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Cytoprotection of human endothelial cells against oxidative stress by 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im): Application of systems biology to understand the mechanism of action

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ABSTRACT

Cellular damage from oxidative stress, in particular following ischemic injury, occurs during heart attack, stroke, or traumatic injury, and is potentially reducible with appropriate drug treatment. We previously reported that caffeic acid phenethyl ester (CAPE), a plant-derived polyphenolic compound, protected human umbilical vein endothelial cells (HUVEC) from menadione-induced oxidative stress and that this cytoprotective effect was correlated with the capacity to induce heme oxygenase-1 (HMOX1) and its protein product, a phase II cytoprotective enzyme. To further improve this cytoprotective effect, we studied a synthetic triterpenoid, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), which is known as a potent phase II enzyme inducer with antitumor and anti-inflammatory activities, and compared it to CAPE. CDDO-Im at 200 nM provided more protection to HUVEC against oxidative stress than 20 μM CAPE. We explored the mechanism of CDDO-Im cytoprotection with gene expression profiling and pathway analysis and compared to that of CAPE. In addition to potent up-regulation of HMOX1, heat shock proteins (HSP) were also found to be highly induced by CDDO-Im in HUVEC. Pathway analysis results showed that transcription factor Nrf2-mediated oxidative stress response was among the top canonical pathways commonly activated by both CDDO-Im and CAPE. Compared to CAPE, CDDO-Im up-regulated more HSP and some of them to a much higher extent. In addition, CDDO-Im treatment affected Nrf2 pathway more significantly. These findings may provide an explanation why CDDO-Im is a more potent cytoprotectant than CAPE against oxidative stress in HUVEC.

1. Introduction

Mammalian cells are constantly exposed to reactive oxygen species generated from oxidase systems, mitochondria metabolism, and external sources (e.g. UV irradiation, xenobiotics) (Finkel, 2012; Ma, 2010). Reactive oxygen species at low concentration serve as important signaling messengers in the processes of cell division, inflammation, and stress response (Finkel, 2011). Under normal conditions, cells can neutralize extra reactive oxygen species through redox reactions with intracellular antioxidants. However, sustained production of oxidants (pro-oxidants or reactive oxygen species) more than cellular reductants (antioxidants) can counterbalance, leads to oxidative stress. The oxidative damage generated by overproduction of reactive oxygen species exacerbates cardiovascular diseases, cancer, diabetes, chronic inflammation, stroke, septic shock, and neurodegenerative diseases (Heitzer et al., 2001; Ma, 2010). In addition, the theory of free radicals remains a major contributor to aging (Halliwell and Gutteridge, 2006).

In a continuing effort to find new ways to ameliorate oxidative stress-mediated ischemia–reperfusion injury, we identified a natural polyphenolic compound, caffeic acid phenethyl ester (CAPE), as a potential cytoprotectant. Our previous studies showed that CAPE protected human umbilical vein endothelial cells (HUVEC) against menadione-induced oxidative stress and that this protection was largely due to induction of a cytoprotective gene, heme oxygenase-1 (HMOX1), by CAPE (Wang et al., 2010, 2008). The protein product of HMOX1, heme oxygenase-1 (HO-1), usually known to be the rate-limiting enzyme for heme degradation, is now considered as

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a phase 2 detoxification enzyme (Talalay, 2000). To further improve cytoprotection of human endothelial cells, we investigated a recently described inducer of phase 2 enzymes, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), a new synthetic triterpenoid (Place et al., 2003).

CDDO-Im is the imidazolide derivative of its parent compound, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO). CDDO, a synthetic oleanane triterpenoid, was identified through an attempt to design new anti-inflammatory agents from the natural products oleanolic acid and ursolic acid (Honda et al., 1998; Suh et al., 1999). CDDO, CDDO-Im, and methyl ester derivative of CDDO also showed antitumor activities in vitro and in animals (Ito et al., 2000; Kim et al., 2002; Place et al., 2003). A recent study found that CDDO and CDDO-Im induced HO-1 both in vitro and in vivo, and the imidazolide derivative was more potent (Liby et al., 2005). This induction was possibly through the activation of a signaling pathway regulated by transcriptional factor nuclear factor, erythroid 2-like 2 (Nrf2). Upon activation, Nrf2 translocates from the cytoplasm to the nucleus and binds to the antioxidant responsive element (ARE) to initiate transcription of an array of drug metabolism and antioxidant genes. Activation of this Nrf2/ARE pathway leads to an increased elimination of xenobiotics and thus increased resistance to oxidative stress (Ma, 2013).

