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Effects of Dietary Garlic Powder on GST-P Positive Foci and Glucose 6-Phosphatase Activity in Diethylnitrosamine-Initiated Rat Hepatocarcinogenesis

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This study was designed to examine the anticarcinogenic effect of dietary supplementation with garlic powder on rat hepatocarcinogenesis. All rats were initiated by a single dose (200 mg/body weight) intraperitoneal injection of diethylnitrosamine (DEN), and three weeks later, subjected to two-thirds partial hepatectomy. Two weeks after initiation, four groups of rats were given experimental diets supplemented with 0 (control group), 0.5, 2.0, or 5.0% garlic powder for 6 weeks. Rats were sacrificed at eight weeks after initiation. The induction of placental glutathione S-transferase (GST-P) positive foci was significantly inhibited almost equally in all three groups fed garlic diets. Glucose 6-phosphatase (G6Pase) activity was increased in rats fed 0.5% and 2.0% garlic powder, and was negatively correlated with the number and area of GST-P positive foci. Thiobarbituric acid reactive substance (TBARS) contents were decreased in rats fed 2.0% and 5.0% garlic powder. Only 5.0% garlic powder supplementation significantly increased the glutathione content and the glutathione S-transferase activity, compared to the control group. Therefore, all levels of garlic powder, 0.5% to 5.0%, exerted an antipromotional effect during hepatocarcinogenesis. Dietary supplementation with garlic powder seemed to maintain microsomal membrane integrity by increasing G6Pase activities. Glutathione-dependent detoxifying enzymes did not seem to contribute to this protective effect directly. The present study suggests that garlic powder is effective in inhibiting the induction of GST-P positive foci, possibly by stabilizing the hepatic microsomal membrane.

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Introduction

Evidence from epidemiological, clinical, and laboratory studies suggests that a considerable number of naturally-occurring dietary constituents may influence cancer incidence (Wattenberg, 1983). Garlic (*Allium sativum*), one of the most common flavor-enhancing foods, and its ingredients have been reported to be used as folk medicines throughout recorded history (Essman, 1984). Garlic's chemical constituents, mainly organosulfur compounds (OSCs), are now known to exert antibiotic (Cavallito and Bailey, 1944), lipid-lowering (Yeh and Yeh, 1994), detoxificative (Hayes, 1987), hypoglycemic (Chang and Johnson, 1980), and other medical effects in the body.

Recently, attention has been paid to the pharmacological activity of raw garlic and its various extract preparations in the inhibition of carcinogenesis. Epidemiological, animal, and *in vitro* studies indicate that OSCs in garlic have anticarcinogenic effects that inhibit several types of tumors and decrease tumor growth and proliferation, including oral cancer (Meng and Shyu, 1990), esophageal cancer (Wargovich, 1988), gastric cancer (You *et al.*, 1989), colorectal cancer (Reddy and Rao, 1993), breast cancer (Liu *et al.*, 1991), and skin cancer (Rao *et al.*, 1990). Thus, garlic has been proven to possess the potent capability to inhibit cancers of various species, organs, or carcinogens.

The incidence of liver cancer is considerably high in Korea, occupying an important position in major causes of death among the Korean population. Therefore, the prevention or suppression of liver cancers is a primary issue in Korea. Studies of garlic effects on liver cancers to-date have mostly focused on animal experiments treated with several biologically active constituents contained in

garlic, in the form of purified compounds rather than the raw or whole garlic (Jang *et al.*, 1991; Takada *et al.*, 1994). While OSCs, abundantly present in garlic, have been consistently reported to prevent or suppress the process of carcinogenesis in other organs, the observance of garlic effects on hepatocarcinogenesis, especially in the post-initiation period, have been quite varied and controversial with the reason for the discrepancies being unclear (Jang *et al.*, 1991; Takada *et al.*, 1994).

In the present study, the modifying potential of dietary garlic powder was examined in the promotion stage of the DEN-initiated rat liver carcinogenesis using the medium-term bioassay system defined by Ito *et al.* (1988). To investigate the modifying effects of garlic during hepatocarcinogenesis, as a food ingredient, and not as the megadose treatment of purified compounds which have usually been force-fed, this study used garlic powder in the diet. The anticarcinogenic property of garlic was evaluated by measuring the GST-P positive foci, the effective end-point marker for DEN-initiated lesions. Lipid peroxidation of liver microsomes (TBARS content) and activities of G6Pase and glutathione-dependent detoxifying enzymes were measured in order to investigate possible mechanisms that might be involved.

