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RESEARCH

Osteochondral lesion mouse model: An alternative for experimental work[☆]



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KEYWORDS

Osteochondral lesion;
Animal model;
Mice;
Cartilage

Abstract

Objective: To report a reproducible and inexpensive model of critical osteochondral lesion (LOC) in adult mice for experimental studies.

Materials and methods: An experimental study was conducted on 20 BKS mice of 15 weeks old, in which a LOC of 0.5 mm in diameter was made in the trochlear groove. Ten animals were sacrificed at day 7, and the other 10 animals at day 14 of follow-up. To assess the ability of the animal to repair/regenerate, a histological analysis was performed using hematoxylin-eosin and safranin-O stains, and the results were evaluated by the ICRS scale using areas of healthy cartilage from the same joint as control. The Mann-Whitney *U* test was used for the statistical analyses of scores (averages).

Results: Significant differences were found on days 7 and 14 between the LOC area and control areas, but no differences were found between the day 7 and day 14.

Conclusion: This model of LOC in the trochlear groove of adult mice is highly reproducible, and could be used in further studies to obtain better treatments for chondral pathologies.

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PALABRAS CLAVE

Lesión osteocondral;
Modelo animal;
Ratón;
Cartílago

Modelo murino de lesión osteocondral: alternativa para trabajos experimentales

Resumen

Objetivo: Analizar un modelo murino adulto de lesión osteocondral crítica (LOC) que sea reproducible y de bajo costo para estudios de experimentación.

Material y método: Se desarrolló un trabajo experimental en 20 ratones BKS, de 15 semanas, realizando una LOC en el surco troclear de 0,5 mm de diámetro. Diez animales fueron sacrificados a los 7 y 14 días tras la cirugía. Para evaluar la capacidad de reparación o regeneración del

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modelo, se realizó un análisis histológico, mediante tinción de hematoxilina-eosina y safranina-O, evaluando con la escala ICRS-II. Se comparó la zona de lesión con áreas de cartílago sano del mismo animal (zonas control). Las puntuaciones obtenidas (promedios) en cada grupo se compararon entre sí determinándose diferencias significativas.

Resultados: En la evaluación en los días 7 y 14 se encontró una diferencia significativa entre la zona de la LOC y las zonas control, sin existir diferencias entre los 2 periodos evaluados.

Conclusión: El modelo murino adulto de LOC crítica, en la tróclea femoral, es altamente reproducible. La potencialidad de regeneración innata del animal secundaria a la presencia de fisas durante la vida adulta no logró reparar adecuadamente la lesión producida sobre el cartílago articular. Este modelo permitirá la realización de nuevos estudios orientados a obtener mejores tratamientos para las patologías condrales.

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Introduction

Pathologies affecting the joint cartilage, such as chondral and osteochondral (OCL) lesions, which are precursors of osteoarthritis (OA), have generated numerous publications in international literature due to the low capacity for repair of this tissue and the lack of optimal treatment options available.¹ This has led to an increasing interest in the development of experimental strategies which are capable of generating physiopathological knowledge allowing an adequate assessment of new treatments. For this reason, the development of experimental animal models for research work is essential to the development of innovative therapeutic strategies. Among these are currently the use of platelet-rich plasma,² autologous implant of chondrocytes associated to membrane and, more recently, some experiences with pluripotent mesenchymal cells,³ reinforcing the importance of these experimental models.

The literature contains reports of several models in research relating to OCL,⁴⁻⁷ including medium-sized (rabbits and dogs) and large (sheep and horses) animals. Nevertheless, there are very few assessments of rodent models for the study of osteochondral pathologies, despite the fact that their low cost, high reproductive rates and ease of maintenance make them highly suitable candidates. The main concern with regard to these animals is their high rate of spontaneous OCL repair. For this reason, they are mainly used in studies on genetic alterations to generate a spontaneous OA.^{7,8} However, potential repair of OCL in adult mice models has not been validated in any published study. The objective of the present work is to analyze a reproducible and inexpensive adult mouse model with critical OCL for experimental studies.

