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Diallyl sulfide inhibits diethylstilbesterol-induced DNA adducts in the breast of female ACI rats

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Abstract

Diethylstilbestrol (DES) is metabolized to reactive intermediates that produce DNA adducts and ultimately cancer. Diallyl sulfide (DAS) has been shown to inhibit the metabolism of several procarcinogens. The ability of DES to produce DNA adducts in microsomal, mitochondrial, and nuclear *in vitro* metabolic systems and in the breast of female ACI rats, as well as ability of DAS to inhibit DNA adducts were investigated. Microsomes, mitochondria, and nuclei isolated from breast tissue of female ACI rats were used to catalyze oxidation reactions. Female ACI rats were treated i.p. as follows: (1) corn oil, (2) 200 mg/kg DES, (3) 200 mg/kg DES/200 mg/kg of DAS, (4) 200 mg/kg DES/400 mg/kg DAS. DES produced DNA adducts in each metabolic system. The relative adduct levels were 2.1×10^{-4}, 6.2×10^{-6}, and 2.9×10^{-7} in microsomal, mitochondrial, and nuclear reactions, respectively. DAS inhibited DNA adducts in each metabolic system. The percent inhibition ranged from 86% in microsomes to 93% in nuclei. DES produced DNA adducts in mtDNA and nDNA. DAS completely inhibited the DES-induced mtDNA adducts and caused a dose dependent decrease in nDNA adduct formation. These findings suggest that DAS could inhibit DES-induced breast cancer by inhibiting its metabolism.

Keywords: Diethylstilbesterol; Diallyl sulfide; Chemoprevention; DNA adducts

1. Introduction

Among women ages 30–60, breast cancer is the second leading cause of death (Greenlee et al., 2001). It has been demonstrated that exposure to estrogens increase the risk for breast cancer (Adami et al., 1989). Diethylstilbesterol (DES) is a hormonal chemical that mimics the activity of estrogen. DES has been shown to produce cancer in humans, as well as in animals, particularly breast cancer (Herbst, 1984; Greenberg et al., 1984). The mechanism of estrogen-induced breast cancer is not clearly understood. It was believed that the overstimulation of the estrogen receptor resulted in cell tranformation by altering gene regulation, protein synthesis, and cell growth (Jenson, 1992). However, a lack of correlation between estrogenecity and tumor incidence suggest that factors other than estrogen potency are involved in estrogen-induced neoplasm (Roy and Liehr, 1990). Recent data suggests estrogen metabolism plays a significant role in carcinogenesis. DES undergoes redox-cycling which leads to the formation of reactive intermediates, such as DES-semiquinone and DES quinone (Liehr and Roy, 1990; Roy and Liehr, 1992). These reactive intermediates covalently bind to DNA and nuclear proteins forming adducts (Williams et al.,

Abbreviations: DES, diethylstilbesterol; DAS, diallyl sulfide; dAP, deoxyadenosine monophosphate; dGMP, deoxyguanosine monophosphate; RALs, relative adduct levels; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; NP1, nuclease P1; PEI, polyetheleneimine; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; PMSF, phenylmethyl sulfonyl fluoride; PNK, T4 polynucleotide kinase; SPD, spleen phosphodiesterase; TLC, thin layer chromatography.

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1993; Roy and Pathak, 1993; Bhat et al., 1994). These DNA adducts may lead to the initiation of estrogen-induced carcinogenesis. Redox-cycling of DES also results in the formation of reactive oxygen species that induce DNA damage including DNA base oxidation, DNA strand breaks, chromosomal damage, gene mutations, and lipid DNA adducts (Roy and Liehr, 1999; Cavalieri et al., 2000). In addition to initiation, the mitogenic action of estrogen could stimulate the promotion and progression of estrogen induced cancer (Fishman et al., 1995; Hiraku et al., 2001).

Traditionally, metabolic studies have used microsomes. However, it has been demonstrated that mitochondria and nuclei have the metabolic capabilities to metabolize DES to reactive intermediates (Thomas and Roy, 1995; Roy and Thomas, 1994). Recently, we have demonstrated that redox-cycling of DES occurs in microsomes, mitochondria, and nuclei in the breast of female ACI rats, suggesting that DES metabolism occurs not only in the liver, but also in the breast which may subsequently lead to breast cancer (Thomas et al., 2004).

