

## AMINE OXIDASES AS IMPORTANT AGENTS OF PATHOLOGICAL PROCESSES OF RHABDOMYOLYSIS IN RATS

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*In this study we have tested an idea on the important role of amine oxidases (semicarbazide-sensitive amine oxidase, diamine oxidase, polyamine oxidase) as an additional source of oxidative/carbonyl stress under glycerol-induced rhabdomyolysis, since the enhanced formation of reactive oxygen species and reactive carbonyl species in a variety of tissues is linked to various diseases. In our experiments we used the sensitive fluorescent method devised for estimation of amine oxidases activity in the rat kidney and thymus as targeted organs under rhabdomyolysis. We have found in vivo the multiple rises in activity of semicarbazide-sensitive amine oxidase, diamine oxidase, polyamine oxidase (2-4.5 times) in the corresponding cell fractions, whole cells or their lysates at the 3-6<sup>th</sup> day after glycerol injection. Aberrant antioxidant activities depended on rhabdomyolysis stage and had organ specificity. Additional treatment of animals with metal chelator 'Unithiol' adjusted only the activity of antioxidant enzymes but not amine oxidases in both organs. Furthermore the in vitro experiment showed that Fenton reaction (hydrogen peroxide in the presence of iron) products alone had no effect on semicarbazide-sensitive amine oxidase activity in rat liver cell fraction whereas supplementation with methylglyoxal resulted in its significant 2.5-fold enhancement. Combined action of the both agents had additive effect on semicarbazide-sensitive amine oxidase activity. We can assume that biogenic amine and polyamine catabolism by amine oxidases is upregulated by oxidative and carbonyl stress factors directly under rhabdomyolysis progression, and the increase in catabolic products concentration contributes to tissue damage in glycerol-induced acute renal failure and apoptosis stimulation in thymus.*

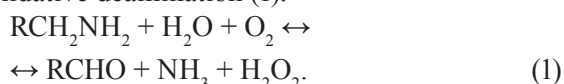
*Key words: rhabdomyolysis, amine oxidases, biogenic amines, polyamines, oxidative/carbonyl stress.*

Enhanced formation of reactive oxygen species (ROS) and reactive carbonyl species (RCS) in a variety of tissues (oxidative and carbonyl stress, respectively) is linked to a number of inflammatory and disease states including rhabdomyolysis [1, 2]. Under rhabdomyolysis, severe muscle injury with release of intracellular contents into the bloodstream and myoglobin appearance in urine (myoglobinuria) testify to structural changes in the kidney due to the nephrotoxic effects of myoglobin [3]. Furthermore, it is believed that iron released from myoglobin and hemoglobin plays a critical role in the pathogenesis of rhabdomyolysis, including glycerol-induced one. Earlier the labile ferric iron pool formation in rhabdomyolysis rat blood plasma was shown, which was completely absent in the intact group of animals. This non-heme iron was not included in transferrin and therefore is redox active, whereas a medicine 'Unithiol' application effectively decreased its (labile iron) concentration [4, 5].

Degradation products of heme proteins display tubular nephrotoxic properties partially mediated by the generation of ROS [6]. Thereby, acute renal failure (ARF) is the main symptom of the disease, though many organs and systems are involved in the process [7]. In particular, rhabdomyolysis induces the immune system response through muscle inflammation [8]. As a consequence, histamine, an inflammation mediator, is released from the cells content [8]. Histamine promotes renal damage through its effect on glomerular and arteriolar endothelium. Oxidative deamination of the primary amino group, catalyzed by diamine oxidase (EC 1.4.3.22; DAO) represents an important catabolic pathway of histamine, mainly in glomeruli. So, histamine and DAO, according to Floris and Mondovi, play critical role in pathogenesis of kidney diseases [9].

It is known that pathological conditions are characterized by elevated levels of different amines. Manifold increase in the concentration of creatine

and sarcosine is typical of rhabdomyolysis. These compounds are the precursors of methylamine, one of the physiologically important semicarbazide-sensitive amine oxidase (EC 1.4.3.21; SSAO) substrates [10]. Although methylamine itself is relatively nontoxic, it becomes very toxic in the presence of SSAO due to simultaneous formation of dangerous products such as formaldehyde and  $H_2O_2$  in reaction of oxidative deamination (1):



SSAO is membrane incorporated enzyme, extremely active in the endothelial cells of highly vascularized tissues including kidney. Yu and Zou [11] have found that namely formaldehyde may be responsible for endothelial injury in the rat kidney through its interaction with proteins. It is known that SSAO is bifunctional enzyme mediating inflammation and ROS production [12]. Under inflammatory conditions the enzyme promotes the infiltration of leukocytes into inflamed tissue, thereby contributing to and maintaining the inflammatory response, and possibly the tissue destruction [13].

