Polish J. of Environ. Stud. Vol. 16, No. 1 (2007), 91-95

Original Research

Effect of α-Cypermethrin and Chlorpyrifos in a 28-Day Study on Free Radical Parameters and Cholinesterase Activity in Wistar Rats

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Received: March 21, 2006
Accepted: September 25, 2006

Abstract

In the current study the influence of single compounds and concurrent exposure to popular insecticides: organophosphate (OP) – chlorpyrifos (CPF) and synthetic pyrethroid (PYR) – α-cypermethrin (CM) on some oxidative stress parameters and cholinesterase (ChE) activity in rats was investigated. Animals received by gavage 10 mg of single compounds or 5 mg of each per kg bw daily in rapeseed oil for 14 and 28 days. Concentrations of total thiols and TBARS, activity of catalase and cholinesterase were measured in tissues. Total thiol concentrations declined in plasma in all experimental groups after 14 and 28 days, while in liver a decrease was noted after only 14 days in animals receiving CPF and after 28 days in rats treated with CM alone with a mixture of pesticides. Lipid peroxidation presented as TBARS concentration was elevated mostly after 2 weeks of exposure in brain and liver but not in plasma in all experimental groups. Catalase activity increased in erythrocytes in all groups treated with insecticides, while in liver CM administered alone reduced the activity of the enzyme. Cholinesterase was markedly depressed to a different degree in plasma and brain of animals receiving CPF alone or in combination, while CM did not significantly elevate brain ChE.

The results of this study seem to indicate that CM and CPF apart from known modes of action demonstrate their toxicity also through free radical mediated mechanisms. It is also evident that CM administered with CPF does not affect the cholinesterase inhibition generated by the latter.

Keywords: catalase, chlorpyrifos, cholinesterase, α-cypermethrin, TBARS

Introduction

Since the 1960s modern generations of pesticides were systematically introduced and with success replaced organochlorine pesticides (OC), very potent but highly resistant to biodegradation and with a high tendency to cumulate in the food chain, which despite its banned use since the 1970s in developed countries are still in relatively high concentrations found in the environment. Moreover, they have been produced in China and Mexico at least up to 1999 [1]. The new insecticides show very good efficiency in pest eradication, a low tendency to cumulate and quite low toxicity to humans – they are likely to be used in large quantities in agriculture for crop protection, veterinary medicine and in households to kill unwanted bugs. Actually, the most popular classes of insecticides are organophosphates (OP) and synthetic pyrethroids (PYR), which are offered in Poland in many formulations. In this experiment, we focus on two insecticides representing these groups, utilized as single compounds or as a mixture of both – α-cypermethrin (CM) and chlorpyrifos (CPF). Although the main mechanisms of action for CM

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and CPF are well known, several studies have been conducted to extend the knowledge and to explain unknowns, especially in the field of neurotoxic long-term effects and developmental toxicity. In recent years, a closer look was taken at free radical mediated damage as an effect of exposure to xenobiotics [2-9]. In the case of OP and PYR, some results are published but to our knowledge, no reports have so far described oxidative stress parameters in rats simultaneously intoxicated with CM and CPF. OP irreversibly affects esterases among which ChE is the most significant, but also inhibits other esterases involved in hydrolyzing the ester bond in pyrethroid molecule. Thus OP noticeably lowers the LD_{50} value for PYR in combined exposure [10]. There is a disagreement in reports considering the cholinesterase activity in PYR exposure; some of them suggest a strong ability for enzyme depression [5] up to seven days in erythrocytes and 14 days in plasma after single dose, while others observed completely opposite results, implying higher brain ChE activity in CM but not in permethrin intoxication [11].

α -CM consists of two *cis*- isomers from the 8 isomers present in cypermethrin. These 2 isomers comprise the most biologically active enantiomer pair. In some studies it is not clearly stated what kind of mixture, by means of *cis:trans* isomer ratio is used, omitting differences in LD_{50} for *cis* and *trans* isomers caused by various metabolic rates of hydrolysis and oxidation [12]. The αCM molecule contains, in contrast to type I pyrethroids, an α-cyano group in the phenoxybenzyl alcohol moiety, which seems to be responsible for production of long-lasting prolongation of sodium permeability; clinically characterized by choreoathetosis and salivation. During metabolism, CM forms cyanohydrines decomposing further to cyanides and aldehydes – substances that can induce production of reactive oxygen species (ROS).

