The Possible Role of Mesenchymal Stem Cells Therapy in the Repair of Experimentally Induced Colitis in Male Albino Rats

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Background and Objectives: Colitis is inflammation of the colon which can be transmural or confined to the mucosa. Colitis may be acute or chronic. In case of serious intestinal discontinuity of epithelium, the regeneration capacity of local stem cells is not enough to complete tissue repair. Bone marrow mesenchymal stem cells (BM-MSCs) migrate into the gastrointestinal wall, where they may contribute to the repair progress. The present study aimed at evaluating the possible therapeutic effect of MSCs on induced colitis in albino rat.

Methods and Results: Twenty male albino rats were divided into 3 groups (control, colitis, MSCs), control group (4 rats), colitis group (8 rats) received once intra-rectal injection of 2 ml of 3% acetic acid. MSCs therapy group (8 rats) injected with MSCs 24 hours after colitis induction. In each group, rats were subdivided into subgroups (a & b). Subgroup (a) corresponds to rats sacrificed 3 days and subgroup (b) corresponds to rats sacrificed 10 days after colitis induction. Isolation and culture of MSCs from rat bone marrow were performed. Colon sections were examined using light and fluorescent microscopy. Colon specimens were subjected to histological, morphometric and statistical studies. In colitis group, ulceration, loss of surface columnar epithelium, disturbed crypts architecture with few goblet cells and huge lymphatic nodule piercing the muscularis mucosa were reported. In stem cell therapy group, MSCs stimulate colonic repair through differentiation into several cells and dampen the inflammation.

Conclusions: MSCs represent future therapeutic hopes for intestinal injury and chronic intestinal inflammatory states.

Keywords: Colitis, Acetic acid, MSCs, PKH26, Rat colon

Introduction

Ulcerative colitis (UC) is a disorder involving the large intestine and is characterized by contiguous inflammation of the colonic lamina propria with subsequent injury and disruption of the mucosal barrier (1). Acetic-acid induced colitis is an easily inducible model of inflammatory bowel disease (IBD), and the similarity of the inflammatory me-

diators profile to IBD suggests that the inflammatory phase bears some resemblance to acute human intestinal inflammation (2). Several major causative factors in the initiation of human colitis such as enhanced vasopermeability, prolonged neutrophils infiltration and increased production of inflammatory mediators are involved in the induction of this animal acetic acid model (3).

The primary functions of the intestinal tract are digestion and absorption, but they also represent a barrier for the luminal pathogens. The intestinal lumen is lined by a simple columnar epithelium whose turnover is highly regulated by local stem cell activity (4). During the rapid regeneration process, intestinal stem cells provide daughter cells in the lower part of crypts. Daughter cells migrate upwards in the crypts to serve different functions and then undergo apoptosis. During their migration, they differ-

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entiate into absorptive or secretory (goblet, enteroendocrine) cells (5). In case of serious intestinal discontinuity of epithelium (i.e. ulcerative colitis or crohn's disease), the regeneration capacity of local stem cells is not enough to complete tissue repair. Many authors in this case suggested that BM-MSC migrate into the gastrointestinal wall, where they may contribute to the repair progress (6-8).

Treatment using mesenchymal stem cells (MSCs) is surfacing as a new method of treatment. MSCs are multipotent non-hematopoietic progenitor cells from adult bone marrow which can differentiate into various cells therefore; MSCs may be involved in tissue regeneration (9). In addition, it was stated that MSCs modulate allogeneic immune cell responses from a pro-inflammatory toward an anti-inflammatory environment by affecting dendritic cells, T lymphocytes and natural killer (NK) cells. The authors further proposed that MSCs also regulate the immune function in inflamed tissues by affecting pro-inflammatory cytokines and chemokine secretion (10).

The present work aimed at evaluating the possible therapeutic effect of MSCs on induced colitis in male albino rat.

