Regenerative Therapy 29 (2025) 177–183

Contents lists available at ScienceDirect

Regenerative Therapy

journal homepage: http://www.elsevier.com/locate/reth

Original Article

Optimal dose of bone marrow mesenchymal stem cell transplantation for experimental ulcerative colitis

Xiaoyun Chen [a], Yan Xia [c], Min Min [d], Lingzhi Qin [e], Yangsheng Liu [b, *]

[a] Department of Pathology, Wuhan No.1 Hospital, Wuhan, 430030, China
[b] Department of Neurology, Xianning First People's Hospital, Zhongnan Hospital of Wuhan University, Xianning Hospital, Xianning, 437100, China
[c] School of Biomedical Engineering and Medical Imaging, Xianning Medical College, Hubei University of Science and Technology, Xianning, 437100, China
[d] School of Clinical Medicine, School of Medicine, Hubei University of Science and Technology, Xianning, 437100, China
[e] Institute of Pathology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

ARTICLE INFO

Article history:
Received 20 November 2024
Received in revised form
9 January 2025
Accepted 19 January 2025

Keywords:
Bone marrow mesenchymal stem cells
Transplantation
Ulcerative colitis

ABSTRACT

Objective: To investigate the optimal dose of bone marrow mesenchymal stem cell-transplantation for the ulcerative colitis rat.
Methods: The BMSC of SD rat were isolated, cultured and labelled with DAPI. SD rats were randomly distributed into 3 groups, Colitis was induced with immune-combined TNBS/ethanol in group A、B、C, 3 groups received caudal vein injection of 1 mL fluids, which contain cell number 1×10^6、5×10^6、1×10^7 separately. 5 rats in each group were sacrificed at day 7 and 14 after injection, Cryostat sections of gut, The number of BMSCs in colon and normal tissue surrounded was observed with fluorescent microscope.
Results: The DAPI marked BMSCs could been seen in the colic mucosa in each group on day 7、14, more cells in colon than the surrounding normal tissue, compared with 1×10^6 group, More cells in 5×10^6 group ($P < 0.05$), there were no significant difference ($P > 0.05$) between 5×10^6 group and 1×10^7 group. There were more cells in colon on 14 day than 7 day, and less in the surrounding normal tissue on 14 day than 7 day.
Conclusions: The density 5×10^6 is proper of bone mesenchymal stem cells for treatment of ulcerative colitis.

1. Introduction

Ulcerative colitis (UC), one of two major forms of chronic inflammatory bowel disease (IBD), is characterized by dysfunction of the innate and adaptive, resulting in chronic inflammation and ulceration of the colonic mucosa. Patients with UC can have severe disease that can result in intestinal bleeding and perforation, requiring surgical treatment, and they are also at the increased risk of developing colorectal cancer [1].

Mesenchymal stromal cells (MSCs) are multipotent stem cells which can differentiate into several tissue lineages originating from the three germinal layers in vitro and in vivo [2,3].These features make MSCs more attractive for cellular therapy, gene therapy, and bioengineering [4]. MSCs have the capacity to differentiate into intestinal epithelial cells, and repairing mucosal injuries. MSC transplantation has been shown to result in migration of stem cells to the ulcerated intestinal mucosa and partially alleviate the symptom of UC [5].

While many researchs report the effectiveness of MSC treatments in attenuating the disease mechanism, some MSC therapies are reported as only demonstrating short-term effectiveness or being ineffective [6–8], various factors, including cellular dose, influence therapeutic efficacy of these cells.There is great variation among experimental models and clinical trials of disease in the injected dosage of MSCs [9,10]. In addition, defining an optimal MSC dose have benefits for both preclinical and clinical studies, such as reduced transplantation costs, less tissue required for proliferation, a reduced likelihood of MSC accumulation in the filtering organs and a lower chance of MSC mutation.In this study, we aim to

* Corresponding author. Department of Neurology, Xianning First People's Hospital, Zhongnan Hospital of Wuhan University, Xianning Hospital, Xianning, 437100, China
E-mail address: yangshengliu@126.com (Y. Liu).
Peer review under responsibility of the Japanese Society for Regenerative Medicine.

https://doi.org/10.1016/j.reth.2025.01.015

investigate at which dose (1×10^6, 5×10^6 or 1×10^7) BM-MSCs are most beneficial in repairing in colitis rats model. Thus, in attempt to improve the efficacy of MSC treatment, our studies aim at evaluating the proper dose of MSC transplantation.

