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ENHANCEMENT OF UDP-GLUCURONYLTRANSFERASE, UDP-GLUCOSE DEHYDROGENASE, AND GLUTATHIONE *S*-TRANSFERASE ACTIVITIES IN RAT LIVER BY DIETARY ADMINISTRATION OF EUGENOL

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Abstract—Male Fisher rats were fed a diet *ad lib.* containing eugenol (4-allyl-2-methoxyphenol) to observe its effects on liver drug-detoxifying enzymes such as UDP-glucuronyltransferase (GT), UDP-glucose dehydrogenase (DH) and glutathione *S*-transferase (GST). Liver weights were not affected significantly by a diet containing 3% eugenol (w/w) for 13 weeks. The activities of GT of liver microsomes toward various xenobiotic substances such as 4-nitrophenol, 1-naphthol, 4-hydroxybiphenyl and 4-methylumbelliferone were enhanced by dietary administration of eugenol, but the activity of GT toward its endogenous substrate, bilirubin, was not changed. Dose–response relationships between the enhancement of GT activities toward these xenobiotics and the dose of eugenol were observed. The induced higher activities of GT toward these xenobiotics were maintained during 13 weeks of eugenol treatment. Similar results on DH and GST activities in the liver cytosol were obtained by dietary administration of eugenol, while no effect on cytochrome P-450 content in the liver microsomes from the rats fed the eugenol diet was observed during 13 weeks. These results suggest that the intracellular content of the active intermediates of various drugs or carcinogens would be reduced by this specific enhancement of drug-detoxifying enzymes in the liver of rats given a diet containing eugenol, as previously described for a diet containing 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) [Y-N. Cha and H. S. Heine, *Cancer Res.* **42**, 2609 (1982)].

Glucuronidation and glutathione conjugation represent the major detoxification reactions involved in the metabolic alteration of xenobiotics and chemical carcinogens [1, 2]. Induction of these conjugation enzymes, such as UDP-glucuronyltransferase (EC 2.4.1.17) and glutathione *S*-transferase (EC 2.5.1.18), performs an important role in the regulation of these detoxification reactions [1, 2].

The experimental production of tumors by a wide variety of chemical carcinogens can be inhibited in a variety of rodent tissues by dietary administration of antioxidants such as 2(3)-*tert*-butyl-4-hydroxyanisole (BHA)† and other phenolic antioxidants [3]. It has been reported that the inclusion of BHA in the diet of mice reduces the mutagenic activities of administered benzo[*a*]pyrene (B[*a*]P) and several other agents [4], and enhances the activities of two important conjugation enzymes, UDP-glucuronyltransferase and glutathione *S*-transferase [5–8], but produces no observable effects on the level of cytochrome P-450 and B[*a*]P hydroxylase activities [8]. Thus, it appears that the protective effects of BHA may be due largely to its ability to enhance the metabolic detoxication process.

We previously reported the enhancement of liver UDP-glucuronyltransferase, UDP-glucose dehydrogenase and urinary glucuronide excretion in the rat, caused by oral administration of eugenol [9]. Recently, we reported that the mutagenicity of B[*a*]P in the Ames test using liver S9 or microsomes prepared from rats treated with eugenol decreases in comparison to B[*a*]P mutagenicity using untreated rat liver S9 or microsomes, and this *in vitro* occurrence is due to the reduction of B[*a*]P hydroxylase activity in the microsomes from eugenol-treated rats [10]. The present study shows that an elevation of activity of detoxifying enzymes, UDP-glucuronyltransferase. UDP-glucose dehydrogenase and glutathione *S*-transferase, was produced by dietary administration of eugenol, and that the dose-dependent activity of eugenol was maintained during 13 weeks of eugenol treatment.

MATERIALS AND METHODS

Materials. Male Fisher rats (F344/N Slc) were obtained from the Shizuoka Laboratory Animal Center, Shizuoka, Japan. Diets (MF) for the rats were from the Oriental Yeast Co., Ltd., Tokyo, Japan. Eugenol (4-allyl-2-methoxyphenol), which was gas chromatographically pure, was purchased from Wako Pure Chemical Industries, Osaka, Japan. Bilirubin was from the Sigma Chemical Co., St. Louis, MO, U.S.A. Cholic acid, purchased from the Nissui Yakuhin Co., was further purified and converted to the sodium salt as described [11].

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† Abbreviations: BHA, 2(3)-*tert*-butyl-4-hydroxyanisole; BHT, 3,5-di-*tert*-butyl-4-hydroxytoluene; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; GT, UDP-glucuronyltransferase; DH, UDP-glucose dehydrogenase; GST, glutathione *S*-transferase; and PCBs, polychlorinated biphenyls.