Materials and Methods

Animals and diets Male Sprague-Dawley rats (50–60 g) were obtained from the Seoul National University Animal Care Facility. Rats were kept in polycarbonate cages in a room with controlled temperature (20 ± 2°C) and lighting (12 h light/dark cycle) and were given food and tap water *ad libitum*. The composition of powdered control diet by weight was as follows: casein, 20%; corn oil, 15%; corn starch, 54.7%; α-cellulose, 5%; AIN-76 mineral mixture, 4%; AIN-76 vitamin mixture, 1%; DL-methionine, 0.3%. For the 0.5, 2.0, and 5.0% garlic test groups, garlic powder (Miwon Co. Ltd., Seoul, Korea) was added in the control diet at the expense of corn starch. All diet ingredients were mixed in our laboratory.

Experimental protocol All rats at six weeks of age were given a single intraperitoneal injection of DEN (200 mg/kg body weight) dissolved in saline to initiate hepatocarcinogenesis. After two weeks on basal diet (Purina rat chow), they were randomized by weight into four groups, and were fed experimental diets supplemented with 0, 0.5, 2.0, or 5.0% garlic powder (freeze-dried) for six weeks. Based on a preliminary study, levels of garlic powder in the diet were determined below the maximum tolerated dose which had made no significant differences in food intake and body weight. Animals were subjected to two-thirds partial hepatectomy at week three to maximize any interaction between proliferation and the effects of experimental diets. All animals were killed at the end of week eight (Fig. 1). Food intake was measured daily and body weight weekly. The relative liver weight was calculated as (liver weight / body weight)×100.

Immunohistochemical staining for placental glutathione S-transferase Immediately upon sacrifice, the excised livers were cut into 2–3 mm thick slices with a razor blade. These liver slices were fixed in ice-cold acetone and processed for embedding in paraffin and subsequent immunohistochemical examination of GST-P positive foci by the avidin-biotin-peroxidase complex (ABC) method described by Hsu *et al.* (1981). After deparaffinization, liver sections were treated sequentially with normal goat serum, rabbit anti-rat GST-P (Medical and Biological Laboratories Co.), biotin-labeled goat anti-rabbit IgG, and ABC. The sites of peroxidase binding were demonstrated by the diaminobenzidine method (Graham and Karonofsky, 1966). Sections were then counterstained with hematoxylin for microscopic examination. The numbers and areas of GST-P positive foci >0.2 mm in diameter and the total areas of liver sections examined were measured using an image analyzer.

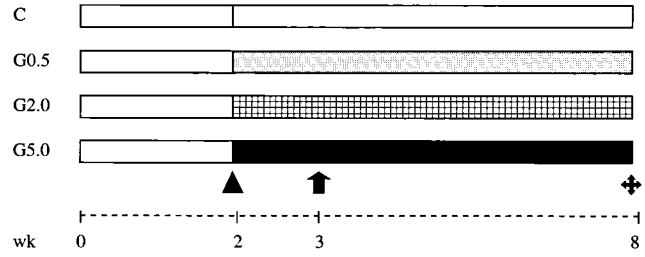

Fig. 1. Experimental protocol. ▲, DEN i.p. injection (200 mg/kg body weight); ⬆, two-thirds partial hepatectomy; ✣, sacrifice; □, control diet; ▨, 0.5% garlic powder diet; ▦, 2.0% garlic powder diet; ■, 5.0% garlic powder diet.

Preparation of microsomal and cytosolic fractions Immediately after animals were sacrificed by decapitation, the livers were excised, trimmed, finely minced, and then homogenized in 50 mM Tris·HCl buffer, pH 7.4, containing 1 mM EDTA. Microsomal and cytosolic fractions were prepared by differential centrifugation and were stored in small aliquots at −70°C until used.

Determination of lipid peroxidation Microsomal NADPH-dependent lipid peroxidation was determined by measuring the formation of TBARS in the hepatic microsomes according to the method of Buege and Aust (1969). Malondialdehyde as the product of lipid peroxidation reacted with thiobarbituric acid and the absorbance of the resulting chromophore was determined at 535 nm. The extinction coefficient for malondialdehyde was taken to be $1.56 \times 105\ M^{-1}cm^{-1}$ (Wills, 1969).

Enzyme assays G6Pase activity was determined by the method of Baginski *et al.* (1983). The inorganic phosphate liberated from glucose 6-phosphate was determined with ammonium molybdate and the absorbance was measured at 700 nm. GST activity was assayed in the hepatic cytosolic fraction using the method of Habig *et al.* (1974). The conjugate of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) was measured at 340 nm using a dual beam spectrophotometer. Calculations were made using a molar extinction coefficient of $9.6\ mM^{-1}cm^{-1}$. Activities of glutathione peroxidase (GPx) and glutathione reductase (GR) in the hepatic cytosolic fraction were measured by monitoring the oxidation of NADPH at 340 nm according to Tappel (1978) and Carlberg *et al.* (1985), respectively.

