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Induction of Hepatic Mixed Function Oxidase System by Endosulfan in Rats

S. R. Tyagi, Yogendra Singh, P. K. Srivastava, and U. K. Misra

Department of Biochemistry, V. P. Chest Institute, University of Delhi, Delhi-110007, India

Both the quality and quantity of dietary proteins is known to modify the response to the pharmacotoxicological activities of drugs and foreign compounds, (Campbell and Hayes 1976; Clinton et al.1980). The biological value of proteins was positively correlated with activity of microsomal mixed function oxidase (MFO) system including cytochrome P-450, aminopyrine N-demethylase, aniline hydroxylase and NADPH-cytochrome C reductase (Kato et al.1981). Besides influencing MFO activity protein malnutrition also affects membrane phospholipid metabolism(Yamada et al.1980).Endosulfan, a chloroinsecticide of cyclodiene group has been reported to be either a weak inducer of hepatic microsomal MFO system(Agarwal etal. 1978) or have no effect on MFO activity (Dorough etal. 1978). The toxicity of endosulfan has been inversely related to the quantity of the protein in the diet (Boyd 1970). In view of the effect of dietary protein on hepatic microsomal MFO activity and its phospholipid metabolism, we have evaluated the effect of endosulfan on hepatic microsomal MFO activity and its phosphatidylcholine metabolism in relation to quality and quantity of dietary protein.

MATERIALS AND METHODS.

Male Wistar rats(weighing 50-60 g) were divided into three groups of eighteen rats each and housed in individual cages. They had free access to water all the time. One group of rats received rice diet without lysine and threonine supplementation (D-diet) for fifteen days. The second group received rice diet supplemented with lysine and threonine (S-diet) for the same period. The third group of rats received 20% casein diet (C-diet) for fifteen days. The diets were

prepared as described by Viviani et al(1964).The rats of supplemented as well as casein group were pairfed against deficient group (ad libitum) to keep the caloric intake constant in all the groups. The above groups were further divided into three subgroups of six rats each. One subgroup was treated with multiple dose of endosulfan (7.5 mg/kg body weight/daily/15 days) and the other one was treated with a single dose of endosulfan (7.5mg/kg body weight)only on the terminal day of feeding. The third group received only vehicle solution and served as control. The weight gain and food consumption of rats during the course of the experiment were recorded. Three hours before sacrifice rats of control and multiple dose of endosulfan groups were intraperitoneally injected with a saline solution of $NaH_2P^{32}O_4$ (10 µci/100g body weight).The rats were sacrificed by decapitation,their liver removed, immersed in ice cold physiological saline, cleaned, wiped and weighed. Microsomes were prepared by the method of Zanoni (1972) and protein was quantified as reported by Lowry et al.(1951).The hepatic Cytochrome P-450 was assayed by the method of Omura and Sato (1964), NADPH-Cytochrome C-reductase by the method of Christensen and Wissing(1972) and aminopyrine N-demethylase was assayed by the method of Kato and Gillette (1965). The lipids from microsomal fractions were extracted by the method of Folch et al. (1957). Phospholipids were fractionated by means of thin layer chromatography on silica gel G (E.Merck, W.Germany) unidimensionally by the method of Abramson and Blecher (1964). The radioactivity of $NaH_2P^{32}O_4$ was determined in a Kontron MR-300 liquid Scintillation Counter. Scintillation cocktail contained 4g 2,5 diphenyl oxazole (PPO) and 200 mg of 2,2 paraphenylene bis 5-phenyl oxazole per litre of pure toluene. The results were statistically analysed and the significance was calculated using student'S "t" test. The results were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION.

Dorough et al.(1978) reported that administration of endosulfan (50 ppm/kg diet) to rats for 28 days did not induce liver mixed function oxidases. On the contrary Gupta and Gupta(1977), Agarwal et al.(1978) reported that endosulfan administration (5 mg/kg body weight) for 7-15 days reduced phenobarbital induced

sleeping time and increased the activities of some enzymes of mixed function oxidase system. The reason for this discrepancy is not clear, but it could be due to the threshold concentration of endosulfan which may be necessary for inducing the activity of mixed function oxidase system. In the present investigation endosulfan(7.5 mg/kg body weight)was administered in two ways:in multiple doses for 15 days and in a single dose on the terminal day of the experiment. It was observed that administration of endosulfan in multiple doses slightly increased liver weight where as a single dose did not show any effect on liver weight as compared to the respective controls(Table 1).Administration of multiple doses of endosulfan significantly increased microsomal protein in supplemented and deficient groups but the administration of single dose of endosulfan did not show any effect as compared to their respective untreated controls (Table 1). Endosulfan administration either as a single dose or as multiple doses did not show any effect on microsomal protein contents of casein group (Table 1). The results indicate that perhaps a threshold concentration of endosulfan in liver appears to be necessary for its inductive effects.

