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Effects of Capsaicin on the Pharmacokinetics of Antipyrine, Theophylline and Quinine in Rats

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Abstract—Capsaicin is the active principal of capsicum fruits, such as hot peppers. The influence of 1-week pretreatment of capsaicin (25 mg kg^{-1}) on the pharmacokinetics of antipyrine, theophylline and quinine was investigated in rats. The drugs were given as an intravenous bolus dose. The control rats received the vehicle solvent (polyethylene glycol) only. Clearance of antipyrine in the capsaicin-pretreated rats was significantly lower than that observed in the control rats (0.241 ± 0.029 vs 0.344 ± 0.034 L h^{-1} kg^{-1}, $P < 0.05$). This is consistent with a prolongation in the elimination half-life of antipyrine in animals pretreated with capsaicin (2.06 ± 0.30 vs 1.61 ± 0.27 h), as the volume of distribution was not significantly changed. In contrast, capsaicin pretreatment had no significant effect on the pharmacokinetics of theophylline and quinine.

Capsaicin is the principal active component of hot pepper (capsicum fruits). An average daily per capita consumption of capsaicin may reach 50 mg in some south-east Asian countries (Buck & Burks 1983). Much effort has been devoted to the study of various aspects of the pharmacology of capsaicin, but relatively limited information is available on the effects of capsaicin on metabolism and pharmacokinetics of drugs. It has been shown that capsaicin competitively inhibits ethylmorphine demethylase activity in-vitro in rat hepatic microsomes, and that it increases pentobarbitone-induced sleeping time in-vivo (Miller et al 1983). An in-vitro inhibitory effect of capsaicin on benzo(a)pyrene metabolism has also been reported in human and murine keratinocyte cultures (Modly et al 1986). It has been suggested that capsaicin inhibits hepatic drug-metabolizing enzymes by interacting with cytochrome P450. The objective of the present study was to examine the effect of capsaicin on the pharmacokinetics of three selected drugs, antipyrine, theophylline and quinine.

Materials and Methods

Materials

Antipyrine and 4-hydroxyantipyrine were purchased from Sigma Chemical Co., St Louis, MO, USA. Norantipyrine and 3-hydroxymethylantipyrine were synthesized in Professor C. R. Clark's laboratories, University of Auburn, AL, USA. Theophylline, 8-chlorotheophylline 1,3-dimethyluric acid, 3-methylxanthine, methyluric acid and 1-methylxanthine were purchased from Sigma Chemical Co. Quinine bisulphate was kindly supplied by Kimia Pharma, Indonesia. Polyethylene glycol 400 and sodium dodecyl sulphate were purchased from BDH Ltd (Poole, UK). All other chemicals used were obtained from usual commercial sources and were of analytical grade.

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Animal protocol

Inbred, male Wistar rats, 280–340 g, were housed in groups of 2–3 animals, in plastic cages with pine-wood shavings as bedding. The animals were fed a commercial rodent diet (F49, Reliance Stock Food Co., Dunedin, New Zealand) and animals were acclimatized for five days before the experiment. During the pharmacokinetic experiment the rats were housed separately in normal or metabolic cages. Food, but not water, was withdrawn the night before drug administration. The animals were provided with food and water during the experimental period. All animals were cannulated with Silastic tubing in the right jugular vein under light ether anaesthesia 24 h before the pharmacokinetic experiment. The study was approved by the Committee of Ethics in the Care and Use of Laboratory Animals, University of Otago, New Zealand.

Experimental design

In each study, the pharmacokinetic experiments were performed with two groups of rats ($n = 6$ for each group). One group served as control and received no capsaicin pretreatment. Instead, they were given a vehicle solvent (polyethylene glycol 400, PEG400) in a volume of 0·2 mL/100 g, once a day for 7 days before drug administration. Another group of rats were pretreated with 25 mg kg^{-1} capsaicin (12·5 mg mL^{-1} in PEG400), directly into the stomach using a thin plastic tube attached to a syringe. The animals received capsaicin once a day for 7 consecutive days before the pharmacokinetic experiment.

Pharmacokinetic studies and drug administration

Drug solutions were freshly prepared by dissolving the drug in normal saline. Drug, in a volume of 0·2 mL/100 g, was injected intravenously via the catheter.

