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THE EFFECT OF DICHLORVOS TREATMENT ON BUTYRYLCHOLINESTERASE ACTIVITY AND LIPID METABOLISM IN RATS

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This paper describes the effects of dichlorvos (DDVP) on butyrylcholinesterase (BuChE) activity with possible consequences for lipid and lipoprotein metabolism in rats. The rats of both sexes were given a single and multiple doses of DDVP (8.0 mg/kg body weight) with two–day intervals between administrations, ensuring the continuous inhibition of BuChE activity without lethal outcome. BuChE activity was measured in plasma, liver, and white and brown adipose tissue. The recovery of BuChE activity was observed only in white adipose tissue of female rats 10 days after treatment. Our results show that DDVP significantly decreases BuChE activity in female and male rat plasma (40–60%; $P<0.05$), and significantly increases triglycerides (60–600%; $P<0.05$) and total cholesterol (35–75%; $P<0.05$). In contrast to the increased HDL–cholesterol (20–30%; $P<0.05$), LDL–cholesterol decreased (30–40%; $P<0.05$). The decrease of BuChE activity and the changes in concentrations of lipids and lipoproteins were observed throughout the experiment. Our results contribute to the hypothesis that BuChE may play a role in lipid and lipoprotein metabolism.

KEY WORDS: *BuChE, DDVP, HDL–cholesterol, LDL–cholesterol, total cholesterol, triglycerides*

Much is known about the irreversible and direct inhibition of the active centre of butyrylcholinesterase (BuChE: EC 3.1.1.8, acylcholine acylhydrolase, plasma cholinesterase) by organophosphorus (OPs) compounds (1, 2). BuChE inhibition is of practical importance, because the exposure to OP compounds in humans relies on laboratory testing of plasma BuChE and red cell acetylcholinesterase (AChE: EC 3.1.1.7) activity. Although BuChE is synthesised in the liver and is released in blood, it has been found in various tissues (e.g. adipose tissue, small intestine, lung and the white matter of the brain). However, it is not known whether the BuChE found in these tissues originates only from blood, or it can be synthesised in those tissues as well (3–5). BuChE is an enzyme without known biological substrate in mammals (6), but it hydrolyses a variety of esters including butyrylthiocholine, butyrylcholine, propionylthiocholine, propionylcholine, and pharmacologically important succinylcholine. It has been suggested that BuChE is the precursor of AChE in the nervous system, with an important role in the regulation of slow impulse conduction in the nervous system (7). Pretreatment with purified human BuChE has a protective effect in mice, rats, and primates exposed to OP compounds (8–10). Some authors suggest that BuChE plays a role in lipid and lipoprotein metabolism (4, 11–12).

In human poisoning with parathion, a decrease in BuChE activity correlated with a decrease in low–density lipoproteins (LDL–cholesterol) and in the total cholesterol concentration (13). With clinical recovery, all parameters returned to normal values. The hypothesis that LDL–cholesterol is formed from very–low density lipoproteins (VLDL–cholesterol) in the presence of BuChE (11) is supported by the fact that increased BuChE activity is associated with abnormal lipid metabolism in humans (14–16). However, the data presented by *Schouten and co–workers* (17) challenge this hypothesis, as they show that BuChE activity in heterozygous familial hypercholesterolemic patients is not related to changes in LDL–cholesterol and high–density lipoproteins (HDL–cholesterol).

Similar changes were observed in animals. Fat Zucker rats that exhibited a marked increase in VLDL–cholesterol had high plasma BuChE activity (18–19). Changes in BuChE activity and lipid metabolism were found in diabetic and normal rats after administration of a specific BuChE inhibitor, iso–OMPA (tetraisopropyl pyrophosphoramide) (19). Rabbits treated with dichlorvos (2,2–dichlorovinyl dimethylphosphate, DDVP) also showed a correlation between decreased BuChE activity and lipid and lipoprotein concentration (15).

OPs compounds are highly toxic substances that have been widely used in agriculture as pesticides for many years. A very limited number of them may be used as drugs in human and veterinary medicine. Metrifonate (Trichlorfon) is used as an antiparasitic drug in the treatment of schisostomiasis (20), and some clinical trials have shown its effectiveness in the therapy of Alzheimer's disease (21). It is known that in a mammalian organism Metrifonate rearranges spontaneously at physiological pH to form the highly toxic DDVP. On the other hand, the widespread use in agriculture and the high vapour pressure of DDVP presents a real danger of poisoning for workers occupationally exposed to DDVP formulations. DDVP is not only esterase inhibitor; according to scarce literature data it may also disturb different metabolic processes through phospolipase and lipase inhibition (15, 22).

