Effect of Hypoxia on the State of Tissues in SAMR1 and SAMP1 Mice with Various Rates of Aging

M. G. Makletsova, G. T. Rihireva*, S. L. Stvolinsky, and V. L. Sharygin*

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Senescence-accelerated SAMP1 mice were more sensitive to the negative effect of hypobaric hypoxia than SAMR1 mice (control). Low-temperature EPR spectroscopy showed that high sensitivity of SAMP1 mice is related to the increased concentration of methemoglobin in the blood leading to hemic hypoxia. Proton magnetic relaxation study showed that the exposure of SAMP1 mice to hypoxia can cause cerebral edema.

Key Words: *senescence-accelerated SAMP1 mice; hypobaric hypoxia; methemoglobin; EPR spectroscopy; simple magnetic relaxation*

It was generally accepted that oxidative stress plays a major role in aging and age-related neurodegeneration [11]. However, recent studies put doubt on this hypothesis. The publication "Did the theory on the role of oxidative stress in aging die?" shows that there is no correlation between the life span of drosophila and mouse strains with knockout genotypes by various systems of antioxidant defense. Knockout animals with a Cu,Zn-SOD (SOD1) deficiency were characterized by a shorter life span [14]. The discrepancy in the results does not disclaim the role of free radicals in age-related changes, but illustrates the complexity of this problem and the existence of multivariate regulatory systems for oxidative stress.

Experiments on SAMP mice (senescence-accelerated mouse prone) showed that accelerated accumulation of age-related changes is associated with persistent oxidative stress in tissues due to deficiency of the antioxidant system [15]. This state is accompanied by accumulation of chromosome aberrations and defects in cellular proteins and lipids [10]. The development of systemic disorders and resultant oxidative stress are related to increased expression of mitochondrial monoamine oxidase B and reduced activity of Mn-SOD, which results in an imbalance between the formation and neutralization of reactive oxygen species and accumulation of various metabolic and functional defects [8].

Here we studied the brain, liver, and blood of adult SAMP1 mice under conditions of oxidative stress due to acute hypobaric hypoxia. SAMR1 mice (senescence-accelerated mouse resistant) served as the control.

MATERIALS AND METHODS

Experiments were performed on 8-month-old male and female (50:50%) SAMR1 (n=12) and SAMP1 mice (n=12). Before the start of the study, all animals were maintained in a SPF vivarium. The experiment was conducted according to the requirements to studies with experimental animals (International Ethics Committee, www.nap.edu/books/0309083893/html/R1.html).

Hypoxia was modeled in a flow altitude chamber to prevent hypercapnia [9]. The pressure in the chamber was reduced to 0.14 atm over 1 min. The mice were maintained in this chamber until respiratory arrest. Then the pressure was increased to 1 atm over 1 min. The animals were rehabilitated at normal pressure for 1 h and then decapitated.

Research Center of Neurology, Russian Academy of Medical Sciences; *N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** mgm52@bk.ru. M. G. Makletsova

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We examined the blood and brain samples. The brain is an oxygen-dependent organ, which determines its high sensitivity to hypoxia. The blood was selected since hypoxia causes significant changes in oxygen transport function of erythrocytes.

The study was performed by means of low-temperature EPR spectroscopy. This method requires minimal treatment of biological samples. Low-temperature EPR spectroscopy allows us to perform direct measurement of specific spectra from EPR paramagnetic metal complexes and free radical compounds that are produced in biochemical systems. Studying the same sample provides information about several biochemical systems in the tissues [5,6]. The brain and blood samples for an EPR study were isolated and immediately frozen in liquid nitrogen.

EPR spectra were recorded on an ER-220D radiospectrometer (Bruker) equipped with an ASPECT-2000 mini-computer at 77 K. The amount of paramagnetic centers in the blood and brain samples was determined from the amplitude of their signals in EPR spectra. The degree of edema in the brain and liver tissue after hypoxic exposure was evaluated by the method of proton magnetic relaxation (PMR) [12,13].

