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Effect of Dietary Capsaicin on Hepatic Drug-Metabolizing Enzyme Activities in Mice

Jung-Mi Kim, Dong-Hyun Kim*, Suck-Young Choe and Rina Yu†

Dept. of Food and Nutrition, University of Ulsan, Ulsan 680-749, Korea
**Bioanalysis and Biotransformation Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea*

Abstract

The effect of dietary capsaicin(8-methyl-N-vanillyl-6-nonenamide, CAP) on drug-metabolizing enzyme activities was investigated in mice. Male ICR mice were divided into 4 groups and fed diets containing 0, 5, 20, 100ppm CAP for 4 weeks. Hepatic drug-metabolizing enzyme activities and serum alanine aminotransferase and aspartate transaminase activities were measured. There was no difference in hepatic alanine aminotransferase and aspartate transaminase activities among the groups. Hepatic microsomal cytochrome P450 dependent dimethylnitrosamine *N*-demethylase and ethoxycoumarin-*O*-deethylase were significantly elevated in CAP fed groups, but *p*-nitrophenol hydroxylase and the cytosolic activity of glutathione *S*-transferase activities were decreased in the dietary CAP supplemented groups compared to the control. These results suggest that the dietary CAP at a low dose differentially modulates drug-metabolizing enzyme activities without causing hepatic toxicity.

Key words: capsaicin, microsome, drug metabolism, cytochrome P450-dependent enzyme

INTRODUCTION

Capsaicin(8-methyl-N-vanillyl-6-nonenamide, CAP) is a major pungent ingredient of hot peppers used as a food additive, preservative, and medicine. Besides enhancing the flavour of foods, CAP exhibits a wide range of neurophysiological and pharmacological properties(1). Biochemical effects of CAP such as the influence of energy metabolism have also been reported(2).

Most xenobiotics are biotransformed and detoxified by the hepatic drug metabolizing system. CAP interacts with microsomal drug metabolizing enzymes *in vivo* and *in vitro*, and thus can affect the metabolism of carcinogens and other xenobiotics. CAP inhibits biotransformation *in vivo* as measured by prolongation of pentobarbital sleep time(3). CAP suppresses the activity of rat epidermal aryl hydroxylase *in vitro*, that is responsible for the metabolism of some carcinogens(4). CAP also exerts inhibitory effects on the metabolism as well as mutagenicity of aflatoxin B_1 and the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone(5-7). The inhibitory effect of CAP on the metabolism of carcinogens has been suggested to be associated with its possible anticarcinogenic property as well as its hepatic toxicity(8). However, these results have been obtained from the view point of pharmacological aspects and dealt with mostly *in vitro* effects. It has not been known whether dietary CAP at a low dose exhibits the potential for altering biotransformation of xenobiotics or causing hepatic toxicity. Since Korean heavily consume the hot ingredient CAP in their daily food, it would be interesting to investigate the interaction of dietary CAP with the microsomal drug-metabolizing system. In this study we have investigated the effect of dietary CAP on selected drug-metabolizing enzyme activities in mice.

MATERIALS AND METHOD

Synthetic CAP(purity 98%) was obtained from Sigma Chemical Co.(St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals and diets

Male ICR mice, weighing about 20g, were fed a commercial powdered diet(Purina Rat Chow, Samyang Co., Wonju, Korea) and allowed free access to both food

†Corresponding author

and water. Rats were randomly divided into 5 groups and fed diets containing 0, 5, 20, 50, 100ppm CAP for 4 weeks. CAP was dissolved in an aliquot of ethanol then mixed throughly with the stock diet ; the ethanol was removed by evaporation. There was no significant difference in body weight or water and food intakes during the experimental period(data not shown), as previously reported(9).

Sample preparation

The animals were sacrificed and the blood samples were allowed to clot at 4°C, and then centrifuged and stored at -20°C until use. Livers were quickly excised, perfused with ice-cold physiological saline and chilled on ice. All procedures were carried out at 0~4°C. Liver (1g) was homogenized in 4 volumes of 0.25M sucrose in 0.01M phosphate buffer(pH 7.4) using a Potter- Elvehjem homogenizer. The homogenates were centrifuged at 14,000×g for 15 min, and the supernatant fraction at 100,000×g for 1 hr to sediment microsomes. The supernatant was used for analysis of cytosolic enzyme activity and the microsomal pellet was resuspended in 0.25M sucrose (microsomes from 1g liver in 1ml). Protein concentration was determined by the Lowry et al. method(10).

