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Xenotransplantation of human adipose-derived stem cells in the regeneration of a rabbit peripheral nerve☆

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Summary Adipose tissue-derived mesenchymal stem cells (AdMSCs) are useful in the regeneration of neural tissues. Furthermore, xenotransplantation of human adipose tissue-derived mesenchymal stem cells (hAdMSCs) into animal models has already been tested and the results encouraged us to study peripheral nerve regeneration in rabbits, in order to test the feasibility of a xenotransplantation of hAdMSCs.

Animals and method: To promote end-to-end nerve fiber contacts of a 4-cm gap in the peroneal nerve of white New Zealand rabbits, an autologous vein conduit was used and three groups of animals were evaluated. In Group I, the gap was repaired with a vein conduit refilled with fibrin. Group II was similar, but the animals were treated with cyclosporine A. In Group III, a fibrin scaffold with hAdMSCs was placed inside the autologous vein conduit, and animals were treated with cyclosporine A. Neurofilament immunohistochemistry results showed 100% nerve regeneration at the vein guidance channel 90 days after the surgery in the hAdMSC-transplanted group but lesser neural regeneration in the neurofilaments of groups I and II. The analysis of variance (ANOVA) test showed statistically significant differences among all groups ($p < 0.04$). Group III exclusively tested positive for human monoclonal anti-mitochondrial antibody. Electron microscopy images showed tiny bundles, with a predominance of nonmyelinated axons. Myelinated axons caused irregular thickness of the myelin sheath, which was especially observed in group III.

Conclusions: Xenotransplantation of hAdMSCs into a fibrin scaffold promoted nerve regeneration through a vein conduit that connected a 4-cm gap created at the peroneal nerve of

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rabbits. Animals treated with hAdMSCs presented negative inflammatory response at the regenerated nerve gaps, but it was demonstrated that hAdMSCs were incorporated to the new nerve creating neural tissue and endothelial cells. However, hAdMSCs required immunosuppression with cyclosporine A to achieve axonal regeneration.

Introduction

Complete nerve sections necessitate surgical anastomosis; in cases of significant gaps, bridging strategies are required to repair the defect.[1,2] An active area of research in peripheral nerve reconstruction is focused on developing techniques to replace nerve grafts, by means of tubular nerve guidance conduits from natural or synthetic materials, which are supplemented with biological cues such as growth factors and regenerative cells. Cell transplantation, including Schwann cells, has been proposed as a method for enhancing axon outgrowth and survival.[3–5] Furthermore, mesenchymal stem cells (MSCs) have the ability to migrate to specific sites of injury or of tissue regeneration where they modulate the immune and inflammatory responses through a series of distinct paracrine mechanisms.[6] MSCs are capable of differentiating not only into tri-lineage mesenchyme cell types, such as adipocytes, chondrocytes, and osteoblasts,[7] but also into neuronal-like cells, including astrocytes, oligodendrocytes, microglial, neurons, and neuroglial cells.[8,9] These properties may be the basis of an early regenerative stage that leads to increased target organ reinnervation through less axonal dieback. However, these cells may present major problems including tumorigenesis in induced pluripotent stem cells and invasiveness.[10,11]

Adipose tissue-derived mesenchymal stem cells (AdMSCs) are another potent source of adult stem cells. In addition, adipose tissues contain higher densities of MSCs than bone marrow.[12] For these reasons, AdMSC-based treatments for a variety of diseases have been investigated for its ability to regenerate neural tissues.[13–16] Xenotransplantation is a promising method for tissue repair in immunologically privileged sites such as the central nervous system; further, xenotransplantation of human adipose tissue-derived mesenchymal stem cells (hAdMSCs) into animal models has already been tested in previous studies.[17–20] The results from these studies encouraged us to investigate a model of peripheral nerve regeneration, to test the feasibility of performing a xenotransplantation of hAdMSCs. To promote end-to-end nerve fiber contacts in a 4-cm gap in the peroneal nerve of adult rabbits, a fibrin scaffold with hAdMSCs was created and placed inside an autologous vein graft conduit. The resulting morphologic recoveries were evaluated across the healing period, 21 days and 90 days after the initial surgery.

Materials and methods

A total of 60 New Zealand white male adult rabbits weighing between 2500 and 3000 g were operated upon throughout this study, following the animal care and experimental procedures according to the European Communities Council Directive (86/609/EEC). The animals were randomized into three experimental groups:

GROUP I: This group consisted of 20 animals in which an autologous vein graft conduit filled with a fibrin scaffold was used to repair a 40-mm peroneal nerve gap. The animals did not receive any type of immunosuppression.

GROUP II: This group was composed of 20 animals in which an autologous vein graft conduit filled with a fibrin scaffold was used to repair a 40-mm peroneal nerve gap. Cyclosporine A was administered subcutaneously to all animals for immunosuppression.

GROUP III: This group consisted of 20 animals in which a vein graft conduit was refilled with a fibrin scaffold containing hAdMSCs. These animals also received immunosuppression by means of cyclosporine A.

In each group, the animals were distributed into two subgroups: 10 animals were sacrificed 21 days after the nerve surgery, and 10 animals that survived 90 days after the initial surgery.

Anesthesia and posttreatment follow-up

The animals were anesthetized by intramuscular injection of 2 ml of a solution of ketamine (Ketalar®, Parke-Davies, 100 mg/kg) and xylazine (Rompun®, Bayer, 10 mg/kg). After surgery, the rabbits were administered a daily dose of intramuscular antibiotics: benzylpenicillin procaine, 200,000 UI; and streptomycin sulfate, 250 mg in doses of 1 ml/10 kg. The animals of groups II and III were treated daily with 25 mg/kg of cyclosporine (Sandimmune®) administered intraperitoneally from the day of surgery until sacrifice.

Surgical procedure

Under general anesthesia and standard procedures of asepsis and antisepsis, the peroneal nerve was exposed by separating the gluteal and biceps muscles. A segment of the peroneal nerve was removed to create a 40-mm gap. A gluteal vein graft was harvested and used to perform an autologous conduit (Figure 1a and b).

The vein graft was then anastomosed to the proximal stump of the peroneal nerve; its lumen was dilated and refilled with fibrin glue, enriched or not with human stem cells, depending on the surgical group. Next, the other end of the vein was sutured to the distal aspect of the peroneal nerve, maintaining the fibrin scaffold into the lumen of the vein. Sutures of 10/0 monofilaments were used. Closure of