ANTITOXIC PROTECTION OF THE BODY USING ANTIDOTES, ANTIOXIDANTS AND OTHER MEMBRANE PROTECTORS

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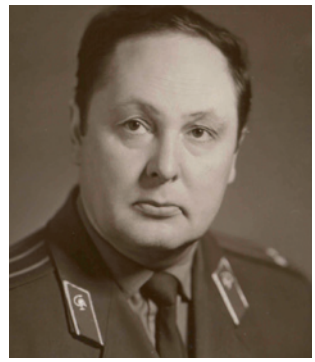

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ABSTRACT — Results obtained in the experimental study on further possibilities of the body's palliative protection against chemical exposures (occupational toxicants) using antioxidants — derivatives of pyrimidine, benzimidazol, succinate-pyrimidine complex compounds, α-tocopherol, ionol and other drugs are presented. Marked antioxidant, hepatoprotective actions of 5-hydroxy-6-methyluracil (oxymethyluracil) and its complex combination with succinate and glycyrrhizinic acid have been determined. Cytolytic and membrane protective effects are confirmed by reduced enzymatic activity of urokaninase, AlAt, AsAt compared with control indicators. The data obtained also allow us to conclude that a disturbance in the lipid peroxidation processes is a common toxigenesis link in the chain: metabolic disturbance – cytolysis and requires adequate correction by an antioxidant regardless its character: primary or secondary. The models of the body damage caused by the action of ethanol, dichlorethanol, polychlorbiphenils, nitrates, chlorphenols and phosphororganic antidotes are used.

KEYWORDS — intoxication, pathogenesis, lipid peroxidation, pyrimidine derivatives, antioxidants, membrane protectors, antidotes.

At present, an important trend of research into chemical safety is associated with "therapeutic protection" [20, 21]. Current knowledge linking advances in molecular and cell biology, general pathology, neurochemistry and neuro-endocrinology to integrated efforts of toxicologists and representatives of other medico-biological disciplines has opened possibilities for identification of fundamental mechanisms underlying cellular toxicity, analysis of hypoxic and free radical mechanisms of cellular necrobiosis [1, 5], development of a methodological concept - the theory of general mechanisms of toxicity [1]. While considering this concept, we used the differentiation and integral approaches to assess toxic effects of the substances studied at different levels of structural and functional organization of living systems: at the whole-organism, organism-tissue and molecular-cellular levels. One of general mechanisms of toxic effects of hepatotropic poisons is activation of lipid peroxidation (LPO) and reduced antioxidant activity, particularly in the liver [1]. Ethanol, dichlorethan (DCE), polychlorbiphenils (PCB), chlorphenols, tetrachlormethan (TCM) and phosphororganic compounds were experimentally used in our trials.

The purpose of the present study is to review major results of experimental studies on further possibilities of the body's palliative protection against toxic damage caused by occupational toxicants based on patho-physiological significance of lipid peroxidation processes.

Complex studies on LPO processes in alcohol intoxication, efficacy of antioxidant correction performed along with assessment of efficiency of correction of survival, metabolic disturbances and the condition of biological membranes show LPO advantages in its pathogenesis. This is confirmed by a high protective effect of antioxidants – ionol, mexidol and certain pyrimidine derivatives in mice intoxication with ethanol at a dose of 8g/kg, a 1.5-fold increase in LPO activity in the liver and erythrocytes of rats after a single administration of the toxicant at a dose of 6 g/kg as well as daily administration of alcohol for a week. It is noteworthy that LPO activation in the liver is preserved during the post-intoxication period at 2-3, 7-8 and 14-15 days following severe intoxication and is accompanied by marked hyperenzymotemia, disorders in erythrocyte membrane permeability. LPO activation and reduced superoxide dismutase (SOD) activity in erythrocytes can be observed within 2 hours after a single ethanol administration to rats [16, 18].

With two-week alcoholization, another sequence of metabolic changes is seen. These changes accompany LPO activation: the period of LPO intensity and

antioxidant enzymes activity stabilizaton is followed by the period of their enhancement. In alcoholization, LPO activity is accompanied by hepatocyte membrane damage and a decrease in mitochondrial energy formation. This is confirmed by a fall in Na-, K-ATPhase, NAD H-dehydrogenase and succinated hydrogenase (SDG) as well as an increase in the amount of TBK-reacting products. We failed to identify a significant change in the number of dien conjugates in the liver and cerebral cortex exposed to alcoholization [18].

