Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells on the radiation-induced GI syndrome

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1. Introduction

The gastrointestinal (GI) tract is one of the most radiosensitive organ systems in the body. Radiation-induced GI injury is described as destruction of crypt cell, decrease in villous height and number, ulceration, and necrosis of intestinal epithelium [1].

Studies show that mesenchymal stem cells (MSCs) treatment may be useful in the repair or regeneration of damaged organs including bone, cartilage, or myocardium. MSCs from umbilical cord blood (UCB) have many advantages because of the immature nature of newborn cells compared to bone marrow derived MSCs [2]. Moreover, UCB-MSCs provide no ethical barriers for basic studies and clinical applications.

In this study, we explore the regeneration capability of human UCB-MSCs after radiation-induced GI injury.

2. Methods and Results

2.1 Animals and treatment

Female Wistar rats (7 weeks old), weighing 200–250 g, were obtained from Central Laboratory Animals (Seoul, Korea) and housed in a room maintained at a temperature of 23±2℃, relative humidity of 50±5%, artificial lighting from 08:00 to 20:00, and 13− 18 air changes per hour. The animals were fed a standard animal diet. All experiments were approved by the Committee for Animal Experimentation of KIRAMS. Anesthetized rats received a single abdominal dose of 15Gy (^{60}Co ɣ -ray) at a dose rate of 0.98Gy/min. PKH-26 labeled UCB-MSCs ($3x10^7$ cells/head) were intravenously injected at 4 hours, 1 day and 2days after exposure.

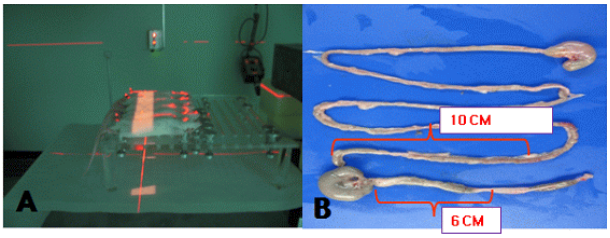

Fig. 1. Abdominal irradiation and tissue sampling sites.

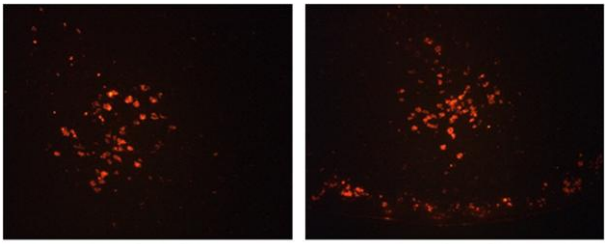

Fig. 2. Human UCB-MSCs localization in rat ileum.

2.2 Peripheral blood count analysis

Blood was collected from tail vein using sterilized syringe and stored in EDTA tube. By using Hemavet 950 (CDC Technologies Inc., Conneticut, USA), total WBC count, differential WBC count, RBC count, and PLT count were analyzed. Previously, WBC and lymphocyte is used as biomarker for radiation injury [3]. Our results suggested that lymphocytes are the most sensitive blood cells to the irradiation in peripheral blood cells but there are no differences between two groups.

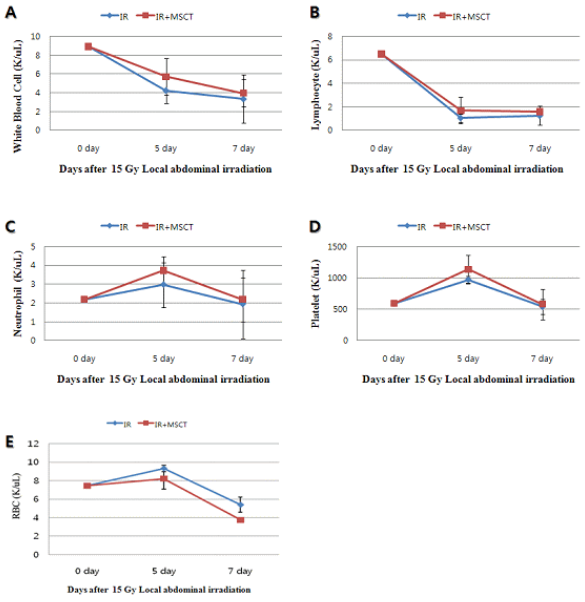

Fig. 3. Peripheral blood count analysis.
Significant decrease of lymphocytes was revealed but there are no differences between UCB-MSCs treatment group and non-treatment group in peripheral blood count results.