Materials and Methods

Twenty male albino rats with average weight 250 grams were used and divided into 3 groups placed in separate cages in the Animal House of Kasr El Aini. The rats were treated in accordance with guidelines approved by the Animal Use Committee of Cairo University. Group I (control group): 4 rats that were subdivided into two subgroups (Ia & Ib), 2 rats for each subgroup and corresponding to the group II & III respectively. Subgroup Ia received intra-rectal injection of 2 ml saline (11) and subgroup Ib received 150 μ l of phosphate buffered saline (PBS) intravenously via tail vein (12), one rat from each subgroup were sacrificed at day 3 and the other at day 10 from the start of experiment. Group II (colitis): 8 rats, received once intra-rectal injection of 2 ml of 3% acetic acid (3). Colitis rats were subdivided into two subgroups IIa and IIb, four rats each that were sacrificed 3 and 10 days of colitis induction. Group III (MSCs) 8 rats, received once injection via tail vein of 5×10^5 cells/150 μ l in Dulbecco's Phosphate Buffered Saline (DPBS, GIBCO, NY, USA) 24 hours after colitis induction (13), rats were subdivided into two subgroups IIIa and IIIb, four rats each that were sacrificed 3 and 10 days of colitis induction.

Induction of colitis

Colitis was induced according to the method described by Millar et al. (14). Briefly, rats were slightly anaesthetized

with ether following a 24 hours fasting, and then 6 cm of soft pediatric catheter size 6F was lubricated with gel then inserted into the anus and the tip was advanced to 8 cm proximal to the anus verge. 2 ml of acetic acid (3% v/v in 0.9% saline) or saline alone (control animals) was instilled into the colon through the catheter; each rat remained suspended in a head down vertical position for one minute to prevent leakage of the intracolonic instillate (3).

Isolation of BM-derived MSCs from rats

Bone marrow was harvested by flushing the tibiae and femurs of 6 weeks old male white albino rats with Dulbecco's modified Eagle's medium (DMEM, GIBCO/BRL) supplemented with 10% fetal bovine medium (GIBCO/BRL). Nucleated cells were isolated with a density gradient [Ficoll/Paque (Pharmacia)] and resuspended in complete culture medium supplemented with 1% penicillin-streptomycin (GIBCO/BRL).

Cells were incubated at 37°C in 5% humidified CO₂ for 12-14 days as primary culture or upon formation of large colonies.

When large colonies developed (80~90% confluence), cultures were washed twice with phosphate buffer saline (PBS) and cells were trypsinized with 0.25% trypsin in 1 mM EDTA (GIBCO/BRL) for 5 minutes at 37°C. After centrifugation (at 2400 rpm for 20 minutes), cells were resuspended with serum-supplemented medium and incubated in 50 cm² culture flask Falcon). The resulting cultures were referred to as first-passage cultures (15).

Morphological identification of BM-derived MSCs

MSCs in culture were characterized by their adhesiveness and fusiform shape and by detection of CD29 one of surface markers of rat mesenchymal stem cell (16).

Labeling of stem cells with PKH26 dye

MSCs cells were harvested during the 4th passage and were labeled with PKH26 fluorescent linker dye. PKH26 is a red fluorochrome, has excitation (551 nm) and emission (567 nm). The linkers are physiologically stable and show little to no toxic side-effects on cell systems. Labeled cells retain both biological and proliferating activity, and are ideal for *in vitro* cell labeling, *in vitro* proliferation studies and long term, *in vivo* cell tracking (17).

Application of mesenchymal stem cells

Labeled MSCs (5.0×10^5) cells/150 μ l in Dulbecco's Phosphate Buffered Saline were loaded in a 1-ml sterile syringe and the contents of the syringe were injected once via tail vein 24 hours after colitis induction through a 26

G hypodermic needle. Animals in non-treated group were received PBS in the same manner (13).

