Journal of Applied Biological Sciences 2 (1): 35-38, 2008
ISSN: 1307-1130, www.nobel.gen.tr

The Effect of Dichlorvos on Glutathione S-Transferase Activity in Some Tissues of Rats

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Received: June 22, 2007
Accepted: September 20, 2007

Abstract

In this study the alteration of Glutathione S-transferase (GST) enzyme activity was examined in the livers, kidneys, brain and small intestine tissues of rats after dichlorvos (DDVP) was applied (4mg.kg^{-1}) intraperitoneally. In all tissues examined for both male and female rats, GST activity increased through DDVP.

GST activity showed similar alterations in liver and kidney tissues. On the other hand, in brain tissues, while significant increases in GST activity started from the 2nd period in male rats, in female rats significant increases were observed at the 4th, 32nd, 64th and 72nd hours. In small intestine tissues the observed activity increase in male rats was significant for all periods except for the initial period, 8th and 72nd hours, while in female rats it was significant for all periods except for the initial period, 8th and 64th hours.

As a result, it was observed that in both male and female rats, GST activity in all tissues that were examined increased through DDVP.

Key words: Dichlorvos, Glutathione S-transferase, Rat, Tissue

INTRODUCTION

Dichlorvos, also known as DDVP, is employed as an insecticide in the keeping and growing of agricultural products, in controlling the internal and external parasites of farm animals, and in the insect control of houses, buildings, aircraft, and external environments. DDVP is an organophosphate compound and shows its effect directly by inhibiting the acetylcholinesterase enzyme [1].

DDVP is an insecticide which has been produced and used all over the world since 1961 and which is effective both as a contact and stomach poison [2]. Among the individuals exposed to the DDVP effect, some symptoms such as increase in salivary secretion, sweating, wetness in the eyes, muscle contractions, shrinking of the pupils, damage to heart rhythm, vomiting, giddiness as a result of diarrhoea and central nervous system, headache, weakness, writhing, and a coma state appear [3]. By inhibiting enzymes regulating ion transition through cell membranes, DDVP spoils membrane permeability and ion transition through these membranes [1].

Toxic materials which enter the system of living organisms via various means encounter the defensive mechanism of metabolism. One of the important enzymes serving this system is glutathione-S-transferase (GST; E.C. 2.5.18). GST is one of the enzymes of xenobiotic metabolism which is multifunctional, and has substrate specificity with a wide spectrum. GST with this feature undertakes a defensive task in living organisms exposed to endogenous and exogenous chemicals. GST is responsible for the activation and inactivation of many electrophilic materials. This enzyme performs its detoxification function by neutralizing toxic components' electrophilic areas through the –SH group of reduced glutathione (GSH). As a product, mercapturic acid dissolving in water appears, and it can be removed from the body in the urine. The most-detected tissues are the cytosol and membrane of many organs such as the small intestines, large intestines, kidneys, lungs, mamma, muscle, spleen, testis, placenta, but especially the liver [4].

In this study, the effect of DDVP on GST activity, one of the important enzymes of xenobiotic metabolism, in liver, kidney, brain and small intestine tissues of rats were investigated. GST reacts with different molecules and its activity changes. By defining the changes in GST enzyme activity, the aim is to make a contribution to studies researching the detoxification metabolic means of DDVP, one of the most important chemicals in the agricultural struggle, and the roles of compounds, which can be dissolved in water, in cells.

MATERIALS and METHODS

Wistar rats (*Rattus norvegicus*), weighing 250-300 g, were used. For each trial period four rats from the control group and eight from the experimental group were used. Control groups were treated with serum physiological while experimental groups were injected intraperitoneally with 4 mg kg^{-1} dose of DDVP. The rats were left without food and water for 24 hours before injection, ensuring the start of metabolism of animals in both groups at the same time. Following injection, food and water were regularly given to the animals until the trial periods were completed. Animals were killed, via cervical dislocation, at 0, 2, 4, 8, 16, 32, 64, and 72 hours after injection. The liver, kidneys, brain and small intestine were quickly removed and perfused in ice-cold 0.15 M KCl. The homogenates were

prepared and homogenized at 2000 rpm in a T-line laboratory stirrer (model No: 136-2) type homogenizer. Each homogenate was centrifuged in a Dupont Instruments Sorvall "RC-5 super speed refrigerated centrifuge" at 48000 g for 30 minutes.

