DOI 10.1007/s10517-017-3742-x

ONCOLOGY

Effects of Bone Marrow Multipotent Mesenchymal Stromal Cells and Their Secretory Products on Microcirculation in the Broad Ligament of the Uterus of Wistar Rats during Experimental Chronic Genital Inflammation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 163, No. 1, pp. 93-97, January, 2017
Original article submitted January 25, 2016

Effects of bone marrow multipotent mesenchymal stromal cells and their secretory products released into the conditioned medium on microcirculatory bed in the broad ligament of the uterus were studied in Wistar rats with chronic genital inflammation. Opposite changes in the parameters of microcirculation and lymphatic drainage in the broad ligament of the uterus were observed after administration of cells and conditioned medium via different routes, which should be taken into account during the treatment of inflammatory and degenerative processes in the pelvic organs.

Key Words: *multipotent mesenchymal stromal cells; conditioned medium; microcirculation; broad ligament of the uterus; chronic inflammation*

Chronic inflammation of the pelvic organs is the most frequent cause of reproductive disorders in women [5]. Chronic inflammatory diseases of the uterus and epididymis taking a long-term and relapsing course can led to infertility, pregnancy loss, and fetus pathologies. Therapy is not always effective, and therefore, the search for new treatment options is of specific interest. Inflammation during chronic salpingo-oophoritis (ICD-10) is associated with impaired microcirculation in the lower pelvis. These impairments correlate to the duration and stage of the inflammatory process [4,7]. As changes in the vascular system play an important role in the pathogenesis of chronic salpingitis [1,10], pathogenic approach to correction of these disorders should be developed. Recently, cellular therapy was effectively applied for the treatment of various pathologies. Published data suggest that the mechanism of action of transplanted bone marrow multipotent mesenchymal stromal cells (BM-MMSC) is based on secretion of bioactive molecules leading to activation of angiogenesis and tissue reparation and reduction of fibrous tissue growth [9,11]. We have previously demonstrated the effects of BM-MMSC and conditioned medium (CM) of BM-MMSC on the blood and lymphatic vessels in the broad ligament of the uterus of intact animals [3].

Here we studied the effects of BM-MMSC and CM on the parameters of microcirculation during experimental chronic genital inflammation.

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MATERIALS AND METHODS

The experiments were performed on female Wistar rats (n=40) weighing 260-330 g in accordance to the Rules for the Use of Experimental Animals and principles of humanity. The animals were kept on standard laboratory diet and had free access to water.

Group 1 consisted of intact rats (control). Chronic genital inflammation was modeled in group 2 females. Female rats of group 3-8 with experimental chronic genital inflammation received the following injections: single intravenous injection of BM-MMSC (2×10^6 cells in 0.5 ml physiological saline (PS); group 3); single lymphotropic injection of BM-MMSC (2×10^6 cells in 0.1 ml of PS under the vaginal mucosa; group 4); single intravenous injection of CM (0.5 ml; group 5); single lymphotropic injection of CM (0.1 ml under the vaginal mucosa; group 6); an intravenous injection of PS (0.1 ml, active control; group 7), single lymphotropic injection of PS (0.1 ml, active control; group 8). Each group consisted of 5 animals.

Inflammation was modeled by administration of daily culture of *Staphylococcus aureus* (strain 24943) in a dose of 3×10^6 microbial bodies in accordance to the opacity standard under the vaginal mucosa [7]. BM-MMSC from the femoral bones of Wistar rats (n=5) and CM from these cells were obtained as described previously [6]. BM-MMSC of passages 2-4 were used. BM-MMSC and CM were administered on day 21 of induced inflammation. On day 9 after the injection of BM-MMSC and CM, the animals were decapitated under light ethaminal narcosis. For evaluation of the effects of BM-MMSC and CM on parameters of microcirculation, the broad ligament of the uterus was mounted on a slide, exsiccated, and stained with hematoxylin and eosin. Film preparations were examined under an Axioplan light microscope (Carl Zeiss) equipped with a digital camera (×50). Images were analyzed using Image-Pro Plus 4.1 software. Vessel diameter was measured in microns. The number of blood and lymphatic vessels in the field of view was calculated in the film samples using a built-in net. The number of lymphoid cell clusters (micro lymph nodes) and structures resembling tissue lymphatic nodes in the tissues of the broad ligament of the uterus were calculated under a light microscope at ×400 in each field of view in the whole sample. The results were recalculated as the mean number of cells and clusters in the field of view.

