BIOGERONTOLOGY

Effect of Histochrome on Brain Vessels and Research and Exploratory Activity of Senescence-Accelerated OXYS Rats

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Changes in the diameter of brain vessels and intensity of collateral blood flow typical of chronic ischemia were detected by magnetic resonance imaging in senescence-accelerated OXYS rats demonstrating reduced (compared to Wistar rats) research and exploratory activity. Histochrome (antioxidant drug) produced positive effects on cerebral vessels in OXYS rats by stimulating collateral blood flow and acting as a vasodilator agent. Analysis of correlations showed that these effects of histochrome were closely related to its capacity to activate research and exploratory activity and reduce anxiety of OXYS rats in the open field test.

Key Words: *magnetic resonance tomography; chronic ischemia; histochrome; early aging OXYS rats*

Chronic ischemia induced by diffuse insufficiency of blood supply to the brain tissue is a cause of reduced functional potential of the brain and changes in the cognitive sphere in elderly people. It is associated with steady increase in the prevalence of cerebrovascular diseases against the background of population aging and increased incidence of acute cerebrovascular disorders of different genesis, which dictates the search for new therapeutic and preventive methods. Discovery of a close relationship between age-associated brain dysfunctions and oxidative stress led to a wide use of antioxidants for their treatment and prevention. However, analysis of their efficiency in chronic ischemia is impeded because of difficulties in objective evaluation of the cerebrovascular status and its reaction to therapy. These problems can be solved by using biological models and unique potentialities of magnetic resonance imaging (MRI), which shows the age-specific changes in the morphological and functional parameters of the cerebral vessels and their reaction to therapy.

We previously demonstrated the prospects of using senescence-accelerated OXYS rats (Institute of Cytology and Genetics) for evaluation of the efficiency of prevention of age-associated cerebral dysfunctions. These animals are characterized by early development of changes in the mental and cognitive spheres typical of aging humans and animals, as well as of cataract, retinal degeneration, osteoporosis, and hypertension [2,4,7,8]. Brain development of OXYS rats is characterized by delayed formation of the vascular bed: delayed pro-

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liferation of capillaries in the pia matter and changes in energy metabolism during the early postnatal period typical of adaptation to tissue hypoxia [9]. By the age of 3 months OXYS rats develop high anxiety, disorders in associative training, and low research and exploratory activity regarded as manifestations of accelerated aging [9]. Subsequent formation of chronic brain ischemia and possible relationship between this condition and changed behavior of OXYS rats were never studied before.

We evaluated the state of cerebral vessels and parameters of cerebral blood flow in OXYS rats by using MRI technique, analyzed its relationship with changes in the research and exploratory activity, and evaluated the possibility of their correction with antioxidant drug histochrome, a natural echinochrome A (2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone) effectively binding iron ions. The drug was created at the Pacific Institute of Organic Biochemistry and successfully used in ophthalmology and cardiology [6]. The effects of histochrome on brain function and age-specific changes were not studied.

MATERIALS AND METHODS

The study was carried out on 12 OXYS rats aged 13 months; 12 Wistar rats of the same age served as controls. The animals were obtained from Institute of Cytology and Genetics (Novosibirsk) and kept 2 per cage. The rats were kept under conditions of natural illumination on standard rations with free access to water and fodder. Histochrome (1% solution for intravenous injections) was injected intraperitoneally (1 mg/kg) for 5 days. The course of injections was repeated after 2 months. Before the therapy and after the first and repeated courses the animals were tested in an open field and cerebral blood vessels were examined by MRI under narcosis (5.5 mg/kg rometar and 37 mg/kg diazepam intraperitoneally). We used a 7 T PharmaScan US 70/16 tomograph for experimental studies 300 MHz frequency, BGA 09P type coil (Bruker) was used. FLASH and RARE_8 pulse sequences were used; layer-by-layer sections in the frontal, saggittal, and axial planes were made for obtaining T1- and T2-weighed images (WI). The parameters of T2-WI were as follows: 256×256 matrix, section thickness 1 mm, 4×4 cm review area, TR/TE 2579.8/44.5 msec. Occipital 3D-reconstruction of the vascular basin was realized using Head_Angio (3D-TOF) program with *ParaVision 3.0.2* software attached to the tomograph. Maximal intensity projection (MIP) was carried out (reconstruction of vessels for detecting vascular abnormalities).

The diameter of cerebral arteries (CA) was measured using 2 points determined by the MRI cursor: at the adventitium/medium interface of the arterial lateral wall and at the medium/adventitium interface of the medial wall. Each measurement was repeated 3 times. Vasomotor reaction to histochrome was evaluated in percent; the diameter of arteries before histochrome injections was taken as 100%. Dynamics of changes in vessel size was estimated using ROI (Region of Instruments) by means of *ParaVision 3.0.2* software attached to the tomograph. The results were analyzed using nonparametric Kruskal—Wallis test [1].

The open field test was carried out as follows. The animals were placed into a corner of a square box (100×100 cm, 100 squares) with 40-cm plastic walls, illuminated with a shadowless 100-W lamp positioned at a height of 100 cm above the center of the field. Motor activity was recorded for 5 min: latency of visiting the center, number of crossed squares, rearings, explored holes, grooming reactions, and fecal boluses.

