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Aging-Related Changes in the Blood Flow Rate and Oxygen Saturation of the Blood in the Cerebral Cortex of Rats

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Abstract—Male Wistar-Kyoto rats received intracerebral transplantation of syngeneic mesenchymal stem cells (MSCs) at 22–24 months of age. The cognitive function of these animals was tested and the microvasculature density, tissue blood-flow rate, and oxygen saturation of mixed blood in the cortical microvasculature were measured in the sensorimotor cortex of the contralateral brain hemisphere under standard conditions, after norepinephrine application, and in global ischemia 3 weeks after cell transplantation. Rats at 2–3 and 22–24 months of age were used as the control groups. MSC transplantation had a positive impact on microcirculation in the cerebral cortex of old animals: deterioration of the vascular bed was prevented and the level of oxygen saturation of blood in the microvasculature was elevated; therefore, the tolerance of animals to the spasm of pial arterioles and global cerebral ischemia was increased, although aging-related decline of cognitive functions was not alleviated.

Keywords: mesenchymal stem cells, cerebral cortex, blood-flow rate, blood oxygen saturation, cognitive function

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INTRODUCTION

Adequate blood supply is more important for normal functioning of the brain than for that of any other organ. Aging-related changes in the structure of the microvascular network contribute to a reduction of blood flow in various brain regions [6, 7, 11], impairment of oxygen diffusion from the blood into tissues [8, 12], and deterioration of the reactivity of cerebral arterioles to exogenous and endogenous adverse factors [9, 13, 14]. Angina pectoris, hypertension, diabetes mellitus, and atherosclerosis commonly occur in aged people and reinforce age-related pathological changes in the circulatory system [10], often causing blood-vessel spasms and ischemia of brain tissue.

The authors' previous studies [5] demonstrated the efficiency of intracerebral transplantation of mesenchymal stem cells (MSCs) for the prevention of age-related deterioration of brain vasculature. The microvasculature density in the pia mater located above the sensorimotor cortex of old rats was almost doubled after MSC transplantation to these animals and the reactivity of the newly formed pial arteries was the same as that of the native microvasculature of young rats [3]. The microvascular network that formed after intracerebral transplantation persisted over the second half of the lifespan of the animals (from 12 to 24 months of age).

The aim of the present work was to study aging-related changes in blood flow and oxygen saturation rate of blood in the microvasculature of the cerebral cortex of rats and to characterize the effect of intracerebral MSC transplantation on these parameters and on cognitive function in old animals.

MATERIALS AND METHODS

Male Wistar-Kyoto rats of 2–3 months of age ($n = 15$) and 22–24 months of age ($n = 25$) were used in the experiments. The animals were housed under standard conditions with natural lighting and free access to food and water. All procedures with animals were performed in accordance with international rules and standards (European Communities Council Directives of November 24, 1986, 86/609/EEC).

MSC cultures were prepared from bone marrow of 2–3-month old syngeneic rats. Cells of the third passage (P_3) were used for transplantation. Immunophenotype of the MSCs was assessed using a Guava HT easyCyte 8 Flow Cytometry System (Millipore, United States). The cells were stained with antibodies that targeted the CD45, CD44, CD106, and CD90, and against the control isotype IgG (Millipore, United States). The GUAVASOFT 2.2.3 software package (Millipore, United States) was used to analyze the data.

