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Polycyclic aromatic hydrocarbon-induced CYP1B1 activity is suppressed by perillyl alcohol in MCF-7 cells

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Abstract

Perillyl alcohol (POH) is a dietary monoterpene with potential applications in chemoprevention and chemotherapy. Although clinical trials are under way, POH's physiological and pharmacological properties are still unclear. In the present study, the effect of POH on polycyclic aromatic hydrocarbon (PAH)-induced genotoxicity, and the related expression were examined in MCF-7 cells. Exposure to environmental toxicant increases the risk of cancer. Many of these compounds are pro-carcinogens and are biotransformed into their ultimate genotoxic structures by xenobiotic metabolizing enzymes. CYP1A1 and 1B1 are enzymes that catalyze the biotransformation of dimethylbenz[*a*]anthracene (DMBA). Our data revealed that 0.5 μM of POH was effective in blocking DMBA-DNA binding. Ethoxyresorufin-*O*-deethylase (EROD) assay indicated that the administration of POH inhibited the DMBA-induced enzyme activity in MCF-7 cells. Enzyme kinetic analysis revealed that POH inhibited CYP1B1 but not CYP1A1 activity. Quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay also demonstrated that the monoterpene reduced CYP1B1 mRNA abundance induced by DMBA. The present study illustrated that POH might inhibit and downregulate CYP1B1, which could protect against PAH-induced carcinogenesis.

Keywords: Perillyl alcohol; Limonene; CYP1A1; CYP1B1; XRE; DMBA

Introduction

Perillyl alcohol (POH) is a dietary monoterpene found in foods such as mints, cherries, and cranberries (Belanger 1998) and is a hydroxyl derivative of limonene. Its protection against chemical carcinogenesis has been demonstrated in several animal experiments. Oral administration of POH inhibits azoxymethane (AOM)-induced colon tumorigenesis in rats (Reddy et al., 1997), 4-(methyl-nitrosoamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in mice (Lantry et al., 1997), and diethylnitrosamine (DEN)-induced liver cancer in rats (Mills et al., 1995). Topical application of POH also deters the formation of skin tumors induced by UV irradiation (Barthelman et al., 1998).

POH also have been found to suppress the growth of breast cancer cells. In both ER positive and negative breast cancer cells, POH blocks cell cycle progression through G1-phase arrest and reduces cyclin D1, E, $p21^{cip1/waf1}$ and PCNA expressions in these cells (Yuri et al., 2004). The monoterpene also triggers cytostasis and apoptosis in rat mammary carcinomas. The underlying mechanisms can be mediated by TGF-β and Smad2/Smad3 proteins (Ariazi et al., 1999). In addition, POH may curb metastasis by inhibiting angiogenesis (Loutrari et al., 2004).

Other evidence does not support POH's chemopreventive effect at the post-initiation stage of chemical carcinogenesis. Low-Baselli et al. (2000) have demonstrated the compound fails to reduce the number and size of placental glutathione 2-transferase positive foci in *N*-nitrosomorpholine-initiated

Abbreviations: FBS, fetal bovine serum; EROD, ethoxyresorufin-*O*-deethylase; AHR, aryl hydrocarbon receptor; XRE, xenobiotic response element; DMBA, 7,12-dimethylbenz[a]anthracene; PAH, polycylic aromatic hydrocarbon.

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hepatocarcinogenesis. POH's protection against carcinogenesis at a specific stage may contribute to these contradictory results.

Polycylic aromatic hydrocarbons (PAHs) are pro-carcinogens and tumor initiators. They can be generated from a number of sources, including industrial emissions, char-broiled meat, tobacco smoke, and overheated cooking oil (International Agency for Research on Cancer, 1983; Environmental Protection Agency, 1990). These compounds can be converted into genotoxic moieties by cytochrome *p*450 (CYP) 1A1 and 1B1 (Gonzalez and Gelboin, 1994). Unlike *CYP1A1*, the structure and function of the *CYP1B1* gene promoter resemble those of constitutively expressed genes (Wo et al., 1997).

The expressions of CYP1A1 and 1B1 are mediated by aryl hydrocarbon receptor (AhR), which is a cytosolic protein that can be activated by PAH. Upon activation, the ligand-bound AhR translocates to the nucleus and dimerizes with AhR nuclear translocator. This protein dimer interacts with a specific gene promoter sequence, which is known as xenobiotic responsive element (XRE), and initiates transcription of the gene. Genes encoding for CYP1A1 and 1B1 contain these elements in their promoter regions, and the transcriptional regulation of these two genes is subject to the activation of AhR (Dertinger et al., 2000; Kronenberg et al., 2000; Safe, 2001). This 'cause and effect' relationship has been exemplified in two animal models. In an AhR-knockout mouse model, benzo[*a*]pyrene fails to induce tumors in these animals (Shimizu et al., 2000). A similar result has been observed in CYP1B1-knockout mice treated with 7,12-dimethylbenz[α]anthracene (DMBA) (Buters et al., 1999). These observations illustrate the importance of the receptor and the enzymes involved in PAH-induced carcinogenesis.

