Rev Esp Cir Ortop Traumatol. 2015;59(4):215–221

Revista Española de Cirugía Ortopédica y Traumatología

www.elsevier.es/rot

RESEARCH

Hyaluronic acid effect on adipose-derived stem cells. Biological *in vitro* evaluation☆

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Received 7 July 2014; accepted 24 October 2014

KEYWORDS
Hyaluronic acid;
Mesenchymal stem cells;
Cell therapy;
Tissular engineering;
Biocompatibility

Abstract
Objectives: To evaluate the *in vitro* effects of hyaluronic acid (HA) on adipose-derived stem cells (ASC) in order to consider the possibility of their combined use in the treatment of knee arthrosis.
Material and methods: The ASC cells were grown both in the presence and absence of AH, and several studies were carried out: proliferation (WST8) and cell viability studies (Alamar Blue® and Trypan Blue), possible chondrogenic differentiation (collagen type 2 expression) by RT-PCR, AH receptor expression (CD44) by flow cytometry and RT-QPCR, and expression of inflammatory and anti-inflammatory factors (IL-6, TGFß, IL-10) by RT-QPCR.
Results: The number of ASC significantly increased after 7 days with HA (158 ± 39%, $p < 0.05$). Additionally, the cell viability of the ASC treated with HA after 1, 3, 5 and 7 days was similar to that of the control cells, being considered non-toxic. There were no changes observed in the expression of CD44 and chondrogenic differentiation. TGFß expression was not modified after AH treatment, but there was a 4-fold decrease in IL-6 expression and IL-10 expression increased up to 2-fold compared to control cells.
Conclusions: Hyaluronic acid favours ASC proliferation without causing cellular toxicity, and inducing an anti-inflammatory profile in these cells. Hyaluronic acid appears to be a suitable vehicle for the intra-articular administration of mesenchymal stem cells.

☆ Please cite this article as: Moreno A, Martínez A, Olmedillas S, Bello S, de Miguel F. Efecto del ácido hialurónico sobre células madre mesenquimales derivadas de tejido adiposo. Evaluación biológica *in vitro*. Rev Esp Cir Ortop Traumatol. 2015:59:215–221.

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PALABRAS CLAVE
Ácido hialurónico;
Células madre mesenquimales;
Terapia celular;
Ingeniería tisular;
Biocompatibilidad

Efecto del ácido hialurónico sobre células madre mesenquimales derivadas de tejido adiposo. Evaluación biológica *in vitro*

Resumen
Objetivo: Evaluar *in vitro* los efectos del ácido hialurónico (AH) en células madre mesenquimales derivadas de tejido adiposo (ASC) ante su posible uso combinado en el tratamiento de la artrosis de rodilla.
Material y método: Las ASC fueron cultivadas en presencia o ausencia de AH, realizando estudios de proliferación (ensayo con WST8) y viabilidad celular (Alamar Blue® y Trypan Blue), posible diferenciación condrogénica de las células (expresión de colágeno tipo 2) por RT-PCR, así como el estudio de la expresión del receptor de AH (CD44) por citometría de flujo y RT-QPCR, y factores pro- y antiinflamatorios (IL-6, TGFß, IL-10) por RT-QPCR.
Resultados: El número de ASC aumentó significativamente tras 7 días con AH (158 ± 39%, $p < 0,05$). Así mismo, la viabilidad de las ASC tratadas con AH a uno, 3, 5 y 7 días fue similar a la de las células control, considerándose que el tratamiento con AH no resultaba tóxico. No se observaron cambios en la expresión de CD44 tras el tratamiento con AH ni tampoco la inducción a la diferenciación condrogénica. La expresión de TGFß no se modificó con el tratamiento con AH; sin embargo, las ASC cultivadas con AH mostraron un incremento de 2 veces en la expresión de IL-10 y una reducción sobre el valour basal de 4 veces en la expresión de IL-6.
Conclusiones: El AH favorece la proliferación de las ASC en cultivo, no presentando toxicidad celular, e induciendo un perfil antiinflamatorio en estas células. Consideramos al AH un vehículo adecuado para la administración intraarticular de células madre mesenquimales.

Introduction

Regenerative medicine is currently presented as one of the most promising alternatives in the treatment of osteoarticular disease. In particular, the use of cells combined with three-dimensional structures and bioactive molecules is showing promising results in tissular regeneration in chondral lesions. Of the different types of mesenchymal stem cells (MSC), those which derive from adipose tissue (ASC) have proved very useful in tissular regeneration due to their chondrogenic and osteogenic potential, which, along with their being relatively easy to obtain and isolate, makes them excellent candidates for use in regeneration studies in orthopaedic and traumatology surgery.[1]

One of the problems facing cellular therapy is determining the vehicle to bring the cells to the injured tissue. Hyaluronic acid (HA) is a basic element of the extracellular matrix and is found in most body tissues and fluids, such as hyaline cartilage and synovial fluid. This is a biodegradable polymer of high molecular weight which acts as scaffolding, and also has important biological functions such as regulating adhesion, mobility, cell differentiation and proliferation. These characteristics, along with its low immunogenicity, make it a very promising biomaterial for use in tissular engineering.[2] HA gels, and substrates derived from it, have been tested for use as support structures for the culture of chondrocytes and the production of cartilage tissue.[3] Recent animal experimentation studies have assessed the possibility of using pluripotential mesenchymal cells from bone marrow along with biphasic substrates of HA and tricalcium phosphate to repair osteochondral defects with encouraging results.[4] Furthermore, recent studies highlight the importance of the interaction between HA and mesenchymal stem cells (MSC) in their therapeutic effect, and the production of HA by the MSC themselves as an essential part of their action mechanism.[5] Finally, it is worth noting that HA has shown a better differentiating effect towards the chondrogenic line of MSC than other products used as scaffolding for their culture.[6]

Arthrosis is one of the most prevalent and widespread osteoarticular diseases and has a very negative impact, not only in terms of how it affects patients' quality of life, but also because of its high economic burden on healthcare institutions.[7] Arthrosis affects joint cartilage and through immunological and inflammatory reactions gradually produces a progressive lesion which eventually results in loss of joint function.[8] Although various joints can be affected by this disease, the knee is one of the most commonly affected. In Spain, it is estimated that 10% of the population present symptoms associated with knee arthrosis.[9]

Taking all these aspects into consideration, the objective of this work was to evaluate *in vitro* the proliferation, viability and possible chondrogenic differentiation of ASC in the presence of HA, and to study HA receptor expression (CD44), the pro- and anti-inflammatory factors in these cells which might be altered in the presence of the polysaccharide, in order to assess the potential of HA as a vehicle for the intra-articular administration of ASC.

Material and method

The ASC were obtained from Inbiobank (San Sebastian, Spain) and came from a young, healthy woman; they were routinely cultured in DMEM medium with 1 g/l D-glucose (Gibco®-Life Technologies, Grand Island, NY, USA), and