

Mitochondrial Mediated Thimerosal-Induced Apoptosis in a Human Neuroblastoma Cell Line (SK-N-SH)

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Abstract

Environmental exposure to mercurials continues to be a public health issue due to their deleterious effects on immune, renal and neurological function. Recently the safety of thimerosal, an ethyl mercury-containing preservative used in vaccines, has been questioned due to exposure of infants during immunization. Mercurials have been reported to cause apoptosis in cultured neurons; however, the signaling pathways resulting in cell death have not been well characterized. Therefore, the objective of this study was to identify the mode of cell death in an *in vitro* model of thimerosal-induced neurotoxicity, and more specifically, to elucidate signaling pathways which might serve as pharmacological targets. Within 2 h of thimerosal exposure (5 μ M) to the human neuroblastoma cell line, SK-N-SH, morphological changes, including membrane alterations and cell shrinkage, were observed. Cell viability, assessed by measurement of lactate dehydrogenase (LDH) activity in the medium, as well as the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay, showed a time- and concentration-dependent decrease in cell survival upon thimerosal exposure. In cells treated for 24 h with thimerosal, fluorescence microscopy indicated cells undergoing both apoptosis and oncosis/necrosis. To identify the apoptotic pathway associated with thimerosal-mediated cell death, we first evaluated the mitochondrial cascade, as both inorganic and organic mercurials have been reported to accumulate in the organelle. Cytochrome *c* was shown to leak from the mitochondria, followed by caspase 9 cleavage within 8 h of treatment. In addition, poly(ADP-ribose) polymerase (PARP) was cleaved to form a 85 kDa fragment following maximal caspase 3 activation at 24 h. Taken together these findings suggest deleterious effects on the cytoarchitecture by thimerosal and initiation of mitochondrial-mediated apoptosis.

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INTRODUCTION

Thimerosal (thiomersal, merthiolate, sodium ethyl-mercury thiosalicylate), an ethyl mercury-containing

antibacterial and antifungal agent, has been used as an antiseptic and preservative in various formulations including paints, topical medications, eye lens cleaners and cosmetics since the 1930s. Concern has been raised by the use of thimerosal in more than 30 vaccines licensed in the United States (Elferink, 1999). With the addition of several important vaccines over the last decade and the use of multi-dose vials, exposure to

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