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Original Article

Potential effect of bone marrow cells for treatment of experimentally induced acute ulcerative colitis in a rat model

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Abstract

Ulcerative colitis (UC) is one of the major inflammatory bowel diseases (IBD) showing an increasing prevalence worldwide. UC patients are exposed to significant alterations in their quality of life due to the remitting and relapsing symptoms of pain, increased defecation frequency, and anemia. Treatment of UC aims to induce and maintain remission, reduce the risk of complications, and improve quality of life. The introduction of biological therapies, such as the anti-tumor necrosis factor alpha, anti-integrin, and anti-interleukin monoclonal antibodies, has led to the identification of important therapeutic targets. However, the high cost of biological drugs, their patent expiration, the potential to develop a loss of response, and the potential for adverse reactions, limit their uses. Nowadays, an alternative therapy for many diseases depends on cell-based approaches, particularly with stem cells. This study is designed to determine the potential effects of bone marrow (BM) cells on healing of ulcerative lesions, and improvement of the oxidative stress, in an animal model of ulcerative colitis. Acute colitis was induced in rats by dextran sulfate sodium and acetic acid. Animals were then treated with BM cells isolated from donor rats. Animals were sacrificed 7 days after treatment and tissue and serum samples were analyzed for measuring levels of the interleukins IL-6, IL-10, and IL-17. Also Caspase-3 was assessed. The group treated with BM cells showed improved disease activity index and pathological features. Both IL-6 and IL-17 were decreased by treatment with BM cells and IL-10 was increased compared to the pathological control group. Caspase-3 levels decreased with BM cells treatment compared to the pathological group. Results of this work showed that BM cells could ameliorate UC in rats. This could pave the way for trials on higher animals before being tested on humans.

Keywords: ulcerative colitis, bone marrow, cell-based therapies

1. Introduction

Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's disease, is a chronic debilitating inflammatory condition for which existing effective and targeted treatments are largely limited due to significant systemic side effects. UC characteristically involves only the large bowel; it begins in the rectum and progressively extends to the proximal colon, and some patients with severe disease experience a tropism for the appendix (Muzammil *et al*., 2023). The natural course of UC

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includes periods of remission interspersed with periods of acute exacerbations or disease flares (Kayal & Shah, 2020). Conventional medication for IBD therapy comprises anti-inflammatory drugs (e.g. 5-aminosalicyclic acid and corticosteroids) and immunosuppressive agents (e.g. azathioprine, 6-mercaptopurine, methothexate, ciclosporin-A and tacrolimus) (Turbayne & Sparrow, 2022).

Despite the efficacy of these drugs, their use is restricted due to non-specific effects on the immune system, which result in short- and long-term debilitating side effects (Wiseman, 2016). Furthermore, anti-inflammatory drugs that are locally active with minimal systemic absorption (5-aminosalicylates) require frequent intake at high doses to exert a measurable clinical efficacy (Wang & DuBois, 2013).

Mesenchymal stem cells (MSCs) are multipotent progenitor stromal cells that self-renew and differentiate toward multiple mesenchymal cell lineages (Zaripova *et al*., 2023). MSCs from BM, umbilical cord and adipose tissues are of great interest in regenerative therapy of tissues damaged by various pathological conditions (Hoang *et al*., 2022). MSC function and therapeutic efficacy are regulated by the surrounding niche/microenvironment (Gilchrist *et al*., 2021). Studies on autoimmune diseases have highlighted the importance of cell-cell contact and paracrine secretion of MSCs (Ha *et al*., 2020).

In this work, we investigated the potential effects of BM cells on healing of ulcerative lesions in an animal model with ulcerative colitis.

2. Materials and Methods

2.1 Ethical approval

This study was approved by the Research Ethics Committee (REC) at Theodor Bilharz Research Institute (TBRI). All animal experiments were carried out under Institutional Ethical Committee rules for the care and use of experimental animals, which were authorized by TBRI's Animal Ethics Committee in Giza, Egypt (FWA 00010609).

2.1.1 Animal model for colitis

Forty female rats – aged two and a half months old – weighing 120-150 g were used. They were fed with normal diet and sterile water. Experimental acute colitis was induced in 20 rats by oral administration of 3.5% (wt/vol) freshly prepared dextran sulfate sodium (DSS) (~40 kDa) in sterile drinking water for 24 hours, in addition to per rectum single injection of 1 ml of 4% acetic acid (AA) once. After 24 hours one animal was sacrificed to assess the development of acute colitis using pathological examination (counted from the pathological control group).