On the study day, animals were placed individually in metabolic cages. An intravenous bolus single dose antipyrine pharmacokinetic study was performed after oral administration of capsaicin (25 mg kg^{-1}). Antipyrine (10 mg, i.v.) was administered and blood samples (0·2 mL) were withdrawn through a venous catheter at 0, 0·17, 0·35, 0·5, 0·75, 1, 1·5, 2,

Table 1. Effect of capsaicin pretreatment on the pharmacokinetics of antipyrine in rats.

Parameter	Control	Capsaicin-pretreated
Body weight (g)	303 ± 13	305 ± 17
$t_{\frac{1}{2}}$ (h)	1·61 ± 0·27	2·06 ± 0·30*
AUC (mg h L^{-1})	97 ± 13	139 ± 20**
Clearance (L h^{-1} kg^{-1})	0·344 ± 0·034	0·241 ± 0·029**
Volume of distribution (L kg^{-1})	0·802 ± 0·175	0·712 ± 0·116

Results are presented as mean ± s.d. (n = 6). *$P < 0.05$, **$P < 0.005$ compared with control.

3, 4, 5, and 6 h after injection of antipyrine. The same volume of saline (0·9% NaCl) was used as fluid replacement at the end of each blood sampling. Blood samples were collected in heparinized tubes. Plasma was separated after centrifugation and was stored at −20°C pending analysis.

An intravenous bolus dose theophylline pharmacokinetic study was performed in the same manner as described above. Aminophylline, equivalent to 10 mg kg^{-1} theophylline, was injected as a bolus through the jugular vein cannula. Blood samples (0·2 mL) were withdrawn through the cannula at 0, 0·03, 0·07, 0·17, 0·25, 0·5, 1, 3, 6, and 10 h after drug administration. Blood samples were centrifuged and plasma was harvested and stored at −20°C until analysis.

The pharmacokinetics of quinine after an intravenous, bolus dose was performed by injection of quinine (25 mg kg^{-1}) to each animal through the cannula. Blood samples (0·2 mL) were collected at 0, 0·2, 0·5, 1, 2, 3, 4, and 6 h after injection of quinine. Plasma samples were obtained and handled as previously described.

Drug analysis

The antipyrine concentrations in plasma were determined by HPLC (Shen & Wanwimolruk 1991) using a reversed-phase C18 (Nucleosil) column and a mobile phase of acetonitrile/water (25: 75, v/v). The mobile phase was pumped at 1 mL min^{-1}. Detection was by ultraviolet absorption at 254 nm. The assay was selective and no interference was caused by any of the antipyrine metabolites (4-hydroxyantipyrine, norantipyrine and 3-hydroxymethylantipyrine). The coefficient of variation for intra- and inter-assay was less than 10%.

Analysis of theophylline in plasma was performed by an HPLC method developed in our laboratory. The assay employed a reversed-phase C18 microbore column (2 mm i.d. × 100 mm) packed with 5 μm ODS Hypersil (Shandon, Cheshire, UK). 8-Chlorotheophylline was used as an internal standard. Chromatographic separation was achieved by using a mobile phase consisting of 4% acetonitrile and 96% Na_2HPO_4 buffer (10 mM, pH 2) with a flow rate of 0·5 mL min^{-1}. A UV absorbance detector was set at 273 nm. Sample preparation was by a direct precipitation of 50 μL plasma with 75 μL acetonitrile containing 50 μg mL^{-1} of the internal standard. After centrifugation, 25 μL of the clear supernatant was injected onto the HPLC column. The assay was selective and no interference was observed with theophylline metabolites tested (1,3-dimethyluric acid, 3-methylxanthine, methyluric acid and 1-methylxanthine). The calibration curve was linear over the plasma drug concentration range 0·1–100 μg mL^{-1} ($r^2 > 0.99$). The detection limit for this assay was 0·1 μg mL^{-1}. Inter- and intra-assay variability was less than 7% (coefficient of variation).

Plasma quinine analyses were performed by HPLC (Zoest et al 1990). This procedure is selective for quinine and has a detection limit of 18 ng mL^{-1}. The intra- and inter-assay variability was low with a coefficient of variation of less than 10%.

