Possible bendiocarb-induced brain oxidative damage

Sobekova, A.[1], Lohajova, L.[1], Greń, A.[2]

[1] Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice, Slovak Republic

[2] Institute of Biology, Pedagogical University of Cracow, Podbrzezie 3, 31-054 Cracow, Poland

Introduction

Pesticides, in addition to their intended effects like control of insects or other pests, are sometimes found to affect non-target organisms (Sharma et al., 2005). The animals and human beings are daily exposed to these chemicals primarily through the consumption of pesticide contaminated feed and water, leading to long term health hazards (Xavier et al., 2004). Environmental pollutants can generate the production of oxygen radicals. These radicals then can influence and damage biomolecules and thereby induce the oxidative stress. Free radicals are generated in a living organism under physiological conditions by controlled stimulation and they participate in regulation of proper course of many processes.

Reactive oxygen species – ROS

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals (Halliwell and Gutteridge, 2007). Reactive oxygen species are formed and degraded by all aerobic organisms, leading to either physiological concentration required for normal cell function, or excessive quantities, the state called oxidative stress. ROS include a number of chemically reactive molecules derived from oxygen (Nordberg and Arnér, 2001). Some of those molecules are extremely reactive, such as the hydroxyl radical, while some are less reactive (superoxide and hydrogen peroxide). Free radicals can readily react with most biomolecules, starting a chain reaction of free radical formation. In order to stop this chain reaction, a newly formed radical must either react with another free radical, eliminating the unpaired electrons, or react with a free radical scavenger – a chain-breaking or primary antioxidant. The step-wise reduction of molecular oxygen via one-electron transfer can be summarized as follows:

$$O_2 \xrightarrow{+e} O_2^{\bullet -} \xrightarrow{+e} H_2O_2 \xrightarrow{+e} H_2O + HO^{\bullet} \xrightarrow{+e} H_2O$$

Superoxide radical $O_2^{\bullet -}$

The superoxide anion created from molecular oxygen by the addition of an electron is not highly reactive. It lacks the ability to penetrate lipid membranes and is therefore enclosed in compartment where it was produced (Nordberg and Arnér, 2001). The formation of superoxide takes place spontaneously, especially in the electron-rich aerobic environment in vicinity of the inner mitochondrial membrane with the respiratory chain. Superoxide is also produced endogenously by xanthine oxidase, lipoxygenase and cyclooxygenase (Lakari, 2002). Two molecules of superoxide rapidly dismutate to hydrogen peroxide and molecular oxygen and this reaction is further accelerated by SOD (Yu, 1994).

Hydrogen peroxide H_2O_2

H_2O_2 is not a free radical but is nonetheless highly important much because of its ability to penetrate biological membranes. It plays a radical forming role as an intermediate in the production of more reactive ROS molecules including HOCl by the action of myeloperoxidase and, most importantly, formation of hydroxyl radical via oxidation of transition metals (Palchaudhuri et al., 2001).

$M^{n-1} + H_2O_2 \rightarrow M^{n} + {}^{\bullet}OH + OH^-$ **Fenton reaction**

Another important function of H_2O_2 carried out is its role as an intracellular signaling molecule (Sigler et al., 1999; Gottschling et al., 2001). H_2O_2 produced by different mechanisms is removed by at least two antioxidant enzyme systems, namely catalases, and glutathione peroxidases (Nordberg and Arnér, 2001).

Hydroxyl radical $^{\bullet}OH$

Due to its strong reactivity with biomolecules, hydroxyl radical is probably capable of doing more damage to biological systems than any other ROS (Nordberg and Arnér, 2001). The hydroxyl radical is very dangerous radical with very short *in vivo* half-life of approx. 10^{-9} s (Valko et al., 2007). The radical is formed from hydrogen peroxide in a reaction catalyzed by metal ions (Fenton reaction), often bound in complex with different proteins or other molecules. Superoxide also plays an important role in connection with Fenton reaction by recycling the metal ions (Leonard et al., 2004):

$M^{n} + O_2^{\bullet -} \rightarrow M^{n-1} + O_2$

overall

M^{n-1} / M^{n}

$O_2^{\bullet -} + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$ **Haber-Weiss reaction**

Oxidative stress and damage of biomolecules

Oxidative stress is a condition where there is an imbalance between antioxidant defence and the production of reactive oxygen species, so that the defence is overcome by radical formation causing oxidative damage to biomolecules (Halliwell and Gutteridge, 2007). Oxidative damage is usually related to the production of reactive oxygen species by xenobiotics, and therefore antioxidant defence has an important role in the protection of organisms against xenobiotic-induced oxidative stress. Indeed, the maintenance of a high antioxidant capacity in cell may increase tolerance against different types of environmental stress (Thomas et al., 1999).

