ECM occurred only at the depth of 0% (superficial region), but at the depth of 50% (intermediate region) and 100% depth (deep region) did not exist.

Conclusion

Our study demonstrated that the OVX model is a good model to discuss OA, showing histological changes similar to those found in OA, the model demonstrated to have a progressive and slow characteristic since after the OARSI evaluations, prominent evidence was found in the initial manifestations of OA.

Introduction

We can define OA as a multifactorial disease, which is chronic, degenerative and inflammatory (COLLINS et al., 2018), where it affects all joint tissues, including articular cartilage, subchondral bone, synovium, menisci and ligaments (MALFAIT et al., 2020). To further explore and understand OA progression, studies are conducted with different animal models: traumatic, pharmacological or genetic (MALFAIT & LITTLE et al., 2015). Among these models, the ovariectomy (OVX), a procedure responsible for the surgical removal of the ovaries, has shown a decrease in the production of female hormones, simulating the post menopausal state in an animal model (COLLINS et al., 2018). The animal model of OVX in research is widely used as an animal model of osteoporosis, however, there is a scarcity of research that uses the OVX model as an animal model of OA even though previous studies demonstrate a possible relationship

between tissue changes that occur OVX powders are similar to those of OA (FERRÁNDIZ 2015; TODA 2020)

After the induction of OVX, its primary effects permeate between the sharp drop of estrogen, simulating the post menopausal state of the woman, followed by the rapid loss of bone mineral density, which leads to changes in the microarchitecture of the trabecular network, due to the increased remodeling bone caused by lack of estrogen (HOLLAND et al., 2013; YANG et al., 2014). Upon detecting the absence of these hormones, which were previously produced in great abundance by the ovaries, the muscle mass or lean mass more common than the fat mass, is now in inverted situations, thus altering the body composition, which ends up directly impacting bone health (YANG et al., 2014).

. Therefore, our study aims to histologically analyze rats submitted to OVX through the histological evaluation of the Osteoarthritis Research Society International (OARSI), recommendations for histological evaluation in OA of rats, which covers the main tissues of the disease, being the first system of histological evaluation specific for OA models in rats (GERWIN et al., 2010). Therefore, our hypothesis is that OVX reproduced the histological alterations of AO.

Methodology

2.1 Animals

Twelve Wistar rats were used, from the Central Animal Bioterium of the Federal University of São Carlos (UFSCar), which remained for 6 months grouped in plastic cages, with free access to water and feed. The animals were kept in the bioterium with controlled environmental conditions, luminosity: 12h light / dark cycle and ambient temperature between 22 and 24 ° C, in conditions similar to those found in the literature (ZENG, 2018; TANG, 2018). The experiment was conducted according to international ethical recommendations (NATIONAL RESEARCH COUNCIL, 1996) and the project was sent to the Ethics Committee on Animal Experimentation at the UFSCar.

2.2 Experimental Groups

The rats arrived at the bioterium at 9 weeks old. After the period of acclimatization to the vivarium and the inverted cycle, the animals were randomly divided into 2 equal groups: Control Group - C (n = 6) and Osteoarthritis Group - OS (n = 6), where they remained until the end of the experiment. The OVX procedure was performed in only six rats when they were 12 weeks old (TANG, 2018).

2.3 Animal Model of Osteoarthritis

The animal model of OA used was bilateral OVX. Initially, the animals were anesthetized with a mixture of Xylazine 20 17 mg / Kg / body weight and Ketamine 90 mg / Kg / body weight injection, following international ethical recommendations (NATIONAL RESEARCH COUNCIL, 1996). At the beginning of the surgical procedure, the reflexes of the rats were tested to verify the effectiveness of the anesthesia procedure. First, a small bilateral incision of 1.0 - 1.5 cm was made through the skin and the muscular

layer, between the last rib and the thigh, in parallel with the animal's body line. The peritoneal cavity was opened and a ligature was made below the fimbria. The ovaries were removed and the incision made in the skin and sutured muscles. In the hours following the surgical procedure, the rats were kept in individual boxes and heated until they returned from the effect of anesthesia.