Determination of total glutathione content Total glutathione (reduced + oxidized) content was measured by a modification of the method of Griffith (1980), using the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB)-GR recycling procedure. 5-Sulfosalicylic acid was used for protein precipitation and DTNB for color development. The rate of 5-thio-2-nitrobenzoic acid formation was followed at 412 nm and the total glutathione content was expressed as μmol of reduced glutathione equivalents per g liver.

Determination of protein concentration Protein amounts of hepatic microsomal and cytosolic fractions were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Statistical analysis All statistical analyses were carried out using Duncan's multiple range test with the SAS program. Differences were considered statistically significant at $p < 0.05$.

Results

Garlic diets did not influence food intake and body weight gain of rats as in our preliminary feeding trial. The average food consumption of all experimental groups ranged from 19.3 to 20.2 g/day without any significant differences among groups. Liver and body weights were not different between control and garlic-fed animals. However, the relative liver weight, which usually increases with carcinogen treatment, was significantly decreased in garlic-fed groups compared to the control group (Fig. 2).

The induction of GST-P positive foci in rats was significantly inhibited by garlic diets with no statistical differences among garlic-fed groups (Fig. 3). The area and number of GST-P positive foci per unit area of liver slices ($1\ cm^2$) were significantly decreased in garlic-fed groups compared to the control group (Fig. 4). TBARS contents were significantly decreased in rats fed 2.0 or 5.0% garlic powder diets (Fig. 5). G6Pase activities in animals fed 0.5 or 2.0% garlic powder diets were increased significantly compared to those of the control group (Fig. 6). Total glutathione contents were significantly increased compared to those of the control group in the 5.0% garlic powder-fed group only (Table 1). GST activities were significantly increased compared to those of the control group in the 5.0% garlic powder-fed group only, but there were no significant differences among the garlic diet-fed groups (Table 1). There were no significant differences in the activities of GPx and GR among all four groups (Table 1).

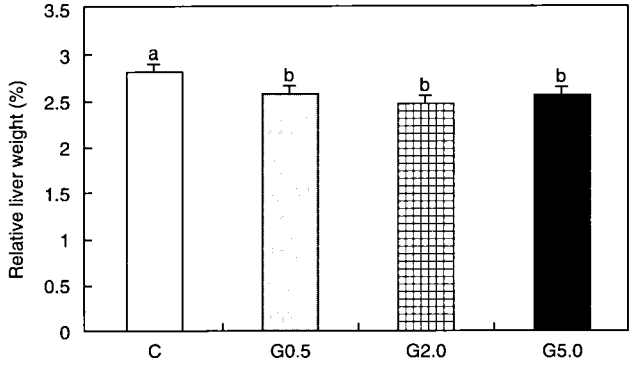

Fig. 2. Effects of dietary levels of garlic powder on relative liver weight in rats treated with DEN and partial hepatectomy. C, control diet + carcinogen treatment; G0.5, 0.5% garlic powder diet + carcinogen treatment; G2.0, 2.0% garlic powder diet + carcinogen treatment; G5.0, 5.0% garlic powder diet + carcinogen treatment. Values are mean ± SE. Means with the same letters are not significantly different at $p<0.05$ by Duncan's multiple range test.

Discussion

The present study clearly demonstrated that dietary garlic powder exerted inhibitory effects on the development of

