

Thimerosal induces oxidative stress in HeLa S epithelial cells

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Abstract

Thimerosal is one of the most widely used preservatives and is found in a variety of biological products, including vaccines, contact lens cleaning solutions, and cosmetics. It has been reported to have harmful effects on epithelial tissues, such as causing conjunctivitis or contact dermatitis. However, the molecular mechanism of its toxicity has not been characterized using epithelial tissues. In the present study, we report that reactive oxygen species play a key role in thimerosal-induced cytotoxicity in HeLa S epithelial cells. Thimerosal significantly reduced HeLa S cell viability and it was associated with a decrease in intracellular glutathione levels. Flow cytometric cell cycle analysis showed a marked increase in the hypodiploidic cell population, indicating apoptosis of thimerosal-treated cells. The apoptotic cell death of epithelial cells was confirmed by observing a significant increase of caspase-3 activity in the cytosolic fraction of the treated cells. Thimerosal also induced a concentration-dependent increase of genomic DNA fragmentation, a biochemical hallmark of apoptosis. Hoechst 33342 nuclear staining demonstrated apoptotic-fragmented multinuclei in thimerosal-treated cells. All the thimerosal-mediated toxic responses observed in the present study were almost completely suppressed by pretreating cells with *N*-acetyl-L-cysteine, a radical scavenger. Taken together, these results suggest for the first time that epithelial cytotoxicity of thimerosal is mediated by oxidative stress.

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1. Introduction

The antiseptic and antimicrobial activities of thimerosal have led to its use as a preservative in biological products, including vaccines, cleaning solutions for contact lenses, and cosmetics since the 1930s. However, there have been several reports that thimerosal has potential side effects, including causing inflammatory diseases (Clarkson, 2002; Lopez Bernal and Ubels, 1991). In particular, it has been speculated that thimerosal preservative in juvenile vaccines is a causative factor of autism in the vaccinated children (van't Veen, 2001); however, this idea is still the subject of much debate. Recently, the mechanisms of this potential side effect have been illustrated in neuronal cells (Ball et al., 2001; Geier and Geier, 2004; James et al.,

2005) and T-lymphocytes (Lebrec et al., 1999; Makani et al., 2002). In a previous study, we have directly demonstrated for the first time that thimerosal can generate reactive oxygen species (ROS), including hydrogen peroxide in cultured mammalian cells (Kim et al., 2002a). The ROS generation stimulates focal adhesion kinase and cytoskeletal rearrangement, resulting in the typical morphological changes observed in thimerosal treatment. It appears to generate not only hydrogen peroxide but also other species of ROS (E. Kim, unpublished data). Apparently, thimerosal-induced intracellular calcium upregulation is also dependent on the generation of ROS by thimerosal (Kim et al., 2002b).

The side effects of thimerosal on epithelial tissues, such as conjunctivitis and contact dermatitis, have been previously reported (Garner, 2004; Belsito, 2002; Pratt et al., 2004; Wantke et al., 1994; Lebrec et al., 1999). However, the molecular mechanism of its toxicity has not been clearly elucidated in epithelial tissue. While the adverse effects of thimerosal on an epithe-

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