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Cerebral Blood Flow in SHR Rats after Transplantation of Mesenchymal Stem Cells

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Intracerebral transplantation of mesenchymal stem cells to 6- and 12-month-old SHR rats induced angiogenesis in the pia mater. In 6-months-old SHR rats, perfusion in the brain tissue after cell transplantation considerably increased, while in 12-month-old rats it remained practically unchanged. We also observed marked activation of regulatory processes in the cerebral vascular system, most pronounced in 12-month-old rats. Neurogenic and myogenic tone of cerebral vessels increased significantly, while endothelium-dependent tone slightly decreased. The increase in neurogenic and myogenic tone of blood vessels in SHR rats at the age of 6 and 12 months after transplantation of stem cells can be explained by the formation of new smooth muscle cells in the pre-existing arteries walls. Greater muscle mass developed stronger force and contributed to narrowing of the arterial lumen, as a result, there was no increase in blood flow despite the downstream angiogenesis. A slight decrease in endothelium-dependent tone can be explained by increased production of vasodilators by newly formed endothelial cells.

Key Words: *intracerebral transplantation of mesenchymal stem cells; perfusion; vascular tone*

Despite significant progress in understanding of the pathogenesis and therapy of arterial hypertension (AH), this pathology remains a major public health problem requiring intensive research to develop new and more effective treatments. During recent decades, cell therapy rapidly develops and stem cell transplantation is now successfully used in the treatment of pulmonary hypertension and severe chronic kidney disease [6]. Cell therapy uses stem cells of the different origins including mesenchymal stem cells, also called multipotent mesenchymal stromal cells that can differentiate into various cells *in vitro*. Mesenchymal stem cells (MSC) produce a pronounced neoangiogenic effect; they secrete a number of bioactive factors involved the formation of new vessels such as vascular endothelial growth factor, hepatocyte growth factor, fibroblast growth factor-2, angiopoietin-1 (Ang-1), *etc*. Moreover, pericytes are viewed as MSC located in all vascularized body tissues [8]. Through tight junctions formed by pericytes and vascular endothelial cells soluble factors and physical signals synergistically participate in the formation of blood vessels and maintenance of their function [3]. Pericytes produce a modulating effect on the endothelial function thereby affecting peripheral vascular resistance and BP [6]. Considering that endothelial dysfunction and decrease in the density of microvessels are important changes in AH [13], improvement of these parameters can help reduce BP in AH.

Here we analyze morphofunctional changes in cerebral vessels of hypertensive SHR rats after MSC transplantation. SHR rats represent a widely used model for the studies of hypertension-related brain damage and approaches to their corrections [10,12]. Age-related BP elevation, decrease in the density of capillary network, brain atrophy, death of nerve cells and changes in glia are the phenomena shared to some extent with hypertensive brain damage in humans [12].

MATERIALS AND METHODS

The experiments were carried out on male SHR rats aged 6 months (n=24) and 12 months (n=24). Wistar-

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Kyoto rats (WKY) served as a control. The animals were kept under standard vivarium conditions at natural illumination with free access to water and food. The experiments were performed in accordance with the "Regulations for Conducting Animal Experiments" (July 19, 2014).

Characteristics of the experimental groups are presented in Table 1. Stimulation of angiogenesis in the rat sensorimotor cortex was performed by intracerebral injection of human MSC [2]. The rats were intraperitoneally narcotized with Zoletil (20 ml/kg; Virbac). Mean BP in the femoral artery was measured throughout the experiment with a device for invasive BP measurement. The bone and dura mater were removed in the skull parietal region above the sensorimotor cortex (a hole with the area of ~1 cm² was formed). The brain surface was continuously sprayed with physiological saline (37°C). Registration Parameters of microcirculation in the cerebral cortex were recorded using a LAKK-M multifunctional laser diagnostic complex (LAZMA). The sensor was placed above the sensorimotor cortex (coordinates AP=2-3 mm from the bregma; SD=1.0 mm lateral from the sagittal suture). LDF parameters were recorded over 10 min; the mean microcirculation index (M) and standard deviation of the mean (σ) were automatically calculated using software supplied with the instrument, and finally, wavelet analysis of the perfusion oscillations on LDF records was carried out. Based on the data on the amplitudes of microcirculation oscillations in different frequency ranges and with consideration of mean BP, the neurogenic (NT), myogenic (MT), and endothelium-dependent tone (ET) were calculated [1]. Along with brain tissue perfusion assessment, intravital measurement of the density of microvascular network in the pia mater of the sensorimotor cortex was performed.

