Effect of organosulfur compounds on the expression of UDP-glucuronosyltransferase and thyroid hormone level in TCDD-treated rats

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Abstract

In this study, we investigated the effect of organosulfur compounds including, diallyl sulfide (DAS), garlic extract, and dially disulfide (DADS) on inducible expression of UDP-glucuronosyltransferase (UGT1) and thyroid hormone level in 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treated rats. We measured UGT1 gene expression in rat liver and lung tissues after treatments. We found that a correlation was found to exist between UGT1 gene expression and active T_4 concentrations. The total concentration of plasma T_4 decreased in TCDD-alone treated male and female rats compared to control animals (TCDD-alone < control). Plasma T_4 concentration in TCDD + DAS, TCDD + garlic extract and TCDD + DADS treated groups were moderately higher than in TCDD alone animals, but lower than control animals. TCDD + DAS, TCDD + garlic extract and TCDD + DADS moderately increased T_4 glucuronidation and moderately reduced total T_4. The nonthyroidal nature of thyroid hormone reduction observed in this study extends the evidence for microsomal enzyme inducers as indirect thyroid tumor promoters in rats. Furthermore, the results of present study suggest protective effects of DAS, garlic extract, or DADS on TCDD-induced changes.

Key words: UDP-glucuronosyltransferase, 2,3,7,8-tetrachlorodibenzo-p-dioxin, dially sulfide, garlic extract, dially disulfide

Introduction

The ubiquitous existance of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans in the environment and their accidental exposure have raised concerns about the possible threat to human health. The potent isomer 2,3,7,8-tetrachloro-dibenzo-p-dioxin (TCDD) is a multi-site carcinogen in rodents and is classified as a tumor promoter in liver and extrahepatic tissues. TCDD increases tumor incidences in the lung, nasal turbinates/hard palate, adrenal cortex, and thyroid tumors in rodents (Huff *et al*., 1991). In particular, the incidence of thyroid tumors in rodents is comparable to or greater than that of liver tumors at equivalent doses (National Toxicology Program, 1982). It is generally believed that TCDD produces its effects by altering gene expression in target cells.

The hepatic microsomal UDP-glucuronosyltransferases (UGTs), a major family of phase II drug-metabolizing enzymes, catalyze the transfer of the glucuronic acid moiety from UDP-glucuronic acid to numerous chemicals containing hydroxyl, carboxyl, amino, imino, or sulfhydryl group (Tephly and Burchell, 1990). Elimination of many hydrophobic xenobiotics and/or metabolites is facilitated by their conjugation with hydrophilic UDP-glucuronic acid, catalyzed by a family of enzymes known as UGTs (Burchell and Coughtrie, 1989). Thus, UGTs may play an important cytoprotective role, either by preventing the accumulation of potentially toxic xenobiotics or metabolites or by avoiding their subsequent bioactivation (Muller *et al*., 1990). The UGTs are divided into two gene families, termed UGT1 and UGT2, based upon a cDNA sequence comparison. The UGT1 family consists of several isozymes with differing amino-terminal domains and an identical carboxyl-terminal domain. The variable amino-terminal portions of UGT1 proteins are thought to be responsible for the aglycone specificity of the isozymes. In rats, UGT enzymes have been tentatively divided into two subfamilies based upon similarities in amino acid sequences of the variable regions (UGT1A and UGT1B). The isozyme UGT1A, which contains the amino-terminal portion encoded by the first exon of 1A, has been identified as a 4-nitrophenol UGT (Emi *et al*., 1995).

Thyroid hormone plays a very important role in brain development (Porterfield and Hendrich, 1993), especially during the early postnatal period (Davenport *et al*, 1976). The presence of adequate circulation of T_4 concentration is especially critical for normal brain development, because T_3 does not cross the blood brain barrier (Ruiz *et al*., 1988;

Calvo *et al*., 1990). The induction of hepatic UGT1 by TCDD and other chlorinated hydrocarbones increases thyroxine glucuronidation (Henry Gasiewicz, 1987; Van Birgelen *et al*., 1993; Lans *et al*., 1993) and biliary excretion of T_4-glucuronide resulting in decreased levels of circulating T_4 (Van Birgelen *et al*., 1994). Although the mechanism by which TCDD induces thyroid tumors in rodents has not been clearly established, studies with other inducers of hepatic microsomal enzymes support the tentative finding that elevated levels of thyroid-stimulating hormone (TSH) lead to thyroid follicular cell hyperplasia and hypertrophy, and that if stimulation is prolonged, these changes may progress to neoplasia (Hill *et al*., 1989; McClain *et al*., 1989).

