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Biochemical Effects of Some Insecticides on the Metabolic Enzymes Regulating Glutathione Metabolism

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Because of the serious environmental problems resulting from the use of pesticides in agricultural sector, several governments are seeking to employ biological and other non-polluting methods of combating farm pests. Several insecticides and/or their metabolites are suggested to be a priori mutagenic and/or teratogenic compounds. The dangers of acute and chronic poisoning have been addressed, to some degree, for a number of insecticides primarily chlorinated hydrocarbons and organophosphorous in terms of acute effects, and chlorinated hydrocarbons in regard to chronic toxicity (Fishbein, 1982).

Glutathione (GSH) has been reported to play an important role in the detoxification of several chemical compounds including carcinogens through conjugation catalyzed by glutathione-S-transferase,(Booth et al., 1961, Jakoby, 1978 and Chasseaud, 1979). The activity of γ-glutamyl transferase is crucial for further processing and ultimate elimination of GSH conjugates (Boyland and Chasseaud, 1969) Glutathione reductase is another enzyme which shares in the regulation of GSH level inside the cell through catalyzing the transformation of oxidized glutathione (GSSG) into reduced GSH (Meister, 1985). The present study was conducted to assess the in vivo effect of the well known insecticides: dimethoate, carbaryl and permethrin on the activity of glutathione-S-transferase, glutathione reductase and γ-glutamyl transferase.

MATERIALS AND METHODS

All fine chemicals used in this study were Sigma products (Sigma Co, St. Louis, Mo, USA). All other chemicals were analytical grade purchased from Merck (Darmstadt, W. Germany).

O,O-Dimethyl-S(N-methyl carbamoyl methyl) phos-phorodithioate (dimethoate) is the product of Fisons Ltd., Agronomical Division, Cambridge, England.

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1-Naphthyl methyl carbamate (carbaryl) was purchased from Union Carbide Corporation, New York, NY, USA. 3-Phenoxybenzyl(IRS)-Cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-carboxylate (permethrin) was obtained from the Imperial Chemical Industries Ltd., Jealott's Hill Research Station, Bracknell Berks, England.

Adult male Swiss albino mice (18-20 g body weight) were kept under observation for one week before insecticide administration. The doses of the different insecticides were calculated in a manner so as to avoid high mortality rate of animals regarding the LD_{50} of each one. All insecticides were dissolved in corn oil and administered orally to the animals in a volume of 0.1 ml. Control animals received the same volume of vehicle only. Single dose was 35, 166.7 and 200 mg/kg body weight of dimethoate, carbaryl and permethrin, respectively. On the other hand, repeated doses for five successive days were 17.5, 83.3 and 100 mg/kg body weight of dimethoate, carbaryl and permethrin, respectively.

Livers of the sacrificed animals were quickly excised, chilled in ice, washed and homogenized in 5 volumes of 0.25 M sucrose in a Potter-Elvehjem homogenizer. The particle- free supernatant was obtained from the homogenate, by ultra centrifugation, as described by Cinti et al, 1980 and the protein content was estimated according to the method of Lowry et al., 1951.

The enzymatic activity was assayed according to the method of Habig et al., 1974. as modified by Baars, et al., 1977. The reaction mixture contained 0.5 mM GSH, 0.5 mM 1-chloro- 2,4-dinitrobenzene (CDNB), 0.13 M phosphate buffer, pH 7.0 and 1 ml of the enzyme source (containing 15 µg protein) in a total volume of 3 ml. The reaction mixtures were preincubated for 2 min. and the reaction was started by adding CDNB in 50 µl of DMSO; incubation took place in a shaking water bath for 5 min. Under these conditions, product formation was linear with respect to time and protein concentration. The reaction was stopped by the addition of 0.2 ml of 33% TCA, and absorbance was read at 340 nm. A complete assay mixture with the enzyme substituted by buffer was used as a blank. The enzyme activity was expressed as units/mg protein/g liver. The enzyme unit was calculated using the molar extinction coefficient of 9.6 $mM^{-1}.cm^{-1}$ for CDNB (Habig et al., 1974).

For glutathione reductase assay 10% (w/v) liver homogenates were prepared in cold 0.05 M Tris-HCl buffer, pH 7.5. The clear supernatant was obtained by ultracentrifugation at 30,000 g (Suojanen et al., 1980). Glutathione reductase activity was assayed as described by Racker, 1955. The assay mixture contained 26 mM GSSG, 0.1 M Tris-HCl buffer, pH 7.6, 0.5 ml of 10 mg/ml bovine serum albumin, 3 mM NADPH and 0.1 ml of the enzyme source in a total volume of 4 ml. The reaction was started by the addition of NADPH. Catalytic oxidation of NADPH by GSSG was followed at 340 nm at 0 and 5 minutes intervals. Blank

was designed in a way that NADPH was substituted by Tris-HCl buffer. Enzyme activity was expressed as units/mg protein.

For γ-glutamyl transferase assay, liver was washed with cold 0.05 M Tris-HCl buffer, pH 7.5, and homogenized in the same buffer to a concentration of 5% (w/v). The enzyme assay was performed according to the method of Szasz, 1969. The reaction mixture contained 4.4 mM L- γ-glutamyl-p-nitroaniline, 22 mM glycyglycine, 11 mM MgCl2 (dissolved in 0.05 M Tris-HCl buffer, pH 8.0), 0.05 M Tris-HCl buffer, pH 7.5 and 1 ml of the enzyme source in a total volume of 3 ml. After incubation for 1 hr, the reaction was stopped by boiling for 3 minutes. The absorbance of the yellow product (p-nitroaniline) was recorded at 405 nm against an appropriate blank. Enzyme activity was expressed as units/mg protein. One unit is defined as the amount of enzyme which catalyzes the production of 1 mg of p-nitroaniline per minute under the assay conditions.

