EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Experimental Study of the Possibility of Metabolic Correction of Maternal, Fetal, and Newborn Hypoxia in Rats Using a New Amino Acid Composition MR-33

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 451-454, October, 1997
Original article submitted June 24, 1996

The status of pregnant rats, their fetuses, and progeny exposed to oxygen insufficiency are compares. By the end of pregnancy the resistance to hypoxia markedly decreases. Newborn rats during nursing are highly resistant to hypoxia. When nursing period is over, the resistance to hypoxia drops, but later is gradually restored. MR-33 preparation produces a pronounced antihypoxic effect. Administration of the drug to pregnant rats not only appreciably improves their resistance to oxygen insufficiency, but also promotes adaptation and compensatory mechanisms in the progeny, thus helping the progeny to better tolerate hypoxia, particularly when its probability is particularly high.

Key Words: *hypoxia; pregnancy; fetus; progeny; prevention; correction*

Experimental and clinical studies demonstrated that even short-term hypoxia during the intranatal period impairs normal growth and development of the progeny. Impaired energy metabolism of a cell, primarily oxidative phosphorylation in the respiratory chain of mitochondria, is the major pathogenetic component of hypoxia [4]. Restoration and maintenance of normal energy metabolism not only improves general status of mother exposed to hypoxia, but can also reduce the risk of posthypoxic complications in the progeny. Metabolic correction of energy metabolism disorders of different origin, including those caused by hypoxia, can be realized by novel metabolism-regulating drugs based on natural metabolites [1,4].

Our purpose was to investigate the possibility of using a new amino acid composition MR-33 for preventing hypoxic damage and protecting the mother, fetus, and progeny against it.

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MATERIALS AND METHODS

Experiments were carried out with females, fetuses, and progeny of outbred albino rats. Individual resistance of animals to acute (high-altitude) hypoxia was assessed in a pressure chamber as described previously [8]. The duration of the period between the moment when the "height" of 11,000 m was reached and the secondary agonal inhalation (the so-called reserve time — RT) was the criterion of animal's resistance to hypoxia. According to the test, the animals were divided into 3 groups: 1) RT less than 3 min — low-resistance animals; 2) RT 3-10 min — medium-resistance animals; and 3) RT more than 10 min — high-resistance animals.

The resistance of females was assessed on day 20 of pregnancy, of the progeny from day 1 to 60 of life.

Fetal compensatory-adaptive mechanisms were investigated on a model of anoxia [5,7]. On day 20 of pregnancy, the fetuses were removed from the uterine horn under total combined narcosis. After ligation of the umbilical cord and attaching pe-

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TABLE 1. Effect of MR-33 on Individual Resistance of Pregnant Rats to Acute Hypoxic Hypoxia (*M*±*m*)

Rats	Total number of animals, *n*	Number of animals in a group, %			Mean RT for a group, min
		highly resistant	medium resistant	low resistant	
Nonpregnant	153	64.05±3.88	29.40±3.68	6.54±2.00	15.85±0.69
Pregnant	36	19.40±6.59*	36.10±8.00	44.40±8.28*	5.68±0.63*
Pregnant+MR-33	10	50.00±15.8	30.00±14.5	20.00±12.6	8.90±1.43*

Note. *$p<0.05$ *vs.* nonpregnant females.

netrating electrodes, the fetus was put in isotonic saline with constant temperature (37.4°C). Functional state of isolated fetuses was assessed from heart rate, the rate of respiratory movements, time of the onset of deep hypoxia (heart rate 50 beats/min), and by the time of survival of fetal heart biopotentials (assessed from the electrocardiogram isoline). All parameters were recorded by an electroencephalograph.

Aqueous solution of the amino acid composition MR-33, based on natural metabolites and developed at the *Biotiki* Medical Complex, was given to pregnant females intragastrally in a dose of 6 mg/kg on days 16-20 of pregnancy. Control animals were administered the same volume of distilled water during the same period.

The results were statistically processed using Student's *t* test.

RESULTS

Hypoxic resistance of pregnant rats is much lower than that of nonpregnant animals (Table 1). RT of pregnant rats is almost 3 times lower than in the control (5.68±0.63 and 15.85±0.69 min, respectively). The number of high-resistance rats decreases, while that of low-resistance ones increases.

MR-33 markedly improved individual reactions of pregnant animals to hypoxia. Although RT did not reach the level of nonpregnant animals (15.85±0.69 min), it was significantly higher than in untreated animals (8.90±1.43 and 5.68±0.63 min, respectively, $p<0.05$). The number of low-resistance rats in this group decreased by 50%, whereas that of high-resistance ones increased by 2.5 times.

The molecular mechanisms of antihypoxic effect of MR-33 are probably associated with its capacity to stimulate the production of endogenous glutathione, an active antioxidant and electron acceptor, influencing energy metabolism in a cell at the level of the respiratory chain [3].