Dietary administration of eugenol. Male Fisher rats (6–7 weeks old) were obtained and were housed in a room with a 12-hr light–dark cycle. These rats had free access to a commercial diet and tap water for a week of acclimatization. Thereafter, diets containing 0 (control), 1, 3 and 5% eugenol (w/w) were fed *ad lib.* to the experimental animals for 23 days. These four groups of rats were dissected on the final day. In the other experiment, one group of rats was the control and another group was fed *ad lib.* on the eugenol diets for a long term. The diet containing 5% eugenol (w/w) affected the appetites and the body weights of the experimental animals. The following schedule of eugenol administration was carried out to minimize adverse effects on the rats for 13 weeks: 1% eugenol (w/w) diet for the first week to adapt to the eugenol diet, followed by 5% for a second week. Since the 5% diet adversely affected feeding and the body weights of the animals, a 3% diet was fed for the next 6 weeks, 1% for the next 2 weeks, 3% for the next 2 weeks, and control diet for the last week.

Preparation of liver microsomes from rats. Liver microsomes were prepared from eugenol-treated rats as described by Imai and Sato [11] as follows. Rat livers, after being perfused with 0.15 M KCl, were minced and homogenized with 4 vol. of the same solution. The homogenate was centrifuged for 15 min at 9,000 *g*. Then this fraction was centrifuged at 105,000 *g* for 60 min to obtain the microsomes.

Assay and other methods. UDP-Glucuronyltransferase activities toward various substrates were assayed by the methods described in the respective references at the aglycone concentrations listed: 1-naphthol (0.5 mM) [12]; 4-nitrophenol (0.5 mM) [13]; 4-hydroxybiphenyl (0.5 mM) [14]; 4-methylumbelliferone (1.0 mM) [15]; and bilirubin (0.1 mM) [16]. UDP-Glucose dehydrogenase activity was assayed by the methods previously described [17]. Glutathione *S*-transferase activity was assayed by