Natural dietary products have recently received much attention as chemopreventive agents. Diallyl sulfide (DAS) is an organosulfur compound present in garlic that has been demonstrated to have potent chemopreventive properties. Diallyl sulfide has been demonstrated to inhibit various forms of chemically induced carcinogenesis (Wargovich, 1987; Wargovich et al., 1988; Sparnins et al., 1988; Hays et al., 1987). The chemopreventive properties of DAS are attributed to the modulation of metabolizing enzymes (Yang et al., 2001; Pan et al., 1993; Dragnev et al., 1995; Guyonnet et al., 1999; Guyonnet et al., 2000). We have also demonstrated that DAS inhibits the redox-cycling of DES in the breast of female ACI rats (Thomas et al., 2004).

In the present study, the ability of DAS to inhibit the formation of DES induced DNA adducts was investigated in in vitro metabolic systems (mitoplasts, microsomes, and nuclei) as well as in female ACI rats. This study provides direct evidence that DAS inhibits the formation of DES-induced DNA adducts. The ability of DAS to inhibit the formation of DES-induced DNA adducts is significant in that it may help explain the role of estrogen metabolism in DES induced cancer. This data will provide a rationale to investigate DAS as a possible chemopreventive agent in estrogen-induced cancers such as breast, cervical and uterine cancer.

2. Materials and methods

2.1. Chemicals

Diethylstilbesterol (DES), diallyl sulfide (DAS), β-naphthoflavone, chloroform:isoamyl alcohol (24:1), phenol:chloroform:isoamyl alcohol (25:24:1), Proteinase K, Ribonuclease A, sucrose, phenylmethyl sulfonyl fluoride (PMSF), micrococcal nuclease, zinc chloride, sodium acetate, sodium succinate, CHES buffer, urea, lithium formate, sodium phosphate, deoxymonophosphate (DAP), and deoxyguanine monophosphate (dGMP) were purchased from Sigma Chemical Company (St. Louis, MO); carrier-free (^{32}P) phosphate in water was purchased from MP Biomedicals (Irvine, CA); nuclease P1 (NP1) and calf spleen phosphodiesterase (SPD) were purchased from EMD Biosciences, Inc. (La Jolla, CA); T4 polynucleotide kinase (PNK) was purchased from US Biologicals (Swampscott, MA); and polyetheleneimine (PEI)-cellulose TLC plates were purchased from Fisher Scientific (Savannah, GA).

2.2. In vitro study

2.2.1. Animals

Female ACI rats weighing 100–115 g (6–7 weeks old) were purchased from Harlan (Indianapolis, IN). The rats were housed in groups of two per cage in a climate-controlled room with a 12-h light/dark cycle. The rats were provided a standard diet (Harlan Teklad, Indianapolis, IN) and water ad libitum. The studies were carried out under established federal guidelines for the care and use of laboratory animals and were approved by the Florida A&M University Animal Care and Use Committee.

Ten female ACI rats were treated with β-naphthoflavone (50 mg/kg i.p.) daily for 4 days. The rats were sacrificed by exposure to carbon dioxide. The breast tissue was dissected and homogenized in 5 volumes of 0.25 M sucrose solution containing 1.0 mM phenylmethyl sulfonyl fluoride. The homogenate was kept on ice at all times until used to isolate nuclei, mitoplasts, and microsomes by centrifugation at 4 °C.

2.2.2. Nuclei preparation

The homogenate was filtered through four layers of cheesecloth and centrifuged for 15 min at $1000 \times g$. The pellet was resuspended in 0.25 M sucrose and underlayed with 4 volumes of 2.3 M sucrose. The nuclei were pelleted by centrifugation at $100{,}000 \times g$ for 60 min. The nuclei were then washed with 0.25 M sucrose.

The purity of the nuclei was assessed by both morphological and biochemical analyses. Nuclei were stained with hematoxylin and eosin. Phase-contrast microscopy confirmed the presence of intact nuclei. The determination of cytochrome *c* oxidase (Wharton and Tzagoloff, 1967), an enzymatic marker of mitochondria, showed very low activity (2 μmol/mg protein/min). The activity in purified mitochondria ranged from 100 to 110 μmol/mg protein/min. Microsomal contamination was assessed by measuring the activity of glucose