2.4 Histological Processing

After the experimental period, all animals were sacrificed and, afterwards, the complete knee joint cartilage (tibia, femur and patella) was removed from each knee joint without disarticulation. The knees were subjected to 7.5% Nitric Acid descaling. glycerin in the same concentration for approximately 7 days (95 hours). This material was processed in paraffin and embedded. With that, a block was obtained for each knee. Subsequently, each block was cut in the frontal plane using a microtome (Leica®). The first 30 cuts of 6 µm were discarded, and from there, cuts of 6 µm were also selected to assemble the blades after every 5 cuts made, giving a total of 40 cuts per joint, over the entire length of the material. The histological slides of the rats' knee joints were stained with hematoxylin and eosin. (GERWIN et al., 2010).

2.5 Histological Evaluation

For the histological evaluation of the tissue, the recommendations of the International Osteoarthritis Research Society were used for the histological evaluation of osteoarthritis in rats, adapted from the article by Gerwin et al (2010). The photomicrographs were made using the 3D HISTECH PANORAMIC DESK Histological Slide Scanner equipment.

2.6. Statistical Analysis

Statistical procedures were performed using Statistic 13 (STATSOFT®). Initially, the data were analyzed in relation to their normality and homogeneity, using the Shapiro-Wilks tests. For all experiments, data will be compared between groups using the ANOVA test with Post Hoc Tukey. For all analyzes, a significance level of 5% ($p \leq 0.05$) will be used for statistically significant differences, in a 95% confidence interval (CI).

Results

Through the histological evaluation of OARSI, the evolution of OA in various tissues of the joint was evaluated. Although the OA group showed noticeable differences from group C, they were not as significant. Thus, only statistically significant favors were presented in the loss of the cartilaginous matrix (OA and C, $p = 0.51$), considering that the changes in the loss of ECM occurred only at the depth of 0% (superficial region), but at the depth of 50% (intermediate region) and 100% depth (deep region) did not exist.

In the other scores of cartilage degeneration such as: extension of total cartilage degeneration, extension of significant cartilage degeneration and depth of lesions by cartilage zone, there was no significant difference between the groups (OA and C, $p = 0.19$; OA and C, $p = 0.13$; OA and C, $p = 0.11$),

In the score of the joint capsule, the evaluation showed a small increase in the thickness of the joint capsule of the OA group, however the groups showed significant differences between them (OA and C, p = 0.03) (Figure 2). In the morphometric evaluation of osteophytes, groups C and OA did not present osteophytes greater than - 200 µm, therefore, by the OARSI tool they are considered marginal and score 0 (OA and C, p = 0,95). In the evaluation of the synovial membrane, only the OA group showed an increase in the number of lining cells and a slight proliferation of subsinovial tissue (Figure 2), however, they were not significant enough for statistically relevant beings (OA and C, p = 0.37).

Discussion

Our study investigated the evolution of OA in the knees of rats submitted to OVX. As expected, the OVX model proved to be a good model for discussing OA since it reproduced the histological changes in OA in rats compared to the control group.

The OVX model as expected showed a slow progression of OA over six months of study. Toda (2020), in his study addresses that the cartilage metabolism may have been influenced by the estrogen deficiency induced by OVX, since the chondrocytes in the articular cartilage have estrogen receptors, which would result in a more slow progression of the disease.

In our study, statistically significant results were found in the most superficial layer of the cartilage where it presented an increase in the condorcites and its migration to the surface was triggered in an attempt to repair and control of the evolution of OA. Ferrándiz (2015) used OVX as an OA model and its main results were structural changes in the cartilage surface, including focal damage and fibrillation, loss of proteoglycans, increased chondrocyte clustering. Thus, we can notice that the results of this study were similar to ours since the results were more present in the initial changes of AO.

Trevisan (2016) describes in his study the evaluation of the evolution of OA in the knees of rats according to the histological evaluation of OARSI, the model of TLCA (transection of the anterior cruciate ligament) the most significant results of articular cartilage, subchondral bone, synovial membrane and joint capsule and osteophytes were found in the groups of nine and twelve months of OA induction. In addition, the study by Araújo (2018) when performing the analysis of OARSI in rats with OA induced by TLCA after collagen supplementation after 2 months of study, it was possible to observe statistically positive results only in superficial layers of the cartilage, synovial membrane and joint capsule. Thus, we can take into account that the tool developed by OARSI is adjustable to the tissues affected by OA, but, on the other hand, it is not sensitive to initial changes in the disease.

