J. Vet. Malaysia (1996) 8(1):21-24

THE INFLUENCE OF DIELDRIN PRETREATMENT ON THE TOXICITY OF PRAZIQUANTEL IN RABBITS

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SUMMARY

Oral administration of dieldrin, a known inducer of drug-metabolising enzyme activity, at a dose rate of 2.5 mg/kg BW produced no pathological changes in the tissues of treated rabbits and resulted in the induction of the activities of hepatic drug-metabolising enzymes aminopyrine-N-demethylase and aniline 4-hydroxylase. The induction of these enzymes might have protected the rabbits from the lethal effect of praziquantel when given at the dose rate of 2000 mg/kg BW (50 times the therapeutic dose) ten days following dieldrin pretreatment.

Keywords: Dieldrin, praziquantel, drug-metabolising enzymes, rabbits.

INTRODUCTION

Induction of drug-metabolising enzymes may alter the efficacy of therapeutic agents or might create unexpected imbalances between rates of toxification and detoxification in organisms exposed to drugs or environmental chemicals (Okey *et al.*, 1986).

Dieldrin is a chlorinated hydrocarbon and widely used insecticide. It is a known inducer of drug-metabolising enzyme activity (Ford *et al.*, 1976), and the exposure of animals to such insecticide is prevalent.

In a previous study, the lethal dose of praziquantel (an anticestodal and schistosomicidal drug) to rabbits was found to be 2000 mg/kg BW, and at this dose level signs of toxicity developed followed by death of the animals. Moreover, reduction in the activity of drug-metabolising enzymes was also observed (Kheir *et al.*, 1995). It was thought of interest to find out whether the treatment of rabbits with drug-metabolising enzyme inducers like dieldrin would modulate the toxic effects of praziquantel in rabbits.

MATERIALS AND METHODS

Experimental animals

The rabbits used in the present study were healthy adult males (5-8 months) and weighed 900-1200 g. They were purchased commercially and kept in our animal house and given a standard diet.

Experimental design

A pilot experiment was performed to determine the dose of dieldrin that causes the optimum level of induction of drug-metabolising enzymes. Optimum induction was obtained 10 days following the administration of 2.5 mg/kg BW. The influence of dieldrin pretreatment on the toxicity of praziquantel was investigated. For the purpose of this study, sixteen rabbits were used. They were divided into four groups (Groups 1-4) of four animals each. Dieldrin was administered orally as a suspension in sesame oil at the concentration of 2.5 mg/mL. Each rabbit received 2.5 mg/kg BW. Rabbits in groups 2 and 3 were sacrificed 10 and 21 days post dosing, respectively. Animals in group 4 received praziquantel orally at the dose rate of 2000 mg/kg BW, ten days following dieldrin administration, and were sacrificed 24 h after dosing. Animals in group 1 served as untreated controls.

Blood collection

Three millilitres of blood were collected from the ear vein using heparinised syringes on day 0, 3, 7, 10, 14 and 21. Plasma was separated and stored at -20°C until analysed for sorbitol dehydrogenase (SD), glutamate dehydrogenase (GD) and aspartate amino-transferase (AST) activity.

Macro-and microscopic examination

Immediately after death, post mortem was conducted and the livers, lungs, kidneys, spleens, muscles and brain were quickly removed and examined for gross pathological changes. A small necropsy specimen from each organ was placed in 10% formal-saline, processed, embedded in paraffin wax, cut at 5 µm and stained with haematoxylin and eosin stain.

Collection of tissue samples

After sacrificing the animals, 5 g of the liver was

dipped in liquid nitrogen until analysis for the determination of the activities of drug-metabolising enzymes.

Measurement of drug-metabolising enzymes in the liver

All steps of the preparation were carried out on ice. The livers were allowed to thaw at 4°C for 15 min, minced with scissors and homogenised in ice-cold, 1.15% w/v KCl solution (pH 7.4) in a Potter homogeniser, for 1 min (6-8 strokes). The crude homogenates (10 to 20% W/V) were centrifuged at 10,000 *g* for 10 min at 4°C in a refrigerated centrifuge. The microsomal-rich supernatant was decanted and used for the enzymatic assays. The microsomes were prepared for the estimation of protein concentration using the calcium aggregation method of Aitio and Vainio (1976). Protein was assayed in the crude homogenates, cytosolic and microsomal fractions of each liver sample by the method of Lowry *et al.* (1951) as modified by Miller (1959).

