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Elevation of 4-hydroxynonenal and malondialdehyde modified protein levels in cerebral cortex with cognitive dysfunction in rats exposed to 1-bromopropane

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ABSTRACT

1-Bromopropane (1-BP), an alternative to ozone-depleting solvents (ODS), exhibits central nervous system (CNS) toxicity in animals and humans. This study was designed to relate CNS damage by Morris water maze (MWM) test and oxidative stress to 1-BP exposure in the rat. Male Wistar rats were randomly divided into 4 groups ($n = 10$), and treated with 0, 200, 400 and 800 mg/kg bw 1-BP for consecutive 12 days, respectively. From day 8 to day 12 of the experiment, MWM test was employed to assess the cognitive function of rats. The cerebral cortex of rats was obtained immediately following the 24 h after MWM test conclusion. Glutathione (GSH), oxidized glutathione (GSSG) and total thiol (total-SH) content, GSH reductase (GR) and GSH peroxidase (GSH-Px) activities, malondialdehyde (MDA) level, as well as 4-hydroxynonenal (4-HNE) and MDA modified proteins in homogenates of cerebral cortex were measured. The obtained results showed that 1-BP led to cognitive dysfunction of rats, which was evidenced by delayed escape latency time and swimming distances in MWM performance. GSH and total-SH content, GSH/GSSG ratio, GR activity significantly decreased in cerebral cortex of rats, coupling with the increase of MDA level. 4-HNE and MDA modified protein levels obviously elevated after 1-BP exposure. GSH-Px activities in cerebral cortex of rats also increased. These data suggested that 1-BP resulted in enhanced lipid peroxidation of brain, which might play an important role in CNS damage induced by 1-BP.

1. Introduction

Ozone-depleting solvents (ODS), such as chlorofluorocarbons (CFCs) and 1,1,1-trichloroethane have been banned in developed countries since 1996 by the Montreal Protocol (D'Souza, 1995; UNEP, 2000). 1-Bromopropane (1-BP) possesses favorable physical properties characterized by high volatility, low flammability, and photochemical breakdown that allows it to have a relatively short atmospheric half-life (17.5–24 days), thus possibly decrease its ozone-damaging capacity (Donaghy et al., 1993; Zhang et al., 2005). It has been introduced as a substitute for ODS, with widespread applications in cleaning agent for metal, electronics and optical instruments. It is also used for intermediates in the synthesis of pharmaceuticals, insecticides, quaternary ammonium compounds, flavors, fragrances, and as a solvent for fats, waxes, or resins (NTP Center, 2004). With its use growing rapidly, the worldwide production of 1-BP was estimated to be 20,000–30,000 metric tonnes in 2007. China was estimated to have produced around 20,000 metric tonnes in 2008, of which approximately 40% were exported. The use for solvent was to be growing at a rate of 15–20% annually in the United States and Asian countries other than China (HSIA, 2010). As a result, the number of workers exposed occupationally to 1-BP expands quickly. However, there is no sufficient toxic data for 1-BP risk characterization until now.

To the best of our knowledge, all types of organic solvents are volatile liquids at room temperature and are lipophilic. After absorption, they may be exhaled unchanged, biotransformed and then excreted, or accumulated in lipid rich tissues such as the brain, myelin, adipose and exhibited negative health effects, of which the central nervous system (CNS) has proved to be the most vulnerable target (Fueta et al., 2004). The evidence indicates that there is a high rate of neurobehavioral disorders in workers exposed to some volatile organic solvents (VOCs) (Broadwell et al., 1995). Recently, the potential CNS neurotoxicity of 1-BP has been revealed. A dozen human cases showing abnormal neurological symptoms or signs in the CNS as well as peripheral nervous system induced by 1-BP exposure have been reported (Ichihara, 2005; Majersik et al., 2007; Samukawa et al., 2012; Sclar, 1999). The surveys on industry workers have also shown memory loss and cognitive dysfunction among those who occupationally exposure to 1-BP (Ichihara et al., 2004a,b; Li et al., 2010). The adverse effects on CNS induced by

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1-BP exposure including loss of brain weight (Ichihara et al., 2000), damage of cortex, hippocampus and cerebellum (Fueta et al., 2002; Mohideen et al., 2009, 2011, 2012; Subramanian et al., 2012) have been demonstrated in experimental animal studies as well. However, little is known about the molecular mechanisms underlying the CNS damages induced by 1-BP.

