Bull. Environ. Contam. Toxicol. (1984) 33:289–294

Bulletin of Environmental Contamination and Toxicology

Subchronic Malathion Treatment Effects on Rat Intestinal Functions

R. K. Wali, R. Singh, P. K. Dudeja,* A. K. Sarkar, and A. Mahmood**

Department of Biochemistry, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India

It is well known that organophosphorous pesticides and their metabolites are potent inhibitors of acetylcholine-esterase system and consequently disrupt the nerve conduction (Metcalf 1971). An exposure to these pesticides also produces hyperglycemia, glycosuria and alter a variety of metabolic processes (Gupta and Paul 1974; Stevens 1974). These insecticides may produce changes in membrane fluidity at concentrations as low as 10^{-7} - 10^{-9}M (Domenech et al 1977). In a previous report from this laboratory, administration of a single oral dose of malathion markedly decreased the levels of brush border enzymes and intestinal uptake of sugar and amino acids in rats (Chowdhury et al 1980). This communication is an extension of these studies, describing the effect of long term (subchronic) malathion (S-1, 2-bis ethoxy carbonyl-O-dimethyl phosphorodithioate) exposure on the digestive and absorptive characteristics of rat intestine.

MATERIALS AND METHODS

Male albino rats (Wistar strain) weighing 80-120 g were used. Animals were maintained on standard rat pellet diet (Hindustan Lever Ltd, Bombay) and had free access to water. Malathion (Cythion malathion technical, Cynamide India Ltd) mixed with corn oil (5 mg/100 g body wt in 0.1 ml oil) was administered by stomach tube daily for 45 days to a group of rats. Animals in the control group received corn oil alone. There were 6 animals in each group. Regular records of the body weights and food intake of the animals were maintained. At the end of the experiment, animals were fasted overnight and killed under light ether anesthesia. Intestines "18-22 cm", starting from the ligament of Treitz were removed, immediately flushed with ice cold saline and everted using a thin stainless steel rod.

* Present address: Gastroenterology Division, Michael Reese Hospital and Medical Center, Chicago, Illinois.

**Send reprint requests to Dr. Akhtar Mahmood, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73126

Intestinal uptake of glucose and amino acids was determined using tissue accumulation method, the details of which have been previously described (Alvarado and Mahmood 1974). Randomly 4 intestinal segments (0.3-0.5 cm) were incubated in 5 ml of oxygenated Kreb's Ringer buffer, pH 7.4 containing 5 mM D-glucose or glycine or L-phenylalanine or L-lysine with trace amounts of U-^{14}C-D-glucose or U-^{14}C-glycine or U-^{14}C-L-phenylalanine or "^{3}H-L-lysine" respectively. The incubations were carried out for 10 min at 37^{o} C. At the end of incubation, tissues were removed, gently blotted, weighed and the amount of radioactivity taken up was measured in Packard scintillation counter. After correcting for the extracellular space, measured separately using ^{3}H-Inulin, the rate of nutrient uptake was calculated and expressed as µmoles/10/g wet tissue.

Brush border membranes were isolated and purified following the method of Schmitz et al (1973). Membranes were suspended in 50 mM sodium maleate buffer, pH 6.5 and exhibited 16-20 fold enrichment of brush border sucrase compared to that in crude homogenate. Sucrase was assayed by the method of Dahlqvist (1964). Alkaline phosphatase activity was determined as described by Bergmeyer (1963). Leucine aminopeptidase activity was assayed by the method of Goldbarg and Rutenberg (1958). For assaying the Na+, K+-ATPase activity, a 5% homogenate of mucosal scrappings was made in 5 mM EDTA-1 mM Tris-HCl, pH 7.4. The homogenate was spun at 700 g for 10 min and the supernatant recentrifuged at 10,000 g for 10 min at 0-4^{o} C. The pellet was suspended in 2.5 mM EDTA and enzyme activity was determined as described by Quigley and Gotterer (1969).