2. Materials and methods

2.1. Animals

SD rat was provided and approved by the Animal Care and Utilization Committee of Huazhong University of Science and Technology, and randomly assigned to experimental groups. All animals were housed in a temperature-controlled environment with 12-h day/night cycles and had ad libitum access to food and water.All animals were treated according to the protocols evaluated and approved by the ethical committee of Huazhong University of Science and Technology.

2.2. Isolation and culture of BMSCs

Rats were killed by cervical dislocation, The fur was disinfected with 75 % alcohol and then the femur and tibia was douched sterilitily with DMEM/F12. After washing twice with D-Hanks, the bone marrow cells were cultured in DMEM/F12 supplemented with 10 % heat-inactivated fetal calf serum, 200 U/ml penicillin G sodium, and 200 U/ml streptomycin sulfate at a density of 5.0×10^5/mL per plastic dish. After 24 h of culture, non-adherent cells were removed, and adherent cells were cultured continually, and the culture medium was replaced twice a week. The dish adherent BMSCs population was expanded after the initial plating. The 3rd to 5th passages cells at a density of 5.0×10^5/mL were examined their expression of CD29, CD45 and CD90 by flow cytometry.

The 3rd to 5th passages cells were cultured in medium contained DAPI with final concentration 10 mg per liter for 30 min, washing six with PBS, to prepare the fluorescent labeled BMSCs for the later cellular transplant.

2.3. Osteogenic and adipogenic differentiation potential of BMSC

To evaluate the differentiation potential of mesenchymal stem cells into adipose and bone tissue, stem cells from the second passage were cultured in special culture medium for 21 days and then stained with Oil red-O and Alizarin Red-S to confirm. To induce osteogenic differentiation, MSCS were cultured in supplemented media with glycerol phosphate (10 mM), dexamethasone (100 mM), and ascorbic acid-2 phosphate (5 g/mL) for 3 weeks. For adipogenic differentiation, MSCs were cultured in complete media supplemented with indomethacin (100 mM), 3-isobuty1-methylxanthine (0.5 mM), dexamethasone (250 mM), and insulin (5 mM) for 21 days [11].

2.4. Model and evaluation of colitis

The homogenate of tunica mucosa coli was prepared, its supernate fluid was obtained by centrifuge, and the protein level in supernate fluid was detected as 20 g/L with Biuret method.

The partes aequales holo-Four's adjuvant was mixed with supernate fluid to be antigen emulsifying agent. For raise of UC model, rat was injected 8 mg antigen twice two week, and then, 0.65 mL mixed liquor which contain TNBS 0.1 mg/g and 500 mL 50 % alcohol per liter was pushed into the colon through rat anus. After one day, for histopathologic analysis, rats were all sacrificed and 10 cm distal colon tissue was collected. The colon specimen was fixed in 10 % buffered formalin phosphate, embedded in sucrose, frozen in dry ice using the OCT compound, and cryosectioned. Section was stained by haematoxylin and eosin (HE) to observe the morphous of colon.

2.5. BMSC treatments

BMSC-treated groups were anaesthetized, and 1 mL liquor was injected through vena caudalis of rat, MSCs were administered at a dose of 1×10^6、5×10^6、1×10^7 fluorescent labeled BMSCs, On the 7, 14 day of transplantation, 5 rats in each group were execute, and their colon were prepared as frozen section. DAPI-fluorescent marked cells were observed with fluorescent microscope, and the fluorescent integral optical density (IOD) in 20 X visual field was randomly calculated with IPP software, each group has 10 times. Same section was stained by haematoxylin and eosin to observe the morphous of colon.

2.6. Statistical analysis

Data are given as mean ± standard deviation (S.D.). Student's t test was used to compare between two groups. Statistical differences were considered significant when $P < 0.05$.

3. Results

3.1. Characterization of BMSCs

The morphology of the BMSCs showed spindle-shaped and was almost uniform under the microscope (Fig. 1A). The markers of BMSCs were authenticated by flow cytometry, The expression of CD29, CD90 and CD45 was 99.26 %, 98.68 % and 0.1% respectively (Fig. 1B), The data demonstrated that BMSCs were cultured successfully.

3.2. Differentiation potential of BMSCs

After 21 days in a supplemented osteogenic induction medium, MSCs were differentiated into bone that confirmed by alizarin red staining (Fig. 2A). Also, the MSCs were cultured in a supplemented adipocyte induction medium and morphological changes from spindle to flat shape was confirmed by Oil red-O staining method (Fig. 2B).

3.3. Pathological changes of colon in UC model rat

Intense colonic inflammation was observed in mucosa and submucosa stratum, with loss of goblet cells, crypt damage, and extensive in the submucosa were observed (Fig. 3).