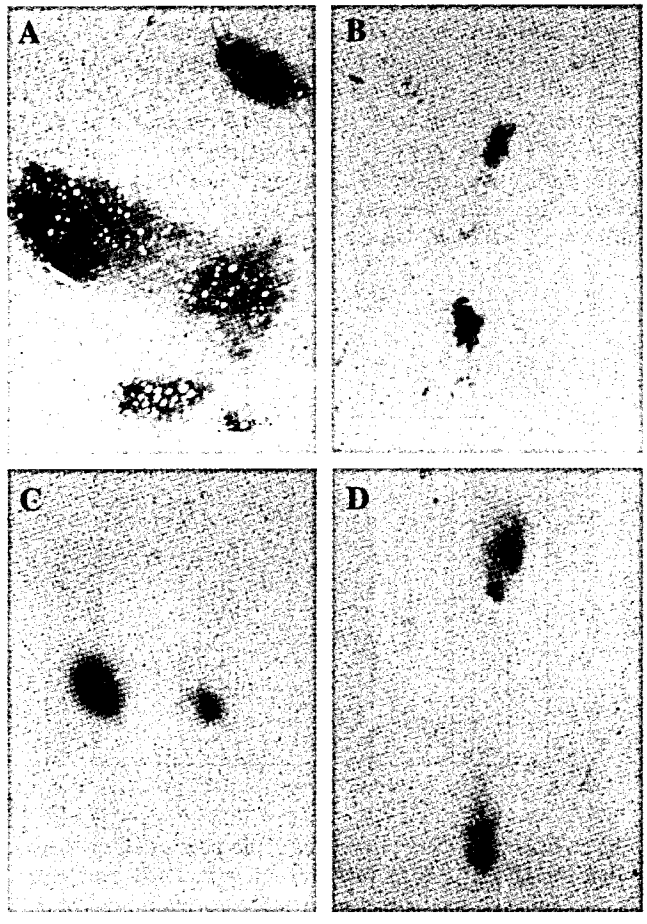

Fig. 3. Immunohistochemical staining of GST-P positive foci in rats treated with DEN and partial hepatectomy (×40). A, control diet + carcinogen treatment; B, 0.5% garlic powder diet + carcinogen treatment; C, 2.0% garlic powder diet + carcinogen treatment; D, 5.0% garlic powder diet + carcinogen treatment.

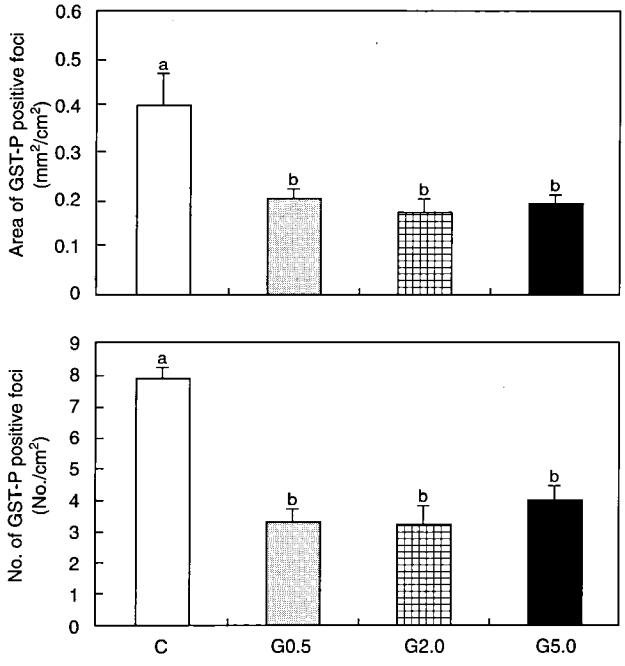

Fig. 4. Effects of dietary levels of garlic powder on area and number of GST-P positive foci in livers from rats treated with DEN and partial hepatectomy. C, control diet + carcinogen treatment; G0.5, 0.5% garlic powder diet + carcinogen treatment; G2.0, 2.0% garlic powder diet + carcinogen treatment; G5.0, 5.0% garlic powder diet + carcinogen treatment. Values are mean ± SE. Means with the same letters are not significantly different at $p<0.05$ by Duncan's multiple range test.

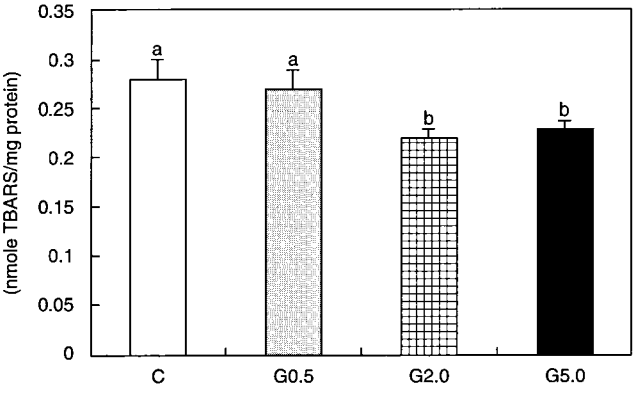

Fig. 5. Effects of dietary levels of garlic powder on the hepatic microsomal TBARS in rats treated with DEN and partial hepatectomy. C, control diet + carcinogen treatment; G0.5, 0.5% garlic powder diet + carcinogen treatment; G2.0, 2.0% garlic powder diet + carcinogen treatment; G5.0, 5.0% garlic powder diet + carcinogen treatment. Values are mean ± SE. Means with the same letters are not significantly different at $p<0.05$ by Duncan's multiple range test.

