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Modification of Hepatic Microsomal Cytochrome P450 2E1 Enzyme by Garlic Powder in Rat Hepatocarcinogenesis

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Abstract : This study was designed to investigate the effects of dietary garlic powder on cytochrome P450 enzymes and membrane stability in murine hepatocarcinogenesis initiated by diethylnitrosamine (DEN). Male Sprague-Dawley rats received a single intraperitoneal injection of DEN (200 mg/kg body wt) dissolved in saline. After 2 weeks on a basal diet, animals were fed diets containing 0, 0.5, 2.0, or 5.0% garlic powder for 6 weeks, and were subjected to two-thirds partial hepatectomy. The areas of placental glutathione *S*-transferase (GST-P) positive foci were inhibited in rats fed with garlic diets. GST-P is the most effective marker for DEN-initiated lesions. Hepatic microsomal lipid peroxidation was significantly decreased in rats fed with 2.0 and 5.0% garlic powder diets compared with that observed in the control animals and hepatic microsomal glucose 6-phosphatase (G6Pase) activity was found to increase significantly in rats fed 0.5 and 2.0% garlic powder diets. Thus as little as 0.5% garlic powder has a positive effect on the stability of hepatic microsomal membranes. *p*-Nitrophenol hydroxylase (PNPH) activity and the level of cytochrome P450 2E1 protein in the hepatic microsomes from rats fed diets containing 2.0 and 5.0% garlic powder were much lower than those of control microsomes. Rats fed 5.0% garlic powder diets exhibited the lowest P450 2E1 activity and protein levels among groups. Pentoxyresorufin *O*-dealkylase activity and immunoblot (cytochrome P450 2B1) analyses were not different between groups. However, the levels of cytochrome P450 1A1/2 protein in rats fed 0.5 and 2.0% garlic powder were significantly induced compared to controls. These results suggest that 2.0% garlic powder is effective in inhibiting the areas of GST-P positive foci, modulating certain isoforms of cytochrome P450 enzymes and stabilizing the hepatic microsomal membrane. Thus, the selective modification of cytochrome P450 enzymes and membrane stability by dietary garlic powder may influence areas of GST-P positive foci and chemoprevention of post-initiation of rat hepatocarcinogenesis.
Key words : cytochrome P450 1A1/2, cytochrome P450 2E1, garlic powder, hepatocarcinogenesis, membrane stability, placental glutatione *S*-transferase (GST-P) positive foci, *p*-nitrophenol hydroxylase (PNPH).

Diet has been suggested to have a significant impact on the cancer process. Garlic (*Allium sativum*), a member of the lily family, is used extensively as a flavoring agent, and has been used for medicinal purposes for centuries. Epidemiological studies revealed that the gastric cancer mortality was about 10 times lower in areas of China where garlic comsumption is high, compared to regions where the intake of garlic is relatively low (You *et al.*, 1989). In addition, several studies suggest garlic powder and/or garlic constituents can reduce the incidence of chemically induced tumors in experimental animals. Thus, Wargovich (1990) found that diallyl sulfide (DAS), a sulfur compound of garlic, reduced the incidence of colon cancer in mice treated with dimethylhydrazine and completely inhibited *N*-nitrosomethylbenzylamine-induced esophageal tumor formation. Liu *et al.* (1992) showed that dietary garlic powder supplementation significantly delayed the onset of tumors and reduced the overall mammary tumor incidence in rats treated with 7,12-dimethylbenz[*a*]anthracene. Also, Cheng *et al.* (1995) reported that 2.5% garlic inhibited the incidence of colon cancer in rats treated with 1,2-dimethylhydrazine.

The mechanism of cancer inhibition by garlic is not clearly understood, but garlic seems to act to modulate some cytochrome P450 enzymes and glutathione *S*-transferase enzyme activity, thereby preventing tumorigenesis induced by various carcinogens. Many studies examining effects of garlic constituents on cytochrome P450 enzymes have been performed with drug (*e.g.*, pyrazine, acetone, *etc.*)-induced rats. Cytochrome

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P450 enzymes are involved in the biotransformation of various endobiotics and xenobiotics, including drug and environmental carcinogens. Many of the P450 enzymes are inducible by a variety of chemicals, and such an alteration of the composition of this enzyme system may have profound effects on chemical toxicity and carcinogenesis (Guengerich *et al.*, 1988).