A portion of the jejunum was homogenized in 50 mM sodium maleate, pH 6.5 and centrifuged at 1000 g for 10 min in the cold. The supernatant was removed and used as such for the assay of various cellular enzymes. Lactate dehydrogenase was assayed as described by Bergmeyer (1963). Glucose-6-phosphatase and transaminases were determined as described by Freedland and Harperi (1959) and Reitman and Frankel (1957) respectively.

All enzyme activities were calculated as units/g protein. One enzyme unit is equal to one µmole substrate transformed in to product per min under standard assay conditions. Protein was determined by the method of Lowry et al (1951) using bovine serum albumin as the standard.

All chemicals used were of analytical grade. The glucose-oxidase peroxidase kit was from Boehringer-Mannheim, FGR. Radioisotopes were obtained from Bhabha Atomic Research Center, Bombay.

RESULTS AND DISCUSSION

Oral intake of pesticides is one of the common routes of pesticide exposure to humans and to various animal species (WHO 1972), but little information is available on the interactions of pesticides with the gastrointestinal tract. Earlier, we reported that acute exposure of malathion to rats severely impairs the digestive and absorptive functions of intestine (Chowdhury et al 1980). But repeated exposures to pesticides for a longer period is of frequent occurrence (WHO 1972). Thus, in the present studies, the effect of subchronic malathion exposure on some of the intestinal

characteristics was investigated. The results presented in Table 1 show that subchronic malathion exposure to rats significantly increased the absorption of glucose, phenylalanine and lysine from intestine; however, the uptake of glycine was not affected under these conditions. A similar augumentation in the uptake of glucose and amino acids was also observed in monkey intestine after chronic DDT exposure (Mahmood et al 1979). An acute exposure to DDT and dieldrin has also been shown to stimulate the uptake of glucose (Mahmood et al 1978,1981; Reymann et al 1983), and of leucine and phenylalanine from intestine (Dudeja and Mahmood 1982).

Table 1 Effect of subchronic malathion exposure on intestinal uptake of D-glucose and certain amino acids in rats

Substrate	Control (μmoles/10 min/g wet tissue)	Malathion Fed	p value
Glucose	7.44 ± 0.16	10.24 ± 0.24	<0.001
Glycine	2.14 ± 0.29	2.66 ± 0.33	NS
Phenylalanine	3.96 ± 0.03	5.19 ± 0.41	<0.05
Lysine	3.33 ± 0.02	5.17 ± 0.50	<0.01

Values are mean ± SD
NS = not significant

Previously we observed that acute malathion exposure to rats greatly depressed the absorption of glucose and glycine (Chowdhury et al 1980). The anomalies observed in the uptake of nutrients in the present studies with that of acute experiments could possibly be attributed to adaptation of animals to repeated doses of malathion for a longer period in subchronic experiments. It has been documented that toxicity of poisonous agents markedly differs depending upon the animal species, dose, duration of exposure, route of administration and nutritional status of the animals (Debruin 1976).

The effect of subchronic malathion exposure on various brush border enzymes was also investigated and these results are presented in Table 2. There was a considerable increase in the levels of sucrase (44%) and alkaline phosphatase (21%) in malathion exposed rats compared to controls, but there was no significant change in leucine aminopeptidase activity under these conditions. Similar results were reported in monkey intestine in response to acute (Mahmood et al 1978) and chronic (Mahmood et al 1979) DDT exposures.

Na+, K+-ATPase system is known to be linked to ion transport processes in various tissues (Skou 1965). In the present studies a significant decrease in Na+, K+-ATPase activity was observed in malathion exposed animals (Table 2) which suggests the disruption of ion transport processes in intestine after pesticide exposure. A similar reduction in the activity of this enzyme system was also observed in intestine of DDT (Dudeja and Mahmood 1982) and endo-

sulfan (Wali et al 1982) treated rats.