Data analyses

Pharmacokinetics of antipyrine, theophylline and quinine were analysed by iterative weighted nonlinear least-squares regression analysis. The computer program utilized was MINIM (courtesy of Dr R. D. Purves, Department of Pharmacology, University of Otago, New Zealand). The data were fitted using one- and two-compartment pharmacokinetic models. The appropriate model was chosen as the one which gave the minimum value according to Akaike's information criterion (Yamaoka et al 1978). The plasma drug concentration-time curves after drug administration were found to be adequately fitted to a standard two-compartment open model (Gibaldi & Perrier 1982) described by the following equation:

$$C = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

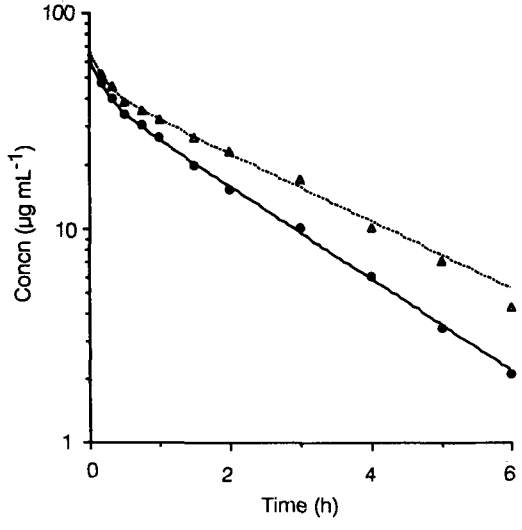

FIG. 1. Effect of capsaicin pretreatment on the elimination of antipyrine in rats. Each point represents the mean (n = 6) plasma antipyrine concentration in control rats (●——●) and rats pretreated with capsaicin 25 mg kg^{-1} day^{-1} (△- - -△) for 7 days before antipyrine administration.

Table 2. Pharmacokinetic parameters of theophylline and quinine in capsaicin-pretreated and control rats.

Parameter	Control	Capsaicin-pretreated
Theophylline		
Body weight (g)	301 ± 11	299 ± 17
$t_{\frac{1}{2}}$ (h)	2·2 ± 0·3	2·4 ± 0·5
AUC (mg h L^{-1})	72 ± 7	71 ± 9
Clearance (L h^{-1} kg^{-1})	0·175 ± 0·017	0·177 ± 0·018
Volume of distribution (L kg^{-1})	0·478 ± 0·026	0·480 ± 0·079
Quinine		
Body weight (g)	314 ± 20	306 ± 20
$t_{\frac{1}{2}}$ (h)	0·8 ± 0·3	1·1 ± 0·4
AUC (mg h L^{-1})	3·8 ± 1·1	4·4 ± 1·4
Clearance (L h^{-1} kg^{-1})	6·9 ± 1·6	6·2 ± 1·7
Volume of distribution (L kg^{-1})	7·7 ± 3·0	8·8 ± 3·7

Results are given as mean ± s.d. (n = 6).

where C is the plasma drug concentration, A and B are mathematical coefficients, α the hybrid rate constant for the distribution phase, and β the hybrid rate constant for the terminal elimination phase.

Drug elimination half-life ($t_{\frac{1}{2}}$) at the terminal phase was calculated as $t_{\frac{1}{2}} = 0{\cdot}693/\beta$. The area under the plasma concentration-time curve (AUC) was estimated as follows: $AUC = (A/\alpha) + (B/\beta)$. The total plasma clearance (CL = dose/AUC) and volume of distribution (Vd = dose/(AUC·β)) were also calculated.

The unpaired Student's *t*-test was used to assess statistical differences in pharmacokinetic parameters between the two groups. In cases where the variance between the two groups was unequal, the non-parametric Mann-Whitney U-test was employed. Differences were only considered significant if $P < 0{\cdot}05$. Results are reported as mean ± s.d.

Results

The kinetics of antipyrine changed significantly after capsaicin treatment. Table 1 summarizes the pharmacokinetic parameters of antipyrine obtained. The profiles of mean plasma antipyrine concentrations vs time are illustrated in Fig. 1. Rats pretreated with capsaicin showed a markedly slower plasma elimination of antipyrine compared with the control animals. After 25 mg kg^{-1} daily dosing of capsaicin for 7 days, the plasma clearance of antipyrine decreased from 0·344 ± 0·034 to 0·241 ± 0·029 L h^{-1} kg^{-1} (Table 1). This caused a significant increase in the elimination half-life ($t_{\frac{1}{2}}$) of antipyrine, as no significant differences were observed in the volumes of distribution. The AUC was also significantly increased in the capsaicin-pretreated animals.

