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Treatment of ischemic colonic anastomoses with systemic transplanted bone marrow derived mesenchymal stem cells

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Abstract. – BACKGROUND: The aim of the study is to investigate the healing effect of the bone-marrow derived mesenchymal stem cells (BM-MSCs) on ischemic colon anastomosis in systemic application and to recovery the adverse effect of ischemia.

MATERIALS AND METHODS: Fourty male Wistar Albino rats weigthing 250-300 g were divided into four equal groups (n=10 Group 1: control; ischemic left colonic anastomoses (4th day); Group 2: control; ischemic left colonic anastomoses (7th day); Group 3: ischemic left colonic anastomoses + systemic transplanted BM-MSCs (4th day); Group 4: ischemic left colonic anastomoses + systemic transplanted BM-MSCs (7th day). BMSCs labelled with bromodeoxyuridine (BrdU) were transplanted into the vena cava. Group 1 and group 3 were killed four days after surgery. In group 2 and group 4 were sacrificed seven days after the surgical procedure. Histopathological features, hydroxyproline levels in the tissue, and anastomotic strength were investigated.

RESULTS: There was no mortality all of the groups.The mean bursting pressures of ischemic colonic anastomoses in group 3 were higher than in control group 1 (4th day). We found significantly higher hydroxyproline values in group 3 and were significantly higher in group 4 than in control groups. We investigated the early period of wound healing (4th day and 7th day). When comparing between group 1 and group 3, we found higher levels for all of the histological parameters except inflammation in group 3. On day 7, when comparing between group 2 and group 4, we found higher levels for parameters of necrosis, collagen deposition.

CONCLUSIONS: BM-MSCs therapy significantly accelerated all of the healing parameters for ischemic colonic anastomosis except for inflammation on fourth day. On the seventh day, BM-MSCs augmented the levels of the hydroxyproline. Histological parameters, necrosis and collagen deposition were also found to be important for healing of ischemic colonic anastomoses. However, they did not accelerate the others histological parameters especially angiogenesis.

Key Words:
Stem cells, Wound healing, Ischemic colon anastomosis.

Introduction

Organ damage and the consequent inflammation responses initiate a series of repair processes, including stem cell proliferation, migration, and differentiation, often in combination with angiogenesis and remodeling of the extracellular matrix. Circulating stem cells may contribute to regenerative responses by migrating into a tissue and differentiating into organ-specific cell types[1]. Cellular transplants involve the injection of cells that have the potential to replace cells in organ that has been damaged by disease, thereby, augmenting the function of that organ[2]. In gastrointestinal surgery, ischemia following hypoxia is very important cause of anastomic leakage. Despite improvements in surgical techniques, the rates of anastomotic leak-

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age remain high; this necessitates investigation of new agents and new methods.

Bone marrow-derived mesenchymal stem cells (BM-MSCs), which are also referred to as stromal progenitor cells, are self-renewing and expandable stem cells. MSCs from several sites, including bone marrow and adipose tissue, may have immunomodulatory, anti-inflammatory, anti-apoptotic properties, thereby, indicating their possible use in regenerative medicine and tissue engineering[3-6]. Recent studies suggest that MSCs play a crucial role in the process of intestinal repair[7]. In this work we aim to investigate the healing effect of the bone-marrow derived mesenchymal stem cells on ischemic colon anastomosis in systemic application and to recovery the adverse effect of ischemia.

Materials and Methods

Isolation and preparation of BM-MSCs

Animals

This study was completed in the Institute of Experimental Medicine at Istanbul University. The study protocol was approved by the Institutional Animal Care and Use Committee at Istanbul University. Animal housing and experiments were approved by the local Animal Care Committee according to the institutional guidelines and National Animal Welfare Act. They were housed under standard conditions before use.

Isolation and culturing of BM-MSCs

Isolation and culture of rBM-MSCs were performed as previously described[8]. Animals Wistar albino rats (male, 8 weeks) were obtained from the Experimental Animal Center of Kocaeli University.Under the sterile conditions, both femurs and tibiae were excised and were cut away. Then a 21-gauge needle that was inserted into the shaft of the bone and marrow was extruded by flushing with MEM-Eagle medium (Biochrom, Berlin-Germany) supplemented with 15% fetal bovine serum (FBS; Invitrogen/GIBCO, Grand Island, NY, USA), as well as 100 IU/ml penicillin and 100 μg/ml streptomycin (Invitrogen/GIBCO) that was included in the growth medium. The marrow plug suspension was dispersed by pipetting, successively filtered through a 70 μm mesh nylon filter (BD Biosciences, Bedford, MA, USA), and centrifuged at 200xg for 10 minutes. The supernatant containing thrombocytes and erythrocytes was discarded, and the cell pellet was resuspended in the medium. The cells from one rat were seeded onto two 25 cm^2 plastic tissue culture flasks (BD Biosciences, San Diego, CA, USA) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 3 days. The mesenchymal stem cells were isolated on the basis of their ability to adhere to the culture plates. On the third day, red blood cells and other non-adherent cells were removed and fresh medium was added to allow further growth. The adherent cells were grown to 70% confluency and were defined as passage zero (P_0) cells. The P_0 MSCs were washed with Ca^{2+}-Mg^{2+} free phosphate-buffered saline (PBS) (Invitrogen/GIBCO) and detached by incubating with 0.25% trypsin-EDTA solution (Invitrogen/GIBCO) for 5-10 min at 37°C. Complete medium was added to inactivate the trypsin. The cells were centrifugated at 200 g for 10 minutes resuspended in 1 ml complete medium, counted manually in duplicate using a Thoma chamber, then plated as P_1 in 75 cm^2 flasks (BD Biosciences) at a density of $1x10^6$ cells/flask. Complete medium was replaced every third day over a 10-14 day period. For each passage the cells were plated similarly and grown to 70% confluency.

