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EFFECT OF ORGANOPHOSPHATE PESTICIDES ON LECITHIN–CHOLESTEROL ACYLTRANSFERASE IN HUMAN PLASMA

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Abstract—Organophosphates—dimethyldichlorovinylphosphate (DDVP), methylparathion and sumithion—inhibited the esterification of cholesterol in sonicated dispersions of lecithin–cholesterol mixtures by the lecithin–cholesterol acyltransferase in human plasma. The order of inhibition by these compounds on the esterification was DDVP > methylparathion > sumithion. This difference in degree of inhibition by DDVP, methylparathion and sumithion was not due to differences in enzymatic degradation of these compounds during incubation. The inhibitory effect of organophosphates on the acyltransferase was not eliminated by the addition of albumin, tri-serine, poly-serine and sulfhydryl protection agents. Poly-serine was a potent inhibitor of the acyltransferase.

MAMMALIAN acetylcholinesterase is known to be inhibited by organophosphates. The inhibition arises from the formation of a phosphorylated enzyme.[1] It has recently been reported that esterases, phospholipase[2] and lipases[3–5] are also inhibited by these compounds *in vitro*.

Human plasma and serum contain lecithin–cholesterol acyltransferase (LCAT), which catalyzes the transfer of an acyl group from the β-position of lecithin to cholesterol.[6]

In this paper, evidence is presented for the inhibitory effect of the organophosphates, DDVP, methylparathion and sumithion, on the LCAT reaction in human plasma.

MATERIALS AND METHODS

Preparation of enzyme. As the enzyme source of LCAT, human plasma was obtained from outdated human blood containing 0·15 vol. anticoagulant solution (citric acid, sodium citrate and glucose) by centrifugation and then was dialyzed against phosphate buffer, pH 7·4, ionic strength 0·1, containing 0·025% ethylenediaminetetraacetic acid (EDTA).

Compounds. Cholesterol was purchased from Kanto Chemical Co. (Tokyo, Japan) and purified by the bromination–debromination procedure.[7] 7α-^{3}H-cholesterol was obtained from New England Nuclear Corp. (Boston, Mass., U.S.A.) and purified by thin-layer chromatography on Silica gel G plates, using as the solvent system *n*-hexane–diethyl ether–acetic acid (70:30:1, v/v). Bovine albumin (essentially fatty acid-free), tri-serine and poly-serine were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Lecithin was prepared from egg yolk by the method of Faure[8] and was purified by silicic acid column chromatography. Thin-layer chromatography of the

purified lecithin on Silica gel G plates with chloroform–methanol–water (65:25:4, v/v) as the developing solvent gave a single spot of lecithin.

Enzyme assays. A sonicated dispersion of lecithin–cholesterol mixture as substrate was prepared in the same manner as described previously.[9] It was confirmed by thin-layer chromatography that no degradation of lecithin to lysolecithin occurred during sonication. The incubation mixture contained 0·1 ml of the dispersion and 0·2 ml of

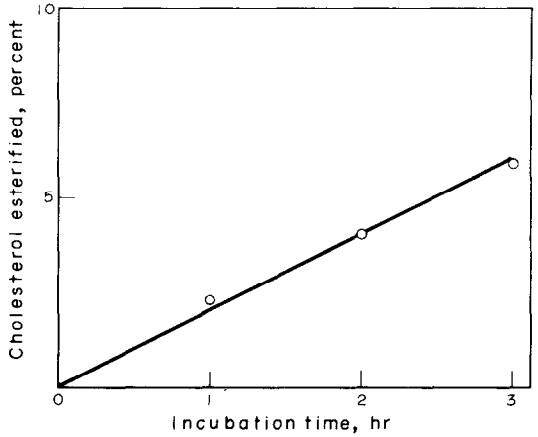

FIG. 1. Time course of cholesterol esterification in human plasma. The medium and conditions of incubation were described in Materials and Methods. The molar ratio of lecithin–cholesterol in the dispersion used was 3·3. The radioactivity and amount of free cholesterol added to the incubation medium as dispersion was 0·2 μCi/0·13 μmole/ml. The percentage of radioactive cholesterol esterified during incubation is given on the ordinate.

human plasma. The final volume was adjusted to 0·5 ml with phosphate buffer, pH 7·4, ionic strength 0·1. The concentrations of the various materials added to the incubation mixture are given in the text. The samples were placed in 15-ml screw-capped tubes, flushed with N_2, sealed and incubated at 37° for 3 hr with mechanical shaking. After incubation, the extraction and separation of lipids and the measurement of radioactivity, protein content and lipid phosphorus were conducted as described previously.[9]

