ОРИГИНАЛЬНАЯ СТАТЬЯ

CHARACTERISTICS OF OXIDATIVE STRESS INDUCED BY MODERATE DOSES OF SODIUM NITRITE IN ISOLATED ERYTHROCYTES IN THE PRESENCE OF SODIUM SELENITE

T.M. Huseynov*, S.Y. Huseynova, R.T. Guliyeva, M.Z. Dadashov, S.M. Rahmanova (Maharramova), F.R. Yakhyayeva, S.A. Jafarova

İnstitute of Biophysics of NAS,
st. Z. Khalilov, 117, AZ1141, Baku, Azerbaijan

ABSTRACT. The effect of low and moderate sodium nitrite doses (final concentration – 0.007, 0.070, 0.15, 0.35, 0.70 and 3.50 mM) on oxidative processes in erythrocytes, glutathione content, changes in catalase and glutathione peroxidase activity and the intensity of lipid peroxidation and the effect of sodium selenite (5 μM) as a possible antioxidant factor affecting the development of oxidative processes *in vitro* (incubation period 30 min, t = 37 °C) was considered. During nitrite-induced oxidation, there is a depletion of intra-erythrocyte glutatione (up to 12% of the initial level), which is in direct proportion to the final concentration of sodium nitrite. Catalase activity at all final sodium nitrite concentrations decreases, reaching a minimum already in the middle of the incubation period. In case of nitrite-induced oxidation, multidirectional changes in the glutathione peroxidase activity take place, which, at low and moderate sodium nitrite concentrations slightly increasein the initial period of incubation and decrease in the middle of the period. Catalase and glutathione peroxidase activity decrease in erythrocytes caused by nitrites is accompanied by an intensive accumulation of methemoglobin and an increase in lipid peroxidation at high concentrations of sodium nitrite (0,70–3,50 mM) by the end of the incubation period. The negative relationship between a decrease in glutathione peroxidase activity and an increase in lipid peroxidation intensity suggests a predominant role of glutathione peroxidase in limiting oxidative stress in erythrocytes.The results obtained indicate the active participation of these antioxidant erythrocytes enzymes in oxidative metabolism under nitrite exposure. Sodium selenite can, to a certain extent, act as a regulator of nitrite-induced oxidative stress.

КЛЮЧЕВЫЕ СЛОВА: erythrocytes, hemoglobin, sodium nitrite, sodium selenite, glutathione peroxidase, catalase, lipid peroxidation.

Abbreviations:

AO – antioxidant factor
CAT – catalase
FC – final concentrations
FCSN – final concentrations of sodium nitrite
GSH – glutathione
GP – glutathione peroxidase
H_2O_2 – hydrogen peroxide
Hb – hemoglobin
LPO – lipid peroxidation
MetHb – methemoglobin
MDA – malondialdehyde
$NaNO_2$ – sodium nitrite
Na_2SeO_3 – sodium selenite
NO – nitric oxide
SPB – sodium phosphate buffer
TBARS – thiobarbituric acid reactive substances

INTRODUCTION

Among the nitrogen-containing substances present in or entering in the human body, the key place is occupied by nitrites, which are the most basic factor of nitrogen impact on the human organism. Nitrites in the body are donors of nitric oxide, which performs a wide range of important regulatory functions (DeMartino, et al., 2019), and depending on the concentration and a number of other conditions can act as an antioxidant, interrupting free radical

* Corresponding aythor:
Tokay Huseynov
E-mail: tokhus@mail.ru

DOI: 10.19112/2413-6174-2021-22-2-25-35

oxidative processes (Wink, et al., 2001) and as a prooxidant (Hogg, 1995). One of the main targets of the nitrites toxic effect is hemoglobin, which has an increased oxidative affinity (formation of MetHb and other oxidative derivatives) for nitrites (Hogg, 1995; Doyle, et al., 1981; Titov, Petrenko, 2005).

During the oxidative reaction between oxyhemoglobin (oxyHb) and nitrites, along with nitrate, MetHb, hydrogen peroxide and $NO_2^{\cdot}$ are mainly formed, that is, radical products capable of serving as intermediates for further oxidative processes, leading to the development of protein and lipid peroxidation, causing cell death (Doyle, et al., 1981; May, et al., 2000; Keszler, et al., 2008). This reaction itself consists of two phases: a slow phase (initiation phase) and an autocatalytic phase, the contribution of which depends on the concentration of nitrites in relation to Hb (Doyle, et al., 1981; Kosaka, Tyuma, 1987; Spagnuolo, et al., 1987; Keszler, et al., 2008). The predominant product in the slow phase is H_2O_2 (Keszler, et al., 2008), while for the autocatalytic phase it is $NO_2^{\cdot}$ radical. In this case, the fraction of the slow phase is significant at low nitrite concentrations, and at high nitrite concentrations, the initiation phase is short or absent, and the reaction becomes autocatalytic (Doyle, et al., 1981; Keszler, et al., 2008).

It should be noted that with a small and moderate effect of nitrite on erythrocytes, stimulation of H_2O_2 breakdown is important for limiting the induced oxidative stress in erythrocytes, which is normally carried out by a system of AO enzymes: superoxide dismutase, GP, CAT, glutathione reductase, peroxiredoxin-2 (Johnson, et al., 2005; Chun-Seok, et al., 2010; Rocha, et al., 2015) and by Hb itself, which has a certain peroxidase activity (Widmer, et al., 2010).

In addition, it is known that nitrites are donors of $NO^{\cdot}$, which, when interacting with the superoxide radical ($O_2\cdot^{-}$), formed during Hb oxidation, generate an aggressive radical product – peroxynitrite ($ONOO^{-}$), capable of oxidizing cellular structures (Kondo, et al., 1997; Denicola, et al., 1998; Osipov, et al., 2007). In this regard, there is extensive information on the use of various antioxidants to neutralize nitrite toxicity, including the destruction of metabolites of nitrite exposure (peroxynitrites, etc.) (Kondo, et al., 1997; Chow, Hong, 2002). In particular, there is data on the AO effect of selenium-containing substances: Se-proteins and Se-amino acids or other selenium compounds (usually acting like SH-containing compounds, but with greater efficiency) (Sies, et al., 1998; Alvazer, Radi, 2003; Storkey, et al., 2015). There is evidence that selenoprotein is able to catalyze degradation of $ONOO^{-}$ at a high final rate of the 2nd order reaction (Sies, et al., 1998; Storkey, et al., 2015). It was suggested that GP acts as a peroxynitrite reductase, reducing and protecting Hb from oxidation and nitration (Sies, Arteel, 2000). At the same time, it was shown that GP selenenzyme is deactivated in the presence of $ONOO^{-}$ which also confirms the importance of Se-proteins and Se-amino acids in the protection against nitrite toxicity, given that nitrites as a source of $NO^{\cdot}$ form with O_2^{-} highly active oxidants – peroxynitrites (Padmaja, et al., 1998).

In addition, there are indications that GP is directly deactivated in the reaction medium in the presence of $NO^{\cdot}$ (Asahi, et al., 1995), which also indicates the importance of selenium-containing compounds in the regulation of nitrite toxicity.

