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Original article

Activity of superoxide dysmutase, catalase and glutathione peroxidase in rats exposed to chlorpyrifos and erofloxacin

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Abstract

The aim of the study was to investigate the influence of administration of chlorpyrifos and/or enrofloxacin on the activity of chosen antioxidative enzymes i.e.: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in erythrocytes of rats. Chlorpyrifos was administered by stomach tube during 28 days at a dose of 3 mg/kg bw (0.02 LD_{50}), and enrofloxacin was administered by stomach tube at a dose of 5 mg/kg bw during 3 subsequent days. It was stated that administration of enrofloxacin at applied dose did not cause any major changes in the activity of investigated antioxidative enzymes. The four-week exposure of rats to chlorpyrifos caused noticeable decrease in SOD and CAT activity in erythrocytes of rats at the beginning of the experiment (up to 24^{th} hour) in comparison with the control group. The activity of GPx during all periods of the experiment was increased. In the group of animals in which both chlorpyrifos and enrofloxacin were applied, the profile of changes in activity of examined enzymes was similar to that one, which was observed after administration of chlorpyrifos exclusively, what may indicate lack of co-action between compounds used in the experiment.

Key words: chlorpyrifos, enrofloxacin, SOD, CAT, GPx, rats

Introduction

Investigations which has been conducted in the last few years indicate the need for studies estimating potential harmful effects of chemical substances and preparations, which are components of plant protection chemicals (pesticides) and medicines. These compounds are omnipresent in contemporary world. Apart from many advantages which result from their application, one has to remember that many of them caused or cause major disorders in the health of people and animals. Paying attention to the toxicological aspects of exposures to these compounds, more frequently we think about the possibility of overdosing and their adverse effects. An additional problem are adverse interactions, that is the occurrence of mutual co-action of several substances which are administered at the same time. They can sometimes change in important way effects of action of given compounds.

Among pesticides, chlorpyrifos deserves the special attention. This is a phosphoroorganic insecti-

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cide, which has a wide range of application in plants protection and sanitary hygiene (Lemus and Abdelghani 2000, Maroni et al. 2000). The mechanism of toxic action, similarly to other phosphoroorganic substaces, mainly results from inhibition of enzymes from the group of esterases i.e.: acetylcholinesterases and butyrylcholinesterases (Amitai et al. 1998, Shenouda et al. 2009). One to the action of enzymes, mainly cytochrome P450, chlorpyrifos is converted to an oxygen analoque – chlorpyrifos oxone, which acts more strongly in comparison with maternal substance – chlorpyrifos (Sams et al. 2004, Mutch and Williams 2006). That insecticide is quickly absorbed from alimentary and respiratory tracts and comparatively slowly through skin. In the body, it is quickly metabolized and excreted with urine and faeces. It does not show tendencies to cumulation (Cochran et al. 1995). Main ways of metabolic biotransformations of chlorpyrifos are processes of oxidative desulphuration and dearylation and hydrolysis (Sultatos and Murphy 1983, Foxenberg et al. 2007). Apart from having effects on the nervous system, that insecticide causes disorders in functioning of liver, circulatory system, immunological system and shows teratogenic, cytotoxic and genotoxic effects (Muscarella et al. 1984, Cetin et al. 2007). Moreover, in the recent years one has proven that exposure of animals to chlorpyrifos, apart from cholinergic effect, causes oxidative stress and intensifies lipid peroxidation, leading to tissue and organ damages (Verma et al. 2007, Tuzmen et al. 2008).