Characterization Studies of BM-MSCs

Flow cytometry

Undifferentiated MSCs after culture were subjected to flow cytometry analysis to determine their phenotypic characteristics. Flow cytometry was performed by FACS Calibur (BD Biosciences) and the data were analyzed with the Cell Quest software. Immunophenotyping of rBM-MSCs was performed with antibodies against rat antigens CD29 (Integrin b_1 chain; FITC), CD45 (Leukocyte Common Antigen; FITC), CD54 (ICAM-1; PE), CD90 (Thy-1/Thy-1.1-FITC), and CD106 (VCAM-1; PE) as well as their isotype controls ($IgG2a_K$; FITC; and $IgG1_K$; PE) (BD Biosciences, San Diego, CA, USA).

Labeling with BrdU of Mesenchymal Stem Cells

BM-MSCs were plated at a density of 13,000 cells/cm^2 and incubated for 2 days until they reached to 50% confluency. The culture medium was refreshed with the same medium containing 10 mM of BrdU (Sigma, St. Louis, MO, USA). After 48 hours of incubation at 37°C and 5%

CO_2, the BrdU-labeled MSCs were trypsinized and evaluated for viability.

Study Design

Fourty male Wistar albino rats weigthing 250-300 g were divided into four equal groups (n=10). The animals were housed at 21°C and were given tap water and standard rat food. Group 1 and group 3 were killed four days after surgery. Group 2 and group 4 were sacrificed seven days after the surgical procedure.

Group 1: control; ischemic left colonic anastomoses (4^{th} day)

Group 2: control; ischemic left colonic anastomoses (7^{th} day)

Group 3: ischemic left colonic anastomoses + systemic transplanted BM-MSCs (4^{th} day)

Group 4: ischemic left colonic anastomoses + systemic transplanted BM-MSCs (7^{th} day)

Preparing for Cell Transplantation

The cryopreserved bromodeoxyuridine (BrdU) labeled BM-MSCs were processed as below and then re-evaluated for cell viability before transplantation. The cells were incubated in a waterbath at 37°C and then cells were washed with phosphate-buffered saline and Dulbecco's Minimal Essential Medium. The cell viability was determined by a trypan blue exclusion assay. Labeled BM-MSCs were stained with trypan blue, whereby viable BM-MSCs with an intact membrane, excluded trypan blue and were not stained. The viable cells were counted on a Beckman Coulter Vi-Cell XR Cell Viability Analyzer (Brea, CA, USA) (1.0×10^6 cells in 1 mL). Immunohistochemistry were performed as previously described[8], to define the immunophenotype of BrdU-positive cells. In addition, transplanted stem cells in the colon tissue samples were identified histologically by staining for proliferating cell nuclear antigen (PCNA) to show the proliferating cells in tissue.

Surgical Procedure and Cell Transplantation

After one night of fasting, the animals were anesthetized by an intramuscular injection of ketamine hydrochloride (50-100 mg per kg of body weight). Abdominal access was achieved through a midline incision 4 cm long, and the left colon was diverted at 3 cm proximal to the peritoneal reflection. In order to establish ischemic colon anastomosis, vessels in the mesocolon between 2 cm proximal and 2 cm distal from the anastomosis line were ligated[8-12]. After the fecal contents had been removed, a standardized end to end anastomosis was made of eight interrupted, inverted sutures of 6/0 polypropylene. After the surgical procedure, group 3 and group 4 received transplanted BM-MSCs very slowly (0.5 mL, $1.0X10^6$ cells) into the vena cava. Group 1 and group 2 (control groups) were injected with physiological serum (0.5 ml 0.9% NaCl) into the vena cava. The bleeding control had been done, 2 cc 0.9% NaCl was injected intraperitoneally and the abdomen was closed with 3/0 continuous silk sutures. Water was given 12 hours and food was given 24 hours later.

