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Cytoplasmic and nuclear toxicity of 3,5-dimethylaminophenol and potential protection by selenocompounds

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ABSTRACT

Most common alkylanilines in the environment are 2,6-dimethylaniline (2,6-DMA), 3,5-dimethylaniline (3,5-DMA), and 3-ethylaniline (3-EA). 3,5-Dimethylaminophenol (3,5-DMAP), a metabolite of 3,5-DMA, is of particular interest, as it is potentially genotoxic. Supplementation with organic or inorganic forms of selenium (Se) may reduce toxicity following exposure to a wide variety of environmental chemicals. This study was designed to evaluate the protective effects of sodium selenite (SS) and selenomethionine (SM) at varying time points of supplementation (24 h and 72 h) against the cytotoxicity, reactive oxygen species (ROS) production, and genotoxicity of 3,5-DMAP in CHO AS52 cells. 3,5-DMAP caused dose-dependent increase of cytotoxicity, ROS production and genotoxicity, and generated free radicals in the nuclei. Thioredoxin reductase (TrxR), catalase and glutathione reductase activities, and glutathione levels were significantly lower while lipid peroxidation and protein oxidation levels were higher after 3,5-DMAP treatment in both cytoplasm and the nucleus vs. control. After 24 h, both SS and SM provided protection in antioxidant/oxidant status of the 3,5-DMAP-treated cells; however other than supplying higher glutathione peroxidase and TrxR activities, 72 h supplementation did not provide advanced improvement. Selenocompounds may be beneficial against cytotoxic and genotoxic potential of 3,5-DMAP and might protect both nucleus and cytoplasm following exposure to alkylanilines.

1. Introduction

Oxidative stress can account for changes that are detrimental to cells. Reactive oxygen species (ROS) are shown to contribute to cellular damage, apoptosis or carcinogenesis (Dalton et al., 1999; Dröge, 2002; Reuter et al., 2010). Currently, there is a growing interest in environmental chemicals that can induce ROS, like alkylanilines (Soory, 2009).

Epidemiological studies have demonstrated that alkylanilines are a group of chemicals that are ubiquitous in the environment. Most individuals experience lifelong exposure to these compounds from occupational exposure or *via* tobacco smoke (Skipper et al., 2010). They are considered factors for the development of bladder cancer (Skipper et al., 2010). Alkylanilines are potentially activated through the P450-catalyzed oxidation of the amino group, subsequently undergoing oxidation of the *N*-hydroxylamine and heterolysis of the N–O bond to produce a reactive nitrenium ion (Chao et al., 2012). The *N*-hydroxylation product of arylamines could form para-*N*-phenyl-hydroxylamine (DMHA) and is isomeric

Abbreviations: 2,6-DMA, 2,6-dimethylaniline; 3,5-DMA, 3,5-dimethylaniline; 3,5-DMAP, 3,5-dimethylaminophenol; 3,5-DMIQ, 4-amino-3,5-dimethylphenol iminequinone; 3-EA, 3-ethylaniline; 6-TG, 6-thioguanine; BCA, bicinchoninic acid assay; CAT, catalase; CHO, Chinese Hamster Ovary cells; CM-H_2DCFA, 5-(and 6-) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate; DMHA, para-*N*-phenyl-hydroxylamine; DMSO, dimethyl sulfoxide; DPNH, 2,4-dinitrophenylhydrazine; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); EMS, ethyl methansulfonate; ESI-TOF-MS, electrospray ionization source time-of-flight and tandem quadrupole mass spectrometry; FBS, fetal bovine serum; *gpt*, xanthine–guanine phosphoribosyl transferase gene; GPxs, glutathione peroxidases; GSH, reduced glutathione; GSSG, oxidized glutathione; HBSS, Hank's Buffered Salt Solution; HNE, 4-hydroxynonenal; HPRT, hypoxanthine–guanine phosphoribosyl transferase; LC, liquid chromatography; MAAs, monocyclic aromatic amines; MDA, malonyldialdehyde; NAC, N-acetylcysteine; NADP, Nicotinamide adenine dinucleotide phosphate; NE-PER, nuclear and cytoplasmic extraction kit; OTM, olive tail moment; p-AP, para-aminophenol; ROS, reactive oxygen species; Se, selenium; SM, selenomethionine; SOD, total superoxide dismutase; SS, sodium selenite; TBARS, thiobarbituric acid reactive substance; TNB, 5-thio-2-nitrobenzoic acid; TrxRs, thioredoxin reductases; XO, xanthine oxidase; XPRT, xanthine-guanine phosphoribosyl transferase.

