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Trichloroethylene exposure aggravates behavioral abnormalities in mice that are deficient in superoxide dismutase

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ABSTRACT

Trichloroethylene (TCE) has been implicated as a causative agent for Parkinson's disease (PD). The administration of TCE to rodents induces neurotoxicity associated with dopaminergic neuron death, and evidence suggests that oxidative stress as a major player in the progression of PD. Here we report on TCE-induced behavioral abnormality in mice that are deficient in superoxide dismutase 1 (SOD1). Wild-type (WT) and SOD1-deficient (*Sod1*$^{-/-}$) mice were intraperitoneally administered TCE (500 mg/kg) over a period of 4 weeks. Although the TCE-administrated *Sod1*$^{-/-}$ mice showed marked abnormal motor behavior, no significant differences were observed among the experimental groups by biochemical and histopathological analyses. However, treating mouse neuroblastoma-derived NB2a cells with TCE resulted in the down regulation of the SOD1 protein and elevated oxidative stress under conditions where SOD1 production was suppressed. Taken together, these data indicate that SOD1 plays a pivotal role in protecting motor neuron function against TCE toxicity.

1. Introduction

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder next to Alzheimer's disease (Bertram and Tanzi, 2005) and more than 90% of cases are sporadic. The pathological hallmark of sporadic PD is a loss of dopaminergic neurons in the substantia nigra (SN). Among the various multifactorial causes, environmental toxins are suspected to play a role (Nagatsu and Sawada, 2006; Shaw and Höglinger, 2008). Trichloroethylene (TCE), which is widely used for industrial applications, has been now implicated as an environmental toxin that elevates risk for PD (Gash et al., 2008; Goldman, 2010; Guehl et al., 1999; Kochen et al., 2003). Animal studies demonstrated that exposure to TCE resulted in the impairment of mitochondrial complex I activity, a decrease in ATP production, and the excessive production of reactive oxygen species (ROS), with the simultaneous loss of dopaminergic neurons

Abbreviations: PD, Parkinson's disease; SN, substantia nigra; TCE, trichloroethylene; ROS, reactive oxygen species; SOD1, superoxide dismutase 1; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP^+, 1-methyl-4-phenylpyridine; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; ATTM, ammonium tetrathiomolybdate; Prx, peroxiredoxi; TH, tyrosine hydroxylase; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin.

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in the SN (Gash et al., 2008; Guehl et al., 1999; Liu et al., 2010).

Oxidative stress, which is caused under situation with a low antioxidative capacity compared to the ROS levels, has been implicated as playing an important role in the development of PD (Dias et al., 2013) and is regarded as an independent risk factor. In fact, dopaminergic neurons in the SN are known to be vulnerable to oxidative insult (Jenner and Olanow, 1998). In addition, the levels of anti-oxidant glutathione in the SN of PD patients are significantly decreased compared with those of healthy subjects (Sian et al., 1994a, 1994b). Superoxide dismutase 1 (SOD1) plays a central role in protection against the damaging process because of its ability to scavenge superoxide anions, which are the primary ROS generated from molecular oxygen (Fridovich, 1995). The SOD1 protein is constitutively expressed in most cells and is localized in the cytosol and partially in the mitochondrial inter membrane space (Okado-Matsumoto and Fridovich, 2001; Kira et al., 2002). In mice, an SOD1 deficiency aggravates the oxidative stress-related damage of organs and tissues under various pathological situations (Ho et al., 1998; McFadden et al., 1999; Didion et al., 2002; Elchuri et al., 2005; Yamanobe et al., 2007; Iuchi et al., 2010).

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is often used to trigger mitochondria-derived neuronal damage and results in the development of PD-linked symptoms. The PD-linked neurotoxicity induced by MPTP is enhanced in mice that are deficient in SOD1 or glutathione peroxidase (Zhang et al., 2000). On the other hand, the overexpression of SOD1 in mice has a protective function against neurotoxicity induced by 1-methyl-4-phenylpyridine (MPP^+), a toxic metabolite of MPTP, and 6-hydroxydopamine (Asanuma et al., 1998; Barkats et al., 2006). In Drosophila, the overexpression of human SOD1 protects dopaminergic neurons in a genetic model of PD (Botella et al., 2008). Although no PD-linked gene mutations have been identified in SOD1 (Bandmann et al., 1995; Farin et al., 2001), the levels of SOD1 mRNA and SOD1 activity are significantly decreased in PD patients (Boll et al., 2008; Bostantjopoulou et al., 1997; Ihara et al., 1999; Kunikowska and Jenner, 2003).