ROS are, due to their high reactivity, potentially toxic, mutagenic, or carcinogenic. The targets for ROS damage include all major groups of biomolecules, nucleic acids, lipids and proteins (Valko et al., 2007). ROS have been shown to be mutagenic; an effect should be derived from chemical modification of DNA (Singal et al., 2000). A number of alterations are due to reactions with ROS. Hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge, 2007). If the DNA-repair systems are not able to immediately regenerate intact DNA, a mutation will result from erroneous base pairing during replication (Nordberg and Arnér, 2001). Permanent modification of genetic material resulting from oxidative damage represents the first step involved in mutagenesis, carcinogenesis, and ageing (Valko et al., 2007).

Generation of ROS results in an attack not only on DNA, but also on other cellular components involving polyunsaturated fatty acids residues of phospholipids. Polyunsaturated fatty acids are, because of their multiple double bonds, an excellent target for free radical attacks (Sigler et al., 1999). Lipid peroxidation is probably the most explored area of research when it comes to ROS.

ROS have been shown to react with several amino acid residues *in vitro*, generated anything from modified and less active enzymes to denatured, nonfunctioning proteins (Sigler et al., 1999). Advanced glycation end products (AGEs) is a class of complex products. They are the result of reaction between carbohydrates and free amino group of proteins.

The intermediate products are known as Amadori, Schiff Base and Maillard products. Most of the AGEs are very unstable, reactive compounds and the end products are difficult to be completely analyzed (Velíšek and Hajšlová, 2009).

Defence against oxidative stress

Exposure to free radicals from a variety of sources has led organisms to develop a series of defence mechanisms. Defence mechanisms against free radical-induces oxidative stress involve: preventive mechanisms, repair mechanisms,

physical defences and antioxidant defences (Valko et al., 2007). Antioxidants are substances that have the ability to inhibit free radical generation, scavenge free radicals and reduced oxidation and damage caused by the radicals. The cellular antioxidant system can be divided into two major groups, enzymatic and nonenzymatic.

A large number of low molecular weight compounds are considered to be antioxidants of biological importance. The main antioxidants are glutathione (GSH), flavonoids, ubiquinones, carotenoids, α-tocopherol, vitamin C, lipoic and uric acid.

One of the most important low molecular weight antioxidant is glutathione (GSH) because of its direct participation in binding with reactive oxygen species. It is most abundant intracellular non-protein thiol in cell and appears in high amounts in the liver (Swiergosz-Kowalewska et al., 2006) because of its synthesis there. GSH also has a significant role as a reductant and substrate for enzymatically catalyzed reactions. Changes in the amount of GSH can be used to detect oxidative damage (Pinto et al., 2003). GSH also serves to detoxify compounds either via conjugation reactions catalyzed by glutathione-S-transferase or indirectly, as is the case with hydrogen peroxide in the GPx catalyzed reaction. Oxidized glutathione (GSSG) is reduced by the NADPH-dependent flavoenzyme glutathione reductase (Peinado et al., 1991).

Another important group of antioxidants are antioxidative enzymes like glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD), which are able to inactivate and remove free radicals (Koivula and Eeva, 2010). The main function of antioxidant enzymes is catalyzing the breakdown of free radicals, therefore the level of enzyme activity indicates the degree of oxidative stress (Ercal et al., 2001; Pinto et al., 2003). In addition to operating as an antioxidant, GSH has an important role in the antioxidant defence in combination with GPx, GR and GST (Sies, 1999). GPx is responsible for oxidizing GSH to GSSG while GR reduces GSSG back GSH to maintain the reduction potential of the cell and hence indirectly supporting the antioxidant defence system (Gurer and Ercal, 2000; Swiergosz-Kowalewska et al., 2006). GST is able to remove hydrogen peroxide produced in reactions of macromolecules and ROS from the cell through GSH oxidation (Koivula and Eeva, 2010). GPx, CAT and SOD are metaloproteins and their function is based on enzymatically detoxifying peroxides and O2•- (Gurer and Ercal, 2000). SOD operates as transformer of superoxide to hydrogen peroxide, which is then catalyzed further by CAT and GPx to water and molecular oxygen. The cooperation of antioxidant enzymes is shown in Fig. 1.

