Microsomal Enzyme Induction By Permethrin in Rats*

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(Received April 20, 1987; Revision Received February 23, 1988;
Accepted March 3, 1988)

ABSTRACT. The synthetic pyrethroid, permethrin, was evaluated for its ability to alter hepatic microsomal drug-metabolizing function. The influence of permethrin (25:75 cis-trans) on plasma antipyrine kinetics and γ-glutamyltranspeptidase (γ-GTP) activity were studied in rats. After 3 days of administration of 90 mg permethrin/kg/day, there was no significant change in the antipyrine half-life and the area under the curve, while the apparent volume of distribution and clearance were significantly increased. Treatment with 190 mg permethrin/kg/day for 3 days decreased antipyrine half-life and the area under the curve, and increased the apparent volume of distribution and the clearance significantly. The γ-GTP activity was significantly increased within 21 days and 14 days after the start of permethrin administration, at doses of 90 and 190 mg permethrin/kg/day, respectively. The antipyrine kinetics results indicate that permethrin is capable of producing a dose-dependent marked enzyme-inducing effect.

Pyrethrins are valuable insecticides. Unlike the chlorinated hydrocarbons, phosphates and carbamates, pyrethrins have the important advantages of being biodegradable, having low vapor pressure, and having low mammalian toxicity. Among the synthetic pyrethroids one of the most promising is permethrin [3-phenoxybenzyl(±)cis, trans-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] (1,2). Permethrin, apart from having the desirable properties of a pyrethrin, is superior in that it is photostable and possesses high insecticidal activity. Already a wide range of applications have been found for the compound (2-4).

Permethrin is used commercially in large quantities. Therefore, studies of the secondary effects of this insecticide in mammals are of great clinical and toxicological importance. Relatively little is known of the effect of permethrin on hepatic microsomal drug-metabolizing function. The pyrethroids appear in general to be either weak inducers (5,6) or noninducers (7). However, marginal induction in rats of microsomal cytochrome P-450 and NADPH cytochrome c reductase occurs at multiple doses of permethrin (8). For further elucidation of the influence of permethrin on the hepatic drug-metabolizing activity, we have investigated the effect of permethrin on other tests of hepatic microsomal enzyme induction.

A number of indices have been proposed, and used to assess the extent of enzyme induction (9). One of the more widely used measures of induction is the change in the rate of elimination of antipyrine. Antipyrine has now been in use for several years as a marker compound of hepatic drug-metabolizing activity (10). Enzyme induction has also been assessed by measuring changes in the disposition of endogenous compounds. γ-Glutamyltranspeptidase (γ-GTP) is an enzyme that catalyzes the transfer of the γ-glutamyl group from γ-glutamyl peptides, particularly from glutathione, to other peptides or L-amino acids, with formation of new γ-glutamylpeptides. A direct role of γ-GTP in the metabolism of drugs is not well established; however, its activity in the liver and in plasma is increased by certain drugs, particularly barbiturates (11,12), after ingestion of alcohol (13), and in most hepatic disorders, particularly when cholestasis occurs (14,15).

In the present study, the influence of prolonged permethrin treatment on the antipyrine kinetics and on plasma γ-GTP activity in rats has been examined.

MATERIALS AND METHODS

Animals

Adult male rats of the Wistar strain weighing 250 g were acclimated for 1 week prior to use. They were housed in well-ventilated cages and kept at a temperature of approximately 24 C. They were allowed food and water ad libitum.

Experimental design

There were 2 studies. In Study 1, the rats were divided in groups of 32 rats/group. There were 3 groups. One group of 32 rats served as control and received antipyrine at a single dose of 18 mg/kg. Antipyrine was administered orally in water after an overnight fast, and food and drink were not taken for a further 4 hr. In these animals the antipyrine kinetics was studied.

*This work received financial support from the University of Leon, Spain.