In addition to the OARSI tool not showing sensitivity in the initial demonstrations of the disease, possible hypotheses for our insignificant results in the deep layers of cartilage are in line with the study by Osman (2020), where OVX resulted in increased erosion of the surface of the cartilage. cartilage, however, did not show results in the deep layer. Gerwin et al (2011), also describes that the OA induction model used in our research, presents a slow evolution with few degenerative changes in the short term

after induction. Therefore, we can again affirm the hypothesis that the non-significant results in deeper layers of the disease were due to the short time of disease induction.

It is evident, therefore, that the OA model induced by OVX proves to be an effective model, as it presented the expected histological changes for OA in the initial phase. However, as expected by the OARSI evaluation, it did not show sensitivity to the initial changes of the disease, emphasizing the reason for our low results in layers and tissues that present changes in OA in more advanced stages.

Conclusion

Our study demonstrated that the OVX model is a good model for discussing OA demonstrating histological changes similar to those found in OA, the model demonstrated to have a progressive and slow characteristic similar to those found in other OA models.

Declarations

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Conflicts of interest/Competing interests: Natália Aparecida Casonato, Camila Marques de Araújo, Mariane Santos Trevisan, Cristina Arrais-Lima and Fernando Augusto Vasilceac confirm have no financial or personal conflicts of interest to declare.

Availability of data and material: Natália Aparecida Casonato, Camila Marques de Araújo, Mariane Santos Trevisan, Cristina Arrais-Lima and Fernando Augusto Vasilceac confirm the availability of the data materials, as well as the transparency of the data.

Code availability: Not applicable.

Authors' contributions: The authors contributions are as follows: Vasilceac, Fernando Augusto contributed to the study design and interpretation of the findings; Casonato, Natalia Aparecida, Araújo, Camila Marques were the main investigator and contributed to the study design, data collection, data analyzes and interpretation of the findings and wrote the manuscript. Arrais-Lima Cristina and Casonato, Natália Aparecida assisted during data collection and statistical analysis.

Ethics approval: The Ethics Committee on the Use of Animals of the Federal University of São Carlos, in the fulfillment of its duties, analyzed and APPROVED the Final Report (version of 07 / July / 2019) of the proposal referred to above.

Consent to participate: Not applicable.

Consent for publication: Consent to submit was received from all co-authors and responsible authorities at the institute / organization where the work was carried out before the work was submitted.

References

1. Collins, KH, Hart, DA, Seerattan, RA, Reimer, RA, & Herzog, W. Obesity induced by a high fat / sucrose diet results in the specific development of osteoarthritis-like degeneration joint in a rat model. Bone and joint research 2018: 274–281.

2. Ferrándiz, ML, Terencio, MC, Carceller, MC, Ruhí, R., Dalmau, P., Vergés, J., ... & Alcaraz, MJ. Effects of BIS076 in a model of osteoarthritis induced by transection of the anterior cruciate ligament in ovariectomized rats. BMC Musculoskeletal Disorders 2015: 92.

3. Gerwin, N. The OARSI histopathology initiative–recommendations for histological assessments of osteoarthritis in the rat. Osteoarthritis and Cartilage 2010: 24–34.

4. GRATAL, P. Chondrocyte enlargement is a marker of osteoarthritis severity. Osteoarthritis and cartilage 2019: 1229–1234.

5. Holland, JC, Brennan, O., Kennedy, OD, Mahony, NJ, Rackard, S., O'Brien, FJ, & Lee, TC. Examination of osteoarthritis and changes in the subchondral bone in the knee joint of an ovariectomized sheep model. Journal of anatomy 2013: 588–597.

6. Malfait, A. M., & Little, C. B. On the predictive utility of animal models of osteoarthritis. Arthritis research & therapy 2015: 225.

7. Malfait, A. M., & Miller, R. E. Why we should study osteoarthritis pain in experimental models in both sexes. Osteoarthritis and Cartilage 2020: 397–399.

8. Osman, FR, Yacoub, MFY, Mohammed, SHAK and Mogahed, OE. Comparative Histological Study between the Effect of Calcitonin versus Hormone Replacement Therapy on Ovariectomized Albino Rat Knee Joint Cartilage. Egyptian Journal of Histology 2019: 515–525.

9. Trevisan, M. S .. Histological evaluation in rat knee osteoarthritis: validation and reproducibility of a new classification. 2018.