2.3 Histopathological analysis

The collected samples were embedded in paraffin wax after perfusion fixation by 4% paraformaldehyde, sectioned at 5-μm thickness. Sections were stained with hematoxylin and eosin (H&E).

Diffuse necrosis was revealed in the ileal mucosal layer on 5days in non-treatment group. In addition, cellular degeneration of neurons and ganglion cells was increased, but muscular necrosis was not observed. However, in UCB-MSCs treatment group, superficial epithelial desquamation was observed. Mucosal necrosis was mild and microvilli structure was also maintained.

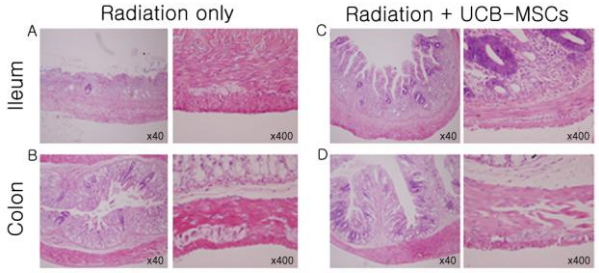

Fig. 4. Histopathological changes of intestine after irradiation.
A. Ileum of non-treatment group on 5days after irradiation
B. Colon of non-treatment group on 5days after irradiation
C. Ileum of UCB-MSCs treatment group on 5days after irradiation
D. Colon of UCB-MSCs treatment group on 5days after irradiation

2.4 Intestinal cell proliferation

The expression of Ki-67 was examined at 5days after irradiation using immunohistochemistry. Sections were incubated with 3% bovine serum albumin for 10 min at room temperature followed by incubation with monoclonal mouse anti-rat Ki-67 (1:40, Zymed, San Francisco, USA) for 70 min at room temperature. Sections were then incubated with Envision TM (Dako, CA, USA). Sections were developed using 3,3'-diaminobenzidine and counterstained with hematoxylin.

On 5day after irradiation, Ki-67 positive cells were limited in gland cells under the injured intestinal epithelium. However, in UCB-MSCs treatment group, there was an enhanced expression of Ki-67 from the gland cells to the mucosal epithelial cells.

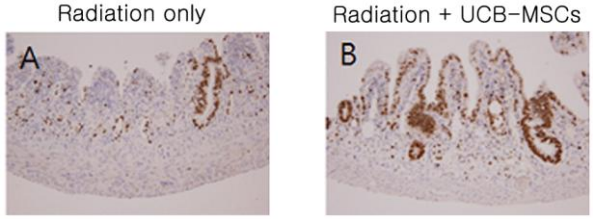

Fig. 5. Intestinal cell proliferation after irradiation.
A. Ileum of non-treatment group on 5days after irradiation
B. Ileum of UCB-MSCs treatment group on 5days after irradiation

3. Conclusions

The purpose of this study is the therapeutic effects of human UCB-MSC on the acute radiation GI syndrome. We found histopathological mechanisms that radiation-induced intestinal injury was characterized by remarkable mucosal injury, edema of neural plexus and cellular degradation of ganglions. Human UCB-MSCs treatment decreased radiation-induced mucosal injury and enhanced epithelial cell proliferation property. Although further study will be needed about time point, route of injection or combined treatment with other drugs, our results suggest that UCB-MSCs treatment is useful for regeneration of intestinal epithelial cell and reconstruction of organic structure against radiation-induced GI syndrome.

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