

Revista Española de Cirugía Ortopédica y Traumatología



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RESEARCH

Use of adipose-derived stem cells in an experimental rotator cuff fracture animal model*



R. Barco^{a,*}, C. Encinas^a, M. Valencia^a, M.T. Carrascal^b, M. García-Arranz^c, S. Antuña^a

- a Servicio de Cirugía Ortopédica y Traumatología, IDIPAZ, Madrid, Spain
- ^b Departamento de Medicina. Escuela Técnica Superior Ingeniaría Industrial, UNED, Madrid, Spain
- c Unidad de Terapia Celular, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Madrid, Spain

Received 19 May 2014; accepted 15 July 2014

KEYWORDS

Cell therapy; Repair; Supraspinatus; Experimental; Adipose-derived stem-cell

Abstract

Aim: Rotator cuff repairs have shown a high level of re-ruptures. We hypothesized that the use of adipose-derived stem cells (ASC) could improve the biomechanical and histological properties of the repair.

Materials and methods: Controlled experimental study conducted on 44 BDIX rats with section and repair of the supraspinatus tendon and randomization to one of three groups: group A, no intervention (control); group B, local applications of a fibrin sealant; and group C, application of the fibrin sealant with 2 \times 10⁶ ASC. At 4 and 8 weeks a biomechanical and histological analysis was performed.

Results: There were no differences in load-to-failure at 4 and 8 weeks between groups. The load-to-failure did increase between week 4 and week 8. Histologically the tendon-to bone union showed a disorganized fibrovascular tissue. Group C showed a different inflammatory pattern, with less presence of neutrophils and more presence of plasma cells.

Conclusion: The use of ASC does not improve the biomechanical or histological properties of the repair site. More studies are needed to improve techniques that enhance the healing site of the repair.

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^{*} Please cite this article as: Barco R, Encinas C, Valencia M, Carrascal MT, García-Arranz M, Antuña S. Uso de células troncales derivadas de lipoaspirado en un modelo experimental animal de rotura de manguito rotador. Rev Esp Cir Ortop Traumatol. 2015;59:3–8.

^{*} Corresponding author.

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PALABRAS CLAVE

Terapia celular; Reparación; Manguito rotador; Experimental; Celula troncal derivada de lipoaspirado Uso de células troncales derivadas de lipoaspirado en un modelo experimental animal de rotura de manguito rotador

Resumen

Objetivo: La reparación del manguito rotador tiene una alta tasa de fracaso. Se investiga si la aplicación de células troncales derivadas de lipoaspirado mejorará la resistencia de la reparación y recreará la entesis original.

Material y métodos: Estudio experimental en 44 ratas BDIX con sección y reparación con sutura del tendón supraespinoso y asignación aleatoria a uno de 3 grupos: grupo A, nada (control); grupo B, aplicación local de vehículo de fibrina; y grupo C, aplicación de 2×10^6 células troncales derivadas de lipoaspirado. Se realiza estudio mecánico en célula de carga y estudio histológico en hematoxilina-eosina.

Resultados: En el estudio mecánico no hubo diferencias entre grupos. La carga hasta el fracaso aumentó de los grupos de 4-8 semanas. En el estudio histológico se observó la unión huesotendón mediante un tejido fibrovascular desorganizado. En el grupo C se observó un aumento de células plasmáticas a las 4 y 8 semanas.

Conclusión: La utilización de células troncales derivadas de lipoaspirado no recrea la organización celular de la entesis ni mejoran las propiedades biomecánicas de la misma. Son necesarios más estudios para investigar técnicas que mejoren la cicatrización del tendón. © 2014 SECOT. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Introduction

The existence of re-ruptures after rotator cuff repairs is still a frequent problem, with some studies reporting an incidence of up to 94% in massive tears.^{1,2} In addition, the functional results of patients with complete repairs are better than those obtained in cases where the repair failed.³

The majority of tears present degenerative changes in the local biology of the tendons which include, among others, an increase in cellular apoptosis and alterations in the levels of matrix metalloproteases and various growth factors. Repair of the rotator cuff does not manage to recreate the native structure of the enthesis or its original biomechanical strength.^{4,5}

The 2 factors affecting healing of the enthesis include the mechanical resistance of the repair and the biological environment, which affects scarring. There has been considerable work focused on increasing the mechanical resistance of repairs, however, there is less information regarding modification of the local biological environment through the use of therapies to improve scarring. The use of mesenchymal stem cells has been one of the strategies explored to biologically improve these repairs. Stem cells are fibroblastic cells which are able to differentiate toward various different cellular types, including osteoblasts and chondrocytes. Their 2 main sources are the bone marrow and adipose tissue, which is more accessible and has greater proliferative power.

Our hypothesis is that the local application of adiposederived stem cells (ASC) in a rotator cuff animal model would improve the mechanical resistance of repairs, as well as the histological structure of the enthesis.

Materials and methods

In order to study tendon-to-bone unions in a rotator cuff model we used a total of 24 syngeneic BDIX rats aged between 6 and 8 weeks in the biomechanical study and 18 animals for the histological study. The work was approved by the Ethics Committee for animal experimentation at our center and we followed all the international guidelines for experimentation with animals (86/609/CEE).

Collection of adipose-derived stem cells (Appendix A)

The method to obtain ASC has been published previously. In short, the cells were extracted from the epiploon and subcutaneous fat of 2 animals and were then submitted to mechanical disaggregation and enzymatic cellular digestion. Next, the cellular phase was selected and the ASC were isolated. We verified the cell lineage through differentiation of the cells into adipocytes, chondrocytes and osteocytes, as well as by flow cytometry studies of membrane markers (positive CD 90, CD 73 and CD 105 and negative CD 34, CD 44). Lastly, we multiplied the cells in an adequate medium and carried out a cell count before application.

Surgical technique

The surgical technique has been described previously. ¹⁰ In short, a longitudinal incision was made on the proximal part of the front leg with horizontal section of the deltoid, exposing the rotator cuff of the animal. Next, the tendon of the trochiter supraspinatus was sectioned and the animals were

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