Measurement of Colonic Bursting Pressure

Four days (group 1 and group 3) and seven days (group 2 and group 4) later, all of the rats were sacrificed. The abdominal incision was reopened and then the anastomotic sutures lines were found. The anastomotic segment was resected preserving the adhesions that were 2 cm proximal and 2 cm distal to the anastomotic line. Bursting pressure was measured in situ without detaching adhesions. The bursting pressure of the anastomotic segment was measured with a mercury manometer and a constant flow pump. Briefly a 16 gauge silastic catheter was inserted via a colostomy in the proximal colon and ligated with a 2/0 silk tie. The rectum distal to the anastomosis was ligated with 2/0 silk and was continuously infused through the catheter via a tube pump at a rate of 4 ml/minute. Bursting pressure was recorded (mmHg) as the peak pressure attained before rupture of the anastomosis, which resulted in an abrupt drop in pressure.

Hydroxyproline Determination

The tissue samples were excised 0.5 cm proximal and 0.5 cm distal of the anastomosis line. Then all of the samples were weighed and homogenized in physiological serum as 20% homogenates using a Potter type glass homogeniser (Heidolphy-RZR 2021, Germany). Homogenates were centrifuged at 1500 r/min for 15 min and the obtained supernatants were hydrolyzed by adding equal amounts of hydrochloric acid for 10-18h. Using a hydrox-

yproline kit (Hipronisticon, Organon, Oss, Netherlands) which was based on the principles of Stegeman and Stadler the hydroxyproline amount was calculated in micrograms per milligram of wet tissue by reading the absorbance of the solution on a spectrometer at 560 nm. Hydroxyproline levels in the tissue were carried out without knowing the identity of the groups.

Tissue Harvesting and Histopathological/ Immunohistochemical Examinations

To determine histological and immunohistochemical assessments, the bursting colon was excised, cleared of surrounding mesentery and fat, and washed with saline. The anastomosis was excised together with 1 cm of the adjacent proximal and distal colon. The tissues were fixed in 10% formalin approximately 24 hours and then embedded in paraffin. Transverse sections of the embedded tissue that were 3 mm in thickness were stained with haematoxylin and eosin, and histological assessment was carried out without knowing the identity of the groups by experienced pathologist. Necrosis, ephithelization, inflammatory processes, fibroblastic activity, and neovascularization at the anastomic site were studied and scored (from 0 to 3, 0: none, 1: slight, 2: moderate and 3: dense). Masson's trichrome staining was used for distinguishing cells from the surrounding connective tissue. Collagen deposition was scored according to the density of the colonic tissue (from 0 to 3, 0: none, 1: slight, 2: moderate, 3: dense).

To perform cell tracing after injection of the BrdU labeled BM-MSCs, an immunofluorescence double staining protocol was performed on the paraffin-embedded tissues. Slides were deparaffinized with two changes of xylene for 5 minutes each and rehydrated in a series of graded alcohol solutions. Endogenous peroxidases were inhibited by incubation with fresh 3% H_2O_2 in PBS buffer. The sections were then treated with a trypsin solution in a moist chamber at 37°C for 10 min. DNA was denatured by incubation with the denaturing solution. Nonspecific staining was blocked with the mixture of two different sera at 1.5% in PBS for 30 min at room temperature (RT). Afterwards, the sections were incubated in a mixture of two primary antibodies in a pair wise fashion with the mouse monoclonal anti-BrdU antibody (Thermo Scientific MS-1058-P) and Vimentin (sc-7557 Santa Cruz) or with CD105 (sc-19793), or with CD31 (sc-1531) and nestin (sc-33677) with PCNA (Proliferating Cell Nuclear Antigen) (MS-106-R7) at appropriate dilutions in antibody dilution buffer for 2 hour at RT. The sections were incubated in a mixture of two fluorescent conjugated secondary antibodies, which included the goat anti-mouse TR (sc-2781) for the BrdU antibody, donkey anti-goat FITC (sc-2024) for vimentin and CD31, CD105, and goat anti-mouse FITC (sc-2010) for nestin at the dilution of 1:500 in PBS buffer for 30 minutes at room temperature and were mounted with mounting medium containing DAPI (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Statistical Analysis

Data were reported as means ± SD. All statistical analyses were performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Data were analyzed using two-way ANOVA and a paired *t*-test. A probability value of $p < 0.05$ was considered to denote statistical significance.

RESULTS

Cell culture of BM-MSC

BM-MSCs attached to the culture flasks sparsely and displayed a fibroblast-like, spindle-shaped morphology during the initial days of incubation. After three to four days of incubation, proliferation started and the cells gradually grew into small colonies. By the time they were six to eight days of old different sized colonies increased in number. As growth continued, adjacent colonies interconnected with each other, and a confluent monolayer was obtained after 12 to 15 days of incubation. In later passages, MSCs exhibited large, flattened or fibroblast-like morphology (Figure 1A) and did not change up to 25 passages. Tests for bacterial and mycoplasm contamination were negative, and the viability was higher than 95%. To confirm the BrdU labeling of cells, we performed immunostaining and imaged them. BrdU-labeled cells could be identified by the green staining in their nuclei seen under fluorescence microscopy (Figure 1B) in addition to diaminobenzidine (DAB) BrdU immunostained cells with a dark gray-black color (Figure 1C). To show the proliferating cells, samples were stained with Proliferating Cell Nuclear Antigen (PCNA) (Figure 1D).

