REDOX STATUS IN WISTAR RAT BLOOD AFTER HYPOXIA

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Abstract

The regulation of tissue oxygen homeostasis is a fundamental and a defence parameter during hypoxia and represents a basic problem of the physical effort, life at altitude adaptation and neonatological pathology.

The integration of the circulation and breathing mechanisms are already known, but the integrative mechanisms which regulate and control the amount of hemoglobin and the response to oxidative aggression are less clarified.

In the present study we monitorized the blood antioxidant parameters in immature Wistar rats exposed to hypoxia. We measured the activity of the antioxidant enzymes (catalase - CAT, superoxide dismutase -SOD, glutahion peroxidase - GPx) and also transaminases (ALT, AST) and lactate dehidrogenase (LDH) as markers of cellular destruction.

Rezumat

Reglarea aportului de oxigen tisular este un parametru homeostazic fundamental şi de apărare în hipoxie şi reprezintă o problemă asociată cu efortul fizic, viaţa la altitudine şi patologia neonatologică.

Mecanismele de integrare a funcţiilor de circulaţie şi respiraţie sunt astăzi binecunoscute, însă, mecanismele integrative, prin care este reglată şi controlată cantitatea de hemoglobină din sânge, şi corelat cu aceasta numărul de eritrocite, factorii ce joacă rolul principal în acest determinism sunt mai puţin clarificaţi.

În acest studiu s-a urmărit monitorizarea parametrilor enzimatici sanguini la şobolanul alb Wistar imatur în condiţiile inducerii hipoxiei. S-a determinat activitatea transaminazelor alanin transaminaza (ALT), aspartat aminotransferaza (AST), a lactat dehidrogenazei (LDH) şi a enzimelor antioxidante superoxid dismutaza (SOD),catalaza (CAT), glutation peroxidaza (GPx) ca markeri ai distrucţiei celulare .

Keywords: erythrocyte, lactate dehidrogenase, catalase, superoxide dismutase, glutathione peroxidase, transaminases, hypoxia.

Introduction

The regulatory mechanisms which provide tissues adaptation to hypoxia have a theoretical and practical importance for the pathology of circulation and respiration but also for the blood transport and release of oxygen [1,2,3].

In the first stage after exposure to hypoxia, oxygen defficiency acts initially on the sino-carotidian area and on the neurons from reticular formation. Following advanced hypoxia, we evaluated the activity of the studied enzymes [4].

Previous studies showed that antioxidant enzyme activity in rats decreased to an hour after the action of the disturbing factor (deprivation of oxygen) or, on the contrary, a general increase of activity of these enzymes at 24 hours after induction of hypoxia [5].

Materials and Methods

All animal procedures were carried out according to the approval of the local ethics committee for animal research from "Carol Davila" University of Medicine and Pharmacy (Bucharest, Romania) and in accordance to the European Communities Council Directive 86 609 EEC.

We used a control group of 20 male Wistar rats, five days old (P_5), healthy, with a weight of 10 to 15 grams to determine the redox status of the red blood cells. The control group was divided in two subgroups: *control 1* included the first ten rats and the *control2* the remaining ten rats. The rats in *Control 1* group were exposed to hypoxia for three hours (hypoxia 3 hours group). The rats in *Control 2* group were exposed to hypoxia for six hours (hypoxia 6 hours group).

Both groups were placed in a experimental hypoxic chamber and exposed to oxygen deprivation (10% O_2) for a period of 3 hours and, respectively, 6 hours.

After the exposure, blood was immediately collected using a vacuum syringe with an anticoagulant vacutainer and enzymatic tests were performed.

Determination of the blood hemoglobin (Drabkin method)

In the presence of Drabkin reactive, hemoglobin is transformed into cyan- methemoglobin, a stable compound, identified by absorbance change at λ=540 nm. Results were expressed in grams of Hb/dL [6].

Determination of the plasma lactate dehidrogenase (LDH)

LDH catalyses the reduction of pyruvate to lactate in the presence of NADH (nicotinamide adenine dinucleotide). The reaction is kinetically monitored by the decrease of the absorbance at 340nm. NADH oxidation is proportional with the LDH activity in the sample. Results were expressed in U/L [7].