Elevated level of polyamines (putrescine, spermidine and spermine) was found in tissues with marked morphological changes, regardless of the nature of the disease process [14]. Products of oxidative deamination of polyamines by polyamine oxidase (EC 1.5.3.11; PAO) are amino aldehydes, hydrogen peroxide and ammonia. Acrolein spontaneously formed from these amino aldehydes is the major uremic toxin [15].

Thereby, catabolic products of biogenic amines and polyamines may be additional source of oxidative and carbonyl stress under different pathological and inflammatory processes. We supposed that enhanced concentrations of biogenic amines and their precursors as well as high levels of amine oxidases activity can be a major reason of kidney and immune system disorders under the rhabdomyolysis. We also assumed that iron acceptor 'Unithiol' application can lead to a decrease in oxidative/carbonyl stress indexes under the rhabdomyolysis just as it was observed with respect to the nitrosative stress indicators [5].

Considering the above mentioned, the work purpose was as follows: 1) to estimate activity of amine oxidases (SSAO, DAO, PAO) and antioxidant enzymes, the catalase (EC 1.11.1.6; CAT), and superoxide dismutase (EC 1.15.1.1; SOD), as oxidative/carbonyl stress indexes, in the rat kidney and thymus under rhabdomyolysis induced by intramuscular in-

jection of glycerol, and to elucidate the influence of 'Unithiol' as metal chelator on the enzymes activity; 2) to clarify the influence of Fenton reaction conditions (hydrogen peroxide in the presence of iron), carbonyl stress factor (methylglyoxal) and combined action of both agents on key amine oxidase and antioxidative enzymes (SSAO and CAT) in the *in vitro* experiment.

## Materials and Methods

**Chemicals.** Phenylmethylsulfonyl fluoride, pargiline hydrochloride, sodium diethyldithiocarbamate trihydrate, semicarbazide hydrochloride, diphenyleiodonium chloride, methylamine hydrochloride, putrescine hydrochloride, spermine tetrahydrochloride, 1,4-diaminobutane (histamine), medium RPMI-1640, HEPES, unithiol (2,3-dimercapto-1-propanesulfonic acid sodium salt), purpald (4-amino-3-hydrazino-5-mercapto-1,2,4-triazole), Bradford reagent and bovine serum albumin, sodium azide were obtained from Sigma (USA). 2-Mercaptoethanol was obtained from Ferac (Germany). All other chemicals were of the highest grade commercially available.

**Animals.** All manipulations with animals before isolation of the kidneys and thymus were performed in accordance with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986) and national requirements for the care and use of laboratory animals. Male Wistar rats (weight approx. 120 g, up to 2 month-old) were used in these studies. Before the experiment Nembutal anesthetic was used.

**Model of glycerol stimulated rhabdomyolysis.** Animals were randomly divided into five groups in an equal number ( $n = 6$ ). Rats in group CG were intact animals, whereas those in groups RMG1, RMG3, RMG3-U and RMG6 were injected with glycerol (10 ml/kg, i.m. in both back legs). After glycerol injection, a medicine 'Unithiol' as 5% solution (10 ml/kg, s.c.) was administered to rats group RMG3-U once a day during 3 days. On the 1<sup>st</sup> (RMG1), 3<sup>rd</sup> (RMG3, RMG3-U), and 6<sup>th</sup> (RMG6) day after glycerol injection, respectively, animals were decapitated under light ether anesthesia. Thymus and kidney tissues were taken from anaesthetized rats.

**Preparation of rat renal cell fractions.** After sacrifice of the animals, the kidneys were removed on ice. All further steps were performed at 0-4 °C.

Cell fractions were prepared by differential centrifugation method as earlier [16]. Briefly, kidneys were homogenized in 10 vol/g of wet tissue weight of 10 mM Tris-HCl buffer (pH 7.2) containing 0.25 M sucrose. The homogenate was filtered through 2 layers of surgical gauze and centrifuged at 700 g for 10 min. The supernatant was centrifuged at 9000 g for 20 min and at 105,000 g for 1 h and used for further investigations as cytosol fraction instantly. The pellet was suspended in the same buffer containing 0.25 M sucrose and centrifuged at 105,000 g for 1 h. The microsomal pellets were resuspended in the buffer (1 ml of buffer per 1 g of wet tissue) and used for further investigations immediately.