Histological study

Rats were sacrificed by lethal dose of ether. A midline incision was done in each animal and the abdomen was opened. The colon was dissected and the distal 3 cm of the colon of each rat was excised. The Specimens were fixed in 10% buffered formalin solution for 24~48 hours, dehydrated in ascending grades of ethanol and embedded in paraffin. Serial sections of 5 μ m thickness were cut and subjected to the followings: Hematoxylin & Eosin (H&E) stain (18), Alcian Blue stain for demonstration of mucin secreting cells (19), Masson's Trichrome stain for demonstration of collagen fibers (20) and Fluorescent detection of labeled mesenchymal stem cells that were observed by fluorescent microscope in unstained sections (21).

Morphometric study

Using Leica Qwin 500 LTD image analysis, assessment of the area percent and optical density of mucin in alcian blue stained sections and area percent of collagen fibers in masson's trichrome stained sections were performed using image menu. The measurements were done in 10 high power fields (HPF) in control and experimental groups.

Statistical analysis

Quantitative data were summarized as means and standard deviations and compared using one-way analy-

sis-of-variance (ANOVA). p-values<0.05 was considered statistically significant. Calculations were made on SPSS software (22).

Results

Haematoxylin and eosin (H&E) stained sections

Control group of the present study recorded the same histological picture in subgroup Ia, Ib. It showed intact mucosal architecture of the colon. This intact mucosa exhibited regularly arranged, closely related crypts. The mucosa and the crypts were lined by columnar cells and goblet cells (Fig. 1). Colitis subgroup IIa revealed ulceration, loss of surface columnar epithelial lining and disturbed crypt architecture with few goblet cells. Huge lymphatic nodule piercing the muscularis mucosa and dilated congested blood capillary were clearly noted (Fig. 2). In MSCs subgroup IIIa, apparently normal surface columnar cells with adjacent area of stratification were demonstrated. Some mucosal crypts were apparently intact. Lymphatic nodule piercing the muscularis mucosa reaching the surface columnar cells was still recorded (Fig. 3).

Following 10 days of colitis, subgroup IIb still showed ulceration, loss of surface columnar epithelial lining and a huge lymphatic nodule piercing the muscularis mucosa (Fig. 4). Examination of H&E stained sections of MSCs subgroup IIIb revealed intact continuous surface columnar epithelium and intact regularly arranged closely related crypts that were lined by numerous goblet cells. Minimal

Fig. 1. Photomicrograph of a section in the colon of a male albino rat of control group (group I) showing intact mucosal architecture of the colon. The mucosa exhibits regularly arranged, closely related crypts (C). The mucosa and the crypts are lined by columnar cells (arrow head) and goblet cells (curved arrow) (H&E, $\times 200$).

Fig. 2. Photomicrograph of a section in the colon of a male albino rat of colitis subgroup IIa showing ulceration (spiral arrow), Loss of surface columnar epithelial lining, disturbed crypts (C) architecture with few goblet cells (curved arrow). Huge lymphatic nodule (LN) piercing the muscularis mucosa (MM) and dilated congested blood capillary (arrow) are clearly seen (H&E, $\times 200$).

Fig. 3. Photomicrograph of a section in the colon of a male albino rat treated with mesenchymal stem cells (subgroup IIIa) illustrating apparently normal surface columnar cells (arrow head) with adjacent area of stratification (right angle arrow). Some mucosal crypts (C) are apparently intact. Lymphatic nodule (LN) piercing the muscularis mucosa (MM) is still observed (H&E, $\times 200$).

Fig. 5. Photomicrograph of a section in the colon of a male albino rat treated with mesenchymal stem cells (subgroup IIIb) showing intact continuous surface columnar epithelium (arrow head). Intact regularly arranged closely related crypts (C) that exhibit numerous goblet cells (curved arrow) are clearly seen. Minimal inflammatory cell infiltration (star) is observed (H&E, $\times 200$).

Fig. 4. Photomicrograph of a section in the colon of a male albino rat of colitis subgroup IIb still illustrating ulceration (spiral arrow), loss of surface columnar epithelial lining and huge lymphatic nodule (LN) piercing the muscularis mucosa (MM) (H&E, $\times 200$).

inflammatory cell infiltration was observed (Fig. 5).

