Effect of Intracerebral Transplantation of Mesenchymal Stem Cells on Pial Microcirculation in Rats

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We studied the effect of intracerebral transplantation of bone marrow mesenchymal stem cells on microcirculation (density of microvascular network and reactivity of arterioles) in the pia mater of 2-3-month-old rats. It was found that after transplantation of mesenchymal stem cells, the density of pial microcirculatory network in the contralateral hemisphere significantly increased (by 1.7 times; $p<0.05$) in comparison with both intact animals and controls. The number of arterioles in the studied area increased most markedly (by ~2.5 times; $p<0.05$) in comparison with other groups. Intracerebral transplantation of mesenchymal stem cells or conditioned culture medium (α-MEM) had no effect on reactivity of pial arterioles.

Key Words: *mesenchymal stem cells; microcirculation; intracerebral transplantation; angiogenesis; reactivity of brain arteries*

The development of new methods for the therapy of age-related brain circulation and microcirculation disturbances is still a pressing problem. Numerous experimental studies have demonstrated the decrease in the number and total length of blood capillaries in the brain of old animals in comparison with young specimens [15,16,18]. The number of narrowed capillaries and capillaries with thickened walls also increases with age, which impairs metabolic processes in the brain [4,7]. However, some authors revealed no differences in the density of the capillary network in old and young animals [3,13]. Published data on age-related changes in the reactivity of arterioles in rats are scanty and contradictory [8,10,12].

Cell therapy with mesenchymal stem cells (MSC) as a possible method of correction of age-related cerebral circulatory disorders is poorly studied. MSC application has a great potential. It has been proven that MSC activate angiogenesis in the ischemic brain tissue [14]. The effect of MSC on reactivity of brain arterioles was never studied.

Here we present the results of the first stage of the study aimed at the analysis of the effect of intracerebral transplantation (ICT) of MSC on microcirculation in the brain cortex of old rats. In this case, ICT of MSC was performed to young animals to demonstrate the stimulating effect of MSC on angiogenesis and arteriogenesis and the absence of the negative effect of ICT on microcirculation in the pia mater.

MATERIALS AND METHODS

Experiments were carried out on 2-3-month-old male Wistar–Kyoto rats (n=30). The animals were kept under standard vivarium conditions at natural illumination and free access to food and water. The study was performed in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986).

Isolation of MSC from syngeneic rat BM, their culturing *in vitro*, and evaluation of the viability of the obtained cells were performed at the Institute

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of Molecular Biology and Genetics, V. A. Almazov Federal Heart, Blood, and Endocrinology Center, using routine methods [1]. MSC were cultured in α-MEM (PanEco) supplemented with 10% embryonic calf serum (HyClone), 2 mM L-glutamine, and 1% penicillin+streptomycin (Invitrogen). Passage 2-3 MSC were provided for further physiological experiments to the Laboratory of Physiology of Circulation, I. P. Pavlov Institute of Physiology within the framework of scientific collaboration.

The animals were divided into 3 groups: intact animals (group 1, n=10); control animals receiving transplantation of 20 μl α-MEM conditioned by SC (group 2, n=10); and animals receiving transplantation of 200,000 MSC in 20 μl conditioned α-MEM (group 3, n=10).

The rats were intraperitoneally narcotized with zoletil (20 mg/kg; Virbac). A hole (1 mm) was drilled in the parietal area of the skull alternately above the right or left hemisphere with a dental drill without damaging the dura matter. The studied preparations were injected with an insulin syringe into the brain cortex at a depth of not more than 2 mm. The skin on the head was sutured.

In 3 weeks after ICT, the animals were narcotized and the parietal bone and the dura mater were removed to visualize the pia mater of the sensorimotor cortex. The brain surface was continuously sprayed with 0.9% NaCl (37°C). During the experiment, the body temperature was maintained at 37°C and mean blood pressure was 105-120 mm Hg.

The animals were placed under a lens of a TV device and the density of the microvascular network in the pia mater was studied (total system magnification ×40 and ×160). Then, a vasoconstrictor norepinephrine (NE) in a concentration of 10^{-6} M or a vasodilator acetylcholine chloride (ACh) in a concentration of 10^{-6} M were applied (solution temperature 37°C). Changes in the diameters of pial arterioles were recorded.