Among cytochrome P450 enzymes, cytochrome P450 2E1 plays an important role in the metabolism of a variety of small organic molecules to reactive intermediates which are capable of covalent binding to critical cellular macromolecules to cause tissue necrosis and/or tumorigenesis (Hong *et al.*, 1987).

DAS has been shown to inhibit chemical toxicity and tumorigenesis in several animal models, presumably by acting as an inhibitor of the cytochrome P450-mediated metabolic activation of the toxic and carcinogenic chemicals. DAS causes a selective alteration of certain forms of cytochrome P450 enzymes in rat liver. For instance, cytochrome P450 2E1 activity (*p*-nitrophenol hydroxylase (PNPH)) and protein levels are decreased significantly whereas cytochrome P450 2B1 (pentoxyresorufin O-dealkylase (PROD)) and protein levels are induced by DAS (Brady *et al.*, 1991). Also, garlic oil suppressed cytochrome P450 2E1 expression (Kwak *et al.*, 1995). Harber *et al.* (1994) showed that DAS as well as diallyl disulfide (DADS) produced an enhancement of the microsomal protein level of cytochrome P 450 1A2 and 2B1/2, with concomitant increase in the enzymatic activities of methoxyresorufin *O*-dealkylase and PROD, respectively. But, most of the studies were done by injection of these sulfur compounds or by forced feeding of garlic flavor compounds in short terms. In addition, studies on modulation of cytochrome P450 enzymes by whole garlic were limited. Therefore, a long-term feeding model of garlic was needed. Thus, the present study was designed to examine the inhibition of placental glutathione *S*-transferase (GST-P) positive foci, effective marker for DEN-initiated lesions (Farber, 1984; Satoh *et al.*, 1989), following a long-term feeding of garlic powder, and also designed to determine the optimal level of garlic powder to be used in the diet that may modulate the hepatic microsomal cytochrome P450 enzymes (P450 2E1, P450 2B1 and P450 1A1/2) and yield chemoprevention against hepatocarcinogenesis in rats treated with DEN and partial hepatectomy.

Materials and Methods

Animals and diet

Male Sprague-Dawley rats (50~60 g) were supplied from Seoul National University Animal Care Facility.

Table 1. Composition of experimental diets (g/100 g diet)

Component/Diet	C
Corn starch	54.7
Casein	20.0
α-Cellulose	5.0
Vitamin mixture[a]	1.0
Salt mixture[b]	4.0
DL-Methionine	0.3
Corn oil	15.0
Garlic powder[c]	–

[a] Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio. Vitamin mixture is composed of; vit. A acetate (500,000 IU/g) 1.8 g, vit. D conc. (850,000 IU/g) 0.125 g, α-tocopherol (250 IU/g) 22.0 g, ascorbic acid 45.0 g, inositol 5.9 g, choline chloride 75.0 g, menadione 2.25 g, p-aminobenzoic acid 5.0 g, niacin 4.25 g, riboflavin 1.0 g, pyridoxine hydrochloride 1.0 g, calcium pantothenic acid 3.0 g, biotin 0.02 g, folic acid 0.09 g, vit. B_{12} 0.00135 g, and dextrose to 1 kg.

[b] Composition of salt mixture, g/kg mixture; $CaHPO_4$ 500 g, NaCl 74 g, K_2SO_4 52 g, potassium citrate monohydrate 220 g, MgO 24 g, manganase carbonate (43~48% Mn) 3.5 g, ferric citrate (16~17% Fe) 6.0 g, zinc carbonate 1.6 g, cupric carbonate (53~55% Cu) 0.3 g, KIO_3 0.01 g, chromium potassium sulfate 0.55 g, $Na_2SeO_3 \cdot 5H_2O$ 0.11 g, sucrose, finely powdered 118.0 g.

[c] Garlic powder was added at the level of 0.5, 2.0 and 5.0% in diet at the expense of corn starch.