In the literature there are a number of indications that Na_2SeO_3 is easily incorporated into erythrocytes (selenium pump), in which it undergoes a complex metabolism, interacting with Hb, influencing its properties, with the subsequent release from erythrocytes into plasma in the composition of various albumins (Gasiewicz, Smith, 1978; Mas, et al., 1988; Suzuki, et al., 1998). Thus, selenium, which is included in erythrocytes, as an active intermediate, can affect oxidative processes induced by nitrites or their metabolites.

The purpose of this study was to examine the patterns of development of oxidative stress, the state of the main antiperoxide enzymes – CAT and GP in isolated human erythrocytes during the development of oxidative stress caused by exposure to low and moderate doses of $NaNO_2$ in the presence of sodium selenite, as a substance with an active AO metabolism in erythrocytes.

MATERIALS AND METHODS

The following reagents were used in the work: sodium selenite ≤ 99% (Sigma-Aldric Chemie Gmbh), reduced glutathione (AppliChem, Germany), 5.5-Dithio-bis (-2-nitrobenzoic acid) (Ellman's Reagent) (Germany), thiobarbituric acid (AppliChem, Germany), ethylenediamine tetroacetic acid (Acros Organics, Belgium), sodium azide (Biochem, France), tert-butyl hydroperoxide (Sigma Aldrich, USA), oxymethyl aminomethane, trichloroacetic acid, sodium nitrite, ammonium molybdate (Russia). All reagents are chemically pure.

In model experiments, the main object of the study was human erythrocytes and Hb. In *in vitro*

experiments, donor blood was used, taken from the cubital vein into tubes with heparin (20 units/ml of blood). Separation of blood plasma from erythrocytes was carried out by centrifugation (800 g for 15 min). To obtain a suspension of erythrocytes, the erythrocyte sediment was washed three times in a tenfold volume of physiological solution – sodium phosphate buffer (0.01 M sodium phosphate + 0.14 M NaCl; pH 7.4; t = 37 °C), centrifuged at 800 g for 15 min, followed by removal of the supernatant liquid. Hemolysis of erythrocytes was achieved by diluting the erythrocyte mass with distilled water in a ratio of 1: 9, followed by freezing, thawing and centrifugation at 10000 g for 10 min. Two series of experiments were carried out to study changes in indicators of oxidative processes in erythrocytes (accumulation of MetHb, GSH content, GP, CAT activity, LPO intensity). In the first series of experiments in an incubation medium with erythrocyte hemolysate, $NaNO_2$ was added, with a final concentration in the incubation medium from 0 to 3.50 mM (0.007; 0.070; 0.15; 0.35; 0.70; 3.50 mM) exposure time 0 to 30 min (aliquots were taken every 5 min), at 37 °C. The second series of experiments were carried out with the simultaneous addition of $NaNO_2$ (0.70 mM) and Na_2SeO_3 (5 μM) to the incubation medium with a 15 minute interval apart and with a total incubation period of 30 min. The dose of 0.70 mM was chosen as the optimal one based on our earlier experiments, where it was found that a significant increase in MetHb corresponds to a noticeable increase in LPO in erythrocytes (Huseynova, et al., 2018; Huseynova, 2019). In the second series of experiments, 5 options were considered: 1 control and 4 experimental. The control sample contained an erythrocyte hemolysate, and in the next 4 experimental samples, $NaNO_2$, Na_2SeO_3, Na_2SeO_3 + $NaNO_2$ and $NaNO_2$ + Na_2SeO_3 were added to the erythrocyte hemolysate, respectively. Sodium selenite was used as a possible antioxidant that affects oxidative processes with high permeability through the erythrocyte membrane and active metabolism in them (Mas, et al., 1988; Suzuki, et al., 1998; Hongoh, et al., 2012). The FC of Na_2SeO_3 (5 μM) in the incubation medium was also selected based on previous experiments (Huseynova, 2019) (see details in the "research results" section).

The accumulation of MetHb was estimated according to semi empirical formulas proposed by J. Szebeni et al. (1984):

$$([MetHb] = 28A_{577} - 307A_{630} - 55A_{560})$$

The content of GSH was carried out by the method, the principle of which is the formation of a colored compound upon interaction of GSH with 5,5-dithiobis (2-nitrobenzoic acid) (DTNBA) (Ellman, 1959).

The activity of GP was determined by the rate of GSH oxidation in the presence of tert-butyl hydroperoxide (Moin, 1986).

Catalase activity was determined by the spectrophotometric method (Korolyuk, 1988), based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts.

The intensity of LPO processes in erythrocytes was assessed by the accumulation of colored products of malondialdehyde, which react in a color reaction with thiobarbituric acid to form a colored trimethine complex with a characteristic absorption spectrum with a maximum at λ = 532 nm (Mengel, Kann, 1966).

All measurements were carried out on an SF-46 spectrophotometer (Russia).

Statistical processing of the obtained results was carried out using the t criterion at a significance level of p = 0.05 (Lakin, 1990). Statistical data processing was performed using the MS Excel 2017 software packages.

RESULTS

Stimulation of the nitrite oxidative process in erythrocytes is accompanied not only by the accumulation of MetHb, but also by accumulation other Hb derivatives and the formation of radical products, including reactive oxygen and nitrogen species, and, as a consequence, by a change in the activity of antioxidant enzymes, primarily CAT and GP as the main antiperoxide enzymes that function under conditions of active H_2O_2 generation (Asahi, et al., 1995; Padmaja, et al., 1998; May, et al., 2000; Chow, Hong, 2002; Nagababy, et al., 2003; Osipov, et al., 2007; Lapinski, et al., 2014; Rocha, et al., 2015; Krych-Madej, Gebika, 2017).

One of the important consequences of radical formation during intracellular oxidation of Hb is the oxidation GSH, proceeding according to the scheme (Stepuro, et al., 1997; Sopyani, et al., 2008; Lapinski, et al., 2014).

$$GS^- + NO_2^\bullet \rightarrow GS^\bullet + NO_2^- \rightarrow GS^\bullet\text{–}GSSH$$

Considering that the change in the ratio of intracellular GSH to the ratio of GSSH, is an indicator of impaired hemostasis in the absence of glucose in an incubation medium that maintains the level of

the most important intra-erythrocyte hemostasis components GSH, NAD, NADPH, ATP, it can be assumed that changes in the transition of GSH to GSSH can serve as an indicator of oxidative stress (Osipov, et al., 2007; Chun-Seok, et al., 2010; Smeyne, Smeyne, 2013). At the same time, the activity of the key complementary antiperoxide enzymes CAT and GP, involved in the regulation of the H_2O_2 level, is related to GSH in different ways, and if the bond for CAT is mediated, then GP is directly related to GSH, because GSH is a substrate for GP. Under these conditions, heme degradation is more dependent on GSH, given that in erythrocytes, GP, at a moderate concentration of hydrogen peroxide, is the main antioxidant protector of Hb (Nagababu, et al., 2003; Chun-Seok, et al., 2010; Rocha, et al., 2015).

In experiments to study the effect of the final concentrations of sodium nitrite in an incubation medium on the content of intra-erythrocyte GSH, it was revealed that nitrites have a significant effect on the depletion of intra-erythrocyte GSH. From Fig. 1. it can be seen that in the first 3-5 minutes of 30-minute incubation there is an insignificant "surge" in GSH content, apparently having a compensatory value inherent in low or moderate FCSN 0.007; 0.07; 0.35 mM and which is absent for 0.70 and 3.50 mM. The rate of GSH depletion is maximum at intervals of 5-10 minutes or 5-15 minutes and depends on the FCSN (the higher FCSN, the higher the GSH depletion rate). After 30 minutes of incubation, the GSH level decreases to 7-25% of the initial (for 0.35; 0.70; 3.50 mM $NaNO_2$) while for lower concentrations, it is about 50%.

