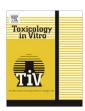


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Increase in intracellular Zn^{2+} concentration by thimerosal in rat thymocytes: Intracellular Zn^{2+} release induced by oxidative stress

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ABSTRACT

Thimerosal (TMR), an ethylmercury-containing preservative in pharmaceutical products, was recently reported to increase intracellular Zn²⁺ concentration. Therefore, some health concerns about the toxicity of TMR remain because of physiological and pathological roles of Zn²⁺. To reveal the property of TMRinduced increase in intracellular Zn²⁺ concentration, the effect of TMR on FluoZin-3 fluorescence, an indicator of intracellular Zn²⁺, of rat thymocytes was examined. TMR at concentrations ranging from 0.3 µM to 10 µM increased the intensity of FluoZin-3 fluorescence in a concentration-dependent manner under external Ca^{2+} and Zn^{2+} -free condition. The threshold concentration was 0.3–1 μ M. The increase in the intensity was significant when TMR concentration was 1 μM or more. N,N,N',N'-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a chelator for intracellular Zn²⁺, completely attenuated the TMRinduced augmentation of FluoZin-3 fluorescence. Hydrogen peroxide (H2O2) and N-ethylmaleimide, reducing cellular thiol content, significantly increased FluoZin-3 fluorescence intensity and decreased 5-chloromethylfluorescein (5-CMF) fluorescence intensity, an indicator for cellular thiol. The correlation coefficient between TMR-induced augmentation of FluoZin-3 fluorescence and attenuation of 5-CMF fluorescence was -0.882. TMR also attenuated the 5-CMF fluorescence in the presence of TPEN. Simultaneous application of H₂O₂ and TMR synergistically augmented the FluoZin-3 fluorescence. It is suggested that TMR increases intracellular Zn²⁺ concentration via decreasing cellular thiol content.

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1. Introduction

Thimerosal (TMR) is an ethylmercury-containing preservative used in vaccines, immune globulin preparations, and other pharmaceutical products. Because it contains 49.55% mercury, it has been hypothesized that early exposure to this preservative is associated with neuropsychological deficits in children (Bernard et al., 2001; Redwood et al., 2001). Although there are many clinical and basic studies against the hypothesis (Ball et al., 2001; Magos, 2003; Verstraeten et al., 2003; Andrews et al., 2004; Burbacher et al., 2005; Zareba et al., 2007; Thompson et al., 2007), some health concerns about the toxicity of TMR remain (Geier et al., 2007; Berman et al., 2008).

The cytotoxicity of TMR has been discussed in the phenomena related to the disturbance of intracellular Ca²⁺ homeostasis. TMR increases intracellular Ca²⁺ concentration by increasing membrane Ca²⁺ permeability and releasing Ca²⁺ from intracellular calcium stores *via* inhibition of Ca²⁺ pump or sensitization of IP₃ receptor at endoplasmic reticulum membranes (Gukovskaya et al., 1992; Thorn et al., 1992; Sayers et al., 1993; Parys et al., 1993). In addition, it has been recently proposed that TMR increases intracellular Zn²⁺ concentration *via* an intracellular Zn²⁺ release (Haase et al., 2009). Zn²⁺ is the second most prevalent trace element and it is involved in the structure and function of over 300 enzymes (Prasad, 1995). Zn²⁺ stimulates the activity of approximately 100 enzymes (Sandstead, 1994). Therefore, an abnormal increase in intracellular Zn²⁺ concentration may cause pathological phenomena.

Intracellular Zn²⁺ is complexed to thiol of protein and nonprotein such as metallothionein and glutathione (Diaz-Cruz et al., 1998; Jacob et al., 1998; Maret and Vallee, 1998; Gelinsky et al., 2003). Oxidative stress releases Zn²⁺ from protein and nonprotein *via* interchange between thiol and disulfide (Maret, 1994; Quesada et al., 1996). TMR induces oxidative stress (Makani et al., 2002; Ueha-Ishibashi et al., 2004; James et al., 2005). Therefore, it is reminiscent of a possibility that TMR increases intracellular Zn²⁺

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