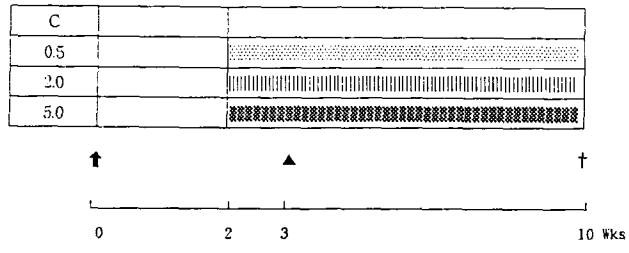

Fig. 1. Experimental design. ⬆, DEN i.p. injection (200 mg/kg body weight); ▲, partial hepatectomy; ┼, sacrifice; □, control diet; ▨, control diet containing 0.5% garlic powder; ▥, control diet containing 2.0% garlic powder; ▩, control diet containing 5.0% garlic powder.

The rats were maintained on a 12 h light and 12 h dark daily cycle, were given food and water *ad libitum*, and were acclimatized to their environment for 2 weeks prior to their use in experiments. Hepatocellular chemical carcinogenesis was induced using the medium-term bioassay protocol (Ito *et al.*, 1992). Animals received a single intraperitoneal injection of diethylnitrosamine (DEN) (200 mg/kg body wt) dissolved in saline. After 2 weeks on a basal diet, they were divided into four groups, and were fed diets containing 0, 0.5, 2.0, or 5.0% garlic powder for 6 weeks (Table 1). Animals were subjected to two-thirds partial hepatectomy (PH)

at week 3, and were killed at week 8 (Fig. 1).

Preparation of microsomal and cytosolic fractions

Animals were killed by decapitation after 12 h of fasting. Livers were immediately removed, finely minced in 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA, and then homogenized. Microsomal and cytosolic fractions were prepared by differentional centrifugation, and were stored in small aliquots at −70°C until used.

Placental glutathione *S*-trasnferase (GST-P) positive foci

At autopsy, livers were excised and sections 2-3 mm thick were cut with a blade. These liver slices were fixed in ice cold acetone for immunohistochemical examination of GST-P positive foci. The avidin-biotin-peroxidase complex method was used to demonstrate GST-P positive liver foci, a putative preneoplastic lesion (Sato *et al*., 1984). Immunohistochemical analysis was carried out with sequential treatments of rabbit anti rat placental glutathione *S*-transferase as a primary antibody, swine anti rabbit IgG antibody as a secondary antibody and peroxidase-antiperoxidase complex. Final visualization of GST-P positive foci was enzymatically activated by 3,3-diaminobenzidine and H_2O_2 as substrates. The areas of the GST-P positive foci >0.2 mm in diameter and total areas of liver sections examined were measured using an image analyzer.

Thiobarbituric acid reactive substances assay

Lipid peroxides of hepatic microsomes were determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) (Buege *et al*., 1978). Malondialdehyde as the product of lipid peroxidation reacted with thiobarbituric acid and the absorbance of the resulting chromophore was measured at 535 nm.

Glucose 6-phosphatase assay

Glucose 6-phosphatase (G6Pase) activity was determined by measurement of the inorganic phosphate liberated from glucose 6-phosphate by the method of Baginski *et al* (1983). The absorbance was determined at 840 nm and the amount of phosphate liberated by enzyme from glucose 6-phosphate was calculated by calibration with the standard (1.15 μmole Pi/volume of the assay mixture).

Determination of total cytochrome P450 content

Total cytochrome P450 content was determined by the method of Omura and Sato (1964). To the fresh liver microsome was added sodium dithionate and the reduced hemoprotein was combined with carbon monooxide by bubbling CO through the solution. The characteristic absorbance at 450 nm was determined by dual beam spectroscopy.

PNPH assay

PNPH was assayed according to Reinke *et al* (1985). Reaction mixtures contained 100 mM potassium phosphate buffer (pH 6.8), 1.0 mM ascorbic acid, 1 mM NADPH, 1 mg hepatic microsomes and 100 μM *p*-nitrophenol in a total volume of 1.0 ml. The 4-nitrocatechol formed was determined spectrophotometrically.