3.4. Histological analysia shows that intravenous MSCs infusion reduces colon damage

On the 7th day of postgraft, the colon mucosa in groups A, B and C appeared striking oedema, inflammatory exudation and scattered bleeding. Blood vessel in proper layer increased, and there was copious neutrophil, lymphocyte and plasma cell infiltrating in submucousa. The ulcer and necrotic tissue covered with cellulose, leucocyte, Inflammatory exudates could be seen (Fig. 4A). On the 14th day of postgraft, there was no epithelium on the surface of granulation tissue of mucosa ulcer in groups A (Fig. 4B); But compared with groups A, the oedema, inflammatory exudation in colon of groups B and C was mitigated, and the ulcer was repaired, covered by anagenetic epithelial cells (Fig. 4C and D).

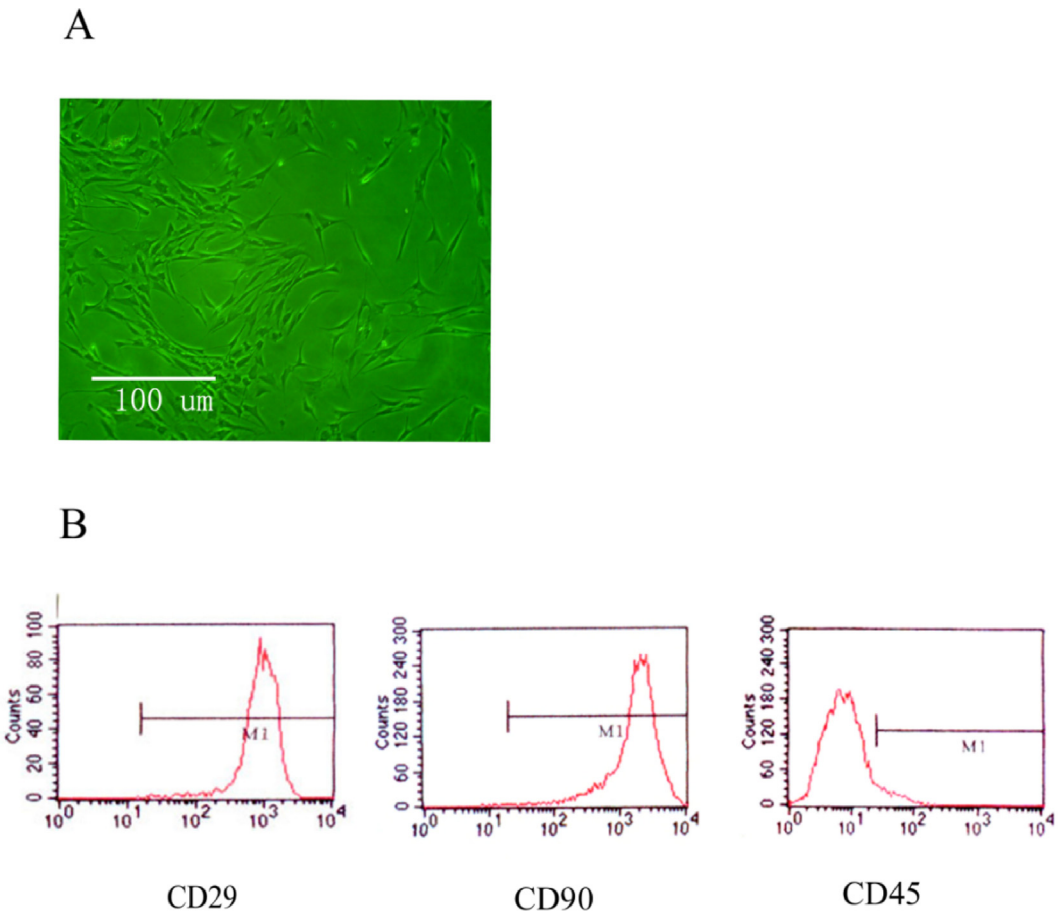

Fig. 1. Morphology and identification of MSCs. (A) The primary BMSCs(after 1 and 3-days growth) showed a long spindle shape. (B) BMSCs surface antigen identification by flow cytometry.Positive antigen: CD29, CD90; Negative antigen:CD45.

