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ORIGINAL ARTICLE

Influence of concentrated platelets on the reconstruction of cartilage defects in the lamb knee joint

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KEYWORDS
Platelets;
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Abstract
Objective: To study the influence of platelets on cartilage growth in articular defects in the sheep knee.
Material and methods: Male Rasa Aragonesa sheep (6 months) were operated under general anaesthesia. A 4mm diameter and 3mm deep defect was made in the femoral trochlea in both knees. The right knee defect was filled with platelet concentrate 5min after being activated with ClCa in group A (n=6), and similarly activated platelets + collagen scaffold in group B (n=6). Platelets were obtained by centrifuging 10ml arterial blood from the sheep prior to the surgical procedure. The left knee defect was not filled. The sheep were sacrificed 10 weeks after surgery. Macroscopic and microscopic studies were performed.
Results: In group A, hyaline cartilage was observed in the right knee defect at the end of the experiment in four cases. None of the defects of the left knees showed hyaline cartilage growth. In group B, hyaline cartilage was not observed in any right knee defect. However, in group B, all sheep showed better chondral cellularity and regeneration and lower fibrosis in the defects treated with platelets than in non-treated ones.
Conclusions: This technique for articular defect reconstruction with platelets has shown satisfactory results in our study. However, collagen scaffolds may decrease this positive effect.

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PALABRAS CLAVE
Plaquetas;
Defecto articular;
Cartílago;
Rodilla

Influencia del concentrado de plaquetas sobre la reconstrucción de defectos de cartílago articular en la rodilla de cordero

Resumen
Objetivo: Analizar el efecto de las plaquetas sobre el crecimiento de cartílago en los defectos articulares provocados en la rodilla ovina.
Material y método: Se provocó un defecto de 4mm de diámetro y 3mm de profundidad en la tróclea femoral de ambas rodillas en corderos macho de 6 meses de edad. La distribución de los grupos fue: grupo A (n=6): el defecto de la rodilla derecha se rellenó con concentrado de plaquetas 5min después de ser activado con ClCa. Grupo B (n=6): el defecto se rellenó con colágeno y plaquetas.
Las plaquetas se obtuvieron por centrifugación de 10ml de sangre arterial obtenida de cada animal antes de la cirugía. En los defectos de la rodilla izquierda no se administraron plaquetas. Las ovejas fueron sacrificadas 10 semanas después de la cirugía. Se realizaron estudios macro y microscópicos.
Resultados: En el grupo A, se observó cartílago hialino en 4 de los defectos de la rodilla derecha a las 10 semanas de la cirugía. Ninguno de los defectos de la rodilla izquierda mostró crecimiento de cartílago hialino. En el grupo B, no se observó cartílago hialino en nigún defecto. No obstante, todos los defectos presentaron mejor celularidad condral y menor fibrosis en los defectos tratados con plaquetas que en los no tratados.
Conclusiones: Esta técnica para la reconstrucción con plaquetas de defectos articulares de oveja ha mostrado en nuestro estudio resultados esperanzadores que empeoran combinadas con un andamiaje de colágeno.

Introduction

Joint cartilage has scant ability for self-repair, so untreated lesions usually degenerate into arthrosis.[1-5] In fact, after suffering injury, the joint surface often forms fibrocartilage, which has worse biomechanical characteristics than hyaline cartilage.[6] For this reason, lesions to joint cartilage have a poor prognosis.

Platelets are rich in growth factors and have been shown to have positive effects on the *in vitro* differentiation and proliferation of cartilage.[7-10] The BB platelet-derived growth factor (PDGF-BB) promotes the proliferation of chondrocytes in polyglycolic matrices.[8] Fibroblastic growth factor 2 (FGF2) encourages the *in vitro* formation of cartilage in particles of hydroxyapatite with stromal cells from human bone marrow.[9] Kaps et al.[10] saw that the supernatant human platelets stimulated the growth of articular chondrocytes and of the nasal septum. Positive effects have also been shown on the reconstruction of joint defects in some experimental models with rabbits.[11,12]

Among the techniques for repairing joint defects, the most outstanding is the implantation of autologous cells in suspension or with a wide variety of matrices for cell support. The matrices provide a three-dimensional structure that may help in joint reconstruction. Several authors have used collagen matrices in joint defects.[13-18] Type I collagen is used in human clinical treatments and is considered as cytocompatible material with integrin recognition sites.[19] Pound et al.[20] observed significant synthesis of cell matrix in bioreactors with bone marrow and type I collagen as well as an increase in cell proliferation. Collagen activates platelets and may generate a positive environment for cartilage repair. For all these reasons, the working hypothesis mooted was that the concentrated platelets might favour repair of cartilaginous defects created in lamb knee joints. Consideration must also be given to the possibility that the collagen matrix associated with the platelets might be used as a support.

