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TOTAL ANTIOXIDANT STATUS – A POSSIBLE MARKER OF ENVIRONMENTAL INFLUENCES ON ANIMAL ORGANISMS

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ABSTRACT

Environmental influences on human and animal health status are in the scope of modern biomedical research. In the present work the effects of two environmental factors – heavy metal intoxication and gamma-irradiation were assessed to evaluate the applicability and availability of total antioxidant status (TAS, one of the oxidative stress parameters) as a possible marker of the influence of environmental factors on animal organisms. We found out that TAS is a very sensitive parameter, which may be used as a marker of some environmental influences (heavy metal intoxication, gamma-irradiation), as well as their time- and dose-dependent effects in animals.

Key words: total antioxidant status; heavy metal intoxication; gamma-irradiation

INTRODUCTION

The environmental contamination and its consequences on living organisms are in the forefront of the scientific as well as laic interest (Nováková et al., 2007). The effects of various environmental pollutants on human and animal organisms were analysed in details in many papers. At present, very important issue is a finding of sensitive parameters for early recognition of these harmful effects.

Various possible mechanisms have been suggested to explain the damage induced by an environmental pollution including oxidative stress.

Reactive oxygen species are produced in living organisms via many reactions, which are necessary for the maintenance of normal metabolism (respiratory chain in mitochondria, arachidonic acid metabolism). On the other hand, reactive radicals may be produced in many pathological processes (inflammation, reperfusion injury), as well as under the influence of exogenous factors (radiation, toxins) (Han et al., 2005).

Living organisms have developed a complex antioxidant network to counteract reactive species (Leonard et al., 2004). Plasma is not a simple chemical system as regards antioxidant activity. In particular, non-enzymatic antioxidants, such as albumin, alpha-tocopherol, ascorbic acid, uric acid, glutathione, bilirubin and flavonoids form a network of plasma antioxidants. It is essential to measure these antioxidants in assessing *in vivo* the antioxidant status. However, the number of different antioxidants in plasma makes it difficult to measure each antioxidant separately. Possible interactions among them could also make a measurement of any individual antioxidant less representative of the overall antioxidant status. Therefore, several methods have been developed to determine the antioxidant capacity of

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various biological samples (Cao et al., 1998).

The method of total antioxidant status, reported first by Miller et al. (1993), has been developed for the measurement of antioxidant activity of pure antioxidants or drugs that can be water-soluble or body fluids. Contribution of human plasma antioxidants expressed in percentage of TAS is represented in Fig. 1. The principle of this method is based on peroxidase activity of met-myoglobin. Following exposure to hydrogen peroxide, met-myoglobin is oxidized to ferrylmyoglobin, which oxidizes compound ABTS (2,2´- Azino-di-[3-ethylbenthiazoline sulphonate]) to a relatively long-lived radical cation $ABTS^{\bullet+}$. It has blue-green colour, which is measured at 600 nm. Antioxidants in the added sample suppress this colour production to a degree that is proportional to their concentration. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is standard in this method. The unit of the TAS is Trolox equivalent antioxidant capacity (TEAC), which is defined as the concentration (mmol/l) of Trolox having the equivalent antioxidant capacity to a 1.0 mmol/l solution of the substance after investigation.

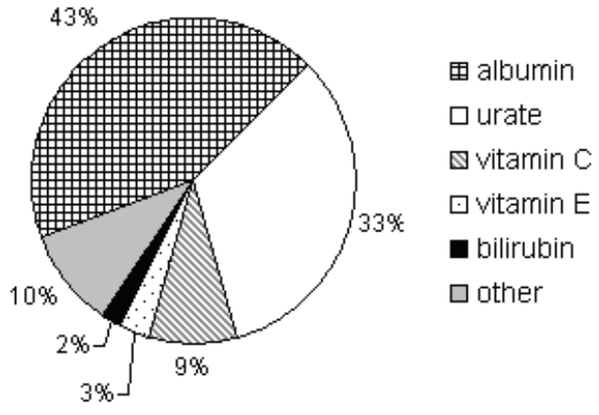


Fig. 1: Antioxidant content of blood plasma in percentage of TAS (Miller et al., 1993)

MATERIALS AND METHODS

Experiment 1: heavy metal intoxication

One hundred and thirteen Wistar albino rats of 3-month age (91 ± 2 days) at average weight of 308 ± 34 g were used for the experiment. The animals were housed under conventional conditions and they were fed *ad libitum* with a commercial fodder for laboratory rats. The experiment was organized in two series.

Fifty-three rats (age - 91 ± 2 days, weight - 318 ± 29 g) were used in the first series of experiments. The animals were divided into 6 groups, each of 8 to 10 animals. Cadmium was given to the experimental groups as a cadmium chloride compound ($CdCl_2$ x $2H_2O$, Sigma) in the tap water at doses ranging from 1.83 to 29.27 $mg.kg^{-1}.day^{-1}$ for 30 days (Tab. 1).