With dichlorethan-induced liver damage, imbalance in the liver pro-oxidant-antioxidant is detected. A hepatoprotective effect of pyrimidine derivatives in acute and sub-acute dichlorethan intoxication was first studied. Positive effects of 1,3,6-trimethyl-5-hydroxyuracil and 5-hydroxy-6-methyluracil (oximethyluracil) on cells, metabolic processes, LPO activity and rats' longevity were shown.

The model of sub-acute intoxication by polychlorbiphenils (PCB) demonstrated that LPO activation process in the liver is due to a reduction in natural antioxidant factors — superoxide dismutase and glutathione peroxydase. A certain advantage of oxymethyluracil over α-tocopherol was revealed. In comparative experiments, a higher hepatoprotective efficiency of oxymethyluracil compared with carsil – a standard hepatoprotector — as well as other referent agents – methyluracil, tykveol, Liv-52, methionin and heptral was shown. In contrast, essentiale increased PCB hepatodamaging effect [8, 12, 15, 19].

To provide strong evidence that LPO activation is a leading pathogenesis link of chemically-induced liver damage by PCB (except for a significant decrease in LPO activity following administration of great toxicant doses to the body) trials were performed. Antioxidants (α-tocopherol and ionol) were profilactically administered (before intoxication) and simultaneously with the toxicant for 4 weeks. The data obtained have shown that α-tocopherol and ionol unlike carsil (silymarine) not only stabilize LPO but prevent lethality. This fact confirms pathogenetic significance of LPO processes in PCB intoxication. By inhibiting LPO activity, the antioxidants under discussion significantly prevent enzymotemia and necrobiotic changes in hepatocytes [12, 18].

Meanwhile, despite differences at the beginning of the free-radical mechanism of hepatocyte damage, its final stages are similar and interconnected with a breakdown in bioenergetic mechanisms. This is confirmed by a progressing reduction in liver ATPh content under conditions of long-term PCB intoxication [17]. That is why we think that for the purposes of restorative correction of toxic hepatopathia, agents combining antioxidant and antihypoxic properties are more preferable. To implement this research trend, an experimental study on their hepatoprotective activity and related pharmacologic properties of the above agents was launched. Preliminary promising results were obtained [16, 19].

The next few experiments showed that in some forms of intoxication by phosphororganic compounds (POC) there was a dysregulation of free-radical oxidation processes resulting in oxidative stress [19]. Oxidative stress is a poorly studied link in the pathogenesis of FOC severe intoxications. The most significant for POC toxigenesis are cytrochrom P-450-dependent monooxygenase reactions, contributing to the generation of reactive oxygen species (ROS), formation of free-radical xenobiotics with subsequent LPO activation. We may suppose that with POC intoxication having a high level of hydrophobicity (carbophos, mercaptophos), oxidative stress mechanisms are involved before their own biotransformation in the liver monooxygenase system and interaction with active acetylcholiesterase (ACE) centres. Meanwhile, armin or phosphacol being more hydrophil and rapidly metabolizing poisons, interact selectively first of all with ACE with subsequent development of hypercholinergic effect and circulatory hypoxia as a leading pathogenesis link. With the current intoxication forms, the possibility of oxidative stress development and disturbance of LPO process activity should be further explored.

Using models of acute carbophos and armin intoxication the antioxidant system (AOS) state, LPO reactions in target organs during toxigenic and somatogenic intoxication phases, ACE activity and some integral indicators of the experimental animals' organism condition (survival/death) were studied. Acute carbophos intoxication was induced by a single enteral administration to rats of the poison at a dose of 0,9 LD50 (320mg/kg), acute armin intoxication – by a single intramuscular toxicant administration at a dose of 0,75 mg/kg (0,9LD50). LPO products: dien (DC), trien conjugates (TC) and shif bases (SB) were determined in lipid extracts of the cortex and myocardium using the spectrophotometric method. Superoxide dismutase (SOD) activity was determined by the V.N. Chumakov method, acetylcholinesterase — the Elman method. A variety of variation statistics methods were used for the analysis of the data obtained.

