Inhibition of Hepatic UDP-Glucuronyltransferase Activity by Organophosphate Insecticides and by Carbon Disulfide in Mice

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We have shown that fenitrothion and other organophosphate insecticides containing P=S group inhibited hepatic drug metabolizing enzymes when administered to mice and the mechanism for this inhibition was due to a preferential decrease in microsomal cytochrome P-450 content(UCHIYAMA et al. 1975; YOSHIDA et al. 1975a, 1975b). Organophosphate insecticides containing P=S group decreased the cytochrome P-450 content when incubated in vitro with microsomes in the presence of NADPH, suggesting that the oxidative metabolism of these insecticides is necessary to decrease in cytochrome P-450 content(YOSHIDA et al. 1975b). Several investigators have demonstrated that administration of carbon disulfide to rats resulted in a decrease in cytochrome P-450 content(BOND and DEMATTEIS 1969; DEMATTEIS and SEAWRIGHT 1973; SOKAL 1973). Evidence has recently been reported that organophosphate insecticide, parathion and carbon disulfide are metabolized in a similar manner by hepatic drug metabolizing enzyme system, namely, sulfur atom is released in a reactive form and bound to microsomes during their oxidative desulfuration, and that this situation of microsomes resulted in a decrease in cytochrome P-450 content(NORMAN et al. 1974; DALVI et al. 1974; DEMATTEIS 1974). Since organophosphate insecticides and carbon disulfide seems to modify microsomal cytochrome P-450 and/or its microenvironment when administered to animals, we are interested in their effects on microsomal UDP-glucuronyltransferase(EC 2.4.1.17) which is linked to cytochrome P-450 and is an important enzyme catalyzing the transfer of a glucuronyl group from UDP-glucuronic acid to various endogenous and exogenous substrates.

EXPERIMENTAL

Male ddY mice weighing 22-25 g were used. Organophosphate insecticides and carbon disulfide dissolved in corn oil were given intraperitoneally to mice. Control mice were given the vehicle only. Mice were killed by decapitation and livers were removed and homogenized in 4 vol of 1.15% KCl using Potter-Elvehjem homogenizer with

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a Teflon pestle. The homogenates were centrifuged at 2,000 x g for 20 min. The resultant 2,000 x g supernatant fraction was used as a enzyme source.

UDP-glucuronyltransferase activity was determined by the method of HOLLMAN and TOUSTER (1962) with p-nitrophenol(0.1 μmole) as the aglycone and a 0.2 μmole of UDP-glucuronic acid(ammonium salt, 98%, obtained from Sigma Chemical Co.). Protein content was estimated by the method of GORNALL and BARDAWILL (1949).

RESULT AND DISCUSSION

When time course study was carried out, there was a significant inhibition of UDP-glucuronyltransferase activity soon after the administration of fenitrothion at a dose level of 100 mg/kg and the activity was returned nearly to control values within 12 hr. The maximum inhibition was observed in 4 hr after the administration of fenitrothion. Administration of carbon disulfide to mice resulted in a inhibition of UDP-glucuronyltransferase activity in a way similar to that observed with fenitrothion. Therefore, in further studies, the 4-hr interval was chosen for examination. Table I shows the effect of organophosphate insecticides and carbon disulfide on UDP-glucuronyltransferase activity in mice when measured 4 hr after their administration. Administration of fenitrothion to mice produced a dose-related inhibition of UDP-glucuronyltransferase activity. Other organophosphate insecticides containing P=S group except parathion also inhibited UDP-glucuronyltransferase activity, although the degree of inhibition differed according to each compound. DDVP(O,O-dimethyl O-2,2-dichlorovinyl phosphate) containing P=O group showed no inhibition. This result is compatible with that of the inhibitory effect of these insecticides on hepatic drug metabolizing enzyme as reported previously(UCHIYAMA et al. 1975). However, UDP-glucuronyltransferase activity responded to the treatment with these insecticides to a lesser extent than that observed with drug metabolizing enzyme activity. Administration of carbon disulfide to mice exhibited an inhibition of UDP-glucuronyltransferase activity although the degree of inhibition was less than that of drug metabolizing enzymes observed in rats by BOND and DEMATTEIS(1969).

Since it has been suggested that UDP-glucuronyltransferase is located in the deep layers of the microsomal membrane(HÄNNINEN and ALANEN 1966; HÄNNINEN and PUUKKA 1971), and is perhaps covered by the drug metabolizing enzyme system(ITO and SATO 1969; VAINIO 1973), the inhibitory response of UDP-glucuronyltransferase to organophosphate insecticides containing P=S group, except parathion and carbon disulfide, observed by the present investigation may have occurred in a similar way to that of drug metabo-

lizing enzymes. That is, the binding of sulfur to microsomes during their oxidative desulfuration may result in the inhibition of UDP-glucuronyltransferase activity. Although the nature of sulfur bound to microsomes is obscure in detail, it may be that the sulfur blocks sulfhydryl groups which are functionally involved in drug metabolizing enzyme system and in UDP-glucuronyltransferase just as sulfhydryl reagents inhibit drug metabolizing enzymes (CONNEY et al. 1957; ORRENIUS 1965; NETTER and JENNER 1966; FRANKLIN and ESTABROOK 1971) and UDP-glucuronyltransferase (STOREY 1965). However, this and other possible explanations for the inhibitory effect of organophosphate insecticides and of carbon disulfide on UDP-glucuronyltransferase activity remain to be experimentally proved.

TABLE I

Treatment	Activity % of control
Experiment I	
Control	100
Fenitrothion	
25 mg/kg	84.1
50 mg/kg	73.6
100 mg/kg	63.2
Experiment II	
Control	100
Parathion(2.5 mg/kg)	104.9
Methylparathion(12.5 mg/kg)	82.2
Diazinon(25 mg/kg)	91.9
Diazinon(50 mg/kg)	78.2
DDVP(25 mg/kg)	110.0
Experiment III	
Control	100
Carbon disulfide	
0.1 ml/kg	77.4
0.3 ml/kg	73.8
0.5 ml/kg	61.1

Mice were injected intraperitoneally with organophosphate insecticides and carbon disulfide at the doses indicated. The control values in experiment I, II and III were 8.42 ± 0.64, 8.27 ± 0.47 and 9.23 ± 0.71 of p-nitrophenol glucuronide in nmoles/mg protein/30 min, respectively.

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