For the second series of experiments 60 rats were used (age 91 ± 2 days, weight 298 ± 35 g). The rats were divided into 6 groups, each of 10 animals. Mercury was administered to the experimental groups as a mercury chloride compound ($HgCl_2$, Lachema, Brno) at doses from 0.15 to 2.47 $mg.kg^{-1}.day^{-1}$ for 30 days (Tab. 2).

The rats of control groups (CdC, HgC) were drank with a pure tap water.

After 30 days of the treatment, rats from all groups were anaesthetized (Sodium pentobarbital, Pentobarbital Spofa, 50 $mg.kg^{-1}$ i.p.). The blood was collected from the heart into heparinized Eppendorf tubes. Samples were centrifuged at 1500 g for 10 min and the blood plasma was used for the TAS assay.

Experiment 2: gamma irradiation

For the second experiment 57 chickens (broiler crossbreeds, age 28 days) were used. The chickens were divided into two experimental (each n = 24) and one control (n = 9) groups. The experimental, as well as the control birds, were fed with a chicken fodder BR_2 and water was provided *ad libitum*. The experimental birds were irradiated by gamma rays (^{60}Co source, Chisostat, Chirana, SSD = 95 cm) at a dose rate 0.3 $Gy.min^{-1}$, using whole body irradiation. The first experimental group (n = 24) was exposed to a total dose of 2 Gy and the second one (n = 24) was exposed to a total dose of 5 Gy of gamma rays. The irradiated chickens were examined in groups of 6 chickens 6, 24, 48 and 72 hours after the irradiation.

Blood was collected immediately after decapitation into heparinized Eppendorf tubes. Samples were centrifuged at 1500 g for 10 min and the blood plasma was used for the TAS assay.

The total plasma antioxidant status was determined by a spectrophotometric assay (Miller et al., 1993) using RANDOX-Kit (Total antioxidant status, RANDOX laboratories, UK). Measurements were carried out on an automatic spectrophotometric analyser Cobas Mira S (Roche, Switzerland).

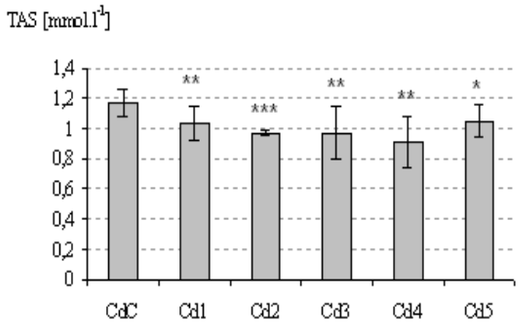
The statistical significance of differences between the groups was determined by Turkey-Kramer ANOVA test; p-values of less than 5 % ($p < 0.05$) were considered as significant.

RESULTS

Data presented in Tab. 1 and Fig. 2 show that daily administration of cadmium chloride for 30 days led to a significant reduction of plasma TAS in all cadmium-treated groups compared to the control group.

Table 1: Dose of $CdCl_2$ a and TAS in Cd-treated rats

Exp. group	n	Dose of $CdCl_2$ $mg.kg^{-1}.day^{-1}$	TAS $mmol.l^{-1}$
CdC	8	-	1.17 ± 0.09
Cd1	10	1.83	1.03 ± 0.11
Cd2	8	3.66	0.97 ± 0.02
Cd3	9	7.32	0.97 ± 0.17
Cd4	9	14.63	0.91 ± 0.17
Cd5	9	29.27	1.05 ± 0.11

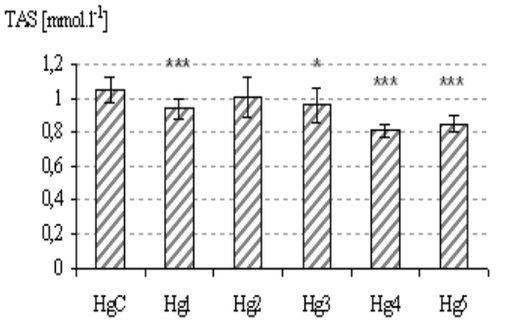


*- p < 0.05, **- p < 0.01, ***- p < 0.001 compared to the control group

Fig. 2: The TAS changes after $CdCl_2$ administration in rats

Table 2: Dose of $HgCl_2$ and TAS in Hg-treated rats

Exp. group	n	Dose of $HgCl_2$ $mg.kg^{-1}.day^{-1}$	TAS $mmol.l^{-1}$
HgC	10	-	1.05 ± 0.08
Hg1	10	0.15	0.94 ± 0.06
Hg2	10	0.31	1.01 ± 0.12
Hg3	10	0.62	0.96 ± 0.10
Hg4	10	1.23	0.81 ± 0.04
Hg5	10	2.47	0.85 ± 0.05



