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COMPARISON OF THE EFFECTS OF PIPERINE ADMINISTERED INTRAGASTRICALLY AND INTRAPERITONEALLY ON THE LIVER AND LIVER MIXED-FUNCTION OXIDASES IN RATS

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SUMMARY

Piperine, a major pungent constituent of black and red peppers, was administered to rats intragastrically and intraperitoneally to study whether it alters the activities of hepatic mixed-function oxidases (MFO) and serum enzymes as specific markers of hepatotoxicity. An intragastric dose of 100 mg/kg of piperine to adult, male Sprague-Dawley rats caused an increase in hepatic microsomal cytochrome P-450 and cytochrome b$_5$, NADPH-cytochrome c reductase, benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase 24 h following treatment. On the other hand, a 10 mg/kg dose given i.p. exhibited no effect on the activities of the aforementioned parameters of the hepatic drug-metabolizing enzyme system. However, when the intragastric and intraperitoneal doses were increased to 800 mg/kg and 100 mg/kg, respectively, the black pepper alkaloid produced a significant decrease in the levels of cytochrome P-450, benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase 24 h after treatment. None of the treatments significantly elevated the activities of serum sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and isocitrate dehydrogenase (ICD), suggesting that piperine is not a hepatotoxic agent.

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INTRODUCTION

Piperine is the principal pungent constituent of black and long, red peppers which are common ingredients of many spices consumed by a large number of people all over the world /1,2/. Safrole, a carcinogen, and piperine are both methylene-dioxybenzene (MDP) derivatives. In recent years piperine has received a lot of attention and has been studied extensively from a toxicological point of view. Toxic doses of piperine have been shown to produce respiratory paralysis and multiple dysfunctions of organs characterized by severe hemorrhagic necrosis and edema in rats, mice and hamsters /3/. At pharmacological doses, it causes CNS depressant and analgesic activity in mice while antipyretic and anti-inflammatory activity has been found in rabbits and rats, respectively /4/. This compound is also known to possess an antifertility effect /5/. Biochemically, piperine inhibits oxidative phosphorylation and calcium transport but stimulates ATPase activity in isolated rat liver mitochrondria /6/. Piperine, which has been reported to be biotransformed by the hepatic cytochrome P-450-dependent monooxygenases /7/, does not affect the mixed-function oxidases at lower concentrations /4/, but at higher concentrations it produces both inhibitory as well as stimulatory effects on the hepatic microsomal enzymes /8-10/.

Since piperine is metabolized by hepatic mixed-function oxidases to a number of metabolites, it would be interesting to study the effect of this generally recognized as safe substance on the liver. The present study was performed to examine and compare the effect of piperine administered intragastrically and intraperitoneally in low and high doses on the liver and several components of the hepatic cytochrome P-450-dependent mixed-function oxidase system. The doses (100 and 800 mg/kg, i.g.; 10 and 100 mg/kg, i.p.) were chosen with respect to past data and reported LD_{50} values for female rats (LD_{50} = 514 mg/kg, i.g.; 33.5 mg/kg i.p.) /3/.

MATERIALS AND METHODS

Piperine, cytochrome c and standard kits for SDH, ALT, AST and ICD were obtained from Sigma Chemical Co. (St. Louis, MO). Benzphetamine hydrochloride was received as a gift from Upjohn

Co. (Kalamazoo, MI). Glucose-6-phosphate, glucose-6-phosphate dehydrogenase and nicotinamide adenine diphosphate (NADP) were bought from Boehringer Mannheim Corp. (Indianapolis, IN). All other chemicals used in these studies were of analytical reagent grade.

Adult, male Sprague-Dawley rats weighing 200-250 g were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN.

In the first experiment, the animals were divided into 3 groups of 5 animals each. Animals from groups 2 and 3 were given piperine intragastrically at a dosage level of 100 mg/kg and 800 mg/kg, respectively. Group 1 received an equivalent amount of corn oil, also intragastrically, and served as a control group.

In the second experiment, the animals were divided into 3 groups of 5 animals each. Animals from groups 2 and 3 received 10 mg/kg and 100 mg/kg of piperine i.p., respectively, while group 1 was injected with an equivalent amount of corn oil i.p. and served as a control group.

