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Oxidative preconditioning promotes bone marrow mesenchymal stem cells migration and prevents apoptosis

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Abstract

Reactive oxygen species (ROS) play essential roles in apoptosis and in the regulation of several transcription factors under both physiological and pathological conditions. However, the effects of ROS on MSCs are not well known, and therefore we have investigated the effects of preconditioning with hydrogen peroxide (H_2O_2) on the level of expression of the chemokine receptor, CXCR4, stromal cell-derived factor-1α (SDF-1α)-dependent migration and apoptosis in MSCs. Preconditioning with 20 μM H_2O_2 significantly increased the level of expression of CXCR4 mRNA and protein, and MSCs migration toward SDF-1α; increased expression of CXCR4 and SDF-1α-induced MSCs migration was attenuated by extracellular signal-regulated kinase (ERK) inhibitor PD98059. Preconditioning with 20 μM H_2O_2 significantly protected MSCs against apoptosis induced by 500 μM H_2O_2. These results suggest that preconditioning with H_2O_2 can increase MSCs migration toward SDF-1α and protect MSCs against apoptosis.

Keywords: Hydrogen peroxide; CXCR4; ERK; Marrow stromal cell; Migration; Apoptosis

1. Introduction

Reactive oxygen species (ROS), including superoxide anions (O_2−), hydroxyl radicals (OH•), and hydrogen peroxide (H_2O_2), induce apoptosis under both physiological and pathological conditions. ROS also have crucial roles in the proliferation, migration and differentiation of microvascular endothelial cells (Ushio-Fukai and Nakamura, 2008). ROS are also important in signal transduction pathways regulating chemokine expression. For example, they regulate the level of CCR2, CCR5 and CXCR4 in human monocytes (Saccani et al., 2000; Sannohe et al., 2003). These effects may be mediated in part through the action of ROS on redox-sensitive proteins, such as extracellular signal-regulated protein kinase, ERK (Cao et al., 2008), or some upstream protein kinases (Lee et al., 2007).

Mesenchymal stem cells (MSCs) from bone marrow possess tremendous potential for cell therapy in several disease processes (Umezawa and Terai, 2005; Francois et al., 2006; Sykova and Jendelova, 2007). However, the retention rate of exogenously administered cells within the injured tissues remains low, and the mechanistic processes underlying stem cell therapy remain unclear. Effective therapies will depend on strategies to increase migration of MSCs toward infarcted tissues and to increase their survival time. Short-term exposure of multipotent stromal cells to hypoxia increases expression of CXCR4 and the ability of migration (Hung et al., 2007), and others demonstrate that MSCs cultured in hypoxic conditions increase their migration rates (Rosova et al., 2008).

Although hypoxic preconditioning has been extensively studied, little is known about the effect of preconditioning with H_2O_2 on the apoptosis and migration of MSCs. We demonstrate that H_2O_2 enhances the ability of MSCs to migrate via the upregulation of CXCR4 and the ERK pathway, and protects MSCs against lethal oxidant injury.

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2. Materials and methods

2.1. Preparation of bone marrow stromal stem cells

MSCs were isolated as previously described (Deng et al., 2006). MSCs were separated from the femurs and tibias taken from Sprague–Dawley rats (male, weighing 80–100 g). Cells were centrifuged at 1000 *g* for 5 min and suspended in Dulbecco's modified Eagle medium with low glucose (L-DMEM, GIBCO, BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, kibbutz beit haemek, Israel), glutamine, penicillin, and streptomycin. Cells used in these experiments were harvested from four passages.

2.2. Nuclear staining for assessment of apoptosis

Chromosomal condensation in the nucleus was observed using the chromatin dye Hoechst 33258. The MSCs were fixed with 4% paraformaldehyde for 10 min. After three rinses with PBS, the cells were stained with 5 mg/l Hoechst 33258 for 10 min, and then analyzed under a fluorescence microscope (BX50-FLA, Olympus, Tokyo, Japan).

2.3. Flow cytometric analysis of stromal cell apoptosis

MSCs' apoptosis was analyzed by staining for Annexin-V-positive (apoptotic) and propidium iodide (PI)-positive (necrotic) cells using the Annexin-VFITC apoptosis detection kit (BD Pharmingen), according to the manufacturer's protocol. Briefly, cells were suspended in 1× binding buffer at 10^6 cells/ml. Annexin-VFITC (5 μl, BD Pharmingen, San Diego, CA, USA) and PI (2 μl, BD Pharmingen) were added and incubated with cells at room temperature for 15 min. Next, 500 μl of 1× binding buffer were added to cells and data were acquired immediately using a FACSCalibur (Becton Dickinson) flow cytometer.