The curves of changes in CAT and GP activity under $NaNO_2$ supply to an incubation medium in the time interval 0–30 minutes are shown on Fig. 2 and 3.

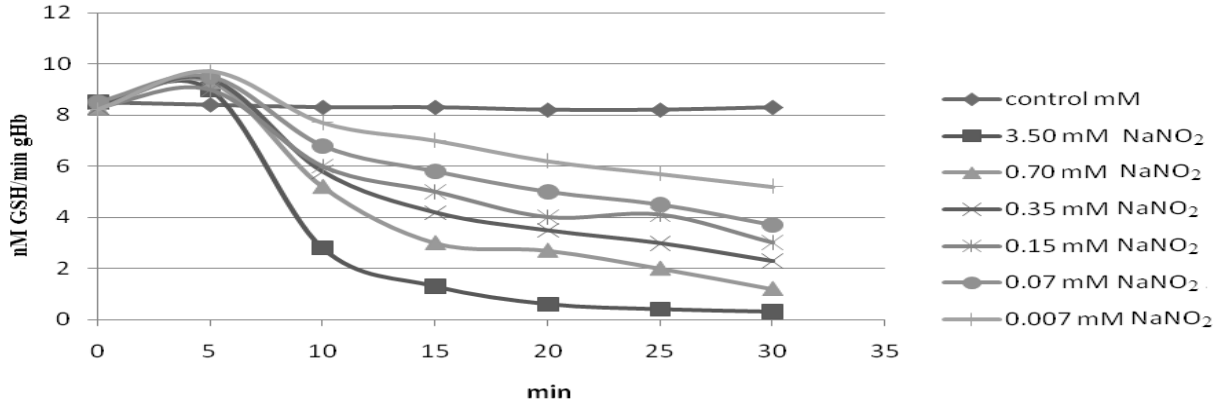

Fig. 1. *Change in the content of GSH in human erythrocytes treated with NaNO2 (the incubation medium contains 10 mM SPB, 0.14 M NaCl, pH 7.4, t = 37 °C)*

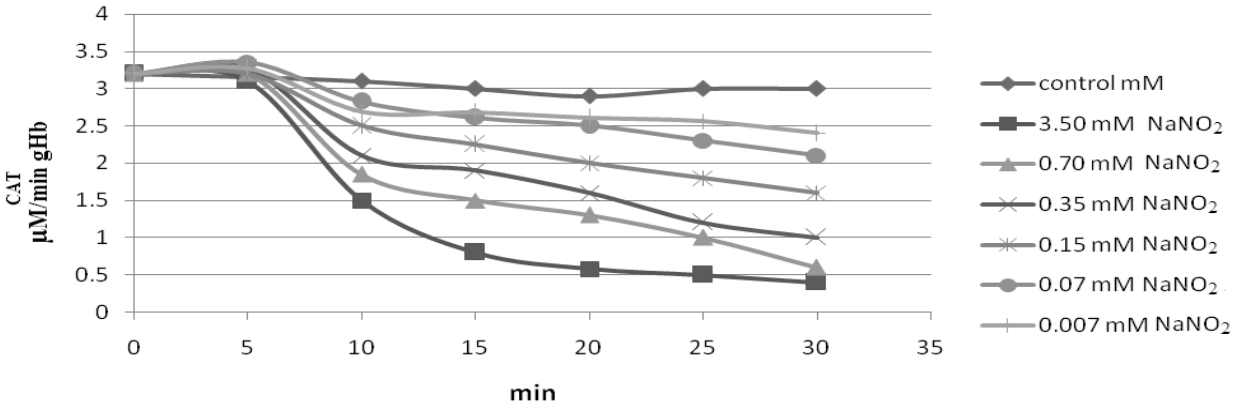

Fig. 2. *Changes in CAT activity in human erythrocytes treated with $NaNO_2$ (the incubation medium contains 10 mM SPB, 0.14 M NaCl, pH 7.4, t = 37 °C)*

* footnote – Under normal conditions (under physiological conditions), oxidative processes associated with the utilization of hydrogen peroxide, including those occurring in the near-membrane space, are regulated by peroxiredoxin-2, the content of which is an order of magnitude higher in comparison with GP and especially with catalase. However, having a low rate constant, this enzyme is not very effective in the case of induced oxidative processes and the associated flow of hydrogen peroxide.

Fig. 2 shows that the activity of CAT at all FC decreases, reaching a minimum already in the middle of the incubation period: 50–60% of the initial level (at 0.35 and 0.70 mM $NaNO_2$ concentrations) and higher activity levels for lower $NaNO_2$ concentrations (less than 0.35 mM).

In contrast to CAT, the GP activity (Fig. 3) at low and moderate concentrations of $NaNO_2$ in the initial period of incubation slightly increases (compared to control), after which it is established at the control level (lag period), and then the activity begins to decrease, ≈ from the middle of the incubation term. However, at higher FC a decrease in GP activity occurs, similar to CAT. Thus, it can be concluded from Fig. 1, 2, 3 that the nitrite treatment of isolated erythrocytes decreases the intra-erythrocyte content of GSH, the activity of CAT and GP (if we exclude the initial increase in the activity for low and moderate FCSN).

In this case, a decrease in the GP activity occurs when the lag period is exhausted, the length of which depends on the FC of nitrites. Apparently, this is due to the fact, that GSH content exceeds the requirements of GP for GSH as an oxidation substrate (Floe, 1988). After passing the "red line", depletion of the GSH level begins to affect the loss of GP activity.

The decrease in the activity of the main anti peroxide enzymes of erythrocytes, CAT and GP, caused by nitrites, is accompanied by an intensive accumulation of MetHb and other oxidative radical products that can lead to the development of protein and lipid peroxidation, and as a consequence to hemolysis (Hogg, 1995; Ansari, et al., 2015). Our experiments have shown that nitrites already in minimal FC – 0.007 mM have an oxidative effect on HbO_2, which increases with the growth of FCSN, reaching already in the first 15–20 minutes of incubation (for the case of 0.35-0.70 mM) 40% and above of the MetHb content mark (Fig. 4). At the same time, there is a relatively weak "motivation" for MDA accumulation: no significant changes (weakening of LPO), were found in the 0-15 minute interval of time and only a 50-70% increase in the MDA level in 30 minutes incubation time for higher FCSN (0.70-3.50 mM) (Fig. 5).