Materials and methods

Animals

After obtaining approval from the bioethics committee at our center, we obtained 20 female mice of the BKS strain

aged 15 weeks from the vivarium, in accordance to the animal care regulations of the US National Institute of Health.⁹

Surgical model of osteochondral lesion

The animals were anesthetized through inhalation of a 3%/32% sevoflurane/oxygen mixture. We conducted the same surgical procedure on both knees of each animal. Using a number 15 scalpel we carried out a medial parapatellar longitudinal approach in extension of the lower limbs until the extensor apparatus was exposed. We then carried out a medial parapatellar arthrotomy with lateral digital dislocation of the patella, thus exposing the condyles and the femoral trochlea. Visualizing with surgical magnification equipment, we identified the trochlear groove and, using a 25G needle (0.5mm diameter), we carried out a single OCL in the central region, penetrating into the subchondral bone (Fig. 1). Under direct visualization, we carried out a reduction of the extensor apparatus, confirming adequate mobility of the patellofemoral joint. The medial parapatellar arthrotomy and the skin approach were carried out with 2

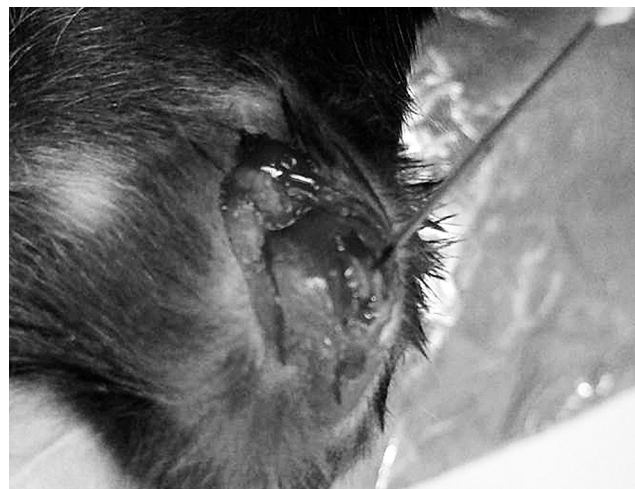


Figure 1 Trochlear groove and creation of 0.50 mm diameter OCL with a 25 G needle. The lesion was created with the knee in flexion to improve manipulation.

Table 1 Semiquantitative histological scale measuring repair of the joint cartilage according to the International Cartilage Repair Society.

Category	Score
<i>Percentage of defect filled</i>	
100	4
75	3
50	2
25	1
0	0
<i>Continuity of the joint surface</i>	
Smooth and continuous	3
Rough but continuous	1
Discontinuous	0
<i>Restoration of osteochondral architecture</i>	
Clearly differentiated	4
Not clear (heterogeneous)	2
Poor (bone-chondral compartmentalization)	1
Nonexistent	0
<i>Integration of repair tissue</i>	
Complete	3
Partial	1
Poor	0
<i>Cellular morphology of the regenerative tissue of the joint cartilage</i>	
Hyaline with regional architecture	5
Hyaline without regional architecture	3
Hybrid fibrohyaline cartilage	2
Fibrocartilage	1
Fibrous tissue	0
<i>Staining of matrix (metachromasia)</i>	
Normal (compared to adjacent cartilage)	3
Slightly reduced	2
Notably reduced	1
Without metachromatic staining	0

planes of prolene 6.0 in continuous suture. Inhaled anesthesia was stopped and 10 mg/kg of sublingual tramadol was administered for analgesia. The animals were allowed *ad libitum* mobility immediately after the surgery concluded. There were no surgical complications and all the animals survived. After waking from anesthesia, the animals did not suffer severe difficulties for movement on their 4 limbs (Table 1).

Sacrifice and collection of surgical specimens

A total of 10 animals were sacrificed on day 7 and another 10 on day 14 after the surgery through an intraperitoneal injection of 100 mg/kg of sodium pentobarbital. The surgical approach mentioned previously was repeated, exposing the extensor apparatus up to the middle third of the thigh. The flexor and extensor musculature was sectioned, freeing it from bone insertions from proximal to distal, thus achieving forward displacement of the entire extensor apparatus and exposing the area of the femoral condyles. Using surgical magnification, we proceeded to carefully disarticulate the proximal region of the knee, avoiding damage to the

joint cartilage during the process. Fracture of the femoral condyles during collection of the surgical specimens took place in 1 knee in the day 7 group and 1 in the day 14 group. The samples obtained were fixed in 4% paraformaldehyde for 48 h, prior to their inclusion in paraffin.

