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Potential of mesenchymal stem cell in stabilization of abdominal aortic aneurysm sac

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ABSTRACT

Background: In their origin, abdominal aortic aneurysms (AAAs) are related to an inflammatory reaction within the aortic wall, which can lead to weakness and degeneration of this structure. One of the most widely accepted treatment modalities for AAAs is the placement of stent grafts. Nevertheless, in some patients blood re-enters the aneurysm sac, creating so-called leaks, which constitute a renewed risk of rupture and death.This study explores the possibility of filling aneurysm sacs treated by endovascular aneurysm repair with adipose tissue–derived mesenchymal stem cells (ASCs) in a porcine model.

Methods: We developed a porcine model using 22 animals by creating an artificial AAA made with a Dacron patch. AAAs were then treated with a coated stent that isolated the aneurysm sac, after which we introduced allogeneic ASC into the sac. Animals were followed-up for up to 3 mo. The experiment consisted of the aforementioned surgical procedure performed first, followed by computed tomography and echo-Doppler imaging during the follow-up, and finally, after sacrificing the animals, histologic analysis of tissue samples from the site of cell implantation by a blinded observer and the detection of implanted cells by immunofluorescence detection of the Y chromosome.

Results: Our findings demonstrate the survival of ASCs over the 3 mo after implantation and histologic changes associated with this treatment. Treated animals had less acute and chronic inflammation throughout the study period, and we observed increasing fibrosis of the aneurysm sac, no accumulation of calcium, and a regeneration of elastic fibers in the artery.

Conclusions: The combination of endovascular aneurysm repair and cell therapy on AAAs has promising results for the stabilization of the sac, resulting in the generation of living

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tissue that can secure the stent graft and even showing some signs of wall regeneration. The therapeutic value of such cell-based therapy will require further investigation.

1. Introduction

Arterial diseases have a common denominator, namely, arterial wall inflammation in response to various noxious stimuli. This response can be associated with the accumulation of degradation products and other substances in the subendothelial region (obstructive disease, intimal hyperplasia) or degradation and weakening of the wall (aneurysms).

Abdominal aortic aneurysms (AAAs) are a public health problem, with prevalence among individuals over 65 y reaching 9% in men and 1% in women. Endovascular aneurysm repair (EVAR) has become a standard approach to AAAs. The goal of EVAR is to prevent aneurysm rupture [1,2]. Several clinical trials [3–5] and experimental studies have shown increased benefits of EVAR over the short and medium term *versus* open aneurysm repair. However, the initial advantage of EVAR is lessened in part by its suboptimal durability, with a 30% rate of reoperations seen in some series [6].

Aneurysm sac shrinkage is a sign of treatment success. In contrast, migration of the stent or the appearance of leaks indicates a new risk of rupture.

The critical difference between the two treatment modalities is that in EVAR the aneurysm sac apparently maintains its mural thrombus, meaning that the inflammatory process may continue after surgery. Aneurysm sac growth may occur in up to 40% of patients and can alter the prognosis of the disease [7]. In the presence of this growth, it is important to combine EVAR with other actions such as embolization of the tributary vessels of the aneurysm or extension of the endoluminal coating proximally and distally or between devices [8].

A different approach being investigated consists of filling aneurysms of the aorta with material that allows the passage of blood to the aneurysm while maintaining normal blood pressure; various types of material have been used, including elastomers and inert materials, and a specifically designed prosthesis has also been tested [9]. Aneurysm sac embolization with inert substances is another treatment option when some types of leaks occur [10].

Cell therapy might enhance the stability of the aneurysm sac, reducing the inflammatory process and developing new reinforced arterial layers. Mesenchymal stem cells (MSCs) have potential to differentiate into endothelial and smooth muscle cells [11]. Recently, interest in these cells has grown because they can contribute significantly to vascular repair processes, neoangiogenesis, the inflammatory response, and stabilization of injuries [12,13]. In our study, we decided to use adipose-derived stem cells (ASCs), a type of MSC that has been shown to have an anti-inflammatory and angiogenic capacity, the ability to release a range of growth factors (especially vascular and fibroblast), and play a regulatory role in the secretion of metalloproteinase, thus making them ideal candidates for the type of treatment that we endeavored to explore. Additionally, ACSs have considerable myogenic potential [14,15] and are easy to obtain in large numbers in a short time.

2. Materials and methods

2.1. Animals

Twenty-two Landrace-Large White pigs weighing 23–28 kg were used, one male to obtain MSCs from subcutaneous adipose tissue and the rest females for the intervention. All procedures were performed in the Experimental Surgery Department of the Hospital Universitario La Paz (Madrid, Spain). We followed the protocol approved by the Animal Welfare Ethics Committee and complied with the EU Directive on experimental animals (63/2010 EU) and related Spanish legislation (RD 53/2013).

2.2. Isolation of adipose-derived MSCs

ASCs were obtained from subcutaneous fat tissue of the one male Landrace-Large White pig after a protocol previously used in humans [16] with minor modifications. The cells were grown in standard culture conditions to a confluence of 70%–80%. At passage 4, the cells were stored at −80°C. Before the experiment, the cells were thawed and plated for 96 h in standard conditions.

2.3. Flow cytometry

Cell characterization studies were carried out before and after cryopreservation by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA). We analyzed the expression of the surface markers, CD45 (fluorescein isothiocyanate), CD90 (allophycocyanin), CD34 (phosphatidylethanolamine [PE]), CD105 (fluorescein isothiocyanate), CD29 (PE), and HLA DR (PE), conjugated with Alexa Fluor 647 (Serotec, Madrid, Spain) to confirm the ASC phenotype of the cultured cells.

2.4. Cell differentiation

2.4.1. Adipogenic differentiation

Cells were plated in 6-well plates and, when they reached confluence, were induced to differentiate into adipocytes by DMEM supplemented with 0.5 mM isobutylmethylxanthine (Sigma–Aldrich, Saint Louis, MO), 1 μM dexamethasone (Sigma–Aldrich), 10 μM insulin (Actrapid; Novo Nordisk A/S, Bagsværd, Denmark), and 200 μM indomethacin (Sigma–Aldrich) for 14 d. The cells were subsequently cultured for 4 d in Dulbecco's Modified Eagle Medium (DMEM) supplemented only with insulin 10 μM (maintenance medium). ASC negative controls were cultured in DMEM alone for the same period.