2.1.2 Isolation of BM-derived cells

On the day of transplantation, BM was harvested by centrifugation of the tibiae and femurs of two rats (counted from normal control group). A 5 ml dose of ammonium-chloride-potassium (ACK) lysing buffer were added to the pellet for 10 min at 4°C with shaking. Cells were washed twice with phosphate buffered saline (PBS) by centrifugation at 1,800 rpm for 10 min. Cells were counted and tested for viability using trypan blue exclusion test.

2.1.3 Animal study groups

The animals were divided into four groups.

1. Healthy (normal) control group (Group I): 8 healthy rats, not subjected to DSS or AA, which were intravenously injected with 100 µl PBS alone into tail vein, at the same interval of cell transplantation in other groups.

2. Healthy (normal) BM cell treated group (Group II): 10 healthy rats intravenously injected with 1×10^6 BM cells in 100 µl PBS per animal into tail vein.

3. Pathological control group (Group III): 10 Rats subjected to ulcerative colitis induction by DSS and AA, which were intravenously injected with 100 µl PBS alone into tail vein at day 1 after colitis induction.

4. Pathological BM cell treated group (Group IV): 10 Rats subjected to ulcerative colitis induction by DSS and AA, which were intravenously injected with 1×10^6 BM whole cells into tail vein in 100 µl PBS at day 1 after colitis induction.

2.1.4 Sample collection and disease evaluation

On day 7 after treatment with BM cells, rats were anesthetized and sacrificed by neck dislocation, blood samples were collected left to clot at room temperature for 20 min, then centrifuged at 2000 rpm. Sera were collected and kept at -20°C till used. The entire colon was excised and washed in PBS and sent for pathological assessment.

2.1.5 Disease activity index

To examine the severity of colitis, feces consistency, occult blood in feces, rectal bleeding, and weight loss were evaluated on a 0 to 4 points scale and averaged for overall ulcerative colitis disease activity index (DAI) (Table 1) (Atta *et al*., 2019; Omar *et al*., 2022).

Table 1. Parameters of disease activity index (DAI) scoring (Alex *et al.*, 2009; Gonçalves *et al.*, 2013)

Score	Weight loss %	Feces consistency	Bleeding
0	0	Normal	Normal
1	1-5%	-	-
2	5-10%	Loose	Occult
3	10-15%	-	-
4	More than 20 %	Diarrhea	Gross+ mucus

2.1.6 Pathological assessment

1) Macroscopic

On the day of sacrifice the colon length was measured and ulcers were assessed (Atta *et al*., 2019).

2) Microscopic

Sections of the distal colon 1 cm long were cut out longitudinally and fixed in 10% formalin for at least 24 hours, and the fixed tissue was dried out in ascending concentrations

of ethanol, cleared in xylene, and then embedded in paraffin blocks. Distal colon paraffin sections of 4 μm thickness were prepared, stained by hematoxylin and eosin (H&E), and examined microscopically to address the degree of inflammation (Kellermann & Riis, 2021).

2.1.7 Immunological assessment

1) Assessment of the immunomodulatory effect of BM cells

This was performed by measuring interleukins (IL-6, IL-10, and IL-17) in serum samples by ELISA technique, using commercially available kits (SunLong, China).

3. Results

3.1 Disease activity index

Groups I and II (Healthy control and Healthy BM cell treated) showed no fecal bleeding or change in feces consistency. There was also no weight loss.

Group III (Pathological control) showed severe diarrhea with extensive fecal bleeding and decrease in body weight. Group IV (Pathological BM cell treated) showed normal fecal consistency with minimal rectal bleeding and no weight loss.

Parameters and DAI are illustrated in Table 2.

3.2 Pathological assessment

3.2.1 Macroscopic

The average lengths of colon of rats of Groups I and II (Healthy (normal) control and Healthy (normal) BM cell treated) were 15.45±2.33 cm, and 14.95±1.18 cm, respectively, with no significant difference. The mucosa of the 2 groups appeared normal with no edema, hyperemia or ulcers.

While group III (Pathological control) showed shortening of colon length to an average length of 13.5±0.34 cm with sever edema, hyperemia and longitudinal bleeding ulcers, on the other hand Group IV (Pathological BM cell treated) showed average length of 15.31±1.35 cm with mild edema, hyperemia and few longitudinal ulcers (Figure 1).

3.2.2 Microscopic

The microscopic pictures of H&E stained specimens of Groups I and II (healthy control and healthy BM cell treated) showed no difference. The two groups showed intact histology, no glandular destruction, cryptitis, crypto abscesses, lymphocytic infiltration or ulceration.

