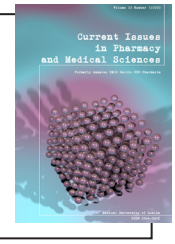
DOI: 10.2478/cipms-2020-0023 Curr. Issues Pharm. Med. Sci., Vol. 33, No. 3, Pages 125-131

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Effects of histamine and sodium hypochlorite on prooxidand state in the rats erytrocytes

Nataliya Harasym*, Svitlana Mandzynets, Dmytro Sanahursky

Ivan Franko National University of Lviv, Ukraine

ARTICLE INFO

Received 18 December 2019
Accepted 20 April 2020

Keywords:
histamine,
sodium hypochlorite;
erythrocytes,
lipid peroxidation – LP,
sodium hypochlorite – SH,
thiobarbituric acid – TBA.

ABSTRACT

We studied the simultaneous influence of histamine and sodium hypochlorite (SH) on lipid peroxidation processes, as well as the level of structural changes in membranes (via the content of sialic acid) in rat erythrocytes. We established that histamine affects lipid peroxidation processes with the formation of lipid hydroperoxides, damages proteins and reduces the content of sialic acids, which leads to changes in the surface charge of red blood cells. However, the simultaneous action of histamine and low SH concentration has a positive effect in that it corrects the pro-oxidant state of erythrocytes. Hence, the content of lipid hydroperoxides, TBA-active products, carbonyl groups of proteins and sialic acids were mainly reduced after the simultaneous action of histamine and SH at all studied concentrations during the rehabilitation period.

INTRODUCTION

Oxidative stress manifests itself in a stable shift of balance of prooxidant-antioxidant processes and causes cytotoxic disorders. It leads to damage of the most important cell biopolymers: nucleic acids, proteins and lipids. High concentrations of active forms of oxygen and lipid hydroperoxides also damage DNA, affect the cell division and can activate apoptosis [1].

The receptors net on the surface of the cell membrane is one of the most important systems that provide cell utility. The number of receptors in different conditions of cell functioning may change, but their structure remains relatively stable. They are represented predominantly by glycoproteins, the carbohydrate part of which in the form of oligosaccharide chains forms supramembrane complexes that play a key role in the mechanisms of migration, adhesion, proliferation and apoptosis of cells. However, in various pathological conditions, changes in the quantitative and qualitative characteristics of surface glycoconjugates of cells [2], in particular, sialic acids, occurs.

The pathological processes in tissue membranes even in the early stages of their development affect the state of the erythrocyte membrane, therefore this forms an informative object for the diagnosis of pathology [3]. Erythrocytes have high sensitivity to the action of chemicals, which allows determining certain specificity of their action, as well as establishing the degree of severity of morpho-functional changes of red blood cells from the intensity of harmful effects [4]. The absence of organelles makes erythrocytes a good model for studying the effects of harmful factors directly on the cell membrane. Erythrocyte membranes exhibit high sensitivity to activation of LP, which rapidly and dramatically changes their properties. Moreover, accumulation of hydrophilic groups in the hydrophobic membrane layer promotes the formation of pores in the membrane and disturbs membrane transport, including selectivity. The damage of membrane-bound enzymes due to inhibition of their activity and changes in the properties of membrane carrier proteins are additional mechanisms of the pathological effect of LP. Reducing the strength of erythrocytes and increasing hemolysis of cells is one of the links in the inclusion of the erythrocyte in the "chain" of changes that are important in the development of thrombotic formations [3].

Today, the action of histamine on erythrocytes (that have both regulatory and pathological effects on various organs and tissues of the body) remains unexplored. While it is known that histamine effects an organism via the histamine receptors (H1, H2, H3, H4), not much is known about such receptors on the erythrocyte membrane. In medicine, although SH is used to detoxicify the body after poisoning, as this substance is a powerful oxidizer, the issue of the combined effect of histamine and sodium hypochlorite on erythrocytes, in particular, in the pro-oxidant state remains unexplored. Thus, the purpose of our work was to investigate the effect of the simultaneous action of histamine and SH on lipoperoxidation processes, the oxidative modification

* **Corresponding author**
e-mail: garasymnataly@gmail.com

of proteins, and the content of sialic acids as indicators of the degree of damage to red blood cells in rats.

MATERIALS AND METHODS

In our experiments we used 21-day-old non-pedigree male white rats (*Rattus norvegicus*), initially weighing 180-220 g. The animals were divided into groups of 20. The 1st group included intact animals. Animals in groups 2 and 3 received subcutaneous injections of histamine solutions in dosage 1 and 8 μg/kg (made up of 0.01% histamine dihydrochloride) for 14 days, respectively. Such dosage is known to induce pathological changes under experimental conditions [5]. Animals in the 4th and 5th group during this period received a solution of SH (5 and 20 mg/l, respectively) in their drinking water. In addition, four more groups were formed, and the animals were simultaneously administered histamine and SH. At 1, 7, 14 and 21 days (rehabilitation) days, 5 animals from each group were decapitated under a light etheric anesthesia, following the requirements of the European Convention for the protection of vertebrate animals used for experimental and scientific purposes (Strasbourg, 1986) and according to "General principles of work on animals", approved by the 1st National Congress on Bioethics (Kyiv, Ukraine, 2001). At this time, the rats weighed 180 grams, and had received 17 milliliters of water per day. This dose corresponds to the recommended daily dose of water for rats. That is, the animals had not thirsted.