The total number of all vessels and the number of arterioles per area unit were evaluated using Photo M

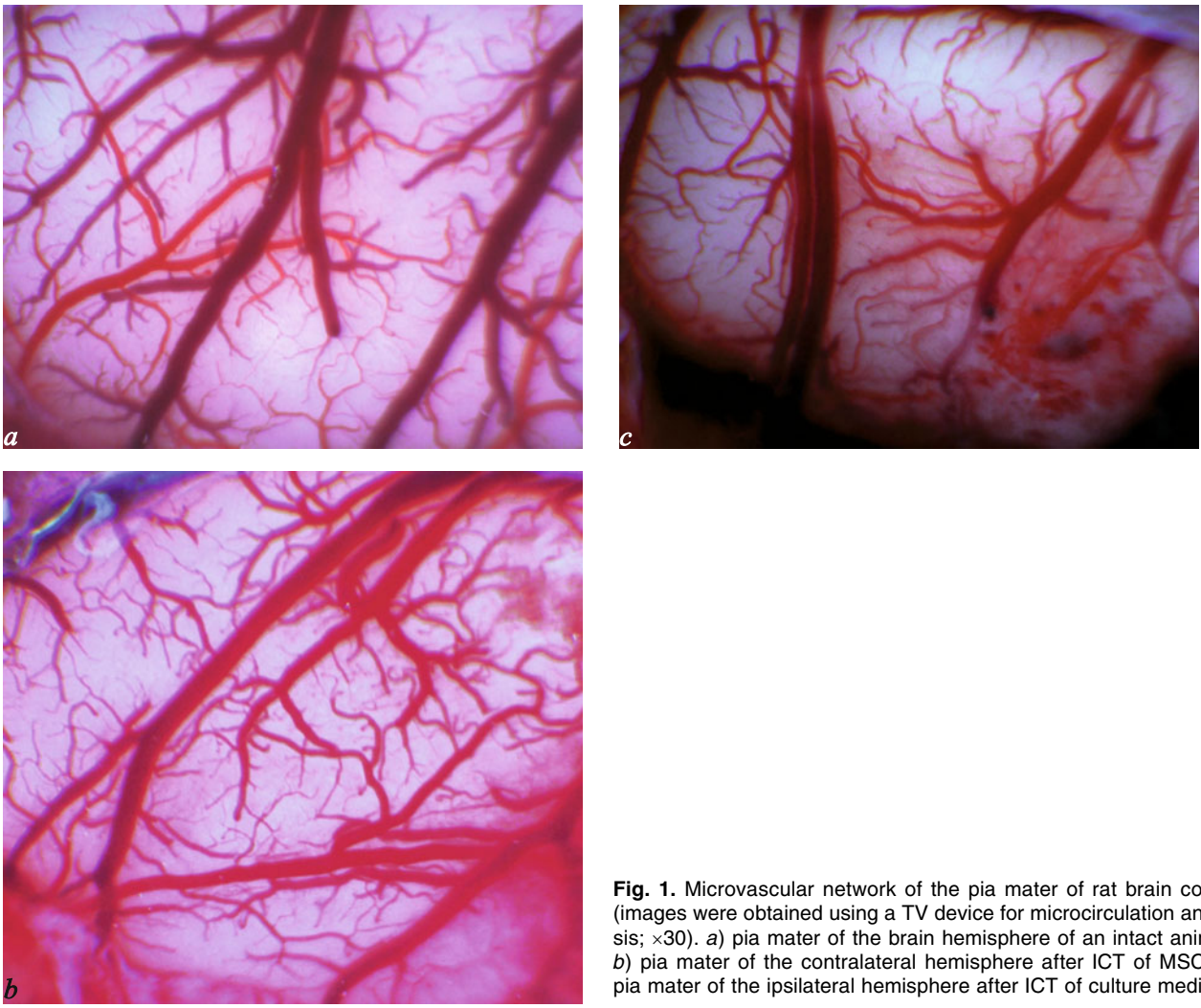

Fig. 1. Microvascular network of the pia mater of rat brain cortex (images were obtained using a TV device for microcirculation analysis; ×30). *a*) pia mater of the brain hemisphere of an intact animal; *b*) pia mater of the contralateral hemisphere after ICT of MSC; *c*) pia mater of the ipsilateral hemisphere after ICT of culture medium.

software; changes in the diameter of arterioles caused by NE and ACh were measured.

Significance of differences was evaluated using the Mann–Whitney test. The differences were significant at $p<0.05$.

RESULTS

ICT is the only possible way to deliver the cell material into the brain in circulatory disorders not associated with blood-brain barriers impairment (Scholtz syndrome, multi-infarct dementia, hypertension, and diabetic microangiopathies). ICT is more traumatic procedure than intravenous MSC transplantation and is associated with injury to the brain cortex. Here we studied the positive results of ICT of MSC and negative effects of this procedure.