Histopathologic picture of colon specimens of Group III (pathological control) showed moderate glandular destruction, moderate cryptitis, mild crypto abscesses, moderate lymphocytic infiltration and extensive ulceration.

Table 2. DAI in different test groups

gp	Weight loss score	Feces score	Blood score	DAI
I (Healthy control group)	0±0	0±0	0±0	0±0
II (Healthy BM cells treated group)	0±0	0±0	0±0	0±0
III (Pathological control group)	4±2.69	4.6±0.52	4.4±0.52	4.3±0.34
IV (Pathological BM cells treated group)	2±4.52	0.5 ± 0.71	0.2±0.42	0.9±0.96

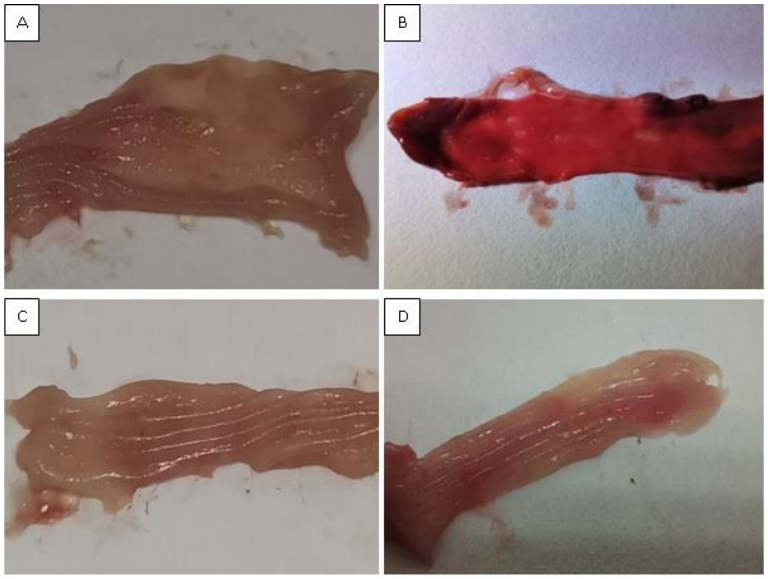


Figure 1. Macroscopic appearance of colon specimens from different groups: A) Group I Normal colonic mucosa, B) Group III Severe ulcerative colitis with severe hyperemia, edema and ulcerations, C) Group II normal appearing colonic mucosa with no hyperemia, edema or ulcerations, D) Group IV Colitis with mild to moderate activity with some hyperemic lesions, mild edema and few ulcers

Examination of Group IV (pathological BM cell treated) showed mild glandular destruction, mild cryptitis, absent crypto abscesses, mild lymphocytic infiltration and minimal ulceration (Figure 2).

3.3 Immunological Assessment

The immune parameters were measured in different groups and the results are expressed as mean± SD.

IL-10 levels were low in pathological group (III) when compared to healthy control group (I) with significance and returned to normal levels after treatment with BM cells (group IV) (P<0.005).

IL-6 was higher in pathological group (III) when compared to healthy control group (I) and treatment with BM cells (group IV) decreased its levels (P<0.005).

IL-17 was found to be significantly decreased after treatment with BM cells (group IV) in comparison to the pathological group (III) (P<0.005). Results of immune parameters are summarized in Figure 3.

3.4 Apoptotic marker

Caspase-3 in the pathological group (III) showed significantly high levels (4.39±.22 ng/ml) compared to groups I and II (Healthy control and Healthy BM cell treated) (3.78±0.50 ng/ml and 3.36±0.26 ng/ml respectively), while its levels were decreased significantly by treatment with BM cells in group (IV) (3.23±.45 ng/ml) (P<0.005).

