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CHARACTERISTICS OF BIOCHEMICAL INDICES CHANGES IN THE BLOOD OF RATS WITH OPIOID EFFECT

Abstract. *The comparative analysis of the metabolite content of lipid peroxidation and enzyme activity of the antioxidant protection in the blood of experimental animals after the use of opioid analgesic confirms statistically reliable difference in the groups of animals which received different doses of the agent during certain periods of time. It should be noted that disorders in the processes of free radical oxidation found in the blood of experimental animals are evidenced histologically as the result of development of destructive changes in the biological cellular membranes of the mucosa and cartilages of the larynx of rats in case of a long effect of opioid analgesic in increased doses, which appeared to be a sensitive indicator of disorders in the functional-metabolic processes in them. Therefore, investigation of free radical oxidation processes and antioxidant protection is rather reasonable in order to determine the deepness and character of injury, quality control in the course of compensatory reactions.*

Key words: *opioid effect, biochemical indices, rats.*

Introduction. The results of the study corresponds to the plan of scientific research of Danylo Halytskyi Lviv National Medical University and is a part of the scientific research conducted at the Department of Normal Anatomy «Structural Organization, Angioarchitectonics and Anthropometric Features of the Organs during Intrauterine and Extrauterine Periods of Development under Conditions of Exo- and Endopathogenous Factors» (state registration № 0115U000041) during 2015 – 2019.

A number of external toxic factors promote disorders of biochemical body status. Free radical oxidation is known to play an important role in maintenance of electron transport in the respiratory part, induction of pores formation in the mitochondrial membrane regulating association of respiration with oxidation phosphorylation, and forms the basis of oxidation processes in the mitochondria. Oxidation processes with participation of activated oxygen metabolites are an integral part of existence of higher living forms [1, 3]. Oxidation-reduction (redox) processes are found to be activated in case of extreme effects in the body resulting in the formation of lipo- and hydroperoxides, which further decay promotes formation of endogenous oxygen essential for vital activity [2, 3]. Superoxide is one of the main pro-oxidants in the cell. Therefore, superoxide dismutase plays a key

role in anti-oxidant protection of the body. Catalase function is breaking down of toxic hydrogen peroxide formed in the process of different oxidation reactions in the body [4].

Due to this fact, the objective of our study was investigation of changes of free radical oxidation and antioxidant protection, and their effect on the mucous membrane and structural organization of the laryngeal cartilaginous components under experimental opioid effect.

Materials and methods. 64 mature inbred male rats with the body weight of 80-135 grams, aged from 4,5 to 7,4 months were used in the study. All the animals were kept in vivarium, and the work concerning their keeping, care, marking and other manipulations were performed according to the principles of “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” [Strasburg, 1985], “General Ethical Principles of Experiments on Animals” approved by the First National Congress on Bioethics [Kyiv, 2001], the Bioethics Board of Danylo Halytskyi Lviv National Medical University, and ethical requirements according to the Order of the Ministry of Health of Ukraine № 231 dated 01.11.2000 (minutes № 10 dated 26.12. 2011).

Blood of the experimental animals collected during the experiment was used for biochemical examination. Biochemical methods of

investigation correspond to the common technique.

The first group included 5 intact rats in order to examine biochemical indices of the norm (Malone aldehyde, diene conjugates, catalase and ceruloplasmin). The second group included 5 rats after 1 week of opioid effect. The third group included 5 rats after 2 weeks of opioid effect. The fourth group included 5 rats after 3 weeks of opioid effect. The fifth group included 5 rats after 4 weeks of opioid effect. The sixth group included 5 rats after 5 weeks of opioid effect. The seventh group included 5 rats after 6 weeks of opioid effect. The eighth group included 5 rats after 8 weeks of opioid effect. The ninth group (control) included 24 rats receiving intramuscular injections of physiological solution during 56 days at the same period of time (10-11 a.m.). The terms of observation of experimental animals were from 1 to 56 days. The drug Nalbuphine was injected intramuscularly every day once a day at the same period of time (10-11 a.m.) during 42 days (6 weeks). Every year injections of Nalbuphine were given in increasing succession: 1st week – 8 mg/kg, 2nd week – 15 mg/kg, 3rd week – 20 mg/kg, 4th week – 25 mg/kg, 5th week – 30 mg/kg, 6th week – 35 mg/kg. Chronic opioid effect was modeled by means of the suggested method – the condition was formed that manifested by changes of behavioural reactions of animals and the data obtained presenting evidence of biochemical indices disorders against the ground of chronic effect of the agent. The suggested model was applied to simulate opioid effect [5].