2.4. Real-time RT-PCR

Total RNA was isolated from 2×10^6 MSCs by using TriZol Reagent (Roche, USA) following the manufacturer's instructions. First strand cDNA synthesis and amplification were performed using an MBI RevertAid First Strand cDNA Synthesis Kit (MBI, Lithuania). The real-time PCR was done with an iQ5 Multicolor Real-Time PCR Detection System (Bio-rad Laboratories, Hercules, CA, USA) in 96-well plates, using SYBR-Green Master Mix (Paragon Biotech Co., LTD., Guangzhou) in 25-μl reaction mixtures. The thermal profile for the real-time PCR was 95 °C for 3 min followed by 40 cycles of 95 °C for 20 s, 52 °C for 30 s, and 60 °C for 30 s. Results were analyzed by the relative quantification method (ΔΔCT) using the iQ5 Optical System Software Version 2.0 (Bio-rad Laboratories). Cycle threshold (Ct) values were obtained from the ABI 7000 software. Fold change of relative mRNA expression was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The primers were as follows:

CXCR4 (U54791.1),
forward 5′-GCTGAGGAGCATGACAGACA-3′
reverse 5′-GATGAAGGCCAGGATGAGAA-3′
β-actin (BC063166),
forward 5′-TGTCACCAACTGGGACGATA-3′
reverse 5′-GGGGTGTTGAAGGTCT-3′.

2.5. Flow cytometry analysis of CXCR4 receptors expression

CXCR4 surface receptors on MSCs were analyzed by indirect immunofluorescence staining. Briefly, MSCs (1×10^6) were incubated in 2% fetal bovine serum/PBS at 4 °C for 30 min with 1 μl of monoclonal antibody specific for CXCR4 (Boster Biotechnology), washed extensively with PBS, and then incubated at room temperature for 30 min with the secondary antibody (goat antimouse IgG Ab) FITC-conjugated affinity-purified F(ab′)2 Ab (Chemicon, Boronia

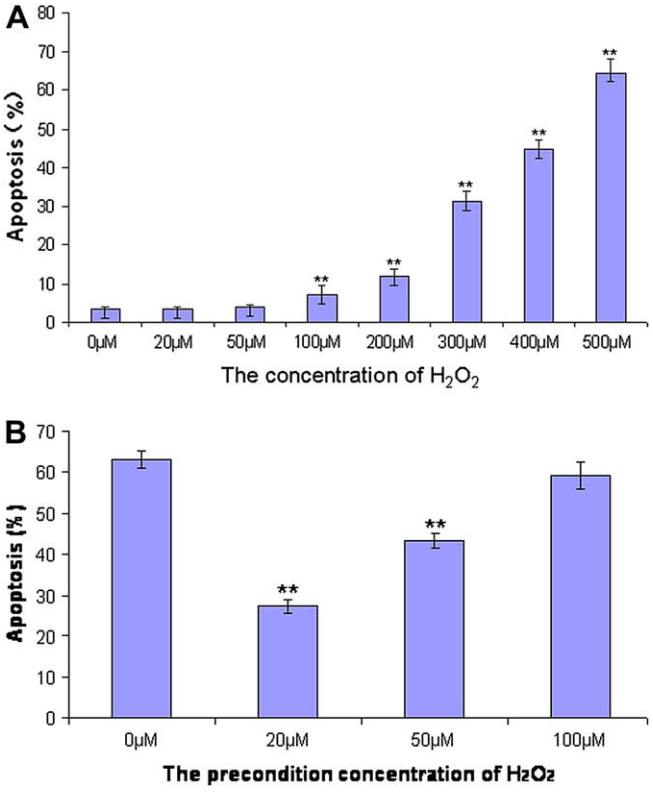

Fig. 1. Flow cytometry analysis for MSCs apoptosis induced by different doses of H_2O_2. MSCs were exposed to different doses of H_2O_2 for 24 h, respectively. (A) The results show that H_2O_2 at 100–500 μM H_2O_2-induced apoptosis in MSCs in a concentration-dependent manner $^{**}P < 0.01$ vs. control ($n = 5$). (B) Cytoprotection of preconditioning with H_2O_2 in MSCs. MSCs were, respectively, preconditioned with 0, 20, 50 and 100 μM H_2O_2 for 24 h, followed by 12 h recovery, and subsequent exposure to 500 μM H_2O_2 for 24 h. $^{**}P < 0.01$ vs. control (preconditioned with 0 μM H_2O_2); $n = 5$.