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Diallyl sulfide induces the expression of estrogen metabolizing genes in the presence and/or absence of diethylstilbestrol in the breast of female ACI rats

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Abstract

Diethylstilbestrol (DES) induces mammary tumors in female ACI rats and is associated with an increased risk of developing breast cancer in humans. Diallyl sulfide (DAS) has been shown to prevent cancer in animals. Previously, we have shown that DAS inhibits the production of DES induced DNA adducts when given prior to DES. We hypothesize that DAS alters the expression of genes responsible for DES metabolism. To test this hypothesis, four groups of 10 female ACI rats were treated daily for four days as follows: (1) corn oil, (2) 50 mg/kg DES, (3) 50 mg/kg DAS, and (4) 50 mg/kg DAS + 50 mg/kg DES. RNA was isolated from breast tissue and mRNA levels of CYP1A1, CYP1B1, glutathione-S-transferase (GST) and superoxide dismutase (SOD) were analyzed by real-time PCR. DES, DAS, and DES/DAS treatments increased the expression of CYP1A1 by 2.1-, 4.7-, and 12.7-fold, respectively. Similar results were seen for CYP1B1. DES decreased the expression of GST by 23%, whereas DAS and DAS/DES treatments increased the expression of GST by 12- and 16.7-fold, respectively. Similar results were seen with SOD. These results suggests that DAS may prevent the formation of DES induced DNA damage by altering the expression of DES metabolizing genes.
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Keywords: Diethylstilbestrol; Diallyl sulfide; Breast cancer; Chemoprevention; Garlic; Metabolism

1. Introduction

Diethylstilbestrol (DES) is a hormonal chemical that mimics the activity of estrogens and has been shown to increase the risk of breast cancer in women (Marselos and Tomatis, 1992). DES has also been shown to produce breast cancer in female ACI rats (IARC, 1979). The mechanism of estrogen-induced cancer is not clearly understood. However, recent data suggest that estrogen metabolism plays a significant role in the etiology of breast cancer (Williams and Phillips, 2000). We have demonstrated that microsomes, mitochondria and nuclei isolated from the breast of female ACI rats catalyze oxidation and reductions of DES (Thomas et al., 2004). These reactions produce reactive oxygen species and DES quinones, which damage DNA resulting in genomic instability (Gladek and Liehr, 1989; Roy and Thomas, 1994). In addition, DES has been shown to decrease the levels of phase II metabolism enzymes such as glutathione-S-transferase (GST), which plays a role in eliminating reactive intermediates that

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damage DNA (Segura-Aguilar et al., 1990; Kondo et al., 2002).

Epidemiological studies have shown that the consumption of garlic is associated with decreased incidence of breast, stomach, and colorectal cancers in humans (Challier et al., 1998; Dorant et al., 1995; Fleischaurer et al., 2000). Diallyl sulfide (DAS), an organosulfur compound found in garlic, has been shown to inhibit various types of chemically-induced cancers in several animal models (Wargovich et al., 1988; Sumiyoshi and Wargovich, 1990; Srivastava et al., 1997). One proposed mechanism by which DAS exerts it's anticarcinogenic activity is through the metabolic modulation of phase I enzymes (Guyonnet et al., 2002). DAS has also been demonstrated to induce phase II enzymes, including GST, uridine diphosphate glucuronosyl transferase (UGT), and epoxide hydrolases resulting in increased detoxification (Hu and Singh, 1997; Wu et al., 2002).

The female ACI rat is a good model for studying estrogen-induced breast cancer because they develop breast cancer upon exposure to estrogens, including 17-beta estradiol and DES, and have a low rate of developing spontaneous tumors (IARC, 1979). We chose DES as our carcinogenic test compound because it has estrogenic activity similar to that of 17-beta estradiol and has been shown to be associated with breast cancer in both animals and humans (Marselos and Tomatis, 1992).