Statistical analyses were performed using the Student's t-test. The probability values "P" less than 0.05 were considered significant.

RESULTS AND DISCUSSION

It was found that none of the three insecticides significantly changed the protein content of mouse liver when administered as a single dose whereas a significant increase in the liver protein content was recorded following repeated administra tion of dimethoate, carbaryl and permethrin (Table 1).

The results revealed insignificant changes in glutathione-S-transferase and γ-glutamyl transferase activities after single dose adminstration of the three insecticides (Table 2). While dimethoate significantly increased glutathione reductase activity (53.6%, $P<0.05$) and the other studied insecticides did not alter the enzyme activity (Table 2). Concerning the effect of repeated adminstration of these insecticides on glutathione-S-transferase, a significant increase was recorded for dimethoate (59.6%; $P<0.05$), carbaryl (76.8%; $P<0.05$) and permethrin (70.7%; $P<0.05$) as shown in (Table 3). Moreover, repeated administrations of dimethoate significantly increased glutathione reductase activity (103.5%; $P<0.05$) and decreased the activity of γ-glutamyl transferase (19.1%; $P<0.05$). On the other hand, carbaryl and permethin treatments did not cause any significant changes in these enzymatic activities (Table 3).

Repeated administration of the organophosphorous insecticide (dimethoate) increased significantly the activity of glutathione-S-transferase and glutathione reductase, but decreased the activity of γ-glutamyl transferase. This could be understood in view of the fact that organophosphates consume GSH through glutathione-S-transferase catalyzed reaction as a major way of detoxification of these chemicals (Hutson 1981; Anonymous 1987; Ridgeway et al., 1978). On the

Table 1. Effect of oral administration of single and repeated doses of dimethoate, carbaryl and permethrin on liver protein content of male mice (mg protein/gm liver)**.

Insecticide	Signle dose (n = 10)	Repeated doses (n = 8)
Control	272±56	213±37
Dimethoate	286±71	283±35*
Carbaryl	250±56	255±34*
Permethrin	267±43	246±34*

Table 2. Effect of single oral adminstration of dimethoate, carbaryl and permethrin on the activities of glutathione-S-transferase, glutathione reductase and γ-glutamyl transferase enzymes**.

Insecticide	GSH-S-transferase (n = 10)	GSH reductase (n = 9)	γ-glutamyl transferase (n = 9)
Control	1.09±0.22	14.05±3.37	2.05±0.64
Dimethoate	1.39±0.64	21.58±5.15*	2.03±0.63
Carbaryl	1.02±0.19	16.99±5.37	1.61±0.37
Permethrin	1.13±0.21	12.40±2.70	1.94±0.13

Table 3. Effect of repeated oral adminstration of dimethoate, carbaryl and permethrin on the activities of glutathione-S-transferase, glutathione reductase and γ-glutamyl transferase enzymes**.

Insecticide	GSH-S-transferase (n = 8)	GSH reductase (n = 8)	γ-glutamyl transferase (n = 8)
Control	0.99±0.23	12.56±2.46	1.78±0.29
Dimethoate	1.58±0.33*	25.56±7.40*	1.44±0.13*
Carbaryl	1.75±0.51*	11.97±2.41	1.88±0.28
Permethrin	1.69±0.57*	12.54±2.37	1.83±0.19

* *These values are statistically significantly different compared to the corresponding control values ($P<0.05$).*

** *The value are expressed as Mean ± S.D.*

contrary, other classes of insecticides such as carbamate may utilize GSH in conjugation reaction but only in minor amounts compared to organophosphate (Hutson 1981). Moreover, knowing that glutathione reductase is the enzyme responsible for providing reduced GSH from its oxidized form (GSSG) (Meister 1985; Change et al., 1978; Reed 1986) and that γ-glutamyl transferase activity is important for the resynthesis of GSH inside the cell (Meister 1981; Arias annd Jakoby 1976). It is not surprising, therefore, to detect an increase in glutathione reductase activity as a possible consequence of the decrease in the activity of γ-glutamyl transferase. Moreover,since the activity of glutathione-S-transferase has increased in order to detoxificate the doses of dimethoate repeatedly injected, the liver is expected, to induce therefore, the activity of glutathione reductase as compensatory mechanism for the decrease in GSH concentration inside the cell.

On the other hand, while repeated administration of carbaryl and permethrin, significantly increased glutathione-S-transferase activity, they did not alter the activity of glutathione reductase or γ-glutamyl transferase. In this case, the amounts of GSH inside the cell as the substrate for glutathione-S-transferase are expected to be enough to cope with the increased activity of the enzyme. Therefore, there was no metabolic need for the induction of glutathione reductase activity.

Also, the significant decrease in γ-glutamyl transferase activity observed after repeated administration of dimethoate could be explained on the basis of the properties of this enzyme. It is well known that γ-glutamyl transferase requires the participation of GSH as well as a wide range of amino acids as substrates (Meister 1981; Arias and Jakoby 1976). Since amino acids content in the metabolic pool is expected to decrease as a result of insecticidal conjugation (Hutson 1981), it is not surprising, therefore, to find a decrease in the activity of γ-glutamyl transferase which utilizes amino acids.

As an overview of the present results, the GSH detoxification system may have the ability to manage single dose adminstration with minor changes in one of the enzymes regulating its metabolism and without the need for any adaptational changes in other enzyme system. While repeated administration of these insecticides induced disturbances in the activities of the enzymes regulating GSH metabolism. By far, the most effective insecticide was found to be dimethoate. The hazard effect of repeated exposure to this insecticide is much serious than that of single dose while carbaryl was less effective and permethrin had the least hazardous effect.

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