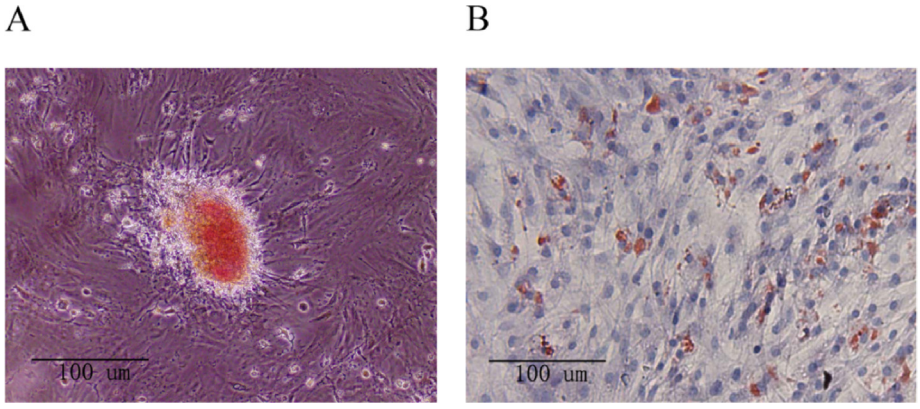

Fig. 2. Multipotential differentiation of MSCs.When cultured in the differentiation medium, the bone marrow-deriver MSCs differentiated into osteogenic and adipogenic lineage cells. (A) Cells dyed with Alizarin Red. (B) Cells dyed with Oil-Red O.

3.5. Dose-dependent effects of MSC treatment

The DAPI marked BMSCs could been seen in the colic mucosa in each group on day 7、14, mainly distributed in the mucosa and submucosa, occasionally seen in the muscularis and tunica adventitia (Fig. 5A). More cells in colon than the surrounding normal tissue (Fig. 5B), compared with 1×10^6 group, More cells in 5×10^6 group ($P < 0.05$), there were no significant difference ($P > 0.05$) Between 5×10^6 group and 1×10^7 group, there were more cells in colon on 14 day than 7 day, and less in the surrounding normal tissue on 14 day than 7 day.

4. Discussion

The treatment of UC is to repair the damaged colonic mucosa. MSCs have therapeutic potential in tissue regeneration and repair due to their differentiation capacity [12]. Many studies focused on the regenerative properties of MSCs, and evidence indicating that

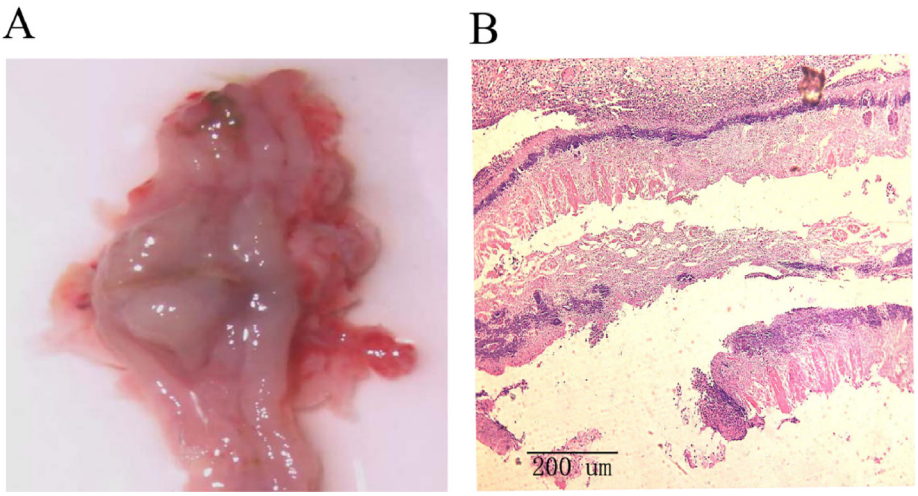

Fig. 3. Pathological changes of colon in UC model rat (A) Mucosal ulcer. (B) Mucosal ulcer lesion stained by HE.

