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Differential roles of 3*H*-1,2-dithiole-3-thione-induced glutathione, glutathione *S*-transferase and aldose reductase in protecting against 4-hydroxy-2-nonenal toxicity in cultured cardiomyocytes

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Abstract

4-Hydroxy-2-nonenal (HNE) plays an important role in the pathogenesis of cardiac disorders. While conjugation with glutathione (GSH) catalyzed by GSH *S*-transferase (GST) has been suggested to be a major detoxification mechanism for HNE in target cells, whether chemically upregulated cellular GSH and GST afford protection against HNE toxicity in cardiac cells has not been investigated. In addition, the differential roles of chemically induced GSH and GST as well as other cellular factors in detoxifying HNE in cardiomyocytes are unclear. In this study, we have characterized the induction of GSH and GST by 3*H*-1,2-dithiole-3-thione (D3T) and the protective effects of the D3T-elevated cellular defenses on HNE-mediated toxicity in rat H9C2 cardiomyocytes. Treatment of cardiomyocytes with D3T resulted in a significant induction of both GSH and GST as well as the mRNA expression of γ-glutamylcysteine ligase catalytic subunit and GSTA. Both GSH and GST remained elevated for at least 72 h after removal of D3T from the culture media. Treatment of cells with HNE led to a significant decrease in cell viability and an increased formation of HNE–protein adducts. Pretreatment of cells with D3T dramatically protected against HNE-mediated cytotoxicity and protein–adduct formation. HNE treatment caused a significant decrease in cellular GSH level, which preceded the loss of cell viability. Either depletion of cellular GSH by buthionine sulfoximine (BSO) or inhibition of GST by sulfasalazine markedly sensitized the cells to HNE toxicity. Co-treatment of cardiomyocytes with BSO was found to completely block the D3T-mediated GSH elevation, which however failed to reverse the cytoprotective effects of D3T, suggesting that other cellular factor(s) might be involved in D3T cytotprotection. In this regard, D3T was shown to induce cellular aldose reductase (AR). Surprisingly, inhibition of AR by sorbinil failed to potentiate HNE toxicity in cardiomyocytes. In contrast, sorbinil dramatically augmented HNE cytotoxicity in cells with GSH depletion induced by BSO. Similarly, in BSO-treated cells, D3T cytoprotection was also largely reversed by sorbinil, indicating that AR played a significant role in detoxifying HNE only under the condition of GSH depletion in cardiomyocytes. Taken together, this study demonstrates that D3T can induce GSH, GST, and AR in cardiomyocytes, and that the above cellular factors appear to play differential roles in detoxification of HNE in cardiomyocytes.

Keywords: 4-Hydroxy-2-nonenal; 3*H*-1,2-Dithiole-3-thione; Glutathione; Glutathione *S*-transferase; Aldose reductase; Cardiomyocytes; Cytotoxicity

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It is increasingly recognized that oxidative stress induced by augmented formation of reactive oxygen species (ROS)[1] may play a causal role in the pathophysiology of various cardiac disorders, including myocardial ischemia and ischemia–reperfusion injury, congestive heart failure, cardiac hypertrophy, and cardiomyopathy [1–4]. In cardiac tissue, ROS may be generated from various sources, including vascular endothelial cells, infiltrated inflammatory cells, and cardiomyocytes [1]. Increased ROS levels may lead to oxidative damage to cellular constituents, including lipids, proteins, and nucleic acids. Oxidation of membrane lipid molecules can result in the formation of a number of reactive metabolites, such as unsaturated electrophilic aldehydes [5,6].