Table 2 Effect of subchronic malathion administration on brush border enzymes in rat intestine

Enzyme	Control (Units/g protein)	Malathion Fed	p Value
Sucrase	343.8 ± 8.2	495.9 ± 15.6	<0.001
Alkaline phosphatase	1085.3 ± 12.4	1316.2 ± 50.1	<0.05
Leucine amino-peptidase	335.4 ± 42.4	399.2 ± 15.9	NS
Na+, K+-ATPase	88.7 ± 6.9	72.7 ± 7.9	<0.01

Values are mean ± SD
NS = not significant

Most transport proteins and digestive enzymes (those involved in terminal events of digestion) are known to be intrinsic membrane proteins, which span the lipid bilayer. Further there is considerable evidence that many functions of biological membranes are influenced by the chemical composition and physical state of the membranes (Lee 1975; Melchior and Steim 1976; Sanderman Jr 1978). Malathion has been shown to affect the red cell membrane fluidity at concentrations as low as 10^{-7}M(Domenech et al 1977). It may be surmised that subchronic malathion treatment may produce alterations in microvillus membrane composition leading to observed changes in intestinal functions. Alterations in chemical architecture of intestinal brush borders in response to DDT exposure has been described (Mahmood et al 1979; Dudeja and Mahmood 1982).

Intestinal epithelium is among the most actively replicating tissues in the body with a life span of 36-48 hr in rats (LeBlond and Stevens 1948). Thus observed perturbations in intestinal functions in subchronic malathion toxicity may result either due to altered cell replication or due to the formation of hypercellular tissue (increased cell size). Analysis of tissue DNA, RNA and protein (Mahmood et al, unpublished data), did not reveal any change in these parameters, this suggested that pesticide administration does not affect the cell size or cell number.

In addition to brush border enzymes, the effect of subchronic malathion administration on some of the cellular enzymes of rat intestine was also studied. These results are presented in Table 3. The activities of lactate dehydrogenase and glucose-6-phosphatase were significantly increased in malathion treated rats compared to control group. However, the activity of transaminases was reduced in pesticide toxicity. Increase in serum transaminase levels has been described by Luckens and Phelps (1969) in DDT and Lindane treated rats.

Table 3 Effect of Subchronic Malathion Administration on Certain Cellular Enzymes of Rat Intestine

Enzyme	Control (Units/g protein)	Malathion Fed	p value
Lactate dehydrogenase	329.9 ± 6.3	486.4 ± 48.3	<0.05
Glutamate oxalo-acetate transaminase	456.3 ± 4.2	397.5 ± 4.9	<0.001
Glutamate pyruvate transaminase	512.4 ± 10.7	477.0 ± 9.2	<0.05
Glucose-6-phosphatase	82.5 ± 5.2	108.6 ± 5.1	<0.01

Values are ± SD

Malathion is one of the most commonly used organophosphorous pesticides in agriculture and is known to be least toxic to mammals as compared to insects (Debruin 1976). The results presented in this communication indicate that exposure to this pesticide may produce alterations in intestinal functions; however, the mechanism of pesticide interactions with intestinal epithelium remains to be elucidated.

ACKNOWLEDGEMENT. We wish to thank Ms. Barbara Bonda for typing the manuscript.

REFERENCES

Alvarado F, Mahmood A (1974) Cotransport of organic solutes and sodium ions in small intestine. A general model: Amino acid transport. Biochemistry 13:2882-2890

Bergmeyer MVC (1963) Methods of enzymatic analysis. Academic Press, Inc., New York, p 783

Chowdhury JS, Dudeja PK, Mehta SK, Mahmood A (1980) Effect of a single oral dose of malathion on D-glucose and glycine uptake and on brush border enzymes in rat intestine. Toxicol Lett 6:411-415

Dahlqvist A (1964) Method for the assay of intestinal disaccharidases. Anal Biochm 7:18-25

