Bull. Environm. Contam. Toxicol. 24, 875-878 (1980)

Inhibition of Sheep Liver Arginase by Malathion

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Malathion is an extensively employed insecticide, effective against a variety of pests (ALDRIDGE 1971). It is a competitive inhibitor of cholinesterase and poses several toxic hazards in non-target animals, like liver damage and hepatocyte necrosis (SAKAGUCHI 1972, ANEES 1978). However detailed work on mammals that are directly or indirectly in contact with fields or food treated with organophosphorus pesticides are less frequent (OBERHEU et al. 1970, DEUBERT et al. 1976). Hence the present study is designed to elucidate a possible damaging effect of malathion inflicted on sheep hepatic arginase activity, one of the key enzymes of urea cycle which is majorly involved in the detoxification mechanism.

MATERIALS AND METHODS

Procurement of material: After decapitation of sheep, the liver was excised expeditiously and brought to the laboratory from local slaughter house. It was kept in deep freeze until further use. After washing thoroughly with cold KREBS-HENSELIETT (1932) Ringer's solution, the peritonial membrane was gently peeled off to prepare 1% homogenate of the tissue (W/V) in cetyl trimethylammonium bromide medium for the assay of arginase activity. After centrifugation at 2500 rpm for 15 min the supernatants were dialysed against the media in which homogenate was prepared, and was utilised in the further study.

Chemicals: Technical grade of malathion with 95% purity was obtained from Cyanamide India Ltd., Bombay, while all the other chemicals used were of A.R. grade products of either Sigma Chemical Company or British Drug House.

Assay of arginase activity: The enzyme activity in the hepatic tissue extracts was assayed by the method of CAMPBELL (1961) by determining the content of urea colorimetrically as described by ARCHIBALD (1945). Ex-

0007-4861/80/0024-0875 $01.00

perimental tubes received 100 ug of malathion after due standardization, while the control tubes received distilled water in the place of malathion in addition to the contents of reaction mixture. The enzyme incubation was made at 37°C with substrate concentrations varying from 0.5 to 5 mM at pH 9.5. The maximal velocity (Vmax) and the Michaelis-Menten constants (Km) were calculated by the method of least squares (FISHER 1970). The protein content in the enzyme extracts was determined by the method of LOWRY et al. (1951).

RESULTS

The enzyme assay was made representing initial velocities. The maximal velocity of experimental enzyme showed a decrease of 19% while the Michaelis-Menten constant showed an increase of 40% over the control enzyme, as revealed by the Lineweaver and Burke double reciprocal plot (Fig. 1) for hepatic arginase activity in sheep.

TABLE 1

In vitro effect of malathion on substrate dependent kinetics of hepatic arginase of sheep

Sample	Kinetic parameters	
	Vmax	Km
Control	3.4×10^{-6} M	0.5×10^{-3} M
Experimental	2.7×10^{-6} M	0.7×10^{-3} M
Percent change	(-) 19%	(+) 40%

Vmax values are represented in moles of urea/ mg protein/h.

DISCUSSION

The results presented above indicate that the concentration of pesticide used in the present study was sufficient enough to trigger alterations in the kinetic parameters. The decreased maximal velocity of the arginase in the presence of malathion suggests a decrease in the active site density perhaps by masking some of the enzyme active sites by inducing masking reactions. Similarly the increased Michaelis-Menten constant for