During the experiment, each rat of the 4th group received 0.085 mg of sodium hypochlorite (0.066 mg NaCl) in 17 ml of water, which corresponds to a concentration of 5 mg/l, while each rat in the 5th group received 0.34 mg of sodium hypochlorite (0.267 mg NaCl) in 17 ml of water, corresponding to a concentration 20 mg/l. The 1st day of the experiment reflected the rapid changes due to the short term action of histamine and SH. In contrast, the 7th and 14th days of the experiment reflected changes that occurred due to long-term exposure of these substances. The experiments were repeated five times.

After sampling, the erythrocytes were removed from heparinized blood and hemolysis was performed with distilled water in a ratio of 1:5. The protein content of each sample was measured by the Lowry method. The intensity of lipid peroxidation processes was estimated by the content of primary and secondary products of LP – hydroperoxides and TBA-active products (products that interact with thiobarbituric acid) [6,7]; the state of the surface receptor apparatus in the erythrocytic membrane was assessed by the content of sialic acids via the Hess method [8]. Oxidative modification of proteins was estimated by the content of carbonyl groups of neutral and basic nature [9]. As a result of the oxidation of proteins, depending on the predominance of neutral amino acids in their molecules (valine, leucine, isoleucine, etc.) or the basic (lysine, arginine, etc.), the aldehyde- or ketone derivatives are neutral or basic. For determination of neutral derivatives of amino acids we used light wavelength 370 nm and for basic amino acids – 430 nm.

The statistical analyses were performed using „Excel-2010" for Windows (Descriptive statistics, Two-way ANOVA). Statistical significance was determined using Student t-test. $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$ was considered to indicate a statistically significant difference.

RESULTS AND DISCUSSION

The content of lipid hydroperoxides increased by the action of histamine in the dose of 1 and 8 μg/kg in rat erythrocytes at the 1st day of the experiment when the amount of TBA-active products decreased only at a lower concentration. At the 7th day, the intensity of lipid peroxidation processes returned to the control limits, but at the next investigated time point, the lipid hydroperoxide content increased (by 127%) by the action of histamine at low dose and decreased (by 19%) at the high dose (Fig. 1a, b). After the rehabilitation period, the intensity of accumulation of secondary LP products increased an average 10 times by the action the biogenic amine in the two studied dosages when the content of primary lipid peroxidation products showed the same trend to increasing at a high dose. Consequently, histamine caused changes in the accumulation of hydroperoxides of lipids (primary products of LP), that was of a dynamic nature.

At the 7th day of the experiment, the action of histamine leveled off. These changes indicated that the histamine caused oxidative stress in the erythrocytes. Polyunsaturated fatty acids, such as arachidonic and linoleic acids, important components of cell membranes and lipoproteins, are the main targets for LP, during which the primary products, lipid hydroperoxides are formed, and then were oxidatively degraded to various aldehyde secondary products [10]. The presence of TBA-active products at the control level (by the content of malonic dialdehyde), or even lower, indicates that the chain of the LP process was interrupted at the level of the primary products by an antioxidant protection system. Such a system is well developed in Erythrocyte cells. Significant increasing of the content of TBA-active products after the rehabilitation period likely occured due to a violation of the erythrocyte prooxidant-antioxidant system. The accumulation of LP products can lead to structural and functional changes in the physical properties of cells, even before their death [11]. In the scientific literature, there is no information on the presence of receptors of histamine on erythrocyte membranes, therefore, the action of histamine is likely to be mediated due to functional changes of other formed blood elements, in particular, neutrophils, eosinophils, thrombocytes. Of note, histamine, depending on the concentration, causes an increase or decrease in the release active forms of oxygen by neutrophils [12].

