Steroids 92 (2014) 96–100

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Diallyl sulfide inhibits diethylstilbestrol induced DNA damage in human breast epithelial cells (MCF-10A)

Michael L. McCaskill [a,b,*], Eleanor Rogan [c], Ronald D. Thomas [d,1]

[a] Pharmacology/Toxicology Department, Basic Pharmaceutical Sciences Division, College of Pharmacy and Pharmaceutical Science, Florida A&M University, Tallahassee, FL 32307, United States
[b] Global Environmental Health Sciences, School of Public Health and Tropical Medicine, Tulane University, New Orleans, LA 70112, United States
[c] Environmental Agricultural and Occupational Health Department, College of Public Health, University of Nebraska Medical Center, Omaha, NE 68198, United States
[d] Pharmacology/Toxicology Department, Basic Pharmaceutical Sciences Department, College of Pharmacy and Pharmaceutical Science, Florida A&M University, Tallahassee, FL 32307, United States

ARTICLE INFO

Article history:
Received 18 September 2013
Received in revised form 22 July 2014
Accepted 18 September 2014
Available online 30 September 2014

Keywords:
DNA damage
Diallyl sulfide
Diethystilbestrol
Breast epithelial cells chemoprevention

ABSTRACT

Breast cancer is the second leading cause of cancer deaths in women in the United States. Diethylstilbestrol (DES) is a synthetic estrogen that has been shown to cause cancer in animals and humans, altering cell viability as well as inducing DNA damage. Diallyl sulfide (DAS) is a garlic organosulfide that has been shown to inhibit both the initiation and promotion phases of cancer *in vivo* and *in vitro*, as well as reduce the risk of cancer in epidemiological studies. MCF-10A cells, regarded as a normal breast epithelial cell line, were treated with varying concentrations of DES, DAS or various dose combinations of DES and DAS concomitantly, and assessed for cell viability, DNA strand breaks, and lipid peroxidation. DES (10 μM) in combination with 1, 10, or 100 μM DAS resulted in a 31%, 34%, or 36% respective increase in cell viability compared to the DES treatment alone, after 24 h. At the same time point, 1, 10, and 100 μM DAS were all effective in significantly reducing DES (100 μM)-induced strand breaks to near that of the vehicle control. Additionally, 1 μM DAS was effective in significantly reducing DES (100 μM)-induced lipid peroxidation after 3 h. The results of this research suggest that DAS is effective in recovering cell viability, attenuating DNA strand breaks, and decreasing lipid peroxidation in MCF-10A cells.

1. Introduction

Historically, researchers have referred to the cell growth promoting attributes of estrogen to explain cell transformation and tumorigenesis in *in vitro* and *in vivo* studies. Although, estrogen acts as a cell growth promoter in estrogen responsive tissue, many of the observed deleterious effects of estrogens in humans, animal models, and estrogen receptor-negative cell cultures cannot be solely attributed to the increases in cellular growth [1–4]. Oxidative metabolites of estrogen have been shown to react with DNA thereby leading to DNA damage [5,6]. These metabolites have been observed to covalently bind to DNA and form stable and depurinating DNA adducts. The resultant DNA damage can lead to genetic errors increasing the risk of cancer initiation in estrogen responsive and non-responsive tissues.

Diethylstilbestrol (DES) is a synthetic estrogen that mimics the metabolism of endogenous estrogens. DES metabolizes into reactive intermediates, including its semiquinone and quinone metabolites [7,8] The primary metabolite, catechol 3'-OH-DES is oxidized into DES-3',4'-quinone [8,9], and attacks DNA [10] by forming depurinating 3'-OH-DES-6'-N3Ade and 3'-OH-DES-6'-N7Gua adducts. DES metabolism can also lead to the formation of peroxides, which have the potential to attack electrophilic sites in nucleic acids and lipids [8,11,12], which models the actions of metabolized endogenous estrogen [6,13,14].