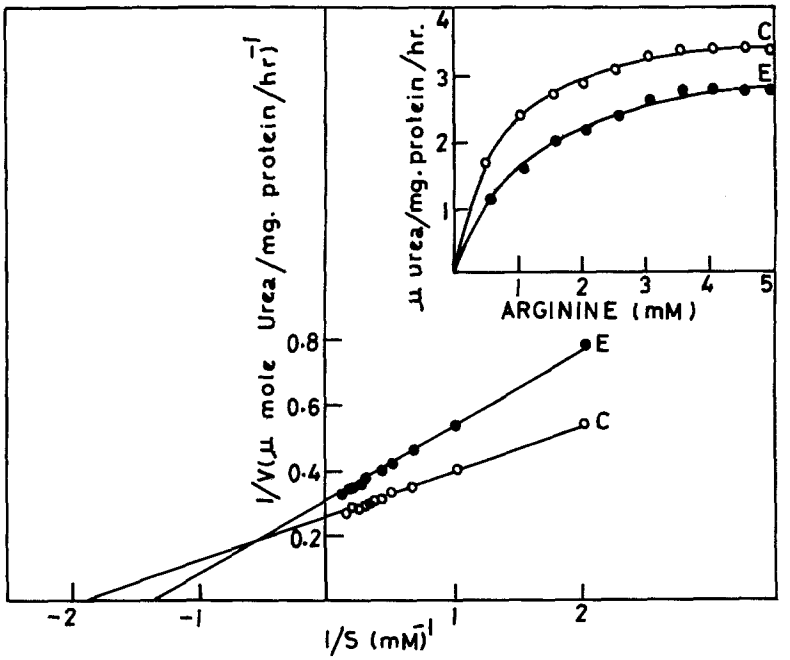

Figure 1. Double reciprocal plot showing control (C) and competitively inhibited sheep hepatic arginase activity by malathion (E) along with an inset figure of substrate concentration verses enzyme activity.

the experimental arginase reflects the decreased affinity of the enzyme for the substrate, consequently E-S complex formation is low and this accounts for the low catalytic activity. Since, these are the two kinetic factors responsible for the observed catalytic activity of an enzyme, an alteration in these kinetic parameters results in the modulation of enzyme expression. This type of modulation as obtained in the present study is a characteristic feature of mixed type of inhibition in which both maximal velocity and the Michaelis-Menten constants are affected. To be more precise, in the present investigation the 40% increase in Michaelis-Menten constant in contrast to 19% decrease in maximal velocity probably indicates that malathion induces a competitive inhibition kinetics than that of non-competitive inhibition kinetics, similar to the malathion inhibition of acetylcholinesterase as reported by several earlier workers (FUKUTO 1971, METCALF 1971, KABEER AHMED et al. 1978).

From the present kinetic study it is known that malathion decreases the overall catalytic efficiency of arginase by exerting a competitive inhibitory modulation and it seems likely that administration of higher doses of arginine might protect the arginase activity

by reducing the inhibitory effect during malathion poisoning in non-target animals, since the competitive inhibition can be overcome in the presence of higher concentrations of the substrate (FROMM 1975). However, the conclusive demonstration for the effectiveness of arginine during malathion toxicity is still under investigation.

REFERENCES

ALDRIDGE, W.N.: Bull W.H.O. 44, 25 (1971).

ANEES, M.A.: Bull. Environ. Contam. Toxicol. 19, 524 (1978).

ARCHIBALD, R.M.: J. Biol. Chem. 157, 507 (1945).

CAMPBELL, J.W.: Archs. Biochem. Biophys. 93, 448 (1961).

DEUBERT, K.H. and R.S. GRAY: Bull. Environ. Contam. Toxicol. 15, 613 (1976).

FISHER, R.A.: Statistical methods for Research Workers, Oliver and Boyd Ltd. Edinburgh (1970).

FUKUTO, T.R.: Bull. W.H.O. 44, 31 (1971).

HERBERT, J.F.: in "Initial Rate Enzyme Kinetics" Springer-Verlag, Berlin, Heidelberg, New York (1975).

KABEERAHMED, I., K.V. RAMANA RAO and K.S. SWAMI.: Ind. J. Exp. Biol. 16, 258 (1978).

KREBS, H.A. and HENSELIETT: J. Physiol. Chem. 210, 33 (1932).

LOWRY, O.H., N.J. ROSEBROUGH, A.L. FARR and R.J. RANDALL: J. Biol. Chem. 193, 265 (1951).

METCALF, R.L.: Bull. W.H.O. 44, 43 (1971).

OBERHEU, J.C., R.U. SOULE and M.A. WOLF: Down to Earth 26, 12 (1970).

SAKAGUCHI, H.: Bull. Jap. Soc. Sci. Fish. 38, 555 (1972).