Administration of endosulfan in multiple doses significantly increased liver microsomal cytochrome P-450 contents and the activities of NADPH-cytochrome C-reductase and aminopyrine N-demethylase in rats of all the dietary groups as compared to their respective untreated controls (Table 2). On the contrary administration of endosulfan in a single dose did not show any effect on cytochrome P-450 contents and activities of NADPH-cytochrome C reductase and aminopyrine N-demethylase in rats of all the dietary groups as compared to their respective untreated controls(Table 2). Based on these observations it can be suggested that the discrepancy about the inducing capacity of endosulfan reported by Dorough et al (1978) and Gupta and Gupta (1977), Agarwal et al.(1978) is perhaps owing to the available concentration of endosulfan in liver. It is known that multiple dosing of phenobarbital, a classical inducer of mixed function oxidase system is also required before its inductive effects on MFO are observed. The results also reveal the marked influence of quality and quantity of dietary protein on mixed function oxidase system. The values of microsomal protein, Cytochrome P-450 contents and the activities of NADPH-Cytochrome C reductase and amino-

Table 1. Effect of endosulfan on liver weight, microsomal protein and phosphatidylcholine contents of rats. Values are mean ± SE from six animals in each group.

Dietary groups	Endosulfan treatment (mg)	Dose	Liver wt (g)	Microsomal protein (mg/g liver)	Microsomal PC (µg PCP/g liver)	$NaH_2P^{32}O_4$ incorporation into PC (CPM/µg PCP)	(CPM/g liver)
D	None		2.20±0.09	10.50±0.87	73.88±2.58[a]	401.37±9.90	30286±1528
D	7.5	Multiple	2.20±0.13	15.29±0.45[a,f]	90.89±3.22[a]	511.74±11.26[a]	46341±5077[a]
D	7.5	Single	2.10±0.15	11.12±0.77	—	—	—
S	None		2.39±0.09	17.84±1.06[a]	83.98±4.83	483.06±15.69[a]	42473±3700[a]
S	7.5	Multiple	2.62±0.08	22.98±0.81[b,e]	95.98±3.26	565.83±11.68[b]	51906±1292[b]
S	7.5	Single	2.46±0.11	16.83±0.56	—	—	—
C	None		3.13±0.14[a,b]	18.22±0.55[a]	91.98±4.24[a]	605.05±10.65[a]	56329±2995[a]
C	7.5	Multiple	3.21±0.18	20.71±1.07	129.26±3.44[c]	570.99±25.37	71854±4810[c]
C	7.5	Single	2.85±0.02	17.15±0.74	—	—	—

a = significantly different from untreated D group; b = significantly different from untreated S group, C=significantly different from untreated C group, e = significantly different from single dose S group and f = significantly different from single dose D group. p value $\leq$ 0.05 has been considered significant. PC=Phosphatidylcholine; PCP=PC Phosphorus, CMP=Counts per minute; —: Not done, D = rats fed deficient diets, S=rats fed supplemented diet, C=rats fed casein diet.

Table 2. Effect of endosulfan on hepatic microsomal mixed function oxidase components of rats. Values are mean ± SE from six rats in each group.

Dietary Groups	Endosulfan treatment (mg)	Dose	Cytochrome P-450 (n moles/mg protein)	NADPH-cytochrome C reductase, (n moles cytochrome C reduced/mg protein/min)	Aminopyrine N-demethylase, (n moles HCHO/mg protein/h.)
D	None		0.52±0.06	47.56±2.13	54.87±4.64
D	7.5	Multiple	0.86±0.05[a,f]	64.12±3.94[a,f]	96.85±4.33[a,f]
D	7.5	Single	0.41±0.03	44.02±3.54	68.08±4.14
S	None		0.61±0.05	65.50±5.08[a]	53.77±4.20
S	7.5	Multiple	0.88±0.05[b,e]	86.03±4.66[b,e]	70.68±3.63[b,e]
S	7.5	Single	0.48±0.02	68.33±3.36	50.74±3.37
C	None		0.86±0.05[a,b]	74.10±3.34[a]	68.31±2.49[b]
C	7.5	Multiple	1.03±0.06[d]	88.78±3.49[c]	81.05±5.83[d]
C	7.5	Single	0.79±0.07	79.89±2.93	61.40±3.19

d = significantly different from single dose C group ($p \leq 0.05$).
See table 1 for symbols a,b,c,e,f,D,S and C.

pyrine N-demethylase were lowest in the deficient group and highest in the casein group. These results are similar to those reported by earlier workers. Though the intial values in deficient group were lower but the induction of Cytochrome P-450 was highest in this group (65%) followed by the supplemented group (44%) and the casein group (19%). Similar effect of endosulfan was observed on the activities of NADPH-Cytochrome C-reductase and aminopyrine N-demethylase. These results are similar to those of Boyd et al.(1970) who observed that the endosulfan toxicity was inversely related to dietary protein contents. The results further show that the inductive capacity of endosulfan was also inversely related to the quality of dietary protein.

Like microsomal protein and the mixed function oxidase system, microsomal phosphatidylcholine was lowest in the deficient group followed by the supplemented and the casein group (Table 1). However, the administration of multiple doses of endosulfan caused the highest increase in microsomal PC contents in the deficient group followed by supplemented and casein groups (Table 1). These results are similar to those observed above on the inductive capacity of endosulfan on mixed function oxidase system (Table 2). There appears to be a direct relationship between increase in microsomal phospholipids and the induction of hepatic microsomal mixed function oxidase system activity by endosulfan.

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