The state of antioxidant protection system in cows under the influence of heavy metals

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Abstract

A highly relevant problem of modern veterinary science is the study of features and mechanisms of combined action of the most common heavy metals – cadmium and plumbum and their influence on the body of humans and animals in the regions of Ukraine under technogenic pollution. The purpose of the work was to study the influence of heavy metals on the state of the antioxidant protection system of cows, in particular on the content of lipid peroxidation products (malonic dialdehyde, lipid hydroperoxides and diene conjugates), and activity of antioxidant enzymes (glutathione peroxidase and superoxide dismutase), depending on the distance to the mine heaps located in the Lviv-Volyn coal basin. The study objects were cows of black-and-white breed at the age of 3–7 years. It was established that this parameter in the place with the highest concentration of diene conjugates in the blood of cows was by 25.8 % higher compared to the place of low concentration and 12.1 % higher compared to the place with medium concentration. In the place with the highest content of lipid hydroperoxides in the blood of cows the parameter was 23.7 % higher compared to the cows from the place with the low content. The concentration of lipid hydroperoxides in the blood of cows from the place with the medium content was 16.7 % higher compared to the cows from the place with the low content. The parameter from the place with the lowest content of lipid hydroperoxides in the blood of cows was 12.1 % lower compared to the place with the highest content. The level of malonic dialdehyde in the blood of cows from the technogenic pollution zone in the place with the largest amount was higher by 36.2; 34.0 and 18.8 % – compared to places with medium and low levels, respectively. The activity of superoxide dismutase in the blood of cows in the place with its highest activity was 0.284 ± 0.0099 % block. reac/g Hb, and in the place with the lowest activity – 0.23 ± 0.0051 % block. reac/g Hb. The activity of glutathione peroxidase in the blood of cows in farms of the technogenic pollution zone depended on the distance to the mine. These researches will further develop effective methods of treating cows under the influence of heavy metals, in particular regarding the antioxidant system.

Keywords: cadmium; plumbum; diene conjugates; malonic dialdehyde; lipid hydroperoxides; glutathione peroxidase; superoxide dismutase.

Introduction

The increase of anthropogenic pollution, which began in the mid-twentieth century, is a real danger to all living organisms. The intensity of anthropogenic emissions, as a result of industrial and agricultural development, poses a risk to life in certain regions of Ukraine (Sliivinska, 2007; Sachko et al., 2016; Gutyj et al., 2017b; Hashemi, 2018). The dependence of animal and human diseases on adverse environmental changes has been noted by scientists in centuries past. However, at that time there was no concept and corresponding knowledge of biogeochemical processes, agroecosystems, so the nature of mass diseases related to the environment was difficult to explain. In disadvantaged ecosystems, various wastes were accumulated, and self-purification mechanisms were not capable of eliminating these processes, which disrupt the life of the living system and leads to its death (Zasiekin, 1999; Rahimi, 2013; Cunty et al., 2014; Gutyj et al., 2017a).

The increasing anthropogenic impact on ecosystems has led to pollution by heavy metals, which creates a number of important problems for world science: to prevent the spread, accumulation and control of their content in soil, water, feed, animals and animal products. Heavy metals are dangerous pollutants that through toxic stress cause various disturbances in the functional state of animals and humans. Getting into the body in small doses, for a long time, and accumulating in various organs and tissues, heavy metals can cause toxicosis, accompanied by disturbances of biochemical processes, structure and function of cells, including the permeability to chemical components of the internal environment (Velychko, 2007; Marenko et al., 2018; Sliivinska et al., 2019; Marenenko & Portiannik, 2019; Gutyj et al., 2019). Some heavy metals, and combinations thereof, which act specifically as poisons of individual functional groups, have a polytopic effect, inhibiting various biologically important groups. The combined action of toxic agents of different nature and modification with other environmental factors remains an ever increasing form of technogenic pressure on the animal and human body and the environment as a whole.