The results were processed using Microsoft Excel. The data are presented as *M*±*SE*. Significance of differences was evaluated using nonparametric Mann—Whitney test. The differences were significant at $p<0.05$.

RESULTS

Direct BP measurement revealed no changes after MSC transplantation in both 6- and 12-month-old rats (Table 1).

Changes in the diameter and lumen of cerebral arteries, basal blood flow, and vascular reactivity were studied not once by the different methods *in vivo* and *in vitro*. It was reported that basal blood flow in the brain of SHR rats is similar [15], lower [6], or higher [5] than in normotensive animals. These differences can be related to the use of different methods of blood flow assessment. We evaluated perfusion of the brain tissue, an integral parameter characterizing the effectiveness of cerebral circulation.

As most patients with hypertension are above 50, studies on old rats are important. BP increase and changes in the structure of arterial walls in SHR rats most actively occur at the age of 9-12 weeks. By this age, the number of convoluted vessels significantly increases [14]. At the age of 24 weeks, pronounced hypertrophy of vascular smooth muscles, wall thickening, and a significant narrowing of the vascular lumen are observed in the arteries of SHR rats [11]. We analyzed vascular changes in SHR rats at the age of 6 and 12 months. By this age, the animals develop adaptive myogenic response to protect the downstream microvessels from increased BP, the vascular walls undergo remodeling and hypertrophy which results in media thickening and narrowing of the arterial lumen [9].

Vascular density in the pia mater of 6-month-old SHR rats was by 14.50±2.11% lower than in age-matched control WKY rats and the density of pial arteries was lower by 42.00±5.32% (Fig. 1). Reduced density of arterial vessels and high levels of ET, MT, and NT led to a decline in brain tissue perfusion (Figs. 2, 3). Pronounced increase in ET, NT and MT in pial vessels of SHR rats (by more than 2 times in comparison with WKY rats) is a manifestation of cerebral blood flow autoregulation, a mechanism protecting exchange-type blood vessels from elevated transmural pressure. Impairment of this protective vasoconstriction can induce damage to the brain—blood barrier, brain edema, and cerebrovascular pathology.

Intracerebral transplantation of MSC induced angiogenesis in 6-month-old SHR rats. In 3 weeks after MSC transplantation, the density of pial vessels in SHR rats increased by 29.8±3.43% and exceeded the corresponding parameter in WKY rats. The density of arterial vessels increased proportionally and approximated that in WKY rats (Fig. 1). Significant reorganization of blood vessels was accompanied by an increase in brain tissue perfusion that reached the value typical of control WKY rats. The effective perfusion index and variation coefficient also increased (Fig. 2). These changes attested to activation of regulatory

TABLE 1. BP in Experimental SHR Rats (*M*±*SE*)

Group	Mean BP, mm Hg
6-month-old intact SHR (*n*=6)	165.6±5.2
6-month-old rats receiving human MSC (*n*=18)	165.1±3.4
12-month-old intact SHR (*n*=6)	167.4±6.4
12-month-old rats receiving human MSC (*n*=18)	166.5±11.1