The aim of this study was to assess the impact of cypermethrin and chlorpyrifos administered separately and in combination with free radical parameters and cholinesterase activity.

Experimental

The study was performed on male Wistar rats weighing about 300 g. The animals were kept under standard conditions with free access to water and rat chow (Labofeed B, Wytwórnia Pasz, mgr inż. A. Morawski, Kcynia, Poland). All the procedures were approved by the Local Bioethics Committee of the Medical University of Gdańsk. Insecticides used in this experiment were administered in doses exhibiting no distinct toxic effects. Animals were randomly divided into four groups of twelve rats each (except for control, n=18):

I. C – Control animals receiving 0.5 mL of rapeseed oil,
II. CM – Animals receiving 10 mg CM/kg bw in rapeseed oil,
III. CPF – Animals receiving 10 mg CPF/kg bw in rapeseed oil,
IV. CMCPF – Animals receiving 5 mg CM and 5 mg CPF/kg bw in rapeseed oil.

All groups of rats were dosed intragastrically 7 days a week, always in the morning. Solutions of pesticides were prepared weekly to correct the dose.

Additionally, during 96 hours after administration of single compounds (10 mg/kg bw CM or CPF) or binary mixture of pesticides (5 mg/kg bw each), blood from the tail vein was sampled at intervals to monitor ChE activity.

On the 13th and 27th days of the experiment, 6 animals from each group directly after dosing were placed in metabolic cages and deprived of feed but had free access to water in order to collect urine for metabolic studies. Next morning animals received the last dose of pesticides and 4 hours later were exsanguinated by cardiac puncture in light ethyl ether narcosis. Blood from hearth was collected into cooled, heparinized tubes. Tissues (liver and brain) were rapidly dissected and washed with ice-cold saline to remove traces of blood and stored on ice until processing. Blood was centrifuged for 10 minutes (2800 rpm) at 4°C to separate red blood cells (RBC) and plasma. Tissues were homogenized in a glass Potter-Elvehjem tissue grinder to obtain 20% homogenates in Tris-HCl buffer pH 7.4, followed by centrifugation for 10 minutes at 4°C (3000 rpm). Obtained post-nuclear fractions were used for biochemical assays. For CAT determination, RBC were washed thrice with ice-cold saline and centrifuged for 10 minutes (2800 rpm) at 4°C and then haemolyzed in 4 volumes of bi-distilled water, liver homogenates and haemolyzed RBC were diluted 500-fold before determination of activity. TBARS concentration was determined with the method of Rice-Evans et al. [13], catalase activity according to Aebi [14], total thiols with Sedlak and Lindsay method [15], cholinesterase activity assessed by the method of Ellman et al. [16] and protein concentration according to Lowry et al. [17]. The Assayed Human Sera Level 2 – 296UN from RANDOX was used as a reference standard for the ChE activity and protein assay. Data are shown as means ± standard deviation. Normal distribution of the results was ascertained with Shapiro-Wilk's test. Analysis of variance (ANOVA) has been performed and Student's t-test was used to compare individual experimental groups to control and the multiple comparisons were evaluated with Tukey's post-hoc test. All statistical calculations were done using Statistica 7.0.

Results and Disscussion

During the experiment, animals from all groups were checked daily for any signs of toxicity. Slight choreoathetosis and hypersensitivity were noticed in rats dosed with 10 mg CM/kg bw about 4-5 hours after dosing and these signs gradually declined after next few hours; similar effects to a lesser extent we observed in the CMCPF group. No visible adverse effects were observed in the CPF group.

Table 1. TBARS and total thiol concentrations and catalase activity in rats dosed with CPF and CM alone or in combination. Values are presented as a mean ± SD.

