TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2020, vol. 5, No 1(8): 48–52

BlOOD ANTIOXIDANT/OXIDANT PARAMETERS IN RATS EXPERIMENTALLY INFECTED WITH *FASCIOLA HEPATICA* AND EXPOSED TO CADMIUM

V. Nanev, I. Vladov, M. Gabrashanska

Institute of Biology and Immunology of Reproduction „Acad. K. Bratanov , Bulgarian Academy of Sciences, Sofia, Bulgaria
E-mail: veselinnnanev@gmail.com

ABSTRACT

The aim of this study was to investigate antioxidant/oxidant status of blood of rats experimentally infected with *Fasciola hepatica* combined with chronic cadmium administration. Parameters of antioxidant/oxidant status were malondialdehyde (MDA), superoxide dismutase (CuZnSOD) and glutathione peroxidase (GPx) in blood of rats experimentally infected with *Fasciola hepatica*. The level of cadmium (Cd) was determined as well. The data showed that oxidative/ antioxidative imbalance was developed due to an increased MDA level, reduced enzyme activity in double treated animals compared to control animals and these with only one treatment. Cd level was significantly increased in all rats received Cd (non-infected or infected with *F. hepatica*). Our study leads us to conclude that co-exposure to Cd and helminths causes a more pronounced increase in the blood oxidative stress in the hosts.

Key words: cadmium, *Fasciola hepatica*, oxidative/antioxidative imbalance.

Introduction

Heavy metals are non-degradable environmental pollutants that can negatively affect the human and animal health. One of them is cadmium (Cd), which is a common environmental pollutant with widespread distribution and highly toxicity. Cadmium levels in environment have increased substantially during the last decades. Lower total antioxidant capacity, high lipid peroxidation and deviations in the activity of antioxidant enzymes have been reported for experimental rat models of Cd- toxicity (Erdem et al., 2016). Parasites are widely distributed in animals and humans and can interfere with bioindicative processes including oxidative parameters in their host. Under environmental conditions organisms are exposed not only to parasites but are also confronted with a variety of other endogenous and exogenous factors. The information about a combined effect of helminths and Cd on oxidative status of animals is very short.

Fasciolosis is a neglected water- and food-borne disease. It is an economically important helminth disease, caused by two trematode species: *Fasciola hepatica* (Linnaeus, 1758) and *Fasciola gigantica* (Cobbold, 1855). Fasciolosis has an important worldwide distribution due to parasite proliferation in a wide range of freshwater snail species and domestic as well as wild mammals, including human.

It is known that parasites strongly interact with pollutant- induced biomarker responses of their hosts by influencing their physiology in a multitude of different ways. The host responds to *F. hepatica* infection stimulating immune cells and activating reactions associated with the generation of reactive oxygen species, including superoxide radicals, causing oxidative stress (Bottari et al. 2015).

Commonly markers used in oxidative stress assessment include the evaluation of oxidant parameter (malondialdehyde, MDA) and antioxidant enzymes (Superoxide dismutase (CuZnSOD),

Glutation peroxidase (GPs) and catalase (CAT). Reactive oxygen species (ROS) - mediated oxidative damage is believed to be an important mechanism of Cd – mediated toxicity. Cd is antagonistic to bioelements copper (Cu) and zinc (Zn) and competes with them (Erdem et al., 2016). Many studies were done on the acute effects of Cd intoxication on the antioxidant status but not on chronic exposure to low dose of Cd combined with helminthoses, which is similar with environmental exposure.

The aim of this study was to evaluate antioxidant/oxidant status of blood of rats infected experimentally with *Fasciola hepatica* combined with oral chronic cadmium administration.

Materials and methods

The experiment was carried out on 32 male Wistar albino rats aged 30 days, divided into 4 groups: The control group with healthy animals is a group 1; a group 2 – *F. hepatica* infected rats ; groups 3 – rats with Cd administration and the group - 4 rats experimentally infected with *F. hepatica* and Cd administration. Every one control or experimental group consists of 8 animals. The animals were kept under standard laboratory conditions (temperature 22± 2ºC, relative humidity 50±10%, natural light-dark cycle). They were fed with commercial standard pellet diet. Food and water were available *ad libitum*.

The laboratory animals were orally infected with 15 viable *F. hepatica* L. encysted metacercariae per animal, suspended in dechlorinated water and passed through a stomach tube on the 1st day of the experiment. Metacercariae were obtained from experimentally cultivated snails *Galba truncatula* after experimental infection with 5 miracidiae per snail, hatched from eggs of mature *F. hepatica* L. The freshwater snails from family Lymnaeidae *G. truncatula* are intermediate hosts of *F. hepatica* L.