The results of studies of the drug effects on behavior and cerebral bloodflow parameters were analyzed by ANOVA analysis of dispersions, the animal genotype and the drug were used as independent factors.

RESULTS

The comparison of MRI of CA in Wistar and OXYS rats (superficial temporal, internal maxillary, internal carotid, shpenopalatine, and ophthalmic artery) showed signs of chronic ischemia in OXYS rats (Fig. 1). In contrast to Wistar rats, their cranial superior alveolar artery was short, without discernible branches. Paired related comparisons failed to show differences between the hemispheres in the diameters of the corresponding cerebral vessels in any of animal groups (Table 1). On the other hand, the drug and the genotype affected the diameters of all studied CA, the interaction between the factors attested to differences in the reactions of Wistar and OXYS rats to histochrome. Before therapy the diameters of the anterior and middle CA in OXYS rats was less than in Wistar rats, while the diameter of the posterior CA was greater. For the anterior right CA $F_{2.54}$=45.8, p<0.0001, $F_{1.54}$=12.6, p<0.001, and $F_{2.54}$=12.3, p<0.0001, respectively.

Histochrome significantly increased the diameters of all CA in OXYS rats as soon as after the first course of treatment. In Wistar rats the diameters of the anterior CA, right middle and posterior CA increased after the first course, the diameter of the left middle CA increased only after the

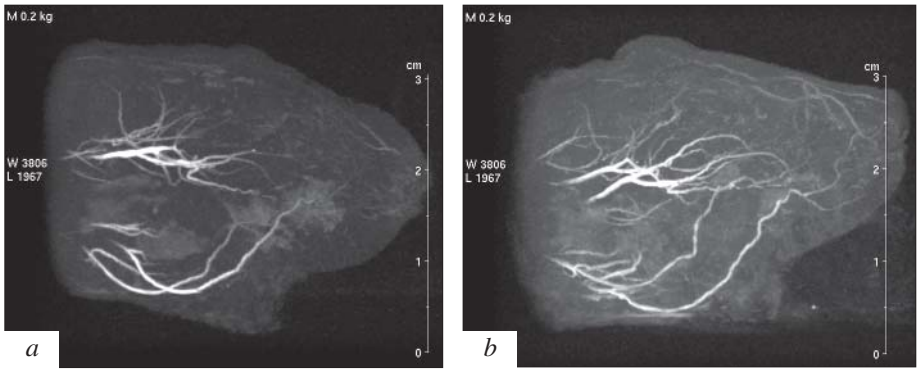

Fig. 1. Tomograms of the brain of OXYS rats before (*a*) and after two 5-day courses of histochrome treatment (*b*).

second course of histochrome, while the diameter of the right posterior CA did not change. As a result, the differences between the strains in the diameters of both anterior CA and left middle CA disappeared, while the diameters of the right middle and posterior CA became greater in OXYS compared to Wistar rats.

The collateral blood flow depended on the genotype ($F_{1.54}$=32.9, p<0.000). Before therapy it was less in OXYS rats compared to Wistar ones. Histochrome modified this parameter ($F_{2.54}$=78, p<0.000), but the interaction between the factors ($F_{1.54}$=66.3, p<0.000) indicated differences in the reaction of this parameter to histochrome treatment in Wistar and OXYS rats. The drug did not change the collateral bloodflow in Wistar rats, while in OXYS rats this parameter increased by 15% after the first course and by 24% after the second course of treatment. As a result of treatment, the collateral bloodflow in OXYS rats became more intense than in Wistar rats treated with histochrome.

During the first presentation in the open field, (Table 2) OXYS rats, as was expected, demonstrated significantly lower horizontal ($F_{1.18}$=182; p<0.001) and vertical activities ($F_{1.18}$=183; p<0.000, *vs.* Wistar rats) and more passively explored the holes ($F_{1.18}$=485; p<0.001). Histochrome modified horizontal ($F_{2.54}$=8.4; p<0.001) and vertical ($F_{2.54}$= 4.7; p<0.013) activities of animals and changed the number of explored holes ($F_{2.54}$=131; p<0.001). However, the reactions of Wistar and OXYS rats to the drug were different. After the first course of histochrome, horizontal activity of Wistar rats decreased by 17% and after the second course by 33% in comparison with the initial testing. Rearing decreased during the third testing, though less significantly. This is obvious: activity of animals in the open field decreased with each subsequent test because the novelty of the situation was lost. This trend was more pronounced in OXYS rats [5]: horizontal activity of Wistar rats decreased 2-fold during repeated testing, while in OXYS rats it decrea-

TABLE 1. MRI Study of the Effects of Histochrome on CA and Collateral Blood Flow in the Brain of Wistar and OXYS Rats (M±m; n=10; 30 measurements per point)

