Free Radical Biology & Medicine 52 (2012) 1937–1944

Contents lists available at SciVerse ScienceDirect

Free Radical Biology & Medicine

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

Original Contribution

Molecular mechanisms of ALDH3A1-mediated cellular protection against 4-hydroxy-2-nonenal

William Black [1], Ying Chen [1], Akiko Matsumoto, David C. Thompson, Natalie Lassen, Aglaia Pappa, Vasilis Vasiliou *

Molecular Toxicology and Environmental Health Sciences Program, Department of Pharmaceutical Sciences, University of Colorado AMC, Aurora, CO 80045, USA

ARTICLE INFO

Article history:
Received 24 February 2011
Revised 17 February 2012
Accepted 28 February 2012
Available online 8 March 2012

Keywords:
Aldehyde dehydrogenase
ALDH3A1
Metabolism
Lipid peroxidation
4-Hydroxy-2-nonenal
Cornea
Free radicals

ABSTRACT

Evidence suggests that aldehydic molecules generated during lipid peroxidation (LPO) are causally involved in most pathophysiological processes associated with oxidative stress. 4-Hydroxy-2-nonenal (4-HNE), the LPO-derived product, is believed to be responsible for much of the cytotoxicity. To counteract the adverse effects of this aldehyde, many tissues have evolved cellular defense mechanisms, which include the aldehyde dehydrogenases (ALDHs). Our laboratory has previously characterized the tissue distribution and metabolic functions of ALDHs, including ALDH3A1, and demonstrated that these enzymes may play a significant role in protecting cells against 4-HNE. To further characterize the role of ALDH3A1 in the oxidative stress response, a rabbit corneal keratocyte cell line (TRK43) was stably transfected to overexpress human ALDH3A1. These cells were studied after treatment with 4-HNE to determine their abilities to: (a) maintain cell viability, (b) metabolize 4-HNE and its glutathione conjugate, (c) prevent 4-HNE–protein adduct formation, (d) prevent apoptosis, (e) maintain glutathione homeostasis, and (f) preserve proteasome function. The results demonstrated a protective role for ALDH3A1 against 4-HNE. Cell viability assays, morphological evaluations, and Western blot analyses of 4-HNE-adducted proteins revealed that ALDH3A1 expression protected cells from the adverse effects of 4-HNE. Based on the present results, it is apparent that ALDH3A1 provides exceptional protection from the adverse effects of pathophysiological concentrations of 4-HNE such as may occur during periods of oxidative stress.

Solar ultraviolet radiation (UV-R) is a well-documented environmental insult to biological systems. Absorption of UV-R by organic molecules, including nucleic acids, proteins, fatty acids, and others within living cells, can result in cellular damage through the formation of reactive oxygen species (ROS). ROS can initiate lipid peroxidation (LPO) by reacting with polyunsaturated fatty acids (PUFAs) in the cell membrane bilayer and thereby lead to the formation of: (a) lipid radicals that propagate the reaction and (b) lipid hydroperoxide reaction products. It is the degradation of the lipid hydroperoxides that yields a variety of breakdown products including more than 200 species of aldehydes [1]. Compared with the ROS that initiated LPO, the aldehydes are relatively stable, allowing them to diffuse intra- and intercellularly from the site of their generation. Several of these aldehydic breakdown products are electrophilic in nature, including acrolein, malondialdehyde, and 4-hydroxy-2-nonenal (4-HNE). These aldehydes readily react with cellular nucleophiles (including nucleic acids, proteins, and phospholipids) to elicit their cytotoxic and genotoxic actions [1].

4-HNE has received significant attention over the past 30 years after it was identified as the most cytotoxic breakdown product generated from LPO [2]. It is also one of the most abundant α,β-unsaturated aldehydes generated from LPO through β-cleavage of hydroperoxides originating from the ω-4 and ω-6 PUFAs arachidonic and linoleic acid, respectively [2,3]. The high reactivity of 4-HNE is attributed to its three main functional groups (the carbon–carbon double bond and the carbonyl and the hydroxyl groups), which appear to react synergistically with biomolecules containing amino and thiol groups [4,5]. The cellular consequences of 4-HNE synergistic reactivities include growth inhibition, decreases in glutathione (GSH) levels, decreases in sulfhydryl- and thiol-containing proteins, inhibition of enzyme activities, inhibition of calcium sequestration by microsomes, inhibition of protein synthesis and degradation, and alterations in signal transduction and gene expression profiles [3,6–12]. Intracellular regulation of 4-HNE is controlled primarily through several biotransformation pathways [13–17]. Excessive production of 4-HNE during periods of oxidative stress may lead to the saturation, inhibition or degradation of these normal metabolic pathways responsible for maintaining its intracellular homeostasis