Due to quick elimination and long–term inhibition of BuChE activity, we considered DDVP appropriate for studying the relationship between BuChE activity and lipid metabolism. The involvement of BuChE in lipid metabolism may be important in the professional exposure to DDVP.

By comparing BuChE plasma activity with that in the liver and white and brown adipose tissue, we wanted to find evidence of the enzyme's synthesis in adipose tissue.

MATERIAL AND METHODS

Chemicals

Dichlorvos, (2,2–dichlorovinyl dimethylphosphate, DDVP); obtained from "Cosmochemia" Otočac, Republic of Croatia, purity 93%) was dissolved in glycerol formal (methylidinoglycerol; 50.0 mg/ml).

Treatment of animals

Male and female adult Wistar rats (240–280 g body weight) were fed on a standard diet for laboratory rodents (Sljeme, Zagreb, Republic of Croatia). Animals had free access to water and were kept in macrolone cages under controlled conditions (room temperature 21 °C, light and dark cycle exchanging every 12 hours). The animals (30 males and 30 females) were randomly divided in three control groups (n=5 each), and three treated groups (n=5 each). In our preliminary study, we established the intraperitoneal (i.p.) DDVP LD_{50} for rats of 16.0 mg/kg body weight. The dose of 12.0 mg DDVP/kg body weight (3/4 LD_{50}) resulted with high mortality of rats. The dose of 4.0 mg of DDVP/kg body weight (1/4 LD_{50}) inhibited only about 10–20% of plasma BuChE activity without changes in lipid and lipoprotein plasma levels. For this study we chose 1/2 DDVP LD_{50} (8.0 mg /kg of body weight) because it inhibited about 50% of BuChE activity in rat plasma with a 100% survival.

One treated group received a single dose of DDVP and was sacrificed 48 h afterwards. Two groups of animals were treated twice (at 48 h intervals) and sacrificed 48 h and 10 days after the last treatment, respectively. The controls were given a solvent according to the same experimental schedule.

All animals were sacrificed with coal gas; blood samples were obtained directly from the heart, and adipose tissue was isolated from the epididymal (males) or parametrial (females) fat depot. Liver tissue was rinsed with saline. Plasma and tissue samples were stored and frozen immediately after sampling at –20 °C until further processing. The tissue samples were homogenized (200 mg tissue/ml saline) and supernatant was obtained by centrifuging for 15 min at 2800 G.

BuChE

The BuChE activity in the plasma and tissues (liver, white and brown adipose tissue) was determined by spectrophotometry (23) using butyrylthiocholine (0.9 mM) (Sigma ChemCo, St. Louis, USA) as a substrate. Since liver tissue contains BuChE and AChE, the BuChE assay was carried out with and without the specific BuChE inhibitor ethopropazine hydrochloride in final concentration of 1.7 μM (Sigma ChemCo, St. Louis, USA). BuChE activity was calculated indirectly as the difference of the two measurements. The activity of the enzyme is expressed as μmol of substrate hydrolysed/min/ml of plasma, i.e. μmol of substrate hydrolysed/min/g of tissue. Since there was no difference between control groups of the same sex in the activity of BuChE either in plasma or in all tissues, the mean BuChE activity in all males or in all females (n=15) was taken as control value. Relative changes in the enzyme activity in the treated animals are presented as the percentage of activity of the respective control group.

Lipids and lipoproteins

The concentrations of total cholesterol in the plasma were determined using a cholesterol enzymatic colorimetric test ("Herbos Dijagnostika", Sisak, Republic of Croatia) (24–25). Triglyceride concentrations were determined using a triglyceride enzymatic colorimetric test – so called GPO–PAP method ("Olympus Diagnostica" GmbH, Hamburg, Germany) (26–27). HDL–cholesterol levels were determined using the method of *Burstein and co–workers* (28). Friedwald's formula was used for calculating LDL–cholesterol levels from total cholesterol, triglycerides and HDL–cholesterol values (29). The concentrations of lipids and lipoproteins were measured in duplicate, and expressed as mmol/l of plasma. Since there was no difference in the plasma concentration of lipids and lipoproteins between control groups of the same sex, the mean concentration in all males or in all females was taken as control value. Relative changes in lipid and lipoprotein concentrations in treated animals are shown as the percentage of the respective control group.

Statistical analysis

Data are shown as the mean plus standard deviation (SD). The mean, medians and SDs were calculated using a software package Statistics® for Windows Version 5.0 A. The statistical significance was determined by parametric *t*–test and non–parametric Kolmogorov–Smirnov two–sample test, where appropriate. The differences discussed in this paper were considered significant at $P<0.05$ level of significance.