Superoxide dismutase (SOD) EC 1.15.1.1

In the reaction catalyzed by SOD, two molecules of superoxide form hydrogen peroxide. The reaction is extremely efficient, limited only by diffusion:

SOD

$2\ O_2^{\bullet -} + 2\ H^+ \rightarrow H_2O_2 + O_2$

In eukaryotic cells, superoxid can be metabolized to hydrogen peroxide by two metal containing SOD isoenzymes, tetrameric MnSOD present in mitochondria, and the cytosolic dimeric Cu/ZnSOD. Bacteria contain both MnSOD and FeSOD (Matés and Sánchez-Jimenéz, 1999) and in some cases also Cu/ZnSOD (Youn et al., 1996). In mitochondria, superoxide is formed in relatively high concentrations due to the leakage of electrons from the respiratory chain. The strictly mitochondrial MnSOD is obviously essential and expression of MnSOD is, in contrast to Cu/ZnSOD, induced by oxidative stress (Järvinen, 2001). A larger Cu/ZnSOD distinct from the cytosolic form can also be found extracellularly (Lakari, 2002).

Catalase (CAT) EC 1.11.1.6

Catalases of many organisms are mainly hem-containing enzymes (Matés and Sánchez-Jimenéz, 1999). The predominant subcellular localization in mammalian cells is in peroxisomes where they catalyze the dismutation of hydrogen peroxide to water and oxygen:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

Catalase also has function in detoxifying different substrates (phenols, alcohols) via coupled reduction of hydrogen peroxide. One role of catalase is to lower the risk of hydroxyl radical formation from H_2O_2 via the Fenton reaction catalyzed by copper or iron ions (Nordberg and Arnér, 2001).

Glutathione peroxidases (GPx)

All glutathione peroxidases may catalyze the reduction of H_2O_2 using glutathione as substrate. They can also reduce other peroxides to alcohols.

$GSH + H_2O_2 \rightarrow GSSG + 2\ H_2O$

$2\ GSH + ROOH \rightarrow GSSG + ROH + H_2O$

There are at least four different GPx in mammals, all of them containing selenocysteine (Bordoni et al., 2003). GPx-1 and GPx-4 (phospholipid GPx) are both cytosolic enzymes abundant in most tissues (Li et al., 2000). GPx-4 has been found to have dual function in sperm cells by being enzymatically active in spermatids but insoluble and working as a structural protein in mature spermatozoa (Nord-

berg and Arnér, 2001). GPx-2 (gastrointestinal GPx) and GPx-3 (plasma GPx) are mainly expressed in the gastrointestinal tract and kidney (Chu et al., 1993).

Some data indicated that GPx should be of high antioxidant importance under physiological conditions while others place the enzymes as important only at events of oxidative stress (Nordberg and Arnér, 2001).

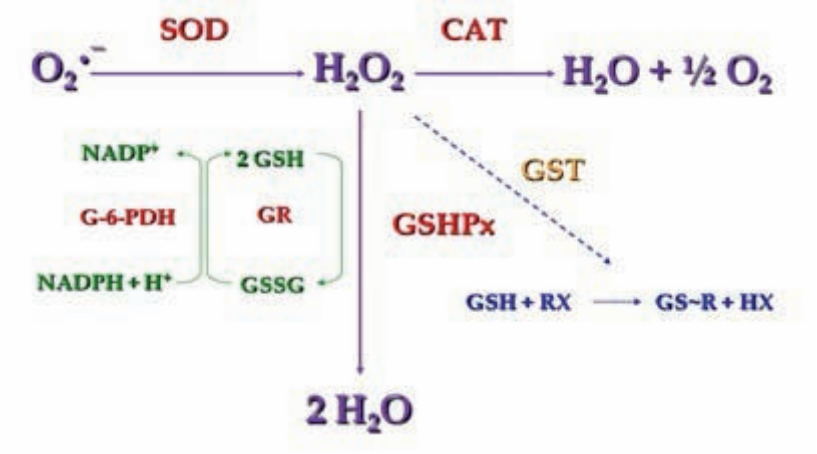

Fig. 1. Cooperation of antioxidant enzymes

The effect of bendiocarb on the activity of antioxidant enzymes in brain of rabbit