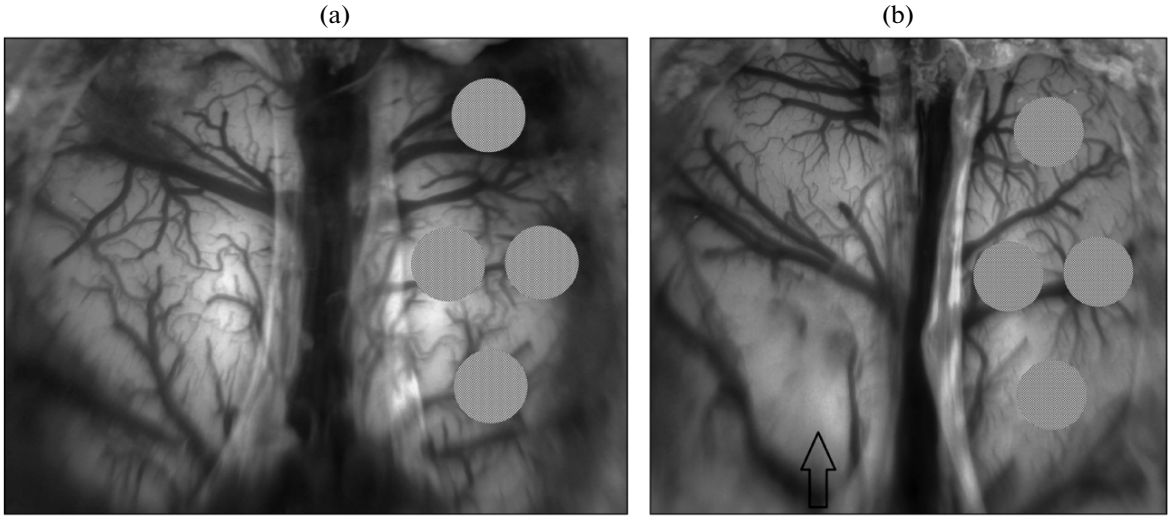

Fig. 1. Original images of the cerebral cortex of experimental animals (at a 25× magnification). (a) Control animal; areas of perfusion (MI) and mixed blood saturation (SO_2) measurement in the microvascular network of the sensorimotor cortex are marked in gray; (b) an animal 3 weeks after intracerebral transplantation of MSCs; the MSC injection area is marked by an arrow, areas of perfusion (MI) and mixed blood saturation (SO_2) measurement in the microvascular network of the sensorimotor cortex are marked in gray.

Intracerebral transplantation was conducted under zoletil (20 mg/kg, Virbac, France) anesthesia. A hole of 1 mm in diameter was drilled in the parietal region of the skull without damaging the dura mater. MSC suspensions (200000 cells in 20 μL α-MEM stem-cell culture medium) were injected into the cerebral cortex to a depth of not more than 2 mm using insulin syringes and the skin on the animal's head was sutured.

The animals were divided into the following groups: 1 (control), young animals (2–3 months of age); $n = 15$; 2 (control), old animals (22–24 months of age); $n = 15$; and 3, animals of 22–24 months of age that received intracerebral MSC transplantations 3 weeks prior to the analysis of the microvasculature of the pia mater of the cerebral cortex, $n = 10$.

The density of the microvasculature of the pia mater that overlies the sensorimotor cortex and that of the arterial component of the vasculature in the contralateral (right) hemisphere of rats from group 3 and in the right hemisphere of the control rats was quantitated using Photo-M software.

The tissue blood-flow rate in the sensorimotor cortex and blood saturation (SO_2) in the microvascular bed were measured using a multi-function LAKK-M laser diagnostic complex (NPP Lazma, Russia). The microcirculation index (MI), a dynamic parameter of blood microcirculation, was defined as the change in blood flow in a given tissue fragment (with a volume of approximately 1 mm^3) per unit time, as measured using laser Doppler flowmetry and expressed in arbitrary perfusion units (pf). Blood SO_2 in the microvasculature of the same tissue fragment was assessed using optical tissue oximetry. The tissue fragment contained both oxyhemoglobin (in the arterioles) and deoxyhemoglobin (in the venules); therefore, the SO_2 value characterized the state of the mixed blood.

The parietal bone and dura of an experimental animal were removed under zoletil anesthesia prior to MI and SO_2 measurements; thus, the pia mater over the sensorimotor cortex was exposed. The brain surface was continuously superfused with physiological saline warmed to 37°C. MI and SO_2 were recorded from four sites in the sensorimotor cortex (Fig. 1) of the contralateral (right) hemisphere of the animals from group 3 and the corresponding hemispheres of rats from the control groups. The body temperature of the animals was maintained at 37°C throughout the experiment. The mean blood pressure was 105–120 mm Hg in young animals and 120–130 mm Hg in old animals. The changes of MI and SO_2 in all of the animals were assessed under standard conditions after the application of the vasoconstrictor noradrenaline (NA, at 10^{-3} M) to the brain surface and upon occlusion of both carotid arterioles for 5 min (global ischemia). The significance of the differences was determined statistically using the nonparametric Mann–Whitney test at a significance threshold of $p \le 0.05$.