Another group of compounds, which are more and more often used in human and veterinary medicine are fluoroquinolones. These are synthetic antibacterial chemotherapeutics, with wide spectrum of action used in the treatment of illnesses caused by microorganisms, especially of alimentary, respiratory and urogenital systems diseases, as well as secondary infections in viral diseases (Martínez et al. 2006, Gotfried and Grossman 2010) and in veterinary dermatology (Ihrke et al. 1999, Hillier et al. 2006). Among that group of compounds special attention should be paid to enrofloxacin, which belongs to the second generation of quinolones and has strong antiseptic effect and wide spectrum of action. Thanks to these properties, enrofloxacin is used in treatment of general and local infections caused by microorganisms which are sensitive to its action i.e.: Gram-positive and Gram-negative bacteria, mycoplasmas and chlamydia (Boothe et al. 2006, Elmas et al. 2006, Grobbel et al. 2007). Owing to its lipophilic properties, enrofloxacin is rapidly absorbed from the alimentary tract, quickly reaches therapeutic concentration (De Lucas et al. 2008, Bimazubute et al. 2010) and easily penetrates into the tissues and cells (Anadon et al. 1999, Wiuff et al. 2002). In the body of different species of animals enrofloxacin is subjected to metabolic transformation into ciprofloxacin, which chemotherapeutic activity is comparable to the maternal substance (Riddle et al. 2000, Grobbel et al. 2007). The mechanism of action of enrofloxacin consists of inhibition of bacterial DNA gyrase, which plays basic role in the process of DNA replication, leading to inhibition of synthesis of bacterial proteins (Vancutsem et al. 1990, Wang et al. 2010). The metabolism takes place in the liver through the N-oxidation, N-dealkylation and deethylation processes. Enrofloxacin is mainly excreted with urine and bile (Vancutsem et al. 1990). Even though during therapy temporary gastrointestinal as well as biochemical and hematological disorders are observed, enrofloxacin is classed as a drug relatively safe and similarly to the whole group of fluoroquinolones it has no shown toxic effects (Tras et al. 2001). Investigations conducted in the recent years suggest that the negative effect of fluoroquinolones administration could be generation of reactive oxygen species and disorders in oxidative body balance (Becerra et al. 2004, Altinordulu and Eraslan 2009).

The high incidence of bacterial infections and widespread use of organophosphorus pesticides in the environment create the risk of undesirable and unexpected interactions between multiple xenobiotics.

Taking into consideration the large extent of chlorpyrifos use for pest control in crops and sanitary hygiene, as well as enrofloxacin in the treatment of bacterial infections and also lack of data concerning impact of these compounds in an organism on oxidative processes, it was decided to investigate their influence on activity of selected parameters of antioxidative barrier. For that reason the aim of the study was to determine the influence of chlorpyrifos and enrofloxacin, administered separately, or in combination on some indicators of enzymatic antioxidant defense cell system i.e.: activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).

Materials and Methods

Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) obtained from the Institute of Industrial Organic Chemistry in Warsaw was used in this study and according to the producer contained 99.0% of pure O,O-diethyl-O-3,5,6-trichloropyridyl phosphorothioate. Enrofloxacin, in the form of preparation of ENFLOCYNA® SOL was produced by the company Biowet Puławy, and contained 50 mg/ml of the active substance.

Studies were carried out on 120 Wistar rats (males), with initial body weight of 180 ± 10 g. The

animals originated from the Animal Breeding Center in Brwinów (near Warsaw) and during the acclimatization and experimental period were kept under standard environmental conditions (12 hours artificial day cycle, room temperature of 22 ± 1°C, air humidity of 70 ± 10%, gravitational-mechanical ventilation). The rats received standard chow "Murigran". Rats were divided into three experimental groups (I-III) and a control group (C). The animals from the group I were given enrofloxacin by a stomach tube at the dose of 5 mg/kg bw during three subsequent days. The rats from the group II received during 28 days by chlorpyrifos a stomach tube at the dose of 3 mg/kg bw. In group III animals were administered mentioned above substances at the same dose and period, but enrofloxacin was applied during the last three days of intoxication with chlorpyrifos. The animals from the control group received the chow and water *ad libitum*.

The experiment conducted on rats followed provisions of the "Act on Animal Protection" and recommendation of the Local Ethical Committee for Animal Experiments of the University of Warmia and Mazury in Olsztyn (Opinion no 49/N 2002).

At a different time after intoxication, i.e.: 3, 6, 24 hours as well as 3 and 7 day, samples of blood from six randomly selected rats were taken from particular groups for enzymatic assays. Assays were: activity of catalase (CAT) according the kinetic method of Aebi (1984), superoxide dismutase (SOD) and glutathione peroxidase (GPx) using the kinetic method with RANSOD and RANSEL analytical kits (RANDOX Lab. Ltd. UK).

Data were analysed statistically by a one-way analysis of variance ANOVA followed by the Newman-Keuls t-test. All data are expressed as mean ± SEM. A differences with $p \leq 0.05$ were regarded as statistically significant.

Results

The results of SOD, CAT and GPx activity in erythrocytes of rats in particular groups and time intervals are presented in Tables 1-3.

