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The experimental research on the treatment of rabbits with acute cornea alkali burn by taking bone marrow mesenchymal stem cell

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ABSTRACT

Objective: To study the effect of bone marrow mesenchymal stem cell(BMSCs) on angiogenesis and inflammation in rabbits with acute cornea alkali burn. **Methods**: New Zealand White Rabbit were chosen, while model of cornea alkali burn was made and divided into BMSCs group and control group. Rabbits in the BMSCs group were injected with BMSCs and those in the control group received PBS Solution via ear vein. Cornea turbidity and the angiogenesis area were measured at 20 d, 40 d, 60 d. The expression of angiogenic and inflammation response cytokines were measured at 60 d. **Results**: At 20 d, 40 d, 60 d, the cornea turbidity in BMSCs group was lower than that in control group, and the angiogenesis area in BMSCs group was smaller than that in control group; At 60 d, the mRNA expression of HIF-1 α , VEGF, MMP2, MMP9, TLR2, TLR4, IL-1 α ,IL-1 β , TNF- α , ICAM-1 in cornea of BMSCs group was substantially Lower than that of control group, while BMSCs group was substantially higher than control group on mRNA expression of PEDF, TIMP1, TIMP2. **Conclusions**: BMSCs has inhibitory effect on angiogenesis and inflammatory response so that it is conducive to the healing of cornea alkali burn.

1. Introduction

Caused by contact with alkaline chemicals, cornea alkali burn is a common type of cornea injuries clinically[1]. Bone marrow mesenchymal stem cell(BMSCs) belonging to marrow stromal cell lines, is a kind of non-hematopoietic adult stem cells, which can repair tissues with its self-renewal and multiple differentiation potential. In cornea treatment, BMSCs can not only become the source of seed cells, but also secrete varieties cytokines in injury part reactively. Participating in multiple links of tissue damage repair in the treatment of corneal injury[2-3]. In recent years, BMSCs have displayed a good effect on the treatment of corneal injury, acute lung injury, acute myocardial infarction, liver injury, intervertebral disc injury and other diseases[4-6]. Besides cell apoptosis and injury caused by morbidity factors, angiogenesis and inflammation are important factors that influence recovery of cornea, and result in falling eyesight. In the following study, we analyzed the value of BMSCs in the treatment of acute rabbit corneal burn with acute rabbit corneal alkali burn as the model, and from the perspective of angiogenesis and inflammatory response.

2. Materials and methods

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2.1.Experimental materials

The New Zealand white rabbit was selected as an experimental objective, and the experiment was approved by the hospital ethics committee. Fetal bovine serum for cell culture, and DMEM F12 culture medium were purchased from Hyclone, The RNA extraction kit, reverse transcription kit and fluorescence quantitative PCR kit from Takara. The gene specific primers were synthesized by Shanghai shenggong company.

2.2. Modelling method

The corneal alkali burn model was prepared by injection of sodium pentobarbital via ear vein, and the method is as following: The circular filter paper (inner diameter 8 mm, outer diameter 14mm) was soaked in 1 mol/L sodium hydroxide solution and placed in the right eye cornea, then washed for 5 min with normal saline after 45 sec. Model animals with Corneal alkali burn grade III above were used for follow-up study.

2.3. Grouping and intervention methods

Animals with Corneal alkaline burn were randomly divided into BMSCs group and control group. BMSCs group was interfered by one-time injection of BMSCs into the ear vein. The methods is as following: The New Zealand white rabbit, which was 1 month old, was punctured and extracted bone marrow and then cultured BMSCs. After that, the third generation cells were taken and diluted with PBS solution, and the BMSCs were injected with a dose of 3.5×10^6 /kg. The control group was treated with a same dose of PBS solution through one-time injection into ear edge vein.

2.4. Evaluation of turbidity of cornea

After the intervention of 20 d, 40 d and 60 d, 10 animals selected from each group were killed , and the corneal tissue of alkali burn was observed under the slit lamp.

The turbidity was judged by the following criteria: no turbidity was 0 point; light turbidity and iris texture could be seen was 1 point; Medium turbidity and iris texture was not clear 2 points; Heavy turbidity and pupil dimly visible was 3 points; Extremely heavy turbidity and the pupil invisible was 4 points.

2.5. Measuring method of corneal neovascularization

20 d, 40 d, 60 d after the intervention, corneal turbidity observation was finished. Observation under the microscope showed that lamellar sclera edge grew, with continuous small bending and towards the longest vessel in the alkali burn, measuring the length and quantity, and then calculating the area of new blood vessels.

2.6. Detection methods of molecular expression of angiogenesis and inflammatory reaction

At 60 d after intervention, a proper amount of corneal tissue was taken from the alkali burn, then use the kit to separate inside RNA which then synthesised for cDNA through reverse transcription reaction, and then conducted fluorescent quantitative PCR reaction of cDNA. primers used respectively for HIF-1 α , VEGF, PEDF, MMP2, MMP9, TIMP1 and TIMP2, TLR2 and TLR4, IL-1, IL-1 β , TNF- α , ICAM-1, then calculated the amount of mRNA expression based on PCR reaction curve.