Parameter	Wistar			OXYS		
	before treatment	after course 1	after course 2	before treatment	after course 1	after course 2
Diameter of anterior CA, mm	0.92±0.02	0.95±0.02*	0.95±0.01*	0.87±0.02^{+}	0.93±0.02*	0.96±0.02*
	0.92±0.02	0.95±0.01*	0.95±0.20	0.88±0.02^{+}	0.93±0.02*	0.96±0.01*
Diameter of middle CA, mm	0.96±0.10	0.97±0.60	1.01±0.05*	0.92±0.02^{+}	1.00±0.01*	1.03±0.01*
	0.96±0.01	1.02±0.11*	1.00±0.01*	0.92±0.01^{+}	1.00±0.01*	1.03±0.01*$^{+}$
Diameter of posterior CA, mm	1.00±0.01	1.01±0.01	1.01±0.01	1.01±0.01^{+}	1.04±0.01*	1.06±0.01*$^{+}$
	0.98±0.01	1.00±0.01*	1.01±0.01*	1.02±0.01^{+}	1.05±0.01*	1.05±0.01*$^{+}$
Intensity of signal from collateral blood flow, arb. units.	31.30±0.68	31.40±0.52	31.60±0.52	29.00±1.16^{+}	33.40±0.52*$^{+}$	36.1±1.66*$^{+}$

Note. Numerator: left CA; denominator: right CA. *p<0.001 compared to the data before treatment. Here and in Table 2: ^{+}p<0.001 compared to Wistar rats.

TABLE 2. Effect of Histochrome on the Behavior of Wistar and OXYS Rats in the Open Field Test ($M \pm m$)

Parameter	Wistar			OXYS		
	before treatment	after course 1	after course 2	before treatment	after course 1	after course 2
Latent period	11.60±0.54	12.50±0.43	11.10±0.41	39.4±0.5^{+}	30.20±0.73^{+*}	23.00±0.72^{+*}
Crossed squares	13.50±0.52	11.20±0.45**	9.30±0.26*	5.60±0.27^{+}	7.40±0.22^{+*}	7.2±0.2^{+**}
Rearings	11.30±0.52	10.50±0.37	9.4±0.4***	3.8±0.2^{+}	6.70±0.21^{+*}	6.90±0.23^{+*}
Explored holes	13.5±0.5	17.10±0.23*	19.30±0.47*	2.00±0.15^{+}	5.70±0.21^{+*}	6.1±0.1^{+*}
Grooming	5.70±0.26	5.90±0.31	6.50±0.22***	5.50±0.17	3.80±0.13*	3.80±0.13*
Defecations	1.80±0.13	1.80±0.13	1.80±0.12	2.30±0.15^{+}	2.00±0.02	1.80±0.13

Note. *p<0.0001, **p<0.001, ***p<0.05 compared to the data before treatment.

sed 7-fold; vertical activity decreased 4- and 7-fold, respectively. Histochrome significantly modified this trend, more intensely in OXYS rats (Table 2). After the first course of histochrome, horizontal activity of OXYS rats was 29% and after the second course 25% higher than during the initial testing (before treatment). Vertical activity of OXYS rats after the first and second courses of histochrome surpassed the initial values. The number of explored holes (the most demonstrative indicator of exploratory activity) increased during the second and third tests in both rat strains. It is noteworthy that the research and exploratory activity of OXYS rats remained significantly lower than in Wistar rats.

High anxiety of OXYS rats manifested in a more than 3-fold prolonged latency of visiting the center of the open field and increased number of defecation boluses compared to the corresponding parameters in Wistar rats (Table 2). Histochrome reduced anxiety of OXYS rats. Without appreciably decreasing the number of defecation boluses, it leveled the interstrain differences by this parameter. The drug shortened the latency of visiting the center, but OXYS rats remained more anxious by this parameter than Wistar rats. The number of grooming reactions did not differ in experimental animals, but histochrome increased their number in Wistar rats and reduced it 1.4 times in OXYS rats.

The relationship between the effects of histochrome on animal behavior and changes in cerebrovascular status induced by this drug was evaluated by analysis of correlations. This analysis revealed a close relationship between cerebral blood flow parameters and parameters of exploratory activity of OXYS rats, which reached the functional level. The coefficients of correlations between the diameters of various cerebral arteries and number of crossed squares varied from 0.60 to 0.74, between the former and rearing episodes from 0.67 to 0.90, and were maximum for the number of explored holes: 0.73-0.95 (p<0.001 for all cases). A significant, but negative correlation was detected between CA diameter and anxiety parameters: the coefficients varied from -0.71 to -0.90 (p<0.0001) for the latency of visiting the center, from -0.37 to -0.53 (p<0.05) for the number of defecation boluses, and from -0.66 to -0.82 (p<0.0001) for grooming reactions. No regularities of this kind were detected for Wistar rats. Only the number of explored holes positively correlated with the CA diameter, while horizontal and partially vertical activities were in negative correlation with these parameters.

Hence, OXYS rats exhibited structural and functional changes in the cerebral blood flow typical of chronic ischemia and leading to reduction of the research and exploratory activity of these animals and their high anxiety. Stimulation of research and exploratory activity and reduction of animal anxiety with histochrome is due to its positive effect on the cerebral bloodflow.

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