Administration of enrofloxacin (Group I) at applied dose caused a slight increase in SOD activity in comparison with the control group, which remained at this level up to the 3rd day of experiment. After 7th day slight (4.8%) decrease in the activity of that enzyme was noted (Table 1). The decrease in the SOD activity in erythrocytes of rats intoxicated with chlorpyrifos (Group II) was found up to the 3rd day, but between the 3rd and 24th hour that decline was statistically significant in comparison with the control group (Table 1). After the 7th day a slight (7.3%) increase in the activity of the enzyme was registered. In the group which received both chlorpyrifos and enrofloxacin (Group III) decrease in the activity of SOD was also observed in erythrocytes in comparison with the control group, but it was less pronounced. The decrease of SOD activity was between 6-17.5% in particular time periods and it was statistically significant only after 24 hours of the experiment.

The CAT activity in erythrocytes after administration of enrofloxacin (Group I) was only slightly (3-6%) increased in comparison with the control group. 1% decline was registered only in 3rd hour. Decrease in the activity of CAT in the blood of rats intoxicated with chlorpyrifos was noted up to 24 hour. That decrease was statistically significant after 3rd and 6th hour and it was 23.2% and 16.2%, respectively (Table 2). In the later periods i.e. after the 3rd and 7th day the increase in the activity of analyzed enzyme was noted. In the group of animals to which chlorpyrifos and enrofloxacin were given (Group III) a similar profile of changes in CAT activity in erythrocytes was observed as in the group of rats exposed only to chlorpyrifos. However the changes mentioned above were less intensive. A statistically significant decline in CAT activity in comparison to the control was found only after 3rd hour of the experiment (Table 2).

In all experimental groups and time intervals of the experiment, the increase in GPx activity in erythrocytes was noted. In the group of rats exposed to enrofloxacin (Group I) the GPx activity oscillated between 2 and 6%. It was observed that the higher increase of this parameter was in the group of rats which received only chlorpyrifos (Group II). The GPx activity increased gradually and its statistically significant maximum (19.4% – in comparison with the control group) was after the 7th day of the experiment (Table 3). In the group of rats which were given both chlorpyrifos and enrofloxacin (Group III) the profile of changes in GPx activity was similar to that one in the group II.

Discussion

The results of numerous research proved that a lot of xenobiotics posses ability of generation of free radicals, what leads to oxidative stress which causes cell and tissue damage (Albesa et al. 2004, Durak et al. 2009). Among xenobiotics such abilities are shown by, for example, phosphoroorganic insecticides (Karademir Catalgol et al. 2007, Lukaszewicz-Hussain 2008). In the course of evolution, the living organisms have developed a complex defence system which counteracts formation and negative action of reactive forms of oxygen. That system consists of enzymatic

Table 1. Superoxide dysmutase (SOD) activity in rat erythrocytes after administration of enrofloxacin, chlorpyrifos and chlorpyrifos and enrofloxacin (expressed as U/g Hb).

Time after intoxication	Group of animals			
	Control (C) (n = 6)	Enrofloxacin (I) (n = 6)	Chlorpyrifos (II) (n = 6)	Chlorpyrifos and Enrofloxacin (III) (n = 6)
3 h	1701.06 /±41.46/	1783.39 /±26.92/^{cd}	1308.12 /±47.70/^{ab}	1483.49 /± 54.59/^{ab}
6 h	1720.77 /±86.47/	1831.59 /±57.73/^{cd}	1422.73 /±92.47/^{ab}	1472.30 /±36.90/^{ab}
24 h	1633.83 /±83.04/	1693.30 /±82.24/^{cd}	1410.98 /±90.58/^{ab}	1347.58 /±25.38/^{ab}
3 d	1616.09 /±36.64/	1658.27 /±70.53/	1464.50 /±57.15/	1515.25 /±53.70/
7 d	1540.71 /±67.94/	1465.98 /±63.56/	1652.87 /±44.01/	1632.54 /±91.83/

values expressed as means ± SEM
n – the number of rats in the group
p – statistically significant in comparison with: a – control, b – enrofloxacin, c – chlorpyrifos, d – chlorpyrifos and enrofloxacin

Table 2. Catalase (CAT) activity in rat erythrocytes after administration of enrofloxacin, chlorpyrifos and chlorpyrifos and enrofloxacin (expressed as U/g Hb).