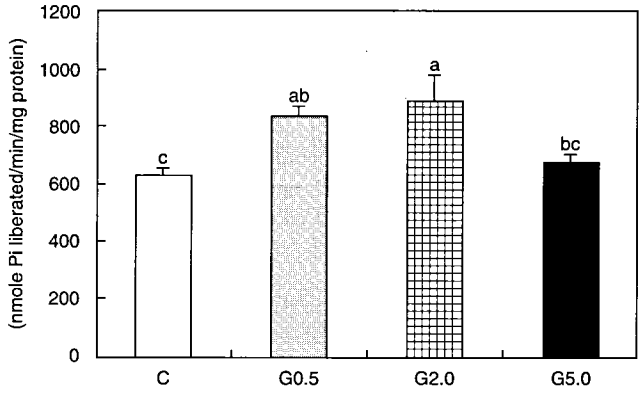

Fig. 6. Effects of dietary levels of garlic powder on the hepatic microsomal G6Pase in rats treated with DEN and partial hepatectomy. C, control diet + carcinogen treatment; G0.5, 0.5% garlic powder diet + carcinogen treatment; G2.0, 2.0% garlic powder diet + carcinogen treatment; G5.0, 5.0% garlic powder diet + carcinogen treatment. Values are mean ± SE. Means with the same letters are not significantly different at $p<0.05$ by Duncan's multiple range test.

GST-P positive foci, the effective end-point marker for DEN-initiated lesions (Sato, 1988), in the rat liver medium-term bioassay system defined by Ito *et al.* (1988). This protocol was established to detect modifying effects of various compounds on hepatocarcinogenesis (Ito *et al.*, 1988). Ogiso *et al.* (1985; 1990) have already proven that the degree of induction of GST-P positive foci and nodules shows direct correlation with the incidence of hepatocellular carcinomas identified in long-term *in vivo* bioassays. Consequently, the results of the present study strongly suggest that dietary garlic powder has anti-promotion effects on hepatocarcinogenesis.

G6Pase is an integral microsomal enzyme, the activity of which largely depends on an intact lipid bilayer. Hence, hepatic microsomal G6Pase has been known to reflect the structural integrity of the microsomal membrane (De Groot *et al.*, 1985; Kim *et al.*, 1990; Kim and Choi, 1994). In addition, G6Pase is also known as one of the specific histochemical markers for preneoplastic lesions in the liver because of the deficiency in its activity of enzyme-altered foci (Farber, 1980). In our present findings, G6Pase activities were not only higher in garlic-fed groups but also negatively correlated with the numbers ($r = -0.62$) and areas ($r = -0.53$) of GST-P positive foci. Dietary supplementation with garlic powder also seemed to maintain microsomal membrane integrity by increasing G6Pase activities.

Lipid peroxidation and a variety of its toxic products have been suggested as major causes of cancer development (Cerutti, 1985). Lipid peroxidation is also known to decrease the fluidity and integrity of microsomal membranes (Eichenberger *et al.*, 1982), resulting in a

Table 1. Effects of dietary levels of garlic powder on the hepatic level of total glutathione and the hepatic GST, GPx, and GR activities in the rats treated with DEN and partial hepatectomy.

Group		Total glutathione content (μmole reduced glutathione eq./g liver)	GST activity (nmole CDNB conjugated/ min/mg protein)	GPx activity (nmole NADPH oxidized/ min/mg protein)	GR activity (nmole NADPH oxidized/ min/mg protein)
C	(5)	6.33 ± 0.16^{b}	1.48 ± 0.04^{b}	651.14 ± 19.25^{a}	61.17 ± 2.19^{a}
G0.5	(6)	6.21 ± 0.45^{b}	1.52 ± 0.03^{ab}	690.37 ± 29.07^{a}	66.94 ± 1.04^{a}
G2.0	(6)	6.16 ± 0.22^{b}	1.63 ± 0.07^{ab}	690.15 ± 29.30^{a}	58.57 ± 5.60^{a}
G5.0	(5)	7.58 ± 0.16^{a}	1.65 ± 0.06^{a}	619.93 ± 17.53^{a}	69.37 ± 4.73^{a}

C : control diet + carcinogen treatment
G0.5 : 0.5% garlic powder diet + carcinogen treatment
G2.0 : 2.0% garlic powder diet + carcinogen treatment
G5.0 : 5.0% garlic powder diet + carcinogen treatment
Values are mean ± SE.
Values with the same superscript are not significantly different at $p < 0.05$ by Duncan's multiple range test.
( ) Number of animals.

decrease or loss of many microsomal enzymes including G6Pase (Koster and Slee, 1980). In this study, although lipid peroxidation did not have a statistically significant correlation with the induction of GST-P positive foci or G6Pase activity, the 2.0 and 5.0% garlic powder levels in the diet did show antioxidative effects.

