STUDY OF GLUTATHIONE PEROXIDASE ACTIVITY IN SOME ENVIRONMENTAL INDUCTION MODELS IN RABBIT

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ABSTRACT

Free radicals are produced continuously in animals. Unless the presence of an efficient protective barrier, these reactive oxygen species induce processes, which end up in cellular damage, dysfunctions. In healthy animals, there is a so-called antioxidant defence system against these processes, which acts through eliminating free radicals. Glutathione peroxidase enzyme family is a prominent member of this system. The typical reaction of these selenium dependent enzyme isoforms is the reduction of reactive oxygen radicals or molecules, while their impaired electron is transferred onto reduced glutathione, which results in glutathione disulfide production. The main purpose of the study presented in the paper was the construction of a model system for studying the role of environmental factors in the regulation of glutathione peroxidase activity. New Zealand white rabbits (n=36) of 10 weeks old were used in the experiments, as they are models for livestock animals and also often used in human research. Four induction models were studied, namely peroxide load (feed was supplemented with rancid oil (2g/kg feed), selenium supplementation (organic selenium sources were added to the diet (0.3mg/kg feed), glutathione depletion (per os bromobenzene treatment (13mM/kg body weight), prooxidant load (alloxan monohydrate injection was applied (0.12g/kg body weight). A non-treated group was also created as control for the treated ones. Blood and tissue samples were taken at certain checkpoints of the experiment for further analysis. Biochemical parameters that are used as indicators of oxidative stress (MDA, GSH-concentration, GSHPx activity) were measured. Induction models resulted in unanimous changes in the enzyme activity of the different observed tissues. According to the measurements, subtherapeutic dose of selenium supplementation has unfavourable effect (8-62% reduction as compared to the control data) on the GSHPx activity, which was significant in liver and testicle tissues. Bromobenzene application has induced drastic reduction in glutathione concentration (11.52±0.65mM/g and 8.04±0.65mM/g in the control and treated group respectively, $P<0.01$), and as a result of the depletion of the enzyme co-substrate, enzyme activity has dropped. Peroxide load has shown correlation with enzyme activity of blood plasma. Alloxan treatment, modelling the prooxidant effect, resulted in total depression (98-100%, $P<0.001$) of enzyme activity in almost all the tissues studied, however this change is related to the increased protein content of tissue samples.

Key words: glutathione peroxidase, peroxidation, prooxidant, GSH-depletion, selenium.

INTRODUCTION

Free oxygen radicals are produced in several biological processes resulting in the induction of physiological or pathological oxidative stress. An antioxidant defence system had been developed in aerobic organisms to prevent deteriorating effects of these *in vivo* produced reactive oxygen species. Selenium dependent glutathione peroxidase enzyme family is a prominent member of it and consequently, these enzymes are responsible for normal health status and performance. At present, five isoforms - cytosol, extracellular, phospholipid, gastrointestinal and liver cytosol forms – of the enzyme are known, but existence of further isoenzymes is presumable. A common characteristic of these biocatalysers is that they have selenocysteine at the active site and they specifically react with hydrogen peroxide and organic hydroperoxides including peroxides of fatty acids (GAMBLE *et al.*, 1997). There are several factors abrogating the activity of selenium dependent glutathione peroxidases. Some of these are internal, individual factors, resulting in significant difference in the enzyme activity of different organs, age groups and sex. However, environmental factors have also definite effect on enzyme activity. Nutrition is one of most essential factors as fat content, fatty acid composition of feed and trace element as well as vitamin status of the animal play crucial role in normal enzyme activity.

Aim of the present research was to study the effects of certain – so far not clearly observed – environmental factors on glutathione peroxidase activity in different tissues. Environmental induction models are willing to mimic natural oxidative environment inducing pathological processes, incidentally. Effects of these conditions were planned to be studied and characterized with certain indicators of antioxidant status such as malonyl dialdehyde, and reduced glutathione concentration and glutathione preoxidase activity.