Material and method

The male lambs used were 6 month-old Aragonese breed. This age corresponds to skeletal maturity in the lamb. The experiment consisted in creating chondral defects in both knees. The lambs were sacrificed 10 weeks after surgery. Macroscopic and microscopic studies were performed.

All the animal experiments were approved by the Ethics Committee of the University of Zaragoza.

Blood samples

Platelets were obtained by centrifugation (1,500 rpm) of 10 mL of arterial blood (citrated to 10%) obtained from each animal 10 minutes prior to surgery. The number of platelets was counted before and after centrifuging by using impedanceometry (ABBOTT® CellDyn 3500R). The mean number of platelets prior to centrifuging was 84×10^9/L and, after centrifugation, 260×10^9/L, i.e. this procedure increased platelet concentration by a factor of three. The supernatant platelets (3 mL) were collected and 1 mL of calcium chloride was added (ClCa 9 mg/mL).

Surgical procedure

Prior to surgery, 1 gramme of amoxicillin and clavulanic acid was administered and repeated 12 h later. Under general anaesthesia (intramuscular: xylazine 0.1 mg/kg, ketamine 5 mg/kg, buprenorfin 0.01 mg/kg and propofol 6 mg/kg) and through a 4 cm medial parapatellar incision, a defect was produced by reaching to the subchondral bone with a 4 mm diameter, 3 mm deep perforation in the femoral trochlea of both knees with a metallic device specially designed for the experiment. In group A (n=6), the defect in the right knee was filled with concentrated platelets 5 min after being activated with ClCa; in group B (n=6), the defect was filled with bovine type I collagen (Hemotese®, Symatese biomatériaux, Chaponost, France) mixed with processed platelets (these were injected onto the collagen filling the chondral defect) the same as in group A.

The defects in the left knees were not treated with platelets and were used as controls.

The joint capsule and the subcutaneous tissue were sutured with 3/0 polyglycolic acid ad the skin with 0 silk. Free deambulation was allowed after surgery. The lambs were sacrificed 10 weeks after the surgery by means of a lethal dose of endovenous pentobarbital (Dolethal®, Vetoquinol S.A., Lure Cedex, France).

Histologic study

After slaughter, the knees were removed and included in formaldehyde at 10% for at least one week, and decalcified with 0.5 M EDTA (pH 7.4) for two weeks. The samples were passed through decreasing graduations of ethanol, rinsed in phosphate buffered saline solution and embedded in paraffin at 60°C. Slices were made with a 7 mm thick microtome and stored at 4°C. These were later stained with haematoxylin-eosin. The preparations were studied under the microscope at 10 and 20 times magnification. The preparations were independently assessed by two of the investigators. A semi-quantitative scale was used to grade the degree of harm and the degenerative changes[21] by giving a score from 0 to 3. A score of 0 corresponded to serious harm or degenerative changes without differentiation of cartilage or signs of proliferation; 1 point: moderate degenerative changes, slight signs of differentiation and proliferation; 2 points: cartilaginous differentiation and signs of proliferation without hyaline cartilage; 3 points: tissue similar to normal hyaline cartilage.

Statistics

The Mann-Whitney U test was used to compare unmatched groups. The Wilcoxon test was used for matched groups. Values of $p < 0.05$ were considered significant.

Results

Hyaline cartilage was observed in group A to be filling in the defect of the right knee 10 weeks after surgery (fig. 1) in four cases. The scores on the semi-quantitative scale for histologic gradation are shown in table 1. In two cases, the defects treated appeared completely filled with new tissue, in two cases the reconstruction was irregular, covering only 75% of the defect's surface, in one case the new tissue was excreting slightly and in another case the new tissue filled only 50% of the defect. In the left knees (untreated defects) (fig. 2), the histologic outcomes were worse. The differences between the scores for the right and left knees were significant (p=0.034). Three of the untreated defects presented complete filling of the defect, one of the untreated defects presented filling of close to 75%, and two defects appeared filled to less than 50%. There was no correlation between the semi-quantitative damage scale and the greater or lesser infill of the defect with new tissue in the right or left knees.