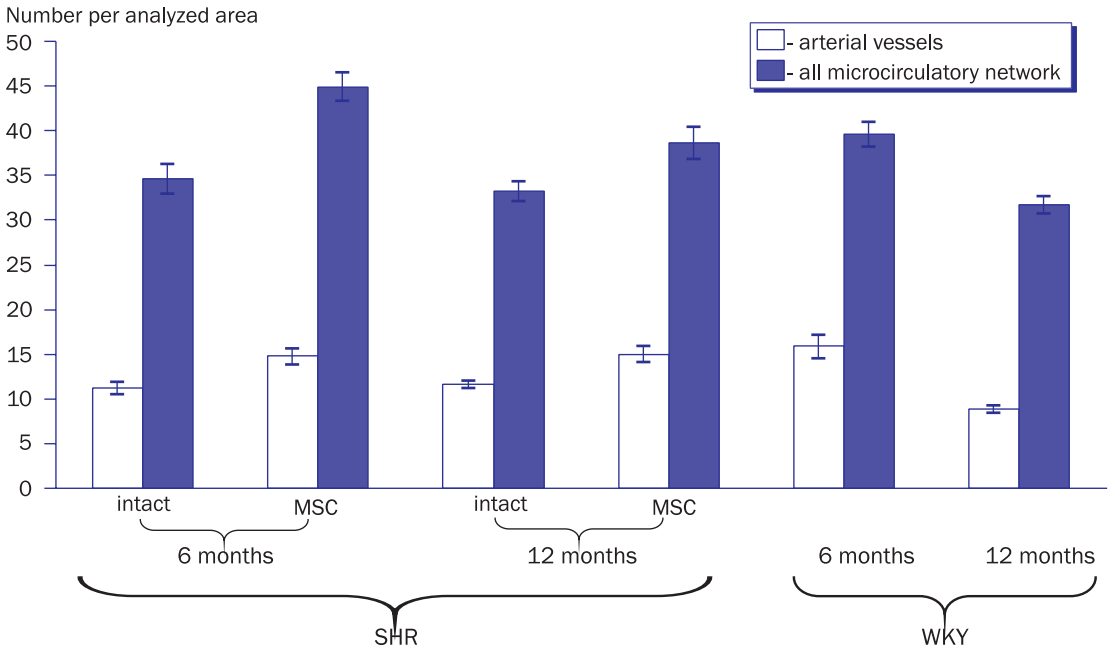

Fig. 1. Vascular density in the pia mater in 6- and 12-month-old SHR before and after MSC transplantation.