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In 32 other animals (group 2 of Study 1), the influence of a low dose of permethrin on antipyrine kinetics was investigated. These animals received permethrin at a dose of 95 mg/kg once a day for 3 consecutive days. Twenty-four hours after the last dose of permethrin, the animals received antipyrine the same way as in the control group. Finally, 32 other animals (the third group of Study 1) were used to investigate the influence of a higher permethrin dose on antipyrine kinetics. In this case, the animals received 190 mg permethrin/kg once a day for 3 consecutive days. The antipyrine was again administered 24 hr after the last permethrin dose.

The permethrin was dissolved in sunflower oil and injected ip in a volume of 1 ml/kg. Four animals from each of the groups were sacrificed 0.25, 0.5, 1, 2, 4, 6, 8, or 24 hr after the antipyrine dose. To determine the plasma pharmacokinetic parameters of antipyrine blood samples were collected in tubes containing sodium heparin. The blood was centrifuged and plasma was separated and stored at -20 C until analyzed.

In Study 2, the effect of permethrin treatment on plasma γ-glutamyltranspeptidase (γ-GTP) was determined in rats. The animals were divided into 3 groups of 12 rats/group. A control group received 0.3 ml of sunflower oil daily ip. Another group was injected with 0.3 ml of a sunflower oil solution containing permethrin at 95 mg/kg/day, and the third group received injections of 0.3 ml of a sunflower oil solution containing permethrin at a dose of 190 mg/kg/day. The pyrethroid insecticide was injected ip daily for a maximum period of 21 days. Four rats of each of the 3 groups were sacrificed at 7, 14 or 21 days after starting the insecticide or sunflower oil injections, and blood samples were collected in tubes containing sodium heparin. The blood was centrifuged, and the plasma was separated and assayed.

Analytical Procedures

Antipyrine in plasma was assayed using a gas-liquid chromatographic method. Samples were removed from the freezer and allowed to thaw completely to room temperature for assay as previously described (16). Antipyrine was assayed on a 3% OV-17 on 80/100 mesh Gas-Chrom Q column in a Hewlett Packard 5830A chromatograph. The column, injection port and flame ionization detector temperatures were 200, 240 and 250 C respectively. The nitrogen carrier gas flow rate was 50 ml/min.

Plasma antipyrine was assayed as follow: 1 ml samples of plasma were made basic with 0.5 ml of 1 N NaOH and were extracted with 8 ml chloroform. After slow shaking for 10 min, the extracts were centrifuged at 3000 rev/min for 10 min. The aqueous phase was aspirated, and a 6 ml aliquot of the chloroform extract was evaporated to dryness under a nitrogen stream. The sides of the tube were washed down with 1 ml acetone and the solvent was again evaporated. The tube was placed in ice water, the residue reconstitued in 25 µl ice-cold acetone, and a 3 µl aliquot injected into the OV-17 column. For each analysis a standard curve was prepared with concentrations of 0.5, 2, 4, 5, 10, 25 and 50 µl/ml. These standards were prepared in blank plasma and were carried through the entire procedure alone with the unknown samples. Standards were injected both at the beginning and at the end of each run.

Plasma γ-GTP activity was measured by a colorimetric procedure (Sigma Tech, Bulletin No 545) based essentially on the method of Naftalin et al (17). The assay was performed under linear reaction conditions using γ-L-glutamyl-p-nitroanilide as the donor substrate and glycylglycine as the acceptor molecule for the glutamyl group released by the action of γ-GTP. Liberated p-nitroaniline is diazotized and measured at 530-550 nm in a LKB Ultrospec 450 spectrophotometer. Enzyme activity was expressed in units (iu), one unit corresponding to the 1 nmol of p-nitroaniline from the substrate in 1 min at 25 C.

Data Analysis

The plasma data were fitted by a Hewlett Packard 85 computer using the non-linear least squares program ELSFIT (18). The curves of antipyrine concentrations in plasma versus time were fitted to a one-compartment model for kinetic analysis. The half-life of elimination phase ($t_{\frac{1}{2}}$), the apparent volume of distribution (V), the total body clearance (CL), and the area under the plasma concentration time curve (AUC) extrapolated to infinity were calculated. For statistical evaluation all values were compared by Student's t-test.