MATERIAL AND METHODS

Four different models were constructed to study the effects of oxidative stress of environmental origin (Table 1). Six half sib New Zealand white rabbits at 10 weeks of age were involved in each of the four treatments. Animals were slaughtered at 16 weeks of age and *post mortem* blood and tissue (liver, kidney, muscle, pancreas and ovary or testis) samples were taken. Animals were fed on same commercial fattening diet (180g/kg DM crude protein, 117.3g/kg DM crude fibre, 90.2g/kg DM crude fat, 557g/kg DM N-free extract). In the selenium supplementation model 0.3mg/kg SelenoYeast® was added to the normal diet and this diet was fed to the animals for five weeks. Glutathione depletion treatment was obtained with 13mM/kg body weight per os bromobenzene intake, and its effect was analysed 24 hours after treatment. Peroxide loead model was provided by adding rancid commercial sunflower oil to the normal diet in concentration of 2g/kg diet and this diet was fed for 5 weeks. Prooxidant effect was modelled with the use of intravenous alloxan monohydrate (0.12g/kg body weight)

injection. To prevent hypoglycaemic coma 40% glucose solution (2.5ml/kg body weight) was injected to the animals. Laboratory analyses were done 36 hours after treatment in the short term experiment, while long term experiment lasted for 8 weeks. In the latter treatment 0.5 – 1IU/kg body weight insulin was applied to maintain normal glucose concentration in the blood. A normal group fed on normal commercial diet was also created.

Blood and tissue samples were taken from animals in each treatment for laboratory analyses. Selenium concentration of whole blood, liver and feed samples were determined with flameless atomic absorption photometry following hydride generation. Glucose concentration was measured in blood plasma with GPO-PAP method according to TRINDER (1969). GSHPx (glutathione peroxidase) activity (MATKOVICS *et al*. 1988) was measured in each sample, while malonyl-dialdehyde (MDA) concentration (PLACER *et al.,* 1966) was analysed in blood plasma, RBC hemolysate, kidney and liver. In the latter one, reduced glutathione (GSH) concentration (SEDLAK and LINDSAY 1968) was determined, as well. Enzyme activity was referred to protein concentration of the samples. To measure protein concentration Biuret reaction (WEICHSELBAUM, 1948) was applied in blood plasma and RBC hemolysate, while Folin phenol reagent was used for tissue supernatants (LOWRY et al., 1951).
Mean and standard deviation values were calculated for each group and each parameter. Two-way ANOVA test was applied for statistical analysis of the results (SNEDECOR and COCHRAN, 1986).

RESULTS AND DISCUSSION

Results of biochemical analyses in the induction models are presented together in Figures 1, 2 and 3, while the discussion of these data is presented separately for the different treatments for better understanding.

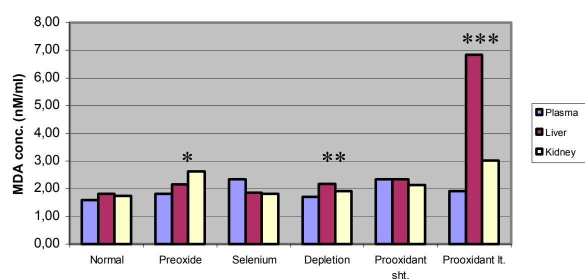

Figure 1. MDA concentration in the blood plasma, liver and kidney samples in the induction models

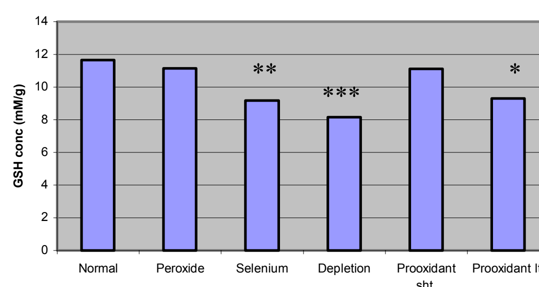

Figure 2. GSH concentration of liver samples in the induction model.

Selenium supplementation model

Selenium concentration of the normal feed was 0.125 mg/kg dry matter (DM), while it was 0.314 mg/kg DM in the supplemented diet. Considering feed intake, daily selenium intake of the treated animals was 13.96 µg/kg BW (body weight), which was significantly higher than the normal value of 4 µg/kg BW (MATEOS and PIQUER, 1994), and it was only slightly lower than the maximum safe 15 µg/kg BW/day intake (WHANGER et al., 1996). Assuming the tissue selenium measurement data (Table 2), selenium concentration in the supplemented group was higher both in the whole blood (41.4%) and liver (16.5%) samples, but the difference was significant (P<0.01) in the whole blood, only.

