

# Cell death and cytotoxic effects in YAC-1 lymphoma cells following exposure to various forms of mercury

Margaret Yole\*, Mark Wickstrom, Barry Blakley

*Department of Veterinary Biomedical Sciences, Western College of Veterinary Medicine, 52 Campus Drive,  
University of Saskatchewan, Saskatoon SK S7N 5B4, Canada*

Received 25 March 2006; received in revised form 29 October 2006; accepted 12 November 2006

Available online 25 November 2006

## Abstract

The effects of 1 min–4 h exposures to four Hg compounds (mercuric chloride [ $\text{HgCl}_2$ ], methyl mercuric chloride [ $\text{CH}_3\text{HgCl}$ ], *p*-chloromercuribenzoate [*p*-CMB] and thimerosal [TMS; ethylmercurithiosalicylate]) on cell death, microtubules, actin, CD3 receptor expression, protein tyrosine phosphorylation (PTyr-P) and intracellular calcium ( $[\text{Ca}^{2+}]_i$ ) levels were investigated in YAC-1 lymphoma cells using flow cytometry. YOPRO-1 (YP) and propidium iodide (PI) dye uptake indicated all forms of Hg tested were toxic at concentrations ranging from 25.8–48.4  $\mu\text{M}$ , with two distinct patterns of effects. Early apoptosis was prolonged for  $\text{CH}_3\text{HgCl}$ - and TMS-treated cells, with more than 50% remaining  $\text{YP}^+/\text{PI}^-$  after 4 h. Both  $\text{CH}_3\text{HgCl}$  and TMS induced complete loss of  $\beta$ -tubulin fluorescence, indicative of microtubule depolymerization and inhibition of tubulin synthesis and/or  $\beta$ -tubulin degradation, while F-actin fluorescence diminished to a lesser degree and only after loss  $\beta$ -tubulin.  $\text{CH}_3\text{HgCl}$  and TMS induced an almost immediate two-fold increase in CD3 fluorescence, with levels returning to baseline within minutes. With continued exposure, CD3 fluorescence was reduced to approximately 50% of baseline values. Both compounds also increased PTyr-P two- to three-fold immediately, with levels returning to baseline at 4 h. Similarly, two- to three-fold increases in  $[\text{Ca}^{2+}]_i$  were noted after 1 min exposure.  $[\text{Ca}^{2+}]_i$  increased progressively, reaching levels five- to eight-fold greater than control values. In contrast, dye uptake was delayed with  $\text{HgCl}_2$  and *p*-CMB, although cell death proceeded rapidly, with almost all non-viable cells being late apoptotic ( $\text{YP}^+/\text{PI}^+$ ) by 4 h. *p*-CMB produced early reductions in F-actin, and after 4 h, complete loss of F-actin with only partial reduction of total  $\beta$ -tubulin was seen with both *p*-CMB and  $\text{HgCl}_2$ .  $\text{HgCl}_2$  reduced CD3 expression and PTyr-P slightly within minutes, while *p*-CMB produced similar effects on CD3 only at 4 h, at which time PTyr-P was increased two- to three-fold. Both compounds increased  $[\text{Ca}^{2+}]_i$  within minutes, though levels remained under twice the baseline concentration after 15 min exposure. With continued exposure,  $[\text{Ca}^{2+}]_i$  increased to levels two- to five-fold greater than control values. These findings indicate the two groups of Hg compounds may induce cell death by distinct pathways, reflecting interactions with different cellular targets leading to cell death.

© 2006 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Mercuric chloride; Methyl mercuric chloride; *p*-Chloromercuribenzoate; Thimerosal; Cytotoxicity; YAC-1 lymphoma

## 1. Introduction

Many of the proposed targets for mercury (Hg) toxicity are also components of the localized supramolec-

ular activation complex (SMAC) or ‘immunological synapse’ formed between antigen-presenting cells (APCs) and responding lymphocytes (Monks et al., 1998). But, while antigenic stimuli are restricted to the localized SMAC, Hg may interact non-specifically with thiol (–SH) groups throughout the cell. Non-localized Hg impacts may mimic antigen-mediated signaling at certain concentrations, but the effects are likely not

\* Corresponding author. Tel.: +1 306 244 5986.  
E-mail address: [yole@sask.usask.ca](mailto:yole@sask.usask.ca) (M. Yole).