Twenty-four h following treatment, animals were stunned and guillotined, blood samples collected in unheparinized tubes, livers weighed and perfused with ice-cold 1.15% KCl solution containing 0.05 mM EDTA. The perfused livers were homogenized with 3 volumes of ice-cold 0.25 M sucrose solution. The homogenates were centrifuged at 9000 x g for 10 min in a refrigerated (5-10°C) Sorvall centrifuge and microsomes from the supernatant fraction were isolated by centrifuging it at 105,000 x g for 1 h in an L8-80M Beckman ultracentrifuge with a temperature range of 5-10°C. The microsomal pellets were washed once with the cold KCl solution containing 0.05 mM EDTA and resuspended in an appropriate amount of buffer for determining the various components of the mixed-function oxidase system.

The levels of microsomal cytochromes P-450 and b_5 were measured following the procedures reported by Omura and Sato /11/. NADPH-cytochrome c reductase, benzphetamine N-demethylase, aminopyrine N-demethylase, and aniline hydroxylase were assayed as described previously /12/. Protein concentrations were determined by the biuret method modified to include deoxycholate in each sample. The activities of sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and isocitrate dehydrogenase (ICD) in serum were determined using the kits purchased from Sigma Chemical Company.

The data were analyzed using Student's t-test. Significance of mean difference was based on $p \leq 0.05$.

RESULTS

As can be seen from Table 1, an i.g. dose of 100 mg/kg of piperine to rats caused a significant increase in the activities of microsomal cytochrome P-450 and cytochrome b_5, benzphetamine N-demethylase and aniline hydroxylase while the increase in NADPH-cytochrome c reductase and aminopyrine N-demethylase activities was not significant at 24 h following treatment. In contrast, an 8-fold higher dose (800 mg/kg, i.g.) of piperine produced a significant decrease in the amount of cytochrome P-450 and the activities of benzphetamine N-demethylase, aminopyrine N-demethylase and aniline hydroxylase. An almost similar inhibitory effect was observed in rats when a 100 mg/kg dose of piperine was administered i.p., while the lower concentration of piperine (10 mg/kg, i.p.) showed no change in the mixed-function oxidase parameters (Table 2). These results indicate that piperine is an inhibitor of selected hepatic mixed-function oxidases at high concentrations (800 mg/kg, i.g. and 100 mg/kg, i.p.) and an inducer at low i.g. concentration. On the other hand, both the i.g. and i.p. doses of piperine did not bring about significant changes in the activities of either SDH, ALT, AST or ICD, suggesting that piperine is not a hepatotoxic agent at the dosage levels used in this study (Tables 1 and 2).

DISCUSSION

The data presented in Table 1 indicate that intragastric treatment of rats with piperine at a lower dose (100 mg/kg) increased the amount of hepatic cytochrome P-450 and enhanced the activities of certain mixed-function oxidases, while a higher dose (800 mg/kg) produced inhibition of the microsomal drug metabolizing enzyme system. Similarly, a lower dose (10 mg/kg) given intraperitoneally did not alter the activity of the mixed-function oxidase system; however, the treatment of rats with a higher dose (100 mg/kg, i.p.) resulted in decrease in the amount of cytochrome P-450 and the activity of certain mixed-function

TABLE 1

Effect of intragastrically administered piperine on hepatic microsomal electron transport components, mixed-function oxidases and serum enzymes

Parameters	Control	Piperine	
		100 mg/kg	800 mg/kg
Cytochrome P-450[a]	0.546 ± 0.06	0.828 ± 0.05*	0.353 ± 0.04*
Cytochrome b_5[b]	0.171 ± 0.02	0.295 ± 0.02*	0.165 ± 0.01
NADPH-cytochrome c reductase[c]	107 ± 11	114 ± 24	92 ± 23
Benzphetamine N-demethylase[d]	5.29 ± 0.19	6.85 ± 0.58*	3.20 ± 0.40*
Aminopyrine N-demethylase[d]	6.01 ± 0.41	6.99 ± 1.03	3.56 ± 0.61*
Aniline hydroxylase[e]	0.400 ± 0.06	0.733 ± 0.11*	0.247 ± 0.09*
Sorbitol dehydrogenase[f]	475 ± 30	457 ± 35	555 ± 52
Isocitrate dehydrogenase[f]	762 ± 25	632 ± 144	590 ± 9
Alanine aminotransferase[g]	45 ± 1	37 ± 7	37 ± 6
Aspartate aminotransferase[g]	140 ± 24	91 ± 6	148 ± 40