Alcian blue stained sections

Subgroups Ia & Ib recorded the same histological picture, it showed alcian blue +ve reaction of the mucin secreting cells lining the crypts. Colitis subgroup IIa and MSCs subgroup IIIa showed apparently few mucin secreting cells with reduction in the intensity of alcian blue +ve mucin (Fig. 6). In colitis subgroup IIb, apparently few mucin secreting cells with reduction in the intensity of al-

cian blue +ve mucin were still observed. However, MSCs subgroup IIIb exhibited numerous mucin secreting cells as well as increase in the intensity of alcian blue +ve mucin (Fig. 7).

Masson trichrome stained sections

Control subgroups Ia & Ib showed the same histological picture in the form of fine collagen fibers in the lamina propria between the crypts. Colitis subgroup IIa and MSCs subgroup IIIa demonstrated very fine collagen fibers in the lamina propria between the crypts (Fig. 8). Colitis subgroup IIb illustrated dense irregularly arranged collagen fibers heavily distributed in between mucosal crypts. On the other hand, MSCs subgroup IIIb showed very fine collagen fibers in between the crypts (Fig. 9).

Fluorescent detection of PKH26 labeled mesenchymal stem cells by fluorescent microscope in unstained colonic sections

MSCs subgroup IIIa showed red fluorescence of MSCs that were heavily distributed at the ulcerated mucosa. Moreover, MSCs labeled with PKH26 lied around the base of the crypts, within and around blood vessels in the submucosa. However, in MSCs subgroup IIIb, regenerated surface epithelium and few MSCs labeled with PKH26 around the regenerated crypts were noted (Fig. 10).

Morphometric results

Statistical analysis by ANOVA test demonstrated sig-

Fig. 6. Photomicrograph of alcian blue stained sections of rat colonic tissues at 3 days of control group (I), colitis subgroup (IIa) and MSCs subgroup (IIIa). (I) demonstrates +ve alcian blue reaction of the mucin secreting cells (curved arrows). (IIa) and (IIIa) illustrate apparently few mucin secreting cells with reduction in the intensity of alcian blue +ve mucin (Alcian Blue reaction. $\times 200$).

Fig. 7. Photomicrograph of alcian blue stained sections of rat colonic tissues at 10 days of colitis subgroup (IIb) and MSCs subgroup (IIIb). (IIb) shows apparently few mucin secreting cells with reduction in the intensity of alcian blue +ve mucin (curved arrows). (IIIb) illustrates numerous mucin secreting cells as well as increase in the intensity of alcian blue +ve mucin (Alcian Blue reaction. $\times 200$).

nificant decrease ($p < 0.05$) in the mean of area % and density of mucin in both colitis subgroups IIa and IIb when compared with the control group. However, MSCs

subgroup IIIb indicated a significant increase in the mean area % and optical density of mucin in alcian blue stained sections following ten days of MSCs treatment compared

Fig. 8. Photomicrograph of masson stained sections of rat colonic tissues at 3 days of control group (I), colitis subgroup (IIa) and MSCs subgroup (IIIa). (I) shows fine collagen fibers (yellow arrows) in the lamina propria between the crypts (C). (IIa) and (IIIa) demonstrate very fine collagen fibers (yellow arrows) in the lamina propria between the crypts (C) (Masson's trichrome. $\times 200$).

Fig. 9. Photomicrograph of masson stained sections of rat colonic tissues at 10 days of colitis subgroup (IIb) and MSCs subgroup (IIIb). (IIb) illustrates dense irregularly arranged collagen fibers (yellow arrows) heavily distributed in between mucosal crypts (C). (IIIb) shows very fine collagen fibers (yellow arrows) in between the crypts (C) (Masson's trichrome. $\times 200$).