Determination of the plasma transaminases

ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were determined using kinetic method. Transaminases catalise the amino groups transfer from an α-aminoacid to an α-cetoacid. Results were expressed in U/L [8,9].

Determination of the antioxidant enzymes activity

Superoxide dismutase determination on erythrocites

Superoxide dismutase (SOD) activity was evaluated using a Ransod kit. SOD accelerate the the dismutation of the toxic superoxide radical produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. The SOD activity is measured by the degree inhibition of this reaction. Results are expressed in U/g Hb.[**10**]

Glutathione peroxidase determination in blood

Glutathione peroxidase (GPx) activity was determined using a Ransed kit. Our method was adapted after Paglia and Valentine [12]; we measured the decrease in absorbance at 340 nm by the consumption of substrate under the action of glutathione peroxidase. Results were expressed in U/g Hb/min.

Catalase determination in erythrocites

Catalase (CAT) activity was determined using the Abei H [13] - adapted method. We used spectrophotometric metod to assess the catalase activity, by substrate (hydrogen peroxide) consumption. Results were expressed in k/g Hb/min.

Results and Discussion

Evaluation of enzymatic parameters in blood after hypoxia allows the determination of erythrocites redox status which indicates the degree of tissular damage in the presence of disorders of oxygen transport and utilization.

Lactate dehidrogenase (LDH) changes in blood serum reflect the whole body metabolic changes.

Serum LDH is constantly released from damaged cells of all tissues and may be useful for diagnosis of different pathological conditions such as myocardial infarction or hepatitis [14]. After hypoxia exposure, we found significantly increased values of the serum LDH activity. (Table I).

Table I
Serum LDH activity in hypoxia depending on time.

	Hypoxia
	LDH activity (U/L)
Control 1	400,60 ± 28,24
3h	477 ± 22,57
Control 2	344,40 ± 23,52
6h	601,60 ± 16,73

Values are mean ± SE, Control 1= first ten rats, Control 2= last ten rats.

The increase in plasma ALT (alanine aminotransferese) and AST (aspartate aminotransferase) [15] were used as parameters of the hepatocellular injury. (see Table II)

Table II
Serum AST and ALT activity in hypoxia depending on time

	Hypoxia	
	AST activity U/L	**ALT activity** U/L
Control 1	24 ± 1,52	20,20 ± 1,39
3h	48,6 ± 3,6	26,4 ± 1,81
Control 2	22,6 ± 2,06	19.2 ± 1,71
6h	91 ± 1,22	45 ± 2,07

Values are mean ± SE, Control1 = first 10 rats, Control2 = last 10 rats.

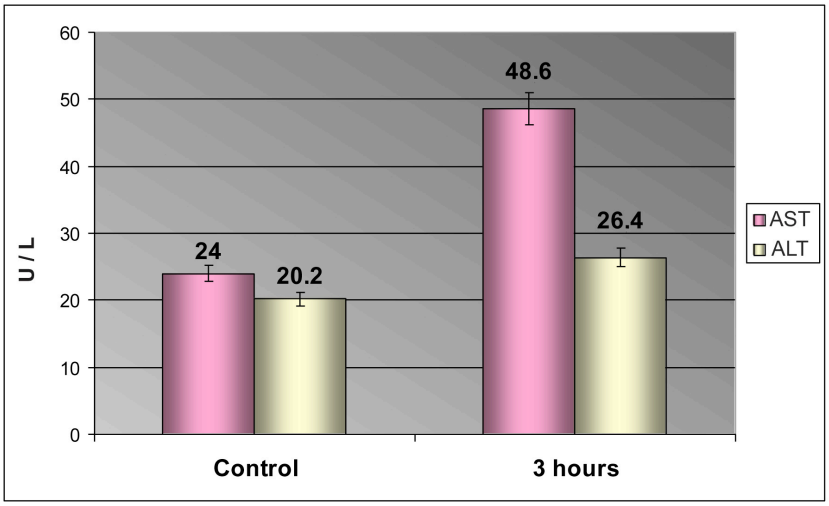

Figure 1.
Dynamic activity of the transaminases in hypoxia after 3 hours.

