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Effect of Bone Marrow Multipotent Mesenchymal Stromal Cells on Blood and Lymphatic Vasculature in Uterine Wall of Wistar Rats during Experimental Chronic Metroendometritis

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The study examined the effect of bone marrow multipotent mesenchymal stromal cells and their products secreted into the conditioned medium on uterine microcirculatory bed in Wistar rats during chronic inflammation. The parameters of blood and lymphatic microcirculation in the uterus changed in various directions in relation to the administration routes of biomedical cell product, which is important when the cell therapy is employed in the treatment of inflammatory-degenerative alterations in the lesser pelvis organs.

Key Words: *multipotent mesenchymal stromal cells; endometrium; myometrium; microcirculation; c*hronic *endomyometritis*

Chronic endometritis ranks first in the structure of intrauterine pathology of female infertility [7]. The course and development of chronic inflammation in organs of the lesser pelvis are mostly affected by microcirculatory deficiency [3]. The consequential hypoxia activates anaerobic flora, down-regulates local immunity, and inhibits the reparative processes. The damage to endometrium caused by inflammation underlies the disturbances of implantation and the development of its pathologic deviations [2].

The treatment of chronic endomyometritis is a challenging and important problem especially in women with reproduction failures in anamnesis [1]. The modern methods of cell therapy are widely employed to stimulate the regeneration processes [11]. There are data attesting to the anti-inflammatory effects of multipotent mesenchymal stromal cells (MMSC) and indicating production of the pro- and anti-inflammatory cytokines by these cells [4,8,9]. MMSC are used in the treatment of hematological, autoimmune, cardiovascular, and osteoarticular diseases [10,12]. However, there are no data on the use of MMSC and their secreted products for treatment of endomyometritis. Unfortunately, current methods employed to cure this pathology are rather complicated and low effective.

This work was designed to study the anti-inflammatory effects of MMSC and their secreted products in rats with experimental *c*hronic endomyometritis.

MATERIALS AND METHODS

Experiments were carried out on female Wistar rats (n=40) weighing 260-300 g in strict adherence to European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The animals were maintained on standard ration with water *ad libitum*.

The rats were randomly assigned to one of 8 groups (5 animals each). The groups 1 and 2 comprised the intact and experimental control rats, respectively. The latter group was examined on day 21 after

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induced chronic endometritis (CE). Other CE rats differed in the mode of administration of the biomedical cell product. Rats of groups 3, 5, and 7 were intravenously injected with MMSC (2×10^6 cell/0.5 ml), conditioned medium (CM) of MMSC culture, or physiological saline (PS), respectively. Groups 4, 6, and 8 received intralymphatic (lymphotropic) injections of MMSC (2×10^6 cells in 0.1 ml), CM, or PS, respectively.

CE was provoked by injection of one-day culture of S. aureus (strain 24943) in a dose of 3 mln microbial CFU according to the turbidity standard [5]. MMSC and their CM were obtained from Wistar rat femora (n=5) according to [4]. In 9 days after combined MMSC+CM injection, the rats were decapitated under a weak sodium etaminal narcosis. To assess the effect of MMSC+CM, the uterus was isolated and fixed in 10% neutral formalin for histological analysis.

The paraffin sections were routinely stained with hematoxylin and eosin to examine under an Axioplan light microscope (Carl Zeiss, eyepiece 10×, objective 5×) equipped with a digital camera. The images were processed with Image-Pro Plus 4.1 software to determine the width of myometrium and endometrium, as well as the diameter of blood and lymph vessels.

The data were analyzed statistically using Statistica 6.0 software. Normalcy of distribution was established by Kolmogorov—Smirnov test. Significance was assessed with Student's t test at $p<0.05$. The results are presented as m±SEM.

RESULTS

The pathogen-induced CE was accompanied by pronounced alterations in the balance between basic uterine structures manifested by a marked thickening of endometrium resulting from an increase in the number of glands, which were clusterized and overfilled with secretions. The destructive processes in epithelium and stroma of the uterine glands were manifested with intensive lymphocytic infiltration and elevation of neutrophils, macrophages, and eosinophils at the background of insignificant decrease of myometrium thickness. The endometrium/myometrium transverse size ratio increased from 0.95 to 1.62 (Table 1).