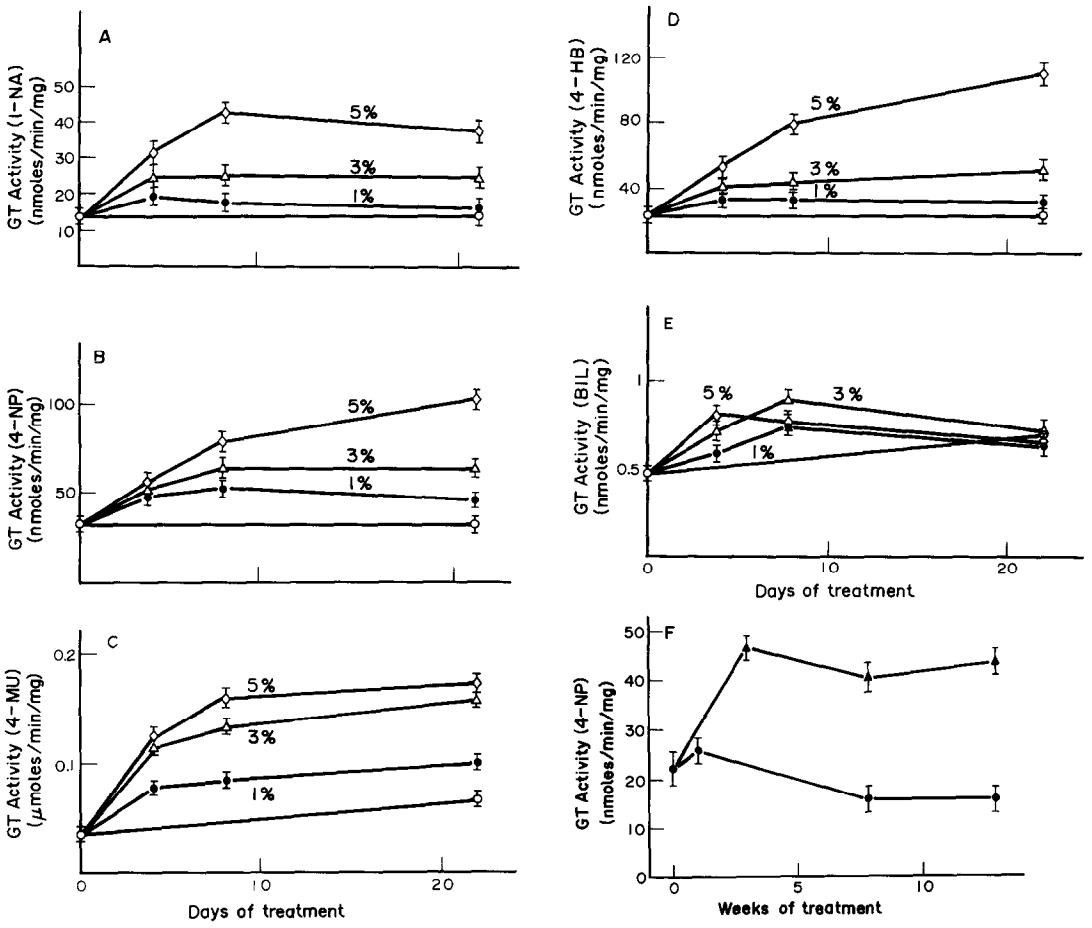

Fig. 1. Effect of dietary eugenol on hepatic microsomal UDP-glucuronyltransferase activity. Liver microsomes were prepared from rats fed a diet containing 1% (●), 3% (△), or 5% (◇) eugenol or the control diet (○) for 22 days. Enzyme activities toward 1-naphthol (A), 4-nitrophenol (B), 4-methylumbelliferone (C), 4-hydroxybiphenyl (D) and bilirubin (E) were determined in these microsomes, after activation by sodium cholate (final concentration in assay medium was 0.02%). (F): GT activities toward 4-nitrophenol in the liver microsomes from rats fed the eugenol diets (▲) or the control diet (●) for a long term. Values represent the means ± SD for three animals.

spectrophotometric methods [18]. Cytochrome P-450 was determined by the method of Omura and Sato [19]. Protein was determined by the method of Lowry *et al.* [20] using bovine serum albumin as a standard.

RESULTS

GT activities toward various substrates in liver microsomes from rats fed eugenol diets are shown in Fig. 1. The activities of GT toward 1-naphthol (Fig. 1A) and 4-nitrophenol (Fig. 1B) increased rapidly with dietary administration of eugenol, as described previously after oral administration of eugenol (200 mg, four times) for 2 days [9, 21]. Furthermore, dose-dependent effects of eugenol on these increases in GT activities were observed, and the increased activities were maintained throughout the term of eugenol treatment (Fig. 1, panels A and B). Activity of GT toward 4-hydroxybiphenyl, which has been reported not to be increased by oral administration of eugenol [21], and activity toward 4-methylumbelliferone were also increased by dietary administration. The dose response and the maintenance of this high activity during eugenol treatment are illustrated in Fig. 1, panels D and C respectively. The high activities of GT resulting from the eugenol diets were maintained for a long time, at least 13 weeks (Fig. 1F). Bilirubin glucuronidation activity in these microsomes, however, was almost unchanged by eugenol (Fig. 1E). Similar effects of eugenol were observed on cytosolic UDP-glucose dehydrogenase activity (Fig. 2, panels A and B). We found that glutathione *S*-transferase activities toward CDNB and DCNB, which enzyme had been reported to play an important role in the detoxification of carcinogens, were increased markedly by dietary administration of eugenol (Fig. 3, panels A and B). The high activity of the conjugation reactions of the xenobiotics continued throughout the period of treatment (13 weeks) (Fig. 3C). In spite of the induction of the two detoxification enzymes conjugating many ultimate carcinogens, the content of cytochrome P-450 in the same microsomes was not increased during the 13 weeks. γ-Glutamyl transpeptidase activity, which is known as one of the most useful hepatic marker enzymes for chemical carcinogenesis, was not changed significantly in our experiments by this treatment (data not shown). The body weights and the liver weights of eugenol-treated rats reached 84 and 93% of control rats by week 13.

DISCUSSION

As shown in Fig. 1, panels A–D, dietary administration of eugenol induced GT activity toward various xenobiotics in addition to activity toward phenolic substances, such as 1-naphthol and 4-nitrophenol, which had been reported previously to be induced by oral administration of eugenol for 2 days (200 mg × four times) and 3-methylcholanthrene treatment [21]. Dietary eugenol also induced activity toward 4-hydroxybiphenyl, which had been reported to be induced by phenobarbital treatment [21], and toward 4-methylumbelliferone. GST, which is known to play an important role in detoxification of xenobiotics and chemical carcinogens, was also induced in liver of rats treated with eugenol (Fig. 3, panels A–C). Inducers such as 3-methylcholanthrene, phenobarbital and PCBs, which increase the conjugation enzyme systems, also induce cytochrome P-450 content and monooxygenase activities. Eugenol is a specific and continuous inducer of the detoxifying enzymes for xenobiotics, since it rapidly increases the GT, DH and GST activities (Figs. 1–3) without increasing the content of cytochrome P-450 as previously described for BHA and BHT [22]. It is well known that dietary administration of BHA and BHT, and of some other food additive antioxidants, protects against tumor induction by chemical carcinogens of diverse structures [23]. Batzinger *et al.* [4] reported that BHA reduces the mutagenic activity arising from B[*a*]P and several drugs. The protective effect of BHA has been attributed to the marked elevation of enzyme activities catalyzing the conjugation reactions, which may lead to a move and shift of the metabolic profile in such a manner as to lower the intracellular concentration of biological reactive intermediates [22]. Recently, we reported that mutagenic activity arising from B[*a*]P is suppressed by liver microsomes from eugenol-treated rats, and this suppression was attributed to the reduction of B[*a*]P hydroxylation reactions catalyzed by specific molecular forms of