RESULTS AND DISCUSSION

Table 1 shows the results of our BuChE assay in plasma, liver, and white and brown adipose tissue of male and female rats. In DDVP–treated rats, the relative inhibition of BuChE was comparable for both sexes (up to 60%) and remained was present throughout the study. The decrease in BuChE activity in white adipose tissue was significant and was similar to the inhibition in the plasma and liver in all but one treated group. The inhibition of BuChE activity in the adipose tissue was significant in all treated males. In female rats treated with a single dose and sacrificed 48 h later and in female rats treated with two doses and sacrificed 48 h after the last treatment the enzyme inhibition in the white adipose tissue was 47% and 70%, respectively, and in the brown adipose tissue 15% and 35%, respectively. The BuChE activity in the adipose tissue collected from female rats treated with two DDVP doses and sacrificed 10 days after the last treatment did not differ from controls. The recovered BuChE activity observed in the last group of female rats may be the consequence of estrogenic effect on the enzyme synthesis in adipose cells. Namely, it is known that in addition to the ovary, sex hormones in females synthesise in the adipose tissue. Unlike in the adipose tissue, BuChE activity did not recover in the liver of either male or female rats after the DDVP treatment ended. Our results of BuChE activity assay showed that, beside the liver, the synthesis of BuChE might occur in the adipose tissue. A relatively poor circulation in the adipose tissue may account for the weaker effect of DDVP on estrogens and on the increase in BuChE activity.

The decrease in plasma BuChE activity and the concurrent significant changes in lipid concentrations were observed in rats of both sexes after single and multiple DDVP treatments. The results are shown in Table 2.

It is known that plasma HDL–cholesterol in rats is much higher than LDL–cholesterol, and that HDL strongly dominates in total cholesterol (30).

The changes in total cholesterol, HDL–cholesterol and triglycerides in the plasma of all treated groups

Table 1 *Catalytic activities and relative changes of BuChE (Mean ± SD) in plasma and tissue of male and female rats after a single dose and two doses of DDVP (8.0 mg/kg b.w.; i.p.)*

Specimens	Units	Control group	Experimental groups Time of sacrifice (days after last dosing)		
			Single dose	Two doses	
		N=15	2 N=5	2 N=5	10 N=5
Plasma					
Male	μmol/min/ml	0.05±0.01	0.03±0.01**	0.03±0.01**	0.02±0.01**
	(%)	(100)	(60)	(60)	(40)
Female		0.15±0.00	0.07±0.02*	0.08±0.03*	0.06±0.01**
		(100)	(47)	(53)	(40)
Liver					
Male		0.36±0.01	0.11±0.03**	0.11±0.04**	0.11±0.01**
		(100)	(31)	(31)	(31)
Female		0.50±0.05	0.12±0.06**	0.10±0.02**	0.13±0.07**
		(100)	(24)	(20)	(26)
White adipose tissue	μmol/min/g				
Male	(%)	0.33±0.17	0.14±0.03^{+}	0.10±0.02^{++}	0.15±0.02**
		(100)	(42)	(30)	(45)
Female		0.45±0.06	0.24±0.04^{+}	0.14±0.04^{+}	0.42±0.20
		(100)	(53)	(31)	(93)
Brown adipose tissue		0.35±0.08	0.28±0.10	0.09±0.01^{++}	0.14±0.03^{+}
Male		(100)	(80)	(26)	(40)
		0.37±0.20	0.32±0.08	0.24±0.10^{+}	0.35±0.08
Female		(100)	(86)	(65)	(95)

Legend: significantly different from controls, * *t*–test, P<0.01; ** *t*–test, P<0.001; $^{+}$ Kolmogorov–Smirnov test, P<0.01; $^{++}$ Kolmogorov–Smirnov test, P<0.001.

of rats do not seem consistent (Table 2). Rats treated with multiple doses of DDVP showed a decrease in LDL–cholesterol. Our results suggest that the changes in BuChE activity affect lipid metabolism in a sex–independent manner, and are in accordance with the hypothesis published by *Kutty and co–workers* (11) that LDL–cholesterol is formed from VLDL in the presence of BuChE.