Materials

Antipyrine and the reagent kit for quantitative colorimetric determination of γ-GTP were obtained from Sigma Chemical Co (St Louis, MO, USA). The pyrethroid compound, permethrin with a dl cis:trans ratio of 25:75, was kindly supplied by Mostyn Chemical Ltd (Stockport, UK). All other chemicals used were obtained from usual commercial sources and were routinely of the highest grade available.

RESULTS

The animals used in Study 1 and 2, where the permethrin effect on antipyrine kinetics and the plasma γ-GTP activity was determined respectively, did not show any toxic signs during either of the prolonged permethrin treatments.

Figure 1 gives the semilogarithmic plasma concentration-time curves for antipyrine. The pharmacokinetic data for antipyrine before and after the prolonged permethrin treatments are given in Table 1. Following permethrin treatment for 3 days at 95 mg/kg/day, the apparent volume of distribution

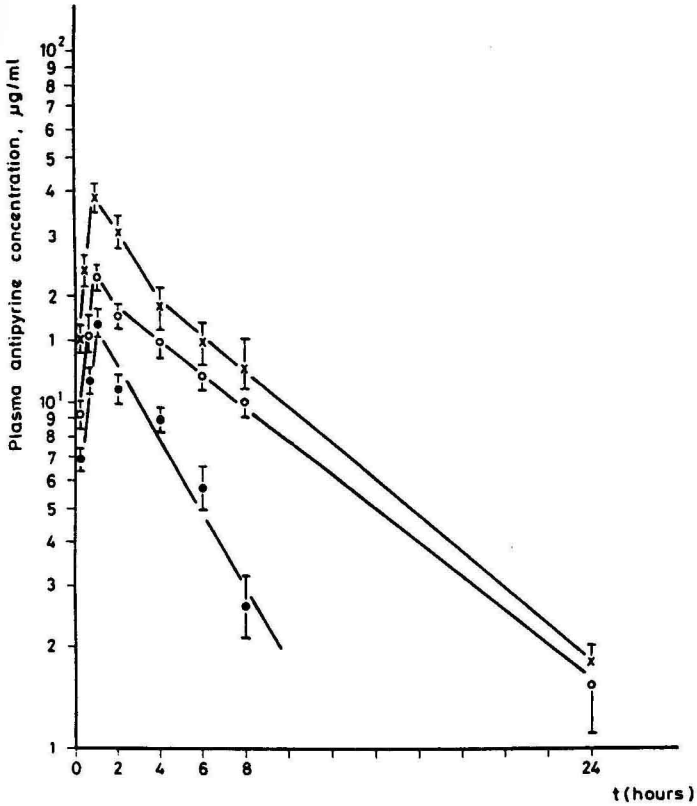

Figure 1. Concentration of antipyrine in rat plasma as a function of time after oral administration of 18 mg antipyrine/kg. One group of rats served as control (x —— x). The other groups were pretreated ip with 95 mg permethrin/kg/day (o —— o) or 190 mg permethrin/kg/day (● —— ●) for 3 days before antipyrine administration. Each point represents the mean of plasma antipyrine concentration in 4 rats. Vertical lines indicate SE.

and clearance were increased, whereas the elimination half-life and the area under the curve were not significantly changed. However, following permethrin treatment for 3 days at 190 mg/kg/day, statistically significant changes in all kinetic parameter were observed. Antipyrine half-life and area under the curve were decreased, and the apparant volume of distribution and clearance increased.

The mean data for the plasma γ-GTP activities are shown in Table 2. Permethrin did not significantly increase plasma γ-GTP activity after 7 and 14 days, but did so after 21 days of administering 95 mg/kg/day. Administration of 190 mg permethrin/kg/day appeared to give slightly greater increases in plasma γ-GTP activity. After 14 and 21 days, treatment at 190 mg permethrin/kg/day caused small by significant increases in γ-GTP activities.

