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Mechanisms of CDDO-imidazolide-mediated cytoprotection against acrolein-induced neurocytotoxicity in SH-SY5Y cells and primary human astrocytes

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HIGHLIGHTS

- CDDO-Im affords a cytoprotection against acrolein toxicity in differentiated SH-SY5Y cells and primary human astrocytes.
- GSH depletion by BSO potentiates acrolein-induced cytotoxicity.
- Upregulation of GSH is a predominant mechanism of CDDO-Im cytoprotection.
- CDDO-Im is a promising novel agent against reactive acrolein-induced neurotoxicity.

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ABSTRACT

Acrolein is a ubiquitous unsaturated aldehyde has been implicated in the pathogenesis of various neurological disorders. However, limited study has been conducted into potential therapeutic protection and underlying mechanism against acrolein-induced cytotoxicity via upregulation of cellular aldehyde-detoxification defenses. In this study we have utilized RA-differentiated human SH-SY5Y cells and primary human astrocytes to investigate the induction of glutathione (GSH) by the synthetic triterpenoid 2-cyano-3,12-dixooleana-1,9-dien-28-imidazolide (CDDO-Im) and the protective effects CDDO-Im-mediated antioxidant defenses on acrolein toxicity. Acrolein exposure to RA-differentiated SH-SY5Y cells resulted in a significant time dependent depletion of cellular GSH preceding a reduction in cell viability and LDH release. Further, we demonstrated the predominance of cellular GSH in protection against acrolein-induced cytotoxicity. Buthionine sulfoximine (BSO) at 25 μM dramatically depleted GSH and significantly potentiated acrolein-induced cytotoxicity. Pretreatment of the cells with 100 nM CDDO-Im afforded a dramatic protection against acrolein-induced cytotoxicity. Pretreatment of BSO and CDDO was found to prevent the CDDO-Im-mediated GSH induction and partially reversed the cytoprotective effects of CDDO-Im against acrolein cytotoxicity. Overall, this study represents for the first time the CDDO-Im mediated upregulation of GSH is a predominant mechanism against acrolein-induced neurotoxicity.

Abbreviations: AR, aldose reductase; BSA, bovine serum albumin; BSO, buthionine sulfoximine; CDDO-Im, triterpenoid 2-cyano-3,12-dixooleana-1,9-dien-28-imidazolide; CDNB, 1-chloro-2,4-dinitrobenzene; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GSH, glutathione; GST, glutathione *S*-transferase; LDH, lactate dehydrogenase release; LDH, lactate dehydrogenase; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium; OPT, *o*-phtalaldehyde; PBS, phosphate buffered saline; RA, retinoic acid; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

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1. Introduction

Acrolein is a ubiquitous environmental toxin formed during the combustion of organic matter. This toxicant is found in many pesticide products and in all forms of smoke, including automobile exhaust and cigarettes (Beauchamp et al., 1985). Acrolein has also been reported to be formed during the metabolism of the anti-cancer drug cyclophosphamide (Norton et al., 1997; Ohno and Ormstad, 1985). An exogenous reactive aldehyde, acrolein, has been found to readily peroxidize lipids including the fatty acid rich nerve cell types (Floyd and Hensley, 2002). Recent studies show that the resulting oxidative damage leads to decreased cell proliferation and increased apoptosis (Agil et al., 2006; Norton et al., 1997). Acrolein can also be formed inside the cell as a product of lipid peroxidation by other forms of reactive oxygen species (ROS) (Kehrer and Biswal, 2000). Neuronal cells exposed to acrolein and other ROS generating compounds are found to be associated with the progression of Parkinson's Disease as well as other neurodegenerative disorders (Calingasan et al., 1999; Lovell et al., 2000). Elevated concentrations of acrolein, up to 40 μM, have been found in the brain tissue of PD patients and are considered a specific marker for lipid peroxidation damage (Agil et al., 2006; Lovell et al., 2001). Formation of these acrolein–protein adducts have been implicated in the pathogenesis of neurodegenerative disorders including PD (Calingasan et al., 1999; Lovell et al., 2000). Acrolein causes damage to biomolecules including lipids, protein, and DNA damage leading to cell death and neurodegeneration (Kehrer and Biswal, 2000). In addition to direct oxidative damage, acrolein also plays a role in the depletion of beneficial antioxidant defenses such as cellular GSH (Norton et al., 1997; Ohno and Ormstad, 1985). Thus, treatment options designed to upregulate the natural cellular antioxidant defenses were found to partially protect against substantia nigra degeneration in animal models (Cadet, 2001a; Jia et al., 2007).