GST activities were estimated spectrophotometrically using the Habig method [5]. Protein concentration was determined with the method of Bradford [6] and bovine serum albumin was used as protein standard.

Statistical analysis

Data were analyzed using SPSS 13.0 for windows. Independent t test was applied between data of control and experimental periods. The significance was calculated using one-way analysis of variance (ANOVA) and Student's *t*-test. A value of $P<0.05$ was taken as statistically significant. The results were calculated as mean with standard error (±SE) values [7]. The results are given in table 1.

RESULTS

An increase in GST activity was found in all tissues studied. However, an insignificant activation in whole tissues of male rats was observed in the first hours ($p>0.05$). In all periods, except for the intial period and the 4th hour, a significant increase was noticed in the liver tissue of male rats ($p<0.05$). These increases reached their maximum levels in the 8th period, approximately as 11.6 times. Similar activations were observed in kidneys, while activations began decreasing through the end of the experimental periods. At the 72nd hour, activation remained at an insignificant level ($p>0.05$). Activity continuously increased in brain tissue, despite starting with an insignificant decrease at the first hour. Although activation in small intestines began rapidly in the first hours, it decreased in the 8th period, followed by insignificant activation in the 72nd experimental period ($p>0.05$) (Table 1).

While significant increases in the liver tissue of female rats occurred in all experimental periods - except the initial period - most activation was observed in the 72nd hour ($p<0.05$). The activation is approximately 7.6 times the activation in the control group. Significant increases occurred in kidney tissue from the 2nd hour until the end of the 72nd hour ($p<0.05$). In brain tissue, insignificant increases and decreases were seen after the first hours, and significant increases were observed at the 4th, 32nd, 64th and 72nd hours ($p<0.05$). Maximum activation, for the control group, was approximately 14 times at the 64th hour. In small intestines, activation was insignificant in the first experimental period ($p>0.05$). However, excluding the 8th and 64th periods, they were significant in other experimental periods ($p<0.05$), (Table 1).

Comparisons of changes in GST activity in male and female rats showed similar increases and decreases in liver and kidney tissue. While activation decreased in liver tissue of males at the 72nd hour, in females it reached a value of 29.36 ± 7.78. Similar results were noticed in kidney tissue. In male rats, activation at the 72nd hour was reduced to insignificant levels, as low as (1.81±0.03) ($P>0.05$), while activation increased in females (Table 1).

While significant increases began in brain tissue of male rats after the 2nd period, in females, the increase was significant at the 32nd, 64th and 72nd hours ($p<0.05$). In male rats, small intestine tissue showed increased activation, becoming insignificant at the initial, 8th, and 72nd hours ($p>0.05$). In females, it is significant in all periods, except for the initial, 8th and 64th hours ($p<0.05$) (Table 1).

DISCUSSION

Dichlorvos affects the antioxidant system, which plays an important role in making xenobiotics entering the body ineffective. The basic reason for this effect is that dichlorvos is made depending on GST which is an essential enzyme in the detoxification of the antioxidant system [8]. The GST enzyme, the activation of which decreases according to pesticide type, makes healthy tissue potentially risky. Decreasing the detoxification process leads to the accumulation of harmful metabolites, and parallel to this, it inevitably causes cytotoxic and genotoxic incidents. Some changes observed in enzyme activities can sometimes be taken under control thanks to vitamins. Oral *et al.* [9] applied vitamin E and C for dichlorvos toxicities in rats in the study they carried out. While an increase in malondialdehyde level was reported in the group to which dichlorvos was applied, when a combine with vitamin was given, a decrease in malondialdehyde level was reported.

