ORIGINAL ARTICLE

Evaluation of the Anti-Oxidative Effects of Monocyte Cells Treated with Bone Marrow Mesenchymal Stem Cells Supernatant on Experimental Colitis in BALB/c Mice

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Abstract

Background: Alternate activation of monocytes could induce anti-inflammatory impacts. This study aimed to investigate whether monocyte cells treated with bone marrow mesenchymal stem cells supernatant (MSC-Sp) could improve anti-inflammatory responses as a cell transfer therapy for colitis. **Materials and Methods:** The induction of experimental colitis was done by acetic acid in four groups of male BALB/c mice, including the control colitis, treated-monocytes, non-treated-monocytes, and mesalazine groups. Following MSCs culture, the supernatant was harvested, and then 50% conditioned media, or negative control media was added to the monocytes for 24 h. After ten days, peritoneal injection of treated or non-treated-monocytes (105 cells/100μL) was performed in animals' relevant groups of colitis. Ten days later, the oxidative stress profile and histopathological evaluation of colon tissue were assessed. **Results:** Treated monocytes showed a significant improvement in the oxidative stress profile, namely myeloperoxidase (0.126±0.008), nitric oxide (0.153±0.01), and malondialdehyde (0.148±0.014) compared to the control colitis group (P<0.05). Also, histopathological results revealed that the rate of damage in the treated-monocytes group was less than in normal mice. **Conclusion:** Our study indicated that the treated monocytes had anti-oxidative potential in colitis mice and were usable as a complementary therapy. **[GMJ.2021;10:e2131] DOI:10.31661/gmj.v10i0.2131**

Keywords: Monocyte; Mesenchymal Stem Supernatant; Colitis; BALB/c Mice

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Introduction

As a chronic inflammatory bowel disease, ulcerative colitis (UC) has an unknown cause that affects the colon and rectum [1]. UC pathogenicity is proposed to result from the contribution of several factors, including genetic context, environmental and luminal factors, and mucosal immune dysregulation [2]. UC has become an encumbrance globally due to a high prevalence in developed countries and a considerable rise in frequency in developing countries [3]. Achieving ideal disease management warrants developing innovative drugs and improving the therapeutic approach by implementing personalized medicine [4]. The bone marrow and most other tissues, including adipose tissue and umbilical cord blood, are normally rich in mesenchymal stem cells (MSCs), dermal tissue, and peri-endothelial areas [5]. During the last few decades, MSCs have been among the broadly discovered multipotent stem cells. Hypo-immunogenic and immunoregulatory properties of MSCs have been reported in their therapeutic impacts on various inflammatory diseases and kidney transplantation [6]. Prior studies established that MSCs were capable of controlling both innate and adaptive immune responses through the release of various mediators (such as immunosuppressant molecules, growth factors, exosomes, chemokines, complement components, and metabolites) upon exposure to inflammatory situations, which thereby promotes the reparation and restoration of injured tissues [7]. Similar to the MSCs, many investigations have demonstrated that the supernatant obtained from MSCs (MSCs-Sp) is therapeutically beneficial in various experimental diseases [7]. Monocytes have a significant contribution to initiating and resolving inflammatory situations [8]. Nonetheless, information is absent or scarce concerning the function of monocytes treated with MSCs-Sp in mitigating oxidative situations. Therefore, the current research aimed to examine whether the MSCs-Sp treated-monocytes could reduce the oxidative condition of experimental colitis in BALB/c mice.

Materials and Methods

Ethical Statement
The procedure was supervised and approved by the Shahid Beheshti University of Medical Sciences ethics committee (approval code: IR.SBMU.RETECH.REC.1398.259). Animal treatment and general guidelines for animal handling were followed by the legislation of the Iranian Ministry of Health and Medical Education. Besides, the least number of mice was assigned to every group with a statistical significance.