Diallyl sulfide (DAS), a lipophilic organosulfide compound found in garlic (*Allium sativum*), has been found to have chemopreventive properties in *in vivo* and *in vitro studies*. Animal models have shown that DAS can induce expression of nuclear excision repair enzymes, and reduce the redox cycling of DES, leading to increased *in vitro* and *in vivo* viability in liver microsomes and rat models [15–17]. The chemopreventive effects of DAS have been attributed to its inhibitory effects on CYP2E1-mediated bioactivation of carcinogenic chemicals [18]. CYP2E1 preferentially catalyzes oxidation of the sulfur atom to form the sulfoxide and the

* Corresponding author. Tel.: +1 504 988 0246.
E-mail addresses: michaellmccaskill@msn.com, mmccaski@tulane.edu (M.L. McCaskill), egrogan@unmc.edu (E. Rogan).
[1] Deceased.

http://dx.doi.org/10.1016/j.steroids.2014.09.005
0039-128X/

sulfone (DASO and $DASO_2$). This final metabolism of $DASO_2$ leads to the autocatalytic destruction of CYP2E1, which is mainly responsible for the chemoprotective effects of DAS *in vivo* [19]. Most importantly, DAS has been shown to inhibit DES induced formation of DNA adducts (*in vivo*) in the breast tissue of ACI rats. [20]. The current study hypothesizes that DAS will prevent DES induced cell death via the inhibiting formation of lipid peroxides and DNA strand breaks in the MCF-10A breast epithelial cell model.

2. Experimental

2.1. Chemicals and reagents

Dulbecco's Modified Eagle's Medium/Nutrient (DMEM/F12) 1:1 mix basal media, horse serum, penicillin, and streptomycin were purchased from Invitrogen (Carlsbad, CA). Hydrocortisone, cholera enterotoxin, human insulin, dimethylsulfoxide (DMSO), Triton X-100, (PBS, NaOH, Trizma base, NaCl, NA_2 Ethylenediaminetetraacetic acid (EDTA), diethylpyrocarbonate (DEPC) ethanol, DNA se/RNAse free water, DES and DAS were all purchased from Sigma Aldrich (St. Louis, MO). Human epidermal growth factor was purchased from BD Pharmingen (San Jose, CA) and fully frosted microscope slides and cover slips were purchased from Fisher Scientific (Pittsburgh, PA).

2.2. Cell culture

MCF-10A human breast epithelial cells were a generous gift from the laboratory of Dr. Thomas Kocarek at Wayne State University (Detroit, MI). The cells were cultured in a humidified incubator at 37 °C under 5% CO_2 atmospheric conditions. The cells were grown, maintained and treated in media containing phenol red free DMEM/F-12 1:1 mix supplemented with human insulin (10 μg/ml), epidermal growth factor (20 ng/ml), cholera toxin (50 μl), hydrocortisone (0.5 μg/ml), horse serum (5%), and penicillin/streptomycin (10,000 units/ml).

2.3. Cell treatments

MCF-10A human breast epithelial cells were treated for 3 and 24 h with DES (10 or 100 μM) and/or varying concentrations of DAS (1, 10 and 100 μM). DES and DAS were both dissolved in DMSO, establishing a 0.1% concentration of DMSO in each treatment. DMSO (0.1%) served as the vehicle control.

2.4. Cell harvesting

Treated cells grown in cell culture flasks were harvested using disassociation media containing RNase free water (450 ml), phosphate buffered saline (PBS) 10× (50 ml), EDTA (0.5 mM) and placed in centrifuge tubes. The cells were then centrifuged at 250G for 5 min to pellet the cells. The disassociation media was decanted off the cells, maintaining the integrity of the pellet, and the cells were resuspended in 1 ml 1× PBS.

