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Changes in the Circulatory and Lymphatic Systems of Internal Genitals in Female Rats after Intravenous and Lymphotropic Administration of Multipotent Mesenchymal Stem Cells and Products Secreted by These Cells

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We studied the effects of intravenous and lymphotropic administration of bone marrow multipotent mesenchymal stromal cells and products secreted by these cells into conditioned medium on the blood and lymph circulation in the uterus and ovaries, as well as on folliculogenesis in female Wistar rats. It was shown that stromal cells and conditioned media of these cells administered via both routes lead to an increase in the number and diameter of blood vessels in the uterine wall and in the cortical layer of the ovaries. Neither mesenchymal stromal cells, not conditioned media affected the ovarian follicular apparatus.

Key Words: *multipotent mesenchymal stromal cells; blood and lymphatic vessels; uterus; ovaries; follicular apparatus*

Recent experimental studies showed that stem cells are a promising material for the therapy of various diseases [7]. Mesenchymal stem cells are used in cell therapy and regenerative medicine, they can be easily isolated and rapidly expanded in culture, can be used for autologous transplantation, and exhibit significant paracrine effects [11].

Stem cells induce angiogenesis and restore the number of follicles and yellow bodies in the ovaries without producing side effects. According to the results of quantitative real-time PCR and immunohistochemical studies, transplantation of stem cells isolated from the adipose tissue increased the expression of VEGF, IGF-1, and HGF in the ovaries of mice treated with cyclophosphamide [9,10]. In previous studies, the use of multipotent mesenchymal stromal cells (MMSC) from the bone marrow improved the condition of the blood and lymphatic vascular network in the tissues of the uterine wall and normalized the cellular composition of the immune system of Wistar rats in experimental chronic endometritis [3,5,6]. However, the effect of stem cells on organs and tissues of the reproductive system not involved in the inflammatory or other pathological process remains unknown. These data are needed to predict possible side effects of cell therapy used to treat diseases not associated with impaired female genital function.

Here we studied changes in the circulatory and lymphatic system of the internal genitals of intact female rats after intravenous and lymphotropic administration of MMSC and products secreted by these cells (conditioned media (CM) of MMSC cultures).

MATERIALS AND METHODS

The experiments were performed on Wistar female rats in accordance with European Convention for the Protection of Vertebrate Animals used for Experimen-

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tal and other Scientific Purposes (Strasbourg, 1986). Female rats (n=25) weighing 200-270 g received standard laboratory diet and had free access to water. MSCS were obtained from the femurs as described elsewhere [4]. Passage 2-4 MMCK were used.

The animals were divided into 5 groups (5 rats per group): intact controls (group 1); single intravenous injection of 2×10^6 MMSC in 0.1 ml saline (group 2); single lymphotropic (under the vaginal mucosa) injection of 2×10^6 MMSC in 0.1 ml saline (group 3); single intravenous injection of 0.1 ml CM of MMSC culture (group 4); single lymphotropic (under the vaginal mucosa) injection of 0.1 ml CM of MMSC culture (group 5).

The experimental animals were decapitated under light ethaminal narcosis in 9 days after administration of MMSC and CM; their body weight was measured. The uterus and ovaries were taken to assess the effect of MMSC and CM. The ovaries were weighed and their weight index was calculated by the formula: organ weight (mg)/body weight (g). For histological examination, the uterus and ovaries were fixed in 10% neutral formalin, paraffin sections were stained with hematoxylin and eosin, the preparations were examined under an Axioplan light microscope (Carl Zeiss) coupled with a digital camera (oc. ×10, ob. ×5). Digital images were processed using Image-Pro Plus 4.1 software. The thickness of the myometrium and endometrium and diameter of blood and lymphatic vessels were measured in microns. The number of vessels in the myometrium and endometrium was determined using a built-in grid (per 1600 mm^2 (numerical density of vessels in the field of view).

The data were processed statistically using Statistica 6.0 software. The arithmetic mean (M), standard error (SE) were calculated. The results were compared using Student's t test as the data distribution fit the normal law (Kolmogorov—Smirnov test). The differences were significant at 95% significance level.

RESULTS

Body weight increased in rats of all experimental groups, but the weight index of the ovaries did not change. After intravenous and lymphotropic injection of MMSC and CM, the diameter of all vessels in the cortical layer of the ovaries increased; the only exception was the diameter of veins after lymphotropic administration of MMSC (Table 1, Fig. 1).

