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

Erika Hashimoto, Toshihisa B. Oyama [1], Keisuke Oyama [2], Yumiko Nishimura [3], Tomohiro M. Oyama [4], Toshiko Ueha-Ishibashi [5], Yoshiro Okano [4], Yasuo Oyama *

Laboratory of Cellular Signaling, Faculty of Integrated Arts and Sciences, The University of Tokushima, Tokushima 770-8502, Japan

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ABSTRACT

Thimerosal (TMR), an ethylmercury-containing preservative in pharmaceutical products, was recently reported to increase intracellular Zn^{2+} concentration. Therefore, some health concerns about the toxicity of TMR remain because of physiological and pathological roles of Zn^{2+}. To reveal the property of TMR-induced increase in intracellular Zn^{2+} concentration, the effect of TMR on FluoZin-3 fluorescence, an indicator of intracellular Zn^{2+}, 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 μM. 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 Zn^{2+}, completely attenuated the TMR-induced augmentation of FluoZin-3 fluorescence. Hydrogen peroxide (H_2O_2) 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 H_2O_2 and TMR synergistically augmented the FluoZin-3 fluorescence. It is suggested that TMR increases intracellular Zn^{2+} concentration *via* decreasing cellular thiol content.

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 Ca^{2+} homeostasis. TMR increases intracellular Ca^{2+} concentration by increasing membrane Ca^{2+} permeability and releasing Ca^{2+} from intracellular calcium stores *via* inhibition of Ca^{2+} pump or sensitization of IP_3 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 Zn^{2+} concentration *via* an intracellular Zn^{2+} release (Haase et al., 2009). Zn^{2+} is the second most prevalent trace element and it is involved in the structure and function of over 300 enzymes (Prasad, 1995). Zn^{2+} stimulates the activity of approximately 100 enzymes (Sandstead, 1994). Therefore, an abnormal increase in intracellular Zn^{2+} concentration may cause pathological phenomena.

Intracellular Zn^{2+} 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 Zn^{2+} 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 Zn^{2+}

* Corresponding author. Tel./fax: +81 88 656 7256.
E-mail address: oyama@ias.tokushima-u.ac.jp (Y. Oyama).
[1] Present address: Faculty of Engineering, Okayama University, Okayama 700-8530, Japan.
[2] Present address: Faculty of Medicine, Saga University, Saga 849-8501, Japan.
[3] Present address: Okayama University Dental School, Okayama 700-8525, Japan.
[4] Present address: Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8512, Japan.
[5] Present address: St. Luke's International Hospital, Tokyo 104-8560, Japan.

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