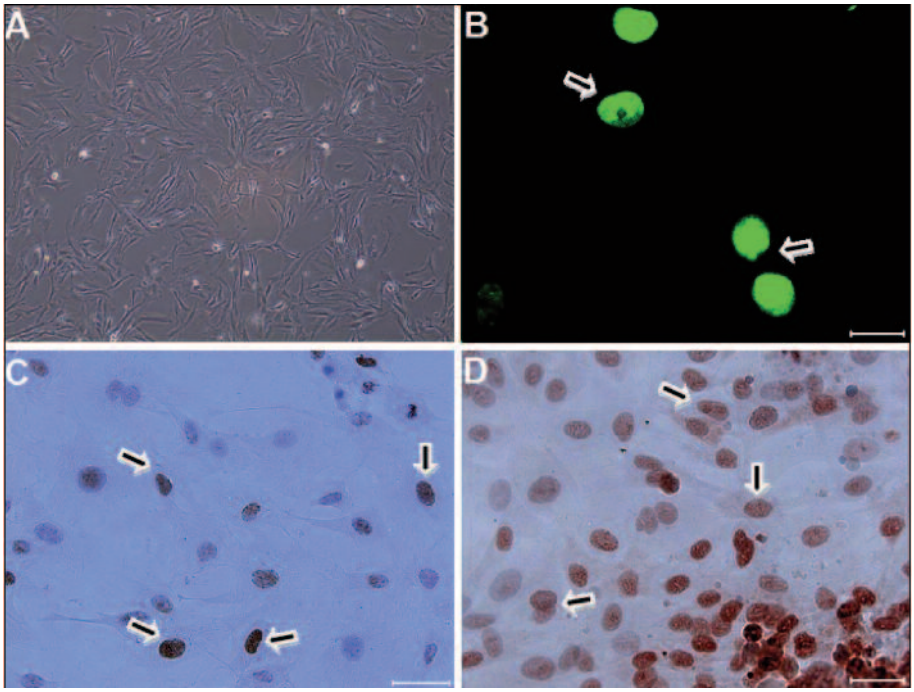

Figure 1. ***A,*** rBM-MSCs (P5) exhibited large, flattened or fibroblast-like morphology (Original magnification: A-X100). ***B-D,*** Representative immunostaining fields are shown for BrdU by immunofluoresence **B,** and immunocytochemical ***C***, techniques. The images shows nuclear localization of BrdU in labelled cells (arrows). In the section ***D,*** rBM-MSCs were positive for PCNA (arrows). (Scale bars: B, C, D, 50 μm).

Flow cytometry identification of BM-MSCs

The general strategy for identifying *in vitro* cultivated BM-MSCs was to analyze the expressions of cell surface markers such as CD29, CD44, CD54, CD90 and CD106[13,14]. In our FACS (Fluorescent activated cell sorting) experiments, BM-MSCs were negative for CD45, a cell surface marker associated with lymphohematopoietic cells (Figure 2). Therefore, there was no evidence of hematopoietic precursors in the cultures. In contrast, the expression of other surface antigens

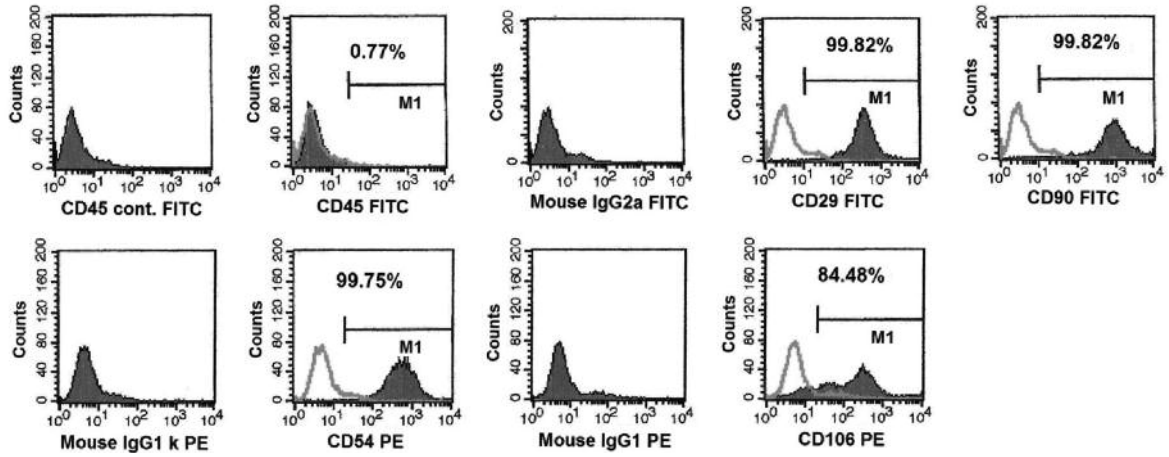

Figure 2. Immunophenotypic properties of rBM-MSCs by flow cytometry. Pre-defined markers that specify MSCs used to define the characteristics of cultured cells. rBMMSCs expressed all mesenchymal stem cell markers including CD29, CD54, CD90 and CD106; but not CD45.