The recent researches indicate that oxidative stress is closely linked to the CNS toxicity of 1-BP (Huang et al., 2011, 2012; Subramanian et al., 2012). Compared to other tissues in the body, the brain is particularly vulnerable to oxidative damage because of the high oxygen consumption rate and metabolic rate with an abundant supply of transition metals and enrichment of polyunsaturated lipids in neuronal cell membranes. In addition, the relatively lower antioxidative capacities such as low to moderate activities of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px) make brain a target for free radical attack (Dringen, 2000; Gilgun-Sherki et al., 2001; Lovell and Markesbery, 2007). There is evidence that GSH plays an important role in the detoxification of reactive oxygen species (ROS) in brain. GSH deficiency induced in newborn rats by application of an inhibitor of γ-glutamylcysteine synthetase, buthionine sulfoximine (BSO), leads to mitochondrial damage in brain (Dringen, 2000). Therefore, GSH content is vital for keeping cells redox homeostasis of the brain, which depends on the GSH balance of generation and consumption. Previous studies indicated that 1-BP significantly decreased the GSH levels in the brain of rats, whether it was long term exposure (Wang et al., 2003) or short term exposure (Wang et al., 2002). Recent proteomic analyses have revealed that the expression pattern of proteins related to oxidative stress was changed in the hippocampus of rats intoxicated with 1-BP (Huang et al., 2011, 2012). However, the CNS toxicity of 1-BP and linkage with oxidative status has not fully been illustrated yet.

Cognitive changes are sensitive to CNS effects of toxicant exposure and helpful to make clinical diagnosis and proper treatment, which often progress into permanent changes after withdrawal from or a decrease in dose of exposure (White and Proctor, 1997). In current study, we gave the report that 1-BP damaged the CNS of rats. The rats exhibited the dysfunction of spatial learning and memory in Morris water maze (MWM) performance. The disturbances of the CNS redox homeostasis were also demonstrated by changes of the oxidative and antioxidative parameters, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) modified proteins as well. The data presented in this study make a contribution to the understanding of the 1-BP toxicity on CNS and provide new insights into the underlying mechanisms.

2. Materials and methods

2.1. Materials

1-Bromopropane (1-BP) (99.99%) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Anti-β-actin monoclonal antibody (clone AC-15) was purchased from Sigma (St. Louis, MO, USA). Anti-malondialdeyde (MDA) (clone 1F83) and anti-4-hydroxynonenal (4-HNE) (clone HNEJ-2) monoclonal antibodies were purchased from JaICA (NOF Co., Tokyo, Japan). Horseradish peroxidase (HRP)-conjugated IgG was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The detection kits for contents of GSH, oxidized glutathione (GSSG), MDA and total thiol (total-SH), the activities of GSH reductase (GR) and GSH-Px were purchased from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China). BCA™ Protein assay Kit and SuperSignal® West Pico Chemiluminescent Substrate Kit were purchased from Pierce Biotechnology, Inc. (Rockford, IL, USA). All other chemicals were of highest quality commercially available.

2.2. Animal treatment

Forty adult male Wistar rats were provided by the Laboratory Animal Center of Shandong University. Animals were housed in plastic cages, and maintained under controlled conditions with 12 h light/dark (22 ± 2) °C room temperature and 50–60% relative humidity. Standard rat diet and tap water were provided *ad libitum*. After 3 days of acclimation, rats were randomly divided into four groups: control group, 200 mg/kg bw 1-BP group, 400 mg/kg bw 1-BP group and 800 mg/kg bw 1-BP group. The exposure levels were selected based on our preliminary experiments, in which toxic effects on the central as well as peripheral nervous systems were exhibited in rats treated with 1-BP (Wang et al., 2012). Rats in 1-BP exposure groups were treated with 1-BP dissolved in corn oil by gavage daily for consecutive 12 days, while rats in control group received equal volume of corn oil. MWM test was employed to assess the cognitive function of rats from day 8 to day 12 of the experiment. Rats were then anesthetized with over-dose pentobarbital sodium and decapitated 24 h after conclusion of the MWM test. The cerebral cortex were quickly removed, frozen in liquid nitrogen, and kept at −80 °C until use. The protocols were approved by the Animal Experimentation Committee of Shandong University.

