Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

An experimental study of preventing and treating acute radioactive enteritis with human umbilical cord mesenchymal stem cells

Rui Wang[1], Wei Yuan[2], Qiang Zhao[2], Peng Song[2], Ji Yue[1], Shi-De Lin[1], Ting-Bao Zhao[1*]

[1]*Jinan Miltary General Hospital, Shandong Province, China*
[2]*People's Hospital of Zhangqiu, Shandong Province, China*

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 15 October 2013
Accepted 15 November 2013
Available online 20 December 2013

Keywords:
Radioactive contamination
Human umbilical cord mesenchymal stem cells
Acute radioactive enteritis
Rat

ABSTRACT

Objective: To test the curative effect of human umbilical cord-derived mesenchymal stem cells on rat acute radioactive enteritis and thus to provide clinical therapeutic basis for radiation sickness. **Methods:** Human umbilical cord-derived mesenchymal stem cells were cultivated *in vitro* and the model of acute radioactive enteritis of rats was established. Then, the umbilical cord mesenchymal stem cells were injected into the rats via tail vein. Visual and histopathological changes of the experimental rats were observed. **Results:** After the injection, the rats in the prevention group and treatment group had remarkably better survival status than those in the control group. The histological observations revealed that the former also had better intestinal mucosa structure, more regenerative cells and stronger proliferation activity than the latter. **Conclusions:** Human umbilical cord-derived mesenchymal stem cells have a definite therapeutic effect on acute radioactive enteritis in rats.

1. Introduction

Radio therapy is an important method to treat malignancy nowadays. However, in the treatment process of abdominal neoplasms, the radials simultaneously cause serious damages to intestinal tract, such as villi damage, mucosal erosion, intestinal vascular embolization and fibrous tissue proliferation which finally lead to intestinal stenosis[1]. The probability of radiation enteritis are increasing gradually in China at present, but it is still in lack of effective means of preventing and treating acute and chronic radioactive enteritis.

Umbilical cord mesenchymal stem cells (MSCs) have such advantages as a variety of sources, easy collection, and convenient transportation. Moreover, they can constantly proliferate *in vitro* without losing any functions, which is superior to other types of MSCs. Therefore, they become an hopeful tool to achieve tissue reconstruction and regeneration[2]. In order to provide new clinical therapeutic MSCs on acute radioactive enteritis, we observed the curative effects of human umbilical cord-derived MSCs in rat model of acute radioactive enteritis.

2. Materials and methods

2.1. Reagents

Mouse antibodies against human CD34-PE, CD45-PE, CD29E, CD44PE, CD73E, CD90E, and CD105-PE were purchased from Cell Signaling Technology (USA).

2.2. Rats and grouping

Sixty male Wistar rats (Laboratory Animal Center of

*Corresponding author: Ting-Bao Zhao, Department of Spinal cord Repairing, Jinan Miltary General Hospital. No. 25, Shifan Road, Tianqiao District, Jinan 250000, Shandong Province, China.
Tel: +86 13153032659
E-mail: doctorzhaotingbao@163.com
Foundation project: This paper was supported by Wu Zuze Science and Technology Development Foundation of Beijing.

Shandong University) with body weight of (200±20) g were divided into six groups randomly, 10 rats in each group, which were blank control group, radiation alone group, normal saline group, stem cells prevention group and stem cells treatment group, and glutamine treatment group.

2.3.Establishment of rat model

The rat model of acute radioactive enteritis was established as described previously[3,4]. The rats were put into about 2 cm thick plastic foam boxes which could not block radioactive radials. Using Elekta Precise linear accelerator (X-rays, Sweden), the irradiation was performed under the following conditions: source-skin distance, 120 cm; absorbed dose rate, 300 MU/min; radiation field, 10 cm ×20 cm; X-ray energy, 6 mV; and total amount, 8 Gy.