Time after intoxication	Group of animals			
	Control (C) (n = 6)	Enrofloxacin (I) (n = 6)	Chlorpyrifos (II) (n = 6)	Chlorpyrifos and Enrofloxacin (III) (n = 6)
3 h	475.86 /±22.56/	471.56 /±22,69/^{cd}	365.36 /±16.93/^{ab}	379.44 /±15.50/^{ab}
6 h	442.39 /±14.76/	464.14 /±19.44/^{cd}	361.78 /±15.43/^{ab}	367.31 /±18.83/^{ab}
24 h	476.01 /±19.83/	501.61 /±19.41/	431.50 /±17.20/	445.26 /±22.85/
3 d	449.74 /±8.69/	475.69 /±37.24/	525.33 /±20.28/	512.38 /±15.93/
7 d	459.07 /±9.18/	475.35 /±15.67/^{cd}	561.68 /±20.81/^{ab}	549.40 /±12.75/^{ab}

values expressed as means ± SEM
n – the number of rats in the group
p – statistically significant in comparison with: a – control, b – enrofloxacin, c – chlorpyrifos, d – chlorpyrifos and enrofloxacin

Table 3. Glutathione peroxidase (GPx) activity in rat erythrocytes after administration of enrofloxacin, chlorpyrifos and chlorpyrifos and enrofloxacin (expressed as U/g Hb).

Time after intoxication	Group of animals			
	Control (C) (n = 6)	Enrofloxacin (I) (n = 6)	Chlorpyrifos (II) (n = 6)	Chlorpyrifos and Enrofloxacin (III) (n = 6)
3 h	492.86 /±5.98/	520.39 /±12.40/	537.30 /±34.39/	540.44 /±28.54/
6 h	477.55 /±47.89/	497.66 /±24.30/	554.82 /±43.28/	545.91 /±31.36/
24 h	450.48 /±26.20/	479.31 /±14.58/	509.76 /±35.00/	502.66 /±19.15/
3 d	489.59 /±8.18/	501.04 /±43.99/	530.58 /±31.81/	556.11 /±30.75/
7 d	458.49 /±18.07/	485.86 /±8.25/^{cd}	547.53 /±17.59/^{ab}	545.73 /±22.86/^{ab}

values expressed as means ± SEM
n – the number of rats in the group
p – statistically significant in comparison with: a – control, b – enrofloxacin, c – chlorpyrifos, d – chlorpyrifos and enrofloxacin

and non-enzymatic systems which cooperate (Kulikowska-Karpińska and Moniuszko-Jakoniuk 2004). The oxidative enzymes, such as SOD, CAT and GPx act protectively against oxidative damage of cells and cooperate to eliminate free radicals and products of their decomposition. Lack or limited ability of an organism to inhibit uncontrolled reactions of free radicals is crucial for development of many diseases (Pitchumoni and Doraiswamy 1998, Dhalla et al. 2000).

Based on the conducted studies it was stated that administration of enrofloxacin and/or chlorpyrifos at applied doses caused the significant decrease in SOD and CAT activity during first hours after exposure and increase in GPx activity which remained at elevated level during all periods of the experiment. The changes in SOD, CAT and GPx activity were particularly noticeable in the group of animals exposed to chlorpyrifos. It may seem that it was a defence reaction of an organism against reactive forms of oxygen which generation was a consequence of administration of a phosphoroorganic insecticide. This hypothesis may be supported by works of other authors, who have observed disturbances of oxidative balance in animals during exposure to phosphoroorganic insecticides, including chlorpyrifos, and changes in the activity of analysed antioxidant enzymes were of different intensity and dependent on the dose of insecticide, time of exposure and the type of examined cells. Mansour and Mossa (2009) in the studies on rats (males and females), intoxicated orally with chlorpyrifos at the dose of 6.75 mg/kg bw during 28 days, stated that there was oxidative stress and it was expressed by the decrease in the activity in the erythrocytes of examined antioxidative enzymes, such as SOD, CAT and GST. That decrease was statistically significant and slightly more intensive in females than in males. In similar research on male rats, Uzun et al. (2010) observed increase in SOD and CAT activity and decrease in GPx in lungs after administration of chlorpyrifos during 28 days. Goel et al. (2005) after exposure of rats to chlorpyrifos during 8 weeks at the dose of 13.5 mg/kg bw (1/10 LD_{50}) noted increase in SOD and GSH-Px and decrease in CAT activity in liver. The increase in SOD activity in animals exposed to chlorpyrifos is explained by the authors with the fact that the production of free radicals was more intensive and could stimulate SOD to copy with intensified oxidative stress. The modulation of SOD activity along with exposure to pesticides can be result of different doses used for experiment. Low doses do not cause increase in the activity of the enzyme, whereas bigger doses can increase SOD activity in various tissues.