It has been shown that in rats with acute carbophos intoxication SOD activity fall in the cerebral cortex, LPO process activation in the brain and heart occur within 2 hours after the poison administration. In the brain, the amount of DK increases after 2-24 hours and by 2–14 days it exceeds control indicators

by 1,5 and 3,3 times. In the myocardium, during the same period DK content increases by 1,4-3,8 times and by 28-30 days it becomes within norm as well as in the brain. However, in this particular case normalization is not veritable since by 42 post-intoxication day DK amount in brain lipids falls reaching negative values and accounts for only 33,3% compared with control. The level of secondary LPO-SO products increases by 1,6-2,5 times, respectively compared with control. Imbalance detected between SOD activity and the number of LPO products occurring between 41 and 43 experimental days [6] precedes the time of rats' mass death [8,18].

The antidote treatment of the intoxicated rats by atropine or atropine and dipiroxyne (dietixymom) does not produce practical effect on intoxication clinical manifestations and biochemical indicators. So, with the first intoxication signs, intramuscular administration of atropine M-cholinomimetic (5 and 10 mg/kg) is not effective similar its long-term use. Co-administration of atropine (10 mg/kg) and dyperoxyme cholinesterase (or dietixim) reactivator at a dose of 25 mg/kg does not impact on the indicators studied, either, including brain and erythrocyte ACE activity. So, after 5 hours, minimal residual ACE activity in erythrocytes of rats intoxicated by carbophos accounts for 32,0±2% while in antidote treated rats it is 36,7± 6% (P>0,1). Meanwhile, addition of antidotes to antioxidants – tonarol or emoxypin (50 mg/kg) is beneficial for SOD activity, DK and SO content in the brain, intoxication clinical symptoms and significantly prevents lethal outcome by 41-43 post-intoxication day [6]. Free-radical oxidation reaction inhibitor – oxymethyluracil as well as benzimidazole derivatives – bemithyl, etomersol and 2-(3,4-dihydroxifenacylsio) having antiradical and antihypoxic activity produce antioxidant effect under conditions of monotherapy [8,18,19]. Since under conditions of mortal carbophos intoxication the use of antioxidants prevents organ LPO/AOS imbalance and mass death of the animals in the post-intoxication period we may conclude that oxidative stress is an important pathogenetic link in carbophos intoxication and demands appropriate pharmacological correction by agents having an antioxidant effect. With acute carbophos intoxication, one of the most possible causes of atropine low efficiency is associated with structural changes in erythrocytes and related microcirculation impairment.

To identify pathochemical mechanisms of erythron damage and to determine methods of its pharmacological correction, rat experiments were conducted. Morphofunctional specificities of erythrocytes under acute carbophos intoxication were studied. An assessment of erythrocyte membrane state, AOS, LPO process activity was performed. The number of erythrocytes and reticulocytes, hemoglobin concentration, hematocrit index, blood colour index (CI), hemoglobin average concentration (HAC), hemoglobin average volume (HAV), hemoglobin average concentration (HAC) in 1 erythrocyte and electric charge of erythrocyte membranes were determined. It has been shown that carbophos at a dose of 0,9 LD50 causes death of 30% of rats during a day and produces a marked toxic effect on the erythron system. In intoxicated rats, an increase in blood reticulocyte content, a decrease in osmotic resistance and electric charge, AOS activity suppression and an increase in concentrations of primary (DC) and secondary LPO products (SO) were identified [13]. Atropin administration prevents death of 30% of rats during the first day, and development of reticulocytosis and limits accumulation of DC in erythrocytes. However, atropinazition doesn't influence on the erythron antioxidant system, the amount of TBA-reacting products, osmotic resistance and membrane electric charge.

The use of actoprotectors – benzimidazole derivatives (bemethyl, tiamazol) having antioxidant, membrane stabilizing effects along with atropine and their subsequent administration to rats under conditions of monotherapy produces marked therapeutic effects on the majority of disturbed indicators including reticulocyte content, SOD and catalase activity, the amount of LPO products, osmotic resistance and an electric charge of erythrocyte membranes. The current study indicates that morphofunctional state disturbance including pro-oxidant-antioxidant balance in erythrone subjected to acute carbophos intoxication is an important disturbance mechanism of erythrocyte membranes. Bemithyl or tiamazol involvement into the treatment regimen prevents pro-oxidant and membrane toxic action of erythron poison [13].