2.3. Morris water maze (MWM) test

MWM test was used to assess the spatial learning and memory (Vorhees and Williams, 2006). The maze consists of a circular tank (1.80 m diameter, 0.6 m depth) with black interior filled with opaque water maintained at a temperature of 25 ± 1 °C. A computerized tracking system (Huaibei ZhengHua Biological Instrument Equipment Co., Ltd., Suixi, China) composed of a video camera mounted directly above the maze and video tracking software (Huaibei ZhengHua Biological Instrument Equipment Co., Ltd., Suixi, China) was used to track and record animal movement and swimming pattern in the maze for all tests.

2.3.1. Spatial navigation test

Spatial navigation test was employed to assess the spatial learning of rats by recording their escape latency (time to reach the hidden platform) and distance traveled (length of the swimming path taken to find the platform). During the test, animals were gently released to the water with head facing the pool wall from a peripheral pseudorandom starting point (N, S, E, or W) to locate the hidden platform (1–2 cm below the water surface) for 120 s. Each rat was given a block of four swimming trials starting randomly from four positions per day for 4 consecutive days. The escape latency and distance traveled were recorded for each trial. Following each trial, rats were removed from the maze and placed in a holding cage for a 30 s intertrial interval. If the rat failed to reach the platform within 120 s, it was gently guided to the platform and allowed to stay for 15 s, and an escape latency time of 120 s was recorded. Data from the four starting positions are pooled to provide summed data per trial block. In assessing the individual swimming path, the search strategy developed by Graziano et al. (2003) was employed to assess the ability of spatial learning as well. Data were expressed as the portion of different searching pattern manifested by the rats in all 4 days. Four representative learning patterns were qualitatively analyzed in current study. Thigmotaxis (rats swim almost exclusively in the periphery) and random searching (the moving trajectories are no longer circular but jagged with sudden changes in direction and velocity) were considered as the ineffective ways, and approaching target (rats adjust its swimming trajectory while approaching the platform) and direct finding (rats swim fast and straight from the starting point to the platform) as effective ways.

2.3.2. Spatial probe trial

On day 5 of the MWM test, spatial probe trial was performed to assess short-term memory by removing the platform from the tank and allowing animals to search for the platform for 90 s. During a probe trial, the spatial accuracy of the animal is determined, represented by the time spent in the target quadrant that had previously contained the platform and by the number of times it crosses the former platform area.

2.4. Measurement of the MDA, total-SH, GSH, GSSG levels and the GR, GSH-Px activities in cerebral cortex of rats

2.4.1. Preparation of the 10% cerebral cortex homogenate

Cerebral cortexes of rats were weighed and homogenized by a glass homogenizer in 9 volume ice-cold buffer containing 0.01 M Tris–HCl, 0.1 mM EDTA–Na_2, 0.01 M saccharose, 0.8% saline at 4 °C. Homogenates were kept in ice-cold water for 30 min, and then centrifuged (2500 rpm, 10 min) at 4 °C. The supernatant was collected and stored at −80 °C until analysis. Protein content was determined by BCA™ protein assay kits (Pierce, Rockford, IL).

2.4.2. Determination of MDA level

The tissue MDA level was determined by measuring thiobarbituric acid reactive substance (TBARS) and expressed as the MDA levels, which was assayed with a commercial kit (Nanjing Jiancheng Institute, China) according to the manufacturer's instruction. The method was used to obtain a spectrophotometric measurement of the color with an absorption maximum at 535 nm produced during the reaction of TBA with MDA at 90–100 °C. The MDA level was expressed as nmol/mg protein.

2.4.3. Measurement of total-SH

The total-SH content was measured by the commercial kit (Nanjing Jiancheng Institute, China) according to the manufacturer's instruction. In brief, the supernatant of cerebral cortex homogenate was added to 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). The chromogen can react with the thiol group to produce a colored compound that absorbs