The relaxation time was measured on a Minispec-P20 device (Bruker) under T1/T2 conditions (operating frequency 20 MHz, 30±1°C). T1 was measured in the following pulse sequence: $180^o - \tau - 90^o$. T2 was measured in the pulse pattern of Carr-Purcell-Meiboom-Gill: $90^o - \tau - (180^o - 2\tau)_n$ (T1>T2) and $180^o - \tau - 90^o$.

The data are expressed as $M \pm m$. The results were analyzed by Statistica 6.0 software. The significance of differences was evaluated by Student's test (comparison of two independent samples).

RESULTS

SAMP1 mice were more sensitive to acute hypobaric hypoxia (AHH) than SAMR1 mice. Respiratory arrest in these animals was observed 20±4 and 50±8 sec after pressure reduction in an altitude chamber, respectively ($p<0.01$). In compliance with different resistance of animals to AHH, we observed some variations in low-temperature EPR spectra of the blood. Two types of EPR signals in the g-factor region (4.3 and 6.0) were revealed in study samples. They are typical of Fe^{3+}-transferrin and methemoglobin (ferri-deoxyhemoglobin in the high-spin state), respectively. Table 1 shows the content of these compounds in the blood of SAMR1 and SAMP1 mice under normal conditions and after hypoxia.

No significant differences were found in Fe^{3+}-transferrin concentration in the blood from mice of both strains. However, Fe^{3+}-transferrin concentration in SAMP1 mice was slightly higher than in SAMR1 mice. Hypoxic exposure was not followed by significant changes in Fe^{3+}-transferrin concentration in the blood from mice of both strains. The total concentration of methemoglobin in SAMP1 mice was much lower than in SAMR1 mice. AHH caused a 62% increase in the total concentration of methemoglobin in SAMP1 mice ($p<0.05$). An AHH-induced decrease in the amplitude of the methemoglobin signal in blood samples from SAMR1 mice was not statistically significant. These data indicate that SAMP1 mice are characterized by a significant activation of oxidative processes after hypoxic exposure. Methemoglobin (Fe^{3+}-hemoglobin) is formed due to hemolysis, which results from destruction of erythrocyte membranes under conditions of strong oxidative processes. The formation of methemoglobin can occur due to direct oxidation of Fe^{2+}-hemoglobin by the hydroxyl radical (H_2O_2 degradation) or ROOH (Fenton's reaction). The increase in the concentration of methemoglobin or hemichromes (degradation products of methemoglobin) is followed by hemic hypoxia, which results in physiological abnormalities of CNS, energy system, and detoxifying system.

These data show that methemoglobin concentration in the blood of SAMP1 mice (most sensitive to hypoxia in the test for breathing cessation) is elevated

TABLE 1. Effect of Hypoxia on the Amplitude of EPR Signals from Fe^{3+}-Transferrin and Methemoglobin in the Blood of SAMR1 and SAMP1 Mice (U/ml blood, $M \pm m$)

Parameter	SAMR1		SAMP1	
	control	hypoxia	control	hypoxia
Fe^{3+}-transferrin	3.07±0.19	2.82±0.32	3.65±0.24	3.29±0.21
Methemoglobin	6.23±0.81	4.69±0.85 (75%)	3.95±0.59	6.36±0.59 (162%)

Note. The percentage of the control is shown in brackets.

TABLE 2. Effect of Hypoxia on the Amplitude of EPR Signals from FRF and ISC in the Brain of SAMP1 and SAMR1 Mice

Mice	Relative intensity, %	FRF	ISC
SAMR1	control	100	100
	hypoxia	58	69
SAMP1	control	65	78
	hypoxia	73	80

1 h after hypoxia, which provides conditions for aggravation of hypoxic injury. Oxidative damage to hemoglobin is accompanied by an increase in the content of free radicals, development of oxidative stress, and inhibition of the antioxidant defense system [2,7]. Methemoglobinemia is followed by a significant decrease in blood respiratory function (2-fold more severe than that observed after the reduction of tissue oxygen concentration) [1].