Cognitive functions were assessed at 3 weeks after transplantation in the Morris water maze, which is a standard test for research on learning and memory. The pool was 145 cm in diameter and 40 cm in height; the depth of the water was 25 cm and the water temperature was 24 ± 1°C. A hidden platform was located in the center of one of the quadrants (the platform diameter was 12 cm, the distance between the platform and the pool wall was 25 cm, and the surface of the platform was 1.5 cm below the water level). The room was lit with diffuse light from two 250-watt lamps. Additional spatial cues (blue and green rectangles)

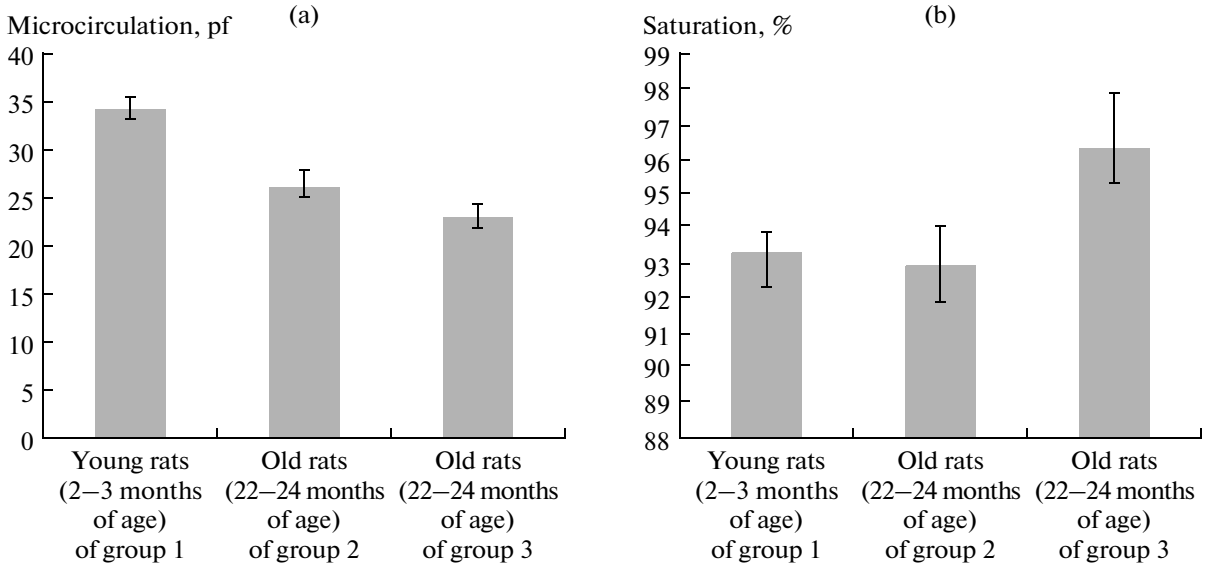

Fig. 2. The rate of tissue blood flow in the sensorimotor cortex of rats of different ages. (a) Perfusion of the sensorimotor cortex; ±CI, $p \le 0.05$; (b) mixed blood saturation (SO_2) in the microvascular network of the sensorimotor cortex; ±CI, $p \le 0.05$.

were mounted on the walls of the testing room; neither the location of the platform nor that of the rectangles changed during the learning period. Each animal was trained daily for 5 days to find the platform. Four 60-second training sessions with four different randomly selected starting points were performed. The rat was placed in a standard cage box in the adjacent room during 30-second breaks between the sessions. The time that was spent searching was averaged over the block of four sessions to obtain a generalized characteristic of the navigation ability of the rat on a certain day. The platform was removed from the maze and a so-called "trial session" of 60 seconds was performed 0.5–1 h after the training period. A video recording of the animal's searching behavior during the trial session was produced and used for the assessment of the parameters of spatial-learning efficiency, viz., the number of entries into the area where the platform had been located and the time of searching in the "target quadrant" (the one where the platform was located during the training sessions). The characteristics of learning in the Morris water maze were analyzed using two-factor ANOVA for repeated measures, and the trial sessions were analyzed using one-way ANOVA. Post-hoc comparison of the means was performed using the Fisher's least-significant difference criterion in all cases.

RESULTS AND DISCUSSION

Flow cytometry showed that the cell culture that was used for transplantation contained 5–8% of $CD45^+$ cells (cells of a hematopoietic lineage) and 90–95% of $CD90^+$ cells (actual MSCs). The authors' previous experiments revealed a 1.8-fold decrease of microvascular density in the pia mater over the sensorimotor cortex of old rats (22–24 months of age) as compared to young animals (2–3 months of age) [1]; that is, aging-related deterioration of the vascular network occurred. Intracerebral transplantation of MSCs to old animals induced an approximately 1.9-fold increase of the density of the microvascular network in this area. Similar results were obtained in the present study.