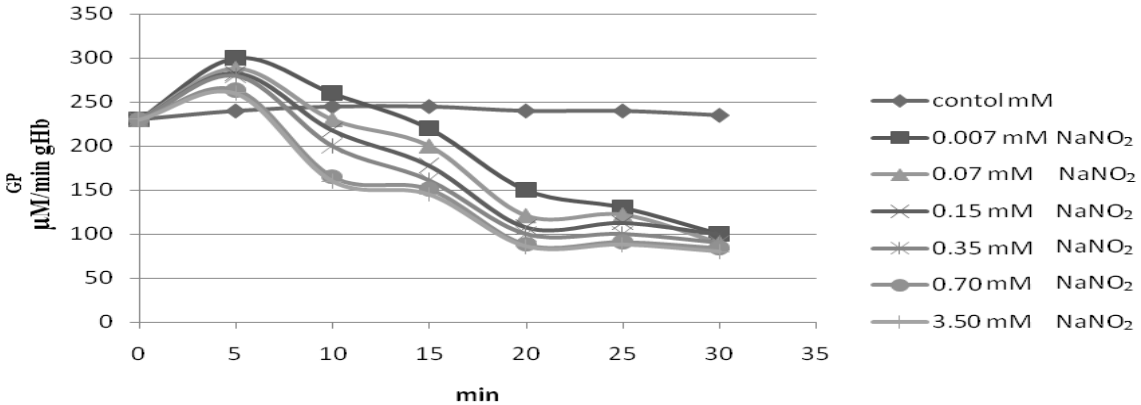

Fig. 3. *Changes in GP activity in human erythrocytes with $NaNO_2$ (the incubation medium contains 10 mM SPB, 0.14 M NaCl, pH 7.4, t = 37 °C)*

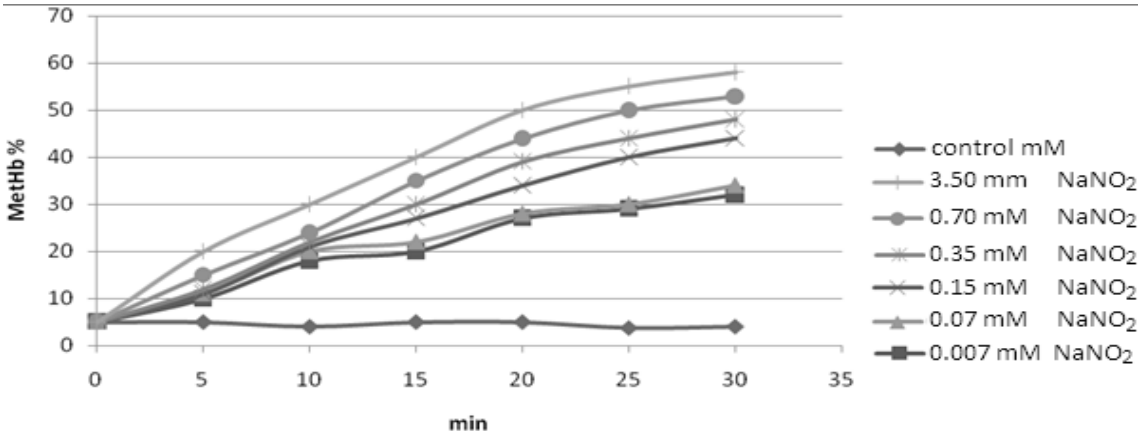

Fig. 4. *Dose-dependent nitrite-induced accumulation of MetHb in an incubation medium containing human erythrocytes*

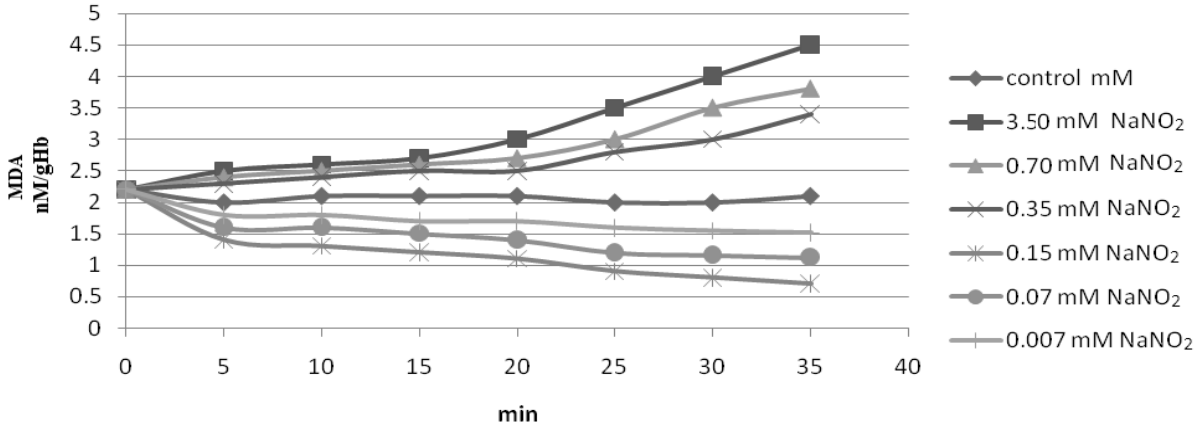

Fig. 5. *Accumulation of TBARS in human erythrocytes incubated in a medium containing $NaNO_2$ (the incubation medium contains 10 mM SPB, 0.14 M NaCl, pH 7.4, t = 37 °C)*

These data show that FCSN has a multidirectional effect on oxidative processes in erythrocytes membrane apparatus. The fact that low FCSN leads to a decrease in LPO with a significant increase in MetHb indicates that MetHb has AO properties. Earlier, in similar experiments, we showed an increase in membrane-bound hemoglobin (Hbm) and its main oxidative derivative MetHbm with an increase in FCSN in an incubation medium containing erythrocytes. At the same time, the ratio as the FCSN grew, between Hbm and MetHbm reached 1: 4 (Shperlink, et al., 2004; Huseynov, et al., 2018; Huseynova, et al., 2018). There is evidence that it is the membrane Hb during oxidative modification, being closely associated with erythrocytes membrane apparatus, stimulates the development of LPO (Shperlink, et al., 2004; Arashiki, et al., 2013; Welborn, et al., 2016).

It can be seen from these experiments that: 1) only at high FCSN (0.70 mM and higher) there is a clear increase in LPO, which is absent (<0.35 mM) or there is a decrease in the accumulation of LPO products at 0.07; 0.15 mM, at the fact that a significant oxidative modification of Hb occurs with all used FCSN; 2) There is a parallelism between a significant decrease in CAT activity at relatively high FCSN (0.35-3.50 mM) and the onset of lipid LPO in erythrocytes. However, the development of LPO occurs in membrane structures and in limiting LPO, GP rather than CAT has a predominant role, since GP also utilizes, along with H_2O_2, organic hydroperoxides, which are participants of LPO (Floe, et al., 1988; Nagababu, et al., 2003). The more important role of GP in comparison with CAT in moderate oxidation of Hb is also indicated by many works (Nagababu, et al., 2003 and references therein); 3) 3) Under the conditions of our experiments (Fig. 4, 5) at FCSN of 0.35 and 3.50 mM, a significant increase in MetHb (tenfold) corresponds to a 50% increase in TBARS, which is probably due to the fact that MetHb itself possesses antioxidant properties and thus limits the development of LPO (Nagababu, et al., 2003). Indeed, according to the literature (Keszler, et al., 2008), at high FCSN (10-50 mM), deep oxidized forms of Hb are formed - ferryl hemoglobins: HbFe (+ IV), $^{\cdot}$HbFe (+ IV), which can lead to triggering the Fenton reaction with all the ensuing consequences of oxidative stress in erythrocytes (Nagababu, et al., 2003).