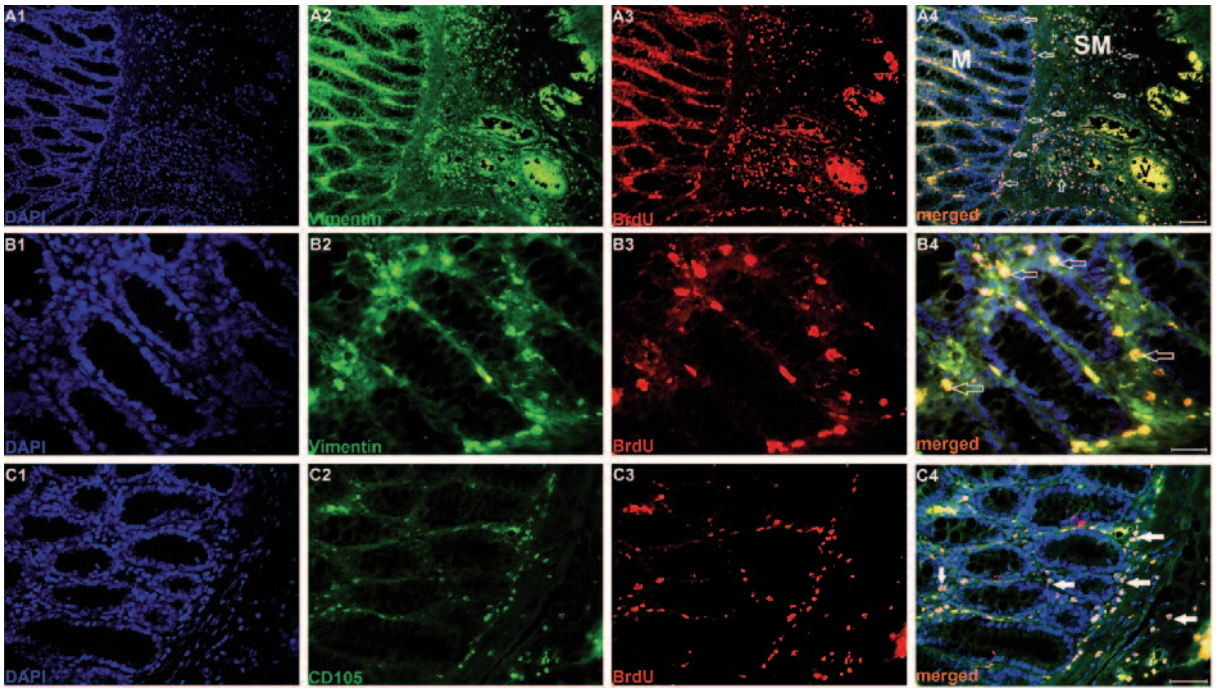

Figure 3. The fluorescence views of paraffin sections from different segments of BM-MSCs transplanted stem cells on 4th day have performed double stainings for BrdU/Vimentin (A1-A4; B1-B4) and BrdU/CD105 (C1-C4). BrdU+ cells (red) were observed in stromal tissues only (signed by arrows) (A3-4, B3-4 and C3-4). (M: Mucosa, SM: Submucosa, V: Vessel) (Scale Bars: A-70 μm; B and C-50 μm).

agreed with the previous reports regarding murine MSCs, and indicated that the cells used in our study had the characteristics of MSCs reported elsewhere[13,14].

Cells Tracing after Injection of the BrdU Labeled BM-MSCs

BMSCs labelled with BrdU were transplanted into the vena cava. Four and seven days later, BM-MSCs were observed in the colon tissues of the recipient rats (Fig. 3 and 4). To confirm this observation, the expression of vimentin (Figure 3 A1-4; B1-4; Figure 4 A1-4) and CD105 (Figure 3 C1-4; Figure 4 B1-4), which are also MSC markers, was shown. We also stained paraffin sections of the colon tissue samples for CD31 and BrdU. CD31, also known as Platelet endothelial cell adhesion molecule (PECAM-1), is a protein that is found on the surface of platelets, monocytes, neutrophils, and some types of T-cells, and makes up a large portion of endothelial cell intercellular junctions. MSCs are negative for CD31, and it was used as negative control. In all analyzed samples, $BrdU^+$ transplanted cells were negative for CD31. In addition, double immuno stainings for PCNA and nestin was performed to determine whether the PCNA immunoreactive cells were mesenchymal stem cells. PCNA is a marker for cells in early G1 phase and S phase of the cell cycle. Nestin is also a stem cell marker. By assaying for the PCNA and nestin expressions, we identified transplanted MSCs (Figure 5 A-D).

Bursting Pressures

The mean bursting pressures of ischemic colonic anastomoses in group 3 were higher than in control group 1 (4^{th} day) ($p < 0.01$) (Table I). Comparison of bursting pressures by the 7^{th} day in group 4 were not significantly different from control group 2 ($p > 0.05$) (Table I).