Analysis

Alanine aminotransferase and aspartate transaminase activities in serum were measured by using a bioassay kit of Asan Pharm. Co., LTD.(Hwasung, Korea)(11). Incubations related to P450 activities were carried out by incubating 0.2mg of liver microsomes with potassium phosphate buffer(pH 7.4, 100mM) and an NADPH-generating system including final concentrations of 10 mM glucose-6-phosphate, 0.5 mM NADP+, and 1 IU of yeast glucose-6-phosphate dehydrogenase/ml. Each P450 activity was measured as follows. *p*-Nitrophenol hydroxylase activity was determined at 37°C by measuring the amount of 4-nitrocatechol formed as described by Koop(12). Dimethylnitrosamine demethylase activity was determined by measuring the production of formaldehyde produced using the Nash reagent as described by Palakodety et al.(13). Ethoxycoumarin *O*-demethylase activity was determined by monitoring the production of flourescent 7-hydroxycoumarine by the method of Greenlee and Poland(14). Cytosolic glutathione *S*- transferase activity toward 1-chloro-2,4-dinitrobenzene was determined by spectrophotometrically monitoring the conjugation of glutathione with 1-chloro-2,4-dinitrobenzene as described by Habig et al.(15).

Statistical analysis

Statistical analysis was performed using ANOVA and Student's *t*-test. Differences were considered to be significant when $p<0.05$.

RESULTS AND DISCUSSION

The effect of dietary CAP which is the spicy principle in red pepper on selected parameters of hepatic drug-metabolizing system was investigated. Concentrations of CAP used in feeding mice in this study were comparable to those ingested by human. Table 1 shows the effect of dietary CAP on changes in weights of various organs. Dietary CAP did not cause an enlargement of liver, which is often observed by inducers of microsomal xenobiotic metabolizing enzymes. As shown in Fig. 1, dietary CAP did not result in hepatic toxicity as judged by the levels of alanine aminotransferase and aspartate transaminase activities in serum. Fig. 2 and Fig. 3 show the effect of dietary CAP on hepatic drug-metabolizing enzymes activities. Dietary CAP stimulated ethoxycoumarin *O*-deethylase and demethylnitrosamine *N*-

Table 1. Effect of dietary capsaicin on the relative weights of various organs

Organs	Dietary capsaicin			
	0	5ppm	20ppm	100ppm
Liver	5.39±0.27	4.93±0.09	5.77±0.32	4.95±0.34
Kidney	1.37±0.07	1.44±0.04	1.46±0.05	1.40±0.10
Adrenal glands	0.08±0.00	0.08±0.00	0.08±0.01	0.09±0.00
Lung	0.73±0.02	1.10±0.11	1.00±0.22	1.10±0.09
Heart	0.46±0.04	0.50±0.07	0.54±0.07	0.51±0.03