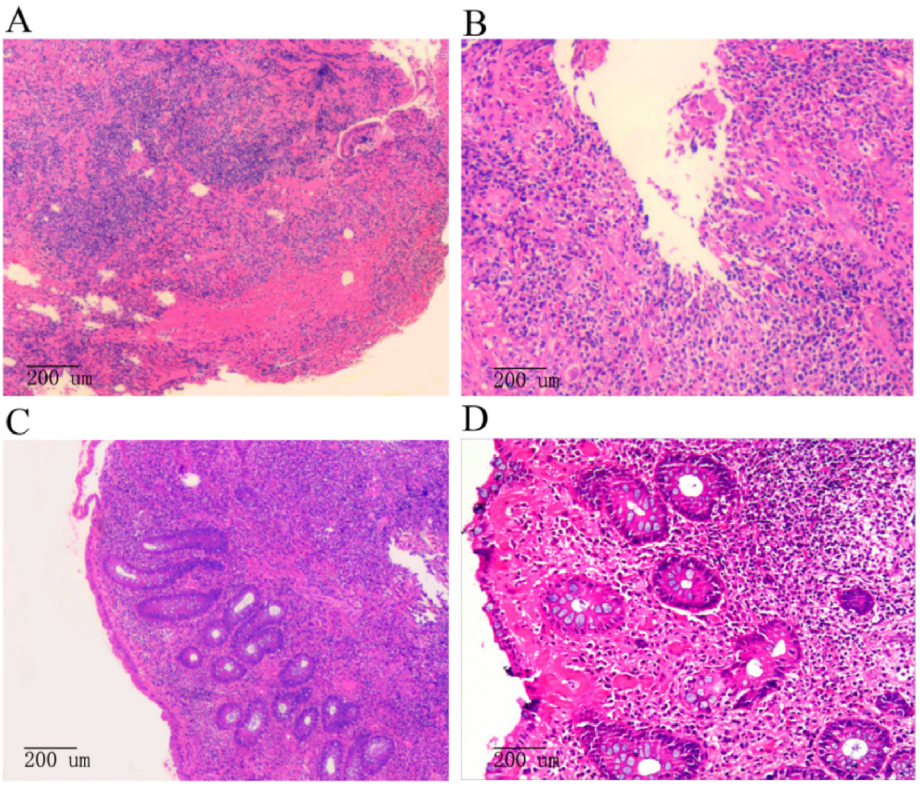

Fig. 4. Effects of MSC treatment on tissue repair (A) Histopathological changes of mucosal ulcer. (B) Histopathological changes of groups A on the 14th day of postgraft. (C) Histopathological changes of groups B on the 14th day of postgraft. (D) Histopathological changes of groups C on the 14th day of postgraft.

MSCs can promote regeneration and repair of injured tissue [13–15].

In our study, we compared different doses of BMSCs for epithelial repair in a rat model of TNBS-induced colitis. Both 5×10^6 and 1×10^7 MSCs treatment demonstrated therapeutic efficacy in the accelerated repair of colonic mucosa.

MSCs have the potential to differentiate into a variety of cell types [16], and in our research, we confirmed that BMSCs could differentiate into osteocytes, adipocytes. Previous some studies have only focused on the use of MSCs in the repair of injured tissue by various mechanisms [17]. But the implantation of MSCs in injured sites is critical for this regeneration and thus there is interest in its application of the treatment of UC [18].

While there are a number of studies report the effectiveness of MSC treatments in attenuating disease, some MSC therapies are reported as only demonstrating short-term effectiveness or being ineffective [7,19,20]. Various factors, including timing of administration of MSCs and cellular dose, influence therapeutic efficacy of MSCs [21]. Hence, it was suggested that different doses of MSCs might have distinct protective or immune effects [22]. There is great variation among experimental models and clinical trials of disease in the injected dosage of MSCs [23], suggesting that MSCs

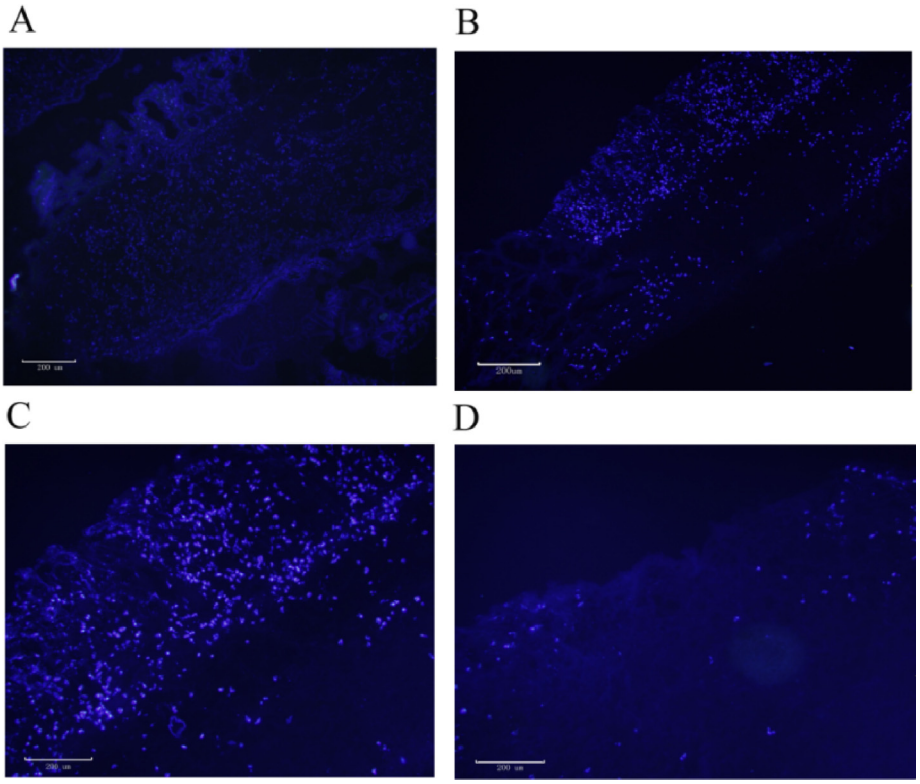

Fig. 5. Location of MSCs in rats on day 7 and day 14 after transplantation (A) The distribution of BMSCs after transplantation. (B) More cell distribution in colon than the surrounding normal tissue. (C) Cell distribution in colon on 14 day in groupB. (D) Cell distribution in the surrounding normal tissue on 14 day in groupB.