The positive results included stimulation of angiogenesis and arteriogenesis in the pia mater of rat brain cortex after MSC ICT (Figs. 1 and 2). We found that the number of vessels in the pia mater of the contralateral hemisphere after MSC transplantation increased by ~1.7 times ($p<0.05$) in comparison with the corresponding parameter in intact and control animals. The number of arterioles in the studied area increased most markedly (by ~2.5 times; $p<0.05$) in comparison with other groups. The fact that despite damage to the brain tissue, the density of microvascular network in the ipsilateral hemisphere did not decrease in comparison with that in intact animals can also be considered as a positive result (Figs. 1 and 2). The effect of MSC transplantation on angiogenesis is now actively discussed. Practically all investigators agree that MSC activates angiogenesis via production of vascular endothelial growth factor (VEGF, the major angiogenesis regulator), basic FGF, and platelet-derived growth factor (PDGF); these substances stimulate proliferation and migration of pericytes and smooth muscle cells and promote the development of the smooth muscle layer in newly formed vascular tubes [9,2,6]. *In vitro*

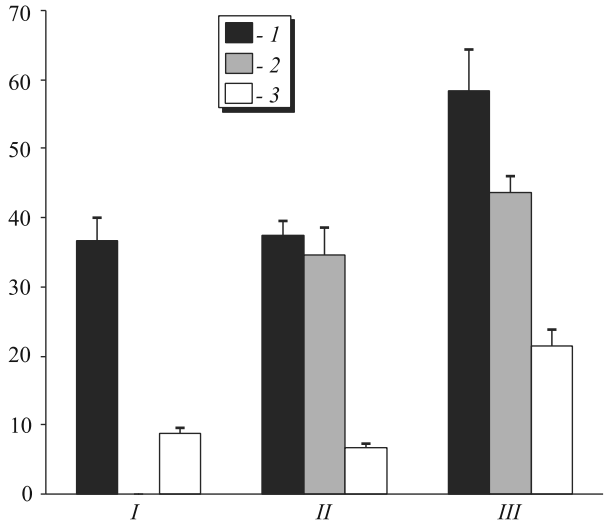

Fig. 2. Density of pial microvascular network of the sensorimotor cortex.

Ordinate: density of microvessels ($p<0.05$). *I*: intact animals; *II*: ICT of α-MEM conditioned by SC; *III*: ICT of MSC. *1*) total number of microvessels in the contralateral hemisphere; *2*) in ipsilateral hemisphere; *3*) number of arterioles in the contralateral hemisphere.

studies showed that MSC promote the formation and stabilization of the vascular network on the matrigel surface [11,17]. Elevation of VEGF content in the brain tissue after MSC transplantation has been experimentally proven [5].

Here we have demonstrated that ICT of MSC stimulates the growth of the arterial compartment of the pial microcirculatory bed. The technique of intravital microscopy of the pia mater allows identification of microvessels (arterioles, venules, capillaries) and evaluation of their diameter, branching order, and number per area unit (Fig. 1).

ICT of MSC led to a significant increase in the relative ratio of pial arterioles that demonstrated a constrictive response to NE and dilatation to ACh in comparison with the corresponding parameters in intact animals (Table 1), except one case: in the ipsi-

TABLE 1. Percentage of Pial Arterioles Responding to NE or ACh*

Group	Contralateral hemisphere						Ipsilateral hemisphere					
	NE			ACh			NE			ACh		
	I	II	III	I	II	III	I	II	III	I	II	III
Intact	46.2	36.4	17.4	53.6	21.2	25.2	46.2	36.4	17.4	53.6	21.2	25.2
ICT of MSC	65.1	21.1	13.8	56.0	32.4	11.6	52.2	28.3	19.6	43.8	46.1	10.1
ICT of α-MEM	44.1	26.9	29.0	47.6	30.9	21.4	61.5	33.3	5.1	62.8	21.8	15.4

Note. *% of the total number of pial arterioles. *I*: vasoconstriction; *II*: vasodilation; *III*: no response.

