

Evaluation of Cytotoxicity Attributed to Thimerosal on Murine and Human Kidney Cells

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Renal inner medullary collecting duct cells (mIMCD3) and human embryonic kidney cells (HEK293) were used for cytoscreening of thimerosal and mercury chloride (HgCl₂). Thimerosal and HgCl₂ acted in a concentration-dependent manner. In mIMCD3 cells the 24-h LC₅₀ values for thimerosal, thiosalicylic acid, 2,2-dithiosalicylic acid, and 2-sulfobenzoic acid were 2.9, 2200, >1000, and >10,000 μM, respectively. The 24-h LC₅₀ value for HgCl₂ in mIMCD3 cells was 40 μM. In HEK293 cells, the 24-h LC₅₀ value for thimerosal was 9.5 μM. These data demonstrate that the higher cytotoxicity produced by thimerosal on renal cells with respect to similar compounds without Hg may be related to this metal content. The present study also establishes mIMCD3 cells as a valuable model for evaluation of cytotoxicity of nephrotoxic compounds.

Public health regulatory agencies are increasingly concerned about the impact of renal diseases produced by commercially used compounds that are nephrotoxic (Thadhani et al., 1996). In particular, renal inner medullary cells are often exposed to high concentrations of common nephrotoxic substances and also frequently are subjected to hyperosmotic and ischemic stress (Burg, 2002; Lee et al., 2002). Little is

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known concerning the cytotoxic effects produced by drugs and toxicants other than nonsteroidal anti-inflammatory drugs (Rocha et al., 2001) on renal inner medullary collecting duct cells (mIMCD3), which are an immortalized cell line derived from mouse renal inner medulla.

Thimerosal (ethylmercurithiosalicylic acid) is a mercury-containing preservative that has been used as an additive for vaccine and biological products for more than 70 years. Thimerosal dissociates as 49.5% ethylmercury by weight and thiosalicylic acid. High-dose, acute or chronic mercury (Hg) exposure of children and adults resulted in nephrotoxicity (Clarkson, 1993; Van Vleet & Schnellmann, 2003). Ethylmercury and thimerosal induce significantly higher Hg concentration in the kidney than in brain (Harry et al., 2004). However, thimerosal has been implicated in neuronal toxicity and autism (Geier & Geier, 2006; Kern & Jones, 2006; Kern et al., 2007).

The colorimetric cell survival assay using the tetrazolium salt MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) has been widely used for measuring cell proliferation and survival (Mosmann, 1983). This assay measures the reduction of a tetrazolium component into an insoluble purple formazan product by the mitochondria of viable cell. The present study was undertaken to investigate cytotoxicity of thimerosal and its structural analogs, and inorganic Hg in mIMCD3 and human embryonic kidney (HEK293) cells.

MATERIALS AND METHODS

Cultures of mIMCD3 and HEK 293 Cells

mIMCD3 cells of passage 19 and HEK293 cells of passage 35 were used for all experiments. All reagents for cell