The current study is motivated by the hypothesis that the identification and exploitation of dietary anticarcinogens may substantially contribute to the prevention of tumor formation. Organosulfur compounds present in garlic and onions, such as allyl sulfide (DAS), allyl disulfide (DADS), and allyl methyl di- and trisulfide, and garlic and onion oil have been found to inhibit carcinogenesis in several organs (Belman, 1983; Wargovich *et al*., 1987; Sparnins *et al*., 1988; Wattenberg *et al*., 1989; Takahashi *et al*., 1992). Inhibition of the P450 enzymes responsible for the metabolic activation of the carcinogenic and toxic chemicals is believed to be a major mechanism of the observed chemopreventive effect of DAS (Takada *et al*., 1994; Burchell *et al*., 1995; Hong, 1997). Several organosulfur compounds inhibit carcinogenesis by increasing the metabolism, detoxification, and elimination of carcinogen through phase I and phase II enzymes (Talalay, 1992; Wattenberg, 1992). This study was designed to examine the effect of TCDD on the thyroid hormone level and UGT1 gene expression. Furthermore, we examined the protective effects of DAS, garlic extract, or DADS on TCDD-induced changes.

Materials and Methods

Chemicals

$[\alpha-^{32}P]$dCTP (3,000 Ci/mmol) was obtained from Amersham (Buckinghamshire, UK). TCDD was perchased from AccuStandard (New Haven, USA). Diallyl sulfide and diallyl disulfide were purchased from Aldrich (Milwaukee, USA). Garlic extract was kindly provided by Bolak Co. (Hwasung-kun. Korea). All other chemicals were obtained from Sigma Chemical (St. Louis, MO, USA).

Treatment of animals and analysis thyroid hormones

Male and female Sprague-Dawley rats weighing 180-200 g were maintained on the standard laboratory chow under the controlled temperature with 12-h light and dark cycles. After an acclimation period of 1 week, the rats were given TCDD (10 μg/kg body weight, in corn oil) to the experimental group and corn oil (5 ml/kg body weight) to the control by oral gavage. After 4 days, DAS (200 mg/kg body weight in corn oil), garlic extract (200 mg/kg body weight in distilled water) or DADS(50 mg/kg body weight in corn oil) was administered orally. At 18 h after the last treatment, the rats were lightly anesthetized with ether and 1.5 ml of blood was drawn from heart with a 22-gauge needle and 0.5 ml syringe. The plasma was separated by centrifugation and stored at -70°C prior to determination of total T_4 and T_3 levels by radioimmunoassay with kits purcharsed from Abbott Laboratories (North Chicago, USA).

Preparation of tissue microsomes and determination of UGT1 activity

After withdrawal of blood, tissues were removed and weighed. Microsomes were prepared by ultracentrifugation (100,000 *g* for 60 min) of the postmitochondrial supernatant (10,000 *g* for 20 min) from a 25% (w/v) tissue homogenate as described (Lu and Levin, 1972) and stored at -70°C. Protein concentration was determined by the method of Lowry (Lowry *et al*, 1951). UGT activity toward 4-nitrophenol was measured by the method of Burchell and Weatherill (1981).

RNA isolation and hybridization analysis

Total RNA was isolated from the liver using a RNA isolation kit (Ultraspec-II RNA, Biotecx Laboratories, Houston, USA). For Northern analysis, electrophoresis was carried out on a 1% agarose gel containing 2.2 M formaldehyde, and then the RNA was transferred on to a nylon membrane (S&S) in $20\times$ SSC and were immobilized by UV irradiation. The membrane was prehybridized in $6\times$ SSC, 50% deionized formamide, $10\times$ Denhardt's solution, 0.5% SDS, 0.4 mg/ml denatured herring sperm DNA at 44°C for 24 h, and hybridization was performed at 44°C for 48 h using the selective cDNA probe for exon 1 of UGT1A1. The cDNA probes were labeled with $[\alpha-^{32}P]$dCTP using the random-primed labeling system. The membrane was washed twice in $2\times$ SSC, 0.1% SDS at 50°C for 20 min. The membrane was exposed for 3 days at -70°C to Kodak XAR-5 film with intensifying screens. The bands on the autoradiographies were quantified by using Bio-imaging analyzer system (BAS 2500, Fuji Photofilm Co.).

Statistical analysis

Results were expressed as mean ± SD. Statistical analyses were performed using the student t-test.