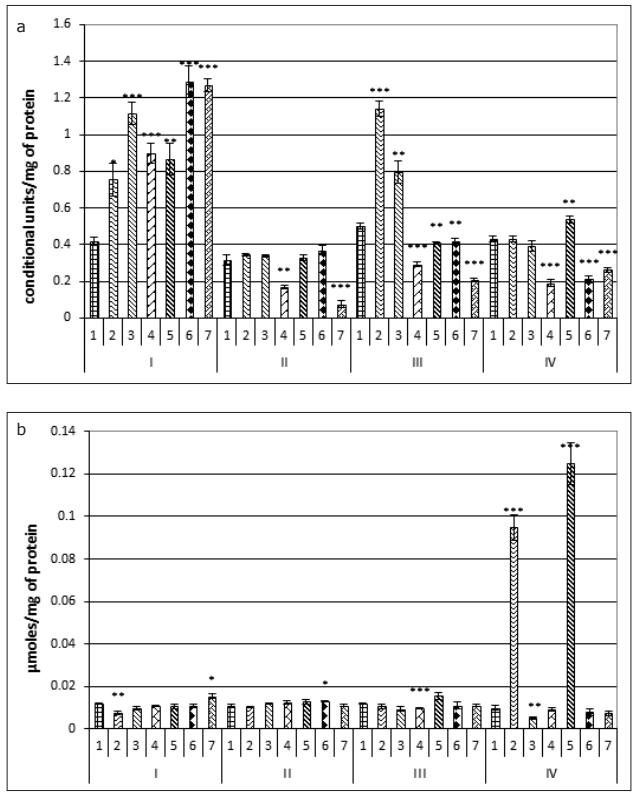
The investigation of the state of oxidative modification of the proteins showed that at the beginning of the experiment, histamine reduced the content of carbonyl groups of the main and neutral nature by approximately 50%. Further subcutaneous administration of the drug caused the intensification of these processes, this indicated free radical damage to them (Fig. 2a, b). Erythrocytes contain 95% (of dry weight) of hemoglobin and only 5% of other compounds (lipids, proteins) [13]. Therefore, it is assumed that histamine damages, first of all, the hemoglobin molecules. As known, histamine changes the rheological properties of blood as

a result of an increase in the permeability of the walls of vessels for water. At the same time, erythrocytes carry out both transport, protective and regulatory functions. The latter is ensured by the presence of hemoglobin – which is responsible for regulation of blood pH, plasma ion composition and water metabolism. Penetrating into the arterial department of the capillary, erythrocytes give water and dissolved O_2, while decreasing in volume, and passing into the venous capillary section, they bind water, CO_2, metabolic products coming from the tissues, and increase in volume. The relative stability of the plasma composition relative to salts and proteins is maintained by erythrocytes [13]. Hence, in the erythrocytes, hemoglobin plays a key role and it is damaged by exogenous influences first and foremost, as the amino acids of hemoglobin are capable of being modified by oxidation, nitration, phosphorylation, etc [14]. Indeed, on the 21st day of the experiment (administration of histamine ceased) the content of carbonyl groups of proteins was significantly reduced by about 70%.

We established that the histamine at a dose of 1 μg/kg led to a decrease of sialic acid by 68, 42, 53% at 1, 7, 14 days, respectively. The decreasing of the content of these products was detected after the action by changing the content of the biogenic amine on the 7th and 14th days, by 58 and 71 %, respectively (Fig. 2c). According to literature, the charge of the surface of the erythrocyte plasmatic membrane depends on the content of the carbohydrate components of membrane glycoconjugates of sialic acids in its structure. These determine more than 60% of membrane total negative charge. Reduction of sialic acid levels can be an absolute (for example, alcohol dramatically reduces the number of sialic acids, viruses have neuraminidase activity) and relative (shielding sialic acids with high molecular weight proteins adsorbed on the surface of erythrocytes from the plasma). The lost sialic acid residues on carbohydrate components lead to the capture of erythrocytes by galactose-specific lectins in the liver and their removal from the blood circulatory system. Damaged erythrocytes are also recognized by macrophage receptors, for which the ligand contains sialo-oligosaccharide chains of clustered or aggregated glycophorin. Changing the number of sialic acids on the surface of the cell leads to an increase in their adhesive properties [2]. Therefore, the decrease in the content of sialic acids indicates damage to erythrocytes by the action of histamine. After the termination of the introduction of this biogenic amine, the content of these investigated acids returned to the control values.

We can conclude that histamine affects the processes of lipid peroxidation with the formation of hydroperoxides, damagings proteins and reducing the sialic acids content, which leads to a change in the surface charge of red blood cells.

The co-administration of histamine and SH into rats in the first day increased the content of lipids hydroperoxides by a factor of three, but the content of TBA-active products remained at the control level, except for the simultaneous action of these compounds at maximum concentrations (content increased by 30%). A combination of histamine at the dose of 8 μg/kg and SH at the concentration of 5 mg/l resulted in a slight increase in the amount of secondary lipid peroxidation products at 7th day. However, SH being



1 – control; 2 and 5 – the action of histamine in the dose of 1 and 8 μg/kg respectively; 3 and 6 – simultaneous effect of sodium hypochlorite (5 mg/l) and histamine (1 and 8 μg/kg, respectively); 4 and 7 – simultaneous effect of sodium hypochlorite (20 mg/l) and histamine (1 and 8 μg/kg, respectively); * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Figure 1. The content of hydroperoxides (a) and TBA-active products (b) in rat erythrocyte haemolysates on the 1st (I), 7th (II), 14th (III) and 21st (IV) days of the experiment under the action of histamine and the combined effects of histamine and sodium hypochlorite