Increasing attention of researchers (Velychko, 2007; Abramov et al., 2009; Al-Attar, 2011; Bigalk et al., 2017) is being drawn to the study of the combined effect of heavy metals on the animal body as an ecopathogenic factor of the environment. One source of such action is mine heaps located in the Lviv-Volyn coal basin.

Due to the intense emissions of industrial enterprises, the pollution of the environment with cadmium and plumbum is constantly increasing. In biogeochemical cycles 300 000 tonnes of Pb and 2000 tonnes of Cd are annually involved. As a result, the contamination of the soil and food products that are grown on them with the experimental elements is also increased. The absorption of these elements in the digestive canal of animals is influenced by age, lactation, and composition of diet. In addition, some polyvalent cations (Ca, Zn, La) reduce the absorption of cadmium and plumbum by reducing their ability to interact with the receptors of apical membranes of enterocytes.

It is known (Hubskyi & Ersteniuk, 2002) that cadmium is able to accumulate in the erythrocytes and stimulate the formation processes of oxidative active forms and lipid peroxidation (LPO) in cells (Valko et al., 2005; Gutyj et al., 2016a). The level of metabolites of free radical processes, which normally flow in all tissues, is an indicator of the activity

of their metabolism. It is ensured by the rapid formation of oxygen active forms and by the antioxidant protection system. The action of oxygen active forms is manifested by free-radical pathology, which results in disruption of cell membranes structure, deoxyribonucleic acid, the accumulation of lipid peroxidation products, and also – their participation in the synthesis of prostaglandins, regulation of proliferation and cell differentiation (Pillai & Gupta, 2005; Goff, 2018). Under physiological conditions, there is a biological equilibrium between the antioxidant system and free radicals (Droge, 2002).

It has been established that an increase in the amount of hydroperoxides in erythrocyte membranes lead to an increase in methemoglobin content and its destruction. Under such conditions, oxidative stress develops, characterized by an imbalance between the rate of free radical oxidation and the activity of antioxidant systems. According to the literature (Shaidkh et al., 1999; Hubškyi & Enstienuk, 2002), the mechanism of destruction of methemoglobin involves several steps: the formation of an unstable intermediate form, possibly hemichrome; breaking the link between heme and globin; cleavage of Fe ions from the porphyrin ring. According to the literary data, the stage that limits this process is the dissociation of the hemoglobin complex. The close relationship between oxidative stress and heme metabolism in mammalian organisms is indicated (Casalino et al., 2002).

Plumbum and its compounds are highly cumulative poisons, which have the ability to exert toxic effects only if they penetrate into different cells and are characterized by slow excretion from body and lithropic action. The degree of damage to the cell itself, and accordingly to tissues, organs and the body as a whole, depends on how much the plumbum accumulates in these subcellular structures. In addition, it is known that even short-lived effects of low-concentration plumbum salts leads to the depletion of the antioxidant protection system, which is accompanied by the accumulation of lipid peroxidation products against the background of reduced activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. Cadmium stimulates the formation of lipid peroxidation products, reduces the activity of catalase and superoxide dismutase (Casalino et al., 2002).

In the mechanism of Cd^{2+} deleterious effect, stimulation of the formation of oxygen active forms and lipid peroxidation in cells plays an important role (Dong et al., 1998; Ercal et al., 2001; Gutyj et al., 2016b), which has a destructive effect on cell membranes and biopolymers proteins and nucleic acids (Waisberg et al., 2003). Cadmium not only inhibits the activity of antioxidant enzymes – catalase and superoxide dismutase (Casalino et al., 2002), but also converts glutathione to its inactive form. The toxic effect of cadmium reduces selenium content (Uetani et al., 2005; Sobolev et al., 2019), by forming inert complex compounds with it, and by preventing inactivation of glutathione transferase and glutathione peroxidase (Sihku et al., 1993).

The purpose of the work was to study the influence of heavy metals (plumbum and cadmium) on the state of the antioxidant protection system of cows, in particular on the content of lipid peroxidation products (malonic dialdehyde, lipid hydroperoxides and diene conjugates), and activity of antioxidant enzymes (glutathione peroxidase and superoxide dismutase), depending on the distance to the heaps of mines in the Lviv-Volyn coal basin.

