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4-Hydroxyestradiol induces oxidative stress and apoptosis in human mammary epithelial cells: Possible protection by NF-κB and ERK/MAPK

Zhi-Hua Chen, Hye-Kyung Na, Yeon-Jin Hurh, Young-Joon Surh*

National Research Laboratory of Molecular Carcinogenesis and Chemoprevention, College of Pharmacy, Seoul National University, Shinlim-dong, Kwanak-ku, Seoul 151-742, South Korea

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Abstract

Catechol estrogens, the hydroxylated metabolites of 17β-estradiol (E_2), have been considered to be implicated in estrogen-induced carcinogenesis. 4-Hydroxyestradiol (4-OHE_2), an oxidized metabolite of E_2 formed preferentially by cytochrome P450 1B1, reacts with DNA to form depurinating adducts thereby exerting genotoxicity and carcinogenicity. 4-OHE_2 undergoes 2-electron oxidation to quinone via semiquinone, and during this process, reactive oxygen species (ROS) can be generated to cause DNA damage and cell death. In the present study, 4-OHE_2 was found to elicit cytotoxicity in cultured human mammary epithelial (MCF-10A) cells, which was blocked by the antioxidant trolox. MCF-10A cells treated with 4-OHE_2 exhibited increased intracellular ROS accumulation and 8-oxo-7,8-dihydroxy-2′-deoxyguanosine formation, and underwent apoptosis as determined by poly(ADP-ribose)polymerase cleavage and disruption of mitochondrial transmembrane potential. The redox-sensitive transcription factor nuclear factor κB (NF-κB) was transiently activated by 4-OHE_2 treatment. Cotreatment of MCF-10A cells with the NF-κB inhibitor, L-1-tosylamido-2-phenylethyl chloromethyl ketone, exacerbated 4-OHE_2-induced cell death. 4-OHE_2 also caused transient activation of extracellular signal-regulated protein kinases (ERK) involved in transmitting cell survival or death signals. A pharmacological inhibitor of ERK aggravated the 4-OHE_2-induced cytotoxicity, supporting the pivotal role of ERK in protecting against catechol estrogen-induced oxidative cell death.
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Keywords: 4-OHE_2; ROS; Oxidative DNA damage; Apoptosis; NF-κB; ERK; MCF-10A cells

Introduction

Endogenous estrogens have been considered as a possible etiological factor in the causation of certain types of human malignancy such as breast, endometrium, ovary, prostate and, possibly, brain cancers (Zhu and Conney, 1998). Estrogen and estradiol can stimulate cancer cell growth by triggering estrogen receptor-mediated signal transduction, resulting in increased DNA synthesis and cell proliferation (Feigelson and Henderson, 1996).

There has been accumulating evidence supporting that catechol estrogens, the oxidized metabolites of estrogens, possess a more potent carcinogenic activity than their parent compounds (Li et al., 2004; Newbold and Liehr, 2000; Rogan et al., 2003). 2-Hydroxyestradiol (2-OHE_2) and 4-hydroxyestradiol (4-OHE_2) are two major hydroxylated metabolites of 17β-estradiol (E_2) formed by cytochrome P450 1A1 and 1B1, respectively (Dannan et al., 1986; Suchar et al., 1995; Zhu et al., 1993). These catechol estrogens are oxidized to quinones accompanying the generation of reactive oxygen species (ROS), capable of causing genotoxicity and tumorigenicity (Liehr and Roy, 1990; Roy and Liehr, 1988; Samuni et al., 2003). The redox cycling of catechol estrogens can be initiated or facilitated by some oxidases (Liehr, 1990; Samuni et al., 2003) or the transitional

* Corresponding author. Fax: +82 2 874 9775.
E-mail address: surh@plaza.snu.ac.kr (Y.-J. Surh).

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metal ions (Li and Trush, 1993a, 1993b). The reduced nicotinamide adenine dinucleotide (NADH) has also been reported to enhance the catechol estrogen-induced generation of ROS and subsequently the oxidative DNA damage (Hiraku et al., 2001; Thibodeau and Paquette, 1999).

It is widely accepted that 3,4-estradiol quinone derived from 4-OHE_2 reacts with DNA to form depurinating adducts which readily leads to genotoxic events while 2,3-estradiol quinone derived from 2-OHE_2 forms stable DNA adducts, which are less genotoxic (Cavalieri et al., 2000; Hayes et al., 1996; Murray et al., 1997). Although the genotoxicity of catechol estrogens has been well defined, little is known about their cytotoxic effects and the underlying molecular mechanisms. In the present study, we have investigated the 4-OHE_2-induced oxidative DNA damage and cell death in human mammary epithelial (MCF-10A) cells. The intracellular signaling events triggered in response to oxidative insult induced by this catechol estrogen have also been explored.