A decrease of the blood-flow rate in various brain structures was previously detected in aging humans

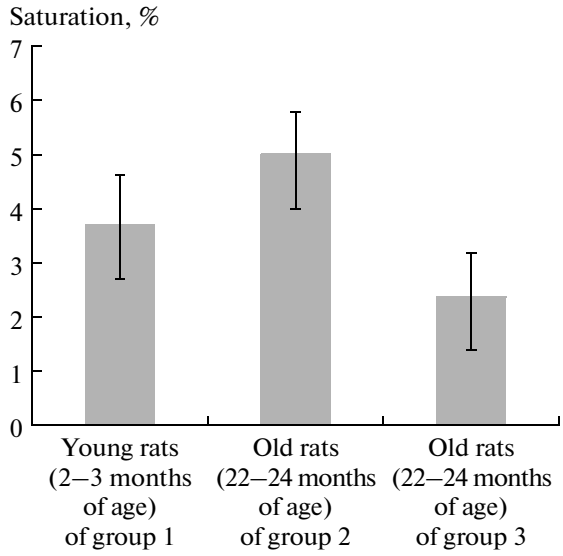

Fig. 3. Changes in the saturation of mixed blood in the microvascular network of the sensorimotor cortex in rats of different ages during global ischemia (±CI, $p \le 0.05$).