Although studies related to a number of disciplines including immunity, blood lipids, prostaglandins, and drug metabolism/cytochrome P450 have been performed previously, the mechanism of cancer inhibition by garlic is not clearly understood. The selective modulation of cytochrome P450 proteins, which are involved in the initial hepatic activation of carcinogens, and the induction of GST activity have been proposed as a possible chemopreventive mechanism (Hayes *et al.*, 1987; Wargovich *et al.*, 1988; Park and Choi, 1997). It may be particularly important in the area of drug metabolism and detoxification that garlic can influence sulfur metabolism. In particular, thiol molecules such as glutathione are potentially protective against toxic, mutagenic, or carcinogenic events involving electrophiles. In addition to conjugation with glutathione by GST pathways, glutathione can exert its antioxidative protection through GPx, the selenium-containing enzyme (Meister and Anderson, 1983). The hepatic total glutathione pool and glutathione-dependent detoxifying enzymes including GST were measured in this study. Only in 5% garlic powder diets were there significantly increased total glutathione contents and GST activities compared to the control diet. No effect was observed on the activities of GPx and GR. Thus, the total amount of glutathione and glutatione-dependent detoxifying enzymes does not seem to contribute directly to the anti-promotion effect observed in DEN-initiated rat hepatocarcinogenesis.

Other previous studies which tested OSCs in bioassay systems similar to this study reported considerably different effects in the promotion stage. While Jang *et al.* (1991) reported significant inhibition of GST-P positive foci induction by allyl sulfide and diallyl sulfide, Takada *et al.* (1994) found enhancing effects of most of the OSCs tested including diallyl sulfide. The reason for this discrepancy still remains unclear. One point of possible relevance is the methodological difference in administration of test compounds. It is noteworthy that Jang *et al.* (1991), who observed inhibitory effects of OSCs, administered test compounds which were incorporated into semi-purified diets and fed to rats as in our present study. On the other hand, rats in the other studies which reported enhancing or no modifying effects received periodical intragastric intubation of comparable doses of test compounds. Moreover, all of the studies with periodical intragastric gavage of OSCs reported significant decrease in body weight and increase in relative liver weight of experimental animals, revealing the definite need to dissociate the enhancing effects from the toxicity of unnatural administration of OSCs. Therefore, even though all of those studies used megadose levels of purified OSCs, this distinct methodological difference in administration deserves attention, especially when assessing the modifying effects of OSCs as naturally-occurring food constituents.

With regard to planning a human study, the dose level is a major consideration. For example, a 200 mg/kg body weight of diallyl sulfide was usually adopted as the administration dosage for rats in many previous studies. However, when extrapolating to a 70 kg man, this would correspond to 14 g (15.8 ml) diallyl sulfide, which would be equivalent to 14,000 garlic cloves (Dausch and Nixon,

1990). This is not a practical amount for humans as Caporaso *et al.* (1983) reported the maximum tolerable dose of fresh aqueous garlic extract in humans to be 25 ml. Moreover, a large dosage of garlic causes the unpleasant odor produced by allicin and toxicity including vomiting and severe burning sensations in the esophagus and stomach, inhibition of spermatogenesis, anemia, weight loss, growth failure, decreased bacterial flora, and decreased serum albumin (Dixit and Joshi, 1982; Caporaso *et al.*, 1983). Therefore, we need a natural feeding model of a practical dose of garlic to examine the dietary effects of garlic on cancer development.

Although direct extrapolation of our animal experiments to a human situation is not justified, an equivalent dose for humans of 0.5% garlic powder, the minimal level that decreased the GST-P induction in this study, was calculated with the rat/human body surface ratio (Dubois and Dubois, 1916; Walker and Mason, 1968). The average daily food intake of rats fed 0.5% garlic powder diet was 19.5 g. Considering a 25% yield of garlic powder from raw garlic, a 500 g rat ate 0.39 g of raw garlic daily. When extrapolating to a 66 kg, 172 cm adult man (reference Korean), this would correspond to approximately 10 g raw garlic per day. This is much less than 20 g/day, the average garlic consumption of certain Chinese populations which showed the markedly lower garlic consumption (< 1 g/day) in the study by You *et al.* (1989). Moreover, Cheng *et al.* (1995) suggested a daily intake of approximately 10 g of raw garlic for an 80 kg human as the optimal dose of garlic to prevent colon cancer, which is strikingly comparable to our efficient dosage. According to the 1995 National Nutrition Survey Report by the Korean Ministry of Health and Welfare in Korea, the nationwide average intake of garlic in the Korean population was 5.9 g/day. Therefore, we believe that our dosage of garlic in this study may be reasonably put to practical use in the typical Korean diet as well as in the high risk groups of liver cancer.

