Evaluation of Effects of Histochrome and Mexidol on Structural and Functional Characteristics of the Brain in Senescence-Accelerated OXYS Rats by Magnetic Resonance Imaging

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The effects of histochrome and mexidol on the morphology and function of the brain and behavior were studied in senescence-accelerated OXYS and Wistar rats. MRI showed that signs of neurodegenerative changes were present in OXYS rats at the age of 3 months and were pronounced at the age of 12 months. Histochrome (1 mg/kg, 5 days) more effectively than mexidol (4 mg/kg, 7 days) reduced anxiety and increased exploratory activity of 1-year-old OXYS rats. Both drugs improved the morphology and function of the brain. Their effects consisting in correction of diffuse changes in the white matter and reduction of edema were comparable; in addition, histochrome reduced the intensity of demyelinization processes.

Key Words: *aging; neuroprotectors; antioxidants; magnetic resonance imaging; OXYS rats*

Aging of humans and animals is associated with gradual reduction of the functional potential of the brain, changes in behavioral and cognitive functions, and higher probability of neurodegenerative diseases. The increase in the number of patients suffering from these diseases is explained by an increase in the number of long living subjects and deterioration of the ecological situation in countries with well-developed economy. Under these conditions, the problem of active longevity and its obligatory condition, retention of the cognitive functions, acquires special significance. Drugs reducing the severity of ischemia and oxidative stress (the leading mechanisms of "physiological" aging of the brain and of the pathogenesis of neurodegenerative processes of different etiology) occupy a special place in the therapy of neurodegenerative diseases.

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Histochrome, a drug from sea products created at the Pacific Institute of Organic Biochemistry, is an effective antihypoxant and antioxidant. This suggests its effective use in cardiology for coronary disease, acute myocardial infarction, angina pectoris, and in ophthalmology as a retinoprotector [5]. Histochrome was synthesized on the basis on a natural echinochrome A (2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone) isolated from urchins. Experiments showed that histochrome effectively protected the brain in acute ischemia caused by hemorrhagic stroke [2]. Histochrome was effective in chronic ischemia: its injections improved the cerebral circulation and exploratory activity of senescence-accelerated OXYS rats [1] characterized by early development of phenotypical manifestations of brain aging [6,7].

We analyzed the effects of histochrome on the structure and functions of the brain in OXYS rats by MRI. Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate), a synthetic antihypoxant with antioxidant effects, was used as the reference drug. Its creation is

acknowledged as an obvious success of the Russian science. Mexidol has many indications for use, but is most often used and most effective as a neuroprotector [3].

MATERIALS AND METHODS

The study was carried out on 54 male OXYS and Wistar (control) rats. The animals were kept 3 per cage at natural light and free access to water and fodder and handled in accordance with the international standards (Council of the European Communities Directive 86/609/EEC).

Control MRI scanning of the brain was carried out at the age of 3 months. Repeated scanning was carried out at the age of 12 months, after which the animals of both strains received a course of intraperitoneal injections of histochrome (Pacific Institute of Organic Biochemistry) in a dose of 1 mg/kg for 5 days or mexidol (Ellara) in a dose of 4 mg/kg for 7 days. After the treatment, animal behavior was evaluated in the open field test [1] and scanning was repeated.

Scanning of the brain was carried out in narcotized animals (5.5 mg/kg rometar and 37 mg/kg seduxene intraperitoneally) on a PharmaScan US 70/16 tomograph for experimental studies (Bruker) at 7.0 tesla magnetic field strength and 300 mHz frequency, with a BGA 09P coil. In order to make an adequate evaluation of changes in the brain status, serial sections in 3 planes were scanned in the SE mode (Spin-Echo) and tomograms of T_1 and T_2 weighted images (WI) were made. The T_1-WI were obtained at TR/TE scanning parameters (1500.8/12.0 msec), T_2 at TR/TE (4200.8/41.0 msec). The matrix for both modes was 256×192, section thickness 1 mm, scanning area 4×4 cm^2. The brain damage focus looked differently in T_1 and T_2-WI: a low signal in T_1-WI and a high one in T_2-WI. The T_1-WI and T_2 FLAIR were used to evaluate the signals from pathomorphological foci, allowing differentiation between the cerebrospinal fluid and demyelination foci. The anatomical topographic structure of the cerebrovascular network of rats and the occipital 3D construction of the vascular basin were studied using the Head_Angio (3D-TOF) program with ParaVision 3.0.2 tomograph software. Reconstruction of the vessels for detection of vascular abnormalities was carried out by MIP (maximal intensity projection) using ROI (Region of Interest) software. The volume of the brain and dynamics of brain tissue structure were calculated using ROI [1].