Study of the individual reactions of fetuses to oxygen insufficiency showed that MR-33 exerts no negative effects on fetal functions and even improves them (Table 2). The absence of differences between the control and experimental groups can be explained by the intrinsic high resistance of the fetus to hypoxia

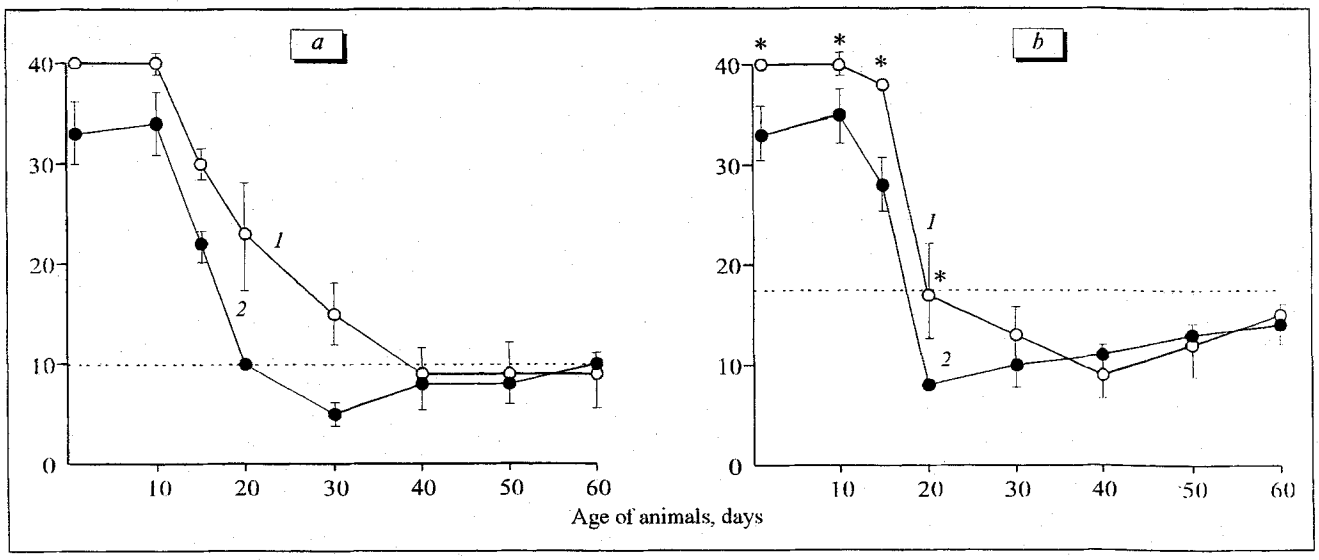

Fig. 1. Individual hypoxic resistance of the progeny of rats treated with MR-33 during gestation. *a*) males; *b*) females; *1*) experiment; 2) control. Dotted line: resistance of adult animals. Ordinate: reserve time, min. *$p<0.05$ *vs.* the control.

TABLE 2. Effect of MR-33 on the Compensatory-Adaptive Mechanisms of the Fetus (*M±m*)

Parameters	Control	Experiment
Heart rate, beats/min	165.7±5.8	173.2±3.7
Rate of respiratory movements	20.2±2.7	24.3±2.1
Time of onset of deep hypoxia, min	23.4±0.5	24.4±0.9
Time of survival of fetal heart biopotentials, min	47.2±3.2	51.6±2.3

Note. All data are statistically insignificant in comparison with the control.

[6,8]; therefore, the protective effect of the drug is not so strong as in animals with low individual resistance (pregnant females). In our case, even if there are no grounds to assert that MR-33 elicits an antihypoxic effect, at least a tendency should be noted.

Individual resistance of the progeny to acute hypoxia is illustrated by Fig. 1. These data confirm a high resistance of newborn animals to oxygen insufficiency. The resistance is the highest immediately after birth and remains at this level during the entire period of nursing. After the nursing period (days 16-20 of life for rats), when maternal milk is gradually replaced by common food rich in carbohydrates, the resistance to hypoxia decreases. This manifests as a many-fold drop in RT of control rats (Fig. 1). In fact, a period of relative physiological instability of the organism takes place. This can be explained by increasing dependence of energy metabolism of rat pups from carbohydrate oxidation [2]. The females show the least resistance to hypoxia on day 20 and the males on day 30 of life. After a critical drop in the resistance to oxygen deficiency, it gradually increases, and by day 60 reaches the level of adult animals.

Administration of MR-33 to pregnant females markedly improved the resistance of the progeny to hypoxia in comparison with the control (Fig. 1). Even during the period of relative physiological instability young rats were sufficiently resistant to hypoxia. In male young rats increased resistant to hypoxia induced by MR-33 persisted during the entire period of postnatal development, and by the 60th day of life it was equal to the control level for adult animals.

Thus, MR-33 administered to a female during the last trimester of gestation stimulates the adaptive-compensatory mechanisms aimed at improving hypoxic resistance in both the female and the fetuses. These protective mechanisms actively function during the early postnatal development, when the probability of hypoxic injury is the highest.

These results allow us to recommend MR-33 as a means for prevention and protection of the mother, fetus, and progeny against hypoxic injuries.

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