Histological analysis

We obtained 5 μ m histological sections of the samples included in paraffin, which were then stained with hematoxylin–eosin and safranin-O. Repair or regeneration of the induced lesions was assessed using the semiquantitative scale for the repair of joint cartilage of the International Cartilage Repair Society II (ICRS II). This scale analyzes 6 variables (percentage of defect filling, continuity of the joint surface, restoration of the osteochondral architecture, integration of the repair tissue, cellular morphology of the chondral regenerative tissue and metachromasia) to provide a score indicating the quality of the repair.¹⁰

Statistical analysis

We conducted a descriptive statistical analysis of the values obtained and used the Mann–Whitney *U* test for continuous samples to assess significant differences ($P < 0.05$) between the groups.

Results

The OCL were macroscopically and microscopically visible in the 38 knees studied (19 knees for each period), both on day 7 and on day 14. The microscopic analysis detected a solution of contiguity in the chondral surface which was compatible with the instruments used to create the 0.5 mm diameter OCL in all the samples analyzed. In addition, the depth of the lesion reached at least the physis in all cases (Fig. 2A and B).

When analyzing the histological sections after 7 days, we found inflammatory connective repair tissue and did not observe the presence of chondrocytes in the damaged region (Fig. 2A). Staining with safranin-O showed that, despite this attempted repair, the characteristics of the extracellular matrix in the damaged region were clearly different from those of the surrounding chondral tissue, with a notable decrease in the amount of glycosaminoglycans. The histological score obtained was of 4.2 ± 0.29 , showing significant differences with the control region (normal cartilage, 22 points, $P < 0.001$).

In the group of animals assessed after 14 days, the histological results were similar to those described above, highlighting a lower presence of inflammatory cells. In 1 knee we observed symptoms compatible with acute osteomyelitis connected to the induced OCL. The histological score obtained was of 4.1 ± 0.33 , with significant difference compared to the control region ($P < 0.001$). Comparison of the results obtained in the 2 periods assessed did not find significant differences (Fig. 3).

Discussion

Different animal models have been proposed for the study of chondral physiology and physiopathology, in particular smaller animals, such as rabbits and dogs, and larger ones,

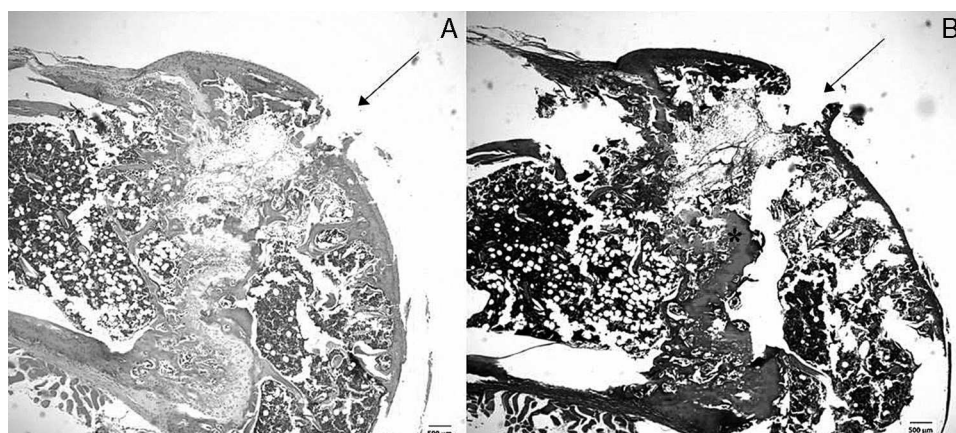


Figure 2 Histological analysis of the OCL produced. Shown as a sagittal section of the trochlear region of an animal sacrificed after 7 days, representing the full sample. (A) Staining with hematoxylin-eosin showing the solution of contiguity of the joint surface. (B) Staining with safranin-O; it is possible to identify the OCL and depth reaching the physis (*).