Rats were exposed to Cd salt dissolved in drinking water in a dose of 15ppm, after 2 week post infestation. $CdCl_2$ was administered 5 days /a week for 4 weeks. The experiments were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations.

The rats were euthanized by anesthesia on the 35th day post infestation. The blood was collected for the further biochemical investigations. Measurements of MDA, CuZnSOD and GPx were determined in blood. Malondyaldehide (MDA), as a lipid peroxidation product was measured by thiobarbituric acid reactive substances according Londero & Lo Greco (1996).

Blood Cd level was determined using ICP-OES Prodigy 7 Teledyne Leeman Labs USA. CuZnSOD activity was established according the method of Misra & Fridovich (1972). GPx was determined according the modified method of Hafeman et al., (1974).

The statistical analysis was carried out on the Prism 4 programme. The distribution of data was determined- a Gaussian one. The determination of the distribution was performed using the test of Kolmogorov- Smirnov and D`Agostino- Pearson. In the Grubb test application, no extreme value have been found (they are strongly differing from the mean one, usually neglible. Variation analysis was used for determining the mean values, the standard deviation (SD) and the significance criterion (P) of four groups. The comparison of the mean values of these parameters was carried out using the Student's t- criterion, Tukey's Multiple Comparison Test.

Results

Parameters of oxidant/antioxidant status are presented in Table 1:

Table 1:

	Serum MDA mmol/L	CuZnSOD (blood) U/ml	GPx (blood) U/ml	Cd blood µg/L
Control	4,85±0,65	115,12±10,20	816,50±44,20	0,96±0,10
Infected	6,17±0,30	81,20±5,16	720,20±30,15	0,95±0,70
Control+Cd	6,70±1,10	83,45±11,22	681,66±18,70	4,50±2,20
Infected+Cd	7,10±1,05	89,00±9,60	722,40±26,10	4,10±1,80

The serum MDA content is significantly increased in rats infected with *F. hepatica* compared with the control level. Similar MDA increase is observed in rats treated with Cd. MDA content in serum from rats with a double treatment (Cd and parasite) is slightly elevated compared to this in rats with only Cd – administration or infected with *F. hepatica*.

CuZnSOD activity is significantly reduced in rats received Cd as well as in infected rats compared to control. The differences between both groups was non-significant. Reduced CuZnSOD activity is seen in the group with combined treatment compared to control but it is higher than that in group 2nd and in group 3rd.

Blood Cd concentration is not changed in infected rats but is significantly increased in groups with only Cd administration and with dual impact (Cd and *F. hepatica*).

Discussion

Blood is the best indicator of internal exposure of an individual to Cd. A lot of studies demonstrated that in rat experimentally infected with *Fasciola hepatica* a high production of reactive oxygen species (ROS) was developed causing oxidative stress (Kolodziejczyk et al., 2006; Anisimova & Gabrashanska, 2007). Increased lipid peroxidation and disturbed antioxidant status were observed in the experimentally infected rats. The increased MDA level is an indication of increased Lipid peroxidation (LPO) oxidative cell injury may occur in the course of fasciolosis. The increased MDA concentration in Cd-treated groups could be due to decrease activity of the defense system protecting tissues from ROS – damage. Rat experimental studies focused on the combined effect of heavy metals and parasites on the antioxidant defence system of the host are very important for pathogenesis of parasitosis. In our experiment rats infected with *F. hepatica* and administered with Cd salt showed an imbalance in antioxidant status compared with those in non-intoxicated rats. The decrease activity of CuZnSOD proved that Cd indeed induced generation of ROS leading to increase ptoduction of MDA which was much higher than the level which could be compensated by the cellular defense systems. So that these compounds may not be converted to less harmful or ineffective metabolites at the sufficient levels (Valko et al., 2005).

The experimentally infected rats only with *F. hepatica* (gr. 2) developed antioxidant disturbances. They were manifested by reduced activity of the main antioxidant enzymes such as CuZnSOD. It was shown that the infection with *F. hepatica* was accompanied by rising level of the superoxide radical (Bottari et al., 2015). Enhancement of lipid peroxidation was observed, shown by MDA and was seen 4 weeks p i. Our finding showed that reduced antioxidant capacity and enhanced generation of ROS are most pronounced during acute stage of fasciolosis.