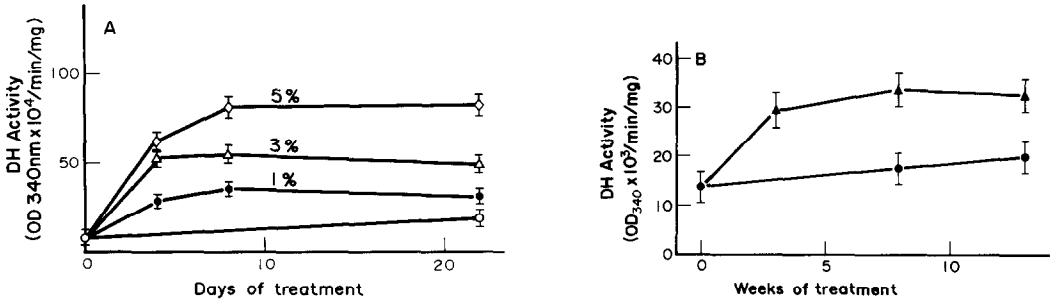

Fig. 2. Effect of dietary eugenol on UDP-glucose dehydrogenase activity in the liver cytosol of rats. (A): The liver cytosol fractions were prepared from rats fed the diet containing 1% (●), 3% (△) or 5% (◇) eugenol (w/w) or the control diet (○) for 22 days. (B): DH activity in the liver cytosol from rats fed the eugenol diets (▲) or the control diet (●) for a long term. Values represent the means ± SD for three animals.

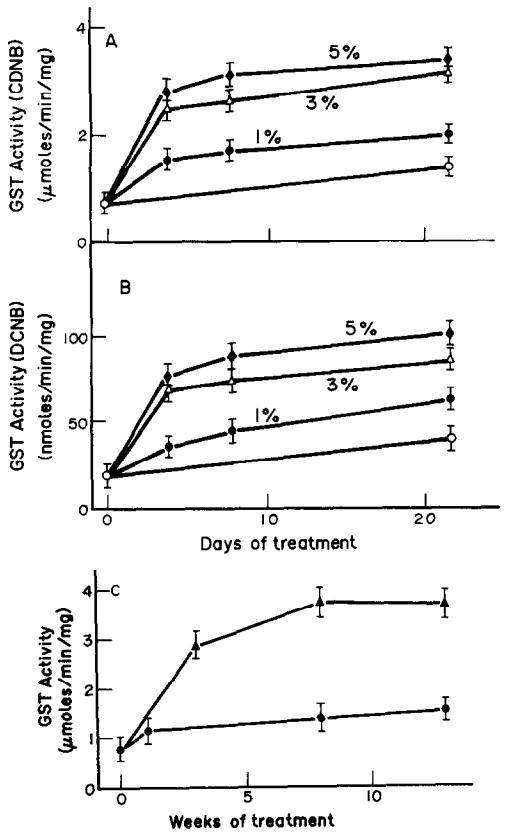

Fig. 3. Effect of dietary eugenol on glutathione *S*-transferase activity in liver cytosol of rats. (A and B): The liver cytosol fractions were prepared from rats fed diets containing 1% (●), 3% (△) or 5% (◆) eugenol (w/w) or a control diet (○) for 22 days. (C): GST activities toward CDNB in the liver cytosol from rats fed the eugenol diets (▲) or the control diet (●) for a long term. Enzyme activities were determined with two substrates: CDNB (A and C) and DCNB (B). Values represent the means ± SD for three animals.

cytochrome P-450 [10]. Decreased monooxygenase [24] and increased epoxide hydrase [25], GT [26] and GST [27] activities during hepatocarcinogenesis have been reported. Elevations by eugenol of these detoxification enzyme systems inherent in living cells may then confer protection from environmental toxic agents including chemical carcinogens such as B[*a*]P.

Eugenol is a widely used flavor additive and chemical intermediate. No evidence of carcinogenicity due to eugenol has been observed in male or female rats, and eugenol did not inhibit or induce a mutagenic response observed in mutagenesis studies using *Salmonella typhimurium* [10, 28]. Eugenol may therefore be a safe and non-toxic inhibitor of carcinogenesis.

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