In group B, we did not find hyaline cartilage in the defects created, although the defects treated in this group

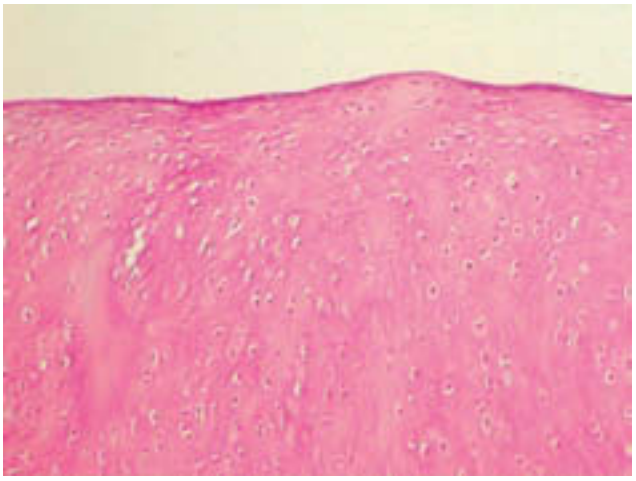

Figure 1 Preparation obtained 10 weeks after surgery for a cartilage defect treated with platelet concentrate (haematoxylin-eosin, ×10).

Table 1 Results of the histologic gradation of the chondral defects 10 weeks after surgery, using a semi-quantitative scale

Group A		Group B	
Right	Left	Right	Left
Platelets	Untreated	Platelets and collagen	Untreated
1	0	1	0
2	2	2	1
3	0	1	0
3	0	1	0
3	0	2	0
3	0	2	0

(0 corresponds to serious damage; 1 point: moderate degenerative changes, slight signs of differentiation and proliferation; 2 points: cartilaginous differentiation and signs of proliferation without hyaline cartilage; 3 points: tissue similar to normal hyaline cartilage).

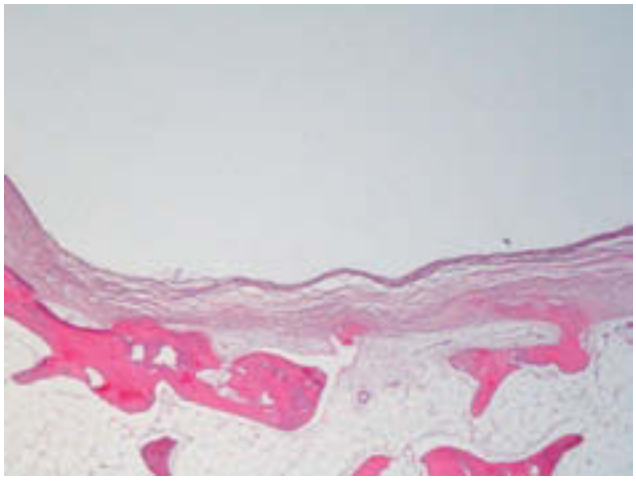

Figure 2 Preparation obtained 10 weeks after surgery for an untreated cartilage defect (haematoxylin-eosin, ×2).

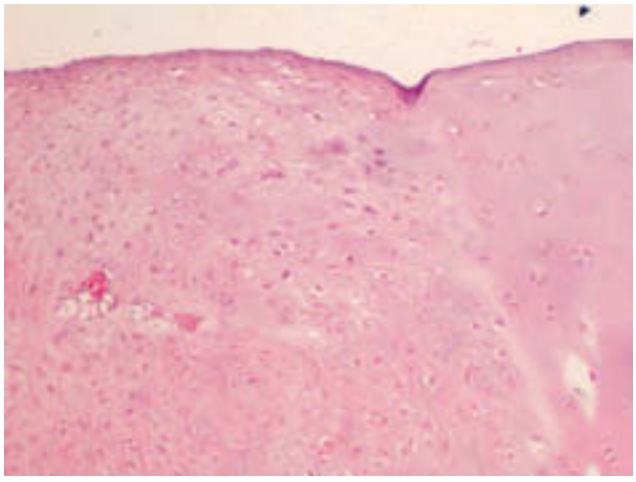

Figure 3 Preparation obtained 10 weeks after surgery for a cartilage defect treated with platelet concentrate and type I collagen (haematoxylin-eosin ×10).