Results

Thyroid hormone concentration

The total concentration of plasma T_4 is shown in Table

1. In male rats, the plasma T_4 concentration was significantly lower for the TCDD-alone animals compared to control group animals (TCDD alone < control). In the TCDD + DAS group and TCDD + garlic extract group, the T_4 concentrations were also decreased as much as in the TCDD-alone treated group (TCDD alone = TCDD + DAS; TCDD + garlic extract). However, the concentration for the TCDD + DADS treated animals was higher than for TCDD-alone group (but still lower than in control group animals; TCDD alone < TCDD + DADS < control). In female rats, the T_4 concentration for the TCDD-alone group was also significantly lower than control group animals. The concentration for TCDD + DAS, TCDD + garlic extract, and TCDD + DADS groups were all moderately higher than TCDD alone animals (but lower than control animals; TCDD alone < TCDD + DADS < control). We compared female rats with male rats in respect to their T_4 concentration. In both, the TCDD + DAS and TCDD + garlic extract groups, the T_4 concentration was higher in female than in male rats.

The total concentration of plasma T_3 is shown in Table 2. The concentration for TCDD-alone group (male) was also much lower than control animals. The concentration for the TCDD + garlic extract group was higher than for TCDD-alone group, but not as high as control group. The

T_3 concentration in female rats was reduced only for TCDD-alone treated group. The TCDD + DAS, TCDD + garlic extract, and TCDD + DADS groups showed much higher T_3 concentration than TCDD-alone as well as the control group.

4-Nitrophenol UGT1 activity and expression

4-Nitrophenol UGT1 activity is shown in Table 3 and Table 4. In male rat liver microsomes, 4-nitrophenol UGT1 activity was significantly increased for the TCDD alone group. Furthermore, the TCDD + DAS group shows higher activity than does the TCDD-alone group. The activity for TCDD + garlic extract and TCDD + DADS groups were similar to TCDD-alone group. In male rat lung microsomes, the activity was also increased, but the amount of increased activity was smaller than in liver microsomes. In female rats, the UGT activity in liver microsomes was much higher than in control group. The activity for TCDD + DAS, TCDD + garlic extract, and TCDD + DADS groups were higher than in TCDD-alone group.

4-Nitrophenol UGT1 gene expression is shown in Figures 1 and 2. We measured UGT1 gene expression in rat liver RNA after treatment. In male rat liver RNA, UGT1 expression was relatively high for the TCDD +

Table 1. The effect of DAS, garlic extract or DADS on plasma total T_4 concentration.

Group	Male (μg/100 ml plasma)	Female (μg/100 ml plasma)
Control	4.08 ± 0.52	3.96 ± 0.24
TCDD	1.27 ± 0.28[a]	0.99 ± 0.20[a]
TCDD+DAS	1.24 ± 0.13[a]	2.56 ± 0.50[b,d]
TCDD+garlic extract	1.54 ± 0.22[a]	2.01 ± 0.16[a,c]
TCDD+DADS	2.16 ± 0.54[b]	1.97 ± 0.47[a,e]

[a] Significantly different from control value, $P < 0.001$; [b] significantly different from control value, $P < 0.01$; [c] significantly different from TCDD value, $P < 0.001$; [d] significantly different from TCDD value, $P < 0.01$; [e] significantly different from TCDD value, $P < 0.05$

Table 2. The effect of DAS, garlic extract or DADS on plasma total T_3 concentration.

Group	Male (ng/100 ml plasma)	Female (ng/100 ml plasma)
Control	84.50 ± 4.07	58.84 ± 5.48
TCDD	64.95 ± 3.55[b]	54.12 ± 2.34
TCDD+DAS	47.28 ± 5.32[a,d]	63.99 ± 1.15[a]
TCDD+garlic extract	70.18 ± 3.49[b]	76.69 ± 5.93[b,c]
TCDD+DADS	59.86 ± 4.03[a]	62.03 ± 7.23

[a] Significantly different from control value, $P < 0.001$; [b] significantly different from control value, $P < 0.01$; [c] significantly different from TCDD value, $P < 0.01$; [d] significantly different from TCDD value, $P < 0.05$.

Table 3. The effect of DAS, garlic extract or DADS on 4-Nitrophenol UDP-glucuronosyltransferase activity in TCDD treated male rat liver, lung microsomes.