*Cell isolation and viability.* The thymus was removed in phosphate-buffered saline (PBS) containing (mM): NaCl – 136.9; KCl – 2.7; Na<sub>2</sub>HPO<sub>4</sub> – 8.1; KH<sub>2</sub>PO<sub>4</sub> – 1.5 (pH 7.2), then small fragmentation was followed by filtration through thrice-folded gauze, centrifugation (at 200 g for 5 min) at room temperature. Cell pellet was suspended in RPMI-1640 medium containing 10 mM HEPES-NaOH buffer (pH 7.3), 0.1% BSA, 100 U/ml penicillin, 100 µg/ml streptomycin, and 50 µM 2-mercaptoethanol. The cells were washed twice and resuspended in RPMI-1640 medium. Cell viability was estimated by trypan blue exclusion assay [17]. Whole cell lysates were prepared in 10 mM Tris/HCl buffer, pH 7.6 containing 5 mM MgCl<sub>2</sub> in the presence of 0.1 mM protease inhibitor phenylmethylsulfonyl fluoride. The cell debris was removed by centrifugation at 6 000 g for 5 min, and the supernatants were used for further assay. The lysates were used for enzymatic activity estimation of PAO, DAO, SOD, CAT, whereas for SSAO activity estimation we used cells suspension in PBS.

*Fenton reaction modeling.* Experimental system based on the Fenton reaction, described by Caillet et al. [18] was used in our study. The reaction mixture contained the following reagents at the final concentrations stated: 20 mM phosphate buffer (pH 7.4), 100 µM FeCl<sub>2</sub>, 104 µM EDTA, 1 mM H<sub>2</sub>O<sub>2</sub> and 100 µM ascorbic acid. Appropriate cell fractions (cytosol and microsomes) in the reaction mixture of 1.5 ml final volume were incubated under agitation at 37 °C for 1 h. Reaction was terminated by cooling on ice, and enzymes activity was determined immediately.

*Assay for amine oxidase activity.* Fluorometric assay was designed to estimate activity of all amine oxidases under the investigation (SSAO, DAO, and

PAO). The method is based on the formation of an intense fluorophore between folic acid and the hydrogen peroxide released during the oxidation of the corresponding amine as substrate [19]. As aforementioned amine oxidases are located in different cellular compartments, we used corresponding cell fractions, namely microsomes for SSAO, cytosol for PAO and DAO, to determine the activity of the enzymes. The enzyme preparations were preincubated with appropriate inhibitors, that are pargiline (1×10<sup>-4</sup> M), semicarbazide (4.3×10<sup>-4</sup> M) or diethyldithiocarbamate (5×10<sup>-5</sup> M), and diphenyleneiodonium (1.5×10<sup>-5</sup> M) at room temperature for 20 min to ensure that activity of MAO, DAO or PAO, respectively, if present, were completely inactivated. The reaction medium contained the enzyme sample, corresponding substrate (0.1 mM methylamine, 0.1 mM histamine/putrescine/spermine), 1 mM NaN<sub>3</sub> and 1.7 mM potassium phosphate buffer, pH 7.2, in a final volume of 250 µl. Following the incubation for 30 min at 25 °C (for SSAO and DAO estimation) or 37 °C (for PAO estimation), the reaction was terminated by addition of 30% ZnSO<sub>4</sub> or 0.6 M HClO<sub>4</sub> (deproteination). After centrifugation at 12,000 g for 10 min supernatants were used for detection of H<sub>2</sub>O<sub>2</sub> released during enzymatic reaction. Samples were aliquoted to a 96-well black plate. Folic acid (1×10<sup>-5</sup> M) and CuCl<sub>2</sub> (2×10<sup>-5</sup> M) solutions were prepared and added to all the wells. After incubation for 60 min at 37 °C in the dark, fluorescence was measured ( $\lambda_{\text{excit}} = 360 \text{ nm}$ ,  $\lambda_{\text{emis}} = 460 \text{ nm}$ ) and H<sub>2</sub>O<sub>2</sub> production was calibrated with H<sub>2</sub>O<sub>2</sub> ( $\epsilon_{240} = 43.6 \text{ M}^{-1}\text{cm}^{-1}$ ), and corrected for the background caused by respiratory substrates in the absence of samples. The developed fluorescence intensity was measured in a microplate fluorometer (FLx800, Biotek, USA). The limit of detection for H<sub>2</sub>O<sub>2</sub> is 0.5 µM. One unit of enzyme activity is defined as nmoles of product formed in a minute per mg protein.

Besides, supernatants were used for detection of aldehydes also released during the enzymatic reaction of amine oxidases. Aldehyde quantity was estimated spectroscopically with 22.8 mM purpald dissolved in 0.5 N KOH. After 20 min incubation at room temperature and 65.2 mM KIO<sub>4</sub> dissolved in 0.5 N KOH addition, the measurements were made at  $\lambda = 550 \text{ nm}$  [20]. Calibration curve for formaldehyde was used for enzyme activity calculation. Sensitivity range is 2-250 µmol of formaldehyde. One unit of enzyme activity corresponds to quantity of

formaldehyde formed per minute per mg of protein. For spectroscopic aldehydes quantity estimation also the Nash reagent was used [16].