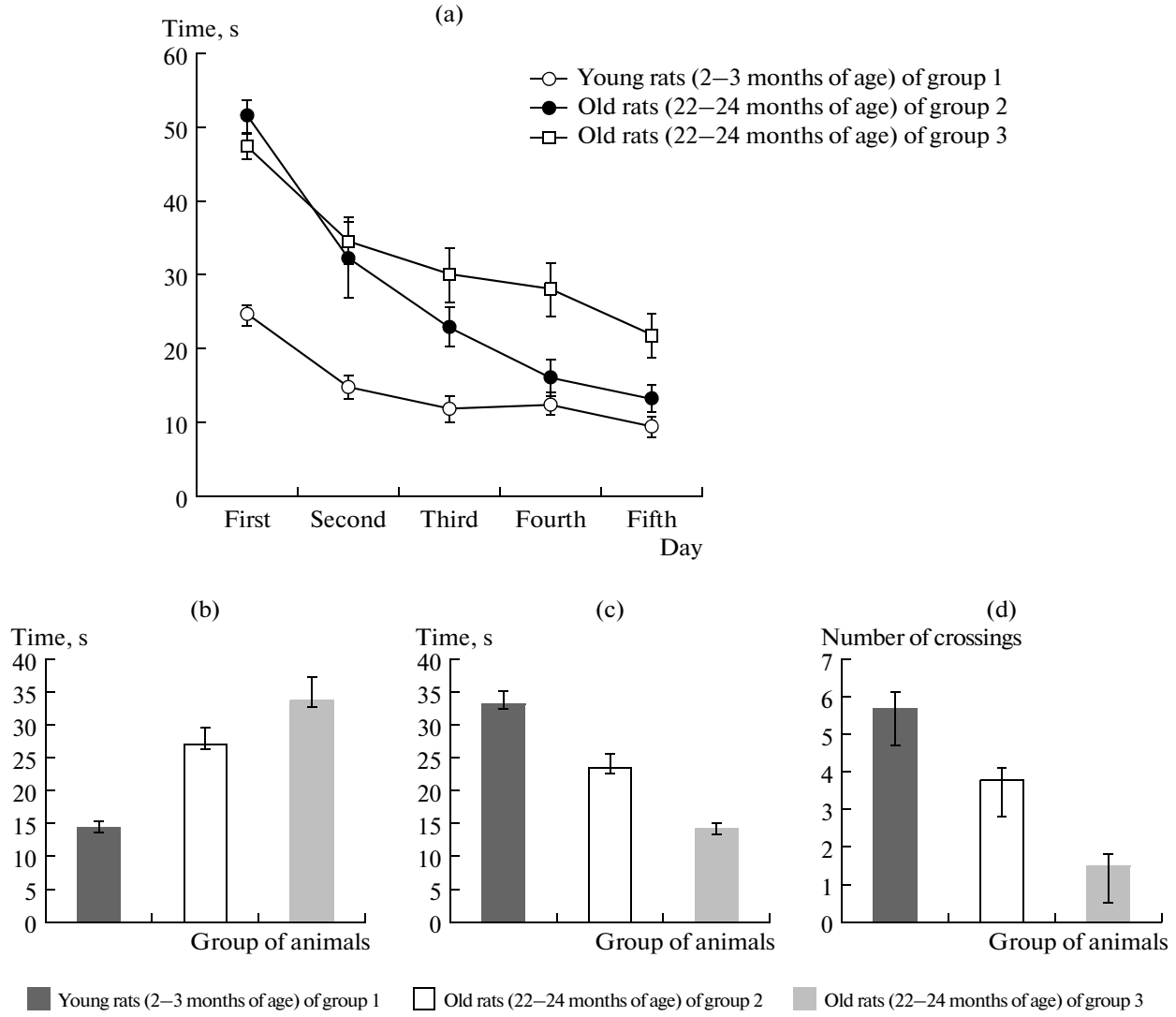

Fig. 4. The assessment of cognitive function in animals of different ages using the Morris water maze. (a) Changes in the time of searching for the platform during 5 days of training (±SEM, $p \le 0.05$); statistically significant differences (Fisher's exact test) between the control groups observed at $p < 0.05$; (b) average time of searching for the platform during 5 days of training (±SEM, $p \le 0.05$); statistically significant differences (Fisher's exact test) between the control groups observed at $p < 0.05$; (c) the time that was spent searching in the target quadrant during the trial session after the learning period (±SEM); (d) the number of crossings of the area where the platform had been located during the trial session after the learning period (±SEM); statistically significant differences (Fisher's exact test) between the second and third group of old animals observed at $p < 0.05$.

and animals [6, 7, 11]. The present study revealed a statistically significant decrease of MI in the sensorimotor cortex of old rats as compared to young animals; a 1.3-fold decrease occurred on average. Intracerebral transplantation of MSCs did not induce an increase in MI of old rats (Fig. 2a). However, the SO_2 of the mixed blood in the cortical microvasculature of animals from group 3 was somewhat higher than in the young and old control animals; the values were 96.3, 93.4, and 91.8%, respectively (Fig. 2b). Pial arteriogenesis was activated after MSC transplantation and the relative abundance of pial and radial arterioles in cortical microvasculature increased [2]; this probably led to an increase of the share of arterial blood with a higher SO_2 in the mixed blood of the microvasculature of the investigated area.

Some of the pial arterioles were constricted in response to application of the vasoconstrictor NA to the pia mater; however, some arterioles expanded and a small part of the arterioles did not react to the stimulus at all [3]. MI in some cortical areas of the experimental animals decreased upon NA application, while MI in other cortical areas of the same animals increased. Both the relative decrease (26.6 ± 2.5 and 25 ± 7.5%) and the relative increase (23.4 ± 7.3 and 25.4 ± 5.6%) of MI in the control groups of animals

were similar; thus, there were no statistically significant changes in the average MI. The decrease in MI in the animals of group 3 was 20.3 ± 4.5%, while the increase that was observed in other areas of the cortex was 40 ± 3.7% and the average MI in the cortex increased by approximately 20% in response to *NA* application.

A 5-minute occlusion of both carotid arterioles caused a reduction of sensorimotor cortex MI by 60.1 ± 5.7; 53.3 ± 3.6, and 79.1 ± 7.6% in groups 1, 2, and 3, respectively. The decrease of SO_2 in the microvasculature blood of animals that received MSCs was the least pronounced in the investigated tissue site (Fig. 3). The higher SO_2 level in the blood of the cortical microvasculature during global ischemia was probably due to the increased density of pial and radial arterioles in the cortex due to arteriogenesis activation (as mentioned above). Pathological conditions associated with spasm of the brain vasculature and/or oxygen deficiency are characteristic of elderly people, and maintenance of the brain oxygen level that is sufficient for the functioning of neurons is very important under these conditions.

Cognitive function tests did not reveal any improvement in memory and orientation in experimental animals that received intracerebral MSC transplants. Both young and old animals demonstrated a capacity to learn and remember spatial cues (the time spent searching decreased concomitantly to the increase in the number of trials) (Fig. 4a). Old animals of groups 2 and 3 spent significantly more time searching for the platform throughout the test period (Figs. 4b–4d). The surgery itself probably caused considerable damage to the cortex of the ipsilateral hemisphere [4] due to the small size of the experimental animals (rats); therefore, the positive impact of MSCs on microcirculation in the cerebral cortex of aged animals was abolished.