Group	Liver (nmol/mg protein/min)	Lung (nmol/mg protein/min)
Control	8.81 ± 0.467	2.17 ± 0.384
TCDD	22.51 ± 0.654[a]	3.67 ± 0.120[a]
TCDD+DAS	31.88 ± 1.447[a,b]	6.64 ± 0.150[a,c]
TCDD+garlic extract	19.22 ± 2.028[a,c]	2.81 ± 0.322
TCDD+DADS	21.28 ± 0.788[a]	3.92 ± 0.455[a]

[a] Significantly different from control value, $P < 0.001$; [b] significantly different from control value, $P < 0.01$; [c] significantly different from TCDD value, $P < 0.01$; [d] significantly different from TCDD value, $P < 0.05$.

Table 4. The effect of DAS, garlic extract or DADS on 4-Nitrophenol UDP-glucuronosyltransferase activity in TCDD treated female rat liver, lung microsomes.

Group	Liver (nmol/mg protein/min)	Lung (nmol/mg protein/min)
Control	8.15 ± 0.289	2.52 ± 0.370
TCDD	24.18 ± 0.404[a]	4.83 ± 0.020[b]
TCDD+DAS	25.87 ± 0.186[a,c]	5.18 ± 0.315[b]
TCDD+garlic extract	26.02 ± 0.092[a,c]	4.35 ± 0.045[b,d]
TCDD+DADS	27.41 ± 0.812[a,c]	6.15 ± 0.785[b]

[a] Significantly different from control value, $P < 0.001$; [b] significantly different from control value, $P < 0.01$; [c] significantly different from TCDD value, $P < 0.01$; [d] significantly different from TCDD value, $P < 0.05$.
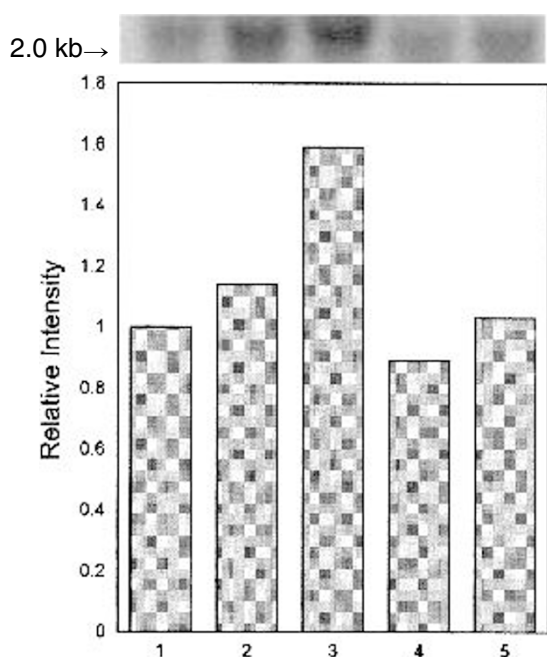
Figure 1 Northern blot analysis for expression of UGT1 mRNA. RNA was extracted from liver tissues from TCDD and TCDD + DAS, garlic extract, DADS treated male rat. RNA (20 μg) was separated in a 1% agarose/2.2M formaldehyde gel, transferred to nylon membrane and hybridized with ^{32}P-labeled cDNA. Lane 1, corn oil; lane 2, TCDD; lane 3, TCDD + DAS; lane 4, TCDD + garlic extract; lane 5, TCDD + DADS.
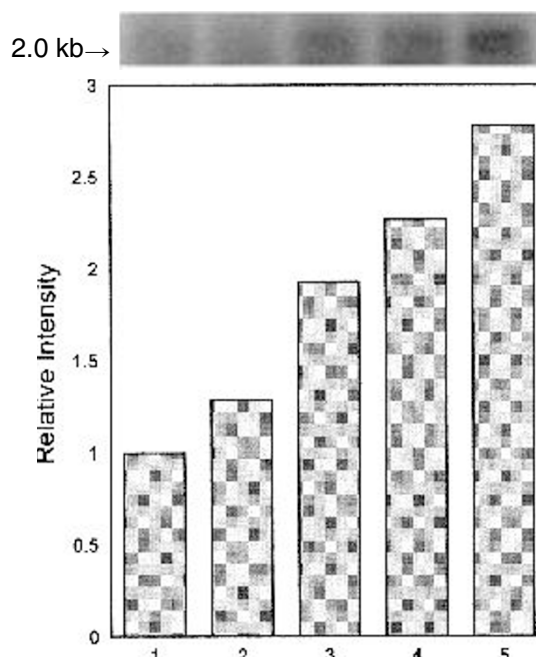
Figure 2. Northern blot analysis for expression of UGT1 mRNA. RNA was extracted from liver tissues from TCDD and TCDD + DAS, garlic extract, DADS treated female rat. RNA(20 μg) was separated in a 1% agarose/2.2M formaldehyde gel, transferred to nylon membrane and hybridized with ^{32}P-labeled cDNA. Lane 1, corn oil; lane 2, TCDD; lane 3, TCDD + DAS; lane 4, TCDD + garlic extract; lane 5, TCDD + DADS.

