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Evaluation of the safety and tolerability of a high-dose intravenous infusion of allogeneic mesenchymal precursor cells

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Abstract

Background aims. Over the past decade, mounting evidence has shown that mesenchymal stromal cells have the potential to exert protective and reparative effects in a variety of disease settings. Clinical trials are being increasingly established to investigate the therapeutic potential of these cells; however, several safety concerns remain to be addressed, of which dosage safety for intravenous administration is paramount. Published safety studies thus far have predominantly been carried out in small-animal models, whereas data for high-dose allogeneic intravenous administration in large-animal models are limited. This study investigates the safety and tolerability of a single high-dose intravenous infusion of 450 million allogeneic ovine mesenchymal precursor cells (oMPCs) in adult sheep. *Methods.* Allogeneic oMPCs ($n = 450$ million) were intravenously administered to 2-year-old castrated male sheep through the use of three different infusion rates. Sheep were intensively monitored for 7 days by means of vital physiological observations (temperature, blood pressure, heart rate, respiratory rate and oxygen saturation) as well as venous and arterial blood analysis. In addition, full *post mortem* examination was performed in all animals. *Results.* A single high dose of intravenously administered cells was well tolerated, with no serious adverse effects reported. No physiologically significant changes in vital signs, oxygen saturation, blood gas analysis or clinical pathology were observed over the duration of the study. *Conclusions.* Intravenous delivery of a single high-dose infusion of oMPCs is well tolerated in a large animal model. This study provides additional safety evidence for their intravenous use in future human clinical trials.

Key Words: *clinical translation, dosage, mesenchymal stromal/precursor cells, safety, stem cell therapy*

Introduction

Regenerative medicine, and in particular stem cell transplantation, has gained significant attention in recent years, with an exponential increase in the number of publications and clinical trials commencing. A major question that still must be addressed is the safety of such cell-based therapies, particularly after high-dose, intravenous administration. The dosage and most efficacious route of administration of mesenchymal stromal cells (MSCs) are routinely debated and depend on both the disease process and target organ [1,2]. It is clear that the most appropriate route for administration of hematopoietic stem cells is intravenous (IV) administration. There is no clear consensus for the route of MSC administration, which will depend on a number of factors. However, IV administration may be the most practical delivery route for treatment of many diseases because of the demonstrated capacity of these cells to migrate to sites of injury [3,4]).

Previous pre-clinical animal studies have evaluated the safety and efficacy of IV delivery of MSCs or their precursor, mesenchymal progenitor cells (MPCs). Doses of up to 2 million MSCs have been shown to be effective in murine and non-human primate models of diabetes [5,6], rheumatoid arthritis [7], stroke [8] and multiple sclerosis [9], without major side effects. Nevertheless, adult humans weigh significantly more than animals previously used in pre-clinical studies (eg, rats and mice typically weigh 200–300 g and 20–40 g, respectively). As such, the cell dosage per kilogram can be equivalent to as high as 100 million cells/kg when extrapolated to humans from small-animal studies; thus, it would result in a significantly higher absolute cell dose. This is particularly relevant to patient

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populations in which obesity is common, for example, type II diabetes.

Currently, 411 clinical trials are listed as investigating MSCs and 75 are listed as using IV delivery (clinicaltrials.gov; "mesenchymal stem cells" AND "intravenous"). The primary outcome for most of these studies is safety; efficacy is a secondary end point. In previous clinical trials investigating the safety of MSC therapy most studies have used dosages of 1 million cells/kg [10,11], 1.5 million cells/kg [12] and 2 million cells/kg [13], which is much lower than that used in pre-clinical studies. A phase II study, conducted by Osiris, investigated the use of MSCs in Crohn's disease and used up to 8 million cells/kg, and no adverse events were reported. Another study that used allogeneic adipose-derived MSCs for stroke delivered 1 million cells/kg IV at 5 mL/min and has not reported any adverse effects [14].

In view of the discrepancy between the high effective doses used in pre-clinical studies in small animals, and the low doses given in clinical trials to assess safety, it is clear that the safety of IV administration of higher doses of MSCs must be further addressed. Knowledge of the physiological response of large mammals to higher doses of MSCs is important before the use of such large doses in clinical trials. Although a few clinical trials have used high cell doses, for example, 8 million/kg, an intensive investigation into the physiological changes after high-cell doses in large-animal models remains to be performed. As such, we evaluated the safety and tolerability of a single IV infusion of 450 million allogeneic ovine (o)MPCs in healthy adult male sheep. Sheep were chosen for this study because they are of similar weight to humans, and arterial and venous catheters can be inserted with ease. Sheep can also be maintained in holding cages to allow for continuous physiological monitoring and regular blood sampling without the need for sedation. In addition to dose, the present study also evaluated the tolerance of three different infusion rates (3.33, 5.0 and 6.6 mL/min); these rates were based on those used in previous clinical trials. Furthermore, the study was designed to demonstrate the feasibility and safety of the specific mode of administration of the oMPCs, including re-suspension in 100 mL of normal saline solution and IV infusion through the filter and catheter system. The results from this study provided valuable safety evidence to support the approval of human clinical trials involving this mode of administration.

Methods

Experimental study design

Animals were randomized to one of three groups, each consisting of three animals per group, to evaluate the following infusion rates: 3.33 mL/min (total infusion time of 30 min; 15 million cells/min; group A), 5.0 mL/min (total infusion time of 20 min; 22.5 million cells/min; group B), 6.66 mL/min (total infusion time of 15 min; 30 million cells/min; group C). All sheep received dosages of cells that equated to 6.4 million cells/kg body weight. Each animal was euthanazed 7 days after the oMPC infusion. Necropsy and tissue collection were performed on all animals under the supervision of a qualified veterinary surgeon.

Description of oMPCs

The ovine STRO-3+ MPCs were obtained under proprietary license from Mesoblast Ltd. Cells were prepared under Good Manufacturing Process conditions by Biotest Laboratories Pty Ltd. The identity of the oMPCs was confirmed by means of multi-lineage differentiation assays and flow cytometry, demonstrating that the MPCs expressed the MSC markers CD29, CD44, CD146, CD166 and lacked expression of the hemopoietic and vascular endothelial markers CD31, CD14 and CD45 [15,16].

The cells used in this study were stored in cryopreserved ampoules containing 60 million oMPCs in 1.2 mL ProFreeze/7.5% dimethylsulfoxide (DMSO)/50% α-modified essential medium (α-MEM). The oMPC manufacturing process involved harvesting bone marrow from healthy sheep, followed by immuno-selection of mononuclear cells for stromal enrichment and then expansion in an *ex vivo* culture system, as previously described [16]. After culture, total cell count and cell viability were determined and oMPCs were resuspended in cryopreservation medium (42.5% ProFreeze/7.5% DMSO/50% α-MEM), cryopreserved and stored until use. The oMPC ampoules were stored in the vapor phase of a long-term liquid nitrogen storage tank. The batch/lot number, manufacture date and storage conditions of each ampoule were recorded for quality control.

Preparation and handling of the cells

Recovery of the frozen preparations was achieved by rapid thawing. A total of nine ampoules (each containing 60 million oMPCs in 1.2 mL of cryoprotectant) were used for each administration. Each ampoule was completely thawed and subjected to a visual inspection to eliminate any samples that exhibited evidence of macroscopic abnormality (such as clumping or discoloration). Cell number and cell viability were determined on an aliquot of the combined oMPC suspension, with the use of a Countess Automatic Cell