Materials and methods

The study was conducted in the Lviv-Volyn coal basin (APC “Ukraine”, Grybovsytysa village, Bilychi village, Zabolotci village, Zastavne village of Ivanychy district of Volyn region). The study objects were cows of black-and-white breed at the age of 3–7 years with a productivity of 5000–5500 kg of milk. The animals were examined clinically by conventional methods (Gupta, 2005). Under physiological conditions, laboratory blood tests were performed. Blood was collected from 10 cows in each of the 4 settlements at different distances from the mines. Blood levels of parameters which characterize the state of lipid peroxidation and antioxidant system were determined by the following methods: malonic dialdehyde content – Korabjennikova (1989), lipid hydroperoxides – V. V. Mironchik (Levchenko et al., 2010); diene conjugates – Staľ’naja (1977); activity of glutathione peroxidase – Moin (1986) and superoxide dismutase – Dubinina et al. (1988).

The research was performed in accordance with the rules for the performance of zootechnical experiments for the selection and keeping of animals-analogues in groups, technology of harvesting, use and accounting of consumed feed. All manipulations with animals were carried out in accordance with the European Convention for the Protection of Vertebrate Animals, used for Experimental and Scientific Purposes (Official Journal of the European Union L276/33, 2010).

The mathematical processing of the research results was worked out statistically using a program package Statistica 6.0 software (Stat Soft, Tulsa, USA). Differences between the mean values were considered statistically significant at P < 0.05 (ANOVA, taking into account the Bonferroni Correction).

Results

Our studies have shown that the concentration of diene conjugates – byproducts of lipid peroxidation – in the blood of cows from APC “Ukraine” of Ivanychi district was in the range of 6.30–8.97 (7.65 ± 0.175) µmol/L. The average blood level of diene conjugates in cows from Grybovsytysa village was 9.62 ± 0.295 µmol/L, which is 25.8% higher than the parameter in cows from APC “Ukraine”.

The blood content of diene conjugates in cows from Bilychi village (8.46 ± 0.220 µmol/L) was 12.1% lower compared to Grybovsytysa village and at the same time it was 10.6% higher compared to animals from APC “Ukraine” (Fig. 1).

Concentration of diene conjugates in the blood of cows from Zabolotci village was almost at the same level as in Bilychi village 8.32 ± 0.209 µmol/L, and it was 10.9% higher, compared to APC “Ukraine”, and 13.5% lower compared to cows from Grybovsytysa village.

In cows from Zastavne village, the blood concentration of diene conjugates ranged 6.98–9.21 (8.13 ± 0.231) µmol/L. – 6.2% higher compared to APC “Ukraine”. The level of diene conjugates was 15.5% lower compared to cows from Grybovsytysa village. The difference with the diene conjugates in cows from other settlements was small (3.9% and 2.9% respectively).

The concentration of lipid hydroperoxides in the cows from APC “Ukraine” was 1.14 ± 0.042 with fluctuations 0.850–1.380 unE480/mL. Their content in cows from Grybovsytysa village was the highest and concluded 1.41 ± 0.097 unE480/mL, which is 23.7% higher compared to cows from APC “Ukraine”.

Concentration of lipid hydroperoxides in cows from Bilychi village ranged 1.10–1.48 (1.33 ± 0.038) unE480/mL, and it was 16.7% higher compared to cows from APC “Ukraine”.

In cows from Zabolotci village, the concentration of lipid hydroperoxides (1.31 ± 0.037 unE480/mL) did not differ from the previous parameter. The lowest content of lipid hydroperoxides was established in cows from Zastavne village – 1.24 ± 0.040 unE480/mL, which is 2.1% lower compared to Grybovsytysa village.