RESULTS AND DISCUSSION

It has been reported that sonicated dispersions of lecithin and cholesterol mixtures served as substrates for LCAT in the residual protein fraction ($d > 1{\cdot}210$ g/cm^3) of human plasma.[9–11]

Figure 1 shows the extent of esterification of cholesterol at various times of incubation by 0·4 ml plasma/ml of incubation medium. The esterification activity for the dispersion with a lecithin–cholesterol molar ratio of 3·3 increased linearly with time up to 3 hr. Thus, by the use of such experimental conditions, the effect of organophosphates on cholesterol esterification was investigated with the results shown in Table 1. The activity was reduced by the addition of DDVP, methylparathion and sumithion. The acyltransferase activity for the dispersions of lecithin–cholesterol at a molar ratio of 6·9 was greater than for that of 3·3, but the degree of inhibition for both substrates was almost the same.

TABLE 1. EFFECT OF ORGANOPHOSPHATES ON CHOLESTEROL ESTERIFICATION*

Addition	Cholesterol esterified (%)					
	Experiment 1		Experiment 2		Experiment 3	
	L/C = 3·3	L/C = 6·9	L/C = 3·3	L/C = 6·9	L/C = 3·3	L/C = 6·9
None	5·00 (100)	6·72 (100)	3·21 (100)	4·42 (100)	4·96 (100)	6·96 (100)
DDVP (1×10^{-4} M)	4·59 (92)	6·38 (95)	3·06 (95)	4·28 (97)	4·13 (83)	6·75 (97)
(5×10^{-4} M)					2·88 (58)	5·16 (74)
(1×10^{-3} M)	1·42 (28)	3·08 (46)	0·85 (26)	1·86 (42)	1·60 (33)	3·65 (52)
Methylparathion (1×10^{-4} M)			3·18 (99)	4·33 (98)	4·59 (93)	6·86 (99)
(5×10^{-4} M)					3·60 (73)	5·83 (84)
(1×10^{-3} M)			1·87 (58)	3·49 (52)	2·49 (50)	3·86 (55)
Sumithion (1×10^{-4} M)	4·83 (97)	6·49 (97)			4·62 (93)	6·77 (97)
(5×10^{-4} M)					4·26 (86)	5·80 (85)
(1×10^{-3} M)	3·19 (64)	3·72 (55)			3·16 (64)	5·02 (72)

* The molar ratios of lecithin/cholesterol (L/C) of the dispersions used was 3·3 (L/C = 3·3), which was the same as the dispersion in Fig. 1, and 6·9 (L/C = 6·9). The radioactivity and amount of free cholesterol in the dispersion having a molar ratio of 6·9 added to incubation medium was 0·2 μCi/0·16 μmole/ml. Each organophosphate was added as 20 μl ethanol solution/ml of incubation medium. The reference sample contained 20 μl ethanol/ml of incubation medium. The other incubation conditions were described in Materials and Methods.

The order of the inhibitory potencies of the organophosphates tested was DDVP > methylparathion > sumithion. The extent of cholesterol esterification varies with different plasma, reflecting the amounts of LCAT or lipoproteins. However, the order of inhibitory potencies of the organophosphates used was not influenced by the source of plasma. The different degrees of inhibition by DDVP, methylparathion and sumithion on the acyltransferase would not appear to be due to differences in enzymatic degradation of these organophosphates during incubation, because we have found that the amount of sumithion recovered after incubation was greater than that of DDVP.

One of the inhibitory effects of organophosphates on acetylcholinesterase is due to the phosphorylation of the serine hydroxyl residue in the enzyme.[1] The mechanism of phosphorylation of the acetylcholinesterase enzyme by organophosphates has been proposed by Krupka;[12] the presence of a basic group (the histidine imidazol moiety), the serine hydroxyl and an acidic group (the tyrosine hydroxyl) in the active site of acetylcholinesterase is required for phosphorylation.

An experiment was carried out to determine whether the inhibition by organophosphates of the esterification of cholesterol was competitively relieved by the addition of tri-serine, poly-serine or albumin (Table 2). The inhibitory effect of sumithion and DDVP was not eliminated by the addition of tri-serine and poly-serine. Poly-serine was an inhibitor of cholesterol esterification regardless of the presence or absence of organophosphates. The inhibitory action of poly-serine on LCAT will be reported elsewhere.