Group	Plasma		CAT (RBC) [k]	Liver			Brain	
	TBARS [nM/g prot.]	SH [μM/g prot.]		TBARS [nM/g prot.]	SH [μM/g prot.]	CAT [k]	TBARS [nM/g prot.]	SH [μM/g prot.]
C 0		8.245 ± 0.288	5.8 ± 0.4		0.149 ± 0.028	12.5 ± 0.8		0.142 ± 0.015
C 14	51.5 ± 2.6	8.362 ± 0.448	5.9 ± 0.6	114.8 ± 8.0	0.170 ± 0.025	12.9 ± 2.3	419.6 ± 55.1	0.149 ± 0.014
CM 14	51.4 ± 4.6	5.801 ± 0.635****	6.1 ± 0.3	158.8 ± 18.8***	0.182 ± 0.018	9.0 ± 1.8*	567.6 ± 63.9***	0.139 ± 0.011
CPF 14	54.8 ± 2.4*	6.511 ± 0.894***	6.9 ± 0.3***	127.8 ± 6.4**	0.128 ± 0.035*	12.8 ± 1.8	509.6 ± 42.7**	0.121 ± 0.015***
CMCPF 14	49.0 ± 0.7	6.231 ± 0.839***	7.4 ± 0.9**	107.2 ± 11.8	0.159 ± 0.012	17.6 ± 2.5***	753.7 ± 227.6**	0.158 ± 0.002
C 28	49.0 ± 0.7	9.455 ± 0.400	5.9 ± 0.2	105.7 ± 9.0	0.216 ± 0.007	14.3 ± 0.8	408.9 ± 36.5	0.139 ± 0.009
CM 28	50.9 ± 2.2	5.591 ± 0.753****	6.5 ± 0.4*	110.1 ± 4.8	0.161 ± 0.026***	12.2 ± 1.1**	410.9 ± 44.6	0.138 ± 0.016
CPF 28	47.6 ± 6.8	7.055 ± 1.694**	5.9 ± 0.2	119.3 ± 17.2	0.191 ± 0.023	13.9 ± 0.9	522.2 ± 71.9***	0.138 ± 0.011
CMCPF 28	49.1 ± 2.5	6.260 ± 0.450****	6.9 ± 0.5**	111.8 ± 11.4	0.145 ± 0.003****	14.8 ± 2.1	378.9 ± 14.3	0.142 ± 0.005

Significance levels:* – p<0.05, ** – p<0.02, *** – p<0.01, **** – p<0.001; experimental groups were compared to adequate control group, respectively C 14 or C 28, with Student's t-test.

The results of TBARS and total thiols concentrations and catalase activity in RBC, plasma, liver and brain are presented in Table 1. Time-dependent changes of ChE activity in plasma of rats intoxicated with single doses of CM, CPF and both compounds are presented in Fig. 1. The activity of cholinesterase in plasma and brain are given in Fig. 2.

The effect of chlorpyrifos on ChE activity was extensively investigated in various schemes of dosing, routes of exposure and on diverse animal species and also in human studies [18, 19]. Some reports also describe effects of mixtures of chlorpyrifos with other pesticides, e.g. cypermethrin [20, 21] and methyl parathion [22] on ChE activity. The obtained results are consistent and unequivocally present CPF as a highly potent nonreversible ChE

Fig. 1. Time course changes of plasma cholinesterase activity after single intragastric dose of pesticides (CM – 10 mg/kg bw, CPF – 10 mg/kg bw, CMCPF – 5 mg/kg bw each).

	plasma			brain	
	single dose	14 days	28 days	14 days	28 days
C vs. CM	ns	ns	ns	ns	ns
vs. CPF	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
vs. CMCPF	p<0.001	p<0.001	p<0.001	ns	p<0.02
CM vs. CPF	p<0.001	p<0.001	p<0.001	p<0.001	p<0.001
vs. CMCPF	p<0.001	p<0.001	p<0.001	p<0.01	p<0.001
CPF vs. CMCPF	ns	ns	ns	p<0.001	p<0.001

Fig. 2. Plasma and brain cholinesterase activity measured 4 hours post-dosing. Values are expressed as a percentage of mean activity in relation to adequate control group (C). In the table, multiple comparisons with statistical evaluation between groups are presented.