GST activity increases in all tissue, but it's higher in liver tissues than in other tissues. DDVP metabolizes in the liver using two metabolic pathways. At the end of one pathway, desmethyldichlorvos forms as glutathione dependent, and at the end of the other pathway, dimethyl phosphate and dichloroacetaldehyde forms [10]. Significant activation occurs in the liver and kidneys of male and female rats from the second hour ($p<0.05$). The reason for this activation is not only DDVP itself, but also its metabolites.

In our study, changes in GST activity may cause oxidative stress. In one research, the effect of a 5mg/kg dichlorvos dose on lipid peroxidation and antioxidant defensive system in different parts of rats' central nervous system was researched, and as a result, it was seen that there was an increase in the activity of superoxide dismutase and catalase enzymes, and a decrease in the activation of lipid peroxidation. Furthermore, it is claimed that there was a significant decrease in glutation peroxidase activity with dichlorvos effect [11].

In a similar study, Hai *et al.* [12] did research about the effects of dichlorvos on antioxidant systems in fish tissues. The researchers employed carp (*Cyprinus carpio*) and cat fish (*Ictalurus nebulosus*) in their study; they looked for superoxide dismutase, catalase, glutation peroxidase activities and other parameters in the tissues of these animals. They concluded that dichlorvos changes antioxidant enzymes and other oxidative and redox parameters. In another study Kanai *et al.* [13] showed that GST is an important enzyme for defence against oxidative stress in E. coli.

Singh *et al.* [14] have researched the effects of pesticides with organophosphate including dichlorvos on erythrocyte antioxidant enzymes. It was found that erythrocyte glycose-6-phosphate dehydrogenase activity decreased after the erythrocytes were affected by organophosphate pesticides, and moreover, GST and glutathione reductase activities increased.

Table 1. The change in GST activities in liver, kidney, brain and small intestine of control and dichlorvos treated group of male and female animals.

	Time Hours [r]		0 Mean±SE	2 Mean±SE	4 Mean±SE	8 Mean±SE	16 Mean±SE	32 Mean±SE	64 Mean±SE	72 Mean±SE
Male	Liver	Control	4.9 ± 0.13	5.25 ± 0.21	5.55 ± 0.01	2.53 ± 0.03	7.07 ± 0.13	8.44 ± 0.13	2.87 ± 0.34	2.97 ± 0.28
		DDVP	7.4 ± 0.51	18.21± 0.59*	8.44 ± 1.82	29.31± 6.89 *	14.2 ± 0.50*	19.46 ± 0.31*	22.54 ± 0.46* *	15.9 ± 3.41*
	Kidney	Control	0.51 ± 0.02	0.53 ± 0.008	0.92 ± 0.14	0.61 ± 0.04	0.84 ± 0.06	0.40 ± 0.02	1.06 ± 0.0	0.33 ± 0.58
		DDVP	0.71± 0.09	2.07 ± 0.005*	4.50 ± 0.16*	7.20 ± 0.51*	5.84 ± 0.35*	7.61 ±0. 36*	4.65 ± 0.0*	1.81 ± 0.03
	Brain	Control	1.34 ± 0.31	0.86± 0.02	1.10 ± 0.002	1.24 ± 0.005	0.80 ± 0.005	0.30 ± 0.04	0.18 ± 0.04	0.44 ± 0.008
		DDVP	1.19 ± 0.06	1.36 ± 0.01 *	1.75 ± 0.08 *	1.55 ± 0.04*	1.77 ± 0.05*	3.95 ± 1.10*	3.06 ± 0.004*	4.36 ± 0.35*
	Small Intestine	Control	0.17 ± 0.002	0.26 ± 0.002	0.35 ± 0.008	0.49 ± 0.005	0.48 ± 0.002	0.28 ± 0.01	0.30 ± 0.02	0.27 ± 0.10
		DDVP	0.31 ± 0.03	1.14 ± 0.02 *	2.17 ± 0.24 *	0.76 ± 0.11	1.62 ± 0.16 *	1.85 ± 0.35 *	0.71 ± 0.05 *	0.40 ± 0.05
Female	Liver	Control	6.4 ± 0.23	5.39 ± 0.19	6.53 ± 0.03	5.55 ± 1.52	7,62 ± 0.01	6.08 ± 0.39	4.49 ± 0.47	3.88 ± 0..23
		DDVP	6.3 ± 0.39	7.29 ± 0.43*	9.53 ± 0.19*	24.11 ± 5.25*	13.45 ± 0.72*	9.45 ± 1.38*	12.7 ± 1.00*	29.36 ± 7.78*
	Kidney	Control	0.56 ± 0.02	0.75 ± 0.02	0.81± 0.002	0.49 ± 0.02	0.57 ± 0.002	1.17 ± 0.08	0.52 ± 0.02	0.60 ± 0.02
		DDVP	0.67 ± 0.13	2.00 ± 0.06*	5.92 ± 0.46*	6.65 ± 0.37*	3.71 ± 0.21*	4.27 ± 0.10*	4.43 ± 0.16*	5.67 ± 0.43*
	Brain	Control	0.98 ± 0.18	0.84 ± 0.01	0.96 ± 0.01	1.06 ± 0.12	0.93 ± 0.02	0.60 ± 0.02	0.25 ± 0.01	0.2 ± 0.005
		DDVP	0.78 ± 0.07	0.85 ± 0.01	0.98 ± 0.04	1.32 ± 0.09	1.12 ± 0.25	1.28 ± 0.08*	3.54 ± 0.12*	2.45 ± 0.13*
	Small Intestine	Control	0.24 ± 0.005	0.44 ± 0.03	0.58 ± 0.05	0.62 ± 0.002	0.58 ± 0.005	0.44 ± 0.002	0.58 ± 0.002	0.31 ± 0.002
		DDVP	0.35 ± 0.008	0.71 ± 0.02*	1.76 ± 0.13*	0.92 ± 0.18	1.28 ± 0.02*	1.13 ± 0.24*	0.63 ± 0.06	1.63 ± 0..27*