Fig. 10. Fluorescence micrograph of unstained section of rat colonic tissue of MSCs subgroup IIIa (at 3 days) and subgroup IIIb (at 10 days). (IIIa) demonstrates red fluorescence of MSCs labeled with PKH26 (arrows) that are heavily distributed at the ulcerated mucosa. Red fluorescence of MSCs at the base of the crypts (C) was also recorded. Note the MSCs within and around the blood vessel in the submucosa (SM). (IIIb) illustrates regenerated surface epithelium (arrow head) and crypts (C). Few MSCs labeled with PKH26 (arrows) around the crypts are clearly seen (PKH26 labeled MSCs immunofluorescence. $\times 200$).

Table 1. The mean $\pm$ standard deviation (SD) of area% and optical density of mucin of control and experimental groups

Group	Mean $\pm$ SD of area% of mucin	Mean $\pm$ SD of optical density of mucin
Control group	24.13 $\pm$ 4.94	0.819 $\pm$ 0.025
Colitis subgroup IIa (3 days)	6.47 $\pm$ 3.239 ^a	0.608 $\pm$ 0.039 ^a
MSCs subgroup IIIa	7.76 $\pm$ 1.648	0.66 $\pm$ 0.085
Colitis subgroup IIb (10 days)	6.99 $\pm$ 3.49 ^a	0.647 $\pm$ 0.065 ^a
MSCs subgroup IIIb	21.97 $\pm$ 5.21 ^b	0.808 $\pm$ 0.031 ^b

^aSignificant ($p < 0.05$) versus group I.

^bSignificant ($p < 0.05$) versus subgroup IIb.

to colitis subgroup IIb. Thus MSCs differentiation was promoting mucin secreting cells formation (Table 1). Morphometric results of colitis subgroup IIa revealed significant decrease ($p < 0.05$) in mean area % of collagen versus the control group. However, colitis subgroup IIb showed significant increase in mean area % of collagen versus the control group. On the contrary, significant decrease of mean area % of collagen in MSCs therapy sub-

group IIIb versus colitis subgroup IIb was recorded. Thus MSCs had the ability to limit the fibrosis (Table 2).

Discussion

The current study demonstrated modulating effect of mesenchymal stem cells therapy on induced colitis in albino rats. This was evidenced by histological and morpho-

Table 2. The mean $\pm$ standard deviation (SD) of area% of collagen of control and experimental groups

Group	Mean $\pm$ SD of area% of collagen
Control group	11.448 $\pm$ 4.146
Colitis subgroup IIa (3 days)	7.357 $\pm$ 3.99 ^a
MSCs subgroup IIIa	7.48 $\pm$ 2.15
Colitis subgroup IIb (10 days)	19.612 $\pm$ 2.05 ^a
MSCs subgroup IIIb	10.02 $\pm$ 1.85 ^b

^aSignificant ($p < 0.05$) versus group I.^bSignificant ($p < 0.05$) versus subgroup IIb.

metric studies.

Control group showed intact mucosal architecture of the colon. This intact mucosa exhibited regularly arranged, closely related crypts. The mucosa and the crypts were lined by columnar cells and goblet cells. Similar results were described by Soliman et al. (23)

In the colitis subgroup IIa (at 3 days), ulceration, loss of surface columnar epithelial lining, disturbed crypts architecture with few goblet cells and huge lymphatic nodule piercing the muscularis mucosa and dilated congested blood capillary were clearly seen. This could be referred to the effect of the aggressor agent (acetic acid) inducing colonic injury resulting in epithelial mucosal damage and subsequent inflammation. The inflammation caused by acetic acid attributed to entry of soluble protonated form of the acid into the epithelium leading to massive intracellular acidification resulting in massive epithelial damage (24). Inflammation is a protective response intended to eliminate the initial cause of cell injury as well as the necrotic cells and tissues resulting from the original insult. Inflammation is accompanied by certain changes as cellular events, emigration of the leukocytes from the microcirculation and accumulation in the focus of injury and vascular changes, vasodilation and increased vascular permeability. Furthermore, ulceration can occur when tissue necrosis and resultant inflammation exist on or near a surface (25).

