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ADIPOSE DERIVED CELLS

Evaluation of function and recovery of adipose-derived stem cells after exposure to paclitaxel

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Abstract

Background aims. Adipose-derived stem cells (ASCs) are considered to play a positive role in wound healing as evidenced by their increasing use in breast reconstructive procedures. After chemotherapy for breast cancer, poor soft tissue wound healing is a major problem. In the present study, the functional capabilities and recovery of ASCs after exposure to chemotherapeutic agent paclitaxel (PTX) using *in vitro* and *ex vivo* models were demonstrated. *Methods.* Human ASCs were isolated from periumbilical fat tissue and treated with PTX at various concentrations. Adult Sprague-Dawley rats were given intravenous injections with PTX. Two and four weeks after the initial PTX treatment, ASCs were isolated from rat adipose tissue. Proliferation, cell viability, apoptosis and cell migration rates were measured by growth curves, MTT assays, flow cytometry and scratch assays. ASCs were cultured in derivative-specific differentiation media with or without PTX for 3 weeks. Adipogenic, osteogenic and endothelial differentiation levels were measured by quantitative reverse transcriptase polymerase chain reaction and histological staining. *Results.* PTX induced apoptosis, decreased the proliferation and cell migration rates of ASCs and inhibited ASCs multipotent differentiation in both *in vitro* human ASC populations and *ex vivo* rat ASC populations with PTX treatment. Furthermore, after cessation of PTX, ASCs exhibited recovery potential of differentiation capacity in both *in vitro* and animal studies. *Conclusions.* Our results provide insight into poor soft tissue wound healing and promote further understanding of the potential capability of ASCs to serve as a cell source for fat grafting and reconstruction in cancer patients undergoing chemotherapy treatment.

Key Words: *adipose-derived stem cells, breast cancer, paclitaxel, wound healing*

Introduction

After radiation and chemotherapy for breast cancer resections, poor soft tissue wound healing can pose a major clinical challenge [1–4]. With recent research advances, adipose-derived stem cells (ASCs) present in clinically applied fat grafts are considered to play a positive role in wound healing as evidenced by their increasing use in breast reconstructive procedures [5–7]. ASCs function as a reservoir for obtaining pluripotent cells with an ability to differentiate into multiple cell lineages, including chondroblasts, osteocytes, adipocytes and endothelial cells, among others [8–10]. However, little is known about the impact that chemotherapeutic agents may have on ASCs in regard to their wound healing properties in this clinical setting. To overcome this problem, a better understanding of ASCs' contribution to wound healing, as well as the degree to which chemotherapy might hinder their ability to participate in normal healing, is needed.

Recent research has focused almost entirely on poor soft tissue wound healing in patients postexposure to chemotherapy and radiation therapy [11–13]. Little is known about the recovery of cell viability and differentiation capability of stem cells after cessation of chemotherapeutic treatment. Emphasis should be placed on the clinical time course of ASC function in relation to chemotherapy applications because the current clinical practice is to provide the patient chemotherapy drugs before, during and after surgical excision of the cancer tumor. Previous work in our laboratory demonstrated

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that *in vitro* direct exposure to tamoxifen had cytotoxic effects through apoptosis on ASCs by decreasing proliferation and multi-potency differentiating abilities [11]. However, we have yet to examine the effect of chemotherapy on ASC outcomes in patient or animal treatment. Paclitaxel (PTX) is another drug that is widely used for chemotherapy and functions by disrupting microtubule organization, causing mitotic arrest and inducing cancer cell death via apoptosis [14,15]. In the present study, we assessed the direct effects of PTX on growth and differentiation capabilities of human ASCs by using an *in vitro* culture system. The effects of PTX on functional capabilities of ASCs were also examined in a rat model conditioned with PTX. The goal of this study was to investigate whether PTX exposure in *in vitro* human ASC populations and injections to rat models modulate overall ASC cellular functions.