Isolation of MSCs and Supernatant Preparation
In short, the femurs and tibias from the sacrificial BALB/c mice were extracted. The cells were rinsed twice and then developed to a concentration of 0.3-0.4 in the Dulbecco Modified Eagle medium (DMEM;Gibo, USA) in 25 cm2 tissue-cropping flake at a concentration of 0.3-0.4 per 106 cells/cm2 enhanced by 15 `% fetal bovine serum (FBS;Gibco, USA) in a 5% CO_2-enriched moistened incubator at 37°C. On the fourth day, unattached cells were washed, and full media were added two days a week to the attachments. After 80% confluence, the bone marrow-MSCs were separated with slight trypsinization using trypsin/EDTA 0.25%(Dacell, Iran), followed by counting and re-plating in a ratio of 1:3 (about 1×106 cells/25-cm2 flask). The cell lines have been pushed up to the third generation. Next, the MSCs were exposed to a medium without FBS for 24 hours. The supernatants were extracted and centrifuged for 10 minutes at 300 g to remove cell remnants. Afterward, a mixture was prepared from the harvested supernatants from every BALB/c mice, passed through a 0.2-μm filter orderly, and then kept frozen at -80ºC before tests [9].

Monocyte Isolation and Treatment
Blood samples were isolated from acutely anesthetized animals using a cardiac puncture in Roswell Park Memorial

Institute-1640 (RPMI-1640, Gibco, USA) medium (1:1) on a Ficoll-Hypaque gradient. The mononucleate cell layer obtained by centrifuging at 400 g for 30 minutes was resuspended in DMEM and grown for 2 hours at 37 °C in 6-well plates (9106 cells/well). This resulted in monocyte adhesion to the plate. Unattached cells (primarily lymphocytes) were scraped using a scraper after repeated phosphate-buffered saline (PBS;Gibco, USA) rinses. Following that, attached monocytes were cultured in DMEM enriched with 10% FBS and 50% bone marrow-MSCs-Sp or DMEM enriched with 10% FBS and 50% bone marrow-MSCs-Sp (negative control media). After 24 hours, the cells were harvested, rinsed three times, and labeled as untreated or treated for in vivo assays [9].

Grouping
Forty male BALB/c mice have abstained for 24 hours and then instilled 4% acetic acid (1 mL, Merk, Germany) into the rectal to induce experimental colitis. After the random assignment of mice to five equivalent isolated groups, the group I was excluded as a negative control without colitis induction. Other BALB/c mice groups received acetic acid for UC induction, which were treated differently after ten days. Groups II, III, IV, and V were treated with normal saline (100 μL, intravenous [IV] injection) as normal colitis, treated-monocytes (105 cells/100 μL/ IV injection), non-treated-monocyte (105 cells/100 μL), and 30 mg/kg of oral mesalazine, respectively. The entire mice underwent euthanasia by CO2 inhalation, and cervical dislocation was performed on all mice and was sampled (blood sampling from hearts and isolation of colon tissue) on day 20.

Evaluation of Myeloperoxidase (MPO) Levels
After adding 2 mL of phosphate buffer comprising 0.5% hexadecyl trimethyl ammonium bromide (HTAB;Sigma-Aldrich, USA) to 100 mg of colon tissue, homogenization on ice was performed six times for 45 seconds, accompanied by sonicating for 10 seconds and freezing with liquid nitrogen three times, centrifugation at 3000 rpm and 4°C for 30 minutes obtained the supernatant. After decanting 2.9 mL of phosphate buffer comprising o-Dianisidine and 0.005% hydrogen peroxide to 0.1 mL of supernatant, 0.1 mL of 1.2 M hydrogen chloride was poured into a funnel until it appeared orange in color. The absorbance of the specimens was determined using the traditional package diagram at 460 nm using an ELISA reader (Biotek, USA).

Measurement of Colon Tissue Nitric Oxide (NO) Level
NO concentrations produced by homogenizing colon tissue were determined in supernatant cells with the Griess reaction. The residues of microscopic cells were discarded via centrifugation of the homogenized colon tissue specimens. The specimens were distilled by PBS contains 0.5% HTAB with a ratio of 2:1, followed by decanting 100 mL of individual specimens into the 96-wells plates triplicated according to the manufacturer protocol (Karmania pars gene, Iran) along with blank and standard samples (Nos. KPG-NO). The absorbance of the sample at 460 nm was measured using a UV–visible spectrophotometer (Eppendorf, Germany) [10].