When using Na_2SeO_3 as an antioxidant, it must be borne in mind that selenite in excess, along with the AO effect, can enhance lipid peroxidation, reduce the content of NADPH, GSH, oxidize SH-groups of Hb and other proteins, and ultimately provoke hemolysis of erythrocytes (Seko, et al., 1989; Sopyani, et al., 2008). In this regard, it was necessary to choose its optimal concentration range of antioxidant effects on erythrocytes.

Previously, from our own experiments, it was found that sodium selenite, with a FC in the incubation medium of up to 10 μM (incubation period 0–30 min), does not have a prooxidative effect, does not oxidize erythrocyte GSH, and at FC within 1.0– 5.0 μM it has a certain AO effect: it slows down the accumulation of MetHb, slightly increases the activity of CAT and GP, with no effect on LPO (Huseynova, 2019).

As can be seen from Fig. 6,7,8 Na_2SeO_3 at this FC in all cases of nitrite action (i.e., at all used concentrations) had an AO effect to one degree or another, reducing the accumulation of MetHb, lowering the level of lipid peroxidation, somewhat reducing the effect of reducing the activity of CAT and GP.

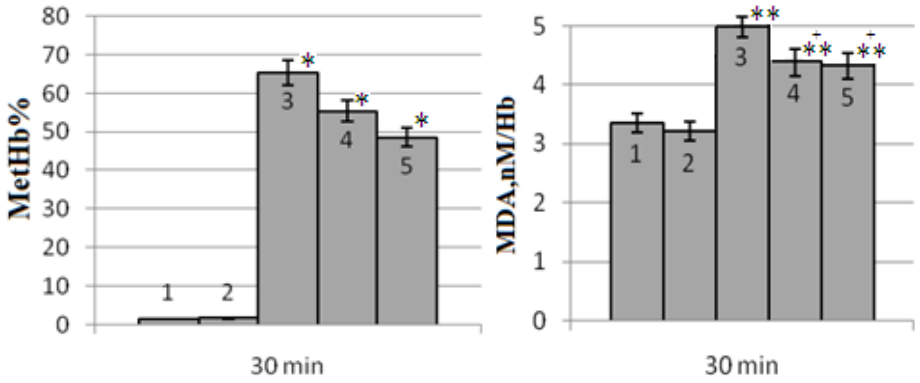

Fig. 6*. Accumulation of MetHb, TBARS, in human erythrocytes incubated (37 ° C) in a medium containing $NaNO_2$ (0.70 mM) and Na_2SeO_3 (5 μM) for 30min.*
Note: $p<0.05$ – *, $p<0.01$ – ** by comparison with control; $p<0.05$ – * by comparison with $NaNO_2$ sample. Values with similar indexes do not differ statistically according to Duncan test at $p<0.05$:
1 – control; 2- Na_2SeO_3; 3 – $NaNO_2$; 4 - Na_2SeO_3+ $NaNO_2$; 5 – $NaNO_2$+ Na_2SeO_3

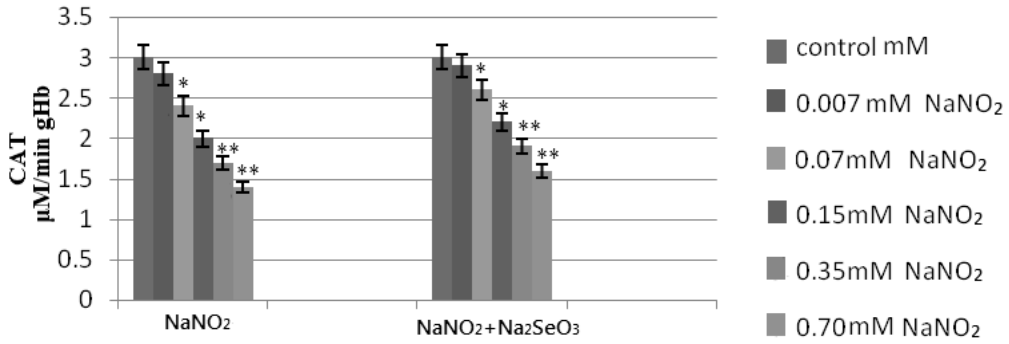

Fig. 7. *Changes in CAT activity in human erythrocytes in an incubation medium containing $NaNO_2$ и Na_2SeO_3.*
(10 mM SPB, 0.14 M NaCl, pH 7.4, t = 37 °C)
Note: $p<0.05$ – *, $p<0.01$ – ** by comparison with control.
Values with similar indexes do not differ statistically according to Duncan test at $p<0.05$

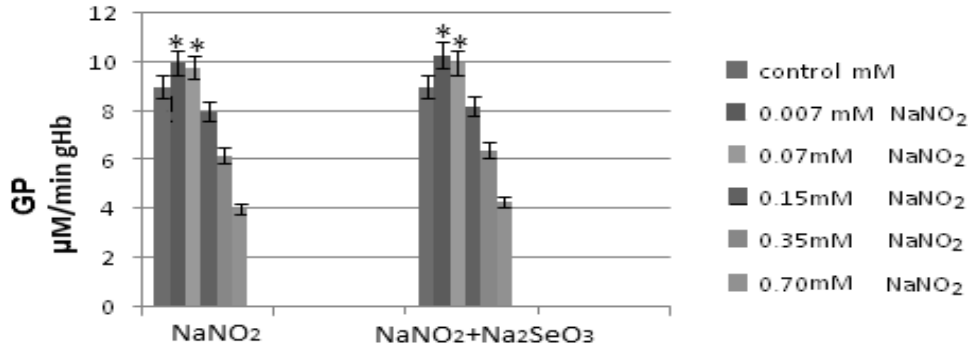

Fig. 8. *Changes in GP activity in human erythrocytes in an incubation medium containing $NaNO_2$ и Na_2SeO_3.*
*(*10 mM SPB, *0.14 M NaCl, pH 7.4, t* = 37 °C)
Note: $p<0.05$ –* by comparison with control.
Values with similar indexes do not differ statistically according to Duncan test at $p<0.05$

DISCUSSIONS

İt is possible to make some assumptions about the possible mechanism of the antioxidant action of Na_2SeO_3 during oxidative stress caused by sodium nitrite. The metabolism of Na_2SeO_3 and $NaNO_2$ has a number of common features: they are both small-sized and uncharged compounds easily penetrate through the membrane into erythrocytes, where they undergo rather complex rapid chemical transformations. This concerns the incorporation of seleni-

um into Hb and its subsequent release from Hb and other SH compounds (GSH, amino acid residues of proteins). The transfer of selenium from erythrocytes to plasma occurs through the membrane (anion exchanger) AE-1 by a complex interaction of membrane SH-proteins, containing transported selenium, with plasma albumin (Suzuki, et al., 1998; Haratake, et al., 2005; Haratake, et al., 2008; Hongoh, et al., 2012). NO *in vitro* / *in vivo* is formed by inherent in Hb nitrite reductase activity according to the scheme (Reutov, et al., 1983):

$Hb + NO_2^- \rightarrow MetHb + NO + H_2O$

On the other hand, NO, as the main metabolite of the NO_2^- ion *in vitro* and *in vivo*, also interacts with Hb in a complex way, entering into a direct bond with heme (nitrosylhemoglobin – HbNO) or is included in the SH group of α- or β-peptide chains (nitrosohemoglobin – SNHb) as nitrosonium NO^+ cations (Pawloski, et al., 2000; Hobbs, et al., 2002; Singel, Stamler, 2005; Böhmer, et al., 2016; Sun, et al., 2018). Of particular interest is the incorporation of NO into the β-chain of Hb at the β-93cys position, which is of significant physiological significance for the performance of the vasodilator function. This circumstance is also interesting because this position also includes selenium from sodium selenite, i.e., selenium, which replaces sulfur in the β-chain of cysteine. In other words, selenium, along with NO, is included in the same site of the β-chain of Hb (β-93cys) (Suzuki, et al., 1998; Haratake, et al., 2005). According to some data the frequency of selenium presence in Hb for healthy humans is 1: 225 (Beilstein, Whanger, 1983), and to other data Se: Hb 1: 300 (Huseynov, et al., 2012). Normally, the frequency of NO inclusion in Hb is NO: Hb 1: 1000 (but in extreme cases it can reach 1: 100), i.e. the number of inclusions in the β-chain is normally higher for selenium than for NO, despite the fact that incorporation of NO directly into the β-chain is even less (≈40%) (Pawloski, et al., 2000; McMahon, et al., 2002; Singel, Stamler, 2005; Osipov, et al., 2007; Vanin, 2016).