Studies have shown that GSH, glutathione *S*-transferase (GST), and aldose reductase (AR) play important roles in the detoxification of acrolein (He et al., 1998a; Petersen and Doorn, 2004). Foremost among these is GSH, a tripeptide thiol consisting of glutamate, cysteine, and glycine, which is found in high levels in neuronal cells (Rice and RussoMenna, 1998). GSH is capable of conjugating reactive species and is also aided by other enzymatic antioxidants to detoxify peroxides in various cells (Zeevalk et al., 1998). It has been suggested that GST utilizes GSH as a cofactor in its detoxification process of electrophilic aldehydes (Yang et al., 2001). Similarly, AR has been shown to utilize a binding site on GSH to detoxify the GSH–aldehyde conjugates (He et al., 1998b; Srivastava et al., 1995). Due to the limited successes using exogenous antioxidants, many studies have been focused on the increase of the endogenous antioxidant capabilities of the cells, including GSH, against oxidative injury. Thus, regulation of GSH, GST, and AR could be an important defense mechanism against acrolein-induced neurological disorders. In our study we proposed a novel strategy for protective intervention of PD through the upregulation of endogenous antioxidant defenses in neuronal SH-SY5Y cells mediated by the synthetic triterpenoid compound 2-cyano-3,12-dixooleana-1,9-dien-28-imidazolide (CDDO-Im). Triterpenoids are steroid like compounds derived from plant extracts which have shown to have numerous protective/therapeutic effects on various cell types (Honda et al., 1998). Various forms of CDDO have also been utilized in clinical trials as an anti-cancer proliferation treatment and an anti-inflammatory agent for rheumatoid arthritis (Gao et al., 2013; Liby et al., 2007; Place et al., 2003; Suh et al., 1999).

We have recently found that CDDO-Im at low concentrations, within an achievable plasma range, significantly induces GSH and NAD(P)H:quinone oxidoreductase 1 (NQO1) in retinoic acid (RA)-differentiated SH-SY5Y cells conferring protection against reactive oxidative and electrophilic species (Speen, 2013). RA-induced differentiation allows the SH-SY5Y cells to more closely mimic primary neuron. Among a variety of transcriptional changes, RA activates the retinoic *x* receptor (RXR) which promotes the transcription of proteins responsible for the development of neurites, formation of neurotransmitter receptors, and alteration of mitochondrial function (Xie et al., 2010; Xun et al., 2012). The effects of CDDO-Im in ameliorating the toxic effects of acrolein, however, remain unknown. Furthermore, it remains unclear the roles of GSH, GST, and AR in CDDO-Im mediated cytoprotection against acrolein toxicity. In this study, using RA-differentiated human SH-SY5Y cells and primary human astrocytes as model systems, we determined the protective effect of CDDO-Im against acrolein-induced cytotoxicity. We further demonstrated that CDDO-Im mediated upregulation of GSH is a predominant mechanism against acrolein-induced neurotoxicity in RA-differentiated SH-SY5Y cells and primary human astrocytes.

2. Materials and methods

2.1. Chemicals and materials

Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin, and fetal bovine serum (FBS) were obtained from Gibco-Invitrogen (Carlsbad, CA). CDDO-Im [2-cyano-3,12-dixooleana-1,9-dien-28-imidazolide] was obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Acrolein, GSH, Retinoic Acid (RA), 1-chloro-2,4-dinitrobenzene (CDNB), *o*-phtalaldehyde (OPT), (±)-α-tocopherol (Vitamin E), 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltertrazolium bromide (MTT) and *N*-ethyleimide were from Sigma Chemical (St. Louis, MO). Tissue culture flasks and 24-well tissue culture plates were from Corning (Corning, NY).

2.2. Cell culture

Human neuroblastoma SH-SY5Y cells (ATCC, Manassas, VA) were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 ug/mL streptomycin in 75 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 80–90% confluence. For experiments, cells were differentiated in medium with 10 μM retinoic acid (RA) for 5–7 days. Human primary astrocytes were purchased from Lonza Research Products (Walkersville, Maryland) and cultured according to the manufacturer's protocol in a recommended medium (Lonza Research Products) at 37 °C in a humidified atmosphere of 5% CO_2. For acrolein treatment the cells were grown in 75 cm^2 tissue culture flasks under the same conditions. Acrolein was diluted in DMEM supplemented with 2% FBS and the cells were treated with acrolein for different time periods based on the specific assays.

2.3. Cell extract preparation

After experimental treatment, cells were pelleted by centrifugation at 300 × *g* at 4 °C for 10 min. Cells were then washed once with phosphate buffered saline (PBS) and resuspended in ice-cold 50 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA and 0.1% Triton X-100. The cell suspensions were sonicated, followed by centrifugation at 13,000 × *g* for 10 min at 4 °C to remove cell debris. The resulting supernatants were collected and the protein concentrations were quantified with Bio-Rad protein assay dye kit (Hercules, CA) based on the method of Bradford using bovine serum albumin (BSA) as the standard. The supernatants were then used for measurement of the antioxidants and phase 2 enzymes.