The effects of the drugs were evaluated by ANOVA. Animal genotype and the drug were regarded as independent factors.

RESULTS

The OXYS rat strain was derived at Institute of Cytology and Genetics by selection and inbreeding of Wistar rats sensitive to the cataractogenic effect of galactose [4]. The formation of behavioral characteristic of aging humans and animals (decrease in motor and exploratory activity, increase of anxiety, disorders in associative training) were regarded as early (by the age of 3 months) aging of the brain in OXYS rats [6,7].

MRI revealed lower brain volume in 3-month-old OXYS rats compared to Wistar rats of the corresponding age (2461±37 and 2569±20 mm^3, respectively), which can be explained by lower body weight of OXYS rats compared to Wistar animals. By the age of 12 months, the volume of the brain in Wistar rats increased by 4% ($p<0.0001$), while in OXYS rats it decreased by 4% ($p<0.0001$). No differences between the two strains in the percent volume of the anterior compartments of the hippocampus and the subarachnoidal space (SAS) liquor volumes in the brain were detected at the age of 3 months (Table 1). On the other hand, ventricular horn cavities in OXYS rats at this

TABLE 1. Morphometric Changes in the Brain (mm^3) of OXYS and Wistar Rats with Aging and under the Effect of Drugs (*M*±*SD*, *n*=9)

Group	Age, months	Anterior compartments of hippocampus		SAS liquor		Ventricular horn cavities	
		Wistar	OXYS	Wistar	OXYS	Wistar	OXYS
Intact	3	139±10.0	133±6.4$^+$	133±4.2	105±10.4	109±6.2	149±7.2
	12	148±8.3*	118±9.4$^+$	135±7.3	120±8.4*	111±6.0*	128±6.0*
Mexidol	12	156±7.1^x	140±6.1$^+$	138±5.7	118±10.1	114±3.1^x	125±6.2^x
Histochrome	12	152±11.0^x	137±4.2$^+$	136±5.8	119.4±9.6	118±4.1	121±5.0

Note. Here and in table 2: $p<0.05$ compared to: *3 months; $^+$Wistar; ^{x}intact animals.