*Assay for SOD activity.* Superoxide was generated from reduction of dissolved dioxygen using xanthine and xanthine oxidase, the well established method used to generate superoxide [21]. The amount of protein required to inhibit the rate of luminol chemiluminescence reaction induced by superoxide anions by 50%, was defined as 1 unit of enzyme activity. The total superoxide dismutase (SOD) and SOD1 activity was measured in rat thymus whole cell lysates and kidney cytosolic fractions, respectively.

*Assay for catalase activity.* The activity was determined at 25 °C by following the decomposition of H<sub>2</sub>O<sub>2</sub> at 240 nm according to the method of Beers and Sizer [22]. One unit decomposes 1.0 μmol of H<sub>2</sub>O<sub>2</sub> per minute per mg of protein.

*Assay for peroxidase catalase activity.* The activity was determined at 25 °C with methanol as the hydrogen donor using the purpald assay developed by Johansson et al. [23] applied to microplate reader. In this assay the formaldehyde produced from methanol reacted with purpald to produce a chromophore. Quantitation was accomplished by measuring the absorbance at 540 nm and comparing the results to those obtained with formaldehyde standards. The series of formaldehyde standards was analyzed each time the assay was performed. One unit forms 1.0 μmol of formaldehyde per minute per mg of protein.

*Protein estimation.* The protein concentration was determined by the Bradford method [24] with bovine serum albumin as a standard.

*Data analysis.* Data were analyzed by Student's *t*-test to determine statistical differences of the means. All results are expressed as mean ± SEM. A value of *P* < 0.05 was considered significant.

## Results and Discussion

Previously, in our laboratory in the *in vivo* animal model of glycerol induced rhabdomyolysis [4], the multiple metabolic alterations associated with muscle damage have been found at the 1<sup>st</sup> day after injection of glycerol (10-fold elevation in total hem and creatine kinase levels in plasma) along with acute renal failure at the 6<sup>th</sup> day after glycerol injection (10-fold increase in blood plasma nitrogenous wastes, uric acid, urea, and creatinine). Furthermore, the results of electron paramagnetic resonance spec-

troscopy revealed the formation in blood an additional pool of labile ferric iron with resonance signal at *g* = 4.3 at the 3<sup>rd</sup> day after injection of glycerol. This ferric iron, which was absent in blood of control animals, appeared at the 3<sup>rd</sup> day, the initial phase of renal injury, and persisted until the 10<sup>th</sup> day of the experiment, and may serve as one of the main reasons for oxidative stress progression in glycerol-injected rats due to Fenton reaction.

In the present experiment we evaluated the extent of oxidative/carbonyl stress as well as antioxidant protection in rat kidney and thymus under glycerol-induced rhabdomyolysis in terms of activity of amine oxidases (SSAO, PAO, DAO) and antioxidant enzymes (CAT and SOD). Results are shown in Tables 1 and 2.

It is known, a key component of antioxidant defense system catalase is a bifunctional enzyme, which may participate either in the catabolism of hydrogen peroxide or in the peroxidatic oxidation of small substrates, such as ethanol etc., depending on the conditions, namely, hydrogen peroxide concentration [25]. In this pathology model, we have shown that peroxidase activity of catalase in kidney cytosolic fraction increased 3-fold at the 1<sup>st</sup> day after glycerol injection as compared with the control (Table 1). In contrast, catalase activity of the enzyme elevated twice only at the 6<sup>th</sup> day after the disease initiation. Apparently, at the initial phase of rhabdomyolysis in the rat kidney the peroxidation dominated, that relates with low H<sub>2</sub>O<sub>2</sub> concentration while later, during the disease progression, the oxidation prevailed due to the high level of hydrogen peroxide and free, not incorporated into the transferrin, ferric iron appearance (Fenton reaction conditions). There was not significant SOD1 activity elevation under the pathology progression. Instead, we observed a slight but significant decrease in the enzyme activity evidently associated with the reducing action of superoxide anions with respect to ferric iron for Fenton reaction progression [26]. As far as the antioxidant activity of CAT (Cat-CAT) and SOD1 is an indirect measurement of oxidative stress under the pathology, their sufficient decrease after 'Unithiol' addition testifies to cells redox state normalization in the rat kidney.