The average level of the final product of lipid peroxidation – malonic dialdehyde in cows from APC “Ukraine” was 2.47–4.15 (3.12 ± 0.122) mmol/L.
In cows, living closest to the mine of Grybovytsya village this parameter (4.90 ± 0.214 nmol/L) was higher (+57.1%) compared to APC “Ukraine”. In cows from Bilychi village the average content of malonic dialdehyde was 4.25 ± 0.099 nmol/L, from Zabolotci village – 4.18 ± 0.086, from Zastavne village – 3.98 ± 0.088 nmol/L. It was higher (by 36.2%, 34.0% and 18.8% respectively) compared to cows from APC “Ukraine”, and at the same time lower, compared to this parameter in livestock closest to the mine of Grybovytsya village.

Superoxide dismutase is the most important level of cellular protection, so determining the content of this enzyme is important for evaluating the whole system of antioxidant protection. The highest activity of superoxide dismutase was in the blood of cows from APC “Ukraine”: 0.256–0.322% block. reac/g Hb (0.284 ± 0.0099) (Fig. 4).

It should be noted that in cows from the area of technogenic pollution the enzyme activity depended on the distance of the mine to the settlement. Therefore, in cows from Grybovytsya village the activity of superoxide dismutase was the lowest and concluded 0.231 ± 0.0051% block. reac/g Hb with fluctuations 0.207–0.256%. The parameter was 18.7% lower compared with cows from the conditionally clean zone – APC “Ukraine”, and its maximum value in animals from Grybovytsya village (0.256% block. reac/g Hb) reached only the minimum value of enzyme activity in cows from APC “Ukraine”.

Superoxide dismutase activity in cows from Bilychi village was 0.254 ± 0.0090% block. reac/g Hb and 10.0% higher compared to cows from Grybovytsya village. However, the parameter was 10.6% lower compared to cows from APC “Ukraine”.

In cows from Zabolotci village the average activity of superoxide dismutase was 0.260 ± 0.0088% block. reac/g Hb (0.224–0.318), which is 8.5% lower compared to APC “Ukraine”, but 12.6% higher compared to cows from Grybovytsya village, and the difference with the parameter in cows from Bilychi village was insignificant (+2.4%).

It should be noted that the highest enzyme activity was in cows from Zastavne village, which is farthest from the mine. The average activity was 0.269 ± 0.0094% block. reac/g Hb (0.234–0.336). This parameter was 16.5% higher compared to Grybovytsya village, and only 5.3% lower compared to cows from APC “Ukraine”.

The activity of glutathione peroxidase in cows from the farms of the technogenic pollution zone depended on the distance to the mine: it was the lowest in cows from Grybovytsya village – 243.2 ± 6.60 μmol/min GSH/g Hb (212.0–270.0) – 25.0% lower, compared to APC “Ukraine” (Fig. 5).

Glutathione peroxidase activity was somewhat higher in cows from Bilychi village – 268.1 ± 4.26 μmol/min GSH/g Hb, which is higher (+10.2%) compared to cows from Grybovytsya village, and 17.3% lower compared to cows from APC “Ukraine”.

In cows from Zabolotci village glutathione peroxidase activity was 275.3 ± 2.82 μmol/min GSH/g Hb. The parameter was 15.1% lower compared to APC “Ukraine”, but 13.2% and 2.7% higher compared to cows from Grybovytsya and Bilychi village respectively.

Glutathione peroxidase activity was the highest in cows from Zastavne village – 281.1 ± 4.34 μmol/min GSH/g Hb, but at the same time it was 13.3% lower compared to cows from APC “Ukraine”. Nevertheless, the activity of glutathione peroxidase was higher 15.6% and 4.8%, respectively, compared to cows from Grybovytsya and Bilychi village. The difference with the parameter in cows from Zabolotci village was insignificant (+2.1%) (Fig. 5).
tis, mastitis etc.) (Gutyj et al., 2019; Grymak et al., 2020). However, there has been insufficient research on the role of lipid peroxidation in cattle from the areas under the technogenic pollution. At the same time, various changes in the oxygen regime of the cells cause the activation of lipid peroxidation and avalanche accumulation of toxic products.

The significant increase in the content of lipid hydroperoxides is indicated by severe lesions and decreased functional capacity of the body’s antioxidant system. Increasing the negative impact of lipid peroxidation products on the body causes the pathological process to spread to adjacent areas of cell membranes and damages them.