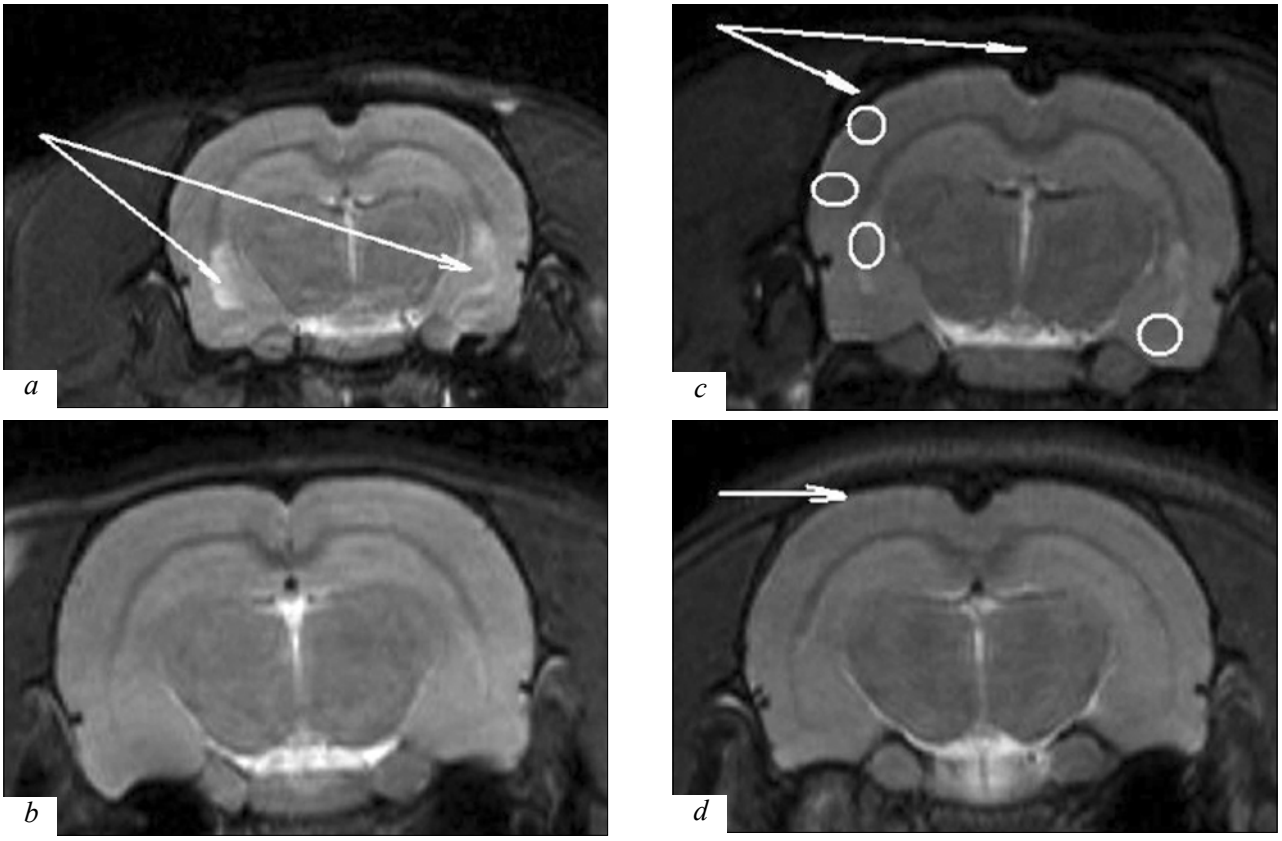

Fig. 1. The brain of OXYS (*a*, *c*) and Wistar rats (*b*, *d*) at the age of 3 (*a*, *b*) and 12 months (*c*, *d*). *a*) enlarged lateral ventricles (arrows) of OXYS compared to Wistar rats; *c*) changed size of SAS (arrows) in OXYS compared to Wistar rats. Focal changes in tissues are shown by circles.

age were larger than in Wistar by 27% ($p<0.0001$). Signs of hydrocephalus of the cerebral lateral ventricles were detected in OXYS rats at the age of 3 months (Fig. 1, *a*, *b*).

By the age of 1 year, the volume of the hippocampal anterior compartments in Wistar rats increased by 6% compared to that at the age of 3 months, while in OXYS rats it decreased by 12% ($p<0.0001$). The volume of SAS liquor in Wistar rats did not change with age, while in OXYS rats it increased by 12% at the expense of dilatation of the lobular external SAS caused by cortical atrophy (Fig. 1, *c*). Excessive accumulation of the liquor caused an increase of its pressure on the brain matter and could lead to development of intracranial hypertension.

The volume of the cerebral ventricular horn cavities did not change with age in Wistar rats, while in OXYS animals it decreased by 14% ($p<0.0005$) by the age of 12 months.

No signs of pathomorphological changes in the brain were detected in 1-year-old Wistar rats, while in OXYS rats of the same age foci of hyperintense signals appeared in T_2-WI and hypointense signals in the T_1-WI and T_1 FLAIR. Focal changes were detected mainly in the cerebral ventricular cortex and anterior horns (Fig. 1, *c*). The foci in the cerebral cortex were presumably caused by hypoxia, disorders in capillary circulation, and changes in the blood brain barrier reactivity. Focal changes in the lateral ventricles indicate endothelial dysfunction of the adjacent vessels. These changes were presumably secondary and resulted from changes in the cerebral liquor dynamics. These severe lesions in tissues paralleled by metabolic disorders in these areas were associated with signs of focal ischemia. The observed morphometric changes were responsible for a 1.4-fold increase of tissue signal intensity in T_2-WI in comparison with the signal in 3-month-old OXYS rats and 1-year-old Wistar rats (Table 2).

Antioxidant therapy had a positive effect on the morphology and function of the brain in animals. The drugs induced no changes in the parameters of the liquor SAS, which depended only on the genotype ($F(1.48)=185$; $p<0.000$), but the relationship between the genotype and drug factors ($F(2.48)=9.3$; $p<0.0004$) indicated strain-specific differences in reaction to therapy.

The drugs did not induce changes in the volume of cerebral ventricular horn cavities in the animal strain, but modified the volume of the hippocampal anterior