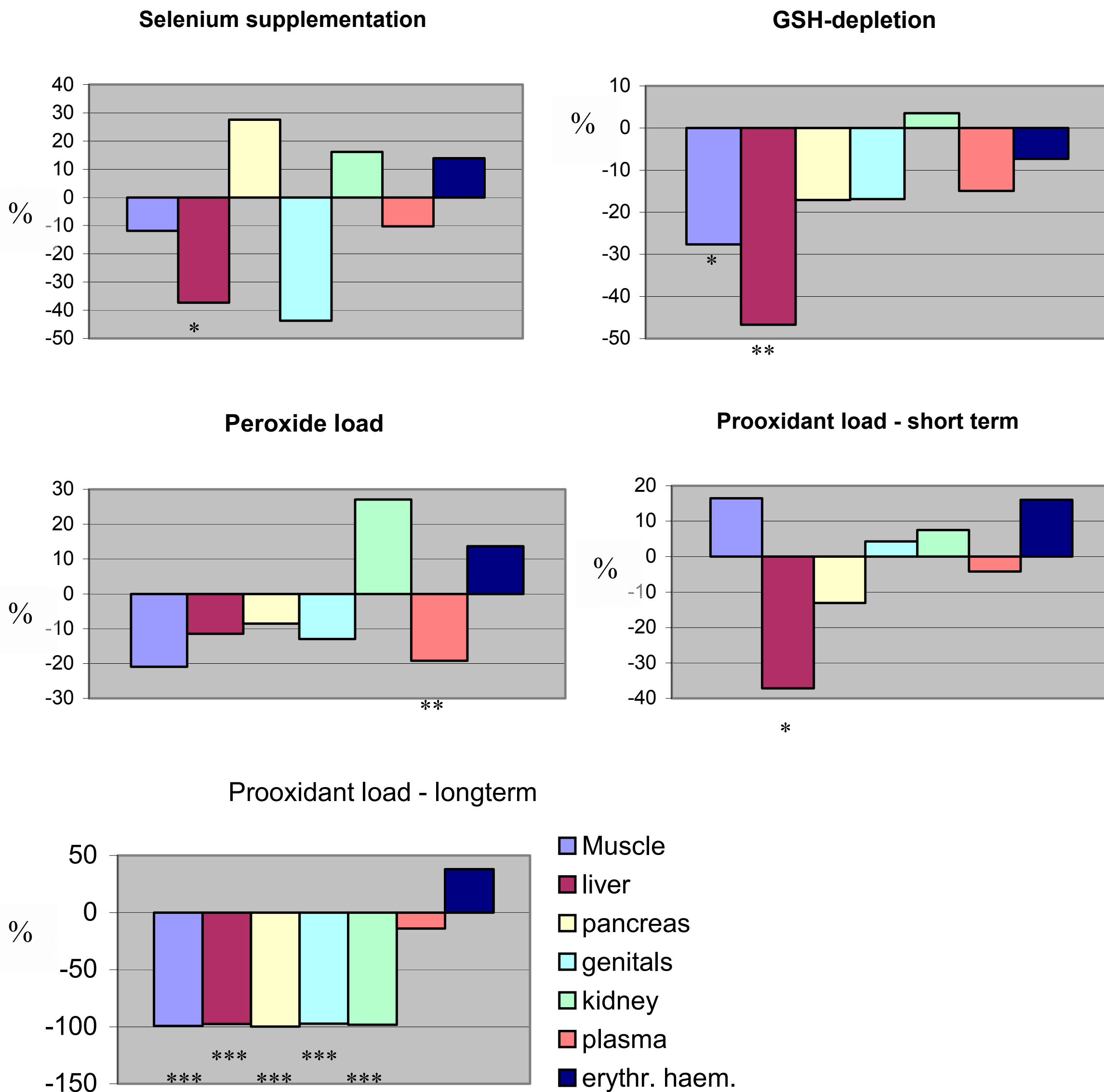

Figure 3. Relative GSHPx activity of the different tissues in the induction models as compared to the normal group

Table 2. Selenium concentration in whole blood and liver samples

Se- concentration	Normal group	Supplemented group
Whole blood (µg/l)	70.55 ± 8.3	99.70 ± 8.30 (P<0.01)
Liver (µg/kg DM)	843.5 ± 135.0	983.0 ± 164.0