Malondialdehyde (MDA) Level Measurement
Briefly, tissue (500 mg) was homogenized in 5 mL of 1.15% cold potassium chloride, and then 3 mL of 1% phosphoric acid and 1 mL of 0.6% thiobarbituric acid (Sigma-Aldrich, USA) were added to 500l of homogenate with sufficient shaking. Then it was heated indirectly to 100o C for 45 minutes, centrifuged at 10,000 rpm for 10 minutes, and the supernatant's absorbance at 532 nm was determined using a UV-visible spectrophotometer (Eppendorf, Germany) [10].

Histological Study
Samples were stained with Hematoxylin and Eosin (H&E; Sigma-Aldrich, USA) stain for inspection under a light microscope of

the histopathologic pattern. The H&E stain is one of the most often encountered tissue stains in histology. It is staining cell nuclei blue, extracellular matrix, and cytoplasm pink, with other structures exhibiting varying tones, hues, and variations of these colors. Thus, a pathologist can clearly distinguish between a cell's nuclear and cytoplasmic components. The staining findings were graded on a scale of 0 to 3. The ratings were as follows: 0=normal; 1=mucosal erythema alone; 2=moderate mucosal edema, minor bleeding, or slight erosion; and 3=extreme ulceration, erosions, edema, and necrosis of the tissue. Finally, the magnitude of the lesions was determined using the numbers derived from each of the parameters collected and analyzed.

Statistical Analysis

The mean and standard deviation (SD) of the whole set of data were recorded. The different findings from the groups were subjected to statistically meaningful analyses using analysis of variance (ANOVA) and the Student-Newman-Keuls test with a statistical significance level of P<0.05 using SPSS21(IBM, USA) software.

Results

MPO, NO, and MDA activities in colon tissue were used to determine the impact of treated and untreated monocytes on anti-oxidative functions.

According to the findings, inducing colitis with acetic acid resulted in significant reductions in total oxidant behaviors as compared to the usual colitis population. MPO (P<0.017, 0.154±0.01 µM/ml, Figure-1), NO (P<0.038, 0.192±0.018 U/g, Figure-2), and MDA (P<0.022, 0.207±0.026 U/mg, Figure-3) levels were significantly increased in control colitis mice as compared to the negative control group. MPO (P<0.041, 0.126±0.008 µM/ml, Figure-1), NO (P<0.038, 0.153±0.01 U/g, Figure-2), and MDA (P<0.046, 0.148±0.014 U/mg, Figure-3) levels of treated monocytes were slightly decreased in contrast to control colitis animals.

After scoring tissue (Figure-4), the pessimistic control group received a score of 0 (the lowest possible score), and the control colitis group received a score of 3 (the highest score possible). The groups receiving treated monocytes and mesalazine demonstrated that the epithelium was not entirely destroyed; hence, they obtained a score of 2 for epithelium loss.

Additionally, the community obtaining treated monocytes and mesalazine had a significantly greater reduction in acetic acid-induced ulcers (P<0.05). Also, the non-treated monocyte group had less colon damage than the control colitis group.
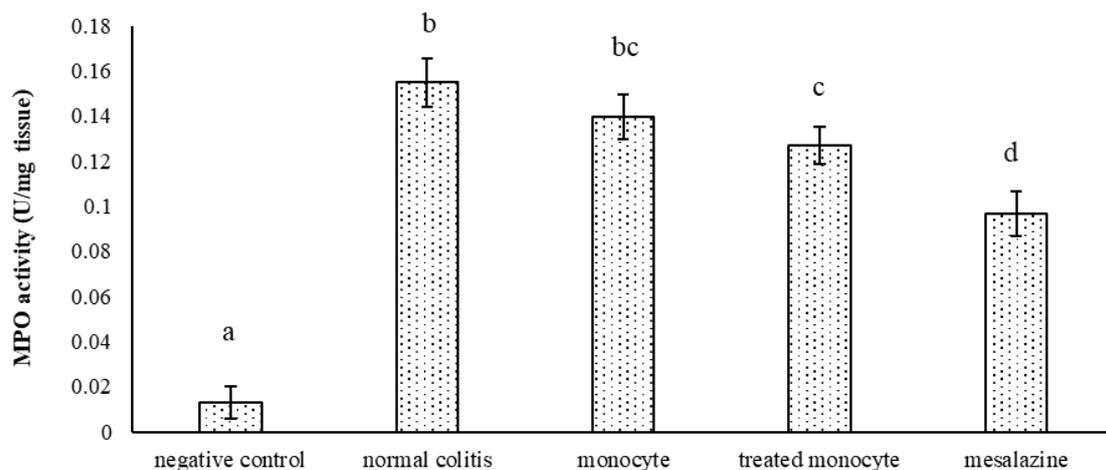

