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Methylmercury alters glutathione homeostasis by inhibiting glutaredoxin 1 and enhancing glutathione biosynthesis in cultured human astrocytoma cells

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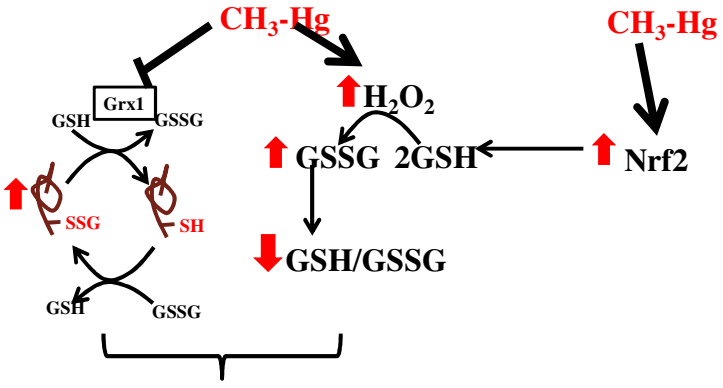
HIGHLIGHTS

- MeHg disrupts glutathione homeostasis and intracellular redox state of cultured human astrocytoma cells.
- Exposure to MeHg results in the accumulation of cellular GSSG which increases the redox potential of GSH/GSSG.
- MeHg directly interferes with glutaredoxin-1 (Grx1) activity prolonging protein *S*-glutathionylation.

GRAPHICAL ABSTRACT



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ABSTRACT

Methylmercury (MeHg) is a neurotoxin that binds strongly to thiol residues on protein and low molecular weight molecules like reduced glutathione (GSH). The mechanism of its effects on GSH homeostasis particularly at environmentally relevant low doses is not fully known. We hypothesized that exposure to MeHg would lead to a depletion of reduced glutathione (GSH) and an accumulation of glutathione disulfide (GSSG) leading to alterations in *S*-glutathionylation of proteins. Our results showed exposure to low concentrations of MeHg (1 μM) did not significantly alter GSH levels but increased GSSG levels by ~12-fold. This effect was associated with a significant increase in total cellular glutathione content and a decrease in GSH/GSSG. Immunoblot analyses revealed that proteins involved in glutathione synthesis were upregulated accounting for the increase in cellular glutathione. This was associated an increase in cellular Nrf2 protein levels which is required to induce the expression of antioxidant genes in response to cellular stress. Intriguingly, we noted that a key enzyme involved in reversing protein *S*-glutathionylation and maintaining glutathione homeostasis, glutaredoxin-1 (Grx1), was inhibited by ~50%. MeHg

Abbreviations: MeHg, Methylmercury; iHg, inorganic mercury; GSH, reduced glutathione; GSSG, glutathione disulfide; Grx1, glutaredoxin 1; Gpx1, glutathione peroxidase 1; Nrf2, Nuclear factor (erythroid-derived 2)-like 2; GS, glutathione synthetase; SdhC, succinate dehydrogenase subunit C; Ndusf1, NADH:ubiquinone oxidoreductase core subunit S1.

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treatment also increased the *S*-glutathionylation of a high molecular weight protein. This observation is consistent with the inhibition of Grx1 and elevated H_2O_2 production however; contrary to our original hypothesis we found few *S*-glutathionylated proteins in the astrocytoma cells. Collectively, MeHg affects multiple arms of glutathione homeostasis ranging from pool management to protein *S*-glutathionylation and Grx1 activity.

1. Introduction

Methylmercury (MeHg) is global pollutant affecting the health of millions of people worldwide (Shapiro and Chan, 2008). MeHg is a well-known neurotoxin and has also been shown to disrupt the function of multiple organs throughout the body (Hansen et al., 1989). Exposure to MeHg has been shown to delay neurodevelopment and exposure has also been associated with development of neurological disorders like Parkinson's disease (Petersen et al., 2008). This is attributed to its capacity to diffuse easily across biological barriers like the blood brain barrier and accumulate in neurological tissues. MeHg neurotoxicity has also been associated with its strong soft acid properties which instils it with the capability to strongly bind soft bases like sulfur residues on cysteines rendering biological thiols inactive (Ballatori and Truong, 1995). Indeed, MeHg has been shown to bind GSH (γ-glutamyl-cysteinyl-glycine) in various biological models including cell culture systems and animal models (Khan et al., 2012; Patrick, 2002).