2.4. Culture of human umbilical cord-derived MSCs

The human umbilical cord-derived MSCs were cultured *in vitro* as previously described[5]. Umbilical cord of the healthy neonatal (about 30-40 cm) was selected in a sterile environment, put into stroke-physiological saline solution containing 0.1% penicillin-streptomycin, and rinsed in 75% (v/v) ethanol. Then it was cut into 1.5-2.0 cm short segments with surgical scissors. After the umbilical cord skin and artery vessels were removed, the residue was washed with normal saline 2-3 times. The obtained umbilical cord was mince up and transferred into a centrifuge tube. Later, two volumes of 2 mg/L collagenase type I and 100 μL of 0.1% penicillin-streptomycin were added, followed by overnight digestion in a 37 ℃ incubator. The next morning, an equal volume of 2.5 mg/L trypsin solution containing 0.5 g/L EDTA was added for further digestion at 37 ℃ for 30 min. After the digestion, the product was diluted about 8 times with normal saline, mixed well, and centrifuged at 3 000 r/min for 20 min. Following the removal of the supernatants, the yielded pellet was suspended with normal saline and centrifuged at 2 000 r/min for 10 min. After the supernatants were discarded again, the cells were re-suspended in an appropriate amount of culture medium, inoculated into culture bottles at a density of 1×10^6 living cells/cm^2, and incubated in a 37 ℃ incubator containing 5% (v/v) CO_2. When the cell layer covered over 90% area of the bottle, the digestion was performed using 0.5 g/L EDTA-containing 2.5 mg/L trypsin solution and PBS which were mixed in a volume ratio of 1:4. Fresh medium was added to stop digestion when most cells became round. The cell suspension was then centrifuged at 1 500 r/min for 10 min. The yielded cell pellet was diluted with an appropriate amount of culture medium and counted under TCS-SPZ fluorescent inverted confocal microscopy (Leica, Germany). Finally, the cells were inoculated at a density of 3 000-6 000/cm^2 and cultured in a 37 ℃ incubator containing 5% (v/v) CO_2. Generally, the first five generations were better for use.

2.5. Isolation of stem cells by flow cytometry

The human umbilical cord MSCs were collected as described above. The cell pellet was suspended in PBS to obtain single cell suspension. Then each centrifuge tube containing 50 μL of the single cell suspension was added 15 μL of mouse antibodies against human CD34-PE, CD45-PE, CD29E, CD44PE, CD73E, CD90E, and CD105-PE, respectively. Following incubation at room temperature in dark for 30 min, 1 mL PBS was added. After centrifugation at 1 500 r/min for 6 min, the supernatant was removed and the pellet was rinsed in 2 mL PBS by centrifugation at 1 500 r/min for 6 min again. Finally, the cells were re-suspended in 400 μL PBS and the targeted human umbilical cord MSCs were isolated using FACSCanto II flow cytometry (BD Medical Equipment Co., Ltd).

2.6. Therapeutic test

The rats with acute radioactive enteritis in the stem cells prevention group were administrated with 0.4 mL human umbilical cord MSCs suspension at a dose of 1×10^6 cells/kg via tail vein 24 h before the radiation, while those in the stem cells treatment group were given the stem cells in the same way after 24 h post the radiation. Those rats in the normal saline group were given 0.4 mL normal saline via tail vein after 24 h post the radiation, while those in the glutamine treatment group received right vein catheterization after abdominal anesthesia and then administrated with 3% glutamine nutrition intravenously according to their weight. The rats in the radiation alone group and blank control group were not given any treatment.

The rats were kept in separate cages under the same conditions, observed and weighed every 3 days. After 72 h post the irradiation, one rat was selected randomly from each group, and intestine tissues were taken in sterile environment and made into slices. HE staining and immunohistochemistry applied proliferating cell nuclear antigen were conducted for observation under the invert microscope.

3. Results

3.1. Morphological observation of human umbilical cord-derived MSCs

As observed under the invert microscope, the human umbilical cord-derived MSCs became adherent after 1 d post the inoculation, and more cells were adherent on day 2. After the removal of dead cells and cell debris, the adherent cells started to proliferate after 3 days and spread into elliptic, short fusiform, polygon and irregular shapes. On day 14, cell colonies appeared and almost cover the bottle bottom. The cells of the 3rd to 5th generations showed a

uniform long shuttle type and arranged into a spiral or radial pattern, which is the typical appearance of MSCs. Using the flow cytometry, the negative expression of CD34 and CD45 and positive expression of CD29, CD44, CD73, CD90 and CD105 were observed in the isolated MSCs.

3.2. Clinical manifestations of experimental rats

On day 1 post the irradiation, the rats in the prevention group and treatment group had the best spirits, bright eyes, swift response, increased food and water intake, active movements and fights with others. They bit the glove when we tried to catch them. However, those in other groups were somnolent and discharged loose stools. On day 2 post the irradiation, the rats in the prevention group and treatment group still had better mental status, and no obvious changes except loose stools were observed in the rats of the glutamine treatment group. Somnolence was observed yet in other groups. On day 3 post the irradiation, loose stools were found in all groups, especially obvious in the radiation alone group. The rats had back furs in various shades of yellow as well as exfoliated skin and irregular spotted in tails. The best spirits were observed in the rats of the treatment group. After 72 h post the irradiation, the number of dead rats was 1, 2, 1, 0, 1, and 1 in the blank control group, radiation alone group, normal saline group, stem cells prevention group, stem cells treatment group, and glutamine treatment group, respectively. The body weight was not significantly different between the groups.