Figure 1. Comparing the mean±SD of MPO level in colon tissues of various investigational groups. The different superscript letters represented the significant statistical differences within the groups in each index (P<0.05).
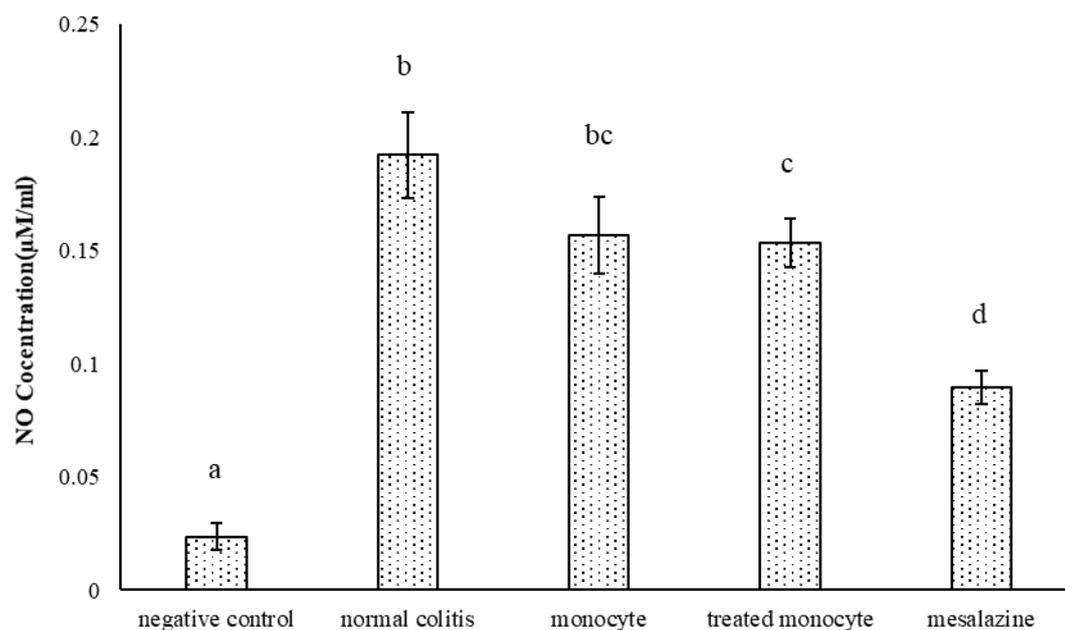
Figure 2. Comparing the mean±SD of NO level in colon tissues of various experimental groups. The various superscript letters represented the considerable statistical differences within the groups in each index (P<0.05).
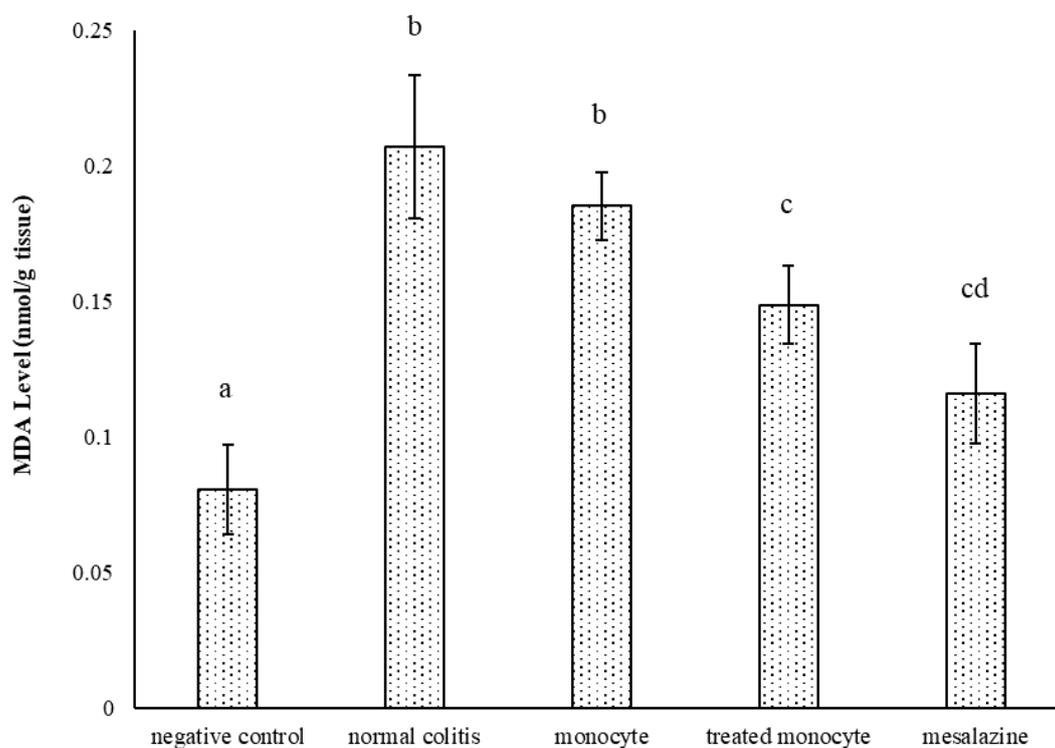