CONCLUSIONS

Intracerebral transplantation of mesenchymal stem cells had a positive effect on microcirculation in the cerebral cortex of aged animals, preventing microvasculature deterioration and increasing the level of blood saturation in the vessels; therefore, the experimental animals showed higher tolerance to spasm of pial arterioles and global cerebral ischemia.

REFERENCES

1. Sokolova, I.B., Sergeev, I.V., Fedotova, O.R., and Dvoretskii, D.P., Age-related changes of microcirculation in pial blood vessels of the sensorimotor cortex of the rat brain, *Adv. Gerontol.*, 2014, vol. 4, no. 2, pp. 145–149.

2. Sokolova, I.B., Sergeev, I.V., Anisimov, S.V., et al., Influence of transplantation of mesenchymal stem cells on density of microvascular network of brain cortex peal membrane of different-age rats, *Kletochnye Tekhnol. Biol. Med.*, 2012, no. 4, pp. 205–210.

3. Sokolova, I.B., Sergeev, I.V., Anisimov, S.V., et al., The effect of intracerebral transplantation of mesenchymal stem cells on reactivity of pial arterioles in old rats, *Kletochnye Tekhnol. Biol. Med.*, 2014, no. 2, pp. 81–84.

4. Sokolova, I.B., Fedotova, O.R., Gilerovich, E.G., Sergeev, I.V., Anisimov, S.V., Puzanov, M.V., and Dvoretsky, D.P., The effect of intracerebral transplantation of mesenchymal stem cells on orientation and exploratory behavior of aged animals, *Adv. Gerontol.*, 2014, vol. 4, no. 1, pp. 22–27.

5. Sokolova, I.B., Fedotova, O.R., Gilerovich, E.G., Sergeev, I.V., Dvoretsky, D.P., Anisimov, S.V., and Puzanov, M.V., The efficiency of mesenchymal cell intracerebral transplantation for corrections of cerebral microcirculation age-related alterations in rats, *Cell Tissue Biol.*, 2014, vol. 8, no. 4, pp. 304–312.

6. Ances, B., Liang, C., Leontiev, O., et al., Effects of aging on cerebral blood flow, oxygen metabolism, and blood oxygenation level dependent responses to visual stimulation, *Hum. Brain Mapp.*, 2009, vol. 30, no. 4, pp. 1120–1132.

7. Bangen, K., Restom, K., Liu, T., et al., Differential age effects on cerebral blood flow and BOLD response to encoding: associations with cognition and stroke risk, *Neurobiol. Aging*, 2009, vol. 30, no. 8, pp. 1276–1287.

8. Brown, W. and Thore, C., Cerebral microvascular pathology in aging and neurodegeneration, *Neuropharmacol. App. Neurobiol.*, 2011, vol. 37, no. 1, pp. 56–74.

9. Mayhan, W., Arrick, D., Sharpe, G., and Sun, H., Age-related alteration in reactivity of cerebral arterioles: role of oxidative stress, *Microcirculation*, 2008, no. 15, pp. 225–236.

10. Najjar, S., Scuteri, A., and Lakatta, E., Arterial aging is it an immutable cardiovascular risk factor?, *Hypertension*, 2005, no. 46, pp. 454–462.

11. Restom, K., Bangen, K., Bondi, M., et al., Cerebral blood flow and BOLD responses to a memory encoding task: a comparison between healthy young and elderly adults, *Neuroimage*, 2007, vol. 37, no. 2, pp. 430–439.

12. Shao, W., Li, C., Chen, L., et al., Stereological investigation of age-related changes of the capillaries in white matter, *Anat. Rec.* (Hoboken), 2010, vol. 293, no. 8, pp. 1400–1407.

13. Uchida, S. and Hotta, H., Cerebral cortical vasodilatation mediated by nicotinic cholinergic receptors: effects of old age and of chronic nicotine exposure, *Biol. Pharm.*, 2009, vol. 32, no. 3, pp. 341–344.

14. Uchida, S., Suzuki, A., Kagitani, F., and Hotta, H., Effects of age on cholinergic vasodilatation of cortical cerebral blood vessels in rats, *Neurosci. Lett.*, 2000, vol. 294, no. 2, pp. 109–112.

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