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Toxicological effects of thiomersal and ethylmercury: Inhibition of the thioredoxin system and NADP$^+$-dependent dehydrogenases of the pentose phosphate pathway

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ABSTRACT

Mercury (Hg) is a strong toxicant affecting mainly the central nervous, renal, cardiovascular and immune systems. Thiomersal (TM) is still in use in medical practice as a topical antiseptic and as a preservative in multiple dose vaccines, routinely given to young children in some developing countries, while other forms of mercury such as methylmercury represent an environmental and food hazard. The aim of the present study was to determine the effects of thiomersal (TM) and its breakdown product ethylmercury (EtHg) on the thioredoxin system and NADP$^+$-dependent dehydrogenases of the pentose phosphate pathway. Results show that TM and EtHg inhibited the thioredoxin system enzymes in purified suspensions, being EtHg comparable to methylmercury (MeHg). Also, treatment of neuroblastoma and liver cells with TM or EtHg decreased cell viability (GI_{50}: 1.5 to 20 μM) and caused a significant ($p < 0.05$) decrease in the overall activities of thioredoxin (Trx) and thioredoxin reductase (TrxR) in a concentration- and time-dependent manner in cell lysates. Compared to control, the activities of Trx and TrxR in neuroblastoma cells after EtHg incubation were reduced up to 60% and 80% respectively, whereas in hepatoma cells the reduction was almost 100%. In addition, the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were also significantly inhibited by all mercurials, with inhibition intensity of $Hg^{2+} > MeHg \approx EtHg > TM$ ($p < 0.05$). Cell incubation with sodium selenite alleviated the inhibitory effects on TrxR and glucose-6-phosphate dehydrogenase. Thus, the molecular mechanism of toxicity of TM and especially of its metabolite EtHg encompasses the blockage of the electrons from NADPH *via* the thioredoxin system.

Introduction

Mercurial compounds have shown a wide range of toxicological effects on human beings, involving especially the central nervous system, causing damage to the brain, but also to the kidneys, the cardiovascular and immune systems (Clarkson et al., 2003; Dórea et al., 2013). Exposure to mercurial compounds such as methylmercury (MeHg) and mercuric mercury (Hg^{2+}) at levels above the toxicity threshold occurs either by regular fish consumption or occupational contact, respectively, and represents a major concern in toxicology (Clarkson et al., 2003; Carvalho et al., 2008a; Nunes et al., 2014). Not less important is mercury exposure in dental practice for both dentists and patients due to the use of dental amalgam fillings that release mercury vapour (Clarkson et al., 2003). Even though the use of mercury compounds such as thiomersal (TM) in medicines and antiseptics is decreasing it is still used as a preservative in some formulas, namely in vaccines (Sykes et al., 2014).

Although mercurial compounds are not new toxicants, there is a significant lack of knowledge about their molecular mechanisms of toxicity, especially about TM and its breakdown product ethylmercury (EtHg). TM, a mercury derivative composed of EtHg and thiosalicylic acid has been widely used as a preservative in vaccines, dermatological (topic) and ocular preparations. Indeed, vaccines with TM are the main route of mercury exposure in clinics (Bigham and Copes, 2005) and while children in most of the developed countries receive normally TM-free vaccines, children in developing countries may receive several doses of different

Abbreviations: 6PGDH, 6-phosphogluconate dehydrogenase; EtHg, ethylmercury; G6PDH, glucose-6-phosphate dehydrogenase; Hg^{2+}, mercuric mercury; MeHg, methylmercury; Se^{2-}, selenide; SeO_3^{2-}/Se (IV), selenite; Se, selenium; TCV, thiomersal-containing vaccines; TM, thiomersal; Trx, thioredoxin; TrxR, thioredoxin reductase.

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