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

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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 (Slivinska, 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; not capable of eliminating these processes, which disrupt the life of the environment with cadmium and plumbum is constantly increasing. Increasing attention of researchers (Velychko, 2007; Abramov et al., 2009; Al-Attar, 2011; Bigalke 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 & Erstenik, 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

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

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 place 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 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 0.28 ± 0.009 % block. rea/(g Hb, and in the place with the lowest activity – 0.23 ± 0.005 % block. rea/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.
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 (Shalik et al., 1999; Hubskyi & Ersteniuk, 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 mechanisms of Cd²⁺ deletory effect, stimulation of the formation of oxygen active forms and lipid peroxidation in cells plays an important role (Dong et al., 1998; Erical et al., 2001; Gutyi 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 (Sidhu 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”, Grybovytsya village, Bilychi village, Zabolotci village, Zastavne village of Ivanychi 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 counting 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 research was performed in accordance with the rules for the performance of zootechnical experiments for the selection and keeping of animals-anaugologs 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 Grybovytsya 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 Grybovytsya 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 Grybovytsya 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 Grybovytsya 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 Grybovytsya village was the highest and concluded 1.41 ± 0.097 unE₄₈₀/mL, which is 23.7% higher compared to cows from APC “Ukraine”. The 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 (1.51 ± 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 Grybovytsya 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) nmol/L.

Fig. 1. The concentration of diene conjugates in the blood of cows from the areas under technogenic pollution (µmol/L, x ± SE, n = 10)
In cows, living closest to the mine of Grybovtsya village this parameter (4.90 ± 0.214 μmol/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 μmol/L, from Zabolotci village – 4.18 ± 0.086, from Zastavne village – 3.98 ± 0.088 μmol/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 Grybovtsya 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. In cows from Grybovtsya village the average activity of superoxide dismutase was 0.269 ± 0.0099% 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 Grybovtsya 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 Grybovtsya 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 Grybovtsya 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 Grybovtsya village, and the difference with the parameter in cows from Bilychi village was insignificantly (+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 Grybovtsya 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 Grybovtsya 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 Grybovtsya 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 Grybovtsya 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 cows from APC “Ukraine”. Nevertheless, the activity of glutathione peroxidase was higher 15.6% and 4.8%, respectively, compared to cows from Grybovtsya and Bilychi village. The difference with the parameter in cows from Zastavne village was insignificant (+2.1%) (Fig. 5).

Discussion

According to the literature data (Wood, 2004), the course of any pathological process in the body depends on the intensity of lipid peroxidation. The intensification of lipid peroxidation is considered (Pavan & Prasad, 2004; Valko et al., 2005; Gutyj et al., 2016b) as one of the complex mechanisms of disorganization of the structural and functional integrity of various biological substances. The processes of lipid peroxidation, on the one hand, can be considered as a non-specific adaptive reaction of the body, on the other – they lead to damage of cell membranes, inhibition of enzyme activity. The accumulation of lipid peroxides is accompanied by damage to membranes, first of all their molecular structure (Peng et al., 2015; Slivinska et al., 2018b). The antioxidant protection system is one of the key regulatory systems of the animal body, as it counteracts the processes of lipid peroxidation and thus helps to preserve the structural characteristics of membranes (Velychko, 2007; Abramov et al., 2009; Gutyj et al., 2019).

Today a considerable number of reports is dedicated to the role of lipid peroxidation in the development of many diseases of noninfectious and infectious etiology (encephalomalacia and exudative chicken disease, white muscle disease, gastrointestinal, respiratory and metabolic diseases, in particular microelementosis, kidney and liver damage, endometriosis).
on cell membranes and biopolymers—proteins and nucleic acids (Nehru et al., 2019). 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 in erythrocytes is not possible due to the lack of a protein biosynthesis system (Droge, 2002). However, rapid replenishment of enzyme molecules is responsible for regulating the intensity of radical formation and the 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 antioxidant enzymes. 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 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).

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 dialdehyde, 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).

The increase in the level of intermediate and final products of lipid peroxidation is due to the fact that in the mechanism of chronic hemorrhage development stimulation of lipid peroxidation plays an important role. The oxygen active forms include free superoxid-anion-radical (O$_2^-$), singlet-oxygen (O$_2^*$), perhydroxide radical (HO$_2^*$), hydrogen peroxide (H$_2$O$_2$), hydroxyl 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 & Silvinska, 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.

Regul. Mech. Biosyst., 2020, 11(2)
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