As mentioned above, NO is an active producer of $NaNO_2$ (Wink, et al., 2001; DeMartino, et al., 2019), and Se is a producer of Na_2SeO_3 (Seko, et al., 1989; Sopyani, et al., 2008), which, individually, can exhibit, depending on the concentration, both oxidative and prooxidant properties. At the same time, their joint presence can have various effects on oxidative processes in Hb and in erythrocytes already in other concentration limits. In particular, low or close to physiological concentrations in the incubation medium of both NO_2^- and $Se_2O_3^-$ impart antioxidant properties to Hb that has bound NO or Se, and thus has a positive effect on the AO status of erythrocytes.

However, in some cases, when dietary conditions change (nitrite poisoning, or deficiency of nitrogen consumption) of both nitrite and selenium (excess or deficiency in the diet), the NO: Hb ratio = 1: 1000 and Se: Hb = 1: 300 can significantly change, especially for nitric oxide due to the widespread use of nitrates / nitrites in agricultural and food industries. In this case, excess NO can stimulate oxidative stress as one of the manifestations of nitrite toxicity. Thus, the incorporation of selenium at the same site of (β-93cys) Hb can create competition for NO and thereby reduce the oxidative loading on Hb, in addition to the action of GP as a natural protector against oxidation (Floe, 1988).

In addition, recently, using transgenic mice, it was shown that the amino acid residue β-93-cys itself confers certain AO properties to erythrocytes upon stimulation of the formation of Hb ferryl forms by hydrogen peroxide (Vitturi, et al., 2012; Kassa, et al., 2017). Earlier, a similar idea was put forward by Mansouri (1979) in the study of sodium-dependent oxidation of Hb, that β-93-cys performs a protective AO function for Hb. As for selenium, we have previously shown that a 2-hour incubation of human erythrocytes with Na_2SeO_3 doubles the selenium content in the Hb fraction, enhancing the AO properties of both Hb and erythrocytes (LPO decrease) (Huseynov, et al., 2012). The authors explain this by the fact that selenium atoms are more electronegative than sulfur atoms, which it replaces (Viswanathan, et al., 2013).

The question of how such low NO inclusions in Hb can have significant physiological effects remains unclear (Sun, et al., 2019), despite the impressive progress in this area (recognition of NO as a gas molecule, etc.). To a certain extent, this also applies to selenium, the content of which in Hb is comparable to NO, but its role as an AO factor, apart from its role as a Se-depot, has not been disclosed. And the fact that a significant part of NO in Hb is located at the same site together with selenium suggests a close interaction of these two ligands, which are in comparable proportions, which makes it interesting to study this issue.

CONCLUSION

1. The relatively low degree of LPO intensification (40–70%) against the background of a signif-

icant oxidative modification of Hb (accumulation of MetHb), which occurs already from the lowest FCSN (0.007 mM), indicates that MetHb has antioxidant properties that prevent the accumulation of LPO products.

2. Oxidative stress induced by $NaNO_2$ has a dose-dependent effect on the activity of the main anti peroxide enzymes (CAT and GP). The activity of CAT decreases monotonously under an increase in FCSN. However, for GP there is a two-phase character change in the activity: low doses stimulate an increase in GP; high - a decrease.

3. The changes in GP activity negatively correlate with intensification in erythrocytes lipid peroxidation, that indicates the dominant role of GP selenenzyme and selenium as an endogenous AO in the regulation of erythrocyte LPO under nitrite exposure.

4. The use of Na_2SeO_3 in a FC of 5μM in an incubation medium containing erythrocytes as a possible antioxidant leads in all cases to a decrease in the oxidative effects of sodium nitrite: a decrease in the intensity of LPO of erythrocytes, the accumulation of MetHb, a decrease in the inhibition of GP and CAT activity.

REFERENCES / ЛИТЕРАТУРА

Alvarez B., Radi R. Peroxynitrite reactivity with amino acids and proteins. Amino Acids. 2003; 25(3–4): 295–311.

Ansari F.A., Ali S.N., Mahmood R. Sodium nitrite-induced oxidative stress causes membrane damage, protein oxidation, lipid peroxidation and alters major metabolic pathways in human erythrocytes. Toxicol in Vitro. 2015; 29(7): 1878–1886.

Arashiki N., Kimata N., Manno S., Mohandas N., Takakuwa Y. Membrane peroxidation and methemoglobin formation are both necessary for band 3 clustering: mechanistic insights into human erythrocyte senescence. Biochemistry. 2013; 52(34): 5760–5769.

Asahi M., Fujii J., Suzuki K., Seo H.G., Kuzuya T., Hori M., Tada M., Fujii S., Taniguchi N. Inactivation of glutathione peroxidase by nitric oxide. Implication for cytotoxicity. J. Biol. Chem. 1995; 270(36): 21035–21039.

Beilstein M.A., Whanger P.D. Distribution of selenium and glutathione peroxidase in blood fraction from humans, rhesus and a querel monkeys, rats and sheep. J. Nutr. 1983; 113(11): 2138–2146.

Böhmer A., Pich A., Schmidt M., Haghikia A., Tsikas D. Evidence by chromatography and mass spectrometry that inorganic nitrite induces S-glutathionylation of hemoglobin in human red blood cells. J. Chromatogr. B. 2016; 1019: 72–82.

Cho C S., Lee S., Lee G.T.,Woo H. A., Choi E.J., Rhee S. Irreversible inactivation of glutathione peroxidase 1 and reversible inactivation of peroxiredoxin II by H_2O_2 in red blood cells. Antioxid Redox Signal. 2010; 12(11): 1235–1246.

Chow C.K., Hong C.B. Dietary vitamin E and selenium and toxicity of nitrite and nitrate. Toxicology. 2002; 180(2): 195–207.

DeMartino A.W., Kim-Shapiro D.B., Patel R.P., Gladwin M.T. Nitrite and nitrate chemical biology and signalling. Br J Pharmacol. 2019; 176(2): 228-245.

Denicola A., Souza J.M., Radi R. Diffusion of peroxynitrite across erythrocyte membranes. Proc Natl Acad Sci USA. 1998; 95(7): 3566–3571.

Doyle M.P., Pickering R.A., DeWeert T.M., Hoekstra J.W., Pater D. Kinetics and mechanism of the oxidation of human deoxyhemoglobin by nitrites. J Biol Chem. 1981; 256(23): 12393–12398.