In acute and chronic intoxication by sodium nitrate, there are certain prerequisites for oxidative stress development. They are oxidant properties of the toxicant itself, its methemoglobinuri effect supplemented by inhibiting effect on enzymes of mitochondria respiratory chain [9, 18, 19]. Sodium nitrate in toxic doses brings about methemoglubinemia, LPO process enhancement and metabolic process disturbance in erythrocytes. This is confirmed by a significant increase in methemoglobin, DK amounts, a decrease in enzyme activity of AOS-SOD, catalase and glucose-6-phosphatdehydrogenase (G-6-PDG). An increase in DK content is also observed in the cerebral cortex and liver of nitrite mice and rats. Maximum DK accumulation is seen after 6 hours following sodium nitrate administration (0,9 LD50). It exceeds control indicators by 75,8% in the cerebral cortex and 67,2%

— in the liver. An elevated DK level is preserved in both organs after 12–24 hours and by 7–14 days during post-intoxication period. The same regularity is revealed in rat experiments during the first 48 hours. A maximum DK increase in the cerebral cortex and liver occurs after 6 hours following toxicant administration. In erythrocytes, this shift in LPO activity is observed in 2 experimental hours: DK amount in intoxicated animals exceeds as much as 2 times control indicators. However, SO amount in the brain and liver is preserved at the control level. This is probably due to the fact that the toxicant under current experiment conditions does not cause stitching in amino-phospholipid membranes underlying the reversible character of their damage [14].

Antioxidants — pyrimidine derivatives (oxymethyluracil) as well as cystamine limit methemoglobin accumulation in blood of intoxicated mice. The above agents as well as bemithyl and mexidol decrease DK level in mice brain and liver during the first 24 hours and by 7, 14 days following sodium nitrate administration.

In rat experiments, oxymethyluracil prevents a decrease in activity of catalase, SOD, G-6-PDG and reduces the elevated number of DK in erythrocytes to the normal limits. Cytoprotective and antioxidant effects of oxymethyluracil are also detected under conditions of long-term nitrite intoxication.

Thus, the results of the present study suggest that in experimental intoxications by hepatotrope poisons – ethanol, dichlorethan, PCB, as well as POS and sodium nitrate – the major consequences of disturbances in pro-oxidant-antioxidant balance are:

— oxidative stress;
— LPO disturbance (activation or activity suppression);
— permeability disturbance and electric charge change in biological membranes;
— enzyme activity disturbance;
- methemoglubinemia and hypoxia;
— bioenergetic process disturbance;
— disruptions in the body state integral indicators (lethal effect).

The use of certain pyrimidine and benzimidazole derivatives with antioxidant, antihypoxic, actoprotective activity as pharmacological correctors in monotherapy or in combination with antidotes is beneficial for pro-oxidant-antioxidant balance in the toxically damaged organs and tissues. It significantly limits or prevents development of hazardous consequences of this damage. Taking into account the complex character of pro-oxidant-antioxidant imbalance during different intoxication stages we may conclude that pharmacological agents with a broad spectrum of protective-restorative activity influencing on basal cellular processes, determining cell resistance and ability to reparation, increasing the body's general adaptation possibilities are necessary for their correction.

The results of studies on hepatoprotective effects of the agents under conditions of chemically-induced liver pathology demonstrate that agents referring to different pharmacological groups having antioxidant activity are effective (Table). Effects of acetylcysteine, oxymethyluracil and its derivatives as well as α-tocopherol are well marked on liver damage models accompanied by a high level of LPO activity (models 1, 2, 3). Mexidol, the well known antioxidant, produces marked hepatoprotective effect on ethanol-induced hepatosis-hepatitis model (model 2), liver fibrosis induced by a combination of sovtol and alcoholization (model 3) [2, 11, 17] as well as age differentiated models of tetrachlormetan hepatitis (models 4, 5). Synthetic analogues of purine based nucleic acid - benzimidazol derivatives (bemithyl, thyetazol, ethomerzol) as well as cytomak - an agent with an antioxidant action mechanism are effective on chlorphenol and trichlormetaphos-induced hepatitis models (models 6,7) [3, 4, 19]. With the liver injured by high doses of tetrachlormetan, ethanol, polychlorbiphenyls, dichlorethan, POS, the liver damage accompanied by suppression of LPO activity and liver functional-metabolic state develops. Antioxidant monotherapy is less effective. Co-administration of antioxidants and antioxidants with direct energizing activity is beneficial [19].