Pentoxyresorufin *O*-dealkylase assay

Pentoxyresorufin *O*-dealkylase activity was measured using a fluorometric method (Lubet *et al*., 1985), with excitation and emission wavelengths set at 530 nm and 585 nm, respectively. Reaction mixtures consisted of 2.0 ml of 0.05 M Tris buffer, pH 7.5, 10 μM pentoxyresorufin, and 20 μg of microsomal protein. Reactions were initiated by addition of 125 μM NADPH to the cuvette and stopped by addition of cold methanol. The formation of resorufin was caculated by comparing amounts of standard resorufin.

Immunoblot analysis

SDS-PAGE analysis was performed according to Laemmli (1970) using the BioRad Mini-protean II apparatus. Microsomal proteins were separated by 10% SDS-PAGE and electrophoretically transferred to Immobilon PVDF transfer membrane, which was immunoblotted with anti-P450 2E1, anti-P450 2B1, and anti-P450 1A1/2 antibodies. Biotinylated goat anti-rabbit IgG was used as the secondary antibody. Detection of immunoblot was performed by incubating the membrane in equal volumes of detection reagents 1 and 2 for 1 min, putting film on the top of blot and exposing the film.

Protein assay

Protein amounts of hepatic microsomes were determined by Lowry's method (1951).

Statistical analysis

All statistical analyses were carried out by Duncan's multiple range test using the SAS program. A P value of <0.05 was selected as a limit of statistical significance.

Results and Discussion

In our preliminary feeding trial, diets containing 0, 0.5, 2.0, and 5.0% garlic powder did not influence food in-

takes and body weight gains in experimental animals. Food intakes of groups during this experiment ranged from 19.3 to 20.2 g/day. In addition, liver and body weights were not different between garlic fed and control animals.

Garlic powder inhibited GST-P positive foci development in the rat hepatocarcinogenesis (Fig. 2). The choice of this experimental design was based on its established application for detecting modifying effects of many chemicals on liver carcinogens (Ito *et al.*, 1988). Several types of GST forms are known to be elevated, but GST-P is the most effective marker for DEN-initiated lesions (Farber, 1984; Satoh *et al.*, 1989). Ogiso *et al.* (1990) have proved that the degree of induction of GST-P positive foci and nodules in this bioassay protocol for liver carcinogens directly corresponds with the incidence of hepatocellular carcinomas revealed in long-term *in vivo* systems. Consequently, the present result strongly suggests that garlic powder would inhibit liver tumor development.

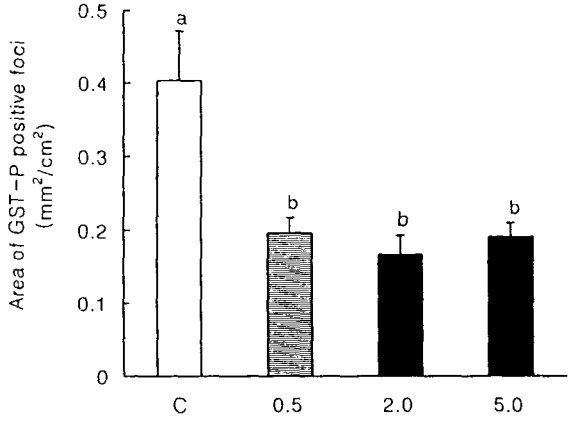

Fig. 2. Effect of dietary garlic powder on the area of GST-P positive foci in rat hepatocarcinogenesis. C, control diet + carcinogen treatment (DEN + partial hepatetomy); 0.5, diet containing 0.5% garlic powder + carcinogen treatment (DEN + partial hepatetomy); 2.0, diet containing 2.0% garlic powder + carcinogen treatment (DEN + partial hepatetomy); 5.0, diet containing 5.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy). Values are mean ± S.E. Means with the same subscripts are significantly different at $p < 0.05$ by Duncan's multiple range test.