The activity of aminopyrine N-demethylase was estimated by measurement of formaldehyde following the procedure of Nash described by Mazel (1971). The activity of aniline 4-hydroxylase was determined by measuring the quantity of p-aminophenol produced (Mazel, 1971). UDP-glucuronyltransferase activity was estimated by the method of Dutton and Storey (1962).

Before measuring the enzyme activities optimum conditions for the enzyme assays were determined.

Plasma enzymes

The activity of sorbitol dehydrogenase (SD) was estimated by the colorimetric method of Ford (1967), glutamate dehydrogenase (GD) activity was determined colorimetrically according to Ford and Boyd (1962) and the activity of aspartate aminotransferase (AST) was estimated colorimetrically as described by Reitman and Frankel (1957). Total protein concentration in plasma was determined by the Biuret method as described by Weichselbaum (1946).

Statistical analysis

Values reported are means ± SEM (number of animals). Comparisons were made using Student's T-test with a significance level of $P<0.05$.

RESULTS

Clinical picture

Animals in groups 1, 2 and 3 showed no clinical signs throughout the experimental period. Animals in group 4 that received the lethal dose of praziquantel (2000 mg/kg BW), started to show signs of toxicity 2 to 5 h post dosing. These signs comprised shivering, incoordination and did not eat, which lasted for about 3 h after which period the rabbits recovered.

Biochemical parameters

No significant changes were observed in the plasma protein concentration or the activity of GD in all rabbits belonging to groups 2, 3 and 4 compared to the control group. Rabbits in groups 2, 3 and 4, showed a significant increase in the activities of SD and AST three to seven days following the administration of dieldrin as compared to the control group ($P<0.05$). In group 4, maximum increase of these enzymes was observed following the dosing of praziquantel ($P<0.001$).

Necropsy findings

On post mortem examination, the livers of rabbits belonging to groups 2, 3 and 4 were enlarged and dark in colour and the kidneys were congested. No post mortem changes were observed in other organs. The liver sections from rabbits in groups 2 and 4 showed variable degrees of congestion. The livers and brains of rabbits in group 4 were slightly congested and there were aggregation of black granules in the livers and lungs. No histopathological changes were observed in other organs.

Drug-metabolising enzymes

The concentration of protein in whole homogenate, cytosolic and microsomal fractions of rabbit livers belonging to groups 1, 2, 3 and 4 are shown in Table 1.

The cytosolic protein concentration was significantly elevated ($P<0.01$) in rabbits belonging to group 2. Moreover, there was a significant increase in the microsomal protein concentration in rabbits belonging to groups 2 ($P<0.001$), 3 ($P<0.01$) and 4($P<0.05$). The activities of drug-metabolising enzymes are shown in Table 2. Aminopyrine N-demethylase activity was significantly increased in rabbits in groups 2 ($P<0.001$), 3 ($P<0.002$) and 4 ($P<0.05$), when compared to control animals. A statistically significant increase in the activity of aniline 4-hydroxylase was observed in animals of groups 2 ($P<0.01$), 3 ($P<0.01$) and 4 ($P<0.05$), compared to that in control rabbits. There was no significant change in the activity of UDP-glucuronyltransferase in all groups.

DISCUSSION

The activities of SD and AST were significantly increased in rabbits given dieldrin. This increase might have been due to stress in the liver cells caused by the drug, since dieldrin is a hepatotoxic compound and causes liver cell injury. Hurkat (1977) reported an increase in the activities of plasma enzymes AST and GPT in rabbits dosed with 2.5 mg/kg BW of dieldrin on alternate days over a period of 100 days. The significant increase in the activities of SD and AST of rabbits in group 4 was indicative of tissue injury which might be due to toxic effect of praziquantel given at

Table 1. The effect of dieldrin on the protein concentration in whole homogenate, cytosolic and microsomal fractions of rabbit livers (mean ± SEM)

	Control	Dieldrin		Dieldrin + Praziquantel
	Group 1 (n = 4)	Group 2 (n = 4)	Group 3 (n = 4)	Group 4 (n = 4)
Whole homogenate	174.990 ± 6.245	252.776 ± 10.067	192.432 ± 13.439	177.232 ± 7.192
Cytosolic fraction	75.770 ± 4.048	95.455 ± 1.126**	87.723 ± 5.774	78.736 ± 3.167
Microsomal fraction	16.889 ± 0.828	37.601 ± 2.146***	20.979 ± 0.632**	20.061 ± 0.794*