* - p < 0.05, *** - p < 0.001 compared to the control group

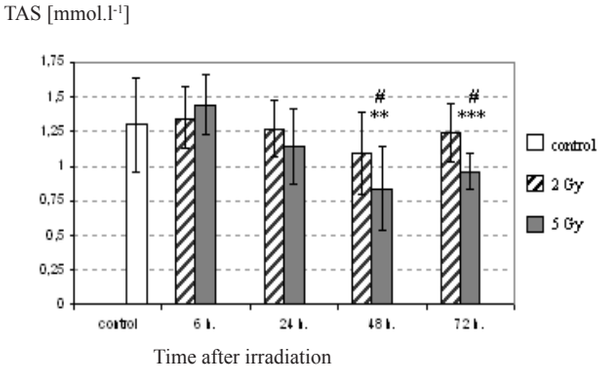
Fig. 3: The TAS changes after $HgCl_2$ administration in rats

In mercury chloride-treated rats (Tab. 2 and Fig. 3), TAS also decreased in the experimental groups compared to the control group. Highly significant reduction of the TAS value was revealed in groups Hg1, Hg4 and Hg5.

In the second experiment (Tab. 3 and Fig. 4), six hours after the irradiation TAS values were elevated at both doses of gamma rays. 24 hours after the irradiation TAS value was decreased and 48 hours after irradiation TAS decrease was even more evident. The TAS was again increased 72 hours after the irradiation. The changes of the TAS values were more evident in the groups of chickens irradiated by a dose of 5 Gy of gamma rays.

Tab. 3: TAS after gamma irradiation

Exp. group	n	Dose Gy	Blood collection	TAS [$mmol.l^{-1}$]
control	9			1.30 ± 0.34
2Gy6	6	2 Gy	6 h after irrad.	1.35 ± 0.22
2Gy24	6	2 Gy	24 h after irrad.	1.27 ± 0.21
2Gy48	6	2 Gy	48 h after irrad.	1.09 ± 0.30
2Gy72	6	2 Gy	72 h after irrad.	1.24 ± 0.21
5Gy6	6	5 Gy	6 h after irrad.	1.44 ± 0.22
5Gy24	6	5 Gy	24 h after irrad.	1.14 ± 0.27
5Gy48	6	5 Gy	48 h after irrad.	0.84 ± 0.30
5Gy72	6	5 Gy	72 h after irrad.	0.96 ± 0.13



- p < 0,05 compared to the control group

** - p < 0,01;

*** - p < 0,001 compared to the group 5Gy6

Fig. 4: The TAS changes in chickens after gamma irradiation

DISCUSSION

Radiation and heavy metal intoxication are well known factors that affect an antioxidant status and increase a free oxygen radical generation.

Metals have a special affinity toward -SH groups of proteins. By covalent binding to -SH groups, metals can block the functional sites of the catalytic or binding domains of enzymes or modify antioxidant protein conformation. Possibly, cell damage may be the result of the generation of ROS by metals. Transition metals (primarily Fe^{2+} and Cu^{+}) are known to be able to generate extremely reactive oxygen species (hydroxyl radical) by the Fenton reaction (Halliwell et al., 1990). We may assume an analogous catalytic effect of other transition metals.

In the first experiment, we analysed the influence of two heavy metals, cadmium and mercury on the plasma total antioxidant status. We observed the influence of various doses of metals on the intensity of response.

In the cadmium chloride-treated rats, the TAS was significantly lower in comparison to the control group. In groups Cd1 – Cd4, the TAS value declined depending on the elevation of the Cd dose.

In the mercury chloride-treated rats, the TAS decrease also depended on the dose of Hg. The decrease was most evident at two highest doses of Hg (Hg4 and Hg5 groups).

The decrease in TAS could be a response of plasma antioxidants to elevated production of reactive oxygen species. In particular, exhaustion of -SH groups of proteins (albumin) and peptides (glutathione) could be at the bottom of the reduction of the TAS.

ROS produced by the irradiation damage the vitally important biological molecules including DNA, proteins and lipids. The most damaged structures are C-H and C-C bonds. Radiation toxicity occurs mainly by generating free radicals by radiolysis of water (Parihar et al., 2006; Robbins et al., 2004).

Our investigations revealed that 6 hours after irradiation the TAS increased depending on the dose of gamma rays. We consider that this elevation was caused by an escape of intracellular antioxidants from damaged cells. 24 and 48 hours after irradiation TAS decreased in both experimental groups. This decrease might be a result of exhaustion of plasma antioxidants after previous elevation. 72 hours after irradiation the TAS increased again at both doses of gamma rays, in the group irradiated with a dose of 2 Gy the TAS almost reverted to control values.

CONCLUSIONS

In this study we have shown, that one of oxidative stress parameters 'Total antioxidant status' is a very sensitive marker, which can be used for the assessment of environmental influences on animal and human organisms. This marker is useful not only for monitoring heavy metal or radiation influence on organisms, but also for the assessment of their time- and dose-dependent effects.

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