Lipid peroxidation and toxicity associated with oxygen radicals have been suggested as major causes of cancer. Therefore, the lipid peroxidation was reported to be increased in rats treated with various carcinogens and xenobiotics (Kagawa *et al.*, 1986). Lipid peroxidation decreased the microsomal membrane integrity and influenced the G6Pase activities (Lucy and Wills, 1976), and was associated with the promotion of carcinogenesis (Slaga *et al.*, 1981). Hepatic microsomal lipid peroxidation determined by TBARS of rats fed 2.0 and 5.0% garlic powder diets was significantly decreased compared to that observed in the control (Table 2). Therefore 2.0% of garlic powder in the diet was enough to decrease the lipid peroxides formed from DEN-induced hepatocarcinogenesis in this study.

Hepatic microsomal G6Pase has been known to reflect the stability of the microsomal membrane (McBrien and Slater, 1982; Kim and Choi, 1994). The decrease in canaculi adenosine triphosphate (ATPase) and G 6Pase was observed in many altered foci (Dragon and Pitot, 1992). The activities of G6Pase were found to increase significantly in rats fed 0.5 and 2.0% garlic powder diets compared to those in animals on the 5.0% garlic powder and the control diets (Table 2). And, the activities of G6Pase are negatively correlated with GST-P positive foci ($r = -0.5335$, $p < 0.05$). This finding indicates that as little as 0.5% garlic powder has a positive effect on the stability of hepatic microsomal membranes and this stability may contribute to decrease of GST-P positive foci, whereas the higher amount of garlic powder (5.0%) exerts a negative effect on membrane

Table 2. Effect of dietary garlic powder on the hepatic microsomal thiobarbituric acid reactive substances, G6P activities, and total cytochrome P450 in rat hepatocarcinogenesis

Group	Number of rats	Thiobarbituric acid reactive substances (nmol TBARS/mg protein)	G6Pase (nmol Pi liberated/ min/mg protein)	Total cytochrome P450 (nmol cytochrome P450/mg protein)
C	7	0.2818 ± 0.0171^{a}	618.87 ± 23.03^{c}	2.64 ± 0.26^{a}
0.5	6	0.2677 ± 0.0163^{a}	825.87 ± 31.95^{ab}	1.44 ± 0.18^{b}
2.0	7	0.2216 ± 0.0088^{b}	882.70 ± 84.63^{a}	1.38 ± 0.12^{b}
5.0	7	0.2266 ± 0.0104^{b}	668.15 ± 34.18^{bc}	1.50 ± 0.14^{b}

C : control diet + carcinogen treatment (DEN + partial hepatectomy)
0.5 : diet containing 0.5% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
2.0 : diet containing 2.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
5.0 : diet containing 5.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
Values are mean ± SE. Means with the same subscripts are not significantly different at $p < 0.05$ by Duncan's multiple range test.

stability.

Total cytochrome P450 contents of rats fed garlic powder diets were significantly lower than the control (Table 2). And, total cytochrome P450 contents were negatively correlated with the areas of GST-P positive foci ($r=0.526$, $p<0.05$). Garlic powder diet reduced the total cytochrome P450 contents. Similar result was observed when the rats were fed DAS (Harber *et al.*, 1994).

PNPH activities in the hepatic microsomes from rats fed 2.0 and 5.0% garlic powder diets were significantly inhibited (Table 3). Immunoblot analyses of hepatic microsomes showed that 2.0 and 5.0% garlic powder significantly suppressed the level of cytochrome P450 2E1 protein (Fig. 3(A)). Decreased activity of PNPH reflected the decreased level of cytochrome P450 2E1 protein. In contrast, 0.5% garlic powder diet failed to suppress P450 2E1 levels. It is probable that garlic powder inhibits cytochrome P450 2E1 enzyme and the suppression of cytochrome P450 2E1 is partly associated with the inhibition of hepatocarcinogenesis in rats.

Many studies examining inhibitory effects of garlic components on cytochrome P450 2E1 enzyme have been performed. DAS has been shown to inhibit cytochrome P450 2E1 activity and protein levels (Brady *et al.*, 1991; Kwak *et al.*, 1995). It seems likely that DAS would decrease cytochrome P450 2E1 via a suicide inhibition mechanism involving diallyl sulphone, a metabolite of DAS (Brady *et al.*, 1991). DADS and allyl methyl sulfide (AMS) have the same effect as DAS (Reicks and Crankshaw, 1996), in another study (Haber *et al.*, 1994), however, DAS and DADS lowered the cytochrome P450 2E1 protein levels while the PNPH activity was enhanced. Already mentioned inhibition of cytochrome P450 2E1 activity and suppression of its level in microsome may contribute to the chemopreventive effects of garlic components in rat hepatocarcinogenesis. But, the degree of inhibition of cytochrome P450 2E1 enzyme activity needs to be determined with relation to anticarcinogenic efficacy of dietary garlic.