Oxidant and antioxidant parameters were influenced after Cd exposure. It is an adaptive process protected the cell from toxic effects of free Cd. Cd induce oxidative effects and lipid peroxidation by either depletion of glutathione or inhibition of antioxidant enzymes CuZnSOD and GPx (Facino et al., 1993; Jomova & Valko, 2011). Our data showed that exposure to Cd induced imbalance of antioxidant enzymes CuZnSOD and GPx. Free radical damage after Cd administration was demonstrated by increased MDA level. Activity of CuZnSOD and GPx was reduced in the supplemented animals compared to the controls. CuZnSOD activity may be reduced as a result of the response against an oxidative challenge. Over accumulation of Cd in blood resulted in a decrease of Zn and Cu, which are in an active sites of SOD. This alterations and interactions have been reported by Nzengue et al., (2011) and Erdem et al., (2016). Our results are consistent with the last authors as well as with Renugadevi & Prabu (2010), who suggested that oral exposure to Cd for 4 weeks had induced decreased levels of CuZnSOD and GPx enzyme. Increased blood Cd concentration clearly suggests that toxic manifestation are due to increased Cd absorption. It is consistent with earlier finding by Valko et al., (2005). Helminths may be induced gastrointestinal permeability modulations and they may contribute to increased Cd absorption and finally high blood Cd burden. Synergistic reactions which may be attributed to 1. high body Cd burden; 2. essential metal deficiency (Ca, Mg) caused due to helminthic exposure; 3. Cd and helminths facilitate ROS production her independently or though common mechanisms causing oxidative stress and GSH depletions either via direct thiol binding or via ROS (Jomova & Valko, 2011).

Conclusion

Our study leads us to conclude that co-exposure to Cd and *F. hepatica* causes a more pronounced increase in oxidative stress. It shows that increased of free radicals generation due to dual impact of Cd and *F. hepatica*, causes disturbances in the body metabolism including oxidant-antioxidant imbalance in the host in a higher degree.

Acknowledgements

This work was supported by the Bulgarian Ministry of Education and Science under project DN -14/7.

References

1. Anisimova, M., M. Gabrashanska. (2007). *Antioxidant status in rats after Fasciola hepatica infection and metal compounds treatment*. XIV. Aktualne Problemy Humannej Parazitologie. Proceedings book. (Eds. R. Holkova, I. Tomo, A. Totkova, M. Klobusicky), Bratislava. 2007, 23–27.
2. Bottari, N., R. Mendes, N. Lucca, C. Schwertz, I. Henker, D. Olsson, A. S. Da Silva. (2015). *Oxidative stress associated with pathological lesions in the liver of rats experimentally infected by Faciola hepatica*. Experimental Parasitology, 159:24–28.
3. Erdem O., N. Yazihan, M. K. Kocak, A. Sayal, Ethem Akcil. (2016). *Influence of chronic cadmium exposure on the tissue distribution of copper and zinc and oxidative stress parameters in rats*. Toxicology and Industrial Health, 32(8): 1505–1514.
4. Facino, M., M. Carini, G. Aldini, R. Ceserani, I. Ceserani, Cavaletti, L. Vederio. (1993). *Efficacy of glutathione for treatment of fascioliasis. An investigation in the experimentally infected rat*. Drug Res, 43: 455–460.

5. Hafeman D. G, Sunde R. A., Hoekstra W. G. (1974). *Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat*. Journal of Nutr., 1974;104:580–7.
6. Jomova K., M. Valko. (2011). *Advances in metal-induced oxidative stress and human disease*. Toxicology, 283 (2–3): 65–87.
7. Kolodziejczyk L, E. Siemieniu, E. Skrzydlewska. (19950. *Antioxidant potential of rat liver in experimental infection with Fasciola hepatica*. Parasitol Res, 96: 367–372.
8. Londero D., P. Lo Greco. (1996). *Automated high-performance liquid chromatographic separation with spectrofluorometric detection of malondyaldehyde-thiobarbituric acid adduct in plasma*. Journal of Chromatography 729: 207–210.
9. Misra F., I. Fridovich. (1972). *The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase*. J Biol Chem 247: 3170–3175.
10. Nzengue Y, S., Candeias, S., Sauvaigo. (2011). *The toxicity redox mechanisms of cadmium alone or together with copper and zinc homeostasis alteration: its redox biomarkers*. Journal of Trace elements in Medicine and Biology, 25: 171–180.
11. Renugadevi J., S. M. Prabu. (2010). *Cadmium –induced hepatotoxicity and renal toxicity and protective effect of naringenin*. Experimental Toxicology and Pathology, 62: 171–181.
12. Valko M, M. Morris, M. Cronin. (2005). *Metals, toxicity and oxidative stress*. Curr. Med. Chem. 12 (10): 1161:1208.