In the present study we found that amine oxidases activity elevated at the 6<sup>th</sup> day (SSAO, PAO) and the 3<sup>rd</sup> day (DAO), the onset of ARF, and was not normalized by 'Unithiol' treatment. On the contrary the activity was significantly (2-3-fold) increased

Table 1. Activities of antioxidant enzymes and amine oxidases in the rat kidney under rhabdomyolysis progression and treatment with 'Unithiol' ( $M \pm m$ ,  $n = 6$ )

Enzymes	Animal groups				
	CG	RMG1	RMG3	RMG3-U	RMG6
Cat-CAT, $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ of protein	191 $\pm$ 21	197 $\pm$ 25	113 $\pm$ 13*	79 $\pm$ 14*#	403 $\pm$ 60*
Cat-Prx, $\mu\text{mol FA}/\text{min/mg}$ of protein	1.15 $\pm$ 0.11	3.47 $\pm$ 0.58*	0.88 $\pm$ 0.15	0.43 $\pm$ 0.09*#	2.96 $\pm$ 0.48*
SOD1, U/mg of protein	298 $\pm$ 27	298 $\pm$ 26	314 $\pm$ 22	192 $\pm$ 17*#	221 $\pm$ 17*
SSAO, $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ of protein	0.24 $\pm$ 0.04	0.12 $\pm$ 0.04*	0.33 $\pm$ 0.05	0.62 $\pm$ 0.12*#	0.78 $\pm$ 0.23*
PAO, $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ of protein	0.52 $\pm$ 0.10	0.31 $\pm$ 0.07	0.23 $\pm$ 0.04*	0.75 $\pm$ 0.13#	0.85 $\pm$ 0.22
DAO, $\mu\text{mol H}_2\text{O}_2/\text{min/mg}$ of protein	0.30 $\pm$ 0.06	–	0.64 $\pm$ 0.09*	0.71 $\pm$ 0.12*	–

\*  $P < 0.05$  (compared to CG); #  $P < 0.05$  (compared to RMG3)

compared with that in RMG3 state, perhaps due to creatinine, the precursor of methylamine, and polyamines content elevation in the injured tissue [4, 27]. In our opinion, the enhancement of amine oxidase activity in the pathologic kidney can be considered as significant reason of ARF, since elevated levels of amines deamination products (acrolein, formaldehyde and methylglyoxal) are major uremic toxins. Oxidative stress related with labile ferric iron appearance is likely not a direct amine oxidases inducer, since 'Unithiol' addition has a corrective effect only on antioxidant enzymes activity of CAT (Cat-CAT) and SOD1 but not amine oxidases. Possibly, the activity enhancement of the amine oxidases is mainly induced by the elevated quantity of amines and polyamines.

We have found the significant correlation ( $r = +0.5$ ,  $P < 0.05$ ) between catalase activity of CAT and SSAO activity at the 3<sup>rd</sup> day after glycerol injection in the kidney. This suggests the relationship between the redox reactions and catabolism of biogenic amines and polyamines.

In view of the fact that immune system function is altered by rhabdomyolysis conditions we examined aforementioned enzymes activities in thymocytes, the data are presented in Table 2. The rat-derived thymocytes received *ex vivo* at the 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> days after glycerol injection were used in our studies. We registered peroxidase activity of the enzyme, since we had shown earlier that catalase activity was undetectable in thymus [28]. It remained

unchanged after glycerol injection and decreased after administration of 'Unithiol', unlike SOD activity which elevated and peaked at the 6<sup>th</sup> day after glycerol injection. Taking into consideration our data obtained previously [28], the 3-fold elevation in the level of total SOD activity, related to superoxide content enhancement under rhabdomyolysis, may be a reason for apoptosis stimulation in thymocytes. It should be noted that the values of amine oxidase activity in the thymus were of the same order and the highest among all the investigated organs (10-fold higher than in the kidney and 100-fold higher than in the liver, data are not shown). It testifies that the metabolism of amines and polyamines is of great importance for T-lymphopoiesis. We again observed multiple rises in activity of amine oxidases under investigation (2-4.5 times) already at the 3<sup>rd</sup> day after glycerol injection that indicate the increase in the content of amines and polyamines under the pathology (the potential threat of thymocytes apoptosis). 'Unithiol' addition influenced only Cat-Prx activity. Thereby, the oxidative stress induced by free ferric iron has no effect on the behavior of SOD and amine oxidases.