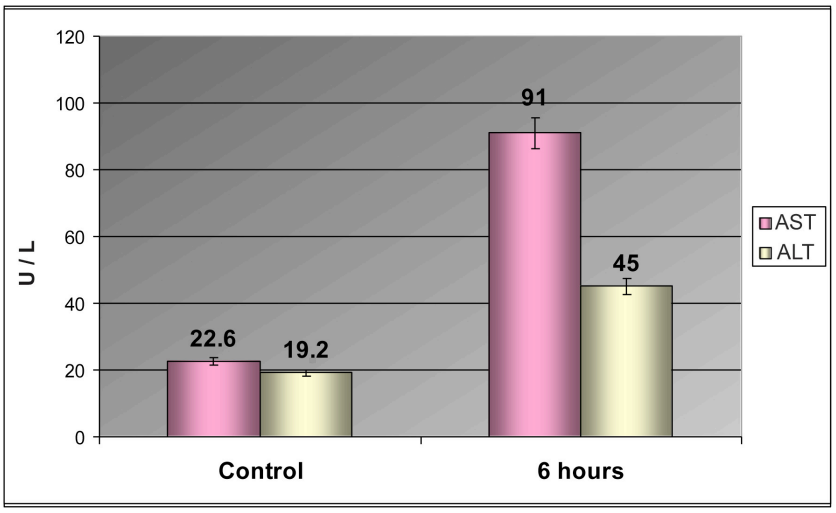

Figure 2.
Dynamic activity of the transaminases in hypoxia after 6 hours.

Hypoxic exposure resulted in a significant change in the activities of the antioxidant enzymes. After 3 hours of oxygen deprivation, the antioxidant enzymes activity (CAT, SOD, GPx) slightly increased and after 6 hours we found significantly reduced values versus control group animals. (see Table III)

Table III
Antioxidant enzymes (CAT,SOD,GPx) activity in hypoxia depending on time

	Hypoxia		
	CAT k/g Hb/min	**SOD** U/g Hb/min	**GPx** U/g Hb/min
Control 1	165.8 ± 5.3	1245.20 ± 142.11	50.23 ± 2.20
3h	223.6 ± 8.77	1399.56 ± 127.65	64.21 ± 3.75
Control 2	161 ± 7.2	1462.00 ± 152.90	49.38 ± 5.90
6h	109,8 ± 3,97	1313,69 ± 126,90	31,45 ±1,82

Values are mean ± SD, Control 1 = first 10 rats, Control 2 = last 10 rats.

As a response to hypoxia the glycolysis is activated at the tissular level, assuring for a while all the energy needs of the cell. Later, the cell initiates the anaerobic glycolysis , lactic acid is produced and acidosis increases the rate of dissociation of oxyhemoglobin and the complete disposal of oxygen to tissues.

We suspect an increase of the antioxidant enzyme activity in the blood at 3 hours as a consequence of acidosis (Figure 3). Some *in vitro* studies showed that medium acidosis may be induce a protective effect to the hypoxic excitotoxicity [16].

Early hypoxia induces a simpatoadrenergical reaction with peripheral vasoconstriction and reduced blood flow in territories like: skin, muscle, intestine, liver, kidney. The adaptive response is a slight increase in activity of antioxidante enzyme at 3 hours. As hypoxia is developing metabolic changes appear and the cell is affected in the conformity with the specific organ, so the activity of antioxidant enzymes begin to decrease.