Hydroxyproline Contents

We found significantly higher hydroxyproline values in group 3 than in control group 1. On the 7^{th} day, the values of hydroxyproline were significantly higher in group 4 than in control group 2.

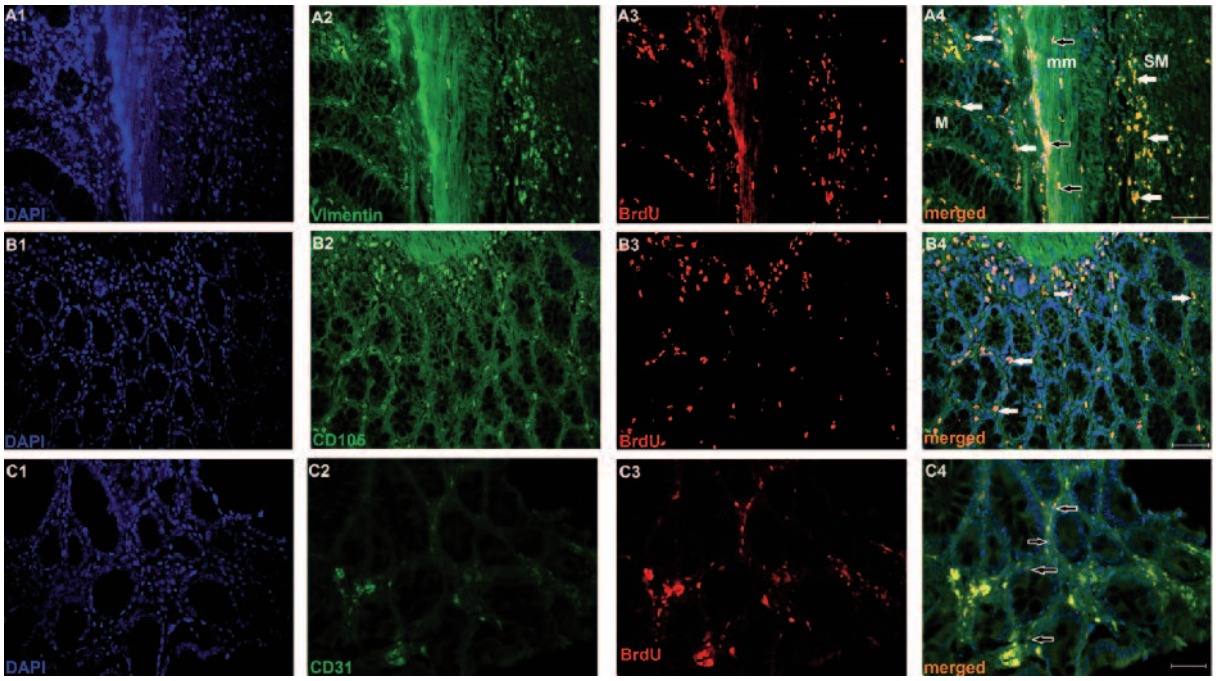

Figure 4. Representative panels of immunofluorescence detection of some markers on different segments of BM-MSCs transplanted stem cells on 7th day have performed double stainings for BrdU/Vimentin (red/green) (A1-A4), BrdU/CD105 (red/green) (B1-B4), and BrdU/CD31 (red/green) (C1-C4). In A4, BrdU+ cells were observed in mucosa (M) (white arrows), muscularis mucosa (mm) (black arrows) and submucosa (SM) (white arrows). In C4, no BrdU/CD31 double positive cells were detected in mucosa of the colon tissue. We could determined only BrdU+ cells (black arrows) in this segment. CD31 also known as Platelet endothelial cell adhesion molecule (PECAM-1) is a protein that is found on the surface of platelets, monocytes, neutrophils, and some types of T-cells, and makes up a large portion of endothelial cell intercellular junctions. As it observed in here, transplanted cells were negative for CD31. In contrast, they were positive in both vimentin and CD105 which are marker for MSCs.

Hydroxyproline levels were increased upon transplanted stem cell therapy in group 3 and in group 4. Hydroxyproline values for all groups are given in Table I.

At necropsy, all of the anastomosis were severed with either omentum or small bowel loops and lacked evidence of leakage or peritonitis. There was no mortality all of the groups. We investigated the early period of wound healing (4^{th} day and 7^{th} day). When comparing between group 1 and group 3, we found higher levels for all of the histological parameters except inflammation in group 3 (4^{th} day) compared to the control group 1. On day 7, when comparing between group 2 and group 4, we found higher levels for parameters of necrosis, collagen deposition.