This result implies good intestinal absorption of selenium-enriched yeast, which results in steady increase of blood selenium concentration (CLAUSEN and NIELSEN, 1988). There were no considerable changes in the MDA concentration of any tissue samples, while reduced glutathione concentration was significantly (P<0.01) lower in the supplemented group than in the control one. Divergent changes were found in the GSHPx activity of the different tissues. It declined significantly in the liver, testis and blood plasma, which was not anticipated. This process is probably the consequence of seleno-diglutathione formation from excessive selenium and reduced glutathione (COMBS and COMBS, 1984), which results in enzyme co-substrate deficiency and consequently blocks enzyme action.

Glutathione depletion model

MDA concentration was higher in each analysed samples owe to the treatment and the difference was even statistically significant (P<0.01) in the liver. However, reduced glutathione concentration was significantly (P<0.001) lower in the liver of treated individuals as compared to the normal ones. Glutathione peroxidase activity was reduced in every observed tissues of treated animals excluding kidney and the reduction was statistically significant in the liver and blood plasma (P<0.01, P<0.05 respectively). These findings suggest that bromobenzene intoxication induces inhibition of glutathione peroxidase via depletion of reduced glutathione, the co-substrate of the enzyme.

Peroxide loading model

Peroxide- and acid-value of the experimental fodder have exceeded considerably the maximum limit values (25meq/kg fat for peroxide-value, 50 meq/kg fat for acid-value) declared in the Hungarian Codex Alimentarius (1990) as a result of treatment. Peroxide load caused elevated MDA concentration in tissues analysed and the difference between control and treated samples was even statistically significant (P<<0.05) in the liver, whereas, no considerable changes were found in reduced glutathione concentration. Glutathione peroxidase activity was decreased in each studied tissues, except kidney and RBC hemolysate. Activity of blood plasma in the experimental group was significantly (P<0.01) lower than that of the normal. The divergent enzyme activity changes found in the different tissues are, assumable, the result of their different oxidative sensitivity, and consequently, the different activation/exhaust status of their glutathione redox system at sampling.

Prooxidant model

Although plasma glucose concentration increased both in the short and long term experiments, individual sensitivity to alloxan was well divergent and concentration changes occurred with considerable time shift after treatment. In the short-term experiment, 36 hours after alloxan injection plasma glucose concentration increased only slightly. However, uniform hyperglycaemia occurred only 60 hours after treatment even in the long-term experiment. There were no significant changes in the malonyl-dialdehyde concentration in the short-term experiment, while in the long run it increased significantly in the liver and kidney. Reduced glutathione concentration in the liver declined considerably only in the long run ($P<0.05$). Enzyme activity changes were rather divergent in the different tissues in the short-term experiment. Only liver enzyme activity changed significantly ($P<0.05$), while in the long term experiment enzyme activity has almost disappeared in each observed tissues, excluding blood samples ($P<0.001$). The drastic activity reduction was related - at least in part - to protein concentration changes in the tissues, which probably caused by insulin as it stimulates protein synthesis. Consequently, 36 hours duration of short-term experiment was not long enough for development of hyperglycaemia or for total induction of lipid peroxidation.

CONCLUSIONS

Comparing the different models, liver was confirmed to be the most sensitive organ to oxidative effects as malonyl-dialdehyde concentration has changed drastically as a result of treatments. This is well supported by the findings that reduced glutathione concentration and GSHPx activity of liver in each models changed and much of these changes were significant. Kidney and red blood cells are tissues having the highest oxidative stability of the studied ones as no significant alteration were found for the measured parameters of antioxidant status in them. The effects of environmental factors on glutathione redox system can be best described with simultaneous measurement of enzyme activity of liver and blood plasma. Subtherapeutical dose of Se-supplementation induces harmful oxidative conditions, which might be prevented by the antioxidant defence system. However, the applied dose of Se resulted in poorer performance of the animals. Glutathione depletion resulted in drastic changes in the GSH-redox system through elimination of its key element, the reduced glutathione. Peroxide load caused significant depression in the antioxidant indicators of blood plasma. Last but not least, effects of prooxidant environment have shown time dependence and have acted mainly through GSHPx activity defect.

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