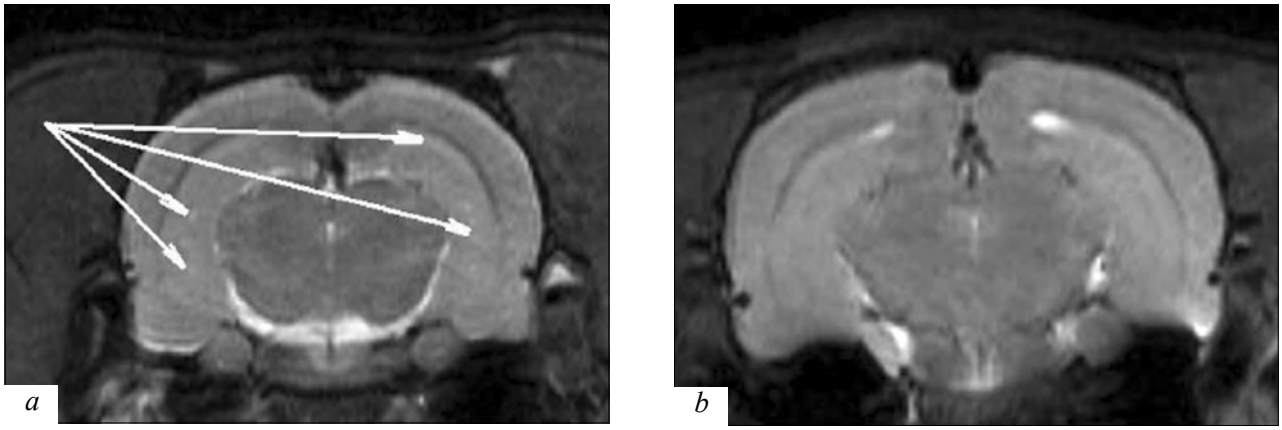

Fig. 2. Changes in cerebral tissue structure in 1-year-old OXYS rats in T_2-WI: before (*a*) and after histochrome treatment (*b*). Arrows: demyelination foci.

horns (F(2.48)=12.8; $p<0.00004$). Mexidol and histochrome therapy led to an increase in the volume of the hippocampal anterior horns: by 5% in Wistar and by 12 and 13%, respectively, in OXYS rats (Fig. 2).

Control scanning of diffuse foci of the cerebral median structures in OXYS rats after therapy showed lower intensity of the signal from modified foci detected in T_2-WI, the focus borderline was blurred and poorly identified in the ROI. Mexidol reduced the signal intensity from the hippocampus projection in T_2-WI by 15%, histochrome reduced it by 17%. The signal intensity in T_2-WI was reduced by the drugs by 17 and 19%, respectively.

Hence, MRI showed the location and size of tissue damage focus and perfusion changes after antioxidant therapy.

Analysis of open-field behavior confirmed the positive effects of mexidol and histochrome on the brain status of OXYS rats (Fig. 3). Both drugs reduced the latency of visiting the center of the open field in OXYS rats: mexidol by 1.2 ($p<0.001$) and histochrome by 1.3 times ($p<0.0001$), which attested to reduction of anxiety. Despite the still much more passive behavior of OXYS compared to that in Wistar rats, the drugs improved horizontal activity of OXYS rats: mexidol by 1.5 ($p<0.0002$) and histochrome by 1.7 times ($p<0.0001$). The number of rearing episodes, reflecting the exploratory component of behavior, increased by 27% ($p<0.032$) only after histochrome treatment. The fact that the drugs were effective only for the motor but not exploratory components of behavior was confirmed by the number of explored holes: the drugs did not modify this parameter.

It was previously reported that the effects of histochrome were determined largely by its effect on the cerebral bloodflow (improvement of collateral circulation and reduction of manifestations of chronic cerebral ischemia in OXYS rats) [1].

Different effects of the antioxidants on the morphology and functions and bloodflow recovery indica-

TABLE 2. Changes in Signal Intensity (SI) from the Cerebral Median Structures and Hippocampal Structures of OXYS and Wistar Rats with Aging and after Antioxidant Therapy (*M*±*m*; rel. units)

Group	Age, months	T_2-WI				T_1-WI			
		SI from hippocampus		SI from brain median structures		SI from hippocampus		SI from brain median structures	
		Wistar	OXYS	Wistar	OXYS	Wistar	OXYS	Wistar	OXYS
Intact	3	146±2.2	162±3.4^{+}	92±6.1	105±1.6	100±6.1	116±3.6	84±6.1	95±1.6
	12	169±4.5	224±3.3^{*+}	106±5.4	143±3.3^{*+}	99±1.7	43±3.3^{*+}	72±1.4	52±3.3^{*+}
Mexidol	12	154±1.4	193±7.4^{+x}	99±3.4	118±1.3^{+x}	89±3.1	78±1.3^{+x}	73±3.4	64±1.3^{x}
Histochrome	12	156±3.6	182±2.1^{+x}	97±2.1	116±1.4^{+x}	94±2.3	86±2.4^{+x}	79±2.1	69±1.4^{+x}

