

Thiol-Modulated Mechanisms of the Cytotoxicity of Thimerosal and Inhibition of DNA Topoisomerase II α

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Thimerosal is an organic mercury compound that is widely used as a preservative in vaccines and other solution formulations. The use of thimerosal has caused concern about its ability to cause neurological abnormalities due to mercury accumulation during a normal schedule of childhood vaccinations. While the chemistry and the biological effects of methylmercury have been well-studied, those of thimerosal have not. Thimerosal reacted rapidly with cysteine, GSH, human serum albumin, and single-stranded DNA to form ethylmercury adducts that were detectable by mass spectrometry. These results indicated that thimerosal would be quickly metabolized in vivo because of its reactions with protein and nonprotein thiols. Thimerosal also potently inhibited the decatenation activity of DNA topoisomerase II α , likely through reaction with critical free cysteine thiol groups. Thimerosal, however, did not act as a topoisomerase II poison and the lack of cross-resistance with a K562 cell line with a decreased level of topoisomerase II α (K/VP.5 cells) suggested that inhibition of topoisomerase II α was not a significant mechanism for the inhibition of cell growth. Depletion of intracellular GSH with buthionine sulfoximine treatment greatly increased the K562 cell growth inhibitory effects of thimerosal, which showed that intracellular glutathione had a major role in protecting cells from thimerosal. Pretreatment of thimerosal with glutathione did not, however, change its K562 cell growth inhibitory effects, a result consistent with the rapid exchange of the ethylmercury adduct among various thiol-containing cellular reactants. Thimerosal-induced single and double strand breaks in K562 cells were consistent with a rapid induction of apoptosis. In conclusion, these studies have elucidated some of the chemistry and biological activities of the interaction of thimerosal with topoisomerase II α and protein and nonprotein thiols and with DNA.

Introduction

Thimerosal (Figure 1) is an organic mercury compound with bactericidal and fungicidal properties that is widely used as a preservative in multiuse vials of vaccines, ophthalmic, otic, nasal, and topical products (1–3). There has been a public perception that thimerosal use in vaccines is unsafe after suggestions that it caused a predisposition to autism in children (1, 4). However, recent epidemiological studies have not supported this hypothesis (4). On the basis of the risk assessment assumption that the dose–effect and dose–response relationships of ethylmercury, the presumed metabolite of thimerosal, and methylmercury were the same, thimerosal was removed from most pediatric vaccines in the United States in 2001 (1, 3). Prior to 2001, by 18 months of age, a child in the United States undergoing a routine schedule of immunizations would have received a cumulative dose of 200 μ g of mercury (3). The fact that the cumulative exposure to mercury from thimerosal in infants undergoing immunization during the first 6 months of life could exceed U.S. Environmental Protection Agency guidelines provided impetus for the removal of thimerosal from pediatric vaccines (3).

Much of what is known about chronic low-dose human methylmercury toxicity causing neurologic abnormalities comes from poisoning episodes and environmental exposure (1, 3). Far

less is known about the effects of thimerosal or its presumed metabolite, ethylmercury (1, 3, 5, 6). The initial distribution of ethylmercury in neonatal mice is similar to that of methylmercury, but they differ sharply in their tissue deposition and their metabolism to Hg²⁺ (4). This suggests that the data on methylmercury may not be suitable for risk assessment for thimerosal (1, 5). Methylmercury reacts rapidly with and has a very high affinity for protein and nonprotein thiols (1, 78), and ethylmercury is likely similar in this regard.

Thus, to elucidate some of the basic chemistry and biochemistry of thimerosal, the reactions of thimerosal with nonprotein and protein thiols and the cellular effects of thimerosal have been studied. While the reaction of thimerosal with thiols has been assumed to be an exchange reaction to yield an ethylmercury-thiol adduct (Figure 1), this does not seem to have been shown. In this study, we showed by MS that thimerosal undergoes an exchange reaction with cysteine, GSH, and human serum albumin (HSA)¹ (Figure 1) and forms an ethylmercury adduct with single-stranded DNA.

¹ Abbreviations: Annexin V-FITC, annexin V-fluorescein isothiocyanate conjugate; 6-mer DNA, DNA with the sequence 5'-CACGTG-3'; 20-mer DNA, self-complementary hairpin DNA with the sequence 5'-TAT-GATATTTTATATCATA-3'; BSO, buthionine sulfoximine; DTT, dithiothreitol; ESI-MS, electrospray ionization mass spectrometry; FCS, fetal calf serum; HBSS, Hank's balanced salt solution; HSA, human serum albumin; IC₅₀, 50% inhibitory concentration; kDNA, kinetoplast DNA; thimerosal-DNA, thimerosal-treated and washed kDNA; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; OTC, (–)-2-oxo-4-thiazolidinecarboxylic acid.

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