Results. In the course of the investigation a comparative analysis of the parameters was performed characterizing the activity of processes of lipid peroxidation and the enzymes of antioxidant protection in the blood of the experimental animals when opioid analgesics are administered. To assess the parameters of the lipid peroxide oxidation and antioxidant protection systems the content of lipid peroxidation products was determined: Malone aldehyde, diene conjugates. The state of the antioxidant protection system was assessed by the activity of the enzymes with antioxidant action – catalase and ceruloplasmin. Experimental administration of an opioid analgesic during 42 days in increasing doses was associated with an increased concentration of Malone aldehyde manifested by a reliable activation of free radical oxidation processes in the blood of experimental

animals depending on the duration of the agent introduction and its doses (Table 1). When the dose of an opioid analgesic increased, the processes of lipid peroxide oxidation intensified. On the 6th week with the dose of 35 mg/kg the concentration of Malone aldehyde became 11,69% higher than that of the norm ($p < 0,001$). The level of Malone aldehyde in the blood of rats increased mostly on the sixth week ($p < 0,001$) of the experiment. When the agent was withdrawn on the eighth week peroxide processes delayed which is evidenced by reduced Malone aldehyde level even lower than that of the norm on 4,5 %. Gradual decrease of Malone aldehyde level occurred at the expense of activation of the antioxidant system.

Table 1

Changes of Malone aldehyde concentration in the blood of animals depending on the duration of opioid administration, nmol/ml

Weeks	M±m
norm	5,644±0,014 ^{▼°}
1 st week of opioid effect	5,688±0,004 [°]
2 nd week of opioid effect	5,690±0,038 ^{°•}
3 rd week of opioid effect	5,848±0,010 ^{°▼•}
4 th week of opioid effect	6,126±0,041 ^{▼°•}
5 th week of opioid effect	6,278±0,007 ^{▼°}
6 th week of opioid effect	6,586±0,008 [▼]
8 th week of opioid effect	5,440±0,005 ^{▼•}
10 th week, withdrawal	5,390±0,007 ^{▼•}

[°] probable reliability of difference ($p < 0,05$) #

[•] probable reliability of difference ($p < 0,01$) ^m

[▼] probable reliability of difference ($p < 0,001$)

The results of the study are indicative of the fact that long administration of opioid analgesics reliably increases the activity of lipid peroxidation processes. It is the evidence of an increased amount of intermediate metabolites of lipid peroxide oxidation in the blood – diene conjugates. The concentration of diene conjugates differed reliably on the sixth week, it became 23,58 % ($p < 0,001$) higher than that of the norm. Therefore, the most considerable changes are found on the sixth week compared with the control and the first week of the experiment. As the result of withdrawal of the agent on the eighth and tenth weeks of the experiment the indices of diene conjugates level decreased considerably and were close to the norm respectively (43,566±0,00543₅ 42,424±0,003, norm -

424±0,009 mcmol/L) (Table 2). There is statistically reliable linear correlation link between the norm and level of diene conjugates on the second and fifth weeks ($p < 0,05$).

Table 2

Changes of diene conjugates concentration in the blood of animals depending on the duration of opioid administration, mcmol/L

Weeks	M±m
norm	42,422±0,003 ▼
1 st week of opioid effect	42,662±0,010 ▼
2 nd week of opioid effect	45,562±0,008 ▼
3 rd week of opioid effect	54,080±0,003 ▼•
4 th week of opioid effect	63,92±0,060 ▼•
5 th week of opioid effect	64,154±0,012 ▼•
6 th week of opioid effect	64,238±0,012 ▼•
8 th week of opioid effect	43,566±0,005 ▼•
10 th week, withdrawal	43,424±0,009 ▼•

- probable reliability of difference ($p < 0,05$)
- probable reliability of difference ($p < 0,01$)
- ▼ probable reliability of difference ($p < 0,001$)

The most substantial increase ($p < 0,001$) of diene conjugates level in the blood of experimental group of animals was found on the sixth week in comparison with the control group and the first week as well as concerning the eighth and tenth weeks. Statistically reliable correlation ($p < 0,05$) was found between the indices of the norm and the second week, first and fifth weeks.

Thus, catalase activity in the blood of experimental animals decreased in comparison with the norm on 19,24 %, on the eighth week – 21,50% lower than that of the norm, and on the tenth week – 11, 46 % (Table 3). The difference between mean values of all the indices is statistically reliable ($p < 0,001$). Catalase activity changed most of all in comparison with the norm on the eighth, sixth and fifth weeks. All the indices concerning the norm reliably changed as well as between themselves ($p < 0,001$). Statistically reliable correlation is found between catalase activity on the 1st and 6th weeks and between the data of the 2nd and 3rd weeks ($p < 0,05$). Inversely proportional correlation exists between the enzyme activity on the 1st and 8th weeks.

