Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 184–192
Journal of Nutritional Biochemistry

Effects of 2(*RS*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid on 4-hydroxy-2-nonenal-induced apoptotic T cell death

Weiyuan Chang[a], Craig J. McClain[a,b,c], Marcia C. Liu[a], Shirish S. Barve[a,b], Theresa S. Chen[a,*]

[a]*Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY 40292, USA*
[b]*Department of Internal Medicine, University of Louisville, Louisville, KY 40292, USA*
[c]*Division of Gastroenterology, Louisville VA Medical Center, Louisville, KY 40292, USA*

Received 25 October 2006; received in revised form 31 January 2007; accepted 6 February 2007

Abstract

4-Hydroxy-2-nonenal (HNE), the aldehydic product of lipid peroxidation, is associated with multiple immune dysfunctions, such as HIV and hepatitis C virus infection. HNE-induced immunosuppression could be due to a decrease in $CD4^+$ T lymphocyte activation or proliferation. Glutathione (GSH) is the most abundant endogenous antioxidant in cells, and an adduct between HNE and GSH has been suggested to be a marker of oxidative stress. Our earlier studies showed that HNE induced cytotoxicity and Akt inactivation, which led to the enhancement of FasL expression and concomitantly decreased cellular FLICE-like inhibitory protein (c-$FLIP_S$) levels. In this study, we found that HNE caused intracellular GSH depletion in Jurkat T cells, and we further investigated the role of 2(*RS*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid (PTCA), a GSH prodrug, in attenuating HNE-induced cytotoxicity in $CD4^+$ T lymphocytes. The results show that PTCA protected against HNE-induced apoptosis and depletion of intracellular GSH. PTCA also suppressed FasL expression through increasing levels of Akt kinase as well as antiapoptotic c-$FLIP_S$ and decreasing the activation of type 2 protein serine/threonine phosphatase. Taken together, these data demonstrate a novel correlation between GSH levels and Akt activation in T lymphocyte survival, which involves FasL down-regulation and c-$FLIP_S$ expression through increasing intracellular GSH levels. This suggests that PTCA could potentially be used in the treatment of oxidative stress-induced immunosuppressive diseases.

Keywords: Hydroxynonenal; Glutathione; PTCA; Apoptosis; Fas ligand; $CD4^+$ T lymphocytes

1. Introduction

There is an increasing body of evidence showing that reactive aldehydic molecules generated as a consequence of lipid peroxidation are causally linked in most of the pathophysiological effects associated with oxidative stress in cells and tissues [1]. 4-Hydroxy-2-alkenals represent the most prominent lipid peroxidation-specific aldehydes [2]. Among them, 4-hydroxy-2-nonenal (HNE) is believed to be largely responsible for the cytopathological effects observed during oxidative stress in vivo [1]. Clinical observations show that HNE is associated with multiple pathophysiological conditions involving immune dysfunction, such as HIV and hepatitis C virus infection [3–5]. HNE plays a central physiological role in the down-regulation of cell proliferation and in the modulation of T cell differentiation [6,7]. In T lymphocytes, thiols play an important role in regulating cell proliferation and programmed cell death. The restoration of cellular glutathione (GSH) levels is known to modulate the effect of inflammatory cytokines and increase the $CD4^+$ cell count of HIV-infected patients [8–10]. Several studies demonstrated an increase in human $CD4^+$ T lymphocyte count after supplementation with antioxidants such as N-acetyl cysteine (NAC), oxothiazolidine carboxylic acid and vitamin E among patients with immune diseases, indicating that oxidative stress can affect human $CD4^+$ T lymphocyte survival [11,12]. NAC, a prodrug of GSH, has been used in HIV infection to prevent the activation of nuclear factor kappa-B and the replication of HIV [10]. Taken together, these results suggest an important role of GSH in oxidative stress-induced T cell toxicity and dysfunction.

* Corresponding author. Department of Pharmacology and Toxicology, School of Medicine, University of Louisville, Louisville, KY 40292-0001, USA. Tel.: +1 502 852 7887; fax: +1 502 852 7868.
E-mail address: tschen01@louisville.edu (T.S. Chen).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.02.008

2(*RS*)-*n*-Propylthiazolidine-4(*R*)-carboxylic acid (PTCA) is a sulfhydryl-protected compound containing a cyclized form of L-cysteine (Cys) that can be nonenzymatically converted into Cys at physiological pH and room temperature levels [13]. Like NAC, PTCA has been shown to attenuate acetaminophen- and para-aminophenol-induced hepatic GSH depletion in mice [14] and hamsters [15]. Whether PTCA can protect against HNE-induced cytotoxicity and depletion of GSH has not been investigated.