We have shown that DAS inhibits DES-induced lipid peroxidation and DES induced DNA adducts in the breast of female ACI rats (Gued et al., 2003; Green et al., 2005).

Furthermore, we have shown that DAS inhibits the metabolism of DES *in vitro* (Thomas et al., 2004). We propose that DAS inhibits DES induced lipid peroxidation and DES induced DNA adducts by altering the expression of genes related to estrogen (DES) metabolism. Although, the effects of DAS on metabolizing genes have been investigated in both hepatic and extrahepatic organs (Wu et al., 2002; Haber et al., 1994) the effect of DAS on the regulation of metabolizing genes in the breast has not been investigated. Therefore, the objective of this study was to investigate the effect of DAS on the expression of phase I and phase II metabolizing genes involved in estrogen metabolism in the breast of female ACI rats.

2. Materials and methods

2.1. Chemicals

DES, RNAlater and DAS were purchased from Sigma Chemical Company (St. Louis, MO). Trizol LS reagent was purchased from Invitrogen (Carlsbad, CA). Brilliant SYBR Green Real-Time PCR Master Mix (2X) and Stratascript First Strand cDNA Synthesis System were purchased from Stratagene (La Jolla, CA). Rat cytochrome P450 1A1 (CYP1A1) and cytochrome P450 1B1 (CYP1B1). glutathione-S-transferases pi (GSTpi), and superoxide dismutase (SOD) primers were ordered from SuperArray, Inc. (Frederick, MD). Female ACI rats (5–6-weeks-old) were purchased from Harlan Sprague Dawley Inc. (Indianapolis, IN).

2.2. Animal treatment

Four groups of 10 ACI rats were analyzed for the expression of phase I metabolizing genes (CYP1A1 and CYP1B1) and phase II metabolizing genes (GST and SOD). The rats were treated daily for four days *via* i.p. injections as follows: (1) corn oil (control), (2) 50 mg/kg DES, (3) 50 mg/kg DAS, and (4) 50 mg/kg DAS + 50 mg/kg DES. The rats were sacrificed on day 5. The breast tissue was removed and immediately placed in RNAlater and stored in at −80 °C. Total RNA was isolated from breast tissue and analyzed for expression using real-time PCR.

2.3. RNA isolation

Fifty milligrams of breast tissue were weighed and homogenized in the presence of 0.75 ml of Trizol LS Reagent. Following homogenization, the samples were incubated at room temperature for five minutes. Chloroform (0.2 ml) was added to the homogenate and vortexed vigorously for one minute. The homogenate were incubated at room temperature for 15 min followed by centrifugation at 12,000 × *g* for 15 min at 4 °C. The upper aqueous layer containing RNA was transferred to a new RNase free tube without disturbing the interphase layer. RNA was precipitated by adding 0.5 ml isopropyl alcohol. The samples were incubated at room temperature for 10 min and centrifuged at 12,000 × *g* for 10 min at 4 °C. The RNA pellet was washed with 1ml of 75% ethanol. The pellets were air-dried and reconstituted in 25 μl of RNase-free water. The quality of RNA was determined by measuring the $OD_{260/280}$ ratio with UV–vis spectrophotometry. RNA purified by this method resulted in $OD_{260/280}$ ratio of >1.7. The quality of RNA was further determined by formaldehyde agarose gel electrophoresis. The presence of 18S and 28S rRNA indicated high quality with low degradation.

2.4. cDNA synthesis

The cDNA was synthesized using the Stratascript First-Strand cDNA Synthesis System for RT-PCR. Ten micrograms of RNA was adjusted to a volume of 38 μl with DEPC-treated water. Three microliters of Oligo (dT) (100 ng/μl) was added to the reaction and mixed gently. The reaction was incubated at 65 °C for 5 min. The reactions were slowly cooled at room temperature (∼10 min) to allow the primers to anneal to the RNA. The first cDNA strand was synthesized by adding 5 μl of 10×

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