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Intracutaneously injected human adipose tissue-derived stem cells in a mouse model stay at the site of injection☆

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Summary The aim of this study was to evaluate the local behavior of intracutaneously injected human mesenchymal stem cells from adipose tissue and to determine the safety of a cell-based cutaneous therapy in an animal model.Human mesenchymal stem cells from adipose tissue were labeled with red fluorochrome and were injected intradermally in the paravertebral area in immunodeficient BalbC/nude mice ($n = 21$). As a control, cell culturemedium was injected in the same fashion on the contralateral paravertebral side. Four weeks, 6 months, and 12 months after the injection, seven mice were examined. In addition to the injected areas, the lungs, kidneys,spleens, and brains were excised and processed for histological evaluation. Serial sections of all the tissues excised were evaluated for adipose tissue-derived stem cells by means of emerging red fluorescent signals.The injected stem cells could be detected throughout the follow-up period of 1-year at the injection site within the dermal and subcutaneous layers. Bar these areas, adipose tissue-derived stem cells were not found in any otherexamined tissue at any point in time. The adipose tissue-derived stem cells showed a slow transition to deeper subcutaneous adipose tissue layers and, in part, a differentiation into adipocytes. No ulceration, inflammation, ortumor induction could be detected.The present study shows that intracutaneously injected human mesenchymal stem cells from adipose tissue stay at the site of injection, survive in vivo for up to 1-year, and partly differentiate into adipocytes. This is a new andvery important

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finding needed to safely apply therapies based on such stem cells in fat transplants in regenerative medicine.

Introduction

Fat transplants for volume augmentation have long been performed by plastic surgeons with varying results.[1–5] The most frequent complications are the development of oil cysts due to dedifferentiation and delipidation of adipocytes, apoptosis of transplanted adipocytes with consecutive inflammation and fibrosis, and loss of graft volume. Within the last several years, more and more stem cell-enriched fat transplants have been carried out making use of the anticipated positive effects of a higher stem cell concentration. These include an increased volume in the long term, a higher graft take, an increased vascularization, and the suggested whitening effect through an inhibited melanin synthesis.[6–11] Adipose tissue-derived stem cells (ADSCs) are known to survive the initial phase of hypoxia, low nutrients, and mechanical stress better than mature cells, such as adipocytes. All these properties make them a promising tool for skin repair, rejuvenation, or wound healing. In preliminary studies, mesenchymal stem cells (MSCs), such as ADSCs, have shown promising results.[12–14] In a minipig model of cutaneous radiation syndrome, the intradermal injection of MSC led to an improved vascularization and wound healing.[15,16] The subcutaneous administration of ADSCs induced an increase in collagen synthesis, dermal thickness, and collagen density as well as an improvement of ultraviolet B rays-induced wrinkles in hairless mice.[17,18] In two patients with recessive dystrophic epidermolysis bullosa, the intradermal administration of allogeneic MSC associated with type VII collagen replenishment at the dermal–epidermal junction prevented blistering and improved wound healing.[19] In six patients, an increase in the volume of the nasolabial folds was reached 2 months after the subcutaneous injection of autologous ADSCs, lasting up to 10 months.[20] However, up to now, there have been no standardized long-term results and histological evaluations that confirm the safety and efficacy of such MSC injections for skin therapy. Above all, the question whether the injected cells remain at the site of injection over a longer period of time or migrate toward other organs has not yet been sufficiently addressed.

Currently, it is known that the technique of cell administration widely influences the distribution of cells injected into the body. Studies using mice and rats have demonstrated that after an intravenous injection, stem cells can be found throughout the body: predominantly in the lungs and the liver but also in the spleen and other organs. In addition, ischemic or injured tissue areas attract stem cells presumably through paracrine mechanisms.[21–26] Even if the stem cells are mainly injected locally and not administered in a systemic fashion, it is important to analyze what happens to them and how they are distributed. This is particularly true as a widespread dissemination of cells to different organs after local administration to ischemic or necrotic cardiac tissue has been established.[27] An intradermal injection could be helpful whenever the dermis is the desired focus of treatment (e.g., in a wound-healing, antiaging, or dermatological setting). In such circumstances, a very potent, precise, and locally limited application is desired since there is no need to apply larger volumes as with regular fat transplantations.

To further evaluate this issue, we investigated the use of intracutaneously administered ADSCs with a focus on their long-term behavior at the injection site and a possible distribution to other selected organs. This is important with regard to the safety of a local intracutaneous administration of stem cells, for example, in wound healing or skin rejuvenation and regeneration.

Materials and methods

All chemicals, if not noted separately, were purchased from Sigma–Aldrich, Munich, Germany.

Donor specification

All participants gave informed consent prior to participation in this study. This procedure as well as the informed consent form was approved by the ethics committees. The study was conducted under the guidelines and with the approval of the ethics committees of the University of Heidelberg and of the medical association of the local district of Baden-Wuerttemberg, Germany. Freshly excised subcutaneous adipose tissue of six adults (two male, four female) with an age range between 21 and 57 years (median age 32.2 years) undergoing elective plastic surgery, that is, abdominoplasty, was used for isolation of MSCs.

Isolation of adult mesenchymal stromal cells from human adipose tissue

ADSCs were isolated from freshly excised subcutaneous fat tissue using a procedure modified from Hauner et al.[28] In brief, after removing fibrous tissue, the adipose tissue was washed in 1% bovine serum albumin/phosphate buffered saline (BSA/PBS), minced, and digested enzymatically by collagenase (collagenase CLS; 220 U/mg; Biochrom AG, Berlin, Germany; 1.5 mg/ml, in 1% BSA/Krebs–Ringer solution) for 45 min under constant shaking at 37 °C. Mature adipocytes and connective tissue were separated by centrifugation (700 × g, 7 min at room temperature). The sedimented cells were resuspended, passed through a 100-μm mesh filter (Neolab, Heidelberg, Germany), and washed twice with 1% BSA/PBS. After erythrocyte lysis (3 min;