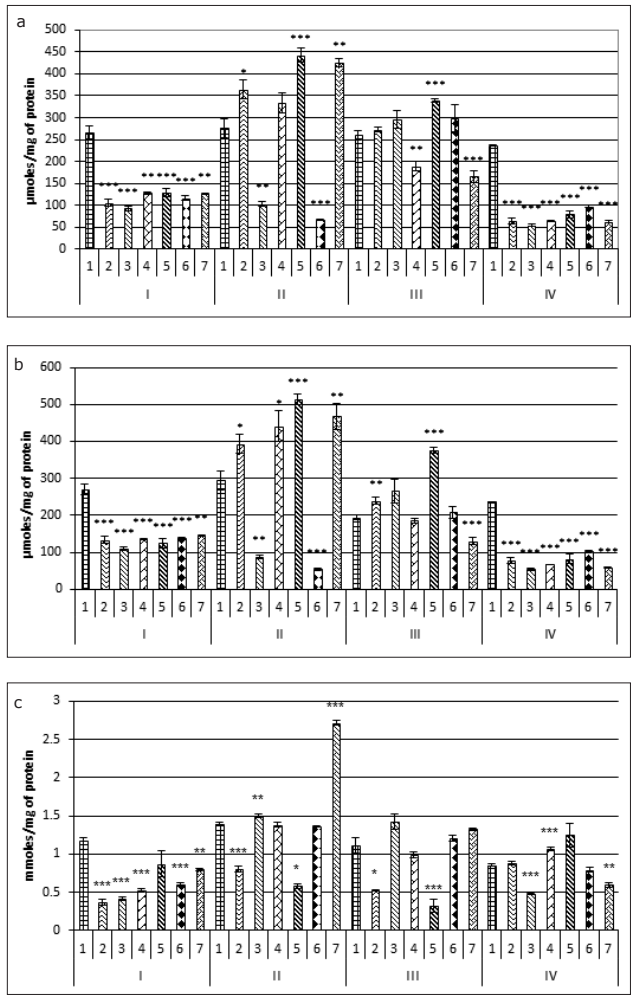
in the drinking water of the rats against the background of action of histamine reduced the content of lipids hydroperoxides. It should be noted that the hypodermic administration of histamine alone on this day of the experiment did not influence the intensity of the LP processes (therein remaining at the same level as the control). The decrease in the content of lipids hydroperoxides was fixed both on the 14th and 21st (rehabilitation) days of the experiment with the simultaneous effect of histamine and SH co-administration at low concentrations over a two-week period. At that time, the content of primary lipid peroxidation products increased by 59% (Fig. 1a, b). Thus, SH in the background of histamine action initially intensifies, and then slows down the processes of lipid peroxidation, as confirmed by the content of the primary products of the LP. It is known that the formation of lipid hydroperoxides in the membranes occurs in the presence of biologically important oxidizing agents such as the metal ions, peroxynitrite, HOCl and cytochrome c [15]. Moreover, it is recognized that the interaction of SH with water may produce the formation of HOCl. Further decreasing in the intensity of the LP processes could be due to the reaction of SH with histamine and the leveling of the effect of the previous dose. This could come about because SH improves the rheological properties of blood, binds with products of lipid peroxidation processes and than converts them into hydrophilic compounds that could be easily washed out of the membranes, causing their renovation.

The predominant reduction in the number of carbonyl groups of proteins was detected under the simultaneous action of histamine and SH during the experiment, in addition to the effect of biogenic amine and oxidant at a dose of 20 mg/l on the 7th day of the experiment, namely, the content increased by 50% (Fig. 2a, b). The deceleration of the intensity of the oxidative modification of proteins occurs by activation of the proteasomal complex, which is responsible for the proteolytic degradation of damaged proteins, underlying the adaptation processes [16].

We found a decrease in the content of sialic acids in rat erythrocytes on the 1st day of the experiment after the combined action of histamine (1 and 8 μg/kg) and SH (5 and 20 mg/l), by 32-64 %. However, already on the 7th day, the effects of these substances in low and high concentrations increased the content of sialic acids (by 8 and 94%, respectively). Subsequent administration of the tested substances to the rats returned the amount of these acids to the control values (Fig. 2c). Consequently, the combined action of histamine and SH positively affects the glycocalyx of membranes of red blood cells. As indicated in the literature, sialic acids play an important biological role. First of all, they determine the basic biophysical effects of the cell. Sialic acids cause repulsion between cells, taking into account their negative charge and the ubiquitous expression on the surface of cells of vertebrates, and this affects the biophysical properties of cellular interactions. Available in the extreme outer position of cell surface glycans, sialic acids are ideally positioned to facilitate various cases of immune recognition mediated by the sensitive receptors of the host cells or by the pathogenic factors. These acids act as "self-associated molecular pathways" that react to "self-regulatory recognition receptors". Accordingly, in some blood groups, there are antigenic variants of sialylated glycans that promote allogenic differences between populations of the same species (for example, MN blood groups in humans and A/B blood groups in cats). The sial's "cloak" also serves as biological masking to reach potentially antigenic glycoconjugates (ie, crypto antigens) or to block interactions between certain host receptors and the underlying glycans. For example, the removing of sialic acids by endogenous sialidases may uncover a terminal galactose that will promote the mechanism of clearance of the galectin through its cross-linking of surface molecules or by binding to asialoglycoprotein liver receptors. Moreover, the adding of finite residues of sialic acids to glycoproteins effectively increases their half-life or changes their mode of action [17].

