

# Thimerosal-Induced Apoptosis in Mouse C2C12 Myoblast Cells Occurs through Suppression of the PI3K/Akt/Survivin Pathway

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## Abstract

**Background:** Thimerosal, a mercury-containing preservative, is one of the most widely used preservatives and found in a variety of biological products. Concerns over its possible toxicity have reemerged recently due to its use in vaccines. Thimerosal has also been reported to be markedly cytotoxic to neural tissue. However, little is known regarding thimerosal-induced toxicity in muscle tissue. Therefore, we investigated the cytotoxic effect of thimerosal and its possible mechanisms on mouse C2C12 myoblast cells.

**Methodology/Principal Findings:** The study showed that C2C12 myoblast cells underwent inhibition of proliferation and apoptosis after exposure to thimerosal (125–500 nM) for 24, 48 and 72 h. Thimerosal caused S phase arrest and induced apoptosis as assessed by flow cytometric analysis, Hoechst staining and immunoblotting. The data revealed that thimerosal could trigger the leakage of cytochrome c from mitochondria, followed by cleavage of caspase-9 and caspase-3, and that an inhibitor of caspase could suppress thimerosal-induced apoptosis. Thimerosal inhibited the phosphorylation of Akt<sup>ser473</sup> and survivin expression. Wortmannin, a PI3K inhibitor, inhibited Akt activity and decreased survivin expression, resulting in increased thimerosal-induced apoptosis in C2C12 cells, while the activation of PI3K/Akt pathway by mIGF-I (50 ng/ml) increased the expression of survivin and attenuated apoptosis. Furthermore, the inhibition of survivin expression by siRNA enhanced thimerosal-induced cell apoptosis, while overexpression of survivin prevented thimerosal-induced apoptosis. Taken together, the data show that the PI3K/Akt/survivin pathway plays an important role in the thimerosal-induced apoptosis in C2C12 cells.

**Conclusions/Significance:** Our results suggest that in C2C12 myoblast cells, thimerosal induces S phase arrest and finally causes apoptosis via inhibition of PI3K/Akt/survivin signaling followed by activation of the mitochondrial apoptotic pathway.

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## Introduction

Thimerosal is a water-soluble derivative of thiosalicylic acid. Due to its antimicrobial properties, it is widely used as a preservative in vaccines, ophthalmic products and cosmetics [1]. The safety of thimerosal has recently been questioned based on a number of studies that indicate to its possible risk of toxicity [2–5]. Thimerosal has been shown to cause a number of immunological and neurotoxic changes in microglia and astrocytes [1,6–9], and also been shown to induce apoptosis of SK-N-SH human neuroblastoma cells via the c-Jun N-terminal kinase pathway [10] and induce DNA breaks, caspase-3 activation, membrane damage and cell death in cultured human neurons and fibroblasts [11]. Woo et al. found that it could induce G<sub>2</sub>/M phase arrest in human leukemia cells via the generation of reactive oxygen species and release of cytochrome C [12], while Makani et al. indicated that thimerosal could induce apoptosis in T cells via the mitochondrial pathway [13]. More recently, thimerosal has been

classified as the second most common allergen after nickel [14–18], and also been shown to induce epithelial cytotoxicity via oxidative stress in HeLa S epithelial cells and apoptosis in human SCM1 gastric cancer cells via activation of the p38 MAP kinase and caspase-3 [19].

When people were vaccinated intramuscularly, thimerosal in vaccine directly contacts and might cause injury to skeletal muscle cells; this might be the reason for inflammation or amyotrophy at the injection site. However, little is known about the acute reactions of skeletal muscle tissues and cells following short-term exposure to thimerosal at nanomolar concentrations. Repair of degenerated muscles depends on a small group of skeletal muscle stem cells known as satellite cells [20]. Satellite cells form a group of quiescent muscle precursor cells that reside beneath the basal lamina and provide the predominant source of additional myonuclei for muscle growth [21,22]. Once activated, satellite cells give rise to myoblasts that proliferate, differentiate, and fuse