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Possible involvement of galectin-3 in microglial activation in the hippocampus with trimethyltin treatment

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ABSTRACT

Trimethyltin (TMT) is an organotin neurotoxicant with effects that are selectively localized to the limbic system (especially the hippocampus), which produces memory deficits and temporal lobe seizures. Galectin-3 (Gal-3) is a beta-galactoside-binding lectin that is important in cell proliferation and regulation of apoptosis. The present study evaluated the temporal expression of Gal-3 in the hippocampus of adult BALB/c mice after TMT treatment (i.p., 2.5 mg/kg). Western blotting analyses showed that Gal-3 immunoreactivity began to increase 2 days after treatment; the immunoreactivity peaked significantly within 4 days after treatment but significantly declined between days 4 and 8. Immunohistochemical analysis indicated that Gal-3 expression was very rare in the hippocampi of vehicle-treated controls. However, Gal-3 immunoreactivity appeared between 2 and 8 days after TMT treatment and was primarily localized to the hippocampal dentate gyrus (DG), in which neuronal degeneration occurred. The immunoreactivity was detected predominantly in most of the Iba1-positive microglia and in some GFAP-positive astrocytes of the hippocampal DG. Furthermore, Gal-3 expression co-localized with the pro-inflammatory enzymes cyclooxygenase-2 and inducible nitric oxide synthase in the hippocampal DG. Therefore, we suggest that Gal-3 is involved in the inflammatory process of neurodegenerative disorder induced by organotin intoxication.

1. Introduction

Trimethyltin (TMT) is an organotin that produces significant and selective neuronal degeneration in the limbic system including the hippocampus and induces memory deficits and temporal lobe seizures (Chang and Dyer, 1983; Dyer et al., 1982). Accidental exposure to TMT has been reported to induce disorientation, confusion, memory deficits, aggressiveness, and seizures in humans (Besser et al., 1987; Feldman et al., 1993; Fortemps et al., 1978). In mice, TMT treatment causes seizures, hyperactivity, memory deficits, and neuronal loss, especially in the hippocampal dentate gyrus (DG), that is characterized by localized dentate granule cell death with sparing of *Cornu Ammonis* (CA) pyramidal cell death (Fiedorowicz et al., 2001; Harry et al., 2008). TMT-induced neurodegenerative processes are common to most neurodegenerative disorders, such as selective neuronal death and neuroinflammation (Geloso et al., 2011). Several studies using the TMT murine model of brain damage have indicated an associated inflammatory response, including increases in interleukin (IL)-1 (Bruccoleri et al., 1998; Fiedorowicz et al., 2001), cyclooxygenase-2 (COX-2) (Shirakawa et al., 2007), inducible nitric oxide (iNOS) (Reali et al., 2005), tumor necrosis factor (TNF)-α (Harry et al., 2008), and glial cell activation (Brabeck et al., 2002; Pompili et al., 2004). In primary glial cell culture, TMT treatment elevates mRNA and protein expression of TNF-α, IL-1-α, and IL-6 (Harry et al., 2002). Additionally, voluntary exercise ameliorates TMT-induced hippocampal DG cell death and expression of TNF-α, TNF receptor 1, myeloid differentiation primary response gene 88, transforming growth factor-β chemokine (C–C motif) ligand 2 (CCL2), and CCL3 in mice (Funk et al., 2011).

Galectin-3 (Gal-3) is an endogenous beta-galactoside-binding animal lectin (galectin family) that plays an important role in pathological processes as an inflammatory mediator as well as in cell proliferation and the regulation of apoptosis (Almkvist and Karlsson, 2004; Liu et al., 2002). Gal-3 produced by macrophages including activated microglia has strong chemotactic properties

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for monocytes and macrophages as well as neutrophils and contributes to phagocytosis in neutrophils and macrophages, and it is therefore involved in inflammatory processes (Almkvist and Karlsson, 2004; Fernández et al., 2005; Hsu et al., 2000; Walther et al., 2000). Gal-3 has also been associated with microglial activation in the mouse brain under various inflammatory conditions, including mouse models of experimental autoimmune disease, prion diseases, and hypoxia–ischemia (Lalancette-Hébert et al., 2007; Mok et al., 2007; Reichert and Rotshenker, 1999). In hypoxic–ischemic brain injury models, Gal-3 expression was localized predominantly in Iba1-positive microglia and GFAP-positive astrocytes in the hippocampus (Doverhag et al., 2010; Satoh et al., 2011). Gal-3-deficient mice were protected from injury, particularly in the hippocampus and striatum (Doverhag et al., 2010), suggesting that Gal-3 exerts its effects by modulating the inflammatory response in brain injury models. As reported previously (Huong et al., 2011), microglial activation (as indicated by Iba1 level) and neuronal cell death are significantly increased in the TMT-induced brain injury model, and the increases are inhibited by indomethacin, a common non-steroidal anti-inflammatory drug. However, little is known about the role of Gal-3 in microglial activation induced by TMT treatment.