In our study, after the rehabilitation period, the indicators reflecting the detrimental effect of the biogenic factors were mostly decreased by the simultaneous action of histamine and SH in all possible studied concentrations. This indicates that under such conditions, rat erythrocytes do not restore structural-functional properties – which is a negative phenomenon.

The addition of SH at low and high concentration to the drinking water resulted in an increase in the content of lipid hydroperoxides by 67 and 96%, respectively, in red blood cells, in the early stages of the experiment, while at the 7th day of the experiment, the TBA-active products increased by exposure of this substance at a concentration of 5 mg/l



1 – control; 2 and 5 – the action of histamine in the dose of 1 and 8 μg/kg, respectively; 3 and 6 – simultaneous effect of sodium hypochlorite (5 mg/l) and histamine (1 and 8 μg/kg, respectively); 4 and 7 – simultaneously effect of sodium hypochlorite (20 mg/l) and histamine (1 and 8 μg/kg, respectively); * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Figure 2. The content of the carbonyl groups of proteins of neutral (a) and main character (b), sialic acids (c) in hemolysates of rat erythrocytes on the 1st (I), 7th (II), 14th (III) and 21st (IV) day of the experiment under the action of histamine and the simultaneous effects of histamine and sodium hypochlorite

by 58% (Fig. 3). Long-term exposure of the SH in the animals caused a decrease in the intensity of the LP processes, which persisted even after the rehabilitation period (a 24% decrease in the content of lipid hydroperoxides). It should be noted that the tendency to increase initially the accumulation of products of lipid peroxidation with their subsequent decreasing is a similar effect to that found for the simultaneous administration of histamine and SH against rat red blood cells. This demonstrates the dominant influence of the SH on the processes of LP.

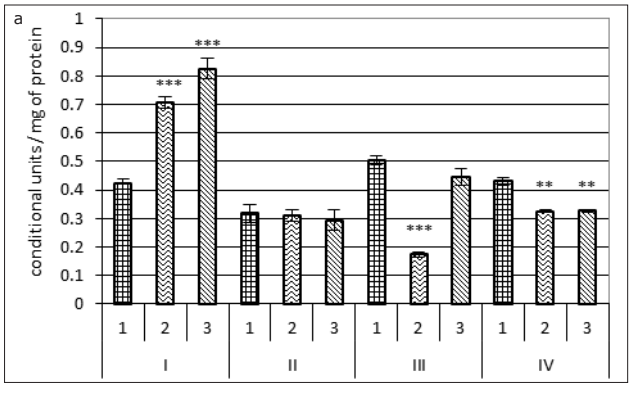
Literature states that lipid hydroperoxides produce free radicals, induce lysosome labialization and the release of proteolytic enzymes. This, in turn, generates highly toxic products that accumulate in the cell (alcohols, ketones and aldehydes) which damage membrane-bound enzymes, affect the membrane transport and, consequently, cause cell death [18]. However, the decrease in the intensity of the LP processes is also a negative phenomenon, since it is known that the physiological levels of oxidizing agents also modulate cellular functions through homeostatic cascades that signal

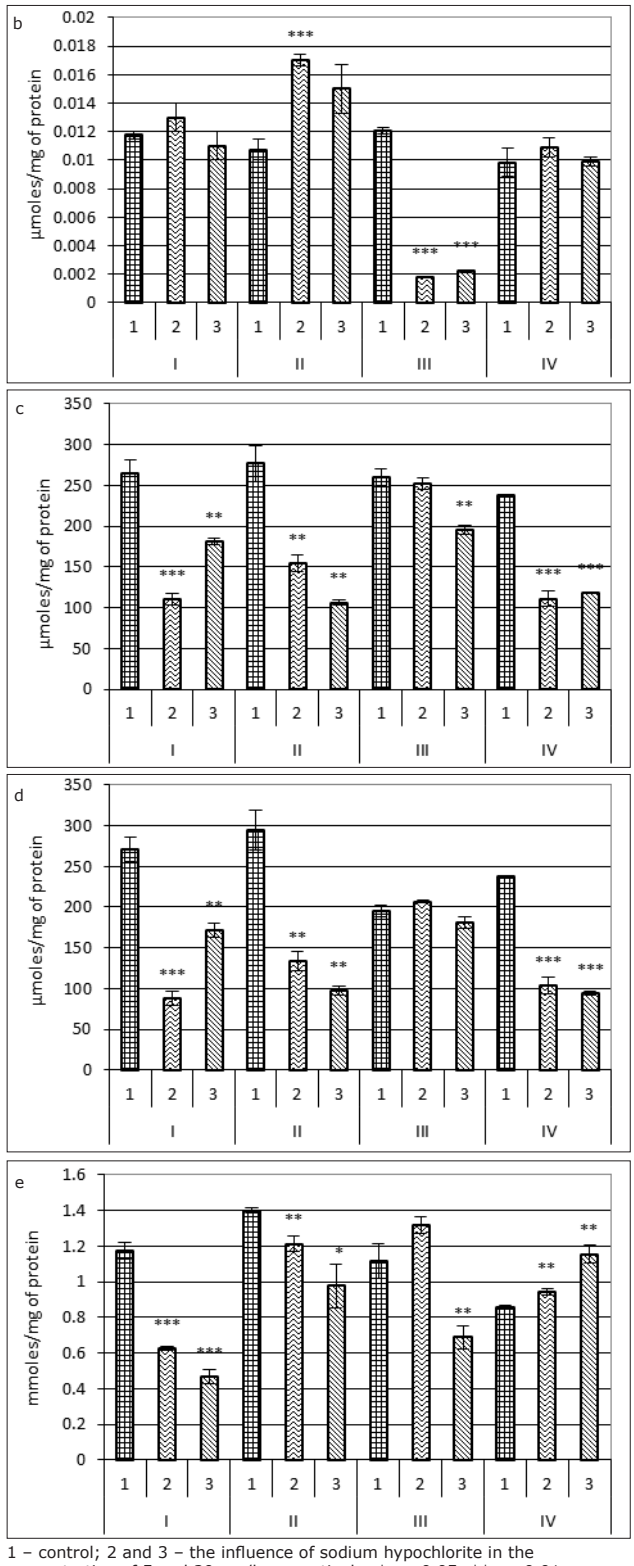
the redox cell sensitivity [19]. LP products are an integral part of a healthy body and contribute to maintaining a stable biochemical status of cells [20]. Lowering the contents of lipid peroxidation products causes a change in the rigidity of the membrane and affects its permeability. This function in erythrocytes is extremely important, as the microviscosity of membranes is lower if the unsaturated fatty acids dominate the lipids composition and higher if the content of saturated fatty acids is high.