3.3. Histological changes of experimental rats

The intestine tissues were taken after 72 h post the irradiation. The rats of the blank control group and normal saline group had regular morphology of intestinal mucosa villi as well as normal size, shape and arrangement of mucosa epithelial cells. No hyperaemia, edema and infiltration of inflammatory cells were observed in the mucosa of these rats (Figure 1A). However, the rats of the irradiation group showed radioactive changes in intestinal mucosa, as demonstrated by irregular morphology of intestinal mucosa villi and abnormal size, shape and arrangement of mucosa epithelial cells. Hyperaemia, edema, infiltration of acute and chronic inflammatory cells and local mucosa anabrosis were seen in the radiated rats (Figure 1B). The rats which were given human umbilical cord MSCs 24 h before the irradiation had reversed changes in the intestine tissues. They showed regular morphology of intestinal mucosa villi, mild anabrosis in focal mucosa, and slight hyperaemia, edema and infiltration of a small quantity of acute and chronic inflammatory cells in some regions of the mucosa (Figure 1C). The rats treated with the stem cells and those given glutamine 24 h after the irradiation had changes of different degrees in the structure of intestinal mucosa compared with the blank control group and normal saline group (Figure 1D and 1E), except that anabrosis and inflammatory cell infiltration to various degrees were observed in the treated rats. The immunohistochemical observation revealed remarkably increased number and proliferation activity of mucosa epithelial cells in the treated rats (Figure 2).

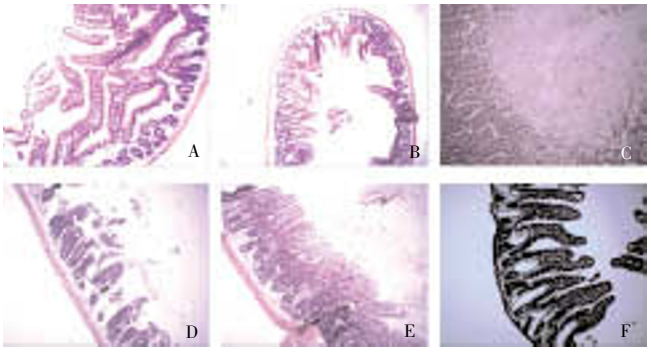

Figure 1. Intestinal mucosa of rats with acute radioactive enteritis after treatment with human umbilical cord-derived mesenchymal stem cells (HE staining, 100×).
A. Blank control group; B. Normal saline group C. Radiation alone group; D. Stem cells prevention group; E. Stem cells treatment group; F. Glutamine treatment group.

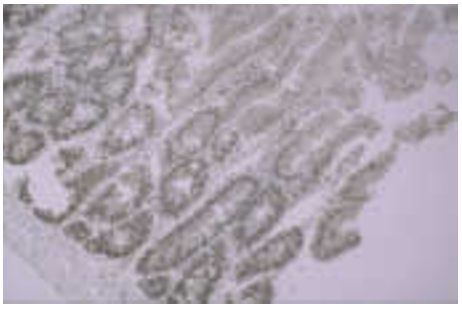

Figure 2. Immunohistochemical test of the rats of the intestinal mucosa of the treatment group (×100).
Proliferating cell nuclear antigen is positive.

4. Discussion

In recent years, the morbidity of intestinal tumor has been at a high level, and radiotherapy has become an important method to treat intestinal tumor. However, radiation enteritis is also accompanied with the treatment. According to the total dose of the radiotherapy, size of radiation field, course time and division method, the course of radiation enteritis can be divided into acute phase and chronic phase[6,7]. The acute radiation enteritis occurs within 1-2 weeks with main manifestations like nausea, emesis, stomachache, acute diarrhea, and tenesmus, while the chronic radiation enteritis generally occurs after several months or years with main manifestations like mucous bloody stool, intestinal stenosis, anal pendant expansion and even intestinal obstruction.

To control symptoms is used to treat acute radiation enteritis, and the measures include the obstruent loperamide, antispasmodic anisodamine, prostaglandins inhibition agent 5-aminosalicylic acid, and intestinal mucosa protective

agent dioctahedral smectite[8]. Pharmaceuticals adjusting the intestinal flora should also be taken at the same time. If the conditions are not well controlled, short-term parenteral nutrition should be given and changed to enteral nutrition later. The measures for the chronic phase include nutritional support, enemas, antioxidant and cytoprotective agents, Chinese herbal medicines, and operation and hyperbaric oxygen therapy if necessary.

Stem cell therapy has hypotoxicity or nontoxicity, and preferable therapeutic effects can be achieved even if the exact pathogenesis of diseases is unclear. Accumulated studies have demonstrated that injection of a certain amount of MSCs before and after radiation can alleviate radials-induced damages to intestinal mucosa, effectively heal intestinal tract and cure radiation enteritis to a certain extent. When *in vivo* environments are suitable, marrow MSCs can replace the necrotic intestinal wall tissues caused by radiation, and they also reconstruct tissues[9]. Zhang *et al* certified the colonization and differentiation capacity of the genetically modified marrow MSCs towards the intestinal tract could be significantly enhanced, and the radioactive damages repair could be promoted[10]. However, it is difficult to acquire and cultivate marrow MSCs.