Among the various forms of aldehydes formed in cardiac tissue under oxidative stress, 4-hydroxy-2-nonenal (HNE) has received extensive attention over the last several years. HNE is a highly electrophilic, α,β-unsaturated aldehyde, which can covalently bind to cellular nucleophiles, particularly the cysteine, histidine, and lysine residues of proteins, leading to the formation of HNE–protein adducts [5–7]. Formation of HNE–protein adducts has been repeatedly observed in cardiac cells/tissue under oxidative stress, and has been suggested to be an important mechanism of oxidative cardiac cell injury [8–10]. In this context, formation of HNE–protein adducts can result in the inactivation of several cellular enzymes, including mitochondrial cytochrome *c* oxidase, pyruvate dehydrogenase, and NADP+-isocitrate dehydrogenase [11–13]. These enzymes are critically involved in energy metabolism in cardiomyocytes.

A number of cellular factors have been suggested to be capable of metabolizing HNE, including glutathione (GSH), glutathione *S*-transferase (GST, EC2.5.1.18), aldehyde dehydrogenase (EC1.2.1.3), and aldose reductase (AR, EC1.1.1.21) [7]. Among them, GSH and GST are most extensively investigated in terms of their roles in detoxification of HNE in target cells. At physiological pH, GSH readily reacts with HNE via Michael addition, yielding a glutathione–HNE conjugate [7]. GST has been found to catalyze this reaction 600-fold faster compared with the spontaneous reaction in cell-free systems [14]. As such, conjugation with GSH catalyzed by GST has been proposed to be a major metabolic pathway leading to detoxification of HNE in target cells [15–18]. However, studies on the roles of GSH and GST in HNE detoxification in cardiomyocytes are currently lacking. In addition, whether upregulation of GSH and GST by chemoprotective agents provides cytoprotection against HNE-induced toxicity in cardiomyocytes has not been reported in the literature. Accordingly, in this study using rat H9C2 cardiomyocytes we have investigated the induction of cellular GSH and GST, as well as AR by D3T, and the protective effects of the D3T-elevated cellular defenses on HNE-mediated cytotoxicity. Our results demonstrated that D3T could potently induce GSH, GST, and AR in cardiomyocytes, and that the above cellular factors appeared to play differential roles in the detoxification of HNE in cardiomyocytes.

[1] *Abbreviations used:* HNE, 4-hydroxy-2-nonenal; GSH, reduced glutathione; GST, glutathione *S*-transferase; D3T, 3*H*-1,2-dithiole-3-thione; BSO, buthionine sulfoximine; ROS, reactive oxygen species; FBS, fetal bovine serum; CDNB, 1-chloro-2,3-dinitrobenzene; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium; PBS, phosphate-buffered saline; LDH, lactate dehydrogenase; γGCL, γ-glutamylcysteine ligase; AR, aldose reductase.

Materials and methods

Materials

D3T with a purity of 99.8% was generously provided by Dr. Mary Tanga at SRI International (Menlo Park, CA) and Dr. Linda Brady at National Institute of Mental Health (Bethesda, MD). 4-Hydroxy-2-nonenal (HNE) was from Cayman Chemical (Ann Arbor, MI). Sorbinil was a generous gift from Pfeizer (Groton, CT). Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, and fetal bovine serum (FBS) were from Gibco-Invitrogen (Carlsbad, CA). All other chemicals and reagents were from Sigma Chemical (St. Louis, MO). Tissue culture flasks and 24-well tissue culture plates were from Corning (Corning, NY).

Cell culture

Rat H9C2 cardiomyocytes (ATCC, Manassas, VA) were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin in 150 cm^2 tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO_2. The cells were fed every 2–3 days, and subcultured once they reached 70–80% confluence.

Preparation of cell extract

Cardiomyocytes were collected and resuspended in ice-cold 50 mM potassium phosphate buffer, pH 7.4, containing 2 mM EDTA. The cells were sonicated, followed by centrifugation at 13,000*g* for 10 min at 4 °C. The resulting supernatants were collected and the protein concentrations were quantified with Bio-Rad protein assay dye (Hercules, CA) using bovine serum albumin as the standard. The samples were kept on ice for measurements of GSH, GST, and AR within 2–3 h, as described below.

Measurement of cellular GSH content

The cellular GSH content was measured using the method of Hissin and Hilf [19], according to the proce-