Since increased generation of hydrogen peroxide in the presence of such metals as iron, in an uncomplexed form in biological systems, as well as enhanced concentrations of RCS may influence the redox system, we tested their cooperative effect to induce antioxidant enzyme catalase and amine oxidase SSAO *in vitro*. These enzymes were cho-

Table 2. Activities of antioxidant enzymes and amine oxidases in the rat thymocytes under rhabdomyolysis progression and treatment with 'Unithiol' ( $M \pm m$ ,  $n = 6$ )

Enzymes	Animal groups				
	CG	RMG1	RMG3	RMG3-U	RMG6
Cat-Prx, nmol FA/min/mg of protein	26.4 ± 5.1	24.9 ± 8.2	39.5 ± 10.1	13.1 ± 2.3* <sup>#</sup>	26.6 ± 4.9
SOD, U/mg of protein	57 ± 7	47 ± 3	101 ± 8*	119 ± 15*	158 ± 12*
SSAO, μmol H <sub>2</sub> O <sub>2</sub> /min/mg of protein	4.82 ± 0.82	1.55 ± 0.42*	9.88 ± 1.59*	9.88 ± 1.16*	10.51 ± 2.4*
PAO, μmol H <sub>2</sub> O <sub>2</sub> /min/mg of protein	1.62 ± 0.46	–	5.62 ± 1.35*	4.17 ± 0.75*	2.09 ± 0.46
DAO, μmol H <sub>2</sub> O <sub>2</sub> /min/mg of protein	1.21 ± 0.27	–	5.50 ± 1.07*	5.34 ± 0.92*	–

\* $P < 0.05$  (compared to CG); <sup>#</sup> $P < 0.05$  (compared to RMG3)

sen because we had found the correlation between Cat-CAT and SSAO activity under rhabdomyolysis. To investigate whether the oxidative and carbonyl stress agents are involved in SSAO and CAT stimulation observed *in vivo* after glycerol injection, we subjected rat liver microsomes and cytosol to Fenton reaction conditions (H<sub>2</sub>O<sub>2</sub>-iron-ascorbic acid) (2, 3)



or methylglyoxal (MG) alone, and their combined action (H<sub>2</sub>O<sub>2</sub>-iron-ascorbic acid +MG). Data are shown in Table 3.

A weak increase in activity of both enzymes under the investigation occurred when the liver cell fractions were incubated only with H<sub>2</sub>O<sub>2</sub>-iron-ascorbic acid, whereas supplementation with MG resulted in the significant 2.5-fold enhancement of their activity. Complicated Fenton reaction conditions (H<sub>2</sub>O<sub>2</sub>-iron-ascorbic acid +MG) significantly induced SSAO activity stronger than MG alone, while CAT

activity enhancement was much lower in this case. Thus, the additive effect of combined action of both carbonyl and oxidative stress agents was observed only for SSAO. These data indicate that only combined action of oxidative and carbonyl stress agents are the regulators of the amine oxidase, whereas antioxidant defensive enzyme was influenced more by the aldehyde. Our studies *in vitro* defined the key role for oxidative stress (iron+H<sub>2</sub>O<sub>2</sub>-Fenton reaction), carbonyl stress (MG) and their combined action in antioxidant defense and notably amine oxidase functioning. Thereby, the changes in activities of antioxidant enzymes and amine oxidases observed in the *in vivo* experiment may be caused by the direct action of oxidative/carbonyl stress factors.

Taking into account the data obtained in our experiments *in vivo* and *in vitro*, we hypothesized that biogenic amine and polyamine catabolism by amine oxidases is upregulated by oxidative and carbonyl stress factors directly under rhabdomyolysis progression. And the increase in hazardous catabo-

Table 3. Effects of ROS (Fenton reaction) and methylglyoxal on rat liver CAT and SSAO activity ( $M \pm m$ ,  $n = 9$ )

Enzymes	Reaction conditions			
	Control	ROS (Fenton reaction)	MG (20 mM)	ROS+MG
Catalase, μmol H <sub>2</sub> O <sub>2</sub> /min/mg of protein	560 ± 29	657 ± 37*	1398 ± 67* <sup>#</sup>	791 ± 69* <sup>§</sup>
SSAO, μmol H <sub>2</sub> O <sub>2</sub> /min/mg of protein	1.09 ± 0.24	1.38 ± 0.23	2.81 ± 0.32* <sup>#</sup>	3.24 ± 0.21* <sup>#</sup>

\* $P < 0.05$  (compared to control); <sup>#</sup> $P < 0.05$  (compared to "ROS"); <sup>§</sup> $P < 0.05$  (compared to "MG")

lic products concentration (releasing RCS and ROS) contributes to tissue damage in glycerol-induced ARF and apoptosis stimulation in the thymus. Possible role(s) of amine oxidases in pathological processes need to be further investigated. Further investigation of the mechanisms of amine oxidases catabolic products toxicity and their participation in the pathological processes is necessary.

*Acknowledgements.* We are very grateful to Dr. Olena G. Alkhimova for the technical advice and critical readings of this article.