During CE, the state of myometrium was characterized by increased diameters of arterial and venous vessels together with diminished diameters of the capillaries and lymph vessels (Table 1). The lumens of vessels contained erythrocytic sludge, leukocytic clusters, and fibrin. Intravenous injection of MMSC and CM restored the arterial diameter to the level of intact group. At this, only solitary leukocytes and lymphocytes were observed in their lumens. In contrast, lymphotropic injection of MMSC increased arterial diameter in comparison with intact, experimental control, and related PS-treated groups. The arterial lumens contained erythrocytic sludge, fibrin filaments, and occasional leukocytes. Lymphotropic injection of CM constricted the myometrial arteries, but the effect was significant only in comparison with PS-treated group. Although diameter of these vessels decreased in comparison with experimental control group, it remained increased relatively to the intact rats. The vascular lumens had no leukocytes, erythrocytic sludge, or fibrin.

In experimental control group, the diameter of myometrial veins increased pronouncedly. Lymphotropic injection of MMSC restored this parameter to the intact level, while after intravenous injection of MMSC, it remained somewhat larger than that in the intact group, although it was smaller than the diameter of myometrial veins in the related PS-group. Interestingly, intravenous injection of CM produced no effect on this parameter. In experimental control rats, the diameters of lymph vessels and capillaries were smaller than the corresponding values in the intact group. Injection of PS increased this parameter, which probably resulted from disturbance of contractile potency of the lymph vessels similar to what is typical under stressful conditions [6]. Both intravenous and lymphotropic injections of MMSC and CM constricted the myometrial lymph vessels in comparison with corresponding PS-groups, although this parameter did not attain the level characteristic of the intact rats (Table 1). It can be hypothesized that both routes of MMSC administration improved the lymphatic drainage of myometrial tissues, because they simultaneously constricted veins and the lymph vessels. In contrast, constriction of the lymph vessels caused by intravenous injection of CM was paralleled with dilation of veins, which suggests persistence of stagnant phenomena in the myometrial circulatory bed.

The state of endometrium during CE was characterized by increased number of vessels, hemocapillary hyperemia, and dilation of arteries and veins. In this layer, the lymphatic system demonstrated increased diameters of vascular and capillary beds probably attesting to lymphostasis (Table 1). Both intravenous and lymphotropic injections of MMSC or CM decreased the thickness of endometrium. It should be noted that CM decreased this thickness to the level of intact rats, whereas MMSC diminished this parameter even more (Table 1). The histomorphological features of endometrium corresponded to that of intact rats: the stroma had no lymphocytic infiltration, and it contained only solitary macrophages and plasmatic cells, while the number of endometrial glands diminished in parallel with recovery of glandular epithelium structure.

During CE, the endometrial arteries dilated. MMSC (intravenous and lymphotropic) and CM (in-

TABLE 1. Effect of Experimental CE and Various Modes of MMSC and MMSC+CM Administration on Uterine Morphometric Parameters (*m±SEM*).

