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Thimerosal Induces DNA Breaks, Caspase-3 Activation, Membrane Damage, and Cell Death in Cultured Human Neurons and Fibroblasts

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Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250-μM concentrations of thimerosal for 45 min to 24 h. A 4′, 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify nonviable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 μM based on the manual detection of the fluorescent attached cells and at a 1-μM level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 μM thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3–dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.

Key Words: **thimerosal; active caspase-3; apoptosis; toxicity; neurons; fibroblasts; DNA breaks; membrane damage; DAPI.**

Thimerosal (sodium ethylmercury-thiosalicylate) is an antibacterial and antifungal mercurial compound used as a preservative in biological products and vaccines, in concentrations ranging from 0.003 to 0.01% (30–100 μg/ml) (Ball *et al*., 2001). Thimerosal contains 49.6 % mercury by weight and releases ethylmercury as a metabolite. In the body, ethylmercury can be converted to inorganic mercury, which then preferentially accumulates in the kidneys and brain (Blair *et al.*, 1975). Inorganic mercury is known to induce membrane and DNA damage (Ferrat *et al*., 2002; Ben-Ozer *et al*., 2000), and in cell culture conditions it was shown to be mutagenic and generate DNA breaks in concentrations below 500 nM (Schurz *et al*., 2000). Ethylmercury can significantly increase the concentration of inorganic mercury in many organs (Magos *et al*., 1985). After *in vivo* administration, ethylmercury passes through cellular membranes and concentrates in cells in vital organs, including the brain, where it releases inorganic mercury, raising its concentrations higher than equimolar doses of its close and highly toxic relative methylmercury (Magos *et al*., 1985).

However, little is known about acute reactions of various types of human cells following short-time exposure to thimerosal in micro- and nanomolar concentrations.

In this paper we used a convenient and easily reproducible combination of fluorescent techniques analyzing various markers of DNA and membrane damage, and investigated the toxicity of micromolar and nanomolar concentrations of thimerosal (125 nM–250 μM) occurring in the first 24 h of exposure in cultures of human cortical neuronal cells and in human fibroblasts.

We found that thimerosal in micromolar concentrations rapidly decreased cellular viability. Within several h after thimerosal administration, cells lost their capability to exclude the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) and developed multiple DNA breaks accompanied by caspase-3 activation and apoptotic morphology. Neuronal cell cultures demonstrated a higher sensitivity to thimerosal compared with fibroblasts.

MATERIALS AND METHODS

Cell cultures. HCN-1A Human cerebral cortical neurons (CRL-10442) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and were cultured according to ATCC recommendations. The line was

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