In conclusion, the results of this study demonstrated the anti-promotion effect of dietary garlic in liver carcinogenesis, although the exact mechanisms involved in its chemopreventive effect are not clearly understood at present. In the promotional stage, dietary supplementation with garlic powder seemed to maintain microsomal membrane integrity by increasing G6Pase activities, but glutathione-dependent detoxifying enzymes did not seem to contribute to this protective effect directly. The present study suggests that all levels of garlic powder, i.e. 0.5% to 5.0%, are protective in the promotional stage of rat hepatocarcinogenesis, with a minimal effective level of around 0.5%. Moreover, this study suggests that a practical feeding model would be obviously necessary when understanding and evaluating the modulatory effects of naturally occurring compounds in the diet. Further studies are needed to clarify the mechanisms by which garlic and its constituents exert their chemopreventive effects and to determine the optimal level of dietary garlic powder to inhibit hepatocarcinogenesis and modulate enzyme activities.

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References

Baginski, E. S., Foa, P. P. and Zak, B. (1983) *Methods of Enzymatic Analysis.* Vol. 2, pp. 876–880, Academic Press, New York.

Buege, J. A. and Aust, S. D. (1978) *Methods in Enzymology.* Vol. 52, pp. 302–310, Academic Press, New York.

Caporaso, N., Smith, S. M. and Eng, R. H. (1983) Antifungal activity in human urine and serum after ingestion of garlic (*Allium sativum*). *Antimicrob. Agents. Chemother.* **23**, 700–702.

Carlberg, I. and Mannervik B. (1985) *Methods in Enzymology.* Vol. 113, pp. 484–490, Academic Press, New York.

Cavallito, C. J. and Bailey, J. H. (1944) Allicin, the antibacterial principal of *Allium sativum.* I. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* **66**, 1950–1951.

Cerutti, P. A. (1985) Prooxidant states and tumor promotion. *Science* **227**, 375–381.

Chang, M. L. W. and Johnson M. A. (1980) Effect of garlic on carbohydrate metabolism and lipid synthesis in rats. *J. Nutr.* **110**, 931–936.

Cheng, J. Y., Meng, C. L., Tzeng, C. C. and Lin, J. C. (1995) Optimal dose of garlic to inhibit dimethylhydrazine-induced colon cancer. *World J. Surg.* **19**, 621–626.

Dausch, J. G. and Nixon, D. W. (1990) Garlic: A review of its relationship to malignant disease. *Prev. Med.* **19**, 346–361.

De Groot, H., Noll, T. and Töll, T. (1985) Loss of latent activity of liver microsomal membrane enzymes evoked by lipid peroxidation. Studies of nucleoside diphosphatase, glucose 6-phosphatase, and UDP glucuronyltransferase. *Biochim. Biophys. Acta* **815**, 91–96.

Dixit, V. P. and Joshi, S. (1982) Effects of chronic administration of garlic (*Allium sativum* Linn) on testicular function. *Ind. J. Exp. Biol.* **20**, 534–536.

Dubois, D. and Dubois, E. F. (1916) Clinical calorimetry. A formula to estimate to approximate surface area if height and weight be known. *Arch. Intern. Med.* **17**, 863–871.

Eichenberger, K., Böhni, P., Winterhalter, K. H., Kawato, S. and Richter, C. (1982) Microsomal lipid peroxidation causes an increase in the order of the membrane lipid domain. *FEBS Lett.* **142**, 59–62.

Essman, E. J. (1984) The medicinal use of herbs. *Fitoterapia* **55**, 279–289.

Farber, E. (1980) The sequential analysis of liver cancer induction. *Biochim. Biophys. Acta* **605**, 149–166.

Graham, R. C. Jr. and Karnofsky, M. J. (1966) The early stage of absorption of injected horse radish peroxidase in the proximal convoluted tubules of mouse kidney: ultrastructural

cytochemistry by a new technique. *J. Histochem. Cytochem.* **14**, 291–302.

Griffith, O. W. (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **106**, 207–212.

Habig, W. H., Pabst, M. J. and Jakoby, W. B. (1974) Glutathione S-transferases. *J. Biol. Chem.* **249**, 7130–7139.

Hayes, M. A., Rushmore, T. H. and Goldberg, M. T. (1987) Inhibition of hepatocarcinogenic response to 1,2-dimethylhydrazine by diallyl sulfide: a component of garlic oil. *Carcinogenesis* **8**, 1155–1157.

Hsu, S. M., Raine, L. and Fanger, H. (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* **29**, 577–580.

Ito, N., Tsuda, H., Tatematsu, M., Inoue, T., Tagawa, Y., Aoki, T., Uwagawa, S., Kagawa, M., Ogiso, T., Masui, T., Imaida, K., Fukushima, S. and Asamoto, M. (1988) Enhancing effects of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rat — an approach for a new medium-term bioassay system. *Carcinogenesis* **9**, 387–394.