Results are expressed as the mean ± standard error of the mean.
* Significantly different ($p < 0.05$) from the control within a parameter.
[a] Nanomoles of cytochrome P-450/mg protein.
[b] Nanomoles of cytochrome b_5/mg protein.
[c] Nanomoles of cytochrome c reduced/min/mg protein.
[d] Nanomoles of formaldehyde liberated/min/mg protein.
[e] Nanomoles of p-aminophenol formed/min/mg protein.
[f] Sigma units/ml of serum.
[g] Units/liter of serum.

TABLE 2

Effect of intraperitoneally administered piperine on hepatic microsomal electron transport components, mixed-function oxidases and serum enzymes

Parameters	Control	Piperine	
		10 mg/kg	100 mg/kg
Cytochrome P-450[a]	0.534 ± 0.04	0.510 ± 0.01	0.292 ± 0.02*
Cytochrome b_5[b]	0.176 ± 0.03	0.206 ± 0.02	0.232 ± 0.03
NADPH-cytochrome c reductase[c]	112 ± 4	121 ± 3	75 ± 4
Benzphetamine N-demethylase[d]	5.59 ± 0.41	5.67 ± 0.22	2.85 ± 0.13*
Aminopyrine N-demethylase[d]	6.65 ± 0.73	6.66 ± 0.69	3.14 ± 0.73*
Aniline hydroxylase[e]	0.389 ± 0.05	0.359 ± 0.04	0.379 ± 0.06
Sorbitol dehydrogenase[f]	523 ± 14	590 ± 7	609 ± 25
Isocitrate dehydrogenase[f]	714 ± 62	774 ± 46	818 ± 51
Alanine aminotransferase[g]	58 ± 3	69 ± 2	42 ± 7
Aspartate aminotransferase[g]	193 ± 23	224 ± 22	173 ± 36

Results are expressed as the mean ± standard error of the mean.
* Significantly different ($p < 0.05$) from the control within a parameter.
[a] Nanomoles of cytochrome P-450/mg protein.
[b] Nanomoles of cytochrome b_5/mg protein.
[c] Nanomoles of cytochrome c reduced/min/mg protein.
[d] Nanomoles of formaldehyde liberated/min/mg protein.
[e] Nanomoles of p-aminophenol formed/min/mg protein.
[f] Sigma units/ml of serum.
[g] Units/liter of serum.

oxidases. These results suggest that piperine is both inhibitory as well as stimulatory to the cytochrome P-450-mediated activities of the microsomal monooxygenases dependent on dose and route of administration. Other investigators /8,9/ have reported that piperine is not an inducer of mixed-function oxidases. However, these researchers had treated their experimental animals with piperine intraperitoneally and not intragastrically. On the other hand, Shin *et al.* /10/ administered piperine (100 mg/kg, p.o.) daily for 7 days to mice and found significant increases in the activities of microsomal aminopyrine N-demethylase and hexobarbital hydroxylase. However, the mechanism by which piperine at a single dose (100 mg/kg, i.g.) or repeated oral doses produces induction of cytochrome P-450-dependent mixed-function oxidases is not known. These investigators further observed that piperine at pharmacologically effective doses did not affect MFO whereas it inhibited both aminopyrine and hexobarbital metabolism at a high dose (100 mg/kg, i.p.).

Although absorbed piperine is predominantly metabolized in the liver, presumably to piperic acid, vanillic acid, piperonyl alcohol and piperonylic acid /7/, the data presented in Tables 1 and 2 suggest that the alkaloid or its metabolites are not hepatotoxic. No studies on the effects of piperine on the liver are available. Bhat and Chandrasekhara /13/ fed piperine to weanling rats in their diets for 8 weeks and found no adverse effects on blood constituents and serum levels of SGPT, also suggesting lack of hepatotoxicity.

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