PROD activity was not different between groups (Table 3). Thus, the results of immunoblot analyses of hepatic microsomes, using an anti-P450 2B1 antibody, were not different between groups (Fig. 3(B)). Dietary garlic is considered to have no effect on cytochrome P450 2B1 level and PROD activity.

Cytochrome P450 1A1/2 protein levels were induced by 0.5 and 2.0% garlic powder diets, but were

Table 3. Effect of dietary garlic powder on the hepatic microsomal PNPH and PROD activities in rat hepatocarcinogenesis

Group	Number of rats	PNPH (nmol catechol/min/ mg protein)	PROD (pmol resorufin/min/ mg protein)
C	7	0.361 ± 0.032^{a}	31.29 ± 2.20
0.5	6	0.328 ± 0.033^{a}	34.92 ± 2.50
2.0	7	0.191 ± 0.015^{b}	28.18 ± 1.44
5.0	7	0.095 ± 0.015^{c}	29.29 ± 2.85

C : control diet + carcinogen treatment (DEN + partial hepatectomy)
0.5 : diet containing 0.5% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
2.0 : diet containing 2.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
5.0 : diet containing 5.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy)
Values are mean ± SE.
Means with the same subscripts are not significantly different at $p<0.05$ by Duncan's multiple range test.

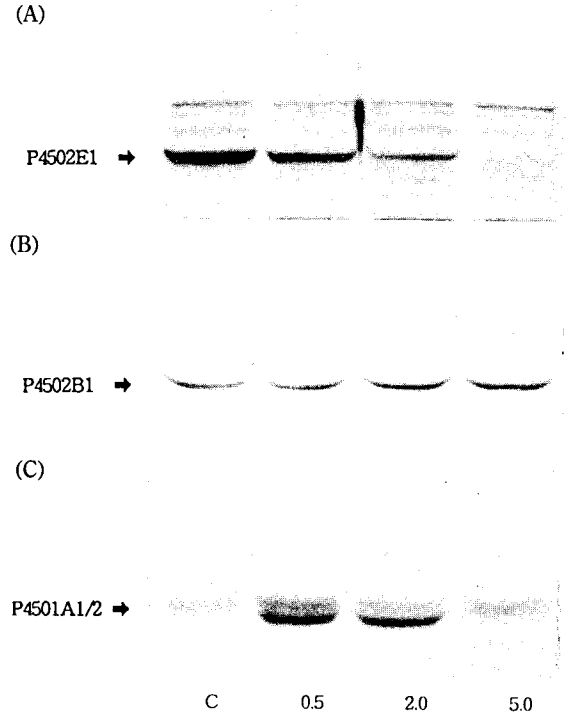

Fig. 3. Immunoblot analysis of the hepatic microsomes from rats fed 0, 0.5, 2.0, and 5.0% garlic powder diets. Each lane was loaded with 60 μg rat liver microsomes. (A) Polyclonal rabbit anti-P4502E1 antibody (diluted 1 : 2000) was used. (B) Polyclonal rabbit anti-P450 2B1 antibody (diluted 1 : 5000) was used. (C) Polyclonal rabbit anti-P450 1A1/2 antibody (diluted 1 : 5000) was used. C, control diet + carcinogen treatment (DEN + partial hepatectomy); 0.5, diet containing 0.5% garlic powder + carcinogen treatment (DEN + partial hepatectomy); 2.0, diet containing 2.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy); 5.0, diet containing 5.0% garlic powder + carcinogen treatment (DEN + partial hepatectomy).