Human umbilical cord MSCs can be induced to differentiate into various types of adult cells, and autologous stem cells transplantation does not cause immunologic rejection. In this study, we tried to treat acute radiation enteritis using the isolated human umbilical cord MSCs. As shown by the HE stainning test, anabrosis and inflammatory cell infiltration in the intestinal mucosa were observed in all groups except in the control. The rats in the radiation alone group had the most serious intestinal lesions. These pathological changes of the intestinal mucosa were also confirmed by the loose stools in clinic. Histological observation revealed that the irradiated rats in the prevention group, stem cells treatment group and glutamine treatment group had regular morphology of intestinal mucosa villi and normal quantity of the mucosa epithelial cells, except that slight inflammatory infiltration was visible. The rats treated with the stem cells 24 h after the irradiation had the mildest intestinal changes. The results indicate that the intestinal mucosa could repair itself with the help of the human umbilical cord MSCs. Previous researches also demonstrated that stem cells could locate in radioactively damaged intestinal tract and help the structural recovery of intestinal tract, thus showing therapeutical effects[11,12]. The immunohistochemical tests also showed the regeneration of intestinal epithelial cells. In addition, although glutamine also help to repair the intestinal tract, its therapeutic effects were not as good as those of the human umbilical cord MSCs.

In summary, we verified the curative effects of human umbilical cord MSCs on acute radioactive enteritis in rats. The prevention with human umbilical cord MSCs before irradiation also showed certain therapeutical effects on radicals-induced intestinal damages. A lot of research works are required for application of MSCs clinically.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This paper was supported by Zu-Ze Wu Scientific and Technological Development Foundation of Beijing.

References

[1] Zhao YZ, Gao CF, Liu CX. Effects of glutamine parenteral nutrition on the proliferation of rat intestinal epithelial cells with radiation enteritis. *Practical J Med Pharm* 2007; **24**: 1493-1495.

[2] Ma XH, Feng K, Shi BY. Biological characteristics and research progress of human umbilical cord mesenchymal stem cells. *Chin J Tissue Engin Res* 2011; **15**(32): 6064-6067.

[3] Shan XZ, Han L, Ma AG, Shang QJ. Effects of beta-carotene on intestine mucosa barrier function in rats damaged by X-ray. *Parent Enteral Nutri* 2009; **16**(6): 346-350.

[4] Ding XF, Li DX, Zhao L. Research of the concentration of nitric oxide and the organization structure variation of rat intestinal mucosa after radioactive damages. *Chin J Cancer Prev Treat* 2003; **10**(3): 283-285.

[5] Fan XB, Liu TQ, Hao YJ, Liu Y, Ma XH, Cui ZF. Optimization for dissociation and culture of mesenchymal stem cells derived from umbilical cord blood. *Prog Biochem Biophys* 2008; **35**(8): 905-913.

[6] Van Halteren HK, Gortzak E, Taal BG, Helmerhorst TJ, Aleman BM, Hart AA, et al. Surgical intervention for complication caused by late radiation damage of the small bowel a retrospective analysis. *Eur J Surg Oncol* 1993; **19**: 336-341.

[7] Carratu R, Secondulfo M, de Magistris L, Daniele B, Pignata S, D'Agostino L, et al. Assessment of small intestional damage in patients treated with pelvic raiotherapy. *Oncol Rep* 1998; **7**(5): 635-639.

[8] Liu MD, Chen LD. Curative effects of mosapride citrate on reflux esophagitis. *Chin J Digest Endosc* 2003; **20**(6): 415.

[9] Song XF. *Study on bone marrow mesenchymal stem cells in repair of radation intestinal*. Public Health, Jilin University 2008.

[10] Zhang J, Zhu WM, Gong JF, Zhang W, Li Ning, Li JS. Role of gene-modified bone marrow mesenchymal stem cells in repair of radiation-induced intestinal injury. *Parent Enteral Nutri* 2008; **15**(3): 65-73.

[11] Liu DQ, Wang FC, Dong SW. Research of the colonization and differentiation of donor bone marrow-derived stem cells in bone marrow chimeramouse intestinal epithelium. *Chin J Gastroenterol Hepatol* 2008; **17**(8): 635-638.

[12] Scmont A, Francois S, Mouiseddine M, Francois A, Sache A, Frick J, et al. Mesenchymal stemcells increase self-renewal of small intestinal epithelium and accel-erate structural recovery after radiation injury. *Adv Exp Mad Biol* 2006; **585**(1): 19-30.