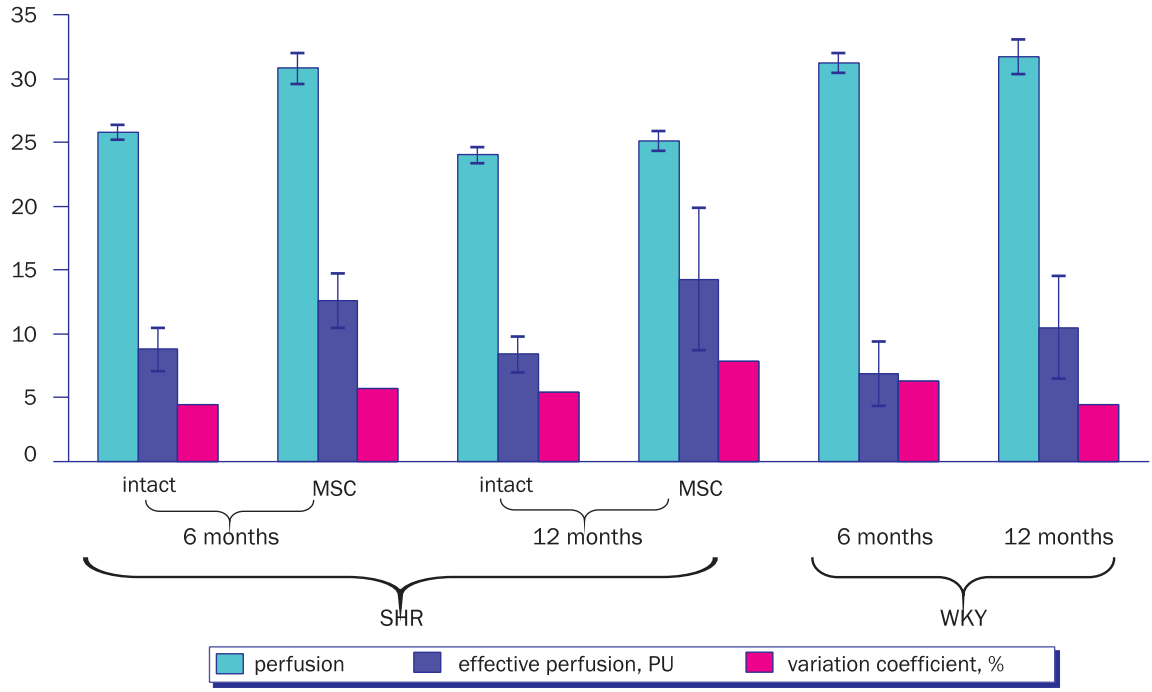

Fig. 2. Perfusion, effective perfusion, and coefficient of variation in the brain tissue of 6- and 12-month-old SHR rats before and after MSC transplantation. PU: perfusion units.

mechanisms, which was confirmed by the increase in NT and MT (Fig. 3). It should be noted that MT predominated before transplantation, which is typical of hypertensive animals. We suppose that these changes in vascular tone after the formation of new arteries are aimed at limiting overabundant blood flow in cerebral vessels. NT also increased after MSC transplantation. It can be hypothesized that newly formed arteries with relatively thin walls (in comparison with hypertrophic arteries typical of SHR rats) are unable to develop high tone and limit high pressure in the downstream exchange-type vessels at the expense of exclusively myogenic mechanisms and the role of neural regulation in the arterial network increased under these conditions. The increase in MT and NT, despite pronounced angiogenesis, stabilized perfusion of the brain

tissue in SHR rats after MSC transplantation and it did not exceed the value typical of WKY rats.

In contrast to MT and NT, ET decreased after transplantation of MSC. We hypothesized that this was related to the formation of new endotheliocytes in newly formed and pre-existing blood vessels. Endothelial function is not impaired in young endothelial cells and they produce sufficient amounts of vasodilators, which lead to ET decrease.

At the age of 12 months, the density of pial and arterial vessels in SHR rats remained almost the same in 6-month-old animals. Perfusion of the brain tissue slightly decreased due to the increase in ET and MT (in comparison with 6-month-old SHR rats). Transplantation of MSC led to less pronounced increase of the density of blood vessels in comparison with 6-month-old rats, whereas the density of arteries in 12-month-old SHR rats increased to the same extent as in 6-month-old rats (Fig. 1). In 12-month-old SHR rats, brain tissue perfusion increased insignificantly after MSC transplantation, despite the increase in the density of pial arteries. The effective perfusion index increased by 70.20±6.52% and coefficient of variation increased by 46.30±4.38% (Fig. 2). These noticeable changes attest to high strain of regulatory mechanisms, which was confirmed by the increase in MT and NT of cerebral vessels. It should be noted that ET of cerebral vessel slightly decreased (approximately to the same extent as in 6-month-old SHR rats) (Fig. 3).

Why brain tissue perfusion in 12-month-old SHR rats remained practically unchanged after MSC transplantation, despite increased density of pial arteries? We hypothesized that restriction of brain blood flow in 12-month-old rats was caused by pronounced morphological changes in the walls of main arteries: significant thickening of the vascular wall and lumen narrowing. Previous analysis of the structure of cerebral arteries in hypertensive showed that stenosis of large arteries develops by the 12th month of life; hypertrophy of the vascular wall and stenosis were most pronounced in the middle cerebral artery [9]. As dilatation of large arteries is almost impossible in stenosis, the formation of downstream vessels has little effect on cerebral blood flow intensity.