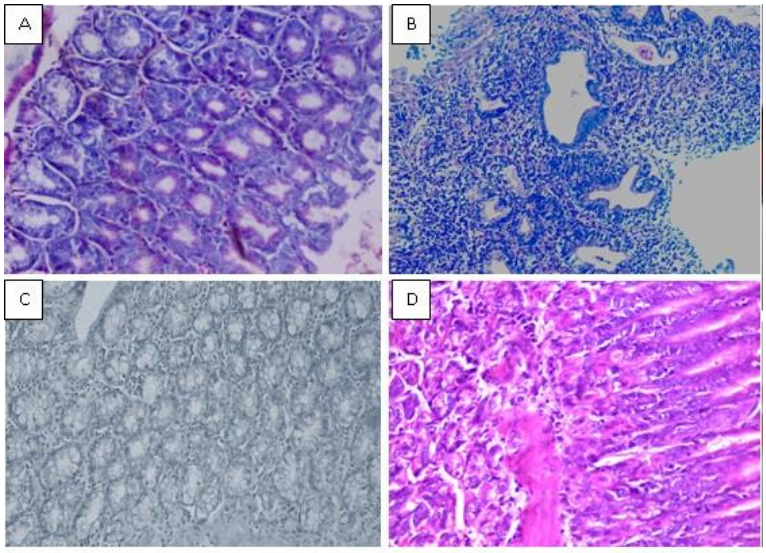
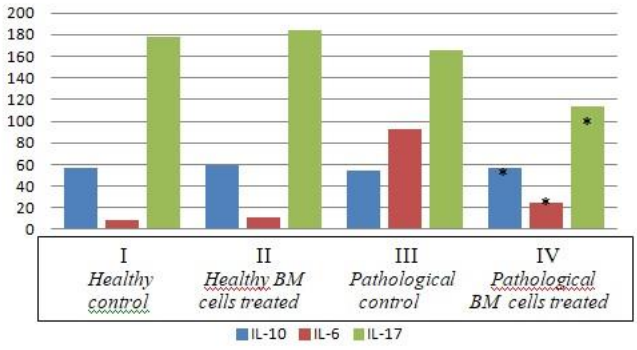


Figure 2. Microscopic appearance of colon specimens from different groups: A) Group I Normal colonic mucosa H&E x40, B) Group III Severe ulcerative colitis with crypt distortion, cryptitis, ulceration, crypt abscesses H&E x40, C) Group II Normal colonic mucosa H&E x40, D) Group IV Colitis with mild to moderate activity, H&E x40



gp	IL-10	IL-6	IL-17
I *(Healthy control group)*	56.82 ±3.04	8.75±1.35	177.50±5.65
II *(Healthy BM cells treated group)*	58.83±1.14	11.37±0.99	184.50±6.64
III*(Pathological control group)*	54.30±2.98	92.05±1.30	165.50±14.56
IV(*Pathological BM cells treated group)*	56.42±2.81*	24.90±5.65*	113.75±44.09*

Figure 3. Levels of the interleukins IL-10, IL-6 and IL-17 in different groups.* Significance *(P<0.005)* (pg/ml)

4. Discussion

Ulcerative colitis is a chronic, relapsing inflammatory gastrointestinal tract disorder with unknown etiology. It is referred to as one of the inflammatory bowel disorders (IBD). Although the etiological factors involved in the evolution of IBD remain uncertain, there is now general consensus that genetic predisposition, immunologic abnormalities, and environmental influences are among the possible etiological factors (Randhawa, Singh, Singh, & Jaggi, 2014).

In this study we aimed to determine the potential effects of BM cells on healing of ulcerative lesions and improvement of the oxidative stress in an animal model of ulcerative colitis

Development of different animal models provides new insights to clarify the onset and the progression of IBD. Various colitis models that closely mimic morphological, histopathological and symptomatic features of human IBD are widely used. Among the colitis models, trinitrobenzene sulfonic acid (TNBS) induced colitis (Duijvestein *et al*., 2011; Ocansey *et al*., 2019), dextran sulphate sodium (DSS) induced colitis (Lee *et al.,* 2016; Mashhouri, Froushani, & Tehrani, 2020) and acetic acid induced colitis models (Atta *et al*., 2019; Hassanshahi, Masoumi, Mehraban, Hashemi, & Zare, 2020) are the most widely used.

Dextran sulphate sodium (DSS) induced colitis is a model that symptomatically and morphologically resembles ulcerative colitis in humans (Eichele & Kharbanda, 2017). However, its high cost may limit its use. DSS causes erosions with complete loss of surface epithelium because of its direct toxic effect on epithelial cells. It causes acute colitis which is morphologically and macroscopically characterized by hyperemia, ulcerations, moderate to severe submucosal edema, symptoms of which are ultimately manifested in the form of bloody diarrhea (Li *et al*., 2022). DSS significantly causes increase in the production of all proinflammatory cytokines in both mid and in distal colon (Randhawa *et al*., 2014).

Acetic acid induced colitis is commonly employed and easily inducible model (Sasaki *et al*., 2000). Acetic acid induced colitis is a model of IBD that bears close resemblance to human IBD in terms of pathogenesis, histopathological features and inflammatory mediator profile (Subramanian, Du, & Tan, 2022). Intrarectal administration of dilute solution of acetic acid causes non-transmural inflammation characterized by increased neutrophil infiltration into the intestinal tissue, massive necrosis of mucosal and submucosal layers, vascular dilation, edema and submucosal ulceration that are noteworthy features of human colitis (Atta *et al*., 2019).