presented better results than the untreated control samples. In this group, all the defects treated with platelets and collagen (fig. 3) presented greater chondral cellularity and regeneration with less fibrosis than the ones not treated with platelets. The differences between the scores on the semi-quantitative histologic gradation scale (table 1) for the treated and untreated defects were significant (group B: p=0,039). We found significant differences between the right knees in group A and group B (U-Mann-Whitney p=0.042). in group B two of the defects treated presented complete infilling of the defect, two defects presented close to 75%, and two defects were filled to less than 50%. Three of the untreated defects presented complete infilling of the defect, one defect presented close to 75%, and two defects were filled to less than 50%. We found no inter-observer differences with respect to the histologic gradation.

Discussion

In previous papers,[11,12] we have published the positive effects of platelet concentrates on the reconstruction of joint defects in an experimental rabbit model. We have not found any similar studies in the literature with non-small animals and so the present paper with lambs was mooted as the lamb model is closer to humans. In this paper, we have observed that the positive effect of the platelet concentrates is also present in the reconstruction of cartilaginous defects of lambs' joints. We believe this to be due to the positive effect of platelet factors, already mentioned in the introduction, on the cells in bone marrow to stimulate the formation of chondral tissue in the context of joints.

No media were used to fix the platelets in the defect. Calcium-activated platelets tend to form a mass confined within the lesion. It is true that, as there is no specific means retaining them part may be lost, yet, despite this, the experiment shows a positive effect on the defects treated with platelets. After reviewing the literature we are of the opinion that the use of type I collagen supports together with concentrated platelets might help in the reconstruction of joint defects. Funayama et al.[17] injected chondrocytes included in a gel of type II collagen in cartilaginous defects in rabbits, finding that the type II collagen gel behaved like scaffolding suitable for the transplant of chondrocytes. Chajra et al.[18] compared the behaviour of bovine chondrocytes cultivated in collagen matrices, one of which was Hemotese®, the same as was used in our case, containing or not hydroxyapatite and glutaraldehyde or EDC/ NHS, obtaining a similar result in the cells in the four types of scaffolding used. Yates et al.[13] pointed out that porous sponges of collagen maintain the viability, shape and activity of bovine joint chondrocytes. Lubiatowski et al.[14] observed the presence in rabbits of cartilage similar to hyaline in penetrating defects in the subchondral structure filled with collagen scaffolding after 4 and 12 weeks. On the other hand, Dorotka et al.[22] found that implanting an isolated collagen matrix did not improve the repair of chondral defects produced in the medial condyle of sheep's femurs. These authors[21] also found that the defects that did not penetrate in the subchondral bone presented the lowest amount of defect infilling, and that the microfracture technique increased the curative response. Malicev et al.[15] used equine type I collagen matrices with human joint chondrocytes. Preparations with human plasma or fibrin gel showed better results than preparations without gel. Chondrocytes implanted directly in collagen scaffolding presented fibroblastic form, whereas those encapsulated in a fibrin gel were spherical. This would indicate that the direct use of collagen scaffolding is not useful without prior processing of the chondrocytes with additional factors. The absence prior cell processing may have influenced the negative result of the collagen group in our paper. In addition, the work by Malicev et al.[15] would show the compatibility of human chondrocytes with collagen matrices from another animal species. Cook et al.[23] found fibrous tissue and fibrocartilage filling the osteochondral defects produced in the femoral condyles of adult dogs treated with 100 mg of bone-derived bovine type I collagen implants. Despite the inter-species compatibility in these

papers, in our work the use of a bovine type I collagen in an ovine model, might have contributed to the negative outcome in the collagen group.

The positive effect of platelets has been demonstrated for the growth of the chondrocytes implanted subcutaneously in rabbits.[24] It is possible that the presence of collagen acts as a barrier between the platelet factors and the cells to be stimulated.

Our study suffers from certain drawbacks. We have not studied other matrices that might contribute to the positive result with platelets on the reconstruction of joint defects. We performed an outcome study after 10 weeks, which has been useful to assess the medium-term effects, however we feel that studies must be undertaken over longer periods of time. For the present study we have used an experimental model based on sheep and, therefore, not all conclusions can be extrapolated to humans.

Platelet concentrate was seen to have a beneficial effect on the growth of chondrocytes in the joint defects in lambs. However, the results worsened when we used type I collagen matrices associated with the platelets.

Conflict of interest

The authors declare they have no conflict of interest.

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