However, after 3 days from mesenchymal stem therapy (subgroup IIIa), lymphatic nodule piercing the muscularis mucosa reaching the surface columnar cells was still observed, longer time might be needed for MSCs to compensate for the mucosal injury and exert their anti-inflammatory effect. However, the mucosa demonstrated apparently normal surface columnar cells with adjacent area of stratification, an attempting of MSCs to compensate for mucosal injury. This could also be explained by the effect of MSCs therapy even as early as 3 days after dextran sulphate sodium (DSS) cessation leading to colonic epithelial

cell hyperplasia reflecting an intense driving force of epithelial cell proliferation (26). Hyperplasia is an increase in cellular number by stimulating resting (G0) cells to enter the cell cycle (G1) to start multiplication as a response to injury (27). Additionally, it was suggested that in an earlier period, (i.e., on day 3 after the colitis induction) engrafted MSCs also differentiated into colonic interstitial lineage cells and produced vascular endothelial growth factor (VEGF) and transforming growth factor beta-1 (TGF- β 1). Therefore, the authors suggested that MSCs may contribute, at least in a part, to the healing of injured colonic mucosa by secreting TGF- β 1 and VEGF (28).

In the present study ulceration, loss of surface columnar epithelial lining and huge lymphatic nodule piercing the muscularis mucosa were still recorded in colitis subgroup IIb (at 10 days). This might be beyond the ability of endogenous stem cells to compensate for the mucosal injury and subsequent inflammation induced by acetic acid. It was suggested that this period of time failed to induce complete improvement of the colonic architecture, as the inflammatory process together with ongoing attempts of regeneration resulted in progressive crypt distortion (29). On the other hand, MSCs therapy subgroup IIIb (at 10 days) of the current study revealed intact continuous surface columnar epithelium and intact regularly arranged closely related crypts that were lined by numerous goblet cells. This could be explained by the capacity of MSCs in tissue repair through differentiation to different types of cells compensating the injured cells. Coinciding with the previous explanation, MSCs accumulate in inflamed regions of the colon and contribute to tissue repair by a differentiation into vascular smooth muscle cells, endothelial cells, pericytes or epithelial cells (30). Moreover, it was proposed that recovery of colitis was significantly promoted by MSCs transplantation due to proceeding cell cycle and inhibiting apoptosis in the epithelia. So MSC is considered an active and beneficial cellular participant in intestinal organ repair by direct stromal cell differentiation (26).

Additionally, subgroup IIIb (MSCs therapy) of the current study demonstrated minimal inflammatory cell infiltration. This could be referred to the anti-inflammatory effect of MSCs. In accordance, as a result of systemic injection of bone marrow-derived MSCs the regeneration became faster, the inflamed area was reduced, the local inflammatory cell number and the crypt damage were decreased in DSS-induced colitis rats although some mild immune cells infiltration was observed (7, 8, 31). Moreover, it was recorded that MSCs are working more effectively in the later part of the inflammation process

and not in the earlier part. This was demonstrated by a significant decrease in alteration, erosion and in the inflammatory process in the rat mesenchymal stem cell (rMSC) transplanted rats on day 10 proving the anti-inflammatory effect of MSCs (13, 26).

Also MSCs possess profound immunosuppressive and anti-inflammatory effects via inhibiting the proliferation and function of several major types of immune cells such as NK cells, dendritic cells and T, B lymphocytes (10). MSC immune-suppressive activity is localized at the sites of inflammation and regulated by cells and factors present in local microenvironment, indicating that greater MSCs homing and infusion close to inflamed sites may enhance therapeutic results (32). Previous studies showed marrow cells differentiate into colonic subepithelial myofibroblasts (SEMFs) and produce pro-inflammatory cytokines and might cause inflammation to flare up (33). In contrast, it was documented that exogenously administered MSCs themselves might have an anti-inflammatory effect before differentiation into various types of cells. Thus the properties of exogenous MSCs were different from those of marrow-derived cells in response to colonic inflammation occurred (7).