Debruin A (1976) Biochemical toxicology of environmental agents. Elsevier/North Holland Biochemical Press, Amsterdam

Domenech CE, Machado de Domenech EE, Balegno HF, Diego-de-Mendoza, Farias RN (1977) Pesticide action and membrane fluidity. FEBS Letters 74:243-246

Dudeja PK, Mahmood A (1982) Effect of a single oral dose of pp' DDT on the absorption of nutrients in vitro and on brush border enzymes in rat intestine. Arch Toxicol 49:131-138

Freedland RA, Harperi AE (1959) Metabolic adaptations in higher animals. J Biol Chem 234:1350-1354

Goldbarg JA, Rutenberg AM (1958) Colorimetric determination of leucine aminopeptidase in urine and serum of normal subjects and patients with cancer and other diseases. Cancer 11:283-291

Gupta RK, Paul BS (1979) Effect of malathion on oxygen consumption and blood choline-esterase of the hen. Arch Environ Health 29: 167-169
LeBlond CP, Stevens CE (1948) The constant renewal of the intestinal epithelium in the albino rat. Anat Rec 100:357-378
Lee AG (1975) Functional properties of biological membrane: A physical-chemical approach. Progr Biophys Mol Biol 29:5-56
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. J Biol Chem 193:265-275
Luckens MM, Phelps KI (1969) Serum enzyme pattern in acute poisoning with organochlorine insecticides. J Pharmaceut Sci 58:569-572
Mahmood A, Agarwal N, Sanyal S, Subrahmanyam D (1978) Effect of DDT (Chlorophenotane) administration on glucose uptake and brush border enzymes in monkey intestine. Acta Pharmacol Toxicol 43: 99-102
Mahmood A, Agarwal N, Sanyal S, Dudeja PK, Subrahmanyam D (1979) Alterations in the intestinal brush border membrane structure and function in chronic DDT exposed monkeys. Pestic Biochem Physiol 12:141-146
Mahmood A, Agarwal N, Sanyal S, Dudeja PK, Subrahmanyam D (1981) Acute dieldrin toxicity: Effect on the uptake of glucose, and leucine and on brush border enzymes in monkey intestine. Chemicobiol Interact 37:165-170
Melchior DL, Steim JM (1976) Thermotropic transitions in biomembranes. Ann Rev Biophys Bioengr 5:205-238
Metcalf RL (1971) in Pesticides in the environment. Stevens WR and Dekker M (eds), Academic Press, Inc. New York, Chapter 1
Quigley JP, Gotterer GS (1969) Distribution of (Na+, K+) stimulated ATPase activity in rat intestinal mucosa. Biochem Biophys Acta 173:456-468
Reitman S, Frankel S (1957) A colorimeteric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28:56-63
Reymann A, Braun W, Drager J (1983) Effects of DDT and dieldrin on intestinal glucose transport and brush border hydrolases. Biochem Pharmacol 32:1759-1763
Sandermann H Jr (1978) Regulation of membrane enzymes by lipids. Biochem Biophys Acta 515:209-237
Schmitz JC, Preiser H, Maestracci D, Ghosh BK, Cerdo JJ, Crane RK: (1973) Purification of human intestinal brush border membrane. Biochem Biophys Acta 322:98-112
Skou JC (1965) Enzymatic basis for active transport of Na+ and K+ across cell membrane. Physiol Rev 45:596-617
Stevens JT (1974) The role of binding in inhibition of hepatic microsomal metabolism by parathion and malathion. Life Sci 14: 2215-2229
Wali RK, Singh R, Dudeja PK, Mahmood A (1982) Effect of a single oral dose of endosulfan on intestinal uptake of nutrients and on brush border enzymes in rats. Toxicol Letter 12:7-12
World Health Organization (1972) Report on health hazards of the human environment. WHO, Geneva, p 205-210
Received September 26, 1983; accepted December 21, 1983