can treat diseases effectively in a dose-dependent manner [24–27]. In addition, defining an optimal MSC dose for both preclinical and clinical studies extends to benefits such as less tissue required for MSC expansion, reduced production costs, reduced the accumulation of MSC in the filtering organs and a lower chance of MSC mutation.

In experimental models of colitis, BM-MSCs derived from rats [28], mice [29],guineapigs [30] and humans [31,32], have been investigated for therapeutic efficacy. And some other studies have assessed adipose MSCs derived from these species [33–35], as well as gingiva, human umbilical cord and umbilical cord blood [31,36,37].Overall, intraperitoneal, local administration and intravenous of MSCs from various species and sources have been reported to ameliorate experimental colitis, however there is no consistency regarding of the most efficacious dose similarly to clinical trials, Studies have report therapeutic efficacy in ameliorating colitis with the doses of 2×10^3 [28], 2×10^4 [38], 5×10^5 [39], 0.5×10^6 [32], 1×10^6 [35,36,40–43], 2×10^6 [31,37], 5×10^6 [27,44], 11×10^7 [45] MSCs.

The therapeutic application of MSCs is their fate post-implantation. In the past, ambiguity seen in the efficacy of MSCs, in both animal studies、preclinical trials and clinical trials, with therapies only temporarily effective or being ineffective-could be due to suboptimal application of MSCs.In this study, we employed MSCs derived from SD rats, surface expression of CD29 and CD90. In our study, BMSCs engrafted into the mucosa at the initial site of TNBS-induced inflammation in all MSC treated groups.Administered 1×10^6、5×10^6 or 1×10^7 MSCs separately through caudal vein injection, the outcomes of the treatment were more pronounced in animals treated with 5×10^6 and 1×10^7 MSCs compared to those treated with 1×10^6 MSCs.

In this study, we can determine that a 1×10^6 dose of BMSCs is not adequate, whereas doses of 5×10^6 and 1×10^7 demonstrate same results in TNBS-induced colitis. Although the 1×10^7 dose MSCs contained double the quantity of cells than the 5×10^6 dose, there is no evident differences between the magnitude of cells homing to and engrafting at the site of tissue injury. This suggests a dose saturation, indicating that although there is a greater number of cells being transplanted in vivo, only the required number migrates and engrafts into the injuried areas. This is consistent with a previous MSC study which revealed that the engraftment of osteoprogenitor cells to be a dose saturated, and concluded that higher doses of cells would be an ineffective strategy to improve engraftment [46].Furthermore, high concentrations of MSCs produced high-dose inhibition of cytokines [47–49].

In this study we have essentially determined an optimal dose of MSCs in TNBS induced colitis. We have demonstrated that repairing effect of BMSCs is dose-dependent in TNBS-induced colitis; BMSCs have the ability to migrat to the intestinal mucosa when administered at a dose of 5×10^6 cells are most beneficial after induction of colitis, with no further benefit gained from a higher dose. The findings of this study are important for further investigations the immunomodulation within the inflamed colon and to improve the efficacy of MSC treatment, further enabling MSC therapy to continue to advance forward in future studies.

Declaration of competing interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and

not under consideration for publication else where, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Acknowledgements

We acknowledge the support from Wuhan No.1 Hospital support: 2017Y05, and the National Natural Science Foundation of China (81600015).