Based on these observations, we hypothesized that ROS produced as the result of exposure to TCE are involved in the development of PD-like symptoms. In the current study, we tested this hypothesis using SOD1-deficient mice that had been exposed to relatively low TCE concentrations after short-term administration, i.e., under moderate conditions. The findings support the notion that TCE-triggered ROS are involved in the neuronal damage because an SOD1 deficiency was found to aggravate the TCE-induced neurotoxicity.

2. Methods

2.1. Mice and TCE administration

Sod1$^{-/-}$ mice used in this study were described previously (Iuchi et al., 2007). C57BL/6 (Wild-type, WT) mice were bred in our institution. They were divided into four different groups: (I) WT mice administered vehicle alone, (II) WT mice administered TCE, (III) *Sod1*$^{-/-}$ mice administered vehicle alone, (IV) *Sod1*$^{-/-}$ mice administered TCE. TCE was purchased from Wako (208-02486) and was dissolved in salad oil. Male mice were intraperitoneally administered with a single dose of 500 mg/kg TCE three times a week for 4 weeks. The animal room climate was maintained under specific pathogen-free conditions at a constant temperature of 20–22 °C with a 12 h alternating light-dark cycle. Animal experiments were performed in accordance with the Declaration of Helsinki under the protocol approved by the Animal Research Committee at Yamagata University.

2.2. Blood test

Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and blood urea nitrogen (BUN) were measured with colorimetric slides using FUJI DRI-CHEM 3500V (FUJIFILM Co., Tokyo, Japan).

2.3. Rotarod test

The rotarod behavior test was carried out using a Rotamex device (Panlab, S.L.). The coordination of the mice was evaluated by increasing the rotation cycle from 4 to 40 rpm over a period of 5 min (cut-off time). The peak time was measured over two trials. The rest period between each trial was 30 min. The mean latency time to fall off the rotating rod for the last two trials was used for the analysis.

2.4. Open-field test

Spontaneous motor activity and anxiety-like behavior were assessed in an open-field chamber consisting of an arena (Toyo Sangyo Co., Ltd, W: 22 × L: 38 × H: 18.5 cm), that was brightly and evenly illuminated by four lamps at 450 lx. One animal was placed in the box and was allowed to explore the arena for 30 min. Results were obtained for each animal during a five-minute period.

2.5. Immunohistochemical analysis of brain sections

Mice anesthetized with ether were fixed transcardially with 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB, pH 7.2). After fixation, the brains were cryoprotected in 30% sucrose/0.1 M PB and cut into 16-μm-thick sections by a cryostat (CM1900; Leica, Nussloch, Germany). All immunohistochemical incubations were performed at room temperature (~18 °C). Immunoperoxidase staining was performed by overnight incubation with a mouse anti-tyrosine hydroxylase (TH) antibody (1:1000; MAB318; Chemicon). Sections were further incubated with a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) for 30 min and an avidin-biotin-peroxidase complex for 30 min using the avidin-biotin-peroxidase complex (ABC) system (Vector Laboratories) with diaminobenzidine (DAB) as a chromogen. Photographs were taken by a light microscope (Leica).

2.6. Quantification of monoamines and their metabolites

Dopamine hydrochloride (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine hydrochloride (3-MT), 5-hydroxyindole-3-acetic acid (5-HIAA) and isoproterenol hydrochloride were obtained from SIGMA (St. Louis, MO, USA). Serotonin-creatinine sulfate monohydrate for standard of 5-hydroxytryptamine (5-HT) was obtained from (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

The levels of monoamines in the mid brain were measured by an HPLC method which was modified from a previous report (Itoh et al., 2006). Brain samples were homogenized in 0.2 M perchloric acid containing 100 μM EDTA-2Na and 100 ng of isoproterenol was added as an internal standard. After centrifugation at 20,000 × g for 15 min, the pH of the supernatant was adjusted to 3 by adding 1 M sodium acetate. Samples were analyzed by an HPLC-ECD system (HTEC-500, Eicom, Kyoto, Japan). The levels of monoamines were separated by using an Eicompak SC-5ODS column (3.0 × 150 mm, Eicom, Kyoto, Japan), which was set at 25 °C. Monoamines were detected using an amperometric electrochemical detector–300 (Eicom) with a WE-3G (GS-25 Eicom) graphite electrode at +750 mV versus Ag/AgCl. The mobile phase