### **РОЛЬ АМІНОКСИДАЗ ЗА РОЗВИТКУ РАБДОМІОЛІЗУ В ЩУРІВ**

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Оскільки за розвитку різних захворювань у тканинах має місце підвищення рівня активних форм кисню та реактивних альдегідів, в цій роботі ми перевіряли припущення про важливість ролі аміноксидаз (семікарбазидчутливої аміноксидази, діаміноксидази, поліаміноксидази), які є додатковим джерелом оксидативного та карбонільного стресу в умовах гліцероліндукованого рабдоміолізу в щурів. У нирках і тимусі, що є органами-мішенями за рабдоміолізу, активність аміноксидаз визначали розробленим нами високочутливим флуоресцентним методом. В експерименті *in vivo* активність аміноксидаз у клітинних фракціях, клітинах та їхніх лізатах вірогідно збільшувалась (у 2,5–4 рази) на 3–6-й день після введення гліцеролу. Зміни активності антиоксидантних ензимів були пов'язані зі стадією рабдоміолізу і залежали від органа. Введення тваринам хелатору металів «Унітіолу» на фоні рабдоміолізу приводило лише до корекції активності антиоксидантних ензимів. Крім того, в експерименті *in vitro* ми з'ясували, що продукти реакції Фентона (залізо в присутності пероксиду водню) не виявляли ефекту на активність семікарбазидчутливої аміноксидази мікросомальної фракції печінки щурів, тоді як присутність метилгліоксалу підвищувала активність ензиму в 2,5 рази. Комбінація чинників оксидативного та карбонільного стресу мала адитивний ефект на

цей показник. Ми припускаємо, що катаболізм біогенних амінів та поліамінів за участю аміноксидаз безпосередньо здатен підсилювати ступінь оксидативно-карбонільного стресу в умовах гліцероліндукованого рабдоміолізу в щурів, а збільшення концентрації продуктів катаболізму в тканинах може бути причиною гострої ниркової недостатності та апоптозу тимоцитів.

**Ключові слова:** рабдоміоліз, аміноксидази, біогенні аміни, поліаміни, оксидативно-карбонільний стрес.

### **РОЛЬ АМІНОКСИДАЗ В РОЗВИТТІ РАБДОМІОЛІЗА У КРЫС**

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Поскольку при различных заболеваниях в тканях повышается концентрация активных форм кислорода и реактивных карбонильных соединений, в данной работе мы проверяли гипотезу о важности роли аминоксидаз (семікарбазидчувствительной аминоксидазы, диаміноксидазы, полиаміноксидазы) – дополнительных источников оксидативного и карбонильного стресса при глицеролиндуцированном рабдомиолизе у крыс. В почках и тимусе, органах-мишенях при рабдомиолизе активность аминоксидаз определяли разработанным нами высокочувствительным флуоресцентным методом. В эксперименте *in vivo* активность аминоксидаз в клеточных фракциях, клетках и их лизатах повышалась (в 2,5–4 раза) на 3–6-й день после введения глицерола. Наблюдаемые изменения активности антиоксидантных энзимов зависели от стадии рабдомиолиза и от органа. Введение животным хелатора металлов «Унитиола» на фоне рабдомиолиза корректировало только активность антиоксидантных энзимов обоих органов. Кроме того, в эксперименте *in vitro* обнаружено, что продукты реакции Фентона (железо в присутствии пероксида водорода) не влияли на активность семікарбазидчувствительной аминоксидазы микросомальной фракции печени крыс, тогда как присутствие метилглюксала в 2,5 раза повышало активность энзима. Комбини-

рование факторов оксидативного и карбонильного стресса оказывало на активность данного фермента аддитивный эффект. Мы предполагаем, что катаболизм биогенных аминов и полиаминов с участием аминоксидаз напрямую усиливает степень оксидативного и карбонильного стресса при глицеролиндукции рабдомиолизе у крыс, а повышенные концентрации продуктов катаболизма в тканях при данной патологии являются причиной развития острой почечной недостаточности и апоптоза тимоцитов.