In the present study, we evaluated the temporal expression of Gal-3 in the hippocampal DG of adult BALB/c mice after TMT treatment to elucidate the correlation between Gal-3 expression and TMT-induced neurodegenerative processes. The results demonstrate the cellular localization of Gal-3 in the hippocampus of TMT-treated mice using cell-type-specific antibodies. Additionally, we examined the co-localization of Gal-3 and pro-inflammatory enzymes, including COX-2 and iNOS, in the hippocampal DG after TMT treatment.

2. Materials and methods

2.1. Animals

Male BALB/c mice, 8–9-week-old mice were obtained from a specific-pathogen-free colony at Orient Bio, Inc. (Seoul, Korea). The Institutional Animal Care and Use Committee of Chonnam National University approved the protocols used in this study, and the animals were cared for in accordance with the Guidelines for Animal Experiments at Chonnam National University.

2.2. Drug treatment and tissue sampling

TMT (Trimethyltin hydroxide; Wako, Osaka, Japan) was dissolved in sterilized 0.9% saline. The time-dependent effects of TMT in the adult mouse hippocampus were observed after intraperitoneal (i.p.) administration of TMT (2.5 mg/kg). The vehicle control group was injected i.p. with 0.9% saline. The mice were sacrificed, and the hippocampi were dissected from each group on days 1, 2, 4, and 8 ($n = 6$ mice/group) after injection (Fig. 1, Arrows). The samples were processed for embedding in paraffin wax after fixation in 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) using routine protocols and stored at −70 °C for biochemical analysis.

2.3. Seizure scoring

Tremor/seizure tests were performed in brightly lit arenas (40 × 40 cm, 250 lux). Behavioral changes were scored as follows: (1) aggression; (2) weak tremor; (3) systemic tremor; (4) tremor and spasmodic gait; and (5) death (Yoneyama et al., 2008).

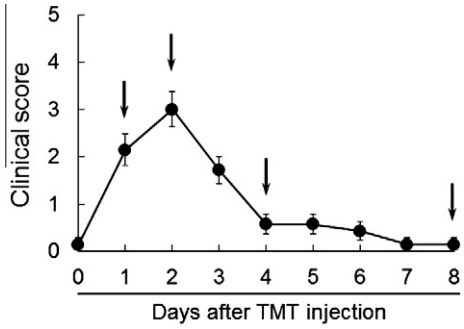

Fig. 1. Clinical scores of tremor/seizure in mice following TMT treatment (2.5 mg/kg). Clinical sign was scored according to 0–5 scale. Animals ($n = 6$) were sacrificed at each time point (days 1, 2, 4, and 8 days after treatment; arrows). The data are reported as the mean ± SE.

2.4. Antibodies

A rat monoclonal anti-galectin-3 antibody (1 mg/ml) was purified by affinity chromatography from the supernatants of hybridoma cells (clone TIB-166™, M3/38.1.2.8. HL.2; American Type Culture Collection, Manassas, VA, USA). Rabbit polyclonal anti-glial fibrillary acidic protein (GFAP; Dako, Glostrup, Denmark), rabbit polyclonal anti-ionized calcium-binding adaptor molecule 1 (Iba1; Wako), and mouse monoclonal anti-NeuN antibodies (Millipore, Temecula, CA, USA) were used to detect astrocytes, microglia, and neurons, respectively. Rabbit polyclonal iNOS (BD Biosciences, San Jose, CA, USA) and mouse monoclonal COX-2 antibodies (Dako, Glostrup, Denmark) were used to detect the appearance of an inflammatory response. A mouse monoclonal anti-β-actin antibody (Sigma–Aldrich, St. Louis, MO, USA) was used to detect β-actin labeling on Western blots.

2.5. Fluoro-jade B staining

Fluoro-jade B (FJB; a high-affinity fluorescent marker for the localization of neuronal degeneration) histofluorescent staining (each group on days 1, 2, 4, and 8 ($n = 6$ mice/group) after injection) was performed according to the method described previously (Schmued and Hopkins, 2000). Briefly, the sections were first transferred to a solution of 0.06% potassium permanganate and then to 0.0004% FJB (Millipore) staining solution. After washing, the sections were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) before being mounted. The FJB-stained sections were examined by immunofluorescence microscopy using a BX-40 microscope (Olympus, Tokyo, Japan) with a ProgRes® CFscan digital camera (Jenoptik, Jena, Germany).

2.6. Western blotting

Mice were sacrificed, and hippocampi were dissected at 1, 2, 4, and 8 days ($n = 3$ mice/group) after injection of TMT. Immunoblotting was performed as described previously (Yang et al., 2010, 2011). Briefly, the resolved proteins were transferred onto membranes, which were then incubated with primary antibodies against rat anti-galectin-3 (1:5,000 dilution), rabbit anti-Iba1 (1:10,000 dilution), or rabbit anti-GFAP (1:5,000 dilution) in PBS containing 0.1% Tween-20 (PBS-T, pH 7.4) overnight at 4 °C. After extensive washing and incubation with horseradish peroxidase (HRP)-conjugated anti-rabbit or rat antibody (1:10,000 dilution, 2 h at room temperature (RT); Thermo Fisher Scientific, Rockford, IL, USA), signals were visualized using a chemiluminescence kit (SuperSignal® West Pico; Thermo Fisher Scientific). For normalization purposes, the membranes were reprobed with anti-β-actin