In WKY rats, the structure of cerebral vascular network underwent considerable changes between the 6th and 12th month of life: the density of all vessels decreased by 19.90±2.24% and the density of arteries decreased by 44.00±4.17%. Interestingly, perfusion of the brain tissue in 12-month-old WKY rats is high despite significant decrease in the density of all vessels and arteries in particular. It is worthy of note that the mechanisms regulating cerebral blood flow in WKY rats are characterized by low strain: at the age of 6 months, NT, MT, and ET values were lower by 2 times than in 6-month-old SHR rats. By the after of 12 months, all types of vascular tone in WKY rats increased, but remained below the levels observed in 6- and 12-month-old SHR rats. Detailed analysis of the mechanisms maintaining high perfusion level in 12-month-old WKY rats was beyond the scope of our research.

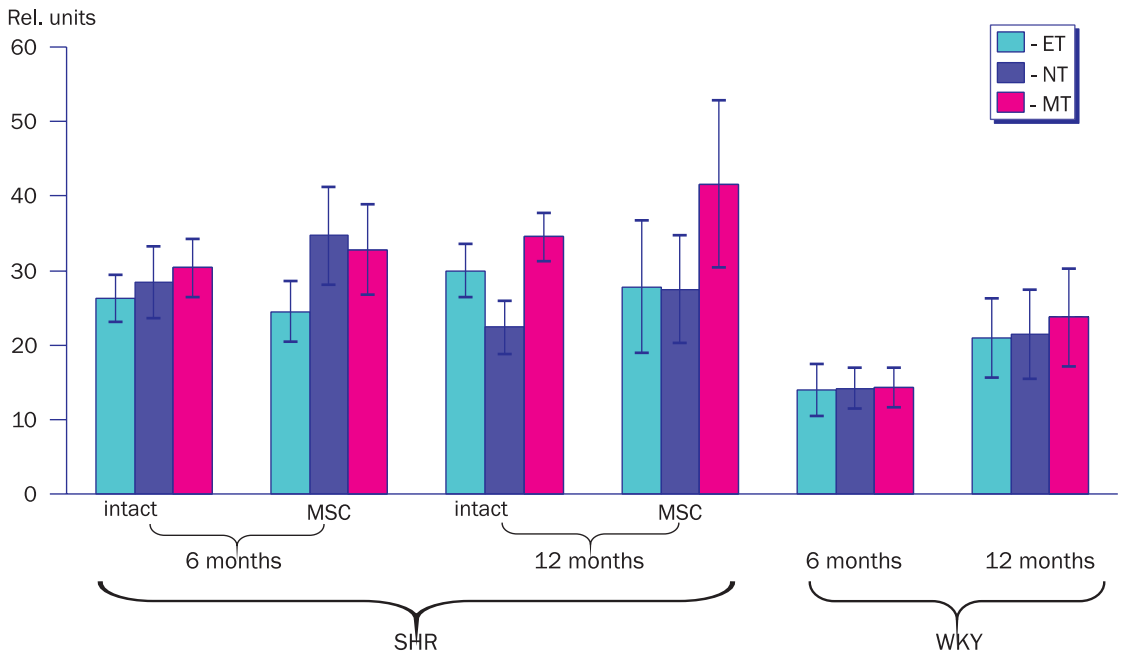

Fig. 3. Endothelial, neurogenic, and myogenic tones of cerebral vessels in 6- and 12-month-old SHR rats before and after MSC transplantation.

Thus, we found significantly reduced density of the cerebral vascular network and, in particular, arterial vessels associated with reduced perfusion of the brain tissue in 6- and 12-month-old SHR rats in comparison with normotensive rats. All types of vascular tone were markedly increased in comparison with those in WKY rats. We assume, that significant increase in ET, NT, and MT in pial arteries of SHR rats reflects an autoregulatory mechanism protecting blood vessels from excessive transmural pressure and cerebral edema. Intracerebral transplantation of MSC did not significantly changed BP, but induced angiogenesis and increased brain tissue perfusion in 6-month-old, but not in 12-month-old SHR rats. The increase in effective perfusion index and coefficient of variation after MSC transplantation attested to a significant activation of regulatory mechanisms, which manifested in the increase in NT and MT. In 12-month SHR, the increase in NT and MT was higher than in 6-month-old animals. The increase in arterial MT in SHR during the period from 6 to 12 months can be explained by hypertrophy of the arterial wall and smooth muscle cells growth in the walls. The same process presumably causes NT increase: high number of myocytes develops greater force at the same intensity of nervous regulation. We think that intracerebral transplantation of MSC leads to not only the formation of endotheliocytes and myocytes in newly formed vessels, but also promotes the appearance of new cells in the pre-existing arteries. This leads to the increase MT and NT by the previously described mechanisms, on the one hand, and contributes to ET decrease through additional production of vasodilators by newly formed endotheliocytes, on the other.