At the point of attachment of peroxide radicals, the fatty acids are broken into fragments at the edges of which are placed aldehyde groups with high reactivity. If the gap passes on both sides, a malonic dialdehyde is formed (Salvatori et al., 2004), which indicates the rate of lipid peroxidation and is one of the indicators of activation of lipid peroxidation processes or reduction of the body’s antioxidant protection.

The increase in the level of intermediate and final products of lipid peroxidation is due to the fact that in the mechanism of chronic hematuria development stimulation of lipid peroxidation plays an important role. The oxygen active forms include free superoxide-anion-radical (O$_2^-$), singlet-oxygen ($\mathrm{O}_2^*$), perhydroxide radical ($\mathrm{HO}_2^-$), hydrogen peroxxide ($\mathrm{H}_2\mathrm{O}_2$), hydroxide radical (OH$^-$). The first two compounds of oxygen active forms are of the greatest importance in lipid peroxidation. In addition, the balance between the activity of oxidants and antioxidants is disturbed, leading to the development of oxidative stress (Shcherbatyi & Slivinska, 2013; Gutyj et al., 2017b). Oxygen active forms are intermediate products of aerobic metabolism, the formation intensity of which in cells is increased by pathological processes and the impact of adverse environmental factors. Oxidative properties of oxygen active forms cause them to participate in various biochemical processes, transduction of regulatory signals in the cell, gene activation, proliferation, differentiation, aging and cell apoptosis.

The processes of lipid peroxidation in the tissues of cows from zones under technogenic pollution are more intense, and therefore in the blood levels of lipid peroxidation products (malonic dihydrole, diene conjugates, lipid hydroperoxides) is higher.

The body constantly produces active forms of oxygen, not as a by-product of metabolism, but as a full accomplice of cellular metabolism. Free-radical oxidation is a prerequisite for cell life, and the whole antioxidant protection system is designed to maintain the intensity of free-radical processes at optimum levels without their sharp activation (Alonso et al., 2004; Gutyj et al., 2017b).

Under the conditions of activation of lipid peroxidation processes, the functional activity of intracellular protective systems plays an important role. First of all, they include a system of antioxidant protection, which is represented by a complex of non-enzymatic antioxidants and specialized enzymes – antioxidants. An antioxidant protection system is a system that is responsible for regulating the intensity of radical formation and the elimination of peroxidation products (Valko et al., 2005).

The body has a system of protection against the action of oxygen active metabolites, which include low molecular weight antioxidants and specialized antioxidant enzymes. Shifting the equilibrium between oxygen active forms and antioxidants toward increasing the formation of oxygen active forms is a potential prerequisite for the development of oxidative stress in biological systems, which plays a leading role in the pathogenesis of many diseases. It is evident that the action of oxygen active forms will lead to the adaptive synthesis of enzymes of the antioxidant protection system (Droge, 2002). However, rapid replenishment of enzyme molecules in erythrocytes is not possible due to the lack of a protein biosynthesis system in these cells. Therefore, it is obvious that erythrocytes are particularly sensitive to the effects of oxidative stress in cows with anemia, including the influence of heavy metal salts, it is known that they, in particular cadmium and plumbum, stimulate the formation of oxygen active forms and lipid peroxidation products in cells that have a destructive effect on cell membranes and biopolymers – proteins and nucleic acids (Nehru & Bansal, 1997). Many scientists have devoted their attention to the study of their effects on lipid peroxidation and the antioxidant defense system, but they are mainly performed on laboratory animals and birds (Valko et al., 2005). Therefore, it was important to study these processes in cows with anemia in the area of technogenic pollution with cadmium and plumbum.