After intravenous and lymphotropic administration of CM, the number of veins in the cortical layer of the ovaries slightly decreased (Table 1). After administration of CM and MMSC via both routes, the number of follicles and yellow bodies did not differ from those in intact controls.

Intravenous MMSC decreased the thickness of the myometrium and endometrium, increased the diameter of arteries, veins, and lymphatic vessels in the endometrium, and the diameter of arteries and lymphatic vessels of the myometrium. Lymphotropic injection of MMSC increased the diameter of arteries, veins, and lymphatic vessels in the myometrium and lymphatic vessels in the endometrium. Intravenous injection of CM increased the diameter of veins and lymphatic vessels in the endometrium and lymphatic vessels in the myometrium. After lymphotropic administration of CM, only the diameter of the lymphatic vessels of the myometrium and endometrium increased (Table 2).

The number of veins and lymphatic vessels in all layers of the uterine wall after administration of MMSC and CM increased; the only exception was the number of myometrial veins (their number did not change after intravenous administration of MMSC). The number of arteries did not change after lymphotropic and intravenous administration of MMSC and CM (Table 3; Fig. 2).

Thus, introduction of both MMSC and CM of MMSC cultures increased the number of veins and

TABLE 1. Diameter and Number of Vessels in the Ovarian Medulla after Intravenous and Lymphotropic Administration of MMSC and CM from MMSC Cultures ($M\pm SE$)

Parameter	Intact control	MMSC		CM of MMSC culture	
		intravenous	lymphotropic	intravenous	lymphotropic
Diameter of arteries, μ	14.20±0.67	27.4±2.3*	29.4±1.6*	27.2±1.2*	27.7±1.3*
Diameter of veins, μ	29.1±1.2	44.3±3.1*	32.0±2.1	36.4±1.2*	41.5±1.6*
Diameter of lymphatic vessels, μ	21.6±1.3	48.8±3.6*	38.5±2.3*	42.7±1.9*	37.4±1.5*
Number of arteries	9.0±0.3	8.7±0.3	8.7±0.4	8.8±0.4	8.0±0.4
Number of veins	8.7±0.3	8.2±0.3	8.1±0.3	7.7±0.4*	7.7±0.4*
Number of lymphatic vessels	7.5±0.3	7.2±0.3	7.6±0.4	6.9±0.3	7.6±0.4

Note. *p<0.05 in comparison with intact control.