Bendiocarb is the carbamate insecticide which is effective against a wide range of pests and disease vectors. The toxicity of bendiocarb is primary connected with the inhibition of acetylcholinesterase (Capcarova et al., 2010). According to toxicological studies bendiocarb does not accumulate in the body. It undergoes to rapid metabolic transformation and then it is rapidly eliminated by kidneys (EHC, 1986). Some intermediates can be toxic at certain conditions (Crespí et al., 2005). It was confirmed that bendiocarb can generate the production of reactive oxygen species. These radicals increase its primary toxic effect (Sobeková et al., 2009). The brain is the organ with the highest oxygen consumption. Its defense mechanism against oxidative stress is insufficient (Achuba, 2005). Superoxide dismutase and ascorbic acid represent the main antioxidative capacity of nerve tissues. Low activity of catalase and glutathione peroxidase was observed in the nervous tissue (Halliwell and Gutteridge, 1986). The aim of this study was to observe the effect of bendiocarb on the antioxidative enzyme system of brain of rabbits.

Material and methods

The experiment was carried out on clinically healthy domestic rabbits (*Oryctolagus cuniculus domesticus*) obtained from an accredited animal farm (Nitra, SR). Experimental animals (6 in each group) were administered bendiocarb (96% Bendiocarb, Bayer) *per os* at a dose of 5 $mg.kg^{-1}$ BW per day. Owing to the adverse side effects of bendiocarb after 10 days of the experiment, the dose mentioned was administered every 48 h. The animals were sacrificed on days 3, 10, 21 and 30 of the experiment. The study was carried out in agreement with the requirements of institutional ethical authority.

The brain of the experimental animals was used to prepare 25% (w/v) homogenates in 5 $mmol.l^{-1}$ TRIS-HCl buffer, pH 7.8. After centrifugation (105 000 g, 1 h, 4°C) the total proteins were determined in supernatants by the method of Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase activity (SOD, EC 1.15.1.1) was determined by measuring the inhibition of the cytochrome c reduction using xanthine/xanthine oxidase $O_2^{\bullet -}$ generating system at 550 nm (25°C) (Flohé and Ötting, 1984). One unit of SOD activity was defined as the amount of enzyme that causes 50% inhibition of cytochrome c reduction under the assay conditions. Catalase (CAT, EC 1.11.1.6) was assayed by monitoring the decrease in absorbance of H_2O_2 at 240 nm (30°C) (Sizer and Beers, 1952). Glutathione peroxidase activity (GPx) was measured by monitoring the oxidation of $NADPH+H^+$ at 340 nm (37°C) as described Flohé and Günzler (1984) in a coupled assay with glutathione reductase using cumene hydroperoxide (GPx-cum, EC 1.11.1.12) or H_2O_2 (GPx-H_2O_2, EC 1.11.1.9) as substrates. Glutathione reductase (GR, EC 1.6.4.2) was determined by following the decrease in $NADPH+H^+$ absorbance at 340 nm (30°C) due to GSSG reduction (Pinto et al., 1984). Glutathione-S-transferase (GST, EC 2.5.1.18) was measured by the procedure of Habig and Jacoby (1981) at 30°C using the substrate CDNB at a final concentration of 1 $mmol.dm^{-3}$; the GSH concentration was 1 $mmol.dm^{-3}$. One unit of enzyme activity (GPx, GR, and GST) was defined as the amount of enzyme that catalyzes the formation of 1 μmol of product per minute under assay conditions.

Lipid peroxidation products measured at 535 nm as thiobarbituric acid reactive substances (TBARS) were determined according to Gutteridge (1984). The content of TBARS was expressed in $absorbance.mg^{-1}$ of protein.

The results are given as means ± SD of at least three independent determinations in six different batches. Statistical analysis was done by Student's t-test.

All reagents were of the highest purity from Sigma, Merck and Boehringer.