Ellman G.L. Tissue sulfhydryl groups. Arch of Biochem Biophys. 1959; 82: 70–77.

Flohe L. Glutathione Peroxidase. Oxygen Radic in Biol Med. 1988; 49: 663–668.

Gasiewicz T.A., Smith J.C. The metabolism of selenite by intact rat erythrocytes in vitro. Chem. Biol. Interact. 1978; 21(2–3): 299–313.

Haratake M, Fujimoto K, Ono M, Nakayama M. Selenium binding to human hemoglobin via selenotrisulfide. Biochim Biophys Acta. 2005; 1723(1–3): 215–220.

Haratake M., Fujimoto K., Hirakawa R., Ono M, Nakayama M. Hemoglobin-mediated selenium export from red blood cells. J. Biol. Inor. Chem. 2008; 13(3): 471–479.

Hobbs A.J., Gladwin M.T., Patel R.P., Williams D.L., Butler A. R. Haemoglobin: NO transporter, NO inactivator or none of the above. Trends in Pharmacol. Sci. 2002; 23(9): 406–411.

Hogg N. Pro-oxidantant antioxidant effects of nitric oxide. Analysis of Free Radic in Biol. Syst. 1995; 37–49.

Hongoh M., Haratake M., Fuchigami T., Nakayama N. A thiol-mediated active membrane transport of selenium by erythroid anion exchanger 1 protein. 2012; 41(24): 7340–7349.

Huseynov T.M, Yakhyaeva F.R, Gulieva R.T. Effect of selenium on the resistance of hemoglobin to photo-oxidative processes. Ukr. Biochem. J. 2012; 84(2): 53–60.

Huseynov T.M., Dadashov M.Z., Gulieva R.T., Huseynova S.Ya., Jafarov A.I., Yakhyaeva F.R. Effect of moderate doses of sodium nitrite on oxidative processes in erythrocytes. Selenium participates in them. Abstracts of the International Scientific Conference dedicated to the 90th anniversary of the National Academy of Sciences of Belarus and the 45th anniversary of the Institute of Biophysics and Cell Engineering, Belarus, Minsk. 2018; p. 150.

Huseynova S.Ya. Oxidative metabolism of sodium selenite in isolated human erythrocytes in vitro. Biomedicine (Azerbaijan). 2019; 17(3): 18–23.

Huseynova S.Ya. Membrane-bound hemoglobin as an indicator of nitric toxicity of erythrocytes. Azerbaijan Chem. J. 2020; 2: 67–73.

Huseynova S.Ya., Huseynov T.M., Dadashov M.Z. Oxidative modification of erythrocytes with moderate doses of sodium nitrite in in vitro experiments. Med biophys biochem chem. 2018; 3(1): 189–195. (in Russ.).

Johnson R.M, Goyette G.Jr., Ravindranath Y., Ho Y.S. Hemoglobin autoxidation and regulation of endogenous H_2O_2 levels in erythrocytes. Free Radic Biol. Med. 2005; 39(11): 1407–1417.

Kassa T., Strader M.B., Nakagawa A., Zapol W.M., Alayash A.I. Targeting βCys93 in hemoglobin S with an antisickling agent possessing dual allosteric and antioxidant effects. Metallomics. 2017; 9(9): 1260–1270.

Keszler A., Piknava B., Sehechter A.N., Hogg N. The reaction between nitrite and oxyhemoglobin a mechanistic study. J. Biol. Chem. 2008; 283(15): 9615–9622.

Kondo H., Takahashi M., Niki E. Peroxynitrite-induced hemolysis of human erythrocytes and its inhibition by antioxidants. FEBS Lett. 1997; 413(2): 236–238.

Korolyuk M.A. A method of measurement of catalase activity. Lab. Delo. 1988; 64(30): 16–17.

Kosaka H., Tyuma I. Mechanism of autocatalytic oxidation of oxyhemoglobin by nitrite. Environ Health Perspect. 1987; 73: 147–151.

Krych-Madej J., Gebicka L. Interactions of nitrite with catalase: Enzyme activity and reaction kinetics studies. J. Inorg. Biochem. 2017; 171: 10–17.

Lakin G.F. Biometrics. M. Higher school. 1990; 352 p. (in Russ.).

Lapinski R., Siergiejuk M., Worowska A., Gacko M. Oxidants and antioxidants of erythrocytes. Prog. Health Sci. 2014; 4(1): 211–219.

Mansouri A. Oxidation of human hemoglobin by sodium nitrite-effect of β-93 thiol goups. Biochem and Biophys res.commun. 1979; 89(9): 441–447.

Mas A., Jiang J.Y., Sarkar B. Selenite metabolism in rat and human blood. Biol. Trace Elem. Res. 1988; 15: 97–110.

May J.M., Qu Z.C., Xia L., Charles E.C. Nitrite uptake and metabolism and oxidant stress in human erythrocytes. Am. J. Physiol. Cell. Physiol. 2000; 279(6): 1946–1954.

McMahon T.J., Moon R.E., Luschinger B.P., Caraway M.S., Stone A.E., Stolp B.W., Cow A.J., Pawlowski J.R., Stamler J.S. Nitric oxide in the human respiratory cycle. Nature Med. 2002; 8: 711–717.

Mengel C.F., Kann H.E. Effect of in vivo hyperoxid of erythrocytes, III in vivo peroxidation of erythrocytes lipid. J. Clin. Invest. 1966; 45(7): 1150–1158.

Moin V.M. Simple and specific method for determining the activity of glutathione peroxidase in erythrocytes. Lab. Delo. 1986, (12): 724–727 (in Russ.).

Nagababu E., Chrest F.J., Rifkind J.M. Hydrogen peroxideinduced heme degradation in red blood cells: the protective roles of catalase and glutathione peroxidase. Biochim. Biophys. Acta. 2003; 1620(1–3): 211–217.

Osipov A.N., Borisenko G.G., Vladimirov U.A. Biologicheskaia rol nitrozilnykhkompleksov gemoproteinov. Uspekhi biol khimii. 2007; 47: 259–292 (in Russ.).

Padmaja S., Squadrito G.L., Pryor W.A. Inactivation of glutathione peroxidase by peroxynitrite. Arch. Biochem. Biophys. 1998; 349(1): 1–6.

Pawloski J.R., Hess D.T., Stamler J.S. Export by red blood cells of nitric oxide bioactivity. Nature. 2001; 409(6820): 622–626.

Reutov V.P., Azhipa Ya.I., Koyushin L.P. News of the USSR Academy of Science biological series. 1983; 3: 408–418 (in Russ.).

Rocha S., Gomes D., Lima M., Bronze da Rocha E., Santos S.A. Peroxiredoxin 2, glutathione peroxidase, and catalase in the cytosol and membrane of erythrocytes under H_2O_2-induced oxidative stress. Free Radic. Res. 2015; 49(8): 990–1003.

Schaer D.J. Hemoglobin can attenuate hydrogen peroxide-induced oxidative stress by acting as an antioxidative peroxidase. Antioxid Redox Signal. 2010; 12(2): 185–198.

Seko Y., Saito Y., Kitahara J. Imura N. Active oxygen generation by the reaction of selenite with reduced glutathione in vitro. In Selenium in Biology and Medicine. WendelA. (Ed.). Springer Berlin Heidelberg. 1989; 70–73.