Discussion

Failure of intestinal healing results in dehiscence, leaks, and fistulas, which carry significant morbidity and mortality. Many factors affect anastomotic healing. The most important factor is the perfusion and oxygenation of the site of anastomosis. Adequate tissue oxygenation is required for normal oxidative function of neutrophils, leukocyte activation, fibroblast production, angiogenesis, and reepithelialization, which are all essential in wound healing[9]. Many studies are related to anastomosis healing[15,16]. Many substances in the literature have been investigated for their ability to eliminate tissue ischemia. Stem cell research has received much attention in recent years, and has shown positive effects on the gastrointestinal system. Indeed, pre-clinical data of animal models and clinical studies revealed positive effects related to transplanted BM-MSCs[8,17]. BM-MSCs potentially represent a novel treatment modality for the repair and regeneration of injured intestinal tissues. In preliminary laboratory and clinical investigations, autologous or allogeneic MSCs either implanted directly into injured tissue or delivered systemical-

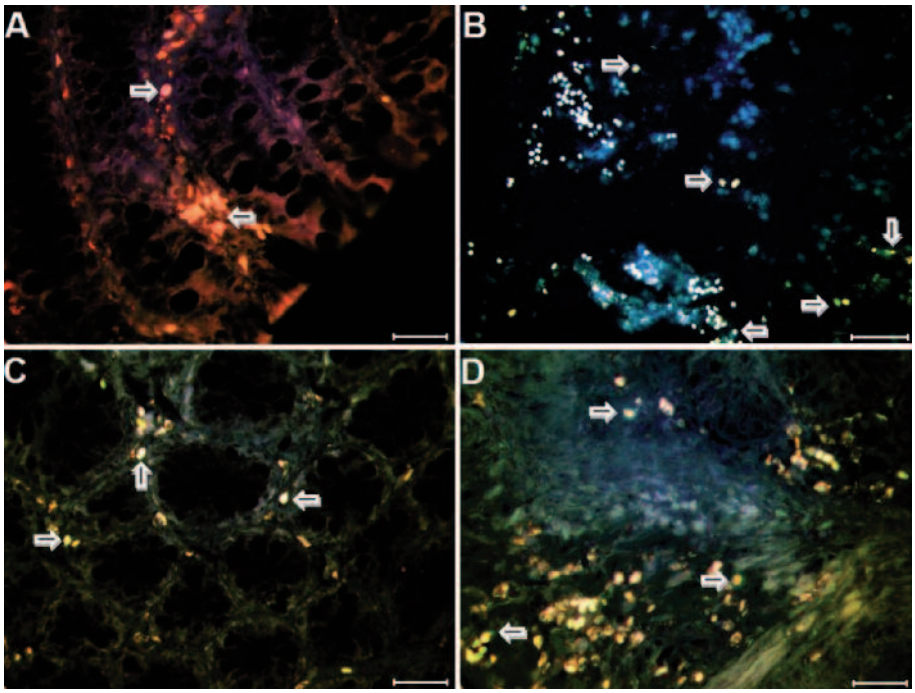

Figure 5. Paraffin sections of the colon tissues from experimental groups (A and C = Mucosa, B and D = Submucosa) were stained for PCNA (red) and nestin (green) and observed by fluorescence microscopy. PCNA is a marker for cells in early G1 phase and S phase of cell cycle. It is used as a proliferation marker, and its staining is localized in nuclei. In this study, we used PCNA and Nestin immunostaining in tissue sections to detect injected BM-MSCs, as supportive method to BrdU. The cells positive for both PCNA and nestin were evaluated as transplanted BM-MSCs. The cells with PCNA positive staining (red) were also stained for nestin (cytoplasmic; green) in colonic sections (arrows) of both transplantation groups (early ischemic groups A, B; late ischemic groups C, D) (Scale Bars: 50 μm).

Table I. Bursting pressures and hydroxyproline contents in groups. p1-3 value upon comparison between group 1 and group 3, p2-4 value upon comparison between group 2 and group 4. NS: non significiant.

Bursting pressure (mmHg)						
	Group 1 (control-4th day)	**Group 3 (BM-MSCs transplanted-4th day)**	**p^{1-3}**	**Group 2 (Control-7th day)**	**Group 4 (BM-MSCs transplanted-7th day)**	**p^{2-4}**
Mean	48.5 (25-95)	69 (46-100)	$p < 0.01$	115 (90-150)	140 (100-180)	NS
Hydroxyproline contents (µg/mg)						
Mean	0.44 (0.38-0.48)	0.77 (0.73-0.80)	$p < 0.001$	0.53 (0.45-0.60)	0.90 (0.84-0.94)	$p < 0.01$

ly via intra-arterial or intravenous infusions have resulted in significant benefits for many types of injured and diseased tissues[8,18].

In the literature, there have been few studies addressing about transplanted systemic BM-MSCs therapy for healing of ischemic colonic anastomosis. This study showed that BM-MSCs engrafted safely and successfully after systemic injection in ischemic colonic anastomoses. The labeled BM-MSCs were found within the anastomotic site. Moreover, the study show that systemic transplanted stem cell therapy significantly accelerated some parameters of the healing process for ischemic colonic anastomosis. Since the importance of the healing process was demonstrated, many studies investigated how to assess

Table II. Histological features characterizing healing of ischemic anastomosis, (all of the parameters were scored from 0 to 3). p1-3 value upon comparison between group 1 and group 3, p2-4 value upon comparison between in group 2 and group 4. NS: non significiant. SCs: Stem cells.

