



## Research Paper

# Impaired cross-talk between the thioredoxin and glutathione systems is related to ASK-1 mediated apoptosis in neuronal cells exposed to mercury



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## ABSTRACT

Mercury (Hg) compounds target both cysteine (Cys) and selenocysteine (Sec) residues in peptides and proteins. Thus, the components of the two major cellular antioxidant systems – glutathione (GSH) and thioredoxin (Trx) systems – are likely targets for mercurials. Hg exposure results in GSH depletion and Trx and thioredoxin reductase (TrxR) are prime targets for mercury. These systems have a wide-range of common functions and interaction between their components has been reported. However, toxic effects over both systems are normally treated as isolated events.

To study how the interaction between the glutathione and thioredoxin systems is affected by Hg, human neuroblastoma (SH-SY5Y) cells were exposed to 1 and 5  $\mu\text{M}$  of inorganic mercury ( $\text{Hg}^{2+}$ ), methylmercury (MeHg) or ethylmercury (EtHg) and examined for TrxR, GSH and Grx levels and activities, as well as for Trx redox state. Phosphorylation of apoptosis signalling kinase 1 (ASK1), caspase-3 activity and the number of apoptotic cells were evaluated to investigate the induction of Trx-mediated apoptotic cell death. Additionally, primary cerebellar neurons from mice depleted of mitochondrial Grx2 (mGrx2D) were used to examine the link between Grx activity and Trx function.

Results showed that Trx was affected at higher exposure levels than TrxR, especially for EtHg. GSH levels were only significantly affected by exposure to a high concentration of EtHg. Depletion of GSH with buthionine sulfoximine (BSO) severely increased Trx oxidation by Hg. Notably, EtHg-induced oxidation of Trx was significantly enhanced in primary neurons of mGrx2D mice. Our results suggest that GSH/Grx acts as backups for TrxR in neuronal cells to maintain Trx turnover during Hg exposure, thus linking different mechanisms of molecular and cellular toxicity. Finally, Trx oxidation by Hg compounds was associated to apoptotic hallmarks, including increased ASK-1 phosphorylation, caspase-3 activation and increased number of apoptotic cells.

## 1. Introduction

Exposure to mercury (Hg) compounds causes neurotoxicity that can range from subtle changes in cognitive and motor development to severe impairment of neuromotor functions, depending on the length and magnitude of exposure [1]. Inhibition of glutamate transport, hindrance of neuronal migration and oxidative stress are known hallmarks of mercury mediated neurotoxicity [2]. On the molecular level, Hg has a high affinity to bind thiols and selenols [3–5] and consequently both the thioredoxin and glutathione systems are targets for mercurials.

The thioredoxin system comprises thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH, and is a major protein disulphide reductase system [6]. The diverse cellular functions relying on the

activity of this system include DNA synthesis, ROS scavenging, regulation of cell signalling and protein folding and repair [7]. Oxidation of Trx results in decreased antioxidant capacity and dysregulation of specific proteins, namely peroxiredoxins (Prx) [8], and activates ASK-1, a MAPKKK that regulates the p38 apoptotic pathway and subsequent downstream molecular signalling, which leads to caspase mediated cell death [9].

The selenoenzyme TrxR is extremely prone to inhibition by mercurials both in vitro [3,4,10] and in vivo [11,12]. For example, in HeLa cells exposed for 24 h to MeHg the  $\text{IC}_{50}$  for TrxR was 1.4  $\mu\text{M}$  whereas for the homologous enzyme, glutathione reductase (GR), it was > 20  $\mu\text{M}$  [3]. This sensitivity is due to the reactivity and position of the selenocysteine (Sec) residue in the open C-terminal of TrxR's active site

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