Triglycerides in plasma are decomposed into free fatty acids by hepatic and plasma lipoprotein lipase (31). According to *Kozlowska and co–workers* (22), a single high oral dose (1/2 LD_{50}) of DDVP significantly inhibits lipoprotein lipase activity in rats sacrificed up to 48 hours after dosing. Repeated small doses of DDVP (1/20 LD_{50}) given consecutively over 10 days did not inhibit the activity of lipoprotein lipase, but the concentration of lipoprotein fraction increased. In our experiment, the increased concentration of triglycerides and the inhibition of BuChE activity were found in plasma of animals given two doses (with 48h interval) of DDVP and sacrificed 2 and 10 days after the last treatment. The high affinity of DDVP for esterase is well known, and it is possible that lipoprotein lipase activity would be inhibited only if a high concentration of DDVP is applied. Therefore, it may be assumed that changes in lipoprotein concentrations in the period of up to 48 hours may be caused by simultaneous inhibition of lipoprotein lipase and BuChE activity. It seems that the increase in triglyceride concentrations found by *Kozlowska and co–workers* (22) in animals

Table 2 *Concentrations and relative changes of plasma lipids and lipoproteins (Mean ± SD) in male and female rats after the administration of a single dose and of two doses of DDVP (8.0 mg/kg b.w.; i.p.)*

Lipids and lipoproteins	Unit	Control group	Experimental groups Time of sacrifice (days after last dosing)		
			Single dose	Two doses	
		N=15	2 N=5	2 N=5	10 N=5
Triglycerides					
Male		1.22 ±0.20 (100)	1.20±0. (98)	1.90±0.04*** (156)	3.30±0.20^{+} (270)
Female		1.10 ±0.10 (100)	1.10±0.04 (100)	2.20±0.20** (200)	6.60±0.20^{++} (600)
Cholesterol					
Male	mmol/l (%)	1.40 ±0.07 (100)	1.90±0.02** (136)	1.50±0.04 (107)	1.18±0.02* (84)
Female		1.60 ±0.10 (100)	1.80±0.20 (112)	1.70±0.10 (106)	2.70±0.05^{+} (169)
HDL cholesterol					
Male		0.99 ±0.15 (100)	1.20±0.10* (121)	1.10±0.05 (111)	1.30±0.10** (131)
Female		1.10 ±0.10 (100)	1.10±0.09 (100)	1.30±0.10** (118)	1.40±0.07** (127)
LDL cholesterol					
Male		0.17 ±0.01 (100)	0.26±0.02** (176)	0.10±0.02* (59)	0.10±0.01* (59)
Female		0.14 ±0.01 (100)	0.31±0.05** (221)	0.10±0.01* (71)	0.10±0.01* (71)

Legend: significantly different from controls, * *t*–test, P<0.05; ** *t*–test, P<0.01; *** *t*–test, P<0.001; $^{+}$ Kolmogorov–Smirnov test, P<0.05; $^{++}$ Kolmogorov–Smirnov test, P<0.01.

treated with low doses of DDVP and our own results for rats sacrificed 10 days after the termination of treatment support the hypothesis that BuChE is involved in the metabolism of lipids.

In conclusion, the inhibition of BuChE activity seems to be connected with changes in lipid metabolism. Judging from experimental animal data, we believe that in addition to the measurement of BuChE activity, it would be appropriate to control lipoprotein levels in workers occupationally exposed to DDVP.

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Sažetak

UČINAK DIKLORVOSA NA AKTIVNOST BUTIRILKOLINESTERAZE I METABOLIZAM LIPIDA U ŠTAKORA

U radu su opisani učinci diklorvosa (DDVP) na aktivnost enzima butirilkolinesteraze (BuChE) i metabolizam lipida i lipoproteina u štakora obaju spolova. Učinci DDVP–a u dozi od 8,0 mg/kg tjelesne težine istraživani su poslije primjene jedne i dviju intraperitonealnih aplikacija. Kod ponovljenih aplikacija DDVP je davan svakih 48 sati kako bi se osigurala kontinuirana inhibicija BuChE u tretiranih životinja, ali bez pojave smrtnosti životinja. Aktivnost BuChE izmjerena je u plazmi, jetri te bijelome i smeđem masnom tkivu. Oporavak aktivnosti BuChE uočen je samo u bijelom masnom tkivu ženki štakora koje su žrtvovane 10 dana nakon zadnjeg tretmana. Nađeno je da DDVP značajno smanjuje aktivnost BuChE u plazmi životinja obaju spolova (40–60%; $P<0,05$) te značajno povećava koncentraciju triglicerida (60–600%; $P<0,05$) i ukupnog kolesterola (35–75%; $P<0,05$). Za razliku od povećane vrijednosti HDL–kolesterola (20–30%; $P<0,05$) uočen je statistički značajan pad koncentracije LDL–kolesterola (30–40%; $P<0,05$). Aktivnosti BuChE, kao i promjene u koncentracijama lipida i lipoproteina primijećene su tijekom cijelog eksperimenta. Naši rezultati govore u prilog hipotezi da BuChE ima važnu logu u metabolizmu lipida i lipoproteina *in vivo*.

KLJUČNE RIJEČI: *BuChE, DDVP, HDL–kolesterol, LDL–kolesterol, ukupni kolesterol, trigliceridi*

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