not induced by 5.0% garlic powder diet (Fig. 3(C)). This result shows that modulation of cytochrome P450 1A1/2 protein levels may be different by dietary level of garlic powder and there is likely to be the optimal level of garlic powder necessary to induce cytochrome P450 1A1/2 protein levels. In the rat liver, cytochrome P450 1A1 and P450 1A2, in the P450 1A gene subfamily, are the major enzymes induced in response to polycyclic aromatic hydrocarbones (PAH) such as 3-methylcholanthrene, *B*-naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Cytochrome P450 1A1, exhibits significant activity in the metabolism of PAH carcinogens; in contrast, P450 1A2 is less active in PAH metabolism but has a high degree of activity in the metabolism of aflatoxin (Kim *et al.*, 1991). Therefore, in future studies, it needs to be determined whether the induction of cytochrome P450 1A1/1A2 by 0.5 and 2.0% garlic powder is beneficial.

DAS as well as DADS induced the microsomal levels of cytochrome P450 1A2, and 2B1/2 proteins, with an increase in the activities of methoxyresorufin-*O*-demethylase, PROD, and benzoxyresorufin-*O*-debenzylase (Harber *et al.*, 1994). Furthermore, cytochrome P450 2B1/2 protein levels and PROD activities in rat liver were induced by DAS, and the mechanism of the induction of cytochrome P450 2B1/2 was shown to be due to transcriptional activation by DAS (Pan *et al.*, 1993). However, Reicks and Crankshaw (1996) reported that benzphetamine demethylase and ethoxyresorufin *O*-deethylase (cytochrome P450 1A1 activity) were not significantly affected by garlic compounds (DAS, DADS, and allyl methyl sulfide) in rats.

As dietary DAS (0.5%) has been shown to inhibit *N*-nitrosodiethylamine-induced carcinogenesis in rat (Jang *et al.*, 1991), it seems important to determine whether allyl sulfide compounds from garlic can modify drug-metabolizing enzymes when given by dietary route. However, the daily doses administered in most of animal studies (approximately 200 mg/kg body weight) are much higher than those normally consumed in a human diet (550~700 μg DAS and DADS per g garlic). Over 20 sulfur-containing compounds are present in garlic extracts (Yu *et al.*, 1989) and in combinations, these compounds may have a substantial inhibitory effect on the activation of toxic chemicals, especially in counteracting low levels of toxic chemicals through competitive inhibition. Epidemiological studies in Shangdong, China, demonstrate that a lower risk of stomach cancer was associated with higher intake of *Allium* vegetables (You *et al.*, 1988). Cheng *et al.* (1995) suggested that a 2.5% garlic is the optimal dose, and may be used mainly as an inhibitor to prevent incidence of colon cancer, and improve survival time. In their experiment, the incidence of colon tumor was significantly decreased in the rats fed 2.5, 5.0 and 10.0% garlic diets, and there was no distinct difference among these concentrations. But in our preliminary study, rats on 10.0% garlic powder did not eat enough diet to be included in the experiment.

The modes of inhibition of cytochrome P450 enzymes by dietary garlic levels were quite different. The inhibition of cytochrome P450 2E1 activity and suppression of its protein level and induction of cytochrome P450 1A1/2 protein levels in rat hepatocarcinogenesis may contribute to the chemopreventive effects of garlic powder. But, the question of whether the excessive induction and suppression of some cytochrome P450 enzymes is beneficial must be investigated, so the balance between the various isozymes of cytochrome P450 may be important in prevention of rat hepatocarcinogenesis. These results suggest that garlic powder might influence hepatocarcinogenesis through the modulation and balance of cytochrome P450 enzymes and membrane stability. Therefore, the mechanism of cancer inhibition by garlic is not clearly understood, but the modification and balance of cytochrome P450 enzymes may be considered to be a part of mechanism for chemoprevention against chemical carcinogenesis. From these results, it is suggested that a 2.0% garlic diet is very effective in inhibiting GST-P positive foci, modulating cytochrome P 450 enzymes, lowering the lipid peroxidation and stabilizing microsomal membrane in rat hepatocarcinogenesis treated with DEN and partial hepatectomy.

Further research is in progress to determine the optimal level of raw garlic in the diet and time (pre- and post-initiation) and duration (6 to 20 weeks) of feeding necessary to be chemopreventive in rat hepatocarcinogenesis.

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