Results

The effect of bendiocarb on the antioxidative enzyme system of brain of rabbits was studied. A significant reduction in activity of superoxide dismutase in the brain of rabbits was observed on the days 3 and 10 of the experiment (Fig. 2). Catalase activity was increased at the same time (Fig. 3). It is assumed that the SOD activity was inhibited either by its own H_2O_2 production or by H_2O_2 production in another metabolic pathways. Glutathione peroxidase activity was statistically increased on the days 10 and 30. Activity of auxiliary enzyme glutathione reductase was significantly decreased on the day 3. Changes in specific activity of glutathione-S-transferase were very similar (Tab. 1). Inhibition of the activity of certain enzymes could be due to accumulation of reactive oxygen species. Oxidative damage of brain was confirmed by increased content of thiobarbituric acid reactive substances (Tab. 1).

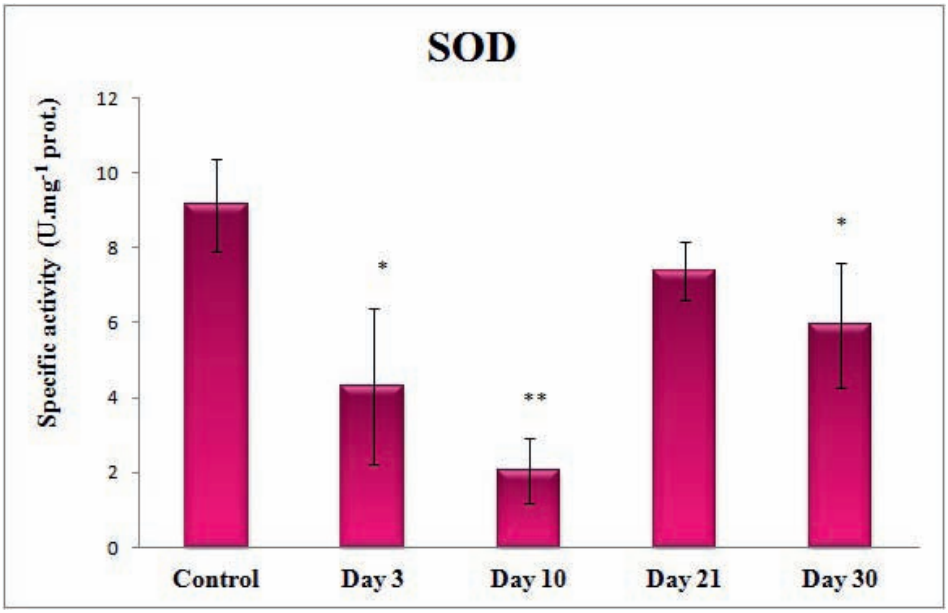

Fig. 2. Specific activity of SOD in brain of rabbits; The values are means ± SD (n=6). Student t-test was used for assessment; * $p<0.05$, ** $p<0.01$

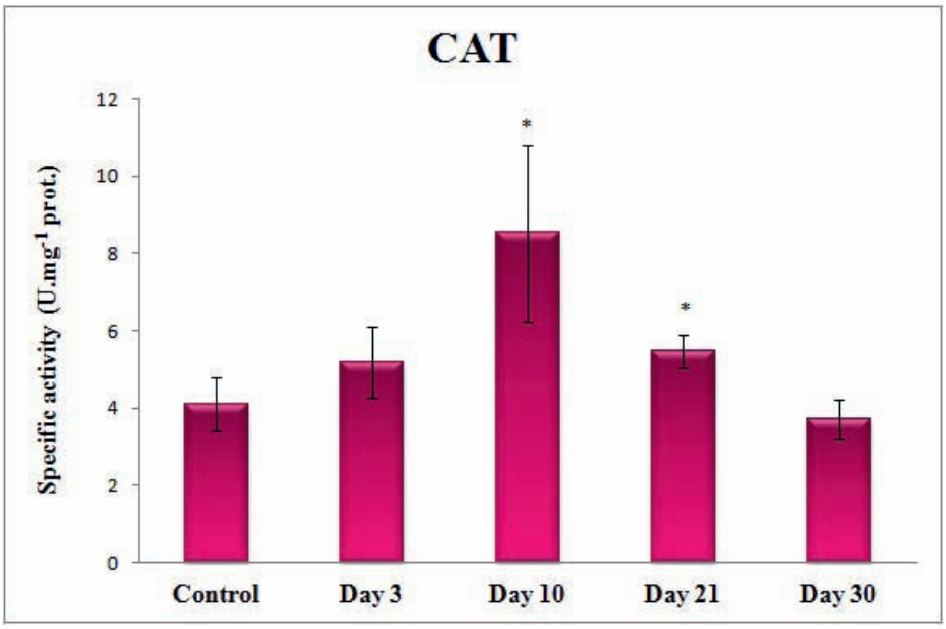

Fig. 3. Specific activity of CAT in brain of rabbits; The values are means ± SD (n=6). Student t-test was used for assessment; p<0.05, ** p<0.01