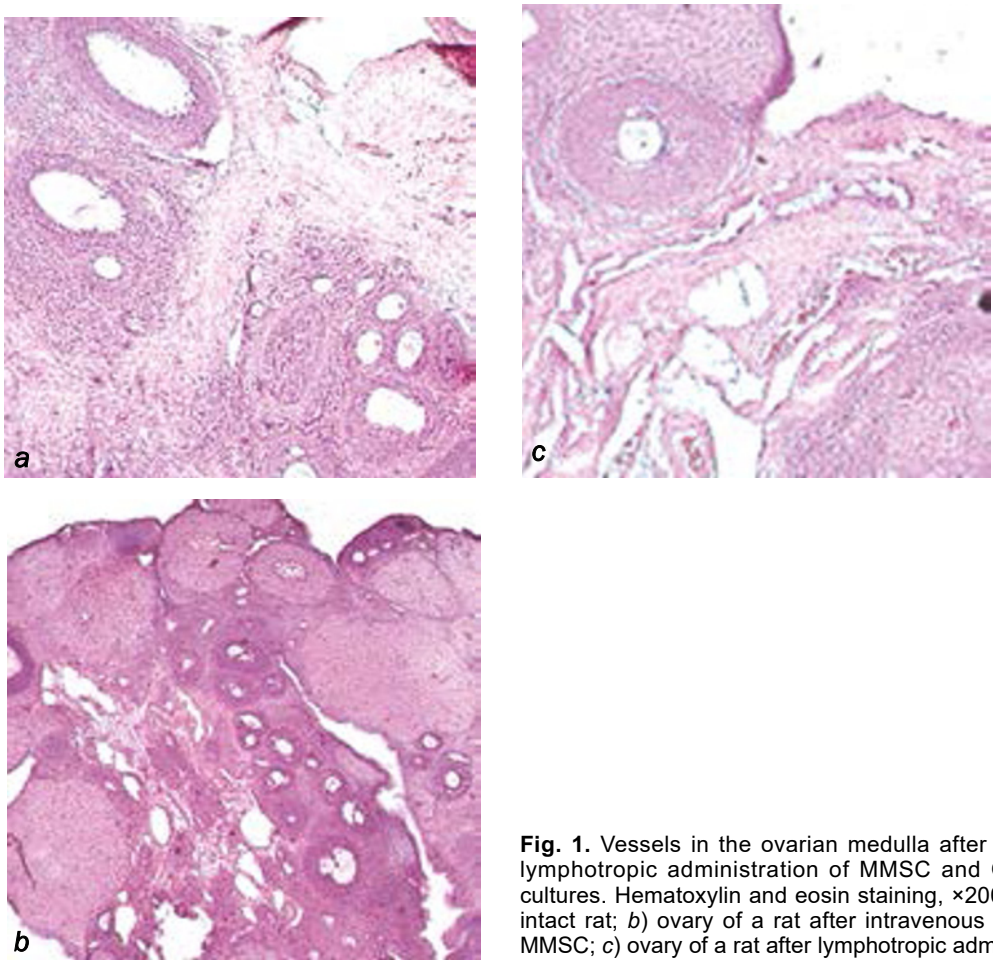

Fig. 1. Vessels in the ovarian medulla after intravenous and lymphotropic administration of MMSC and CM from MMSC cultures. Hematoxylin and eosin staining, ×200. *a*) Ovary of an intact rat; *b*) ovary of a rat after intravenous administration of MMSC; *c*) ovary of a rat after lymphotropic administration of CM.

lymphatic vessels in the myometrium and the number of veins, capillaries, and lymphatic vessels in the endometrium. Intravenous administration of MMSC increased the number of lymphatic vessels, which reflected stimulation of outflow through the lymphatic channel, because the thickness of the myometrium and endometrium decreases in parallel with the increase in the diameter and number of veins and lymphatic ves-

TABLE 2. Diameter of Vessels and Thickness of Myometrium and Endometrium after Intravenous and Lymphotropic Administration of MMSC and CM from MMSC Cultures (μ; *M*±*SE*)

Parameter	Intact control	MMSC		CM of MMSC culture	
		intravenous	lymphotropic	intravenous	lymphotropic
Thickness of myometrium	1130±66	856±59*	977±49	1062±47	937±37
Thickness of endometrium	1076±77	873±50*	791±44*	857±37*	991±95
Diameter of arteries in the endometrium	5.5±0.3	8.5±0.5*	5.5±0.2	6.6±0.3*	6.0±0.3
Diameter of veins in the endometrium	12.10±0.85	15.5±1.0*	14.70±0.95	12.90±0.56	13.9±1.3
Diameter of arteries in the myometrium	15.6±1.7	19.2±1.0*	17.7±0.7*	18.8±1.0*	12.3±0.8
Diameter of veins in the myometrium	17.8±1.0	20.1±1.4	22.9±1.3	28.7±2.6*	18.6±2.0
Diameter of lymphatic vessels and capillaries in the endometrium	8.7±0.3	13.4±0.5*	15.4±0.8*	14.8±0.6*	17.1±1.2*
Diameter of lymphatic vessels and capillaries in the myometrium	11.7±0.6	14.7±0.5*	17.4±0.8*	15.1±0.7*	18.3±1.4*

Note. *$p<0.05$ in comparison with intact control.