The pharmacokinetic parameters of theophylline and quinine in the control and capsaicin-pretreated rats are summarized in Table 2. There was no significant difference in the body weight of animals between the two groups in both studies. In the theophylline and quinine studies, the plasma drug concentrations declined bi-exponentially after an intravenous bolus dose of the drug (data not shown). There were no significant differences in the plasma pharmacokinetic parameters of theophylline and quinine between the capsaicin-pretreated and control rats (Table 2).

Discussion

Capsaicin pretreatment in rats caused, on average, a 30% decrease in the clearance of antipyrine (Table 1), indicating that capsaicin pretreatment impaired the elimination of antipyrine in the rats. Antipyrine, the most widely used model compound for assessment of oxidative xenobiotic-metabolizing enzyme activity in the liver (Vesell 1991), is metabolized by at least three independent pathways yielding 4-hydroxyantipyrine, 3-hydroxymethylantipyrine and norantipyrine. Evidence from animals indicated that their formation is regulated by different isozymes of cytochrome P450 (Danhof et al 1979; Boobis et al 1981). Antipyrine is a low-clearance drug and its elimination is independent of hepatic blood flow (Rane et al 1977). Therefore, our finding of a significant decrease in the clearance of antipyrine, indicates that capsaicin inhibits the metabolism of antipyrine. As the urinary recovery of antipyrine metabolites was not measured in this study, it is not possible to speculate whether the decrease in antipyrine clearance was caused by inhibition of a particular metabolic route. It is necessary to clarify this in order to gain insight into the mechanism of capsaicin inhibition of oxidative drug metabolism. A recent study has demonstrated that capsaicin pretreatment caused a significant reduction in cytochrome P450 content in rats (Iwama et al 1990). It has also been shown that capsaicin exhibited profound inhibition of ethylmorphine *N*-demethylase activity and increased pentobarbitone-induced sleeping time in rats (Miller et al 1983). Capsaicin is a potent in-vitro inhibitor of human and murine epidermal metabolism of benzo(a)pyrene (Modly et al 1986). This evidence supports our findings with antipyrine, a drug which is extensively metabolized by oxidative pathways.

In contrast to antipyrine, capsaicin pretreatment caused no significant change in the pharmacokinetics of theophylline and quinine. Theophylline is metabolized by P450 isozymes to form three major metabolites (Grygiel et al 1979; McManus et al 1988). The cytochrome P450 enzymes catalysing formation of various metabolites of both antipyrine and theophylline are likely to be different. Only the formation of 4-hydroxyantipyrine was found to have a close relation with isozymes responsible for the demethylation of theophylline (Breimer 1983; Schellens et al 1988). P450IA2 appears to have a major role in the demethylation of

theophylline (Campbell et al 1987; Robson et al 1988; Sarkar et al 1990). The P450 isozymes responsible for antipyrine metabolic pathways have not been identified. Similarly the metabolic pathways of quinine have not been fully elucidated. It has been suggested that the biotransformation is carried out largely by P450 isozymes different from P450IID6 (CYP2D6) which is responsible for the metabolism of debrisoquine (Wanwimolruk & Chalcroft 1991). Furthermore, in-vitro studies have shown that quinine is metabolized by a P450 enzyme (P450IIIA, CYP3A) that oxidizes nifedipine but not debrisoquine (Guengerich et al 1986a, b; Smith 1991). These differences in the form of isozymes catalyzing metabolic pathways of these drugs may explain the lack of effect of capsaicin pretreatment on the pharmacokinetics of theophylline and quinine in rats. It may also be possible that the inhibition of capsaicin on oxidative drug metabolism is selective to a certain P450 isozyme or some isozymes may be more susceptible than others at different concentrations of the inhibitor.

In conclusion, this report provides initial evidence of the impairment effect of capsaicin pretreatment on the pharmacokinetics of a widely used model drug, antipyrine. The dose of capsaicin (25 mg kg^{-1}) given to the rats is similar to that used in many previous studies, but it is much greater than that usually ingested (3–6 mg kg^{-1}) by, for example, Thai people (Kawada et al 1986). The data from studies in rats should be extrapolated to man with extreme caution; however, it may be relevant to consider the possible effects of foods containing capsaicin spices, such as chilli, when pharmacokinetic studies are performed in non-Caucasian subjects.

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