Biochemical and Biophysical Research Communications 417 (2012) 346–351

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

The expression and function of vascular endothelial growth factor in retinal pigment epithelial (RPE) cells is regulated by 4-hydroxynonenal (HNE) and glutathione *S*-transferaseA4-4

Rit Vatsyayan, Poorna Chandra Rao Lelsani, Pankaj Chaudhary, Sushil Kumar, Sanjay Awasthi, Yogesh C. Awasthi *

Department of Molecular Biology and Immunology, University of North Texas Health Science Center, Fort Worth, TX 76107, USA

ARTICLE INFO

Article history:
Received 17 November 2011
Available online 1 December 2011

Keywords:
Lipid peroxidation
4-Hydroxy-t-2-nonenal
Glutathione *S*-transferasesA4-4
Vascular endothelial growth factor
Vascular endothelial growth factor receptor
Retinal pigment epithelium cells

ABSTRACT

It is well established that 4-hydroxynonenal (HNE) plays a major role in oxidative stress-induced signaling and the toxicity of oxidants. Surprisingly our recent studies also demonstrate that low levels of HNE generated during oxidative stress promote cell survival mechanisms and proliferation. Since the expression and secretion of VEGF is known to be affected by Oxidative stress, during present studies, we have examined dose dependent effect of HNE on VEGF expression and secretion in a model of retinal pigment epithelial (RPE) cells in culture. Results of these studies showed that while inclusion of 0.1 μM HNE in the medium caused increased secretion of VEGF, its secretion and expression was significantly suppressed in the presence of >5 μM HNE in the media. These concentration dependent hormetic effects of HNE on VEGF secretion could be blocked by the over expression of GSTA4-4 indicating that these effects were specifically attributed to HNE and regulated by GSTA4-4. VEGF secreted into the media showed angiogenic properties as indicated by increased migration and tube formation of HUVEC in matrigel when grown in media from RPE cells treated with 1 μM HNE. The corresponding media from GSTA4-4 over expressing RPE cells had no effect on migration and tube formation of HUVEC in matrigel. These results are consistent with earlier studies showing that at low concentrations, HNE promotes proliferative mechanisms and suggest that HNE induces VEGF secretion from RPE cells that acts in a paracrine fashion to induce angiogenic signaling mechanism in the endothelial cells. These findings may suggest a role of HNE and GSTA4-4 in oxidative stress induced proliferative retinopathies.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

4-Hydroxynonenal (HNE) is the predominant end-product of lipid peroxidation (LPO) that contributes to cytotoxicity of oxidants via electrophilic attack on DNA and proteins [1,2]. It contributes to toxicity by inducing pro-apoptotic signaling through multiple pathways and also by necrosis. We and others have established that HNE is involved in regulation of gene expression and cell cycle signaling in a concentration dependent manner, and that its concentration in cells is regulated through a coordinated action of GSTA4-4, that catalyzes its conjugation to GSH and RLIP76, that transports GS-HNE conjugate out of cells. *GstA4* knock-out mice having impaired HNE metabolism and increased HNE levels in tissues are more sensitive to the toxicity of oxidant chemicals/oxidative stress suggesting the role of HNE in the mechanisms of toxicity of oxidant xenobiotics and a protective role of GSTA4-4 against oxidative stress. However, recent studies have shown that unless subjected to oxidative stress, *GstA4* knock-out mice have a normal phenotype, and surprisingly show a noticeable increase in their life span [3]. Other intriguing aspects of HNE-induced signaling observed in our studies and also reported by other investigators are its concentration dependent effects causing apoptosis at high concentrations, but promoting proliferation at low concentrations. These contrasting concentration dependent effects of HNE are observed in most of the cell types studied so far. The physiological significance of the contrasting hormetic effects of HNE on signaling and the mechanisms responsible for a surprisingly higher life span of *GstA4* knock-out mice are not understood and need to be investigated.

Abbreviations: HNE, 4-hydroxy-2-nonenal; GST, glutathione *S*-transferase; LPO, lipid peroxidation; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; HUVEC, human umbilical vascular endothelial cells; RPE, retinal pigment epithelial cells.

* Corresponding author. Address: Department of Molecular Biology and Immunology, University of North Texas Health Science Center, 3500 Camp Bowie Boulevard, Fort Worth, TX 76107, USA. Fax: +1 817 735 2118.
E-mail address: Yogesh.Awasthi@unthsc.edu (Y.C. Awasthi).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.11.113

Our recent studies show that besides being toxic HNE also induces defense mechanisms against oxidative stress to prevent its own toxic effects and protect the neighboring cells from a 'runaway apoptosis'. These studies have shown that HNE induces defense mechanisms such as transcriptional activation of heat shock factors (HSFs), induction of HSP70, induction of anti-oxidant enzymes, and the activation of Daxx mediated anti-apoptotic mechanisms. More importantly, these studies suggest that oxidative stress (UV radiation or H_2O_2)-induced activation of these defense mechanisms requires HNE. Together, these findings suggest a requirement of HNE for the activation of defense mechanisms against oxidative stress for cell survival. VEGF is a homo-dimeric protein of about 34–45 kDa and it is implicated in angiogenesis in cancers and also in retinal microenvironment [4]. In vaso-proliferative disorders, including ARMD and diabetic retinopathy [5] the retinal pigment epithelial layer has been suggested to be the source of VEGF and it has been shown that oxidative stress causing agents and HNE can induce VEGF secretion from RPE cells. Thus, during present studies we have systematically investigated the dose dependent effect of HNE, an inevitable consequence of oxidative stress, on the expression of VEGF and VEGFR to address the hypothesis that at low concentration HNE activates various survival mechanisms. We have also evaluated the possible physiological consequences of HNE induced secretion of VEGF secreted from RPE cells shows angiogenic effects in an *in vitro* model. Here we show, for the first time that HNE exerts a hormetic (concentration dependent opposite effect) on the secretion of VEGF, i.e. at low levels HNE causes increased secretion of VEGF from RPE cells but at higher concentration, it inhibits VEGF secretion.