DISCUSSION

Based on previous studies, permethrin seemed only to be a weak enzyme-inducing agent in rats (8). Administration of 50 mg permethrin/kg/day po for 8 and 12 days resulted in small increases in microsomal cytochrome P-450 and NADPH cytochrome c reductase. In the present study, another

Table 1. Effect of permethrin prolonged treatment on plasma antipyrine pharmacokinetic parameters

Treatment	$t_{\frac{1}{2}}$(h)	V(l)	CL(l h^{-1})	AUC(mg l^{-1} h)
Control (sun-flower oil)	5.35±0.15	0.129±0.008	0.016±0.002	276±23
Permethrin (95 mg/kg/day) for 3 days	5.95±0.65	0.191±0.007**	0.022±0.002*	202±20
Permethrin (190 mg/kg/day) for 3 days	3.35±0.15***	0.272±0.019**	0.056±0.005***	82±7***

Results are means±SEM, n=4

*P<0.05 ; **P<0.01 ; ***P<0.001 significantly different from controls, Student's t test.

index of hepatic microsomal enzyme induction was used to assess the enzyme-inducing capacity of prolonged permethrin treatment in rats. Under the experimental conditions, permethrin produced significant changes in antipyrine kinetics. The differences in the degree of enzyme induction from permethrin between the present results and those reported by Carlson and Shoening (8) may be explained in part by the difference in the permethrin doses used. We found that permethrin administered ip for 3 days at a dose of 190 mg/kg/day resulted in significant changes in antipyrine kinetics, whereas smallest changes were observed after 95 mg permethrin/kg/day. Interestingly, treatment with 190 mg permethrin/kg/day resulted in a decrease in half-life and the area under the curve from 5.35±0.15 to 3.35±0.15 h (P<0.001) and 276±23 to 82±7 mg l^{-1}h (P<0.001), respectively, and an increase in the apparent volume of distribution and the clearance from 0.129±0.008 to 0.272±0.019 l (P<0.01) and 0.016±0.002 to 0.056±0.005 l h^{-1}, (P<0.001), respectively. This is consistent with the view that hepatic enzyme activity is impaired.

Table 2. Plasma γ-glutamyltranspeptidase activity in rats after 7, 14 and 21 days of permethrin treatment

Treatment	γ-GTP(iu/ml)		
	7 days	14 days	21 days
Control (sun-flower oil)	6.17±0.31	6.11±0.45	6.25±0.35
Permethrin (95 mg/kg/day)	6.86±0.29	8.35±0.85	11.54±2.05*
Permethrin (190 mg/kg/day)	9.62±1.69	7.76±0.09*	10.79±1.42*

Values represent means±SEM, n=4

*P<0.05 significantly different from controls, Student's t test.

Antipyrine half-life was substantially decreased on prolonged permethrin dosing (190 mg/kg/day). This can mainly be attributed to an increasing of its clearance; the apparent volume of distribution was increased. Since antipyrine is distributed in a volume equivalent to that of total body water (19), the increased apparent volume of distribution would indicate an increase in total body water due to the various permethrin treatments.

Our finding that antipyrine clearance increased after permethrin treatment supports the suggestion of induction of the enzymes responsible for drug-metabolism since the clearance of antipyrine depends directly upon hepatic oxidases' activity (10).

In addition, the plasma γ-GTP activity appeared significantly increased following permethrin treatment (190 mg/kg/day) for 14 and 21 days. Increases of γ-GTP in the plasma of treated subjects have been reported with numerous drugs. Besides the measurement of γ-GTP activity in clinical chemistry in cases of liver injuries, particularly in cholestasis, carcinoma and after alcohol consumption, the use of this enzyme as an indicator of induction by certain drugs, particularly those of the phenobarbital group, has been proposed (12). However, plasma γ-GTP activity must be regarded an unreliable index of induction of drug-metabolizing enzymes. Therefore the increase in plasma γ-GTP activity found in our study may indicate other pathological changes that disturb hepatic enzyme activity.

It has been demonstrated in the rat that permethrin changes antipyrine kinetics and increases plasma γ-GTP activity. Although the mechanism and clinical significance of the increased plasma γ-GTP activity require additional study, this investigation supports the idea that permethrin may produce a pronounced dose-dependent enzyme-inducing effect, as indicated by changes in antipyrine kinetics.

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