DAS group.

Discussion

Several hepatic microsomal enzyme inductions have been shown to induce alterations in thyroid hormone levels and thyroid gland function. Microsomal enzyme inducers such as TCDD and polychlorinated and polybrominated biphenyl have been shown to reduce circulating thyroid hormone levels (Allen-Rowlands, 1981; Gorski and Rozman, 1997). This reduction of thyroid hormone levels can lead to a stimulation of the thyroid gland by thyroid-stimulating hormone (TSH) through the normal thyroid feedback loop. It has been postulated that thyroid tumor promotion produced by TCDD is not due to a direct effect on the thyroid gland, but rather due to an alteration of the degradation and elimination of thyroid hormones, leading to a stimulation of the thyroid by TSH (McClain *et al*., 1989). Chronic administration of many microsomal enzyme inducers has been shown to produce thyroid hypertrophy and hyperplasia (Hill *et al*., 1989).

Thyroid hormones are degraded predominantly in the liver and excreted in bile. A major pathway of thyroid hormone biotransformation is glucuronidation of thyroxine (T_4). The microsomal enzyme, UGT catalyzes the forma-

tion of T_4-glucuronide, which is subsequently excreted into bile. The UGTs consist of a family of related enzymes that differ in their substrate specificities and their ability to be induced upon treatment with various chemicals. UGTs are involved in the elimination of thyroxine as T_4-glucuronides, the main pathway for metabolic inactivation of T_4 in rats. Moreover, good correlations have been found between decreased plasma thyroid hormone concentrations and induction of T_4-UGT by 3-methyl-cholanthrene, TCDD and 2,3,3',4,4',5-hexachlorobiphenyl (Van Birgelen *et al*., 1993). Visser *et al.* (1993) reported that UGT1 (phenol UGT) is involved in the glucuronidation of T_4.

It is therefore possible that an increase in the glucuronidation of T_4 is responsible for the observed reduction of plasma levels of UGT activity after treatment with TCDD, TCDD + DAS and TCDD + garlic extract (see Results).

We have analyzed the inducibility of UGT1 gene by TCDD, TCDD + DAS, garlic extract and DADS in rat liver and lung. DAS prevented *N*-nitrosomethylbenzylamine-induced esophageal tumor formation (Wargovich *et al*., 1992), benzo(α)pyrene induced forestomach neoplasia (Sparnins *et al*., 1988), and dimethylhydrazine-induced colonic adenocarcinoma (Wargovichl., 1987) when given by intragastric intubation prior to carcinogen challenge. DADS also prevented *N*-nitrosodiethylamine-induced forestomach neoplasia when given prior to the carcinogen

(Wattenberg *et al*., 1989).

The modulation of metabolism of carcinogen by DAS or DADS was considered as one possible mechanism for the protection against cancer by several author (Wargovich, 1987; Sparnins *et al*., 1988; Reddy *et al*.,1993). Several studies showed that allyl sulfides can modulate hepatic drug-metabolizing enzymes. This study showed that administration of TCDD + DAS, garlic extract or DADS produces different effects in rat liver and lung. The difference in the metabolism of DAS and DADS could also account for their different protective properties on the initiation phase of chemically-induced cancers. It seems that the chemopreventive effects of allyl sulfides on chemical-induced carcinogenesis depend on the compound, the carcinogen, and the organ site investigated.

Both allyl sulfides modified the same parameters in liver and lung. Both allyl sulfides increased phase II enzymes, UGT. The present results, together with previous results from several laboratories, suggest that alteration of thyroid function may be secondary inducible expression gene of UGT1. Thus, microsomal enzyme inducers may reduce thyroid hormone levels by an extrathyroidal mechanism, and their actions may be more complex than previously assumed.

Based on our finding that UGT1 expression and sensitivity for cancer was variable in presence of DAS, garlic extract, DADS according to treated animal gender and tissue (see Results), we conclude that UGT1 gene expression by DAS and garlic extract may contribute to individual variations in cancer suceptibility. By modifying UGT1 expression, DAS and garlic extract may modulate the ability of UGT1 to detoxify carcinogens.

Acknowledgement

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