Statistical analysis of the obtained data was performed using Statistica 6.0 software. The arithmetic mean (M) and standard error of the mean (SE) were calculated using the methods of descriptive statistics. Comparison of results was conducted using Student's t test, as preliminary examination showed normal distribution of obtained data (Kolmogorov—Smirnov criterion). The differences were significant at $p<0.05$.

RESULTS

Chronic inflammation was accompanied by an increase in the diameter of veins, capillaries, and lymphatic vessels. Intravenous administration of BM-MMSC to rats with inflammation was followed by a decrease in the number of veins; the diameter of veins, blood capillaries, and lymphatic vessels decreased to a level of intact control. Under these conditions, the number of capillaries and lymphatic vessels increased and surpassed the control level. Lymphotropic administration of BM-MMSC decreased the number of veins and increased the number of capillaries and lymphatic vessels. The diameter of all vessels decreased to a level of intact control. Intravenous administration of CM led to an increase in the number of blood vessels and a decrease and normalization of the diameters of all vessels. Lymphotropic injection of CM was accompanied by a decrease in the number of blood vessels and normalization of the diameter of blood capillaries, lymphatic vessels, and veins. Intravenous and lymphotropic administration of CM normalized the number of lymphatic vessels and diameter of blood and lymphatic vessels (Table 1).

Lymphotropic and intravenous administration of BM-MMSC led to a decrease in number of in comparison with the model group, intact and active controls. The number of blood capillaries did not change during the inflammation, but increased after all types of administration of BM-MMSC relative to the model group, active and intact controls. The number of lymphatic vessels during chronic inflammation did not differ from the intact control. After intravenous and lymphotropic administration of BM-MMSC, this parameter increased in comparison with the model group, active and intact controls. Vein diameter increased after lymphotropic injection of CM in comparison with the model group, active and intact controls. Diameter of blood capillaries increased during chronic inflammation. This parameter reduced after the intravenous and lymphotropic administration of BM-MMSC comparing to the model group and active control to the level of intact control. Thus, the effects of BM-MMSC administered via different routes on parameters of microcirculation in the broad ligament of the uterus were shown: the number of microcirculatory vessels (blood and lymphatic) increased, but their diameter decreased. This fact reflects intensification of hemolymph circulation and lymphatic drainage, which can be related to the reduction of intensity of inflammatory process. CM administration did not affect these processes.

TABLE 1. Parameters of Microcirculation in the Broad Ligament of the Uterus of Wistar Rats with Chronic Genital Inflammation after Administration of BM-MMSC and CM of BM-MMSC (*M±SE*)

Parameter	Intact control	Chronic inflammation	BM-MMSC, intravenous	BM-MMSC, lympho-tropical	CM, intravenously	CM, lymphotropi-cal	PS, intravenous	PS, lympho-tropical
Number of veins	1.50±0.5	1.33±0.33	0.6±0.3^{+*}	0.14±0.14^{*+o}	1.28±0.50	2.0±0.6^{x}	1.86±0.74^{o}	1.80±0.29^{x}
Number of capillaries	27.75±4.02	32.89±5.62	61.50±4.95^{*+}	71.6±7.7^{*+}	57.86±6.32^{*+}	51.0±3.5^{*+x}	49.71±3.83^{+o}	48.60±4.73^{+x}
Number of lymphatic capillaries	23.75±2.28	31.78±4.01	49.1±4.6^{*+}	58.90±6.12^{*+}	31.14±3.48^{o}	38.83±2.86^{+x}	28.57±2.56^{o}	40.90±4.23^{*+x}
Vein diameter	14.59±0.85	19.24±0.73^{+}	14.20±0.85	13.79±1.66*	13.46±0.6*	18.06±0.8^{+x}	12.84±0.54*	13.85±0.61*
Diameter of capillaries	4.23±0.17	5.48±0.13^{+}	4.40±0.07	4.10±0.09*	5.01±0.10^{*o}	5.11±0.11^{x}	5.25±0.11^{o}	5.63±0.10^{x}
Diameter of lymphatic capillaries	7.85±0.24	12.35±0.36^{+}	8.11±0.16*	7.60±0.15*	7.74±0.23^{*o}	9.97±0.24^{*x}	8.94±0.22*	8.48±0.19*

Note. Here and in Table 2: $p<0.05$ in comparison with $^{+}$intact control, *chronic inflammation group, ^{o}group receiving BM-MMSC intravenously, ^{x}group receiving BM-MMSC lymphotropically.

TABLE 2. Lymphoid Aggregates in the Broad Ligament of the Uterus of Wistar Rats with Chronic Genital Inflammation after Administration of BM-MMSC and MS of BM-MMSC (*M±SE*)

Parameter	Intact control	Chronic inflammation	BM-MMSC, intravenous	BM-MMSC, lymphotropi-cal	CM, intravenously	CM, lympho-tropical	PS, intravenous	PS, lympho-tropical
Micro lymph nodes	1.53±0.10	1.14±0.40^{+}	3.92±0.04^{*+}	3.57±0.60^{*+}	2.83±0.30^{*+o}	3.81±0.10^{*+}	4.26±0.46^{*+}	3.67±0.39^{*+}
Tissue lymph nodes	0	0.04±0.03^{+}	0.20±0.03^{*+}	0.40±0.07^{*+o}	0.10±0.03^{*o}	0.5±0.1^{*+}	0.3±0.1^{*+}	0.30±0.07^{*+}

It should be emphasized that the administration of BM-MMSC has maximal correcting effect on the state of vessel nets (number of blood veins and capillaries of both blood and lymphatic systems) during inflammation. It should be noticed that cellular therapy results in a decrease in venous outflow, which probably increases the accumulation of cells in the inflammation locus. Similar administration of the biomedical product results in a quantitative increase in blood and lymphatic capillary nets, which might be related to the enhancement of their drainage function. CM practically does not affect the study parameters, and this fact does not confirm the current hypothesis on mostly paracrinous activity of used cellular technologies.

Single lymph nodes appeared during chronic inflammation, but the number of micro lymph nodes reduced. Probably, loose micro lymph nodes transformed into more structured lymph nodes during inflammation [8], which may reflect the stimulation of lymphoid tissue by a bacterial antigen. Administration of BM-MMSC and CM intravenously and lymphotropically was accompanied by a significant increase in the number of micro lymph nodes comparing to the model group. This effect was more pronounced after intravenous administration of BM-MMSC comparing to CM (Table 2). Number of lymph nodes increased also after the injection of BM-MMSC and CM by both described methods. This parameter was higher after intravenous administration of BM-MMSC comparing to lymphotropic injection and intravenous administration of CM. Dynamics of changes in the number of lymph nodes in the broad ligament of the uterus after treatment with BM-MMSC and CM did not differ from it in the active control (PS injection). Thus, it might be suggested that the administration of BM-MMSC and CM has non-specific effects on the lymphoid tissue, which is similar to effects of injection-induced stress. Probably, any stress exposure induces defensive response manifested in the generation of additional lymphatic structures for relief of regional lymph nodes [2], and stimulation of local immunity.

Performed study reflects that effects of BM-MMSC and CM of BM-MMSC on the parameter of microcirculation depend on the type of administration (intravenous or lymphotropic). The most pronounced effects of hemo microcirculation and lymphatic drainage are observed after both types of administration of BM-MMSC. CM of BM-MMSC increases only the diameter of veins after lymphotropic administration, which might be considered as venostasis during retaining impairments of microcirculation. These results should be taken into account for estimation of indications and contraindications of treatment and development of new methods of cellular therapy.

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