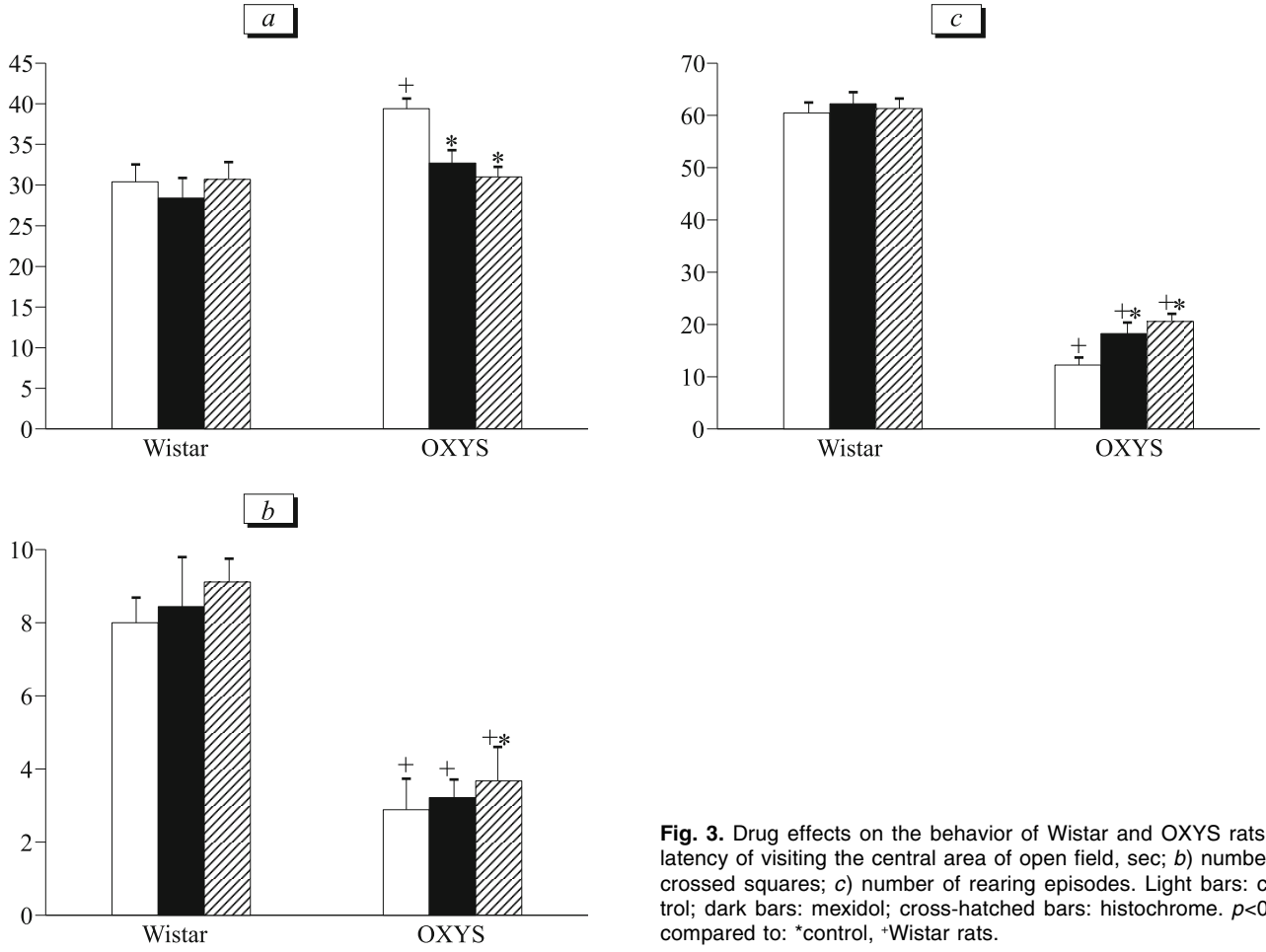

Fig. 3. Drug effects on the behavior of Wistar and OXYS rats. *a*) latency of visiting the central area of open field, sec; *b*) number of crossed squares; *c*) number of rearing episodes. Light bars: control; dark bars: mexidol; cross-hatched bars: histochrome. $p<0.05$ compared to: *control, +Wistar rats.

ted different mechanisms of their action were different. Histochrome in our study acted as an anticoagulant and antiaggregant, which was possible in small foci of cortical location.

The results showed that histochrome and mexidol effectively modulated the morphofunctional characteristics of the brain in OXYS rats. The therapeutic effect of the drugs was due to correction of diffuse changes in the white matter, reduction of edema and volume of the liquor SAS in the cerebral median structures. However, only histochrome reduced the severity of brain tissue demyelinization in OXYS rats. On the whole, our results and published data confirmed good prospects of histochrome use for therapy and prevention of neurodegenerative processes.

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