REFERENCES

1. Lobov GI, Gurkov AS. Modulation of blood flow in the microvasculature of fingers after the formation a radiocephalic arteriovenous fistula. Nefrologiya Dializ. 2014;16(3):364-371. Russian.
2. Sokolova IB, Sergeev IV, Skorobogataya EV, Ufimtseva AN, Polyntsev DG, Dvoretskii DP. Effect of Transplantation of Mesenchymal Stem Cells on the Density of Pial Microvascular Network in Spontaneously Hypertensive Rats of Different Age. Bull. Exp. Biol. Med. 2017;163(1):129-132.
3. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. Circ. Res. 2005;97(6):512-523.
4. Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, Lambrichts I. Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol. Ther. 2014;143(2):181-196.
5. da Silva Meirelles L, Caplan AI, Nardi NB. In search of the in vivo identity of mesenchymal stem cells. Stem Cells. 2008;26(9):2287-2299.
6. de Oliveira LF, Almeida TR, Ribeiro Machado MP, Cuba MB, Alves AC, da Silva MV, Rodrigues Júnior V, Dias da Silva VJ. Priming mesenchymal stem cells with endothelial growth medium boosts stem cell therapy for systemic srterial hypertension. Stem Cells Int. 2015;2015. ID 685383. doi: 10.1155/2015/685383.
7. Heinert G, Casadei B, Paterson DJ. Hypercapnic cerebral blood flow in spontaneously hypertensive rats. J. Hypertens. 1998;16(10):1491-1498.
8. Lee TH, Liu HL, Yang ST, Yang JT, Yeh MY, Lin JR. Effects of aging and hypertension on cerebral ischemic susceptibility: evidenced by MR diffusion-perfusion study in rat. Exp. Neurol. 2011;227(2):314-321.
9. Li Y, Shen Q, Huang S, Li W, Muir ER, Long JA, Duong TQ. Cerebral angiography, blood flow and vascular reactivity in progressive hypertension. Neuroimage. 2015;111:329-337.
10. Meneses A, Hong E. Spontaneously hypertensive rats: a potential model to identify drugs for treatment of learning disorders. Hypertension. 1998 Vol. 31(4):968-972.
11. Sabbatini M, Strocchi P, Vitaioli L, Amenta F. Microanatomical changes of intracerebral arteries in spontaneously hypertensive rats: a model of cerebrovascular disease of the elderly. Mech. Ageing Dev. 2001;122(12):1257-1268.
12. Tayebati SK, Tomassoni D, Amenta F. Spontaneously hypertensive rat as a model of vascular brain disorder: microanatomy, neurochemistry and behavior. J. Neurol. Sci. 2012;322(1-2):241-249.
13. Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? Hypertension. 2004;44(3):248-252.
14. Wang L, Mu Z, Lin X, Geng J, Xiao TQ, Zhang Z, Wang Y, Guan Y, Yang GY. Simultaneous imaging of cerebrovascular structure and function in hypertensive rats using synchrotron radiation angiography. Front. Aging Neurosci. 2017;9:359. doi: 10.3389/fnagi.2017.00359.
15. Zhou Q, Dong Y, Huang L, Yang S, Chen W. Study of cerebrovascular reserve capacity by magnetic resonance perfusion weighted imaging and photoacoustic imaging. Magn. Reson. Imaging. 2009;27(2):155-162.