	Group 1 (control-4th day)	Group 3 (SCs-4th day)	p^{1-3}	Group 2 (Control-7th day)	Group 4 (SCs-7th day)	p^{2-4}
Necrosis	2.8	2	$p < 0.05$	2.4	1.4	$p < 0.05$
Epithelialization	0.6	1.5	$p < 0.05$	1.5	1.8	NS
Collagen deposition	0.7	2	$p < 0.01$	1.3	2.1	$p < 0.05$
Fibroblasts activity	1	2.2	$p < 0.01$	2.1	2.4	NS
Inflammation	2.1	2.4	NS	2.7	2.4	NS
Angiogenesis	1.4	2.4	$p < 0.01$	2.1	2.5	NS

it. Bursting pressure is the most used method to assess anastomic healing[19]. Bursting pressures have been shown to remain low for the first 3-4 days[20,21]. It depends on the collagen density. The lowest level of bursting pressure (48.5 mmHg) was found in group 1 when compared to the other groups on the 4th postoperative day. The bursting pressure was approximately 50% greater in the group of transplanted BM-MSCS (69 mmHg) than in the controls and this difference was statistically significant. In addition, bursting pressure was found statistically different in the group of transplanted BM-MSCS at postoperative day 7. When comparing all groups with each other, the highest explosion pressure was found in group 4 (140 mmHg). Bursting pressure and hydroxyproline levels showed that transplanted stem cells augmented the healing process in ischemic colonic anastomoses in group 3 although in bursting pressure was higher in group 4 than control group, there was no significance. In conjunction with, comparing collagen formation and fibroblastic activity were similar to these results.

Mesenchymal stem cells (MSCs) have demonstrated a great capacity to repair and regenerate injured tissues both through transdifferentiation to tissue-specific cell types[22]. In that reason, they can be therapeutically delivered via systemic infusion and appear capable of homing to and engraftment within sites of injury and inflammation[23,24]. Some reports have examined the use of BM-MSCs for the treatment of inflammatory bowel disease (IBD). Transplanted cells have been reported to improve microcirculation of inflamed areas, which might be important for mucosal repair[25]. MSCs have been used to treath both IBD in humans and induced colitis in mice. Studies showed that these treatments may effectively treat Crohn's disease and ulcerative colitis and lead to profound remission with a median follow-up of 7 years[26]. The major mechanisms of MSC's contribution to wound repair process are thought to be: 1) structural repair of wounds via cellular differentiation; 2) immunomodulation; 3) production of growth factors; 4) mobilization of resident stem cell niche[27]. One of their primary contributions is the secretion of a large number of paracrine factors that are known to be critical to wound healing[28,29]. Furthermore, stem cells have been shown to produce growth factors such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), keratinocyte growth factor (KGF), epidermal growth factor (EGF), and are believed to protect ischemic tissues via the promotion of angiogenesis and the promotion of cellular proliferation[29]. After systemic administration of MSCs, they become entrapped within the lungs as in a homeostatic host, but 1-3 days postinjection MSCs can be found at the wound site. A small percentage of MSCs that home to the wound engraft within the newly formed tissue and can be found there at 2 weeks postinjection[28]. Angiogenesis is very important factor in the wound healing process[8,27]. In our study, necrosis in the BM-MSCS transplanted group was significantly different from the control group on postoperative days 4 and 7. In addition, when comparing rates of angiogenesis, group 3 and group 4 were numerically higher. Although differences in angiogenesis rates were statistically significant on the 4th postoperative day, these differences were not statistically significant on the 7th postoperative day in group 4. Stem cells support important wound healing events blood vessel formation by stimulating increased rate of growth factor production such as VEGF[27].

In clinics, stem cells have been used to treat refractory Crohn's disease in humans. Long term remissions have been reported[29]. Stem cells may also play a powerful role in treatment of short bowel syndrome, necrotizing enterocolitis[30]. Similarly, infusion of human BM-MSCs into irradiated mice accelerated recovery from radiation induced intestinal damage. These studies suggest potential therapeutic benefit for MSCs as enhancers of epitelial repair in a variety of gastrointestinal diseases[30]. Our work showed that epithelialization in the BM-MSCS transplanted groups was significantly enhancer than control groups. There were several criticisms in this work: (1) growth factors were not shown directly; (2) engraftment numbers or percentage of mesenchymal stem cells were not calculated in anastomotic area and were not compared different numbers of MSCs, (3) finally, we have not investigated any adverse effect of MSCs in a long time. Further studies of MSCs based treatments on ischemic colonic anastomoses should investigate effects of which delivery methods better and should determine adverse effects of MSCs in a long term.

Conclusions

BM-MSCs therapy significantly accelerated all of the healing parameters for ischemic colonic anastomosis except for inflammation on fourth day. On the seventh day, BM-MSCs augmented the levels of the hydroxyproline. Histological parameters, necrosis and collagen deposition were also found to be important for healing of ischemic colonic anastomoses. However, they did not accelerate the others histological parameters especially angiogenesis.

Disclosure of Interest

Authors declare no conflict of interest or financial disclosure for this manuscript.

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