Data are mean weight(g/100g BW)±SEM, n=6 for each dietary group

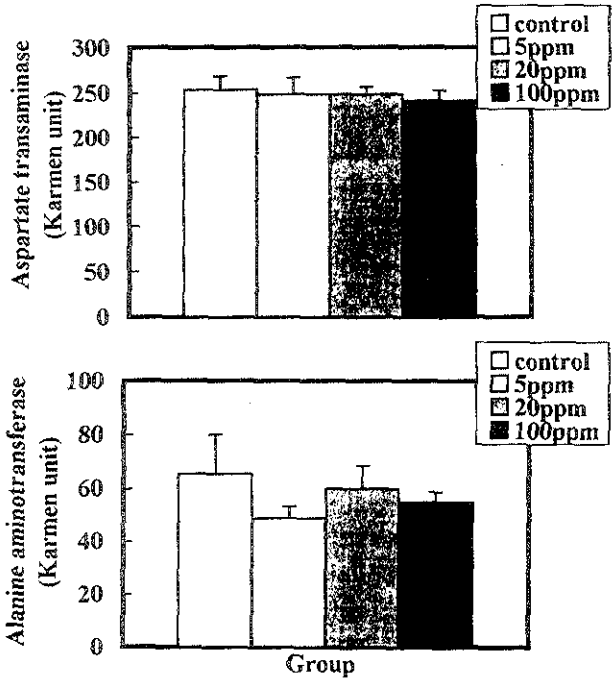

Fig. 1. Effect of dietary capsaicin on serum alanine aminotransferase and aspartate transaminase activities.
Values are the mean±SEM, n=6 for each dietary group.

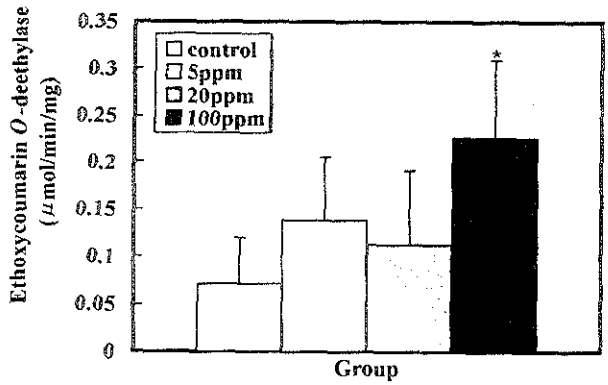

Fig. 2. Effect of dietary capsaicin on hepatic ethoxycoumarin *O*-deethylase activity.
Values are the mean±SEM, n=6 for each dietary group.
*Significantly different from the control($p<0.05$)

demethylase activites, but suppressed the activities of *p*-nitrophenol hydroxylase(Fig. 4). The activity of cytosolic glutathione-*S*-transferase decreased in the group fed dietary CAP compared to control(Fig. 5).

Most foreign chemicals are detoxified through biotransformation by the hepatic drug metabolizing system. CAP has been shown to be hydroxylated to *N*-(4,5-dihydroxy-3-methoxybenzyl)acrylamide by a microsomal mixed function oxidase *in vitro*(3,16). The hepatotoxicity of

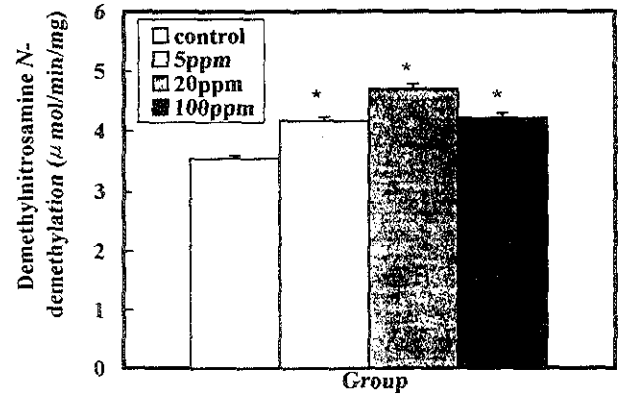

Fig. 3. Effect of dietary capsaicin on hepatic demethylnitrosamine *N*-demethylase activity.
Values are the mean±SEM, n=6 for each dietary group.
*Significantly different from the control($p<0.05$)

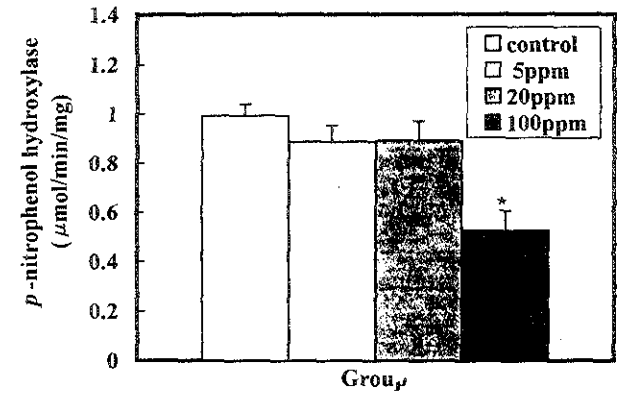

Fig. 4. Effect of dietary capsaicin on hepatic *p*-nitrophenol hydroxylase activity.
Values are the mean±SEM, n=6 for each dietary group.
*Significantly different from the control($p<0.05$)