10. TODA, Toshiya; SUGIOKA, Yuko; KOIKE, Tatsuya. Soybean isoflavone can protect against osteoarthritis in ovariectomized rats. Journal of Food Science and Technology 2020: 1–6.

11. YANG, PY. et al. Effects of tibolone on osteoarthritis in ovariectomized rats: association with nociceptive pain behaviour. European journal of pain 2014: 680–690.

Figures

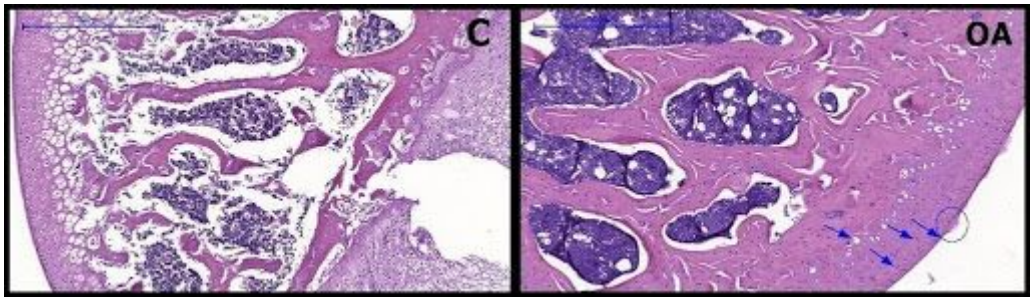

Figure 1

Photomicrographs of the articular cartilage slides, illustrating the changes present in groups C and AO. The arrows on the OA slides signal the proliferation of cells and their migration to the surface in an attempt to repair and control the disease. The circle represents the degradation on the surface of the MEC. (Bar corresponds to 50 μm; 500 times increase).

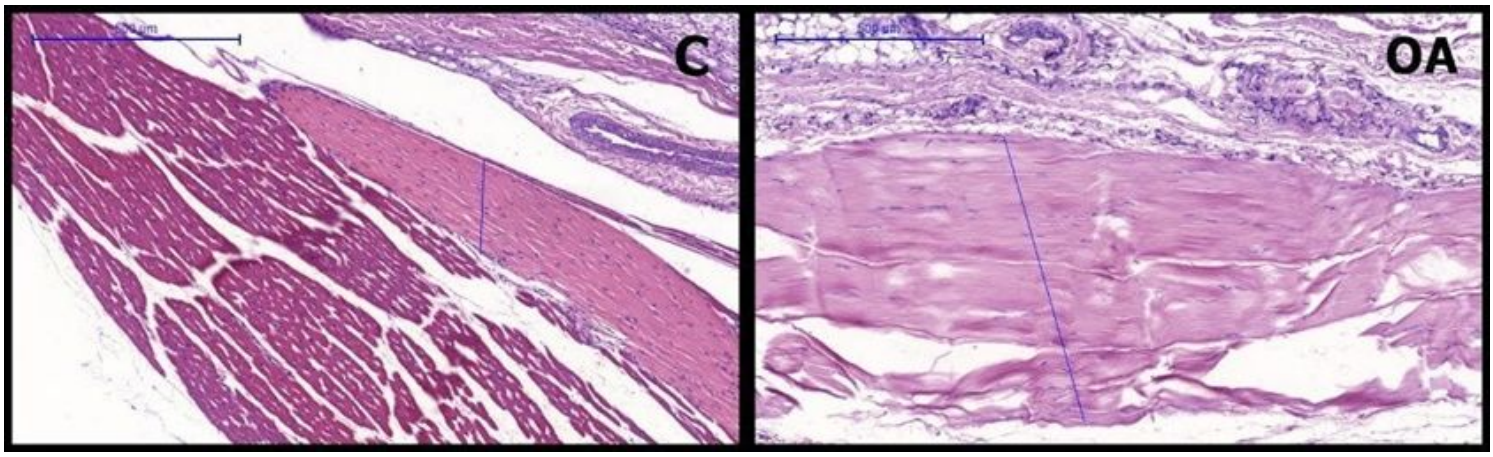

Figure 2

Photomicrographs of the slides of the joint capsule, illustrating the evolution of the degradation of ECM C and OA group. In the blade OA, we can observe the difference in the thickness of the joint capsule of group C and OA. The blue line represents the difference in capsule length between groups. (Bar corresponds to 50 μm; 500 times increase).