References

[1] Lakatos PL, Lakatos L. Risk for colorectal cancer in ulcerative colitis: changes, causes and management strategies. World J Gastroenterol 2008;14:3937–47.
[2] Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
[3] Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41–9.
[4] Caplan AI. Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 2007;213:341–7.
[5] He XW, He XS, Lian L, Wu XJ, Lan P. Systemic infusion of bone marrowderived mesenchymal stem cells for treatment of experimental colitis in mice. Dig Dis Sci 2012;57:3136–44.
[6] Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, et al. Intracoronary bone marrow cell transfer after myocardial infarction. Circulation 2006;113:1287–94.
[7] Badillo AT, Peranteau WH, Heaton TE, Quinn C, Flake AW. Murine bone marrow derived stromal progenitor cells fail to prevent or treat acute graft-versus-host disease. Br J Haematol 2008;141:224–34.
[8] Sudres M, Norol F, Trenado A, Grégoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 2006;176:7761–7.
[9] Li J, Ezzelarab MB, Cooper DKC. Do mesenchymal stem cells function across species barriers? Relevance for xenotransplantation. Xenotransplantation 2012;19:273–85.
[10] Sharma RR, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. Transfusion 2014;54:1418–37.
[11] Yousefi F, Ebtekar M, Soudi S, Soleimani M, Mahmoud S. In vivo immunomodulatory effects of adipose-derived mesenchymal stem cells conditioned medium in experimental autoimmune encephalomyelitis. Immunol Lett 2016;172:94–105.
[12] Mokarizadeh A, Delirezh N, Morshedi A, Mosayebi G, Farshid AA, Dalir-Naghadeh B. Phenotypic modulation of auto-reactive cells by insertion of tolerogenic molecules via MSC-derived exosomes. Vet Res Forum 2012;3:257–61.
[13] Qiu XC, Jin H, Zhang RY, Ding Y, Zeng X, Lai BQ, et al. Donor mesenchymal stem cell-derived neural-like cells transdifferentiate into myelin-forming cells and promote axon regeneration in rat spinal cord transection. Stem Cell Res Ther 2015;6:105.
[14] Liu Y, Nie L, Zhao H, Zhang W, Zhang YQ, Wang SS, et al. Conserved dopamine neurotrophic factor-transduced mesenchymal stem cells promote axon regeneration and functional recovery of injured sciatic nerve. PLoS One 2014;9:e110993.
[15] Hyatt AJ, Wang D, van Oterendorp C, Fawcett JW, Martin KR. Mesenchymal stromal cells integrate and form longitudinally-aligned layers when delivered to injured spinal cord via a novel fibrin scaffold. Neurosci Lett 2014;569:12–7.
[16] Ren G, Chen X, Dong F, Li W, Ren X, Zhang Y, et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. Stem Cells Transl Med 2012;1:51–8.
[17] Jackson WM, Nesti LJ, Tuan RS. Concise review: clinical translation of wound healing therapies based on mesenchymal stem cells. Stem Cells Transl Med 2012;1:44–50.
[18] Ricart E. Current status of mesenchymal stem cell therapy and bone marrow transplantationin IBD. Dig Dis 2012;30:387–91.
[19] Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, Fichtner S, et al. Intracoronary bone marrow cell transfer after myocardial infarction. Circulation 2006;113:1287–94.
[20] Sudres M, Norol F, Trenado A, Grégoire S, Charlotte F, Levacher B, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol 2006;176:7761–7.
[21] Kim N, Cho S-G. New strategies for overcoming limitations of mesenchymal stem cell-based immune modulation. Int J Stem Cells 2015;8:54–68.
[22] Zhang J, Huang X, Wang H, Liu X, Zhang T, Wang Y, et al. The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy. Stem Cell Res Ther 2015;6:234.
[23] Sharma RR, Pollock K, Hubel A, McKenna D. Mesenchymal stem or stromal cells: a review of clinical applications and manufacturing practices. Transfusion 2014;54:1418–37.
[24] Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood 2005;106:1755–61.
[25] Li YP, Paczesny S, Lauret E, Poirault S, Bordigoni P, Mekhloufi F, et al. Human mesenchymal stem cells license adult CD34+ hemopoietic progenitor cells to differentiate into regulatory dendritic cells through activation of the Notch pathway. J Immunol 2008;180:1598–608.
[26] Joo SY, Cho KA, Jung YJ, Kim HS, Park SY, Choi YB, et al. Mesenchymal stromal cells inhibit graft-versus-host disease of mice in a dose-dependent manner. Cytotherapy 2010;12:361–70.
[27] Gonzalez-Rey E, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009;58:929–39.