Dieldrin was given orally at the dose rate of 2.5 mg/kg BW to rabbits of groups 2, 3 and 4. Those of groups 2 and 3 were sacrificed 10 and 21 days following dieldrin administration. Animals of group 4 received praziquantel at the dose rate of 2000 mg/kg BW 10 days after dieldrin treatment and sacrificed 24 h later. Rabbits of group 1 served as untreated controls.
Protein concentration is expressed as mg protein /g of liver
* $P<0.05$ Compared to the control values
** $P<0.01$ Compared to the control values
*** $P<0.001$ Compared to the control values

Table 2. The effect of dieldrin in rabbits on the hepatic activities of aminopyrine N-demethylase, aniline 4-hydroxylase and UDP-glucuronyltransferase (mean ± SEM)

	Control	Dieldrin		Dieldrin + Praziquantel
	Group 1 (n = 4)	Group 2 (n = 4)	Group 3 (n = 4)	Group 4 (n = 4)
Aminopyrine N-demethylase	6.375 ± 0.493	11.549 ± 0.305****	10.634 ± 0.653***	9.893 ± 0.182**
Aniline 4-hydroxylase	0.372 ± 0.027	0.936 ± 0.230**	0.999 ± 0.010**	0.839 ± 0.010*
UDP-glucuronyl -transferase	3.520 ± 0.568	2.820 ± 0.065	3.190 ± 0.090	2.813 ± 0.077

Dieldrin was given orally at the dose rate of 2.5 mg/kg BW to rabbits of groups 2, 3 and 4. Those of groups 2 and 3 were sacrificed 10 and 21 days post dosing, respectively. Animals of group 4 received praziquantel at the dose rate of 2000 mg/kg BW 10 days after dieldrin treatment and sacrificed 24 h later. Rabbits of group 1 served as untreated controls.
Enzymes activities are expressed as n mole/mg of microsomal protein/min.
* $P<0.05$ Compared to the control values.
** $P<0.01$ Compared to the control values.
*** $P<0.002$ Compared to the control values.
**** $P<0.001$ Compared to the control values.

that high level combined with the hepatotoxic effect of dieldrin. Dieldrin premedication resulted in an increase in microsomal protein concentration in rabbits belonging to that group.

There was a significant increase ($P<0.01$) in the cytosolic protein concentration in rabbits of group 2 and similar results were obtained in goats by previous workers (Elsheikh *et al.*, 1991). The activities of aminopyrine N-demethylase and aniline 4-hydroxylase were increased following the administration of dieldrin. These findings are similar to those reported in mice by Virgo and Bellward (1975) & by Ford *et al.* (1976) in sheep and calves respectively. Dieldrin produces a phenobarbitone-like induction in the microsomal cytochrome P-450, that the activities of aminopyrine N-demethylase and aniline 4-hydroxylase concentrations are enhanced (Alvares, 1972). This enzyme induction might have protected the rabbits from the lethal effect of high doses of praziquantel (2000 mg/kg BW) 10 days after receiving the dieldrin.

The toxic effect of praziquantel might have been due to the effect of the parent compound, which is biotransformed into less toxic metabolites facilitated by enzyme induction in rabbits, and the metabolism of praziquantel might be mediated by cytochrome P-450 isoenzymes induced by dieldrin as was reported by Buhring *et al.* (1978).

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RINGKASAN

PENGARUH PRARAWATAN DIELDRIN TERHADAP KETOKSIKAN PRAZIQUANTEL DALAM ARNAB

Pemberian oral dieldrin, suatu bahan yang diketahui pengaruh aktiviti enzim memetabolisme drug, pada kadar dos 2.5 mg/kg berat badan tidak menghasilkan perubahan patologi dalam tisu arnab teperlaku dan membawa kepada pengaruhan aktiviti enzim memetabolisme drug hepar aminopirin-N-demetilase dan anilin-4-hidroksilase. Pengaruhan enzim-enzim tersebut mungkin telah melindung arnab daripada kesan maut praziquantel apabila diberi pada kadar dos 2000 mg/kg berat badan (50 kali lebih tinggi daripada dos terapi) sepuluh hari selepas prarawatan dieldrin.