The decrease in CAT activity which was observed in our studies in rats exposed to chlorpyrifos subchronically is a result of use of that enzyme for transformation of H_2O_2 to H_2O. The difference in CAT and GPx activity is explained with the fact that H_2O_2 in lower concentration is metabolized mainly by GPx.

The literature data provide information that antibiotics, including fluoroquinolones, are metabolized in the liver by enzymes of the cytochrome P450 complex, what leads to generation of free radicals and lipids peroxidation (Albesa et al. 2004, Zhang et al. 2011). Information concerning enrofloxacin and its oxidative properties are not unanimous. Studies on different living organisms have shown that both enrofloxacin and its metabolite ciprofloxacin may cause oxidative stress (Becerra et al. 2004, Tu et al. 2008). Benzer et al. (2009) in the research on chickens intoxicated with enrofloxacin at the dose of 10 mg/kg during 9 days observed oxidative stress, which was expressed by increase in MDA level in serum and intestines, decrease in CAT activity in liver and intestines and GPx in erythrocytes. Yazar and Tras (2001), administering intravenously fluoroquinolones to mice registered significant increase in SOD activity and decrease in GPx in liver after administration of danofloxacin, whereas enrofloxacin caused only decline in GPx activity in the liver. The authors explain the difference in the activity of examined enzymes under influence of applied substances with the fact that the higher dose of danofloxacin could cause production of free radicals, which are directly inactivated GPx, whereas SOD activity was slowly inducted by these radicals. On the other hand Altinordulu and Eraslan (2009) administering enrofloxacin to chickens during 3 days at the dose of 10 mg/kg bw observed significant decrease in CAT activity in erythrocytes of birds in comparison with the control group. However, these changes were observed only on the first day after exposure. During subsequent time periods i.e. – after 3, 5 and 7 days, the CAT activity was higher than in the control group, but this increase was not statistically significant. On the basis of these results the authors suggest the lack of correlation between dose of enrofloxacin and oxidative damage. The results of our investigations also indicate that the administration of enrofloxacin at the dose of 5 mg/kg bw during three subsequent days does not cause essential changes in the activity of analyzed antioxidative enzymes, what may provide the evidence that there is no oxidative damage. Studies of Carrerasa et al. (2004) may support our observations. They also did not observe changes in SOD, CAT and GPx activity in chickens exposed to enrofloxacin at the dose of 50mg/L administered during five days with drinking water.

More and more information concerning co-action of phosphoroorganic insecticides and medicines with different compounds on oxidative processes has been

published recently (Anderson and Zhu 2004, Sivapiriya et al. 2006, Uzun et al. 2010). Most of investigations however, concerns protective action of vitamins and minerals during exposure to phosphoroorganic insecticides. Verma et al. (2007) found significant increase in the activity of CAT and SOD as well as in the level of GSH in various tissues in rats treated with chlorpyrifos and pretreated with mixture of antioxidant vitamins (A+E+C) comparing to the animals given chlorpiryfos only. Similar results were observed by Mansour and Mossa (2009) who stated that zinc alleviates results of oxidative stress caused by chlorpyrifos in rats. Supplementation of zinc at a dose of 227 mg/l^{-1} during 28 days showed alteration in the activity of CAT, SOD and GST in the rats treated with chlorpiryfos. In our previous research on rats (Barski and Zasadowski 2008) we observed the occurrence of oxidative stress after administration of dimethoate and pyrantel embonate, in the group of mixed intoxication the changes in analyzed parameters were more larger, what was not observed in case of exposure to enrofloxacin and chlorpyrifos.

Conclusions

In summary, it was found on the basis of our study that the administration of enrofloxacin at applied does not evoke oxidative action. Subchronic intoxication of rats with chlorpyrifos leads to occurrence of oxidative stress, expressed as significant changes in SOD, CAT and GPx activity, especially in the first period after exposure. In the mixed group, the profile of changes was similar to that one observed in the group of rats intoxicated only with phosphoroorganic insecticide, what indicates the lack of co-action between the compound applied in the experiment. However, according to the authors, because of the lack in the available literature of information concerning directly that subject, the study should be continued further.

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