



## Expression of metallothionein mRNAs on mouse cerebellum microglia cells by thimerosal and its metabolites

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### ABSTRACT

Effects of thimerosal and its metabolites, ethyl mercury and thiosaliclylate, on the expression of metallothionein (MT) mRNAs in mouse cerebellum microglia cell line, C8-B4 cells, were studied. The level of MT-1 mRNA significantly decreased at early hours and recovered time-dependently 24 h after thimerosal was added to the C8-B4 cells. However, MT-2 and MT-3 mRNA expressions did not change from the control group. In contrast, the expression of MT-1 mRNA increased in a mouse neuroblastoma cell line 6 h after incubation with thimerosal. In addition, the level of MT-1 mRNA decreased in C8-B4 cells 6 h after the addition of thiosaliclylate, but ethyl mercury induced MT-1 mRNA expression. When cell viability was compared with thimerosal, thiosaliclylate, and ethyl mercury, the viability of C8-B4 cells decreased dose-dependently 24 h after either thimerosal or ethyl mercury was added; however, the viability increased dose-dependently until 15  $\mu$ M thiosaliclylate was added. From the present results, it is concluded that the expression of MT-1 mRNA may be mediated by different factors than the expression of MT-2 mRNA in C8-B4 cells. The reduction of MT-1 mRNA level by thiosaliclylate may affect the proliferation of C8-B4 cells.

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### 1. Introduction

Thimerosal is recommended as a preservative of vaccines and toxoids (Geier et al., 2007), because it is cheap with supposedly stable preservative action without side effects. However, as thimerosal splits into two compounds, ethyl mercury and thiosaliclylate, in the body (Clarkson, 2002), it was suspected that thimerosal induced autism (Stajich et al., 2000; Bernard et al., 2001; Pichichero et al., 2002; Francois et al., 2005; Mutter et al., 2005). This suspicion was rejected by large-scale epidemiological surveys (Madsen et al., 2003; Clements and McIntyre, 2006; Geier and Geier, 2006; Gallagher and Goodman, 2008; Young et al., 2008), but ethyl mercury has an adverse effect on the central nervous system even at clinical doses of thimerosal (Guzzi and La Porta, 2008).

Metallothionein (MT) is a ubiquitous low-molecular-mass protein. Of the four major isoforms of MT, MT-1 and MT-2 are known to be acute-phase proteins and are induced together in the same tissues in response to various stimuli (Waalkes, 1996; Moffatt and Denizeau, 1997; Miles et al., 2000; Coyle et al., 2002; Haq et al., 2003), and in the brain, it is known that MT-1 and MT-2 are observed in glia and astrocyte cells and MT-3 in neurons (Nishimura et al., 1992; Coyle et al., 2002; Ghazi et al., 2006). For example, MT-1 and

MT-2 are induced simultaneously in the brain when either mercury vapor or methyl mercury affects the brain (Leyshon-Sørland et al., 1994; Yasutake et al., 2003, 2004), and both MTs probably act as detoxification substances (Penkowa and Hidalgo, 2000; Penkowa et al., 2006). In contrast, brain MT-3 acted to protect and repair neurons, but recently, it was reported that MT-3 content increased by IL-3, TGF- $\alpha$ , and EFG and decreased by IL-6, kainate, and dexamethasone, and MT-3 content changed in neuronal diseases such as Parkinson and prion diseases (Yu et al., 2001; Carrasco et al., 2003; Hozumi et al., 2006; Kim et al., 2008).

We previously reported that mercury contents increased in the cerebrum when a high dose of thimerosal was subcutaneously injected into mice, and mercury contents increased in the cerebrum after lipopolysaccharide pretreatment even when the dose of thimerosal was low (Minami et al., 2007). In addition, both MT-1 mRNA and protein levels increased in the cerebellum of mice after the injection of low dose of thimerosal (Minami et al., 2009). It seems that thimerosal and its metabolites enter in a brain even if the injection dose is low; therefore, it is important to clarify the effect of thimerosal on the brain.

Microglia and oligodendrocytes were reported to be devoid of MT-1 and MT-2 (Young et al., 1991; Nishimura et al., 1992; Blaauwgeers et al., 1993; Nakajima and Suzuki, 1995), but Vanguri (1995) demonstrated the presence of MT-2 mRNA in a primary culture of microglia, and Agullo et al. (1998) reported that MT-1 and MT-2 in a primary culture of rat microglia were induced by

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