The significance of CYP1 family enzymes in the etiology of human breast cancer is still unknown. Polymorphisms with augmented CYP1A1 activity in African–Americans (Taioli, 1999) or increased CYP1B1 activity in Asian women (Zheng et al., 2000) are associated with elevated breast cancer risk. A clinical study has also revealed that PAH-DNA adducts found in the non-cancerous breast tissue of female breast cancer patients are higher than those of normal women (Li et al., 1996). However, negative correlations have also been reported (Ahsan et al., 2004; Lee et al., 2003; Thyagarajan et al., 2004).

Clinical trials for the anticancer effect of POH are in progress (Greenwald, 2002). However, the database for its pharmacological and physiological properties is very limited. The present study was carried out to examine the inhibition of POH on two phase-I drug-metabolizing enzymes, which play important roles in the biotransformation of xenobiotics. The breast cancer cell line MCF-7 was used in the present study because these cells are useful for identifying chemopreventive compounds (Smith et al., 2001). These cells have similar expressions of AhR, CYP1A1, and 1B1 as the non-tumorigenic breast cells MCF-10A with simpler culturing conditions and shorter doubling time (Spink et al., 1998a, 1998b).

Materials and methods

Chemicals. POH, ethoxyresorufin, and DMBA were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals, if not stated, were acquired from Sigma Chemicals.

Cell Culture. MCF-7 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI-1640 phenol red free media (Sigma Chemicals) with 10% fetal bovine serum (Invitrogen Life Technology, Rockville, MD) and incubated at 37 °C and 5% carbon dioxide. These cells were routinely subcultured when reaching 80% of confluency. The cell density in each experiment was maintained at 5×10^2 cells/mm^2.

EROD activities in intact MCF-7 cells. The assay method was performed as previously described (Ciolino and Yeh, 1999). In brief, MCF-7 cells in 96-well plates were treated with 1 μM DMBA and various concentrations of POH. The medium was then removed, and the cells were washed twice by 100 μl phosphate-buffered saline (PBS). Ethoxyresorufin-*O*-deethylase (EROD) activity assays were then carried out. Fifty microliters of ethoxyresorufin (5 μM) and salicyclamide (1.5 mM) dissolved in PBS was added in each well and incubated at 37 °C for 15 min. The reaction was stopped by 50 μl of ice-cold methanol, and the resorufin generated was measured by a FLUOstar Galaxy microplate reader (BMG Labtechnologies, Offenburg, Germany) with an excitation wavelength at 544 nm and 590 nm as the emission wavelength. The activities were quantified against resorufin standards.

Enzyme inhibition assays. Recombinant human CYP1A1 and 1B1 proteins expressed in insect cells (Supersomes®) were purchased from Gentest Corp. (Woburn, MA, USA). Two picomoles of protein was incubated in 100 μl PBS, pH 7.2 with 400 nM ethoxyresorufin and POH in different concentrations. The reaction was initiated by 500 μM NADPH and terminated by 100 μl of ice-cold methanol after 20 min of incubation. The fluorescence was measured as described earlier.

Measurement of cell viability. Cell viability was assessed by 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetra-zolium bromide (MTT) staining as described by Mosmann (1983). Briefly, MCF-7 cells were cultured in 96-well plates at 10^4 cells per well and treated with 1 μM DMBA and various concentrations of POH for 24 h. At the end of the treatment, 50 μl MTT (1 mg/ml) was added and incubated at 37 °C for 4 h. Cell viability was determined by the absorbance at 600 nm.

Measurement of DMBA-DNA adduct formation. This assay was performed as previously described (Chan and Leung, 2003). MCF-7 cells were plated in 6-well plates and allowed to attach for 24 h. Then 0.1 μg [^{3}H]-DMBA (Amersham, Arlington Height, IL, USA) per ml was added with or without POH. After 16 h, cells were washed twice with cold PBS, trypsinized and pelleted. Nuclei were separated by incubating the cells for 10 min on ice in lysis buffer A (10 mM Tris–HCl, pH7.5, 320 mM sucrose, 5 mM magnesium chloride and 1% Triton X-100). The nuclei were collected by centrifugation at 5000 rpm after the incubation. The collected nuclei were then lysed by 400 μl lysis buffer B (1% sodium dodecyl sulphate (SDS) in 0.5 M Tris, 20 mM EDTA and 10 mM NaCl, pH 9), followed by treatment with 20 μl Proteinase K (20 mg/ml) for 2 h at 48 °C. The samples were allowed to cool to room temperature, and the residual protein was salted out by adding 150 μl of saturated NaCl. The samples were then centrifuged at 13,000 rpm for 30 min at 4 °C. Genomic DNA was isolated from the supernatant fraction by ethanol precipitation and redissolved in autoclaved water. Ten micrograms of each DNA sample, which attained a 260 nm/280 nm ratio of greater than 1.9, was used for scintillation counting.

Quantitative real-time RT-PCR assay. MCF-7 cells were seeded in 6-well Costar plates and underwent various treatments. After 24 h, total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Carlsbad CA USA). The RNA's concentration and purity were determined by its absorbance at 260/280 nm. First DNA strands were synthesized from 3 μg of total RNA using oligo-dT primers and M-MLV Reverse Transcriptase (USB Corporation, Cleveland, Ohio, USA). Target fragments were quantified by real-time PCR and an ABI prism 7700 Sequence Detection System (Applied Biosystems) was employed for these assays.