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with para-aminophenol (p-AP). The hydroxylamine might also be a source of DMHA, which is known to rearrange to DMAP under mildly acidic conditions (Sone et al., 1981; Fishbein and McClelland, 1987). The mutagenic activity of p-AP was suppressed with the addition of dimethylsulfoxide (DMSO) or catalase (CAT), suggesting the involvement of ROS in the mutagenic activity of p-AP (Yoshida et al., 1998).

3,5-Dimethylaminophenol (3,5-DMAP), a metabolite of 3,5-DMA, is of particular interest, as it is potentially genotoxic by a mechanism involving non-enzymatic oxidation to the quinoneimine and quinone (Jefferies et al., 1998; Yoshida et al., 1998; Ye et al., 2012). Chao et al. (2014) showed that 3,5-DMAP caused apoptosis *via* generation of H_2O_2 and hydroxyl radical (·OH) and upregulation of caspase 3 in Chinese Hamster Ovary (CHO) AS52 cells (Ye et al., 2012; Chao et al., 2014). Therefore, it can be suggested that one of the major causes underlying the toxicity of 3,5-DMAP is oxidative stress.

There is considerable interest in developing strategies to prevent the cytotoxicity and genotoxicity induced by alkylanilines and their metabolites, with minimal risk or toxicity. If not counteracted by cellular antioxidants, high levels of ROS can cause acute injury and damage of important biomolecules including cellular proteins, lipids and DNA and this phenomenon may lead to cell death (Martindale and Holbrook, 2002; Kern and Kehrer, 2005). Published data associates both low antioxidant status and genetics as contributing factors to the risk of several types of malignancies (Baliga et al., 2007; Lee, 2009). Trace elements like selenium (Se) are a key component of several antioxidant enzyme systems. Se-dependent glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs) protect the body from cellular metabolism's endogenous by-products, which are associated with DNA damage, mutagenesis, and carcinogenesis (Ganther, 1999; Jablonska et al., 2009). Se was found to be protective against the toxic potential of different agents both *in vitro* and *in vivo* (Erkekoglu et al., 2010a, 2010b;Sharma et al., 2014), through different pathways some of which need to be discovered.

Research has shown that low, non-toxic supplementation with either organic [selenomethionine (SM)] or inorganic [sodium selenite (SS)] selenium forms can reduce cancer incidence following exposure to a wide variety of carcinogens (el-Bayoumy et al., 1991). Se supplementation was shown to reduce the risk of many types of neoplasia, including bladder cancer. The results from seven epidemiological studies conducted in different populations indicate that high levels of serum Se provided a significant and 39% decreased risk of bladder cancer (Amaral et al., 2010).

Based on this background information and taking into account the importance of Se in the antioxidant system, this study was designed to evaluate the protection offered by supplementation with different selenocompounds, SS and SM, at varying time points of exposure against 3,5-DMAP-caused cytotoxicity, ROS production, and genotoxicity in CHO AS52 cells.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals including SS, SM, 6-thioguanine (6-TG), dimethyl sulfoxide (DMSO), ethyl methansulfonate (EMS), DL-isocitric acid, triethanolamine, vinylpyridine, protease inhibitor cocktail were purchased from Sigma–Aldrich (St. Louis, MO). Nicotinamide adenine dinucleotide phosphate (NADP) was purchased from Boehringer Mannheim (Indianapolis, IN). Cell proliferation reagent WST-1 from Roche Applied Science (Indianapolis, IN). 5-(and 6-)Chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (CM-H_2 DCFA) ROS dye, molten normal melting point agarose, low melting point agarose, SYBR Gold and Hoechst 33258 were obtained from Molecular Probes/Invitrogen (Eugene, OR).

