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Adipose tissue-derived mesenchymal stromal cells as part of therapy for chronic graft-versus-host disease: A phase I/II study

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Abstract

Background aims. Despite the efficacy of allogeneic hematopoietic stem cell transplantation (allo-HSCT), the procedure is still associated with high toxicity in patients with refractory graft-versus-host disease (GvHD). Mesenchymal stromal cells (MSCs) are a new mode of therapy in the context of allo-HSCT. The objective of this study was to evaluate the safety and feasibility of the use of adipose tissue–derived MSCs (AT-MSCs) in patients with chronic GvHD. *Methods.* Fourteen patients with moderate (n = 7) or severe (n = 7) chronic GvHD received 1×10^6/kg (group A, n = 9) or 3×10^6/kg (group B, n = 5) AT-MSCs with cyclosporine and prednisone as first-line therapy. *Results.* Ten of the 14 patients were able to continue under the protocol: 80% were in complete remission, and 100% were off of steroids at week 56. The remaining 4 patients either worsened from chronic GvHD (n = 3) or abandoned the study (n = 1). At the end of the study, 11 of 14 patients are alive (overall survival 71.4%, median survival of 45.3 weeks). No suspected unexpected serious adverse reactions occurred during the trial. Neither relapse of underlying disease nor mortality due to infection was observed in this cohort. Biological studies showed increased CD19, CD4 and tumor necrosis factor-α with a temporary decrease in natural killer cells. *Discussion.* AT-MSCs, in combination with immunosuppressive therapy, may be considered feasible and safe and likely would have an impact on the course of chronic GvHD. More studies are warranted to understand the potential benefits of AT-MSCs in these patients.

Key Words: *adipose tissue–derived mesenchymal stromal cells, allogeneic hematopoietic stem cell transplantation, chronic graft-versus-host disease, GvHD*

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a well-established procedure for curing several hematological malignancies. Acute and chronic graft-versus-host disease (GvHD) are still the main causes of non-relapse mortality and the focus of clinical research. To minimize such complications, several methods have been developed; their controversial effects include a higher risk of relapse, delayed immune reconstitution and infectious complications [1–3]. Given the higher incidence of chronic GvHD in recent years [4], new efforts have been made to better diagnose and classify chronic GvHD [5,6]. Meanwhile, little progress has been achieved in treating patients with aggressive GvHD. Another problem is the long period of exposure to immunosuppressive therapy (IST), which eventually causes toxicity and long-term side effects [7].

The pathology of chronic GvHD is due to inflammatory T cells and, as a consequence, tissue damage with a reduction in number and functionality of B

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regulatory cells and T regulatory cells (Tregs) and reduced interleukin (IL)-10, along with a failure of intrathymic function; by contrast, impaired donor natural killer (NK)-cell reconstitution is associated with a favorable course of GvHD [8].

Mesenchymal stromal cells (MSCs) have immune properties, and their capacity for suppressing the cytotoxic activity of CD4, CD8 and NK, inducing Tregs and inhibiting the proliferation of B cells has been demonstrated *in vitro*. Furthermore, their interference with dendritic cells and macrophages leads to a decreased presentation of antigens to T cells, thus affecting the production of pro-inflammatory (IL-2, tumor necrosis factor [TNF]-α, interferon [IFN]-γ) cytokines [9].

Mesenchymal stromal cells (MSCs) are known to have immunomodulatory effects [10–12], an attractive characteristic in the clinical setting. Several attempts have been made to demonstrate their benefits in the quality of engraftment [13–15], the control of GvHD [16–18] or tissue repair [19,20], although no phase III clinical trial showing consistent results has been published to date. As the source of MSCs, fat has advantages compared with bone marrow, including accessibility and procedural ease for obtaining a large number of MSCs [21].

In February 2010, a randomized clinical trial was initiated with the main aim of demonstrating the safety and feasibility of adipose tissue–derived MSCs (AT-MSCs) in patients with chronic GvHD.

Methods

This was an open, prospective, multicentral and randomized phase I/II clinical trial, involving two experimental arms: group A (standard therapy plus 1×10^6/kg AT-MSCs) and group B (standard therapy plus 3×10^6/kg AT-MSCs). Initially it was designed as a controlled trial, but due to inadequate recruitment, it was necessary to modify the design to a noncontrolled trial. The protocol was performed in accordance with the respective institutional review board policy and the Spanish Medical Agency, and informed consent form was signed by both donor and recipient, according to the Helsinki Declaration. This trial was registered at www.clinicaltrials.gov (# NCT01222039).

At-MSC isolation, quality control and administration

Adipose tissue was obtained from a third party by surgical excision from 10 patients (mean age 43.9 years) undergoing abdominoplasty or liposuction under sterile conditions. Donor suitability was ensured by a study of medical history plus laboratory tests to exclude the transmission of infectious agents (serology and polymerase chain reaction: human immunodeficiency virus, hepatitis B, hepatitis C). Depending on the fat reservoir, 400 to 1000 g of tissue were removed from the donor in the operating room and transferred in physiological serum to the Cell Production Unit. Production and packaging operations were performed in a biosafety cabinet (Class A) in the cleanrooms (Class B). Facilities and methods met Good Manufacturing Practice requirements and were authorized by the Spanish Medical Agency. The tissue was mechanically disintegrated, digested with collagenase, centrifuged and filtered. The cells obtained were cultured in medium containing 10% fetal bovine serum, 2% alanyl-glutamine, gentamycin 0.1 mg/mL, penicillin 100 UI/mL and expanded until obtaining a maximum of three cell passages. AT-MSCs were recovered and packaged with dimethyl sulfoxide for cryopreservation as described elsewhere [22,23]. The AT-MSCs were kept in quarantine until the results of quality control met acceptance criteria (Table I). Not all MSCs were used in this clinical trial because some of them were used for investigational purposes and for clinical treatment by compassionate use.

Table I. Quality control of AT-MSCs.

Test	Method	Acceptance criteria
Sterility	Microbiological control of cellular products (Eur.Ph. 2.6.27)	Absence of micro-organism
	Gram stain	Absence of micro-organism
	Calcofluor stain	Absence of fungal structures
Endotoxins	Bacterial endotoxins (Eur.Ph. 2.6.14)	≤3.33 UE/mL
Mycoplasma	PCR (Eur.Ph 2.6.7)	Absence of mycoplasma
Cell viability	Trypan blue stain	≥80 %
Differentiation	Differentiation to osteocytes and adipocytes	Positive differentiation
Chromosomal stability	Karyotype	Absence of chromosomal alterations
Phenotype	Flow cytometry	CD73, CD90, CD166 ≥80% CD45, HLA DR, CD19, CD11b ≤20%
DNA Fingerprint	PCR	100% correspondence donor blood and expanded cells
Virus	Co-culture in MRC-5, RD and Vero Cells	Absence of virus

PCR, polymerase chain reaction.