2.7. Statistical method

The data was input and analyzed by SPSS20.0. The measurement data was analyzed by *t*-test. Difference was regarded as statistical significance when $P < 0.05$.

3. Results

3.1. Cornea turbidity and angiogenesis area

After the intervention of 20 d, 40 d and 60 d, the corneal turbidity of the two groups and the angiogenesis area were compared as follows: the corneal turbidity of BMSCs group was lower than that in the control group, while angiogenesis area in BMSCs group was smaller than that in control group. The BMSCs group and the control group were statistically significant at 20 d, 40 d and 60 d after intervention ($P < 0.05$)(Table 1).

Table 1
Comparison between cornea turbidity and angiogenesis area. ($\bar{\chi} \pm s$)

Index	Time	BMSCs	Control	*P*
Cornea turbidity	20 d	2.41 ± 0.39	3.24 ± 0.41	<0.05
	40 d	2.12±0.35	2.70±0.38	<0.05
	60 d	1.79±0.24	2.31±0.34	<0.05
Angiogenesis area	20 d	19.83±2.41	26.28±3.61	<0.05
	40 d	16.38±2.14	21.35±3.24	<0.05
	60 d	12.91±1.89	16.78±2.25	<0.05

3.2.The expression of angiogenesis cytokines in cornea

After the intervention of 60 d, the expression of angiogenesis factor HIF-1 α , VEGF, PEDF, MMP2, MMP9, TIMP1 and TIMP2 in two groups of corneal tissues were analyzed as follows: The mRNA expression of HIF-1 α , VEGF, MMP2, MMP9 in cornea of BMSCs group was substantially lower than that of control group, while BMSCs group was substantially higher than control group on mRNA expression of PEDF, TIMP1, TIMP2. The differences in the expression of HIF-1 α , VEGF, PEDF, MMP2, MMP9, TIMP1 and TIMP2 mRNA in the corneal tissues were statistically significant ($P < 0.05$) at 60d after intervention of BMSCs group and control group.

(Table 2).

Table 2
Comparison of the expression of angiogenic factors in the cornea between the two groups. ($\bar{\chi}$ ±s)

Index	BMSCs	Control	*P*
HIF-1 α	0.31±0.08	1.00±0.17	<0.05
VEGF	0.42±0.07	1.00±0.14	<0.05
PEDF	2.31±0.36	1.00±0.11	<0.05
MMP2	0.48±0.09	1.00±0.19	<0.05
MMP9	0.35±0.07	1.00±0.18	<0.05
TIMP1	1.98±0.26	1.00±0.08	<0.05
TIMP2	2.42±0.39	1.00±0.16	<0.05

3.3.Expression of inflammatory response factors in cornea.

After the intervention of 60 d, the expression of inflammatory response factors TLR2, TLR4, IL-1 α , IL-1 β , TNF- α , ICAM-1 in cornea were analyzed as follows: The mRNA expression of TLR2, TLR4, IL-1 α , IL-1 β ,TNF- α , ICAM-1 in cornea of BMSCs group was substantially below that of control group. The differences in the expression of TLR2, TLR4, IL-1 α , IL-1 β , TNF- α , ICAM-1 mRNA in the corneal tissues were statistically significant ($P < 0.05$) at 60 d after intervention of BMSCs group and control group. (Table 3)

Table 3
Comparison of expressions of inflammatory response factors in cornea between the two groups ($\bar{\chi}$ ±s)

Index	BMSCs	Control	*P*
TLR2	0.29±0.06	1.00±0.12	<0.05
TLR4	0.33±0.07	1.00±0.16	<0.05
IL-1 α	0.49±0.08	1.00±0.17	<0.05
IL-1 β	0.22±0.06	1.00±0.12	<0.05
TNF- α	0.41±0.08	1.00±0.10	<0.05
ICAM-1	0.36±0.07	1.00±0.09	<0.05

4. Discussion

Contacting with cornea, alkaline chemical is the reason that cased Corneal alkali burns, having direct damage to cornea. On the one hand, it can destroy corneal cells by producing heat; one the other hand, it can dehydrate cells and cause corneal cell decomposition and necrosis, and severe cases can cause corneal ulcers and perforation and left eye defects[7-8]. In the repairing phase, as a crucial biological link, angiogenesis can provide sufficient oxygen and nutrients for corneal repair, blocking further injury of cornea. However, in the process of continuous repair of injury, angiogenesis mediated by angiogenesis and inflammatory cytokines can cause scar formation of cornea, adhesion of blepharon, which result in further impairment of visual acuity[9-10]. In recent years, BMSCs has been paid more and more attention to the value of tissue damage repair. with multiple differentiation potential, BMSCs can differentiate and become the cell source of tissue repair in the injury part[11-13]. It has been reported that BMSCs can be differentiated into corneal epithelial cells, corneal stromal cells, corneal endothelial cells, and repair corneal injury[14-16]. However, the effect of BMSCs on angiogenesis in the repair of corneal injury is not clear. In the above study, we studied the corneal alkali burn of rabbits and gave BMSCs intervention by means of auricular intravenous injection. From comparison of the corneal turbidity and the angiogenesis area between the intervention group and the control group, we can know: The corneal turbidity of BMSCs group was lower than that in the control group, while angiogenesis area was smaller than that in the control group. This indicates that BMSCs can improve the transparency of corneal alkali burn healing process, reduce the number and area of new blood vessels, and facilitate the repair and healing of cornea.