In our study we combined the use of DSS and acetic acid to induce colitis: the rats were primed for 24 hrs with DSS 1% in drinking water and then 2 ml of 4% acetic acid were injected intrathecal to induce colonic ulcerations. By applying this modification, we could overcome the obstacle of the high cost of DSS and at the same time get the benefit of induction of colitis by two different elements to study the effect of BM cells transplantation both on oxidative stress markers and cytokines. The protocol used in this study succeeded to induce colitis in rats as confirmed by macro and microscopic pictures.

Cytokines are small cell-signaling molecules secreted by various types of cells including immune cells (Roda, Marocchin, Sartini, & Roda, 2011). They play an important role in the pathogenesis of UC (Uchiyama et al., 2024). UC is characterized by an immune response mediated by the classical proinflammatory cytokines, such as IL-1, IL-6, and TNF-α, as well as IL-10 and IL-13 that play a key role in the pathogenesis of UC (Roda *et al*., 2011). IL-17 is also a strong pro-inflammatory cytokine that plays a role in IBD (Prananda, Abdullah, Koesnoe, & Sinto, 2024). Our results showed that the immune markers elucidated increased levels of proinflammatory IL-6 and decreased the anti-inflammatory cytokines IL-10 in colitis group. IL-17 increased with induction of UC. These results are in agreement with Lee, Kwon & Cho (2018); Nikolic *et al*. (2018); Omar *et al*. (2022); Shahini & Shahini (2022) and Song *et al*. (2018).

The term **"**apoptosis**"** describes the morphological changes that include nuclear segmentation, chromatin condensation, cytoplasmic shrinkage, blebbing, and formation of apoptotic bodies (Sakai *et al*., 1997). Apoptosis is closely related with UC (Dias *et al*., 2014; Xu *et al*., 2005). Excessive apoptosis of intestinal epithelial cells leads to intestinal barrier dysfunction, which is not only one of the pathological features of inflammatory bowel disease (IBD) but also a therapeutic target (Geng *et al*., 2024). Caspases are crucial mediators of apoptosis. Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins (Porter & Jänicke, 1999). This is in agreement with our caspase-3 results showing an increase after induction of colitis.

After confirmation of colitis development, rats were transplanted with BM cells. Our results showed great improvement in the overall condition of the rats in comparison to the positive control, as confirmed by the disease activity index, pathological studies, immune parameters and apoptotic markers.

BM cells, being considered as the most extensively studied tissue source (Ouzin & Kogler, 2023), have been used by several researchers in the treatment of experimental colitis using various animal models, including mice, rats, and Guinea pigs (Randhawa *et al*., 2014). Allogeneic transplantation of BM cells in a TNBS-induced colitis rat model was demonstrated to populate the cells in the injured regions of the colon (Hosseini-Asl, Mehrabani, & Karimi-Busheri, 2020).

When the therapeutic effects of BM cells and IFN-γ were compared in the treatment of a TNBS- and DSS-induced colitis mice models, it was shown that BM cells had immunosuppressive effects and could enhance the capacity to inhibit Th1 inflammatory responses and diminish mucosal damage (Duijvestein *et al*., 2011). These immunosuppressive effects could be attributed to MSCs constituting 0.001–0.01% of the total cell number within the BM tissue (Zaripova *et al*., 2023). MSC therapy decreased the expression level of the pro-inflammatory cytokines IL-17, while increasing that of IL-10. These results agree with Kim *et al*. (2013) who documented decreased IL-17 levels in mononuclear cells from the mesenteric lymph nodes and the spleen and elevated IL-10 levels after MSC-CM injection. In addition, the current study corroborates Lee *et al*. (2019) results of weight gain, DAI reduction, and decreased expression of IL-6, and IL-17 after MSC treatment. In agreement with those

findings, our results showed normalization of pro- and anti-inflammatory cytokines in BM cell treated groups when compared to the colitis group.

5. Conclusions

Treatment of UC aims to induce and maintain remission, reduce the risk of complications, and improve quality of life. The diverse disadvantages of biological drugs limit their use. Therefore, there is a growing need for effective and safe therapeutic agents. Nowadays an alternative approach for many diseases depends on cell-based therapies, including BM cells which are the main source of essential BM cells. Results of this work are encouraging and pave the way for trials on higher animals before being tested on humans.

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