Tab. 1. Specific activities of some antioxidative enzymes and TBARS levels in brain of rabbits; The values are means ± SD (n=6); Student t-test was used for assessment; * p<0.05, ** p<0.01

Specific activity	GPxcum ($U.mg^{-1}$)	GPxH_2O_2 ($U.mg^{-1}$)	GR ($U.mg^{-1}$)	GST ($U.mg^{-1}$)	TBARS ($A_{535}.mg^{-1}$)
Control	**0.11** ± 0.01	**0.069** ± 0.008	**0.103** ± 0.009	**0.8** ± 0.2	**0.0175** ± 0.0007
Day 3	**0.13** ± 0.01	**0.10** ± 0.02	**0.09** ± 0.01*	**0.49** ± 0.06	**0.027** ± 0.002**
Day 10	**0.16** ± 0.02**	**0.085** ± 0.007*	**0.10** ± 0.01	**0.5** ± 0.1	**0.023** ± 0.005
Day 21	**0.13** ± 0.02	**0.09** ± 0.03	**0.096** ± 0.005	**0.7** ± 0.1	**0.030** ± 0.008*
Day 30	**0.142** ± 0.008**	**0.10** ± 0.01*	**0.091** ± 0.007	**0.8** ± 0.1	**0.016** ± 0.002

Discussion

Free radicals are generated in a living organism under physiological conditions by controlled stimulation and they participate in regulation of proper course of many processes. The increased steady-state concentration of ROS constitutes the chemical basis of oxidative stress. The natural endogenous protection of

the organism against the generation of ROS is the antioxidant enzyme system. Changes in their activities are specific cells response to contaminants exposure. A significant reduction in activity of SOD in the brain of female rabbits was observed on the day 3 and 10 of the experiment. Catalase activity was increased at the same time. SOD provides primary antioxidative protection of cells against ROS. The extent of the decrease of SOD activity depends on the degree of oxidative stress (Pracasam et al., 2001). The inhibition of the SOD activity may result from the increased production of superoxide anion radical (Sobeková et al., 2009). Inhibition of SOD causes accumulation of superoxide anion radical and leads to damage to the mitochondrial membrane and apoptosis (Huang et al., 2000). Chronic inhibition of SOD induces degeneration of spinal neurons (Rothstein et al., 1994). Lower activity of SOD was found in rat brain in relation to age. Superoxide production in the brain increases with age together with decreased SOS activity. It has been shown that SOD is inhibited by H_2O_2 (Hodgson and Fridovich, 1975). Inactivation of SOD is caused by the reduction of copper ions in the active centre of the enzyme. Catalase and glutathione peroxidases are the predominant enzymes in regulating and controlling intracellular H_2O_2 concentrations. Catalase is especially effective at high H_2O_2 concentration and glutathione peroxidase is capable to utilize hydroperoxides and to metabolize H_2O_2 at its low concentration (Chance et al., 1979). Because H_2O_2 is continuously produced by several enzymes, it can be assumed that the SOD activity was inhibited either by its own H_2O_2 production or by H_2O_2 production in another metabolic pathways. The correlation between decreased SOD activity and increased lipid peroxidation in different parts of the rat brain was shown (Alper, 1998). Oxidative damage of rabbits' brain was confirmed by increased content of thiobarbituric acid reactive substances in our experiment.

The alterations in the activities of antioxidative enzymes and increased TBARS values showed that brain of rabbits was exposed to the action of free radicals. Toxicity and possible oxidative damage of brain depends on the concentration of pesticide and on the time of exposition.

* * *

The study is a part of the project KEGA 020UVLF–4/2012 of the Institute of Medical Chemistry of UVMP in Košice.

References

Achuba, F.I. (2005): Oxidative stress in the brain of rabbits with petroleum-induced hypoglycaemia. Biological Lett., 42(1), 33–39.