In the present study, alcian blue stained sections of group I exhibited alcian blue +ve reaction of the mucin secreting cells. In agreement with the previous finding, the acidic mucins were found to be an indicator of adequate colonic epithelial secretory function. They are mainly composed of sulfomucins and sialomucins which are branched chains of glycoproteins with sialic acid terminals (23).

Subgroup IIa alcian blue stained sections showed apparently few mucin secreting cells with reduction in the intensity of alcian blue +ve mucin which was confirmed morphometrically. This was postulated to the effect of acetic acid as injurious agent destructing the epithelial lining of the mucosa including the mucin secreting cells. This coincided with Soliman et al. (23) who recorded that the mean number of goblet cell containing mucins was significantly decreased in acetic acid induced colitis as compared to the control. Mucin depletion was often associated with IBD-like disease and might limit the effectiveness of the mucous barrier to bacterial infection to the colon mucosa which together with increased colonic permeability might allow bacterial translocation to the colon wall (34). In subgroup IIIa alcian stained sections, apparently few alcian blue +ve mucin secreting cells were observed. Longer time might be needed for stem cell therapy to exhibit its regenerative effect in the replacement of lost mucin cells. In the present study, subgroup IIb still revealed

apparently few mucin secreting cells with reduction in the intensity of alcian blue +ve mucin. This was proved morphometrically. Longer time might be needed for endogenous stem cells to replace the lost mucin secreting cells.

On the other hand, subgroup IIIb recorded numerous mucin secreting cells as well as increase in the intensity of alcian blue +ve mucin. This was confirmed statistically by the significant increase in mean area % and density of mucin in comparison to subgroup IIb. This could be explained by the differentiation of MSCs into mucin secreting cells. Coinciding with the previous finding, it was found that in alcian blue staining sections, the goblet cells were decreased in colonic tissues without MSCs transplantation, but were excessively recovered with transplantation (26). The previous authors concluded that repopulating MSCs could effectively enhance the global regenerative responses, such as promoted proliferation, suppressed apoptosis and corrected secretory cell differentiation in damaged crypts.

Concerning the present study, masson stained sections of group I revealed very fine collagen fibers in the lamina propria between the crypts. Subgroup IIa showed very fine collagen fibers in between the crypts concomitantly with significant decrease in mean area % of collagen versus the control group. This might be due to injurious effect of acetic acid damaging the collagen fibers. Subgroup IIIa revealed very fine collagen fibers in the lamina propria between the crypts. Longer time might be needed for stem cell therapy to compensate for regeneration of connective tissues. MSCs contribute to the maintenance and regeneration of connective tissues through engraftment. Indeed, they have the capacity to engraft into several organs and to differentiate into wound healing myofibroblasts (35).

Based upon Hx. and E, alcian blue and masson results of the current study at 3 days of subgroup IIIa, very minimal improvement was recorded, as longer time might be needed for MSCs to complete their regenerative process.

Masson stained sections of subgroup IIb illustrated dense irregularly arranged collagen fibers heavily distributed in between mucosal crypts. This was confirmed morphometrically by significant increase in mean area % of collagen when compared with control. This could be explained by excessive connective tissue deposition as a consequence of the repair process of the injured tissue. In accordance, there was significant increase of the mean area % of collagen fibers after UC induction by acetic acid as compared to the control group (23). In agreement with the previous finding collagen fibres were clearly disorganized in the injured colon, being diffusely distributed through-

out the colon wall (36). When tissue injury is severe and leading to either damage of parenchymal cells, epithelia as well as the stromal framework or if nondividing cells are injured, repair cannot be accomplished by regeneration alone. Under these conditions, repair occurs by replacement of the non-regenerated cells with connective tissue or by a combination of regeneration of some cells and scar formation. Repair begins within 24 hours of injury by the emigration of fibroblasts and the induction of fibroblast and endothelial cell proliferation (25). Fibrosis is typically associated with mesenchymal cell hyperplasia, tissue disorganization and fibrillar collagen deposition. The transient appearance of activated myofibroblasts is a feature of normal wound healing that disappears by apoptosis at the end of the normal repair process, but the persistence of these cells is associated with excessive collagen deposition and fibrosis (33, 35).