We found that SH in both investigated concentrations led to a decrease in the content of carbonyl groups of proteins, both of neutral and basic. Moreover, the substance at a low concentration caused a more intense decrease in the content of carbonyl groups of proteins (about 60%) than in the high concentration (about 30%) at 1st day (Fig. 3a). The two-week treatment with SH at a concentration of 20 mg/l led to a decrease only in the content of carbonyl groups of neutral proteins by 25% in rat erythrocytes. However, after the rehabilitation period, the intensity of the oxidative modification of proteins was decreased (about 50% of this decrease being in the content of the concerned products). Consequently, SH leads to a predominant decrease in LP and oxidative modification of proteins in rat erythrocytes. SH is recognized as being a potent oxidant that intensifies free radical processes, however, erythrocytes have an excellent metabolism compared to other cells of the body. Thus, a powerful antioxidant defense system is available in these cells, that being devoid of organelles and nuclei, use anaerobic glycolysis as a main way of generating energy (90%). Therefore, there is no "leakage" of free radicals (from mitochondria) in erythrocytes during "respiration", and this effect intensifies the processes of LP and oxidative modification of proteins against the background of action of SH, hence, accordingly, they are oxidant resistant.

In our study, SH reduced the content of sialic acids throughout its administration, except for the concentration of 5 mg/l on the 14th day. At the initial stages of the experiment, the reduction of the content of these compounds was more pronounced (under the action of a low concentration by 47%, and under the effect of the high dose – by 60%), however, the subsequent slowdown of intensity was less pronounced (Fig. 3).

After discontinuing treatment of SH in low and high concentrations, the sialic acid content increased by 9% nd 34%, respectively. The scientific literature reports that the polyclonal antibody S reacts with Met29 glycophorin B of erythrocytes, but may bind to neighboring amino acids.





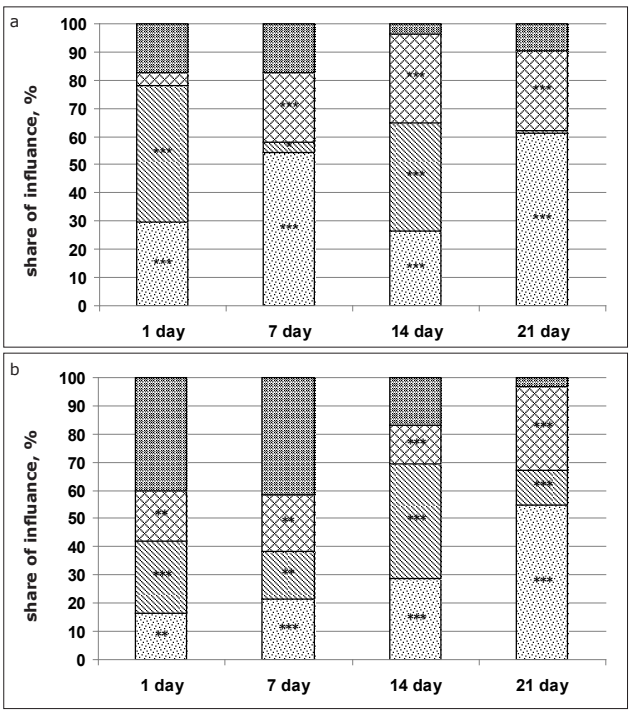
1 – control; 2 and 3 – the influence of sodium hypochlorite in the concentration of 5 and 20 mg/l, respectively; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