Alper, G. (1998): Age-Related Alterations in Superoxide Dismutase and Catalase Activities in Rat Brain. Tr. J. Medical Sciences, 28, 491–494.

Bordoni, A., Biagi, P.L., Angeloni, C., Leoncini, E., Muccinelli, I., Hrelia, S. (2003): Selenium supplementation can protect cultured rat cardiomyocytes from hypoxia/reoxygenation damage. J. Agric. Food Chem., 51, 1736–1740.

Bradford, M.M. (1976): A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72, 248–254.

Capcarová, M., Petrovova, E., Flesarova, S., Danková, M., Massanyi, P., Danko, J. (2010): Bendiocarbamate induced alterations in selected parameters of rabbit homeostasis after experimental peroral administration. Pest. Biochem. Physiol., 98, 213–218.

Crespí, F.L., Luengo, J.B., Quinto, J.O. (2005): Urban pesticide exposure disables workers in Catalunya. Pesticides News, 68, 12–14.

EHC 64 (1986): Carbamate pesticides: a general introduction.

Ercal, N., Gurer-Orhan, H., Aykin-Burns, N. (2001): Toxic metals and oxidative stress part 1: mechanisms involved in metal-induced oxidative damage. Curr. Top. Med. Chem., 1, 529–539.

Flohé, L., Günzler, W.A. (1984): Assays of glutathione peroxidase. Method Enzymol., 105, 114–121.

Flohé, L., Ötting, F. (1984): Superoxide dismutase assays. Methods Enzymol., 105, 93–104.

Gottschling, B.C., Maronpot, R.R., Heiley, J.R., Peddada, S., Moomaw, C.R., Klaunig, J.E., Nyska, A. (2001): The role of oxidative stress in indium phosphide-induced lung carcinogenesis in rats. Toxicol. Sci., 64, 28–40.

Gurer, H., Ercal, N., (2000): Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biol. Med., 29, 927–945.

Gutteridge, J.M.C. (1984): Ferrous ion-EDTA-stimulated phospholipid peroxidation. Biochem. J., 224, 697–701.

Habig, W.H., Jakoby, W.B. (1981): Assays for differentiation of glutathione-S-transferases. Meth. Enzymol., 77, 398–405.

Halliwell, B., Gutteridge, J. (2007): Free radicals in Biology and Medicine, 4th ed., Oxford University Press, New York.

Halliwell, B., Gutteridge, J.M.C. (1986): Oxygen free radicals and iron relation to biology and medicine: Some problems and concept. Arch. Biochem. Biophys., 246, 501–514.

Hodgson, E.K., Fridovich, I. (1975): The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of enzyme. Biochem., 14 (24), 5294–5949.

Huang, P., Feng, L., Oldham, E.A., Keating, M.J., Plunkett, W. (2000): Superoxide dismutase as a target for the selective killing of cancer cells. Nature, 407 (6802), 390–395.

Chance, B., Sies, H., Boveris, A. (1979): Hydroperoxide metabolism in mammalian organs. Physiol. Rew. 59, 527–605.

Chu, F.-F., Doroshow, J.H., Esworthy, R.S. (1993): Expression, characterization and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-gi. J. Biol. Chem., 268, 2571–2576.

Järvinen, K. (2001): Antioxidant enzymes and related mechanisms in malignant pleural mesothelioma. Academic Dissertation, University of Helsinki, Medical Faculty, Department of Industrial Hygiene and Toxicology, ISBN 952-10-0212-3.

Koivula, M.J., Eeva, T. (2010): Metal-related oxidative stress in birds. Environ. Pollut. doi: 10.1016/j.encpol.2010.03.013.

Lakari, E. (2002): Expression of oxidant and antioxidant enzymes in human lung and interstitial lung diseases. Academic Dissertation, University of Oulu, Faculty of Medicine, ISBN 951-42-6662-5.

Leonard, S.S., Harris, G.K., Shi, X. (2004): Redox-active metal ions, reactive oxygen species, and apoptosis. Free Radical Bio. Med., 37 (12), 1921–1942.

Li, S., Yan, T., Yang, J.-Q., Oberley, T., Oberley, L. (2000): The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. Cancer Res., 60, 3927–3939.