CONCLUSIONS

1. The data obtained allow us to conclude that a change in the lipid peroxidation processes is a common toxigenesis link in the chain: metabolic disturbance – cytolysis and requires adequate pharmacological correction by an antioxidant regardless its character: primary or secondary. Not only the lipid peroxidation activation but its suppression may be of pathogenetic value.
2. With intoxication, the most important condition for the lipid peroxidation activation in the organs (tissues) is the weakening of natural antioxidant factors — an impairment of the antioxidant system enzymatic link activity. Among intoxication pathogenesis factors, hypoxia, energetic deficiency, direct membrane-toxic effect are of great importance.
3. Effective therapy for chemical types of pathology is possible if to take into account the basic (specific) and co-factor (nonspecific) pathogenesis. Examples of practical implementation of this trend include new derivatives of benzimidazol,

Table. *The differentiation approach to pharmacological correction of chemically-induced liver pathology due to lipid peroxidation processes disturbance [2–4, 8, 10–12, 15–19]*

Experimental model, pathogenesis	Correction methods
Hepatitis induced by dichlorethan with LPO high level activity, reduced amount of restorative glutathione and SH- protein group	Acetylcysteine, oxymethyluracil, 1,3,6-trimethyl-5-hydroxyuracil, α-tocopherol
2. Hepatosis-hepatitis induced by ethanol, with LPO high level activity, elevated volume of fat dystrophy, high level of transaminase activity	Oxymethyluracil, mexidol, silymarin, a combination of oxymethyluracil with mexidol, sodium succinate
3. Hepatitis-fibrosis induced by combination of sovtol and excessive alcohol, with LPO high level activity, antioxidant deficiency, suppression of Krebs cycle enzyme activity	Oxymethyluracil, its combinations with sodium succinate, mexidol, succinate-pyrimidine complexes
4. Hepatitis-cirrhosis in young and elderly rats induced by TCM, with moderate and high LPO activity level, severe cytolysis and cholestasis events, liver dysfunction	Bemithyl, ethomerzol, thyetazol, mexidol, succinate-pyrimidine complexes
5. Hepatitis-cirrhosis in old rats induced by TCM, with LPO phase dynamics, ATP deficiency, the body reduced general resistance to high-altitude hypoxia	Mexidol, sodium succinate-oxymethyluracil, antihypoxicants, phytopreparations
6. Hepatitis induced by chlorphenils, with moderate LPO activation, suppression of bioenergetic and metabolic processes, oxidation separation and phosphorylation	Thyetazol, cytomak + vitamin B, C, E supplementations; oxymethyluracil
7. Hepatitis induced by trichlormetaphos, with moderate LPO activation, suppression of antioxidant defense system, activation of lysosomal hydrolases	Bemithyl, ethomerzol, thyetazol, antihypoxicants
8. Toxic hepatalgia induced by high doses of TCM, ethanol, PCB, DCE, POS occurring with suppressed LPO activity, liver functional-metabolic state	Desintoxified therapy, antidotes, antihypoxicants of direct energizing action

pyrimidine, complex combinations of pyrimidine derivatives with biologically active substances and their combinations with antidotes.

4. The differentiated approach to metabolic correction of chemically-induced liver pathologies due to lipid peroxidation process disturbances has been developed. In the mechanism of oxymethyluracil protective-restorative action, antiradical activity, an impact on the antioxidant protection enzymes, bioenergetic processes and the biologic membrane state are of great value.