[28] Tanaka H, Arimura Y, Yabana T, Goto A, Hosokawa M, Nagaishi K, et al. Myogenic lineage differentiated mesenchymal stem cells enhance recovery from dextran sulfate sodium-induced colitis in the rat. J Gastroenterol 2011;46:143–52.
[29] Liu W, Zhang S, Gu S, Sang L, Dai C. Mesenchymal stem cells recruit macrophages to alleviate experimental colitis through TGFβ1. Cell Physiol Biochem 2015;35:858–65.
[30] Stavely R, Robinson AM, Miller S, Boyd R, Sakkal S, Nurgali K. Allogeneic Guinea pig mesenchymal stem cells ameliorate neurological changes in experimental colitis. Stem Cell Res Ther 2015;6:263.
[31] Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183:7787–98.
[32] Duijvestein M, Wildenberg ME, Welling MM, Hennink S, Molendijk I, van Zuylen VL, et al. Pretreatment with interferon-γ enhances the therapeutic activity of mesenchymal stromal cells in animal models of colitis. Stem Cell 2011;29:1549–58.
[33] Ando Y, Inaba M, Sakaguchi Y, Tsuda M, Quan GK, Omae M, et al. Subcutaneous adipose tissue-derived stem cells facilitate colonic mucosal recovery from 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats. Inflamm Bowel Dis 2008;14:826–38.
[34] Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Adiposederived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterol 2009;136:978–89.
[35] Anderson P, Souza-Moreira L, Morell M, Caro M, O'Valle F, Gonzalez-Rey E, et al. Adipose-derived mesenchymal stromal cells induce immunomodulatory macrophages which protect from experimental colitis and sepsis. Gut 2013;62:1131–41.
[36] Liang L, Dong C, Chen X, Fang Z, Xu J, Liu M, et al. Human umbilical cord mesenchymal stem cells ameliorate mice trinitrobenzene sulfonic acid (TNBS)-induced colitis. Cell Transplant 2011;20:1395–408.
[37] Kim HS, Shin TH, Lee BC, Yu KR, Seo Y, Lee S, et al. Human umbilical cord blood mesenchymal stem cells reduce colitis in mice by activating NOD2 signaling to COX2. Gastroenterol 2013;145:1392–403.
[38] Yabana T, Arimura Y, Tanaka H, Goto A, Hosokawa M, Nagaishi K, et al. Enhancing epithelial engraftment of rat mesenchymal stem cells restores epithelial barrier integrity. J Pathol 2009;218:350–9.
[39] Fawzy SA, El-din Abo-Elnou RK, Abd-El-Maksoud El-Deeb DF, Yousry AbdElkader MM. The possible role of mesenchymal stem cells therapy in the repair of experimentally induced colitis in male albino rats. Int J Stem Cells 2013;6:92–103.
[40] Gonzalez-Rey E R, Anderson P, Gonzalez MA, Rico L, Buscher D, Delgado M. Human adult stem cells derived from adipose tissue protect against experimental colitis and sepsis. Gut 2009;58:929–39.
[41] He XW, He XS, Lian L, Wu XJ, Lan P. Systemic infusion of bone marrowderived mesenchymal stem cells for treatment of experimental colitis in mice. Dig Dis Sci 2012;57:3136–44.
[42] Chen QQ, Yan L, Wang CZ, Wang WH, Shi H, Su BB, et al. Mesenchymal stem cells alleviate TNBS-induced colitis by modulating inflammatory and autoimmune responses. World J Gastroenterol 2013;19:4702–17.
[43] Gonzalez MA, Gonzalez-Rey E, Rico L, Buscher D, Delgado M. Adipose derived mesenchymal stem cells alleviate experimental colitis by inhibiting inflammatory and autoimmune responses. Gastroenterol 2009;136:978–89.
[44] Tanaka F, Tominaga K, Ochi M, Tanigawa T, Watanabe T, Fujiwara Y, et al. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate sodium-induced colitis via anti-inflammatory action in damaged tissue in rats. Life Sci 2008;83:771–9.
[45] Hayashi Y, Tsuji S, Tsujii M, Nishida T, Ishii S, Iijima H, et al. Topical implantation of mesenchymal stem cells has beneficial effects on healing of experimental colitis in rats. J Pharmacol Exp Therapeut 2008;326:523–31.

[46] Marino R, Martinez C, Boyd K, Dominici M, Hofmann TJ, Horwitz EM. Transplantable marrow osteoprogenitors engraft in discrete saturable sites in the marrow microenvironment. Exp Hematol 2008;36:360–8.
[47] Audet J, Miller CL, Rose-John S, Piret JM, Eaves CJ. Distinct role of gp130 activation in promoting self-renewal divisions by mitogenically stimulated murine hematopoietic stem cells. Proc Natl Acad Sci USA 2001;98:1757–62.
[48] Audet J, Miller CL, Eaves CJ, Piret JM. Common and distinct features of cytokine effects on hematopoietic stem and progenitor cells revealed by dose–response surface analysis. Biotechnol Bioeng 2002;80:393–404.
[49] Viswanathan S, Benatar T, Rose-John S, Lauffenburger DA, Zandstra PW. Ligand/receptor signaling threshold (LIST) model accounts for gp130- mediated embryonic stem cell self-renewal responses to LIF and HIL-6. Stem Cell 2002;20:119–38.