Matés, J.M., Sánchez-Jiménez, F. (1999): Antioxidant enzymes and their implications in pathophysiologic processes. Front. Biosci., 4, 339–345.

Nordberg, J., Arnér, E.S.J. (2001): Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Bio. Med., 31 (11), 1287–1312.

Palchaudhuri, S., Raymond, A., Carlson, E.A., Li, Y., Zelikoff, J.T. (2001): Cytotoxic and cytoprotective effects of selenium on bluegill sunfish (*Lepomis macrochirus*). Phagocytic cells *in vitro*: Pre-treatment with selenium modulates the effects of hydrogen peroxide. Bull. Environ. Contam. Toxicol., 67, 672–678.

Peinado, J., Florindo, J., Garcia-Alfonso, C., Martinez-Galisteo, E., Llobell, A., López--Barea, J. (1991): Metals are directly involved in the redox interconversion of *Sacharomyces cerevisiae* glutathione reductase. Mol. Cell. Biochem., 101, 175–187.

Pinto, M.C., Mata, A.M., López-Barea, J. (1984): Reversible inactivation of *Sacharomyces cerevisiae* glutathione reductase under reducing conditions. Arch. Biochem. Biophys., 228, 1–12.

Pinto, E., Sigaud-Kutner, T.C.S., Leitao, M.A.S., Okamoto, O.S., Morse, D., Colepicolo, P. (2003): Heavy metal-induced oxidative stress in algae. J. Phycol., 39, 1008–1018.

Prakasam, A., Sethupathy, S., Lalitha, S. (2001): Plasma and RBCs antioxidant status in occupational male pesticide sprayers. Clin. Chim. Acta, 310, 107–112.

Rothstein, J.D., Bristol, L.A., Hosler, B., Brown, R.H. Jr., Kuncl, R.W. (1994): Chronic inhibition of superoxid dismutase produces apoptotic death of spinal neurons. Proc. Natl. Acad. Sci. USA., 10, 4155–4159.

Sharma, Y., Bashir, S., Irshad, M., Datta Gupta, S., Dogra, T.D. (2005): Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. Toxicol., 206, 49–57.

Sies, H. (1999): Glutathione and its role in cellular functions. Free Radical Bio. Med., 27, 1164–1170.

Sigler, K., Chaloupka, J., Brozmanová, J., Stadler, N., Hofer, M. (1999): Oxidative stress in microorganisms − I. Microbial vs. higher cells – damage and defenses in relation to cell aging and death. Folia Microbiol., 44 (6), 587–624.

Singal, P.K., Khaper, N., Farahmand, F., Belló-Klein, A. (2000): Oxidative stress in congestive heart failure. Curr. Cardiol. Rep., 2, 206–211.

Sizer, I.W., Beers, R.F. Jr. (1952): A spectrofotometric method for measuring the breakdown of hydrogen peroxid by catalase. J. Biol. Chem., 195, 133–139.

Sobeková, A., Holovská, K., Lenártová, V., Flesarova, S., Javorský, P. (2009): The other toxic effects of carbamate insecticides. Acta Biol. Hung., 60, 45–54.

Swiergosz-Kowalewska, R., Bednarska, A., Kafel, A. (2006): Glutathione levels and enzyme activity in the tissues of bank vole *Clethrionomys glareolus* chronically exposed to a mixture of metal contaminants. Chemosphere, 65, 963–974.

Thomas, D.J., Thomas, J.B., Prier, S.D., Nasso, N.E., Herbert, S.K. (1999): Iron superoxide dismutase protects against chilling damage in the cyanobacterium *Symechococcus* species PCC7942. Plant Physiol., 120, 275–282.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., Telser, J. (2007): Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell B., 39, 44–84.

Velíšek, J., Hajšlová, J. (2009): Chemie potravin I, OSSIS, Tábor, 353–358.

Xavier, R., Rekha, K., Bairy, K.L. (2004): Health Perspective of Pesticide Exposure and Dietary Management. Mal. J. Nutr., 10, 39–51.

Youn, H.D., Kim, E.J., Roe, J.H., Hah, Y.C., Kang, S.O. (1996): A novel nickel-containing superoxide dismutase from *Streptomyces spp*. Biochem. J., 318, 889–896.

Yu, B.P. (1994): Cellular defenses against damage from reactive oxygen species. Physiol. Rev., 74, 139–156.