Figure 3. Comparing the mean±SD of MDA levels in colon tissues of various experimental groups. The different superscript letter represents the considerable statistical differences within the groups in each index (P<0.05).
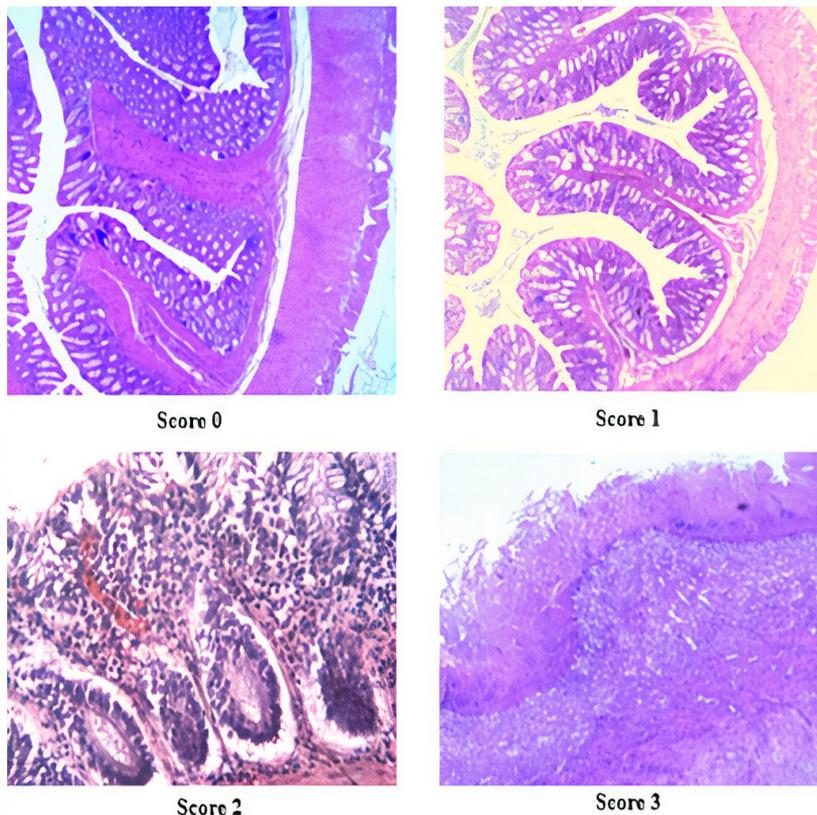
Figure 4. Representative sections depict the scoring system used for histopathological examinations (H&E staining, original magnification ×400).