Thus, catalase activity on the eighth week decreased on 17,49 % in comparison with the first week. Due to withdrawal of opioid analgesic catalase activity was close to the norm which is indicative of the balance between the processes

Table 3

Changes of catalase activity, mmol/ min, MDA in the blood of animals depending on the opioid dose during weeks

Weeks	M± m
norm	7,610±0,006 ▼
1 st week of opioid effect	7,240±0,010 ▼
2 nd week of opioid effect	7,136±0,013 ▼
3 rd week of opioid effect	6,918±0,009 ▼
4 th week of opioid effect	6,846±0,012 ▼
5 th week of opioid effect	6,560±0,009 ▼
6 th week of opioid effect	6,146±0,012 ▼
8 th week of opioid effect	5,974±0,006 ▼
10 th week, withdrawal	6,738±0,014 ▼

- probable reliability of difference ($p < 0,05$)
- probable reliability of difference ($p < 0,01$)
- ▼ probable reliability of difference ($p < 0,001$)

of free radical lipid oxidation and antioxidant protection.

Examination of the blood of experimental animals found that ceruloplasmin activity is considerably lower than that of the norm (on 16,5 %), as well as it is reliably lower (on 10, 76 %) concerning the first week of the experiment ($p < 0,05$) (Table 4). The difference between mean values of all the indices is statistically reliable ($p < 0,001$). The indices of ceruloplasmin activity changed utmost on the sixth, fifth, tenth and fourth weeks of the experiment as compared to the norm. All the indices concerning the norm reliably changed as well as between themselves

Table 4

Changes of ceruloplasmin concentration in the blood of animals depending on the term of opioid administration

Weeks	M±m
norm	320,8±0,37 ▼
1 st week of opioid effect	312,2±1,02 ▼
2 nd week of opioid effect	296,6±1,03 ▼
3 rd week of opioid effect	287,0±0,55 ▼
4 th week of opioid effect	279,4±0,51 ▼•
5 th week of opioid effect	271,4±0,93 ▼•
6 th week of opioid effect	267,2±0,58 ▼
8 th week of opioid effect	293,2±1,24 ▼
10 th week, withdrawal	278,6±1,17 ▼

- probable reliability of difference ($p < 0,05$)
- probable reliability of difference ($p < 0,01$)
- ▼ probable reliability of difference ($p < 0,001$)

($p < 0,001$). Statistically reliable correlation was found between the first and fourth, and between the fourth and fifth weeks of the experiment ($p < 0,05$).

Conclusion: Therefore, comparative analysis of the metabolite content of lipid peroxidation and enzyme activity of the antioxidant protection in the blood of experimental animals after the use of opioid analgesic confirms statistically reliable difference in the groups of animals which received different doses of the agent during certain periods of time. It should be noted that disorders in the processes of free radical oxidation found in the blood of experimental animals are evidenced histologically as the result of development of destructive changes in the biological cellular membranes of the mucosa and cartilages of the larynx of rats in case of a long effect of opioid analgesic in increased doses, which appeared to be a sensitive indicator of disorders in the functional-metabolic processes in them. Therefore, investigation of free radical oxidation processes and antioxidant protection is rather reasonable in order to determine the deepness and character of injury, quality control in the course of compensatory reactions.

Thus, the results obtained are indicative of an important role in the study of lipid peroxidation processes and antioxidant protection in order to

investigate opioid effect on the functional state of the mucous membrane and laryngeal cartilage..

References:

1. Shakirov DF. Sostoyaniye svobodnoradikal'nogo okislennya u rabochikh neftepererabatyvayushchey promyshlennosti. *Meditsina truda i promyshlennaya zkolohiya*. 2001;(1):10-3.
2. Zinchuk VV, Borisyuk MV. Rol' kislородosvyazyvayushchikh svoystv krovi v podderzhanii prooksidantno-antioksidantnogo ravnovesiya organizma. *Uspekhi fiziologicheskikh nauk*. 1999;30(3):38-48.
3. Shakirov DF, Farkhutdinov RR, Zul'karnayev TR. Otsenka sostoyaniya zdorov'ya robotayushchikh s pomoshch'yu khemilyuminestsentnykh metodov issledovaniya. *Gigiyena i sanitariya*. 1999;(3):36-9.
4. Hnid' RM. Rezul'taty doslidzhennya aktyvnosti fermentiv antyoksydantnoho zakhystu rotovoyi ridyny u khvorykh na parodontyt, yaki prozhyvayut' na terytoriyi, zabrudneniy sirkoyu. *Visnyk problem biolohiyi i medytsyny*. 2016;2(127):224-7.
5. Pal'tov YEV, Fik VB, Vil'khova IV, Onys'ko RM, Fit'kalo OS, Kryvko YuYa, inventors; Danylo Halytskyi Lviv National Medical University. *Ukraine patent №76565*. 2013.