The purpose of our present research was to (1) identify more potent cytoprotectants against oxidative stress than CAPE and (2) obtain insight into potential mechanisms of this cytoprotection through a genome-wide systems biology approach. In the study, we examined the cytoprotective effect of CDDO-Im against oxidative stress in HUVEC and compared it to CAPE. A microarray analysis was conducted on HUVEC after a 6-h treatment with either CDDO-Im or CAPE to look for induction of cytoprotective genes. Data were analyzed through the use of Ingenuity Pathway Analysis (IPA) for functional enrichment and pathway analysis of microarray data to identify potential involvement of cellular functions and canonical pathways mediating oxidative stress.

2. Materials and methods

2.1. Chemicals and reagents

CAPE was purchased from Cayman Chemicals (Ann Arbor, MI, USA). CDDO-Im was kindly provided by Dr. Michael Sporn (Dartmouth Medical School, Hanover, NH, USA). Menadione sodium bisulfite (menadione) and dimethyl sulfoxide (DMSO) were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

2.2. Cell culture

HUVEC (Life Technologies, Carlsbad, CA, USA) were cultivated on 1% gelatin-coated 75 cm^2 culture flasks (Corning Incorporated, Corning, NY, USA) in Medium 200 supplemented with 2% fetal calf serum, penicillin (100 units/ml), streptomycin (100 units/ml), and fungizone (0.25 μg/ml) supplied by Life Technologies (Wang et al., 2008). Stock cultures were maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO_2 with medium changes every 2 days till confluent. Prior to an experiment, HUVEC were subcultivated with trypsin/EDTA onto 1% gelatin-coated 48-or 96-well Costar® multiplates (Corning Incorporated) at 10,000 or 5000 cells/well, respectively, grown to confluence, and kept for 72 h to produce a quiescent cell layer. On the day prior to the experiment, the medium was changed. Only the second through fifth population doubling levels of cells were used.

2.3. Cell viability and toxicity assay

Cell viability was estimated in HUVEC using Alamar Blue™ (Life Technologies), which utilizes metabolic conversion of resazurin to fluorescent resorufin by viable cells. As previously described (Wang et al., 2008), menadione was used to induce oxidative injury in HUVEC. Briefly, HUVEC were exposed to a series of doses of menadione for 24 h. The cells were incubated with culture medium containing 10% Alamar Blue™ for an additional 2 h. Fluorescence was measured at 545 nm excitation and 590 nm emission with a SpectraMAX® M2 microplate reader (Molecular Devices, Sunnyvale, CA, USA). A dose of menadione close to its maximum toxicity, causing 80–90% cell death, was chosen for the cytoprotection assay. Nontoxic doses of CDDO-Im in HUVEC were determined as follows. A series of doses of CDDO-Im (50–1000 nM) were incubated with HUVEC for 24 h followed by cell viability assay. Doses of CDDO-Im causing more than 90% cell viability were considered not toxic to HUVEC and used in the following cytoprotection assay.

2.4. Cell protection assay

CDDO-Im and CAPE were dissolved in DMSO and diluted 1000-fold with medium (final concentration of DMSO no more than 0.1%). Confluent HUVEC were pretreated with CDDO-Im and CAPE at nontoxic doses for 6 h. They were then exposed to the preselected dose of menadione for an additional 24 h. Cell viability was measured using the Alamar Blue™ assay.

2.5. mRNA-based microarray expression profiling

HUVEC were incubated with CDDO-Im at 200 nM or CAPE at 20 μM for 6 h. Total RNA was extracted from treated HUVEC grown in 6-well plates with TRI™ reagent according to the manufacturer's protocol (Molecular Research Center, Cincinnati, OH, USA). The amount of isolated RNA samples was quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of isolated RNA samples was assessed using an Agilent Bioanalyzer 2100 (Agilent Technologies. Inc., Santa Clara, CA, USA).

Gene expression profiling of HUVEC treated with CDDO-Im or CAPE versus vehicle control (DMSO, 0.1%) was performed using Agilent 60-mer Whole Human Genome Microarrays (Agilent Technologies). Statistical analysis for differences among treatment groups was performed using BRB Array Tools (Biometric Research Branch, National Cancer Institute, USA, http://linus.nci.nih.gov/BRB-ArrayTools.html). Genes were determined to be statistically altered in their expression with both P value <0.001 and false discovery rate (FDR) <0.001 after class comparison between treated and control groups. In addition, the significant gene lists from either CDDO-Im or CAPE treatment were intersected through BRB Array Tools to identify common genes significantly altered by both compounds.

2.6. Ingenuity pathway analysis

The lists of significant genes from microarray data analysis were submitted to IPA for gene function and canonical pathway analyses (Ingenuity® Systems, www.ingenuity.com). IPA maintains a large knowledge database of modeled relationships among proteins, genes, complexes, cells, tissues, drugs, pathways, and diseases generated from published reports. When a date set containing gene identifiers and corresponding expression values such as fold change was uploaded into IPA application, each gene identifier was mapped to its corresponding gene object in this Ingenuity Pathway Knowledge Base (IPKB). A P value and FDR