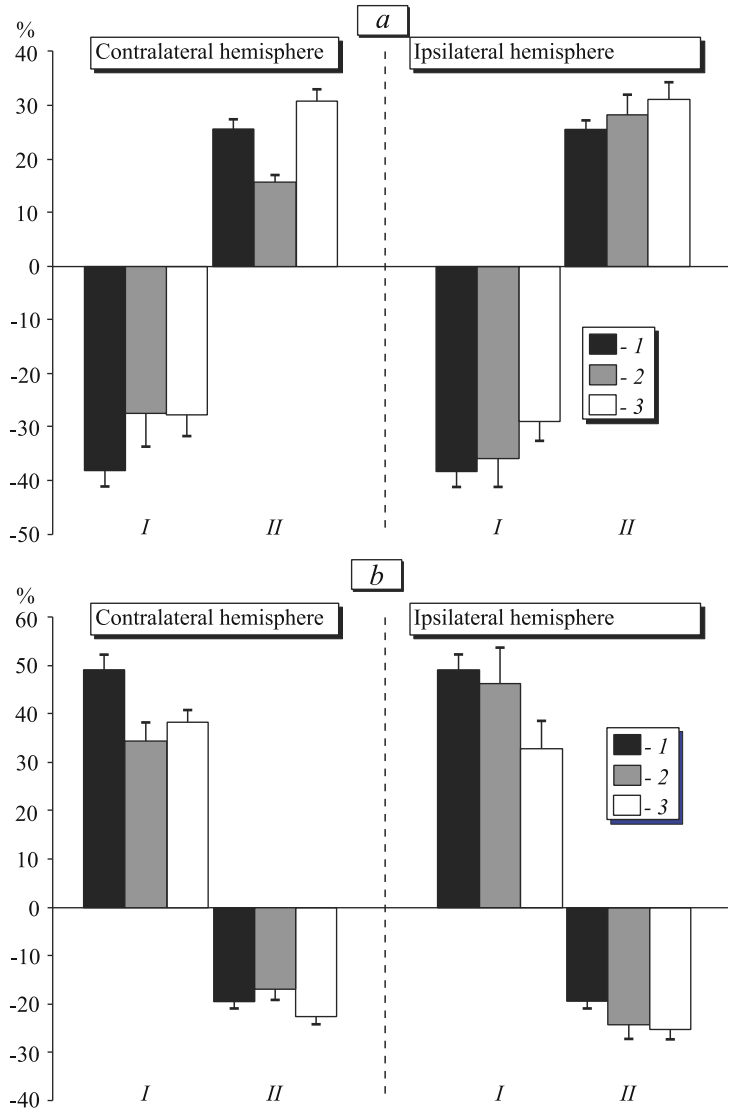

Fig. 3. Changes of the diameter of pial arterioles in response to NE or ACh (%). *a*) response to NE; *b*) response to ACh *I*: vasoconstriction; *II*: vasodilatation. *1*) intact animals; *2*) ICT of α-MEM conditioned by SC; *3*) ICT of MSC.

lateral hemisphere of animals receiving cell therapy (group 3), the number of arterioles responding to ACh by contraction increased by 20% ($p<0.05$), while the number of arterioles responding by dilation decreased by 10% (insignificant changes).

The relative changes in the diameter of vessels responding to NE by constriction and dilatation in both hemispheres did not change significantly (Fig. 3). In group 3 animals (cell therapy), no significant changes in the diameter of arterioles responding to ACh by dilation and constriction in the ipsi- and contralateral hemispheres were found under these conditions.

These findings suggest that ICT of MSC had practically no effect on reactivity of pial arterioles to vasoconstrictors and only slightly impaired their response to vasodilators.

ICT of α-MEM insignificantly reduced the number of arterioles responding to NE by dilatation in the contralateral hemisphere and increased the number of these vessels by 15% in the ipsilateral hemisphere. The number of arterioles responding to ACh by dilatation in the contralateral and ipsilateral hemisphere was higher by 6 and 9%, respectively (Table 1). In the contralateral hemisphere, the amplitude of both the constrictor and dilatation response of arterioles to NE was lower by 10 and 15%, respectively (Fig. 3). The ACh-induced dilatation in this hemisphere was less pronounced (by ~15%) and remained unchanged in constricting vessels. In the ipsilateral hemisphere, the reaction to NE and ACh did not differ from that in intact animals.

The results suggest that ICT of the control substance did not impair reactivity of the pial arterioles. Hence, ICT does no significantly damage the micro-

circulation in the cerebral pia mater even in small animals (rats).

Thus, ICT of MSC to young animals has confirmed a great potential of this method for stimulation of angiogenesis in the pia mater. Despite traumatic procedure of cell transplantation, reactivity of pial arterioles practically did not differ from that in intact animals. This suggests great prospects of using MSC ICT in the therapy of pathologically impaired microcirculation in aging organism. This possibility will be studied in further experiments on old (22-24-month-old) animals.

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