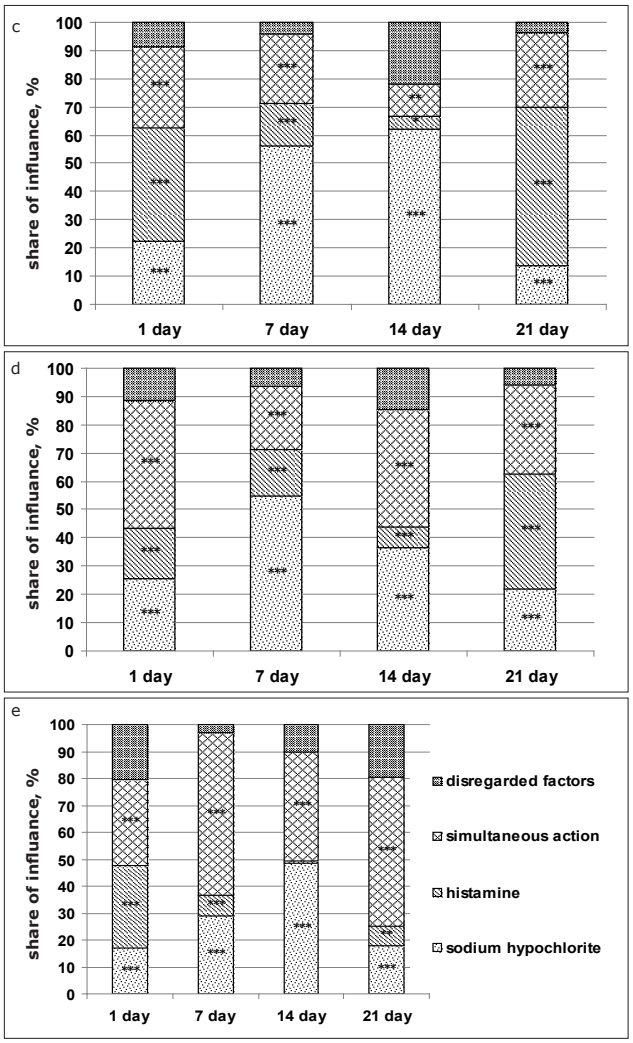
Figure 3. The content of hydroperoxides (a), TBA-active products (b), the carbonyl groups of proteins of neutral (c) and the main character (d), sialic acids (e) in the hemolysates of rat erythrocytes on the 1st (I), 7th (II), 14th (III) and 21st (IV) day of the experiment

SH at various concentrations affects the interaction of the polyclonal antibody S with glycophorin B through the effect on sialic acids that are present on the surface of the latter [21]. Thus, our data correlated with the data of other researchers who reported that SH affects the content of sialic acids on the surface of erythrocytes, which,

in turn, negatively influences the reception of these cells. The increase in the content of sialic acids after the rehabilitation period is a positive phenomenon, which indicates a gradual restoration of the functional properties of erythrocytes after the action of SH.

In our work, Anova two-way analysis was used for estimating the degree of influence of histamine, SH and their joint action. We found that the content of hydroperoxides of lipids was significantly influenced by SH on the 7th and 21st day (by 54% and 61%, respectively), while histamine induced this effect on the 1st and 14th day (by 48 and 38%, respectively; Fig. 4). Moreover, the simultaneous introduction of histamine and SH insignificantly influenced the content of the primary products of lipid peroxidation consistently from the 7th day until the end of the experiment. In addition, histamine significantly affected the content of TBA-active products on the 14th day (flood share 41%), while other substances in various combinations caused a moderate effect on the content of these lipid peroxidation products. This indicates that the primary products of the LP are influenced by SH, but the continuation of the chain reaction of lipid peroxidation is determined by histamine, SH and their combined action. What is more, SH administration was responsible for the acumulation of the content of protein neutral and basic carbonyl groups on 7th and 14th days (approximately 50% of the influence factor). However, in the initial and final stages of the study, histamine had a significant effect on the content of carbonyl groups of proteins of neutral character (a particle of influence of 40 and 56%, respectively). The influence factor of simultaneous administration of histamine and SH against oxidative modification of proteins of the main nature was significant (45 and 42%) at 1st and 14th day of the experiment (Fig. 4). This indicates the unequal effect of histamine by itself and of histamine in combination with SH on the content of carbonyl cultures of proteins of the basic and neutral nature.





* p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001

Figure 4. The results of Anova two-way analysis of the parameters of the prooxidant state (a – hydroperoxides; b – TBA-active products; c, d – carbonyl groups of proteins of neutral and basic character, respectively, e – sialic acids) in hemolysates of rat erythrocytes under the influence of histamine and sodium hypochlorite

Anova two-way analysis allowed us to recognize that histamine and the combined administration of the tested compounds significantly influenced the amount of sialic acids in rat erythrocytes at 1st day. However, in the further stages of the experiment, sodium hypochlorite and the combined effect of histamine and SH had a predominant effect (Fig. 4). These results confirm the indirect effect of histamine on the content of sialic acids on the surface of rat red blood cells.