* Data shown in the vertical column are different from control at 0.05 statistical levels

r All data in the table showed enzyme activities as $U.(mg.protein)^{-1}$

SE: Standard Error.

In addition to this, of the enzymes dependent on membrane, asetylcholinesterase, Na-K-ATPaz and Ca-ATPaz activities decreased too. As a result of these changes in enzyme activities and of the decrease in glutathione level, the researchers claimed that organophosphate pesticides create oxidative stress in erythrocytes.

In all tissues, and possibly as a result of the effect of DDVP on DNA and mRNA, significant and insignificant increases in GST activity were observed. In one research DDVP prevented the repair of damage to DNA by inhibiting DNA acyltranspherase enzymes [15]. In another study examining the effects of DDVP on glucose metabolism, evidence emerged showing that glycokinase mRNA level increases [16]. However, pancreatic glycokinase activity increases and hepatic glycokinase activity decreases. Sarin and Gill [17] reported that DDVP prevents the oxygen transport to tissues, causing a decrease in glycogen level in the brain. The changes in GST activity may stem from increases in protein synthesis or enzyme kinetic properties. One study showed DDVP changed enzyme kinetic properties [18]. The kinetic property of enzyme Km and V_{max} may change through DDVP bound to GST enzyme or one of the substrates of GST enzyme.

One reason for changes in GST activities in our tissues may be the change in cellular membrane permeability or cell degradation. Research shows that DDVP causes changes in the histopathological and cell structure of liver, kidney and cardiac muscles [19]. In another study, some organophostate pesticides are maintained to cause deformation in sizes and surface shapes of erythrocytes [20].

DDVP significantly affects GST activity in the liver, kidney, brain and small intestines. More studies on the kinetics need to be conducted to explain the effects of DDVP on GST activity. DDVP, employed excessively and unconsciously in agriculture, pollutes nature and indirectly results in negative changes to living things. Hence, it is of critical importance to public health that we be careful and selective in using such chemicals, and inform consumers and producers of possible and real damage.

Acknowledgement

We would like to thank Uludag University, Faculty of Medicine, Department of Pharmacology. This study is part of a project and it was supported by the Research Foundation of Uludag University.

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