2. Materials and methods

2.1. Material

HNE was purchased from Cayman Chemical (Ann Arbor, MI). Bradford reagent, bis-acrylamide, and SDS for SDS–PAGE were obtained from Bio-Rad (Hercules, CA). Western blot stripping buffer was obtained from Pierce Co. (Rockford, IL). EGM-2 bullet kit medium was purchased from Lonza (Walkersville, MD). The cell culture medium DMEM, Lipofectamine 2000 transfection reagent, and fetal bovine serum were purchased from GIBCO (Invitrogen, Carlsbad, CA). All other reagents and chemicals including DMSO, G418 (geneticin), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), etc. were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Cell lines

The simian virus SV40-transformed human fetal male RPE 28 cells (Coriell Institute, Camden, NJ) that exhibit epithelioid morphology and retain physiological functions characteristic of the primary human RPE cells were cultured in standard medium containing 10% fetal bovine serum and antibiotics in a humidified incubator at 37 °C in 5% CO_2 atmosphere as described before [6]. The HUVEC (Lonza, Walkersville, MD) cells were grown in EGM-2 bullet kit. All studies were conducted by using cells of passages 10–20 for RPE and 2–6 for HUVEC. The cells were trypsinized and passaged every 3–4 days.

2.3. Cell viability assay

The cytotoxicity of HNE to RPE cells was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium Bromide (MTT) assay as described before [7] with minor modifications. Briefly, 2×10^4 cells in 190 μl of medium were seeded in 96-well microtiter plates and allowed to attach for 24 h. Next day, HNE in 10 μL PBS was added to achieve the desired concentration. After 12 h incubation, 10 μl of a stock solution of MTT (5 mg/ml in PBS) was added to each well and the plates were incubated for additional 4 h at 37 °C, centrifuged, and the medium was decanted. Cells were subsequently dissolved in 100 μl DMSO with gentle shaking for 2 h at room temperature, followed by measuring absorbance at 562 nm in a micro plate reader (El × 808 BioTek Instruments, Inc). A dose–response curve was plotted and the concentration of HNE causing a 50% reduction in formazan crystal formation (IC_{50}) was determined.

2.4. LDH assay

To measure lactate dehydrogenase (LDH) released from the RPE cells, a commercially available cytotoxicity detection kit was used as briefly described below: Cells (2×10^4 in 190 μl of medium) were seeded in 96-well microtiter plates and allowed to attach for 24 h. The next day, 10 μl of PBS containing the desired concentration of HNE was added. After 12 h incubation, total culture medium was collected and centrifuged to remove contaminating cells and cellular debris. The volume of media was then measured. For assay, 100 μl of each sample was transferred to a 96 well microtiter plate, 100 μl of LDH reagent mixture was added to each well and incubated for up to 30 min at room temperature. After incubation 50 μl stop solution was added and the absorbance of samples was measured at 490 nm.

2.5. Transient transfection with hGSTA4

Transient transfection of RPE cells was performed with *hGSTA4* as described by us before [6,13]. Briefly, RPE cells at a density of 5×10^5 cells per 100 mm Petri dish were plated and the dishes having >70% confluent cells were used for the transfection with 24 μg of either empty pTarget-T vector (VT) or the pTarget vector containing the open reading frame (ORF) of the restored Kozak *hGSTA4* sequence (*hGSTA4-Tr*). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) was used for transfection as per the manufacturer's instructions.

2.6. Western blot analysis

The cells with or without specified treatment(s), were pelleted, washed thrice with PBS, re-suspended in RIPA lysis buffer (50 mM Tris–HCl, pH 7.5; 1% NP-40; 150 mM NaCl; 1 mg/ml aprotinin; 1 mg/ml leupeptin; 0.5 mM phenylmethylsulfonyl fluoride; 1 mM Na_3VO_4; 1 mM NaF) at 4 °C for 30 min, and lysed by sonication. Cell debris was removed by centrifugation at 14,000*g* for 30 min at 4 °C to obtain clear extracts. Western blot analyses were performed with the extracts containing 25–75 μg protein. Protein was determined by the method of Bradford [8] throughout these studies.

2.7. Wound healing assay

In vitro wound-healing assay was performed using previously described method [9]. Briefly, HUVECs (8×10^4) were grown to confluence on 12-well tissue culture plates for 48 h, and then starved in serum-free maintenance medium for 4 h. A "wound" was made by scraping the middle of the cell monolayer with a sterile 10 μL micropipette tip. Floating cells were removed by extensive washing with PBS and the conditioned media obtained from RPE cell cultures with or without HNE treatment was added to each of the wells. Cells were stained with crystal violet and photographed using a phase-contrast microscope at 12 h after wounding.

Download English Version:

https://daneshyari.com/en/article/1930125

Download Persian Version:

https://daneshyari.com/article/1930125

Daneshyari.com