inhibitor. Similarly, the results of this experiment show that chlorpyrifos, at a dose 10 mg/kg bw significantly decreased plasma ChE activity to about 15% of control values 12 hours after dosing. Repeated oral exposure to 10 mg CPF/kg bw for 14 days caused further inhibition to 8.3% and a similar level of activity was noted after 28 days – 10.1%. CM given alone in a single dose did not cause any significant changes in plasma ChE during 96 hours after dosing, when binary mixture depressed predosing activity maximally by 76.0% even after 12 hours. Recovery in plasma ChE activity occurred in both groups after 96 hours (rats treated with a single dose of CPF or CMCPF). When CPF was co-administered with CM at a dose of 5 mg/kg bw each, plasma ChE was inhibited to 14.6 and 19.4% of control activity, respectively, after 14 and 28 days of exposure. The results suggest a dose response effect in enzyme inhibition. In a repeated dose 28-day oral study, Jacobsen et al., noted slight but significant inhibition in plasma ChE in male rats exposed to CPF at doses of 0.15 and 0.3 mg/kg bw/day, while lower doses (0.006 and 0.03 mg CPF/kg bw/day) did not produce any depression in enzyme activity [21]. In the same study, females treated in the same manner showed no plasma ChE inhibition. Moreover, brain ChE was not affected in both sexes of rats by administered doses of CPF. The same study included also combined exposure of CPF and α-CM. In animals receiving CPF at doses of 0.006 and 0.03 mg/kg bw in combination with 18 mg/kg bw of α-CM no changes in plasma and brain ChE were noticed. The results of this study did not confirm the hypothesis that inhibition of brain and plasma ChE by CPF could be affected by co-administration of CM. The results of our study are consistent with Rao and Rao [11], who noticed a significant increase in ChE activity in rats dosed with cypermethrin (isomers ratio not specified). Krechniak and Łoboda-Pelplińska [23] studied the effect of several pyrethroids on blood, plasma, liver and brain cholinesterase in rats. In conclusion, only decamethrin caused inhibition in blood ChE, while cypermethrin and permethrin did not depress ChE activity in any of investigated tissues [23]. Latuszyńska et al. performed a study on female rats dermally exposed to a formulation of Nurelle® – a mixture of CPF and CM, for 4 hours, 2 and 4 weeks. The respective doses were: low dose 5.6 mg CPF and 0.5 mg CM per cm^2 and high dose: 27.8 mg CPF and 2.7 mg CM per cm^2. Authors observed a significant decrease in plasma and brain ChE activity [20].

Gultekin et al. [9] in an experiment *in vitro*, studied the impact of CPF on lipid peroxidation and antioxidant enzyme activity in RBC. In the lower concentration range (0.05-0.1 g/L), CAT activity was not affected, whereas TBARS concentration increased. At higher levels (0.4-100 g/L) a concentration and time dependent significant increase in lipid peroxidation and decrease in CAT activity was assessed.

Khan et al. administered a combination of CPF and CM (20 mg/kg bw each) for 15 days to male mice and studied their impact on free radical parameters in the liver. In dosed animals, they found reduced total thiol concentrations and catalase activity [24]. In another experiment [8] performed in rats given 50 mg/kg bw CM for 5 days an induction of lipid peroxidation was found in liver, kidney and brain, but not in plasma. In addition, a significant increase in CAT activity in erythrocytes and decrease in liver was noticed. In our study, a significant increase in RBC catalase activity in CM-treated rats was found after 28 days of exposure, while in animals exposed to both compounds, RBC catalase activity was markedly elevated at both time points. CPF given alone also caused an increase in erythrocyte CAT activity after 14 days but after the next 2 weeks it decreased to control values. Atessahin et al. found in rats treated with CM a decrease in liver CAT activity [8]. The same effect we observed in our experiment after 14 and 28 days of exposure. Surprisingly, in liver the mixture of both insecticides increased CAT activity, whereas CM administered alone produced the opposite effect. After 14 days of exposure to both compounds, a significant elevation in CAT activity was noted, while after the next 14 days it dropped to control level.

The results of this study seem to indicate that both CM and CPF administered as single compounds or in combination cause an impact on different free radical mediated parameters. The exact mechanisms of this action need further investigation.

The present study confirms that the inhibitory effect of CPF on ChE activity is not influenced by co-exposure to pyrethroids.

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