Abbreviations: ALDH, aldehyde dehydrogenase; GSH, glutathione; GST, glutathione *S*-transferase; 4-HNE, 4-hydroxy-2-nonenal; 4-HNA, 4-hydroxy-2-nonenoic acid; LPO, lipid peroxidation; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; TRK, rabbit corneal keratocyte cell line; UV-R, ultraviolet radiation.

* Corresponding author. Fax: +1 303 724 7266.
E-mail address: vasilis.vasiliou@ucdenver.edu (V. Vasiliou).

[1] These authors contribute equally to this work.

0891-5849/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.freeradbiomed.2012.02.050

[18] and thereby result in its accumulation and consequent reactivity with other cellular constituents. As a direct result of the ability of 4-HNE to affect cellular functions, a great deal of research has been conducted over the past 15 years to understand the enzyme systems responsible for its metabolism and disposition [19].

The cornea, an avascular tissue at the anterior surface of the eye, is structurally and functionally organized to serve as a protective barrier between the external environment and the internal ocular elements. Like other surface tissues of the body, it is exposed to a variety of environmental assaults, including UV-R. The cornea comprises three distinct layers: a self-renewing outermost squamous epithelial layer, a collagenous stromal matrix, and a thin endothelial layer. It possesses an armamentarium of nonenzymatic and enzymatic defense systems to combat oxidative stress, including high levels of albumin, ascorbate, ferritin, superoxide dismutase, catalase, glutathione peroxidase, glutathione *S*-transferases (GST), aldose reductase, alcohol dehydrogenases, and aldehyde dehydrogenases (ALDH), which enable this tissue to maintain its structural integrity and functional role in light transparency [19–21].

ALDH3A1 is an enzyme present in the corneal tissues that efficiently metabolizes 4-HNE [13,14,22,23]. This cytosolic, $NADP^+$-dependent protein catalyzes the oxidation of a wide variety of endogenous and exogenous aldehydes to their corresponding carboxylic acids. ALDH3A1 is also considered a mammalian corneal crystallin, attaining high concentrations in this ocular tissue [24]. Its presence has been identified by immunofluorescence in both the epithelial and the stromal matrix layers of the normal human cornea, but not in the endothelial layer [13,25]. It was originally identified as a highly expressed protein in the bovine cornea [26] and has subsequently been shown to constitute 10 to 40% of the total water-soluble protein in the corneas of mammalian species [25,27,28]. The precise role of this enzyme in corneal function remains to be elucidated. However, data suggest ALDH3A1 plays a significant role as an enzymatic antioxidant in the protection of cells from 4-HNE-induced cytotoxicity [22,29]. Such a protective function of ALDH3A1 has also been shown in corneal stromal keratocytes exposed to a variety of oxidative stress-inducing agents, including H_2O_2, the chemotherapeutic agents mitomycin-C and the etoposide VP-16 [30]. The capacity of ALDH3A1 to metabolize 4-HNE by direct conversion to 4-hydroxy-2-nonenoic acid (4-HNA) [13,14] supports the notion that this enzyme is an integral part of the cellular defense mechanisms protecting corneal tissues from UV-R-induced damage [13,14,22]. In addition, conjugation of 4-HNE to glutathione via GST isozymes to form the 4-HNE glutathione conjugate, GS–HNE, has been reported in a number of tissues as a predominant metabolic pathway for detoxification of this aldehyde [15–17]. Given that GSH conjugates may themselves adversely affect cellular function, elimination of GS–HNE may be an important step in driving this detoxification pathway. Accordingly, ALDH enzymes could contribute to this pathway by converting GS–HNE to GS–HNA. To date, no studies have reported a role for ALDH-mediated metabolism in the elimination of GS–HNE, although it has been alluded to in a review [31]. Hence, the demonstration of a metabolic role of ALDH3A1 in eliminating the GS–HNE conjugates amplifies its significance in cellular protection from 4-HNE-induced toxicity.