2.2. Kits

Colorimetric assay kit for bicinchoninic acid (BCA) assay, and spectrophotometric GPx1, TrxR, CAT, total superoxide dismutase (SOD), glutathione reductase (GR), glutathione S-transferase (GST), glutathione (GSH), caspase 3 and caspase 8 kits were obtained from Sigma–Aldrich (St. Louis, MO). Nuclear and Cytoplasmic Extraction kit (NE-PER) were purchased from Thermo Scientific (Rockford, IL). Thiobarbituric acid reactive substance (TBARS) kit and carbonyl assay kit were obtained from Cayman Chemical Company (Ann Arbor, MI). All cell culture reagents and GelBond film were purchased from Lonza (Walkersville, MD). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA). Bottomless 96-well plates for Comet assay were purchased from Greiner BioOne (Monroe, NC).

2.3. Synthesis of 3,5-dimethylaminophenol

2.3.1. Instrumentation

NMR spectra were recorded on a Varian INOVA NMR spectrometer (Varian, Inc., Palo Alto, CA) at 500 MHz for ^{1}H NMR. Analyses were performed in high resolution detection on an electrospray ionization source time-of-flight and tandem quadrupole mass spectrometry (ESI-TOF-MS) (Agilent, Palo Alto, CA). ESI was conducted using a needle voltage of 3.5 kV. Nitrogen was used as the drying (12 L/min) and nebulizer (35 psig) gas with the heated capillary at 325 °C. The ion trap mass spectrometer was operated in full scan. Chromatography was performed using an Agilent 1200 LC system. Liquid chromatography (LC) separations were performed using a Zorbax Eclipse XDB-C18 (2.1 mm × 150 mm; 5 μm; Agilent column with a flow rate of 0.3 mL/min). Solvent A was 0.1% (h/h) formic acid in water, and solvent B was 0.1% (h/h) formic acid in methanol.

2.3.2. 3,5-Dimethylaminophenol

3,5-DMAP was synthesized according to Chao et al. (2012). The yield of final product was 82%, the positive ion in high resolution detection by performed by ESI-TOF-MS: calculated for $[C_8H_{11}NO]H^+$, 138.0913, found 138.0910. ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): 7.17 (s, 1H), 6.15 (m, 2H), 4.3 (s, 2H), 2.02 (s, ^{6}H).

2.4. 4-Amino-3,5-dimethyl quinoneimine (3,5-DMQI) synthesis

The 3,5-DMAP (35 mg) was dissolved in 10 mL of ethyl acetate and mixture was stirred under argon atmosphere. Lead dioxide (350 mg) was added and was stirred for 30 min. The reaction mixture was washed with 10 mL of 5% aqueous $NaHCO_3$. The high resolution detection was performed by ESI-TOF-MS and was calculated for $C_8H_9NO]H^+$, 136.0757 and was found 136.0751. The results for ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C) were 11.59 (s, 1H, OH), 7.05 (s, ^{1}H, Ar–H), 6.87 (s, ^{1}H, Ar–H), 1.94 (s, ^{3}H, Methyl-H), 1.89 (s, ^{3}H, Methyl-H).

2.5. Determination of the conversion rate of 3,5-dimethylaminophenol to 4-amino-3,5-quinoneimine

Cell media including 3,5-DMAP (50 μM) was analyzed by liquid chromatography-ESI-TOF-MS (LC-ESI-TOF-MS) with or without cells at 0 h and 1 h. Media were analyzed by ESI-TOF-MS. The full scans of each sample were extracted with exact mass of the 3,5-DMAP. The consumption rate of the 3,5-DMAP was also measured for 1 h.