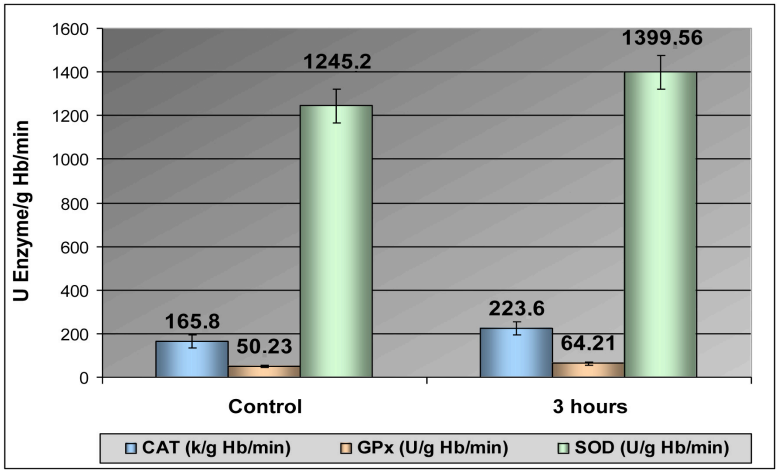

Figure 3.
Dynamic activity of antioxidant enzymes in hypoxia after 3 hours

The evolution of the antioxidant enzymes activity followed a pattern which included a slightly increase followed by a progressive decrease after 6 hours of oxygen deprivation (Figure 4). In conditions of increasing oxidative stress, the studied antioxidant enzymes are consumed, their detoxifying capacity decreasing.

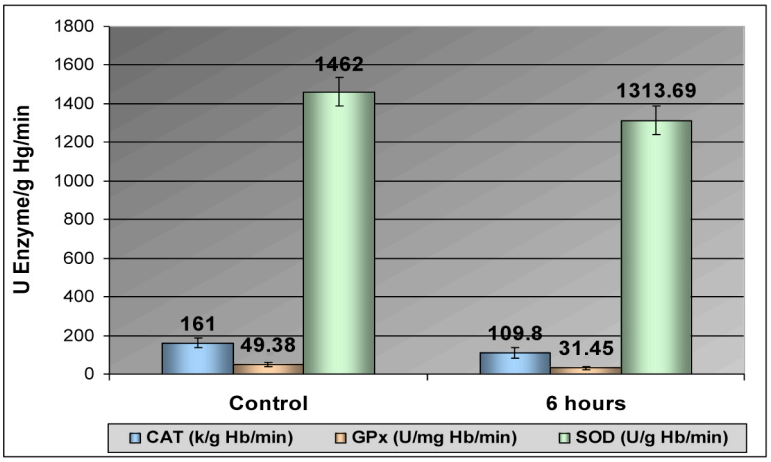

Figure 4.
Dynamic activity of antioxidant enzymes in hypoxia after 6 hours

The evolution of the antioxidant enzymes is different from the transaminases and lactate dehidrogenase activity, which progresively increased. (Table IV)

Table IV

Dynamic activity of enzymes in hypoxia after 6 hours.

	Hypoxia (*p* values*)					
	CAT	**GPx**	**SOD**	**GOT**	**GPT**	**LDH-P**
Contol vs 3h Hypoxia	< 0.001	0.0014	0.0012	< 0.001	0.0015	< 0.001
Control vs 6h Hypoxia	< 0.001	0.0059	0.005	< 0.001	< 0.001	< 0.001

**p* values shown were determined using t-Test: Paired Two Sample for Means.

These events may contribute to the later morphological damage in the brain and indicate that it would be essential to pursue neuroprotective strategies, aimed to counteract oxidative stress, as early as possible after the hypoxia injury.

Conclusions

The basis of all cellular hypoxic injury is the insufficient macroergical phosphates, process which limits the ability of cells to maintain cellular homeostasis. We can assess the level of cell damage after the induction of hypoxia, by monitoring the activity of antioxidant enzymes (protective response) as well as transaminases (liver damage) and lactate dehidrogenase (general cell destruction).

LDH may be considered a *general marker* of injured tissue and an indicator of the existence/severity of the damage (acute or chronic).

Our study confirmed that the liver function was damaged. Increased values for LDH and ALT in liver pathology showed limited injury, while, the simultaneous increase of showed AST indicated a more advanced damage that interests mitochondrial membranes.

Adaptive response to oxygen deprivation is given by the slight increase activity of antyoxidante enzymes at 3 hours after induced hypoxia which decreases as the hypoxia continues its injuring effect (6 hours).

Using immature white Wistar rats as experimental model we investigated the blood redox status changes that occur after oxygen deprivation. Issues related to hypoxia in newborns is actual and requires extensive research in this area.

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Manuscript received: May 14th 2011