GSH (γ-glutamyl-cysteinyl-glycine) is a sulfur containing tripeptide synthesized in virtually all mammalian cells (Dalle-Donne et al., 2009). Two enzymes, γ-glutamylcysteine ligase and glutathione synthetase (GS), catalyze the ATP-dependent biosynthesis of GSH from its constituent amino acids (Pastore and Piemonte, 2012). By virtue of its high concentration and low midpoint reduction potential, it is utilized principally as an antioxidant (Flohe, 2013). Indeed, GSH is utilized ubiquitously throughout nature as a "sink" for H_2O_2, a two electron nonradical generated naturally by biological systems which can induce oxidative stress at high enough quantities. However, the direct reaction of GSH with H_2O_2 is kinetically slow ($\sim 0.89\ M^{-1}\ s^{-1}$) and thus GSH-mediated H_2O_2 sequestration relies on the enzymatic activity of glutathione peroxidase (Gpx) (Winterbourn, 2015). In the presence of two GSH molecules, Gpx catalyzes the removal of H_2O_2 at a rate of $\sim 6 \times 10^7\ M^{-1}\ s^{-1}$ generating GSSG (Mailloux, 2015). Glutathione reductase (GR), in the presence of NADPH, reduces GSSG with high efficiency rejuvenating the GSH pool. Due to the action of GR, the cellular glutathione pool is normally highly reducing with GSH levels varying between 1 and 10 mM and GSSG at ~0.01 mM (Pastore and Piemonte, 2012). Thus in normal cells the GSH/GSSG redox pair is over 100 whereas in models of oxidative stress the ratio approaches 1. MeHg has been shown to alter the ratio of GSH/GSSH either by binding directly to GSH, decreasing the levels of selenium dependent glutathione peroxidase, and causing oxidative stress by disrupting the energy regulation in the mitochondria in various biological systems (Ni et al., 2011; Truong et al., 2015; Wang et al., 2009).

It is now appreciated that cells have an entire *redoxome* where protein functions are modulated in response to fluctuations in cellular redox buffering networks (Mailloux and Treberg, 2015). Various antioxidant networks including glutathione and peroxiredoxins work in tandem to buffer redox environments in response to changes in cellular ROS production. By virtue of its high concentration and negative redox potential, GSH serves as one of the major redox buffers in cells (Mailloux and Treberg, 2015). Glutathione conveys redox signals to proteins through *S*-glutathionylation, a type of covalent modification which involves the formation of a disulfide bridge between glutathione and a protein cysteine thiol (Gao et al., 2013). Historically, formation of protein glutathione mixed disulfides (PSSG) was associated with oxidative stress where a sudden rise in GSSG levels leads to a simple disulfide exchange reaction with protein cysteine residues and the subsequent spontaneous *S*-glutathionylation of a protein (Gallogly et al., 2008). However, it is also now appreciated that protein *S*-glutathionylation proceeds under normal cellular conditions in response to fluctuations in redox environment and mediated by changes in cellular H_2O_2 levels (Mailloux and Willmore, 2014). Glutaredoxins (Grx1; cytosol, Grx2; mitochondria), small heat stable thiol oxidoreductases that are part of the thioredoxin superfamily, catalyze the reversible *S*-glutathionylation of proteins in response to local changes in GSH/GSSG and spatiotemporal changes in H_2O_2 levels (Gallogly et al., 2008). Grx1 and 2 also play important roles in the deglutathionylation of proteins following bouts of oxidative stress. Genetic deletion or inhibition of either Grx1 or Grx2 leads to deregulated cellular *S*-glutathionylation patterns, disruption of cell signaling events, induction of cell death, and dysfunctional cellular metabolism (Kim et al., 2011; Mieyal et al., 1991; Tarrago et al., 2009). Loss of function in either protein has also been associated with various pathological events including heart disease, neurological deficits, obesity, diabetes mellitus, and perturbations in embryonic development (Garcia-Garcia et al., 2012; Mailloux and Willmore, 2014; Picklo et al., 2013).

The effects of oxidative stress as one of the mechanisms of the neurotoxicity of MeHg have been extensively studied (Ni et al., 2010; Ni et al., 2011; Wang et al., 2009). Coupled with this several studies have been able to show that MeHg can induce oxidative stress by disrupting glutathione homeostasis, particularly through the depletion of cellular GSH. However, considering the function of cellular glutathione in mediating cellular redox signals, it is also imperative to decipher if MeHg can disrupt these pathways. In the present study, we examined the impact of different concentrations of MeHg on glutathione homeostasis which includes an in depth analysis of its effects on protein *S*-glutathionylation and Grx1 expression and activity. We hypothesized that exposure to MeHg would lead to a depletion of reduced glutathione (GSH) and an accumulation of glutathione disulfide (GSSG) leading to the prolonged *S*-glutathionylated of various proteins. Considering that MeHg also accumulates in astrocytes, a glial cell that provides metabolic and antioxidant support for neurons, we decided to utilize the human astrocytoma cell line, CCF-STTG1, as a model system to examine the impact of MeHg on glutathione homeostasis and Grx1 activity and expression.

2. Materials and methods

2.1. Cell culture and MeHg treatment

CCF-STTG1 human astrocytoma cells (American Type Culture Collection) were routinely cultured on matrigel-coated T25 cm^2 flasks for up to 25 passages in RMPI-1640 containing 10% (v/v) fetal bovine serum and 2% (v/v) antibiotic-antimycotics. Media was changed every 48 h and cells were passaged every 6 days. For experiments, cells were seeded at high concentration (~50,000 cells/mL) matrigel-coated 100 mm plates or 96-well plates. Upon