CONCLUSIONS

Histamine increased the content of lipid hydroperoxides, decreased the content of sialic acids and changed the intensity of oxidative modification of proteins in rat red blood cells. The combined administration of histamine and SH also induced significant changes – increasing of the content of primary lipid peroxidation products on the 1st day of the experiment, but by the 14th day, their content was lowered. The intensity of the oxidative modification of proteins and the accumulation of sialic acids was preferentially slowed

down under these conditions. Treatment of red blood cells with SH in both studied concentrations led to the same changes in the amount of sialic acids. Anova two-way analysis identified the significant effect of SH on the indices of free radical reactions, and the simultaneous administration of histamine and SH on the content of sialic acids.

ORCID iDs

Nataliya Harasym https://orcid.org/0000-0002-6102-4271
Svitlana Mandzynets https://orcid.org/0000-0003-3053-628X
Dmytro Sanahursky https://orcid.org/0000-0002-8998-7117

REFERENCES

1. Vitushinska M, Matiytsiv N, Chernik Ya. Sensitivity to the oxidative stress conditions lifespan and neurodegenerative changes in the brain structure of Drosophila melanogaster superoxiddismutase mutants. *Visn Lviv Univ Biol Ser.* 2013;62:108-16.
2. Kanyuka OP, Filyak YeZ, Sybirna NO. Peculiarities of structural and functional organization of erythrocyte membranes in mice of wild-type and mice with PTTG gene knockout. *Stud Biol.* 2011;5(1):97-104.
3. Girina OM, Karlova O, Bruzgina TS. Evaluation of lipid profiles of erythrocytes in patients with metabolic syndrome. *Med Chem.* 2007;9(1):72-5.
4. Apikhtina OL. Investigation of the toxic effect of heavy metals on the blood erythrocyte model in vitro. *Mod Probl Tox.* 2011;1-2:65-9.
5. Komarenko VI, Terekhov AA, Vorobyova AP. Investigation of the role of H1-receptor responses in rat liver portal vessels to histamine. *Cherk Nat Univ B Khmelnitsky Biol Sci Series.* 2008;128: 54-8.
6. Oleksiuk NP, Yanovych VG. The activity of pro- and antioxidant systems in the liver of freshwater fishes in different seasons. *Ukr Biochem J.* 2010;82(3):41-8.
7. Timirbulatov RR, Seleznev EI. Method of increase intensity of free-radical oxidization of lipid components of blood and his diagnostic value. *Lab Bus.* 1981;4:209-11.
8. Morozenko DV, Leontieva FS. Research methods markers of connective tissue metabolism in modern clinical and experimental medicine. *Young Scien.* 2016;2(29):168-72.
9. Meschyshyn IF, Poloviy VP. Mechanism of oxidative modification of proteins. *Buk Med Herald.* 1999;3(1):196-205.
10. Kawai Y, Nuka E. Abundance of DNA adducts of 4-oxo-2-alkenals, lipid peroxidation-derived highly reactivgenotoxins. *J Clin Biochem Nutr.* 2018;62(1):3-10.
11. Michael M, Brent R. Lipid peroxidation in cell death. *Biochem Biophys Res Commun.* 2017;482(3):419-25.
12. Iskusnyh AYu, Basharina OV, Artyukhov VG, Alabovsky AA. The influence of histamine on the functional properties of neutrophils and the intensity of the process of peroxide oxidation of lipids in the blood of donors. Vest VSU Ser. *Chem Biol Phar.* 2008;1:93-6.
13. Shaiko LM, Bokut SB. Workshop on medical and biological physics. Section "Biological Physics": Methods of Biophysical Research. Minsk: A.D. Sakharov Moscow State Polytechnic Institute; 2011.
14. Lin YW. Structure and function of heme proteins regulated by diverse post-translational modifications. *Arch Biochem Biophys.* 2018;641:1-30.
15. Miyamoto S, Di Mascio P. Lipid hydroperoxides as a source of singlet molecular oxygen. *Subcell Biochem.* 2014;77:3-20.
16. Andriichuk AV, Tkachenko HM, Kurhaluk NM, Tkachova IV. Dynamics of hematological parameters and oxidative stress markers in Ukrainian warmblood horses under exercise influence The J of V.N. Karazin Kharkiv National *Univ. Ser Biol.* 2013;17(1056):148-60.
17. Anu P, Padler-Karavani V. Evolution of sialic acids: Implications in xenotransplant biology. *Wiley Xenotransplant.* 2018;12424:1-11.
18. Osoba IA. The biological role of lipid peroxidation in ensuring the functioning of fish organism. *Fish Sci Ukr. 2013;*1:87-96.
19. Radi R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. *Proc Natl Acad Sci (USA)* 2018;115(23): 5839-48.
20. Popova E, Koshchiy I. Role of lipids in adaptation of fish to ecological stress. *Fish Sci Ukr.* 2007;1:49-56.
21. Halverson GR, Reid ME, Sutherland J, Rhodes M. Evaluation and comparison of three human monoclonal anti-S, two human polyclonal anti-S, and one murine anti-GPB. *Immunohematol.* 1999;15(4):163-6.