**Ключевые слова:** рабдомиолиз, аминоксидазы, биогенные амины, полиамины, оксидативно-карбонильный стресс.

### References

- Dall-Donne I., Rossi R., Colombo R., Giustarini D., Milzani A. Biomarkers of oxidative damage in human disease. *Clin. Chem.* 2006; 52(4): 601-623.
- Miyata T., Izuhara Y., Sakai H., Kurokawa K. Carbonyl stress: increased carbonyl modification of tissue and cellular proteins in uremia. *Perit. Dial. Int.* 1999; 19(Suppl 2): S58-S61.
- Khan F. Y. Rhabdomyolysis: a review of the literature. *Neth. J. Med.* 2009; 67(9): 272-283.
- Shandrenko S. G. Labile iron pool formation in rat's blood under rhabdomyolysis. *Ukr. Biokhim. Zhurn.* 2012; 84(6): 124-129. (In Ukrainian).
- Chumachenko I. M., Kapustyanenko L. G., Shandrenko S. G. Blood labile iron effect on nitrosative stress development under rat experimental rhabdomyolysis. *Studia Biologica.* 2012; 6(3): 105-114. (In Ukrainian).
- Aydogdu N., Erbas H., Atmaca G., Erten O., Kaymak K. Melatonin reduces nitric oxide via increasing arginase in rhabdomyolysis-induced acute renal failure in rats. *Ren. Fail.* 2006; 28(5): 435-440.
- Bagley W. H., Yang H., Shah K. H. Rhabdomyolysis. *Intern. Emerg. Med.* 2007; 2(3): 210-218.
- Abboud H. E., Ou S. L., Velosa J. A., Shah S. V., and Dousa T. P. Dynamics of renal histamine in normal rat kidney and in nephrosis induced by aminonucleoside of puromycin. *J. Clin. Invest.* 1982; 69(2): 327-336.
- Floris G., Mondovi B. Copper amine oxidases: structures, catalytic mechanisms and role in pathophysiology. CRC Press, 2009; 374 p.
- Yu P. H., Zuo D. M. Oxidative deamination of methylamine by semicarbazide-sensitive amine oxidase leads to cytotoxic damage in endothelial cells. *Diabetes.* 1993; 42(4): 594-603.
- Yu P. H., Zuo D. M. Formaldehyde produced endogenously via deamination of methylamine. A potential risk factor for initiation of endothelial injury. *Atherosclerosis.* 1996; 120(1-2): 189-197.
- Wong M., Saad S., Zhang J., Gross S., Jarolimek W., Schilter H., Chen J. A., Gill A. J., Pollock C. A., Wong M. G. Semicarbazide-sensitive amine oxidase (SSAO) inhibition ameliorates kidney fibrosis in a unilateral ureteral obstruction murine model. *Am. J. Physiol. Renal. Physiol.* 2014; 307(8): F908-F916.
- Duperray A., Mantovani A., Introna M and Dejana E. Endothelial cell regulation of leukocyte infiltration in inflammatory tissues. *Mediators Inflamm.* 1995; 4(5): 322-330.
- Kaminska A. M., Stern L. Z., Russell D. H. Altered muscle polyamine levels in human neuromuscular diseases. *Ann. Neurol.* 1981; 9(6): 605-607.
- Sakata K., Kashiwagi K., Sharmin S., Ueda S., Igarashi K. Acrolein produced from polyamines as one of the uraemic toxins. *Biochem. Soc. Trans.* 2003; 31(2): 371-374.
- Latyshko N., Gudkova O., Dmytrenko M. Semicarbazide as potential source of formaldehyde and nitric oxide formation. *Drugs Ther. Stud.* 2012; 2(1): 43-47.
- Strober W. Trypan blue exclusion test of cell viability. *Curr. Protoc. Immunol.* 2001: appendix3:appendix 3B.
- Caillet S., Yu H., Lessard S., Lamoureux G, Ajdukovic, D; Lacroix, M. Fenton reaction applied for screening natural antioxidants. *Food Chem.* 2007; 100(2): 542-552.
- Hirakawa K. Fluorometry of hydrogen peroxide using oxidative decomposition of folic acid. *Anal. Bioanal. Chem.* 2006; 386(2): 244-248.
- Whiler C. R., Salzman J. A., Elsayed N. M., Omaye S. T., Korte D. W. Jr. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. *Anal. Biochem.* 1990; 184(2): 193-199.
- Eriksson V. J., Borg L. A. Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformation *in vitro*. *Diabetologia.* 1991; 34(5): 325-331.



22. Beers R. F. Jr, Sizer I. W. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 1952; 195(1): 133-140.
23. Johanson L. H., Borg L. A. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal. Biochem.* 1990; 174(1): 331-336.
24. Bradford M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72(1-2): 248-54.
25. Vetrano A. M., Heck D. E., Mariano T. M., Mishin V., Laskin D. L., Laskin J. D. Characterization of the oxidase activity in mammalian catalase. *J. Biol. Chem.* 2005; 280(42): 35372-35381.
26. Prousek J. Fenton chemistry in biology and medicine. *Pure Appl. Chem.* 2007; 79(12): 2325-2338.
27. Bachrach U., Heimer Y. M. The Physiology of Polyamines, Vol. 1. CRC Press, Inc. 1989; 368 p.
28. Latyshko N. V., Gudkova L. V., Gudkova O. A. Effect of polyamines on superoxide dismutase activity under dexamethasone induced apoptosis in rat thymocytes. *Ukr. Biokhim. Zhurn.* 2008; 80(3): 78-83.

Received 29.09.2015