Albumin slightly elevated the degree of esterification in both the presence and absence of organophosphates. We have recently reported that the elevation of acyltransferase activity by albumin may be attributed partly to stabilization of lecithin–cholesterol dispersions.[13] An experiment (Fig. 2) was carried out to determine whether the stimulatory effect of albumin on esterification in the presence of organophosphates is partly due to a hydrophobic binding of organophosphates to albumin. Cholesterol esterification gradually increased upon the addition of increasing

TABLE 2. EFFECT OF ALBUMIN, TRI-SERINE AND POLY-SERINE ON CHOLESTEROL ESTERIFICATION IN THE PRESENCE OF ORGANOPHOSPHATES*

Addition	Expt. No.	Cholesterol esterified (%) None	Albumin	Tri-serine	Poly-serine
None	1	2·31	3·72	2·20	1·50
		(2·39)	(3·21)	(2·07)	(1·38)
	2	2·46	2·70	1·93	1·25
DDVP (1×10^{-3} M)	1	0·62	0·93	0·93	0·43
		(0·81)	(1·10)	(0·95)	(0·43)
	2	0·99	1·27	0·97	0·42
Sumithion (1×10^{-3} M)	1	0·93	1·17	0·94	0·59
		(1·11)	(1·55)	(1·11)	(0·63)
	2	1·29	1·92	1·28	0·67

* The substrate used was the same dispersion as in Fig. 1. The amount of albumin, tri-serine or poly-serine used was 10 mg/ml of incubation medium. The other incubation conditions were the same as in Table 1. The values in parentheses indicate the average per cent cholesterol esterification in duplicate experiments.

amounts of albumin, but the enhancement of esterification by the addition of albumin in the presence of DDVP (curve 3) and sumithion (curve 2) was parallel with that in the absence of DDVP and sumithion (curve 1). Accordingly, the inhibitory effect of organophosphates may not be eliminated by albumin. However, we have recently observed in preliminary experiments* that organophosphate can be bound to albumin. These results suggest that an albumin–organophosphate complex formed can still act as an inhibitor for esterification.

Some organophosphates have alkylating properties on nucleophiles.[14] Acyltransferase contains sulfhydryl residue(s).[15] Table 3 shows the effect of dithiothreithol (DDT) and mercaptoethanol (ME) on acyltransferase activity in the presence of

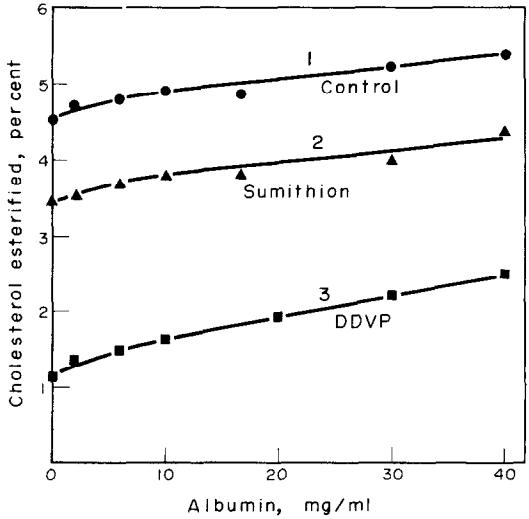

FIG. 2. Effect of albumin on cholesterol esterification in the presence of organophosphates. The substrate used was the same dispersion as in Fig. 1. The concentration of sumithion and DDVP in the incubation medium was 1×10^{-3} M. The other incubation conditions were the same as in Table 1.

* M. Uchiyama, unpublished data.

TABLE 3. EFFECT OF DITHIOTHREITOL AND MERCAPTOETHANOL ON CHOLESTEROL ESTERIFICATION IN THE PRESENCE OF ORGANOPHOSPHATES*

Addition	Cholesterol esterified (%)		
	None	DTT	ME
None	3·66	4·28	4·80
DDVP (1×10^{-3} M)	1·06	0·85	1·04
Sumithion (1×10^{-3} M)	2·87	3·09	3·53

* The substrate used was the same dispersion as in Fig. 1. The concentration of organophosphates, dithiothreitol and mercaptoethanol was 1×10^{-3} M. The other incubation conditions were the same as in Table 1. DTT, dithiothreitol; ME, mercaptoethanol.

DDVP and sumithion. The inhibition of DDVP and sumithion was not restored to the control level in the presence of DTT and ME. Therefore, the alkylation of sulfhydryl residues in LCAT by these organophosphates must be excluded.

Experiments are now in progress to study the mechanism of the inhibitory effect of organophosphates on LCAT in human plasma.

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