To date, recent research has focused almost entirely on chemotherapy-induced cytotoxic acute injury to mesenchymal stromal cells [3,4,16,17], with little attention paid to the recovery of stem cells after exposure to chemotherapeutic agents. For clinical translation, it is necessary to better understand the effects of chemotherapy regarding the possible preservation or destruction of these stem cells in clinical practice [18,19]. The purpose of the present study was also to investigate the recovery of ASC cellular functions after exposure to PTX in both *in vitro* and animal model studies. The study design was to carry out *in vitro* cultures of human ASCs and *ex vivo* cultures of rat ASCs from PTX-treated animals to assess whether ASCs have the potential for recovery of function after exposure to chemotherapeutic agents. The hypothesis for this study was that PTX treatment may influence ASC function via (i) induction of apoptosis, inhibition of cell proliferation and multi-potency differentiating capacity or (ii) patterns of recovery after withdrawal of the drug. We further hypothesized that demonstrating manipulations of the stem cell microenvironment would help to further understand the potential capability of ASCs to serve as a cell source for fat grafting and reconstruction in cancer patients undergoing chemotherapy.

Materials

Isolation and culture of human ASCs

Adipose tissue was obtained by lipoaspiration from patients undergoing reconstruction procedures in accordance with our institutional review board–approved protocol. Liposuctioned adipose tissue was washed and incubated in collagenase I solution with bovine serum albumin (1 mg/mL + 4 mg/mL) for 1 h at 37°C. After centrifugation at 1500 rpm for 10 min followed by a wash with phosphate-buffered saline, the stromal vascular cells were cultured at 37°C, 5% CO_2 in medium M199 (Mediatech) and supplemented with 10% fetal bovine serum and antibiotic/antimycotic solution (Gemini Bio Products). Non-adherent cells were removed after 24 h, and the culture medium was subsequently replaced twice weekly. ASCs were split 3:1 when ~80% confluent.

Paclitaxel treatment and cell viability

PTX was purchased from Sigma and dissolved in dimethyl sulfoxide (Sigma-Aldrich) to make a stock concentration of 2 mmol/L. The stock PTX was then aliquoted into 50 µL per tube and kept in −20°C refrigerator for fresh use each time. ASCs were treated with PTX at different concentrations (0, 0.1, 1., 10.0 µmol/L etc.) as indicated in each experiment. The viability of ASCs was examined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. ASCs were plated at a density of 1×10^4 cells per well in a volume of 1 mL in 24-well plates. At days 1, 3 and 6 the culture media were replaced with a medium containing MTT solution (5 mg/mL, Sigma-Aldrich) and incubated at 37°C with 5% CO_2 for 3 h. The wells were then decanted and the purple formazan crystals formed were dissolved in 200 µL dimethyl sulfoxide. The absorbance of the plate was read on a microplate reader at 570 nm. All assays were performed in triplicate.

Cell proliferation

Proliferation was assessed by constructing growth curves over an 8-day period. ASCs were plated into 24-well plates at 5×10^3 cells /cm^2 and then treated with PTX at low and high concentrations (0.1 and 1 µmol/L) for a period of 8–10 days with replenishment of medium every 3–4 days. At various time points, cells were trypsin-released and counted using a Coulter counter (Beckman Coulter). To evaluate the recovery of ASCs after PTS treatment, we extended the culture time period. ASCs were exposed to PTX for 72 h then removed. The cells were cultured in medium for an additional 9 days, and cell numbers were determined at different time points.

Cell apoptosis assays

For apoptosis analysis, the Annexin V-FITC apoptosis detection kit (BD Biosciences) was used. Briefly, after 3 days of PTX treatment, the ASCs were resuspended in 1 × binding buffer at a concentration of 1×10^5 cells/ml. Approximately 5 µL of annexin V-FITC reagent and 10 µL of propidium iodide (PI) were added to the cell suspension and then incubated for 15 min under room temperature in the dark. Data acquisition and analyses were performed by C6 flow cytometer (Accuri) with FlowJo software. ASCs cultured with ethanol for 24 h were used as the negative control.

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