Depletion of GSH by oxidants or by depleting agents such as diethyl maleate and buthionine sulfoximine (BSO) is known to increase cell vulnerability to oxidative stress and disturb the redox balance [16]. Recently, studies demonstrated that GSH depletion is probably an early signaling event in apoptotic cell death, which is characterized by the activation of protein kinase C-δ [17]. Others showed that cell viability and activation of Mek1 and Akt decreased when *ETV6-NTRK3*-transformed fibroblastic cells were incubated with BSO, suggesting that GSH plays a critical role in Akt activation in the transformed cells [18]. However, there is no direct link between GSH content and the protein kinase B/Akt survival signaling pathway.

The role of Akt kinase in T lymphocyte survival is well documented [19–21]. Activation of Akt plays an important role in the promotion of cell survival and prevention of proapoptosis in the intracellular signaling pathway in T lymphocytes [22]. In contrast to the activation of Akt by PDK1, the mechanisms of the inactivation of Akt have not been clearly delineated. The cellular FLICE-like inhibitory protein (c-FLIP$_S$) is an antiapoptotic cytoplasmic protein with sequence homology to caspase 8 and hence functions as a dominant-negative inhibitor of caspase 8, thereby preventing Fas-induced apoptosis [23]. The data obtained from our previous studies showed that HNE inhibited Akt activation and decreased c-FLIP$_S$ expression with a correspondent increase in caspase-8 activity that led to Fas-mediated apoptosis [22]. Although several studies have identified the antiapoptotic functions of Akt and c-FLIP$_S$ in T lymphocytes, the potential role of GSH in regulating Akt activation and c-FLIP$_S$ expression resulting in Fas-mediated apoptotic signaling has not been examined.

We hypothesized that GSH directly correlates with Akt expression and survival of CD4$^+$ T lymphocytes, as well as Fas-mediated death signaling. Therefore, our aim in this study was to test this hypothesis by modulating GSH status with PTCA and BSO in the HNE-induced cytotoxicity of Jurkat cells. We also explored whether PTCA can attenuate HNE-induced cytotoxicity by blocking the Fas-mediated apoptotic signaling pathway through Akt activation.

2. Materials and methods

2.1. Cell culture

Jurkat (clone E6-1) cells were obtained from ATCC (Rockville, MD, USA) and grown in RPMI-1640 supplemented with 10% fetal bovine serum, 10 U/ml of penicillin and 10 μg/ml of streptomycin in a 5% CO_2 incubator at 37°C.

2.2. Chemicals

HNE and malondialdehyde (MDA) were kindly provided by Dr. Sanjay Srivastava (Department of Internal Medicine). The antioxidant, PTCA, was synthesized and kindly provided by Dr. Herbert T. Nagasawa (VA Medical Center, Minneapolis, MN, USA). BSO, Nonidet P40, sodium orthovanadate and benzamidine were purchased from Sigma (St. Louis, MO, USA). FasL (C-178) and β-actin antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-FLIP$_{\gamma/\delta}$ (191–209) antibody was purchased from Calbiochem (La Jolla, CA, USA). *p*-Akt (Ser 473) and total Akt antibodies were purchased from Cell Signaling (Beverly, MA, USA). Goat antirabbit, antimouse and antirat antibodies conjugated with horseradish peroxidase were purchased from Bioscience International (Rockville, MD, USA). Phenylmethylsulfonyl fluoride was obtained from Boehringer Mannheim (Indianapolis, IN, USA). β-Glycerophosphate was purchased from TCI America (Portland, OR, USA). Fetal bovine serum, penicillin/streptomycin and trypan blue were purchased from Invitrogen (Grand Island, NY, USA).

2.3. Analysis of GSH and other thiols (SH) and disulfides (SS)

Cell pellets were dispersed with 0.25 ml of 4% metaphosphoric acid and centrifuged at 10,000×*g* for 2 min. The supernatants were collected for GSH analysis. Reduced GSH, oxidized GSH (GSSG), cysteine and cystine were simultaneously quantified by high-performance liquid chromatography with dual electrochemical detection according to our previous method [24]. In brief, 20-μl samples were injected onto a 250×4.6-mm and 5-μm C-18 column (Val-U-Pak HP, fully end-capped ODS, ChromTech, Apple Valley, MN, USA). Samples (20 μl) were injected onto the column and eluted isocratically with a mobile phase consisting of 0.1 M of monochloroacetic acid, 2 mM of heptanesulfonic acid and 2% acetonitrile at pH 2.8 and then delivered at a flow rate of 1 ml/min. The compounds were detected in the eluant with a Bioanalytical Systems model LC4B dual electrochemical detector using two Au–Hg electrodes in series with potentials of −1.2 and 0.15 V for the upstream and downstream electrodes, respectively. Current (in nanoamperes) was measured at the downstream electrode. Analytes were quantified from peak area measurements using authentic external standards.

2.4. Trypan blue dye exclusion

Cells were stained with trypan blue dye, counted by light microscopy with a minimum of 100 total cells counted per slide and then scored as cells that were able to exclude the dye (alive) or unable to exclude the dye (dead) to measure their viability [25].