According to the results of our research, the concentration of primary product of lipid peroxidation – lipid hydroperoxides, increases in the blood of cows. It was higher in cows of all settlements (Fig. 2). The primary products of lipid peroxidation are unstable and break down with the formation of secondary ones – alcohols, aldehydes, ketones, epoxides, which, even more than lipid hydroperoxides, disrupt the function of biological membranes. Among them, the most studied is malonic dihydrole. Its content increases in the blood of cows in all settlements of the technogenic pollution zone (Fig. 3). By reacting with the SH and CH$_3$ groups of proteins, malonic dihydrole inhibits the activity of enzymes, in particular cytochrome oxidase, thereby inhibiting tissue respiration. By reacting with amino groups of proteins, malonic dihydrole alters the elastic fibers structure of the lung tissue. Lipid peroxidation products oxidize sulfhydryl groups of proteins, damage deoxyribonucleic acid, can slow and even stop cell division (Valko et al., 2016).

However, it should be noted that lipid hydroperoxides, diene conjugates, and malonic dihydrole are not cellular slag, but normal cell metabolites. Only end products of lipid peroxidation exhibit toxic effects on cell membranes and subcellular structures (Borysiewycz et al., 2006; Stybel et al., 2017; Kovalenko et al., 2020).

Among the components of the antioxidant protection system there are three groups: a) enzymes of antioxidant protection; b) low molecular weight antioxidants synthesized in the body (glutathione, uro acid); c) nature antioxidants that enter the body with food (vitamins C, E, P, carotenoids, flavonoids, etc.). The concerted function of all components of antioxidant protection supports the formation and transformation of free radicals and other potentially dangerous compounds at a constant level (Jornova et al., 2010; Gutyj et al., 2017b; Zhukova et al., 2017).

Enzymes of antioxidant protection system, the level of which the cell maintains through continuous synthesis, repeatedly exceed the specific antioxidative activity of non-protein components and perform the main antioxidative function in the cell. These include superoxide dismutase, which catalyzes the reaction of superoxide anion radical dismutation in H$_2$O$_2$, a catalase that hydrolyzes hydrogen peroxide, glutathione-dependent peroxidases and transferases (Slivinska et al., 2018a). In a healthy body, there is a dynamic equilibrium between the formation of lipid peroxidation products and the antioxidant protection system. However, the influence of cadmium and plumbum salts disturbs this equilibrium. We have studied the enzyme link of antioxidant protection: the activity of superoxide dismutase, which catalyzes the reaction of dismutation of the superoxide anion radical in H$_2$O$_2$, glutathione-dependent peroxidases and reductases. Superoxide dismutase is the most important level of cellular protection. There are three isoforms of the enzyme: cytosolic – C, Zn-superoxide dismutase, mitochondrial – Mn-superoxide dismutase and extracellular – E-superoxide dismutase (Cu, Zn-superoxide dismutase) (Ota et al., 2017).

The activity of superoxide dismutase in the blood of cows from zones under technogenic pollution decreases, that is, the ability of the body to respond adequately to the formation of oxygen active forms is reduced.

It is evident that in the body of cows there is a lack of low-molecular-weight antioxidants (phenolic antioxidants, sulfur-containing compounds, carotenoids, and vitamins A, E, and C) that are required for the effective function of superoxide dismutase (Pavan & Prasad, 2004; Roggeman et al., 2014; Gutyj et al., 2016b).

The enzyme that controls the level of H$_2$O$_2$ in cells is glutathione peroxidase. In addition to H$_2$O$_2$, it catalyzes the reaction of hydrolysis of fatty acid peroxides as well as protein and nucleic peroxides. Its activity in cows of settlements subjected to technogenic pollution is reduced (Fig. 5).

We established a direct correlation between the activity of superoxide dismutase and lipid hydroperoxides ($r = 0.665$), a high degree between the activity of superoxide dismutase and the concentration of diene conjugates and malonic dihydrole ($r = 0.828$ and 0.875 respectively), glutathione peroxidase and lipid hydroperoxides ($r = 0.690$), glutathione peroxidase and diene conjugates and malonic dihydrole ($r = 0.855$ and 0.837 respectively). The correlation coefficient calculations confirm that the activity of antioxidant protection system enzymes is reduced by the action of radioactive elements and heavy metal compounds.

**Conclusion**