Despite the high abundance of ALDH3A1 in corneal tissues, primary culture and established cell lines of mammalian corneal cells lose constitutive ALDH3A1 expression [32]. Therefore, to study the role of ALDH3A1 in the disposition of 4-HNE in the corneal stromal matrix, we utilized the SV-40-immortalized rabbit corneal stromal keratocyte cell line (TRK43), which was stably transfected with human ALDH3A1 [30]. This paper presents the results of a series of studies in which this transfected cell model was exposed to pathophysiological levels of 4-HNE. The objectives were to determine the effects of ALDH3A1 expression on: (i) 4-HNE-induced cytotoxicity, (ii) metabolism of 4-HNE and GS–HNE, (iii) 4-HNE-protein adduct formation, and (iv) cellular glutathione levels and proteasome activity. The results of these experiments demonstrated a role for ALDH3A1 in protecting cells from the deleterious effects of 4-HNE.

Materials and methods

Reagents

All tissue culture media, supplements, growth factors, assay reagents, protein inhibitors, and buffers were purchased from Gibco BRL (Gaithersburg, MD, USA) or Sigma–Aldrich Co (St. Louis, MO, USA) unless otherwise specified. Lipofectamine Plus and hygromycin reagents were purchased from Invitrogen (Carlsbad, CA, USA). The bicinchoninic acid kit was purchased from Pierce Chemical Co (Rockford, IL, USA). The polyvinylidene difluoride membranes were obtained from Immobilon-P Millipore (Bedford, MA, USA). 4-HNE was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). The monoclonal anti-4-HNE antibody was purchased from Oxis International (Portland, OR, USA). Polyclonal anti-4-HNE antibody was provided as a generous gift from Dr. D. Peterson (University of Colorado at Denver, Aurora, CO, USA). The monoclonal anti-ALDH3A1 antibody was developed and described by our laboratory [13]. Horseradish peroxidase-conjugated secondary antibody was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). The chemiluminescence assay kit was obtained from NEN Life Science Products (Boston, MA, USA). The OxyBlot detection kit was purchased from Chemicon International (Temecula, CA, USA). 4-HNE stock concentrations were prepared and confirmed by measurement of UV absorbance at 224 nm (extinction coefficient $\varepsilon = 13750\ M^{-1}\,cm^{-1}$; $A_{224} = \varepsilon \times \text{concentration} \times 1\ cm$). GS–HNE was prepared and measured per the methods described by Tjalkens et al. [33].

ALDH3A1-expressing corneal stromal keratocyte cell line (TRK43)

SV-40-immortalized TRK43 cells were derived from rabbit corneal stromal keratocytes and were provided as a generous gift from Dr. James Jester (University of California at Irvine, Irvine, USA) [34]. Cells were maintained at 37 °C in a humidified 5% CO_2 incubator and grown on 100-mm culture plates in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin solution. Parental TRK43 cells were transfected with the ΔpCEP4Δ mammalian expression vector containing human ALDH3A1 cDNA (ΔpCEP4Δ-ALDH3A1) or the empty vector (ΔpCEP4Δ) using Lipofectamine Plus reagent as described elsewhere [30]. Stably transfected cells were selected in culture medium containing 0.4 mg/ml hygromycin for 4–6 weeks before individual colonies were obtained. Hygromycin-resistant clones were isolated from individual colonies, further expanded, and characterized for ALDH3A1 expression by enzymatic assay, silver staining, and Western immunoblot analysis. The TRK43-ALDH3A1 clone 10 showed high ALDH3A1 expression and therefore was used for all experiments described herein between passages 10 and 20. The mock cells (TRK43-vector) behaved the same as the parental TRK43 cells (data not shown).

Preparation of whole-cell extracts

Cell cultures were washed twice with ice-cold phosphate-buffered saline (PBS), collected by scraping, and resuspended in cell lysis buffer containing 25 mM Tris, 0.25 M sucrose (pH 7.4), protease inhibitors (0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, 1 μg/ml pepstatin, 100 μg/ml phenylmethylsulfonyl fluoride), and 0.1% Triton X-100. Cell lysates were subjected to three cycles of sonication for 5 s on ice using a Branson Sonifier 250 (VWR Scientific, Willard, OH, USA) and cooled on ice for 30 s. The resultant cell lysates were centrifuged