Shperlink I.A., Ryazantseva N.V., Kuprina N.P., Fillipova O.N., Rogova O.A., Akimova V.V., Bas V.V. About the composition of membrane-bound hemoglobin in methemoglobinoemias. Modern science-intensive technol. 2004; 6: 104–105.

Sies H., Klotz L.O., Sharov V.S., Assmann A., Briviba K. Protection against peroxynitrite by selenoproteins. Z. Naturforsch. C. J. Biosci. 1998; 53(3–4): 228–232.

Sies H., Arteel G.E. Interaction of peroxynitrite with selenoproteins and glutathione peroxidase mimics. Free Radic. Biol. Med. 2000; 28(10): 1451─1455.

Singel D.J., Stamler J.S. Chemical physiology of blood flow regulation by red blood cells: the role of nitric oxide and S-nitrosohemoglobin. Annu. Rev. Physiol. 2005; 67: 99–145.

Smeyne M., Smeyne R.J. Glutathione metabolism and Parkinson`s disease. Free Radic. Biol. Med. 2013; 62: 13–25.

Sopyani M., Foller M., Gulbins E., Lang F. Suicidal death of erythrocytes due to selenium compounds. Cell Physiol. Biochem. 2008; 22: 387–394.

Spagnuolo C., Rinelli P., Coletta M., Chiancone E., Ascoli F. Oxidation of human oxyhemoglobin with nitrite: a reexamination. Biochim. Biophys. Acta. 1987; 911(1): 59–65.

Stepuro I.I., Tchaikovskaya N.A., Solodunov A.A., Artsukevich A.N. Generation of NO during the oxidation of hemoglobin ferroforms by nitrite. Biochemistry. 1997; 62(09): 1122–1129 (in Russ.).

Storkey C., Pattison D.I., Ignasiak M.T., Schiesser C.H., Davies M.J. Kinetics of reaction of peroxynitrite with selenium- and sulfur-containing compounds: Absolute rate constants and assessment of biological significance. Free Radic. Biol. Med. 2015; 89: 1049–1056.

Sun C., Yang J., Yang j., Kleschyov A.L., Lunbderg J.O., Zhuge Z., Carlstrom M., Pernov J., Wajih N., Isbell T.S., Oh J.,Cabrales P., Tsai A.G., Townes T., Patel R.P. Hemoglobin β-93 cysteine is not required for export of nitric oxide bioactivity from the red blood cell. Circulation. 2019; 139(23): 2654–2663.

Suzuki K.T., Shiobara Y., Itoh M., Ohmichi M. Selective uptake of selenite by red blood cells. Analyst. 1998; 123(1): 63–67.

Szebeni J., Winterbourn C.C., Carrell R.W. Oxidative interactions between haemoglobin and membrane lipid. A liposome-model. Biochem. J. 1984; 220(3): 685–692.

Titov V. Yu., Petrenko Yu. M. Proposed mechanism of development of nitrite-induced methemoglobinemia. Biochemistry. 2005; 70(4): 575–587 (in Russ.).

Vanin A.F. Dinitrosyl iron complexes with thiol- containing ligands as a «working form» of endogenous nitric oxide, nitric oxide. Biol. Chem. 2016; 54: 15–29.

Viswanathan U.M., Burkholz T., Jacob C. Electrochemistry at the edge of reason: Chalcogen-based redox systems in biochemistry and drug design. Z. Phys. Chem. 2013; 227: 691–706.

Vitturi D.A., Sun C.W., Harper V.M., Williams B., Medellin N., Chacko B.K., Peng N., Dai Y., Wyss J.M.,Townes T., Patel R.P. Antioxidant functions for the hemoglobin β93 cysteine residue in erythrocytes and in the vascular compartment in vivo. Free Radic. Biol. Med. 2013; 55: 119–129.

Welbourn E.M., Wilson M.T, Yusof A., Metodiev M.V., Chris E., Cooper C.E. The mechanism of formation, structure and physiological relevance of covalent hemoglobin attachment to the erythrocyte membrane. Free Radic. Biol. Med. 2017; 103: 95–106.

Widmer C.C., Pereira C.P., Gehrig P., Vallelian F., Schoedon G., Buehler P.W., Wink D. A., Miranda K. M., Espey M. G., Pluta R. M., Hewett S.J., Colton C., Vitek M., Feelisch M., Grisham M.B. Mechanisms of the antioxidant effects of nitric oxide. Antioxid. Redox Signal. 2001; 3(2): 203–213.

ОСОБЕННОСТИ ОКИСЛИТЕЛЬНОГО СТРЕССА, ИНДУЦИРОВАННОГО УМЕРЕННЫМИ ДОЗАМИ НИТРИТА НАТРИЯ В ИЗОЛИРОВАННЫХ ЭРИТРОЦИТАХ В ПРИСУТСТВИИ СЕЛЕНИТА НАТРИЯ

Т.М. Гусейнов, С.Я. Гусейнова, Р.Т. Гулиева, М.З. Дадашев, С.М. Рахманова, Ф.Р. Яхъяева, С.А. Джафарова

Институт Биофизики, НАН Азербайджана
ул. З. Халилова, 117, AZ1141, г. Баку, Азербайджан

РЕЗЮМЕ. Рассмотрено влияние умеренных доз нитрита натрия ($NaNO_2$) (конечная концентрация – 0,007, 0,070, 0,15, 0,35, 0,70 и 3,50 мМ) на окислительные процессы в эритроцитах, содержания глутатиона, изменения активности каталазы, глутатионпероксидазы (ГП) и интенсивности перекисного окисления липидов (ПОЛ) и влияние селенита натрия (Na_2SeO_3) (5 μM) как возможного антиоксидантного (АО)-фактора, влияющего на развитие окислительных процессов *in vitro* (срок инкубирования 30 мин, *t*=37°C). При нитритиндуцированном окислении имеет место истощение внутриэритроцитарного глутатиона (до 12% от исходного уровня), которое находится в прямой зависимости от конечной концентрации $NaNO_2$. Активность каталазы при всех конечных концентрациях $NaNO_2$ уменьшается, доходя до минимума уже в середине инкубационного срока. При нитритиндуцированном окислении имеют место разнонаправленные изменения в активности ГП, которая при низких и умеренных концентрациях $NaNO_2$ несколько растет в начальный срок инкубирования и уменьшается середине срока. Снижение активности каталазы и ГП в эритроцитах, вызванное нитритами, сопровождается интенсивным накоплением метгемоглобина (MetHb), и ростом ПОЛ при высоких концентрациях нитрита натрия (0,70–3,50 мМ) к концу инкубационного периода. Отрицательная зависимость между уменьшением активности ГП и ростом интенсивности ПОЛ позволяет высказать предположение о предоминантной роли ГП в лимитировании окислительного стресса в эритроцитах. Полученные результаты свидетельствуют об активном участии указанных антиокислительных энзимов эритроцитов в окислительном метаболизме при нитритном воздействии. Селенит натрия в определенной мере может выступать в роли регулятора нитритиндуцированного окислительного стресса.

КЛЮЧЕВЫЕ СЛОВА: эритроциты, гемоглобин, нитрит натрия, селенит натрия, глутатионпероксидаза, каталаза, перекисное окисление липидов.