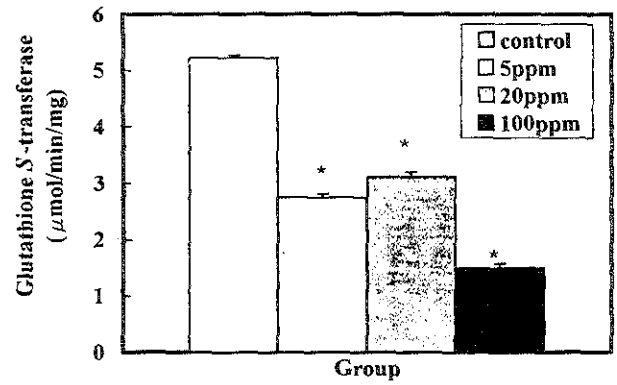

Fig. 5. Effect of dietary capsaicin on hepatic glutathione *S*-transferase activity.
Values are the mean±SEM, n=6 for each dietary group.
*Significantly different from the control($p<0.05$)

CAP is considered to be associated with the bioactivation of CAP that forms an electrophilic intermediate which irreversively binds to nucleophilic molecules, such as protein(8). However, dietary CAP is known to enhance CAP-hydrolyzing enzyme activity in the digestive system and is readily metabolized into vanillylamine, vanillin, vanillyl alcohol, and vanillic acid(17). Therefore, dietary CAP at its lower dose might not form the toxic electrophilic metabolite of CAP, or at least it could possibly be removed by reduced glutathione or via other conjugation reactions(8). In line with this supposition, dietary CAP used in this study did not cause hepatic toxicity. Dietary CAP thus should be distinguished from its pharmacological behavior at high dose levels.

CAP has been shown to inhibit cytochrome P450 that catalyzes metabolic activation as well as detoxification of many carcinogens. CAP inhibits hepatic microsomal P450 dependent ethylmorphine demethylase and aryl hydrocarbon hydroxylase(5). CAP also exerted inhibitory effects on metabolism, mutagenicity and covalent DNA binding aflatoxin B_1 and the toacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone(6,7) .The suppressive effect on metabolic activation of carcinogens is considered to account for chemopreventive activity of CAP(8). However, contrary to the *in vitro* inhibition of drug-metabolizing enzyme activity, our study demonstrates that dietary CAP *in vivo* has stimulated ethoxycoumarin *O*-deethylase and demethylnitrosamine *N*-demethylase activites, but suppressed *p*-nitrophenol hydroxylase and glutathione *S*-transferase activities. This finding is partially in aggreement with *in vivo* modulation of P-450 dependent drug-metabolizing enzyme such as ethoxycoumarin *O*-deethylase by dietary CAP(18,19). CAP has been shown not to affect phase II enzyme such as sulfotransferase and glutathione *S*-transferase activity(20). There might be species differences in those enzyme activities between rat and mouse. Further study is needed to explain the decrease in glutathione *S*-transferase activity. Cytochrome P-450 enzyme activity has been shown to be involved in norepinephrine-induced contraction in rat mesenteric arteries(21). Since CAP evokes catecholamine secretion(22), the modulating effect of CAP on P-450 enzyme might be associated with the activity. It is also possible that dietary CAP at low dose could differentially modulate the drug-metabolizing system. Moreover, the stimulation of certain enzyme activity might result in the synergistic enhancement of detoxification of some xenobiotics. The interaction of dietary CAP with the drug-metaboizing system should be distinguished from the action of this compound at higher dose to inhibit the metabolic activation of carcinogen.

Based on the results from this study, we propose that dietary CAP differentially modulates the microsomal drug-metabolizing system. The capability of biotransforming xenobiotics including carcinogens in people who frequently intake hot pepper containing CAP could be different from that of non-CAP eaters.

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