Materials and methods

Materials. Calf thymus DNA, ΦX174 phage DNA, cupric sulfate, bathocuproinedisulfonic acid (BCS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 4-OHE_2, L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK), 2′-deoxyguanosine (dGuo) and 8-oxo-7,8-dihydroxy-2′-deoxyguanosine (8-oxo-dGuo) were purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum and horse serum were obtained from Gibco BRL (Grand Island, NY). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dichlorofluorescein diacetate (DCF-DA) were obtained from Molecular Probes, Inc. (Eugene, OR). Consensus oligonucleotides of nuclear factor κB (NF-κB) and activator protein-1 (AP-1) were purchased from Promega (Madison, WI). [γ-^{32}P]ATP and [^{3}H]thymidine were the products of NEN Life Science (Boston, MA). All antibodies used were products of Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) except the anti-poly(ADP-ribose)polymerase (PARP) which was obtained from Cell Signaling Technology (Beverly, MA). All other chemicals used were of analytical grade or the highest grade available.

Cell culture. Immortalized human mammary epithelial (MCF-10A) cells kindly supplied by Dr. Aree Moon (Duksung Women's University, Seoul) were cultured with medium containing DMEM/F12, 10 μg/ml insulin (bovine), 100 ng/ml Cholera toxin, 0.5 μg/ml hydrocortisone, 20 ng/ml recombinant human epidermal growth factor, 0.5 μg/ml fungi zone, 2 mM L-glutamine, 100 μg/ml penicillin/streptomycin mixture, and 5% horse serum at 37 °C in a humidified atmosphere of 5% CO_2/95% air.

Determination of DNA strand scission. Induction of DNA strand breaks by 4-OHE_2 was assessed by measuring the conversion of ΦX174 phage DNA to open circular and linear forms. ΦX174 DNA (0.3 μg) was incubated with 4-OHE_2 (20 μM) in the presence or absence of 50 μM Cu^{2+} in a final volume of 30 μl of 10 mM Tris–HCl buffer (pH 7.4). After incubation at 37 °C for 3 h, 6 μl of loading buffer consisting of 100 mM EDTA, 0.1% bromophenol blue tracking dye, and 50% (v/v) glycerol was added, and the resulting mixtures were subjected to 0.8% submarine agarose gel electrophoresis. The gels were stained with ethidium bromide and photographed under a transilluminator.

HPLC analysis of 8-oxo-dGuo formation. Calf thymus DNA (50 μg) was incubated with 4-OHE_2 (20 μM) in a final volume of 100 μl 10 mM Tris–HCl buffer (pH 7.4) in the absence or presence of Cu^{2+} (50 μM). The reaction was carried out at 37 °C for 2 h and subjected to hydrolysis by incubation with 3 units of nuclease P_1 (from *Penicillium citrinum*, Sigma product) at 37 °C for 30 min. After the pH of the solution was adjusted to 8.0 by addition of 10 μl of 1 M Tris–HCl buffer (pH 8.5), 2 units of calf intestine alkaline phosphatase was added, and the incubation was continued at 37 °C for additional 1 h. The reaction was terminated by the addition of 15 μl of 250 mM sodium acetate (pH 5.0) containing 50 mM EDTA. The hydrolysates were centrifuged at 15,000 × *g* for 30 min at 4 °C and analyzed by HPLC equipped with an electrochemical detector (ESA, Inc., Chelmsford, MA) using a C_{18} reverse-phase column (4 μm; 3.9 × 150 mm). Products were eluted with 10% methanol in 50 mM sodium phosphate buffer (pH 5.0) at a flow rate of 0.5 ml/min. The amounts of 8-oxo-dGuo and dGuo were calculated from the corresponding peak areas, and the results were expressed as the ratio of 8-oxo-dGuo to 10^5 dGuo.

Cellular DNA was isolated according to the protocol supplied with the DNA extractor WB kit (Wako Pure Chemicals, Nagoya, Japan). The DNA pellets were dissolved in 100 μl of 20 mM sodium acetate buffer and subjected to hydrolysis following the procedure as described elsewhere (Chen et al., 2004).

Determination of cell viability and proliferation. MCF-10A cells were plated at a density of 4 × 10^4 cells/300 μl in 48-well plates, and the cell viability was determined by the conventional MTT reduction assay. The MTT assay relies primarily on mitochondrial metabolic capacity of viable cells and reflects the intracellular redox state. After incubation, cells were treated with the MTT solution (final concentration, 1 mg/ml) for 2 h. The dark blue formazan crystals formed in intact cells were solubilized with lysis buffer (20% sodium dodecylsulfate in 50% aqueous N,N-dimethylformamide), and absorbance at 540–595 nm was measured with a microplate reader (Molecular Devices, Sunnyvale, CA). Results were expressed as the percentage

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