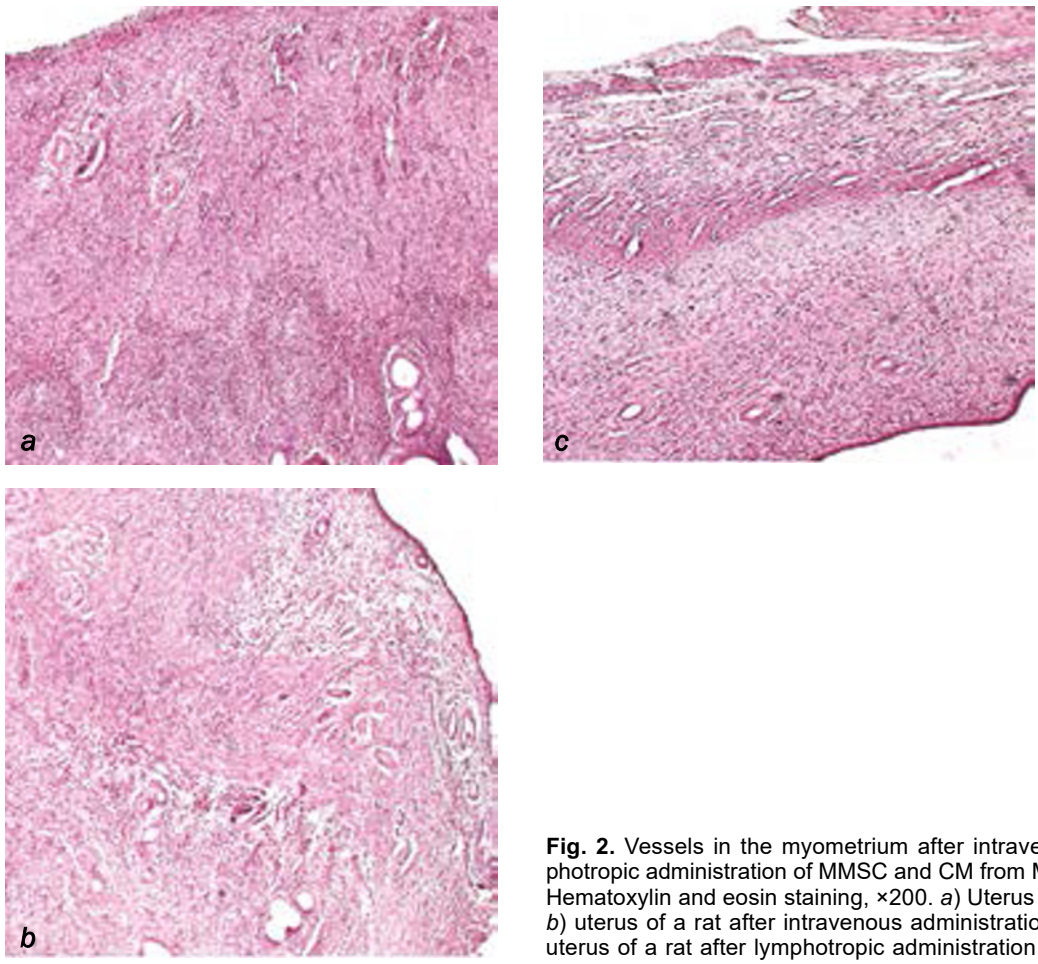

Fig. 2. Vessels in the myometrium after intravenous and lymphotropic administration of MMSC and CM from MMSC cultures. Hematoxylin and eosin staining, ×200. *a*) Uterus of an intact rat; *b*) uterus of a rat after intravenous administration of MMSC; *c*) uterus of a rat after lymphotropic administration of CM.

sels in the endometrium. Vascular reactions were less pronounced after intravenous administration of MMSC and lymphotropic administration of CM.

These results suggest that the main effect of MMSC and their products on the internal genitals in intact female rats is triggering vascular reactions that can include both angio- and neoangiogenesis, which improves tissue nutrition. The increase in the number and diameter of blood vessels can be caused by bioactive molecules and cytokines secreted by MMSC [4,8,5]. An important component in the action of cells and their products on the uterine tissue is the increase in the number of lymphatic vessels, which can be regarded as stimulation of drainage and detoxification functions [1,2]. However, we have previously shown that administration of these cell products to rats with

TABLE 3. Number of Vessels per Field of View in the Myometrium and Endometrium after Intravenous and Lymphotropic Administration of MMSC and CM from MMSC Cultures (*M*±*SE*)

Vessels	Intact control	MMSC		CM of MMSC culture	
		intravenous	lymphotropic	intravenous	lymphotropic
Arteries of the myometrium	16.0±1.1	14.8+2.09	19.50±2.33	16.82±1.45	15.00±1.82
Veins of the myometrium	7.6±0.5	9.0±1.2	14.0±2.7*	15.0±2.1*	12.5±1.1*
Lymphatic vessels of the myometrium	54.2±6.3	89.9±7.2*	145.0±11.6*	96.4±7.3*	120.8±13.5*
Capillaries of the endometrium	267.2±12.1	266.0±10.4	403.8±25.8*	309.5±16.4*	300.0±27.6
Veins of the endometrium	86.20±12.03	212.2±14.1*	288.8±20.7*	228.2±11.5*	217.5±26.8*
Lymphatic vessels of the endometrium	77.0±5.2	331.5±21.0*	335.4±25.9*	303.6±25.9*	351.0±29.3*

Note. *$p<0.05$ in comparison with intact control.

chronic experimental endometritis produced opposite effects on vessels of the internal genitals: the diameter of initially dilated blood vessels decreased [3]. This suggests that this therapy produced a modulating effect depending on the initial state of the vascular bed.

Thus, we showed that MMSC and their products produce a stimulating effect on microcirculation and lymphatic drainage in tissues of the reproductive system in intact animals without visible effect on folliculogenesis in the ovaries.

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