2.5. Cell viability

Cellular viability was quantified by using the CellTiter 96® AQueousOne Solution Cell Proliferation assay (MTS Assay) (Promega, Madison, WI). Cells (5×10^4) were plated per well in flat bottom 96-well plates and treated with DES, DAS or DES/DAS as described above. At 24 h, a 20 μl aliquot of AqueousOne Solution was added to each well and the plate was incubated at 37 °C for 2–4 h to allow for color development. The plates were analyzed on a BioTek Ex800 microplate reader using KC Junior software at 480 nm (Biotek, Winooski, VT).

2.6. Comet assay

The Comet assay was used to detect DNA strand breaks in response to treatment with the carcinogen DES in the presence and/or absence of the chemopreventive agent DAS. This assay sensitively detects DNA damage at the level of individual cells in the form of DNA strand breaks, Cellular DNA undergoes electrophoresis, and as the positively charged DNA migrates to the negatively charged electrode, damaged DNA migrates creating a comet like pattern. The olive tail moment (OTM) is defined as the product of the tail length and the fraction of total DNA in the tail. The OTM integrates the measurement of the smallest detectable size of migrating DNA (reflected in the comet tail length) and the number of relaxed/broken pieces, which creates the intensity of DNA in the tail. MCF-10A cells were treated and harvested from T-25 cm^2 cell culture flasks. A 100 μl aliquot of harvested cells suspended in 1× PBS was mixed with 900 μl of 0.75% low melting point agarose and placed in a 37 °C water bath. A 100 μl aliquot of cell/agarose suspension was placed on three microscope slides pre-coated with 1% normal melting point agarose (NMPA), covered with a coverslip and solidified on ice. Following the removal of the coverslip, a top coat of NMPA was placed on each slide and solidified on ice. The slides were placed in ice-cold lysis buffer (pH = 10) containing 1% Triton X-100 and refrigerated (4 °C) for at least 1 h. After lysis, the slides were placed in a highly alkaline (pH > 13) electrophoresis buffer for 30 min to allow the DNA to unwind followed by electrophoresis for 30 min at 280 A/25 V. The slides were then deactivated by rinsing with a neutralizing buffer (Tris buffer, pH = 7) three times and fixed with 100% ice-cold ethanol for 5 min. In preparation for analysis, the slides were stained with 100 μl of propidium iodide (20 μg/ml) and evaluated under a fluorescent microscope. A total of 150 cell images were examined per treatment under 20× magnification using Kinetic Imaging Komet 5.5 software (Nottingham, UK). The mean olive tail moment (MOTM) was used as a parameter of DNA fragmentation.

2.7. Lipid peroxidation

The determination of the degree of free radicals present in a sample can be measured by the level of peroxides present. Lipid peroxidation is associated with several pathophysical cell and tissue abnormalities, including cancer. Lipid peroxidation is the oxidative degradation of lipids and is considered a basic mechanism of cellular damage induced by free radicals. Lipids were isolated using the Bligh and Dyer method [21]. The isolated lipids were analyzed with the Sigma PeroxiDetect kit. Lipid peroxides are measured with a methanolic reagent containing XO and butylated hydroxytoluene (BHT), an antioxidant that prevents the effects of excess peroxidation. The nmole of peroxide/ml is then calculated from a standard curve with 200 μM tert-BuOOH as a standard. The lipids from cells treated and harvested from T-175 cm^2 cell culture flasks. The cellular lipids from 1×10^6 cells were analyzed for lipid peroxidation by using a PeroxiDetect kit (Sigma–Aldrich). A total of 100 μL of isolated lipids from each sample was placed in a clean microcentrifuge tube, followed by 1 ml of Working Color Reagent (4 mM butylated hydroxytoluene and 125 μM xylenol orange in 90% methanol, with:1:100 volume of Ferrous Ammonium Sulfate Reagent (25 mM ferrous ammonium sulfate in 2.5 M sulfuric acid)). The solution was mixed by vortexing and incubated at room temperature for 30–60 min to complete color formation. A Kayak HP 8453 UV–visible Spectroscopy System (Hewlett Packard, Waldbronn, Germany) was used determine the absorbance of each sample at 560 nm.