such as sheep and horses. However, the complexities associated to the use of these types of animals has led to a limited availability of these models, especially for studies in which the transfer of knowledge from *in vitro* models is increasingly being applied. Smaller animals, including rodents, offer comparative advantages for the development of scientific research, mainly their low maintenance costs, high reproductive rate, short experimental time and reproducibility of results. Furthermore, there are athymic, transgenic and knockout animals for certain pathologies, so they represent an interesting alternative as first animal model.⁸

Among the main disadvantages attributed to experimental mice models are the small size of the joint, narrowness of the joint cartilage and high rate of spontaneous regeneration or repair maintained during their adult life due to the persistence of the physis, which could have a negative influence when assessing therapies.¹¹ However, after reviewing the available literature, we did not find any publications regarding adult mice models which reported spontaneous regeneration or repair of chondral or osteochondral lesions, with only 1 report of OCL focusing on young animals (8 weeks), in which this intrinsic capacity for repair was partially demonstrated.¹² Similarly, adequate studies of therapeutic alternatives for the study of OCL have been

developed in animals which share many of the characteristics described with our model, like rats.¹³

Our results, both at 7 and 14 days, were consistent in their capacity to generate reproducible OCL of a critical size which, although inducing a reparative process by the organism, as illustrated by the presence of inflammatory cells, were not adequately covered by new chondral tissue, as reflected by the histological scores.

Although it has been recognized that, in mice, progenitor cells derived from the physis have the potential to repair damage at the joint level, as is also the case in human beings, the molecular and histological processes which mediate this repair are not fully understood.¹⁴ It is known that embryogenesis of the joint cartilage in mice takes place early, as the joint cartilage can be identified after 2 weeks of embryonic development,¹⁵ thus leading us to believe that the time required to regenerate the damaged cartilage should be in the same order of magnitude. For this reason, the periods assessed in the present work (7 and 14 days of natural evolution after the OCL) should enable us to determine the potential for innate repair in adult animals. We believe that the short follow-up period required represents an advantage of the proposed model. In addition, this is supported by the decrease in the initial reparative response, characterized by a decrease of the cellular component of inflammatory cells seen at 14 days, a similar phenomenon to that published in OCL models in other animal species.¹³ The low histological score obtained in the 2 periods studied agrees with the low capacity of adult animals to repair the critical lesion generated, regardless of the persistence of the physis. We believe that this is crucial, as it proves that our surgical model will enable the observation of significant improvements in relation to the regenerative capacity of certain treatments.

The specific limitations of the proposed animal model include the fact that it does not correspond to the classical OCL observed in clinical practice, both regarding the causal mechanism and the area in which they occur. In human beings, OCL take place mainly in the femoral condyles, which, being a load area, have a different behavior regarding their potential for repair. For these reasons, the adult mouse model for critical OCL at the level of the

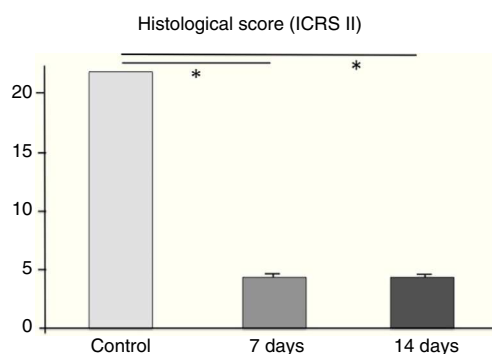


Figure 3 Histological score (ICRS II) with statistically significant differences compared to the control region, with no differences between the 2 periods studied. * $P < 0.05$.

femoral trochlea presented in this study is highly reproducible. In our results, the innate potential for regeneration of the animals secondary to the presence of the physis during their adult life did not manage to adequately repair the lesion generated by the procedure on the joint cartilage. This model will enable the development of new studies aimed at obtaining better treatments for chondral pathologies.

Level of evidence

Level of evidence I.

Ethical responsibilities

Protection of people and animals. The authors declare that this investigation adhered to the ethical guidelines of the Committee on Responsible Human Experimentation, as well as the World Medical Association and the Declaration of Helsinki.

Confidentiality of data. The authors declare that this work does not reflect any patient data.

Right to privacy and informed consent. The authors declare that this work does not reflect any patient data.

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Conflict of interest

The authors have no conflict of interest to declare.

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