In subgroup IIb very fine collagen fibers in between the crypts were recorded. As evidenced by significantly decrease in mean area % of collagen versus the colitis subgroup IIb. This might be related to the antifibrotic effect of MSCs. It was found that injection of MSCs was able to significantly decrease collagen deposition within the colon, at levels comparable to the control group (36). It was added that basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) had been shown to be involved in the prevention of fibrosis. In that study, human and murine BM-derived MSCs and human adipose-derived stem cells were found to express HGF in response to stimulation with bFGF. In a situation of tissue injury, perivascular cells become proliferative and secrete HGF, which in turn mediates anti-fibrotic and immunomodulatory effects (37). Additionally, it was possible that mesenchymal stem cells newly arrived and then integrated into the lamina propria might be less profibrogenic than those mesenchymal cells present during chronic inflammation. While the exact mechanism was unclear, preliminary studies using animal models of hepatic fibrosis support the conclusion that stem cell therapy may be useful antifibrotic treatment (38).

Based upon Hx. and E, alcian blue and masson results of the current study at 10 days and upon explanations of other investigators, MSC could be highlighted as a future therapy of colitis as they are considered as inducers of colon regeneration.

In the present study, immunofluorescence PKH26 stain of subgroup IIIa showed red fluorescence of MSCs that were heavily distributed at the ulcerated mucosa this could be due to homing of MSCs at site of colonic injury. Moreover, MSCs labeled with PKH26 lied around the base

of the crypts, within and around blood vessels in the submucosa. The existence near blood vessels correlates with the route of administration which was intravenous. In accordance, bone marrow transplanted cells engraft with a high efficiency in the areas of mucosal ulceration undergoing regeneration (39). Moreover, systemically delivered MSCs accumulate in injured tissues and localized in the lamina propria, particularly near the bottom of the crypt (7). BM- MSCs migrate to the nearby crypts as mediated by several chemoattractant proteins, which are expressed in injured tissue. The number of BM-MSCs is influenced by the degree of inflammation (31). Another study recorded that infused stem cells observed by GFP+ (green fluorescent protein) fluorescence were confined to the mucosa and submucosa of the inflamed colon and in the close vicinity of newly formed blood vessels, suggesting a contribution to vasculogenesis (40).

Immunofluorescence PKH26 stain of subgroup IIb demonstrated few MSCs labeled with PKH26 around the regenerated crypts. This might be explained by ability of MSCs to differentiate into multiple cell type thereby decreasing the number of MSCs. It was suggested that bone marrow-derived cells are temporally and efficiently recruited into gastrointestinal tissue during the healing process of chemical injury (30). The involvement of the bone marrow-derived cells into gastrointestinal tissue reduces after the damage was completely healed, suggesting that the bone marrow derived cells participate positively in wound healing (28).

Based upon immunofluorescence PKH26 labeled MSCs results of the current study and upon explanations of other investigators, MSCs were numerous three days after MSCs injection for promoting the healing process and then disappeared late at ten days as healing was completed and no further homing of MSCs cells.

It could be concluded that MSCs were considered a cell-based therapy, because of the apparent positive effects of MSCs on the experimental colitis models which includes their ability to home and differentiate at sites of injury replacing lost stromal cells, epithelial and goblet cells as well as stimulating repair and dampen inflammation through harness the inflammatory cell infiltration. These were confirmed by morphometric results.

Potential conflict of interest

The authors have no conflicting financial interest.

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