REFERENCES

1. **Golikov S.N., Sanotskiy N.B., Tiunov I.A.** General mechanisms of toxic effect. – M.: Medicine, 1986. – 279 p.
2. **Ibatullina R.B., Myshkin V.A., Bakirov A.B.** A method of chronic hepatopathy modeling // RF invention patent N 2343556 of 10.01.2009.
3. **Ibatullina R.B., Myshkin V.A., Sergeeva S.A., et al.** Prophylactic efficiency of thyetazol and oxymethyluracil in chlorphenol exposures // Occupational health and industrial ecology. – 2002. – N5. – P.16–19.
4. **Ibatullina R.B., Myshkin V.A.** Antioxidant protective-restorative effects in experimental intoxication by chlorphenols // Occupational health and industrial ecology. – 2002. – N5. – P.28–31.
5. **Kryzhanovskiy G.N.** Basics of general pathophysiology. – M.: MIA, 2011. – 252 p.
6. **Myshkin V.A.** Antioxidant action and pharmacological properties of pyrimidine derivatives // Perspectives of bioorganic chemistry in the development of new medicines: All-Russia Symposium theses. – Riga, 1982, P. 214.
7. **Myshkin V.A., Khaibullina Z.G., Bashkatov S.A., et al.** The impact of methyluracil and oxymethyluracil on free-radical oxidation in model systems // Bulletin of experimental biology and medicine. – 1995. N8. – P.142–145.
8. **Myshkin V.A., Bakirov A.B.** Oxymethyluracil. Essays on experimental pharmacology. – Ufa, 2001. – 218 p.
9. **Myshkin V.A., Bakirov A.B.** Experimental correction of liver chemical damage by pyrimidine derivatives. Its effectiveness and mechanism of action. – Ufa, 2002. – 150 p.
10. **Myshkin V.A., Ibatullina R.B., Simonova N.I., Bakirov A.B.** A method of toxic hepatopathy modeling // Invention patent N 2188457 of 27.08.2002.
11. **Myshkin V.A., Ibatullina R.B., Savlukov A.I.** A method of liver cirrhosis modeling // Invention patent N 2197018 of 20.01.2003.
12. **Myshkin V.A., Ibatullina R.B., Volkova E.S., et al.** The use of sylimarine and α-tocopherol for correction of metabolic and morphofunctional disorders in rat liver intoxicated by polychlorbiphenyls // Toxicology Vestnik. – M.: 2004. N3. P. 30–33.
13. **Myshkin V.A., Gulyaeva I.A., Ibatullina R.B., et al.** The impact of actoprotectors on lipid peroxidation and the state of rat erythrocyte membranes under

carbophos intoxication // Pathological physiology and experimental therapy. – M., 2004. – N 3. – P.10–12.

14. **Myshkin V.A., Igbayev R.K., Ibatullina R.B., et al.** Combined pharmacorrection of hypoxia induced by experimental nitrate intoxication // University science: looking into the future. Proceedings of the KGMU jubilee conference. – Kursk, KGMU. – 2005. – V.2. – P.135–136.

15. **Myshkin V.A., Volkova E.S., Ibatullina R.B., et al.** Morphofunctional indicators of rat liver intoxicated by polychlorbiphenyls //Toxicology Vestnik – 2006. – N6. –P. 159–160.

16. **Myshkin V.A., Ibatullina R.B., Bakirov A.B.** Liver damage by chemicals: functional-metabolic damages, pharmacological correction. – Ufa: Gilem, 2007. – 177 p.

17. **Myshkin V.A., Bakirov A.B.** Polychlorbiphenyls and new models of liver pathology // RAMS Bulletin. – 2009. – N1(65). – P.255–259.

18. **Myshkin V.A.** Lipid peroxidation correction in experimental intoxications by a variety of chemicals. – Doctorate thesis. – Chelyabinsk. 1998. – 393 p.

19. **Myshkin V.A., Bakirov A.B.** Oxidative stress and liver pathology under chemical exposures. – Ufa, 2010. – 176 p.

20. **Ontshchenko G.G.** Urgent issues of chemical and biological safety // Up to-date problems of hygienic science and occupational health. Coll. of sci. papers. All-Russia scientific conference with international participation. – Ufa, 2010. – P.17–19.

21. **Sanotskiy I.V.** The problem of palliative protection measures under chemical exposures // Occupational health and industrial ecology. – 2007. – N2. – P. 10–14.