Jang, J. J., Cho, K. J., Lee, Y. S. and Bae, J. H. (1991) Modifying responses of allyl sulfide, indole-3-carbinol and germanium in a rat multi-organ carcinogenesis model. *Carcinogenesis* **12**, 691–695.

Kim, H. A., Kim, H. D., Choi, H. M. and Lee, J. H. (1990) Effects of fatty acid composition and butylated hydroxytoluene on the lipid peroxide metabolizing enzyme activities in 2-acetylaminofluorene treated rat liver. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **23**, 302–307.

Kim, S. K. and Choi, H. (1994) Effect of N-6, N-3 fatty acid and vitamin E supplement on the induction of preneoplastic lesion in murine hepatocarcinogenesis model. *Korean Biochem. J.* (presently *J. Biochem. Mol. Biol.*) **27**, 125–131.

Koster, J. F. and Slee, R. G. (1980) Lipid peroxidation of rat liver microsomes. *Biochim. Biophys. Acta* **620**, 489–499.

Liu, J. Z., Lin, R. I. and Milner, J. A. (1991) Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors and DNA adducts by garlic powder. *Carcinogenesis* **13**, 1847–1851.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275.

Meister, A. and Anderson, M. E. (1983) Glutathione. *Annu. Rev. Biochem.* **52**, 711–760.

Meng, C. L. and Shyu, K. W. (1990) Inhibition of experimental carcinogenesis by painting with garlic extract. *Nutr. Cancer* **14**, 207–217.

Ogiso, T., Tatematsu, M., Tamano, S., Tsuda, H. and Ito, N. (1985) Comparative effects of carcinogens on the induction of placental glutathione S-transferase-positive liver nodules in a short-term assay and of hepatocellular carcinomas in a long-term assay. *Toxicol. Pathol.* **13**, 257–273.

Ogiso, T., Tatematsu, M., Tamano, S., Hasegawa, R. and Ito, N. (1990) Correlation between medium-term liver bioassay system data and results of long-term testing in rats. *Carcinogenesis* **11**, 561–566.

Park, K. A. and Choi, H. (1997) Modification of hepatic microsomal cytochrome P450 2E1 enzyme by garlic powder in rat hepatocarcinogenesis. *J. Biochem. Mol. Biol.* (fomerly *Korean Biochem. J.*) **30**, 73–79.

Rao, A. R., Sadhana, A. S. and Goel, H. C. (1990) Inhibition of skin tumors in DMBA-induced complete carcinogenesis system in mice by garlic (*Allium sativum*). *Indian J. Exp. Biol.* **28**, 405–408.

Reddy, M. W., Rao, C. V., Rivenson, A. and Kelloff, G. (1993) Chemoprevention of colon carcinogenesis by organosulfur compounds. *Cancer Res.* **53**, 3493–3498.

Sato, K. (1988) Glutathione S-transferases and hepatocarcinogenesis. *Jpn. J. Cancer Res.* **79**, 556–572.

Takada, N., Matsuda, T., Otoshi, T., Yano, Y., Otani, S., Hasegawa, T., Nakae, D., Konishi, Y. and Fukushima, S. (1994) Enhancement by organosulfur compounds from garlic and onions of diethylnitrosamine-induced glutathione S-transferase positive foci in the rat liver. *Carcinogenesis* **54**, 2895–2899.

Tappel, A. L. (1978) *Methods in Enzymology*. Vol. 52, pp. 506–513, Academic Press, New York.

Walker, H. L. and Mason, A. D. Jr. (1968) A standard animal burn. *J. Trauma* **8**, 1049–1051.

Wargovich, M. J., Wood, C., Eng, V. W. S., Stephens, L. C. and Gray, K. (1988) Chemoprevention of N-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. *Cancer Res.* **48**, 6872–6875.

Wattenberg, I. W. (1983) Inhibition of neoplasia by minor dietary constituents. *Cancer Res.* **43**, 2448–2453.

Wills, E. D. (1969) Lipid peroxide formation in microsomes. General considerations. *Biochem. J.* **113**, 315–324.

Yeh, Y. Y. and Yeh, S. M. (1994) Garlic reduces plasma lipids by inhibiting hepatic cholesterol and triacylglycerol synthesis. *Lipids* **29**, 189–193.

You, W. C., Blot, W. J., Chang, J. S., Ershow, A., Yang, Z. T., An, Q., Henderson, B. E., Fraumeni, J. F. Jr. and Wang, T. G. (1989) Allium vegetables and reduced risk of stomach cancer. *J. Natl. Cancer Inst.* **81**, 162–164.