Discussion

As a chronic, incapacitating, and persistent illness, the characteristic of UC is a lifetime therapy and detrimentally influences patients' quality of life. For some decades, UC has been treated using anti-inflammatory medicines (e.g., 5-aminosalicylic acid and glucocorticoids) [11]. Although, a variety of treatments (e.g., stem cell therapy) have been offered to promote our understanding of the pathologic mechanisms of UC exactly. There are reports on the regenerative and immunomodulatory activities of MSCs in UC models [12].

Our research focused on the anti-oxidant impacts of monocyte cells subjected to treatment by bone marrow MSCs-Sp experimental colitis in BALB/c mice. Through delivering treated monocytes to BALB/c mice, we found an effective reduction in colonic oxidative stress.

Colitis is caused experimentally in animal models with several compounds, including 2, 4, 6-trinitrobenzene sulfonic acid, disuccinimidyl suberate, oxazolone, and acetic acid [13]. Acetic acid colitis induction is used as an experimental colitis model since it causes acute inflammation and colonic ulceration like those seen in patients. It is described by bloody diarrhea, weight loss, inflammatory cell invasion, and epithelial cell loss on the surface [14].

Moreover, oxidative stress has a major contribution to the pathogenicity of UC. MPO, NO, and MDA are essential components of cell protection from oxidative injury [15]. Elevated MPO, NO, and MDA activities were found in the colonic homogenate of BALB/c mice with UC compared to the control animals.

Also, the use of monocytes treated with bone marrow MSCs-Sp resulted in significantly declined MPO, NO, and MDA activities as opposed to normal colitis.

However, there are unknown modes of action behind the immune inhibition by MSCs. Broadly available evidence indicates that MSCs inhibit the multiplication of T-cells once stimulated allogeneically or mitogenically [16].

It has been proposed that soluble factors such as indoleamine 2,3-dioxygenase, prostaglandin E2, transforming growth factor-1, interleukin (IL)-6, and heme oxygenase 1 are responsible for this effect [16]. MSCs have also been shown to prevent the proliferation of B cells and IL-2-stimulated natural killer cells and to facilitate the development of regulatory T cells (T reg) [17]. MSCs may also influence immune responses by inhibiting the differentiation of monocyte-derived immature dendritic cells (iDCs) [12].

IL-6 and IL-10 have been shown to suppress monocyte differentiation to iDC. Numerous studies have shown that IL-6 may contribute to MSCs' inhibitory impact on monocyte differentiation to dendritic cells [18]. Nonetheless, these data come from in vitro co-culture assays of MSCs and monocytes that do not recognize the cellular source of cytokine production. To be more precise, the function of these cytokines in suppressing MSC behavior is unknown at the moment [18].

Based on the pathogenicity of disease, neutrophils are the essential immune cells migrating into the colonic tissue after luminal instilment of acetic acid and induce inflammatory damages by the formation of oxygen and nitrogen species [19].

It was previously found that local inflammation would diminish by inserting MSCs into the inflamed tissue through suppression of MPO and NO generation, which improves tissue healing by the reduction of lipid peroxidation. The generation of reactive aldehydes (e.g., MDA) represents lipid peroxidation by oxygen and nitrogen species [20]. Melief *et al*. indicated that multipotent stromal cells influence monocytes to produce anti-inflammatory IL-10 through IL-6 production [21]. Esmaeili *et al*. reported that monocytes grown with supernatant of MSCs might potentially be an approach for cell-based treatments of mice model of asthma [9]. Accordingly, it was formerly documented that MSCs could increase the anti-oxidant capability of small bowel tissue after intestinal ischemia-reperfusion damage [22].

A leading observation in this research was elevated MDA levels in the colonic homogenates of BALB/c mice with colitis and treated monocytes' capability to reduce its levels. Here, it was confirmed that the migration of treated-monocytes into the re-perfused small intestine resulted in declined oxidative stress by these cells due to the impacts of superoxide dismutase, catalase, and glutathione peroxidase, as well as reduction of the MDA levels.

Also, histopathological results in parallel with the above results showed that the rate of degradation in the groups receiving mesalazine and treated monocytes were less than the normal group of colitis.

Conclusion

This study indicated that the monocytes co-cultured with bone marrow MSCs-Sp could inhibit the oxidative condition of experimental colitis. Overall, this procedure can potentially be an approach for cell-based treatments of colitis.

Acknowledgments

Conflict of Interest

The authors have no conflicts of interest.

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