The differences were also revealed in brain samples of experimental animals. Some signals from carriers of mitochondrial respiratory chain were recorded in low-temperature EPR spectra of the brain. These signals were generated by free radicals of flavosemiquinones (FRF, g-factor 2.003) and reduced iron-sulfur clusters (ISC, g-factor 1.94; first component of the respiratory chain). The intensity of these signals in brain samples from SAMP1 mice was normalized by the average level of signals in the corresponding samples from SAMR1 mice. Table 2 shows the relative intensity of EPR signals from FRF and ISC in brain samples of mice. The amplitude of EPR signals in brain samples from control animals (SAMR1 mice) was taken as 100%.

The reduction level of the brain mitochondrial respiratory chain was highest in control SAMR1 mice. Exposure of these animals to hypoxia was followed by a decrease in the reduction level of the respiratory chain. The reduction level of the brain mitochondrial respiratory chain in SAMP1 mice was much lower than in SAMR1 mice. SAMP1 mice exhibited an insignificant response to hypoxic exposure.

AHH-induced variations in the degree of tissue watering were different in animals of these strains. This index was estimated from indirect parameters of the magnetic relaxation time (T1 and T2). They are measured by the PMR method and reflect the volume of water in study tissues. The degree of tissue watering is an important factor, which characterizes the progression of cerebral edema. A PMR study allowed us to identify some structural changes in the brain without histological examination.

Table 3 shows characteristics of proton relaxation (T1 and T2) in the liver and brain of SAMR1 and SAMP1 mice under normal conditions and after hypoxia. T1 and T2 did not differ in liver samples from intact animals of various strains. T2 in the liver of SAMP1 mice was elevated 1 h after hypoxia (by 22% compared to the control, $p<0.05$). T2 in the liver of SAMR1 mice remained unchanged after hypoxia. No statistically significant differences were found in the effect of hypoxia on the relaxation time T2 in the brain of SAMR1 and SAMP1 mice. The relaxation time T1 in brain samples from SAMP1 mice was 14% lower than that in SAMR1 mice ($p<0.02$). The relaxation time T1 in brain samples from SAMP1 mice increased by 11% ($p<0.05$) 1 h after hypoxia. These changes suggest the development of cerebral edema. T1 in brain samples from SAMR1 mice remained unchanged after hypoxia.

Comparative study shows that SAMP1 mice are much more sensitive to the negative effect of hypoxia than control SAMR1 mice (*e.g.*, period to the cessation of breathing). A low-temperature EPR spectroscopic

TABLE 3. Effect of Hypoxia on the Relaxation Time T1 and T2 in the Brain and Liver of SAMR and SAMP Mice (msec, $M\pm m$)

Parameter		SAMR		SAMP	
		control	hypoxia	control	hypoxia
Liver	T1	348±11	335±20	343±21	361±12
	T2	45.8±1.4	45.8±1.9	41.0±1.9	50.2±2.4*
Brain	T1	601±16	566±39	545±11	619±22*
	T2	80.1±2.4	77.7±8.9	80.4±2.5	83.2±2.2

Note. *$p<0.05$ compared to the control.

study allows us to examine the tissues under normal conditions and after deep fixation. In our experiments, this method was used for studying the molecular-and-cellular processes in the brain and blood. We revealed some processes that are probably involved in the negative effect of hypoxia. The concentration of methemoglobin in blood samples from SAMP1 mice increases 1 h after hypoxic exposure. Study tissues of these animals are probably characterized by the transition from the acute hypoxic episode to severe persistent (secondary) hemic hypoxia. These changes contribute to a further increase in the degree of oxidative stress. Methemoglobin is not accumulated in the blood of control SAMR1 mice during this period. Our results suggest that these animals have a strong antioxidant defense system and intensive compensatory-and-reparative processes.

The observed differences in proton relaxation (T1 and T2) in the liver and brain of SAMP1 mice probably serve as an indirect parameter for the probability of cerebral edema and increase in the degree of tissue watering after hypoxic exposure. This hypothesis requires further investigations. Our results confirm the fact that oxidative stress is a pathogenetic factor for hypoxic injury in SAMP1 mice.

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