In the corneal injury repair process, the formation of angiogenesis is related to the abnormal expression of various angiogenic factors. VEGF is the most critical control factor in the process angiogenesis, and it can direct effects on receptors of endothelial surface layer and promote proliferation of endothelial cell and induced the formation of the vascular structure pattern, and then participate in the control during angiogenesis process[17]. Corneal damage local hypoxia environment is one of the important pathological factors inducing VEGF expression increased. Entering into the nucleus, HIF-1 α can activate the transcription of VEGF, increase the stability of VEGF mRNA, and then increase the expression of VEGF, and enhance the angiogenesis effect mediated by VEGF[18]. MMP2 and MMP9 are members of the MMPs family that have hydrolyzed extracellular matrix and effect of promoting cell invasion. In the repair process of corneal injury, it can promote the angiogenesis process by promoting the infiltration of endothelial cells in the wound surface[19-21]. PEDF, TIMP1 and TIMP2 are inhibitors of angiogenesis. PEDF can inhibit the proliferation of endothelial cells mediated by VEGF, and TIMP1 and TIMP2 can inhibit the hydrolysis effect of MMP2 and MMP9[22-23]. In order to further clarify the influence of BMSCs intervention on angiogenesis in corneal alkali burn healing, we analyzed the expression of the above-mentioned angiogenic factors in corneal tissue, and the results showed: The mRNA expression levels of HIF-1 α , VEGF, MMP2 and MMP9 in the cornea tissues of BMSCs were significantly lower than those in the control group, and the mRNA expression levels of PEDF, TIMP1 and TIMP2 were significantly higher than those in the control group. This means that BMSCs in the process of corneal alkali burn healing can inhibit the expression of angiogenic factors and promote the expression of angiogenesis inhibiting factors, which can inhibit angiogenesis process.

The formation of neovascularization in the corneal wound is not only directly regulated by angiogenic factors, but also affected by the

inflammatory response. Inflammation is an important pathological changes throughout corneal alkali burn. On the one hand, a variety of inflammatory cytokine secretion can cause damage to diverse cells within the tissue, on the other hand, it can promote endothelial cell migration, infiltration within the cornea and participate in the formation of angiogenesis[24]. TLR2 and TLR4 are the key upstream regulators of inflammation in local tissues, both of which belong to the pattern recognition receptor. It is able to dissociate the transcription factor NF-kB with the inhibitory factor by downstream MyD88 dependent pathway and non-myd88 after the activation of exogenous pathological factors, and then transfer into the nucleus and launch a variety of inflammatory cytokines[25]. IL-1 α, IL-1 β, TNF- α, and ICAM-1 are downstream cytokines regulated by TLR2 and TLR4, which play an important role in various stages of inflammatory response. IL-1 α, IL-1 β all belong to IL-1, synthesis and release at the beginning of the infection, can promote neutrophil activation, inducing mononuclear macrophage infiltration, basophils and mast cells degranulation and, in turn, cause inflammation cascade activation. TNF- α is a pro-inflammatory cytokine secreted by mononuclear macrophages, which can directly mediate the inflammatory process of local tissues and promote the activation and infiltration of various inflammatory cells. ICAM-1 is an intercellular adhesion molecule, which promotes the adhesion of inflammatory cells and endothelial cells in the local corneal injury, which promotes the cascade amplification of inflammatory response and the formation of angiogenesis. In order to further clarify the effect of BMSCs intervention on the inflammatory response of corneal alkali burn, we analyzed the expression of the above-mentioned inflammatory response factors in corneal tissue and the result is as follows: The mRNA expression levels of TLR2, TLR4, IL-1 α, IL-1 β, TNF- α and ICAM-1 in the corneal tissues of BMSCs were significantly lower than those in the control group. This indicates that BMSCs has inhibitory effect on the expression of inflammatory response factors in corneal alkali burn, which can inhibit the inflammatory response.

Conclusion: BMSCs can improve corneal transparency and reduce the number of angiogenesis. At the molecular level, BMSCs can inhibit the angiogenesis and inflammatory response of corneal alkali burn, which can help to heal the corneal burn.

Conflict of interest statement

The author reports no conflict of interest.

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