



Low molecular weight thiols reduce thimerosal neurotoxicity in vitro: Modulation by proteins

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ABSTRACT

Thimerosal (TH), an ethylmercury complex of thiosalicylic acid has been used as preservative in vaccines. In vitro neurotoxicity of TH at high nM concentrations has been reported. Although a number of toxicological experiments demonstrated high affinity of mercury to thiol groups of the extracellular amino acids and proteins that may decrease concentration of free TH in the organism, less is known about the role of interactions between proteins and amino acids in protection against TH neurotoxicity. In the present study we examined whether the presence of serum proteins and of L-cysteine (Cys), D,L-homocysteine (Hcy), N-acetyl cysteine (NAC), L-methionine (Met) and glutathione (GSH) in the incubation medium affects the TH-induced changes in the viability, the intracellular levels of calcium and zinc and mitochondrial membrane potential in primary cultures of rat cerebellar granule cells. The cells were exposed to 500 nM TH for 48 h or to 15–25 μ M TH for 10 min. Our results demonstrated a decrease in the cells viability evoked by TH, which could be prevented partially by serum proteins, albumin or in a dose-dependent manner by 60, 120 or 600 μ M Cys, Hcy, NAC and GSH, but not by Met. This neuroprotection was less pronounced in the presence of proteins. Incubation of neurons with TH also induced the rise in the intracellular calcium and zinc concentration and decrease in mitochondrial membrane potential, and these effects were abolished by all the sulfur containing compounds studied and administered at 600 μ M concentration, except Met. The loss of the ethylmercury moiety from TH as a result of interaction with thiols studied was monitored by ¹H NMR spectroscopy. This extracellular process may be responsible for the neuroprotection seen in the cerebellar cell cultures, but also provides a molecular pathway for redistribution of TH-derived toxic ethylmercury in the organism. In conclusion, these results confirmed that proteins and sulfur-containing amino acids applied separately reduce TH neurotoxicity, while their combination modulates in more complex way neuronal survival in the presence of TH.

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1. Introduction

Sodium ethylmercurithiosalicylate, commonly known as thimerosal (TH) has been widely used as preservative in vaccines and topical liquid medicines. The presence of TH in vaccines was proposed to be one of factors responsible for autism in children (Bernard et al., 2001; Geier and Geier, 2004), but this concept has not been widely accepted (Madsen et al., 2003; Stehr-Green et al., 2003). However, the autistic children have significantly lower steady-state plasma levels of Met, Hcy, Cys and total glutathione than the control group (James et al., 2004; Geier and Geier, 2006). Beginning from 2001 TH has been phased out from the most of pediatric vaccines in resources-rich countries, but it is still

in use in developing societies (Bigham and Copes, 2005). Other vaccines, including those against influenza, or immunoglobulin anti-D vaccine still contain TH to a maximal concentration of 25 μ g Hg/0.5 ml, corresponding to 250 μ M (Stratton et al., 2001). They have been administered to the wide population, including pregnant women (James et al., 2005; Geier and Geier, 2007).

Tan and Parkin (2000) using primary cultures of cerebellar granule cells (CGC) demonstrated TH neurotoxicity. They found that TH decomposes into ethylmercuric ion (EtHg⁺) and thiosalicylic acid (TSA) and confirmed neurotoxicity of the former compound. Mercuric ions (Hg²⁺) and their alkyl derivatives including ethylmercury are toxic to living organisms because of their strong affinity to protein cysteine thiols (Divine et al., 1999). Low molecular weight thiols, primarily L-cysteine (Cys) and reduced glutathione (GSH) modulate TH toxicity. On the one hand they are important factors in the transport and distribution of mercury throughout the body by means of molecular mimicry (Bridges and Zalups, 2005;

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