Parameter	Intact control	Experimental control	CE+MMSC		CE+CM		CE+PS	
			intravenous	lymphotropic	intravenous	lymphotropic	intravenous	lymphotropic
Myometrium								
Thickness, μ	1129.93±65.90	1002.34±49.77	1066.87±41.22	1102.10±45.47^{x}	1296.10±85.33^{o}	997.68±27.58	1054.50±81.06	972.50±37.46
Diameter of arteries, μ	15.70±1.70	23.61±1.29*	17.04±0.76^{+o}	28.55±1.50^{*+x}	16.55±0.85^{+o}	20.06±1.07^{*+x}	22.13±0.64*	24.87±0.99*
Diameter of veins, μ	17.84±1.02	28.97±1.95*	20.21±0.89^{*+o}	18.65±1.93^{+x}	28.44±1.61*	26.51±1.39*	29.24±0.77*	24.02±1.47*
Diameter of lymph vessels and capillaries, μ	11.71±0.58	9.41±0.88*	14.15±0.47^{*+o}	15.92±1.28^{*+x}	16.10±1.11^{*+}	16.52±1.08^{*x}	19.42±0.64^{*+}	21.68±0.84^{*+}
Endometrium								
Thickness, μ	1076.02±77.10	1625.69±96.72*	856.19±7.53^{*+o}	830.71±29.32^{*+x}	1178.27±50.89^{+o}	1099.8±70.8^{+x}	1379.40±83.06*	1332.19±53.53*
Diameter of arteries, μ	5.46±0.29	8.58±0.37*	6.07±0.25^{+o}	6.23±0.26^{+x}	5.72±0.22^{+o}	7.04±0.26^{*+x}	8.41±0.25*	8.20±0.28*
Diameter of veins, μ	12.05±0.85	16.35±0.76*	13.82±0.55^{+o}	11.52±0.35^{+x}	13.38±0.53^{+o}	16.37±0.82*	15.41±0.63*	14.24±1.08*
Diameter of lymph vessels and capillaries, μ	8.74±0.34	11.99±0.37*	9.29±0.39^{*+o}	8.92±0.29^{+x}	9.71±0.35^{+o*}	9.35±0.49^{+*}	11.30±0.34*	10.24±0.35*

Note. $p<0.05$ in comparison with *intact control, $^{+}$experimental control, ^{o}intravenous administration of PS, ^{x}lymphotropic administration of PS.

travenous) decreased the diameter of these arteries to the level observed in intact rats, which was smaller than that in PS-treated animals. In contrast to PS, lymphotropic injection of CM constricted the endometrial arteries, although their diameter did not attain the intact level. The endometrial veins dilated. MMSC (intravenous and lymphotropic) and CM (intravenous) decreased the diameter of these veins to the intact level, which was smaller than that in PS-treated animals. Lymphotropic injection of CM produced no effect on the vein lumen, which in this group did not differ from the values observed in experimental control or PS-treated groups. The endometrial lymph vessels and capillaries dilated. Their diameter decreased to the intact level only after lymphotropic injection of MMSC. In contrast, intravenous injections of MMSC or CM produced only a negligible constriction and could not restore the diameter of lymph vessels and capillaries to the intact level, although both injections significantly decreased this parameter relatively to the corresponding value in PS-treated rats. So, similar to what was observed in myometrium, intravenous injections of MMSC or CM as well as lymphotropic injection of MMSC synchronously constricted the endometrial veins and lymph vessels attesting to recovery of drainage function of venous and lymphatic systems (Table 1).

Thus, chronic endometrial inflammation results in morphological and hemocirculatory alterations in both endo- and myometrium. Administration of bone marrow MMSC and/or soluble factors produced by these cells resulted in normalization of vascular abnormalities and elimination of endometritis manifestations. Previously we described a similar effect of MMSC and CM on the vessels of the broad ligament of the uterus during experimental chronic metroendometritis [3]. Probably, the cells injected into the lymphatic bed in the inflammatory focus persist in the environmental tissues and produce bioactive substances, so they pronouncedly affect the inflammatory process. Similar studies are increasingly promising. As a rule, the researchers examine the efficacy of injection of autologous MMSC and possibility to modulate the properties of the host stem cells residing in the endometrium [14]. It was recently demonstrated that human MMSC can up-regulate expression of VEGF and FGF2; moreover, they can pronouncedly enhance expression of angiogenin thereby significantly stimulating neovascularization and improving perfusion in the uterus [13]. These data showed that the use of autologous MMSC and the regulator factors produced by them holds much promise in the treatment of chronic endometritis with endometrial hyperplasia. At this,

both intravenous and local lymphotropic injections can be used. Thus, the clinical studies are needed to assess effectiveness and safety of this method of cell therapy in comparison with routine therapeutic-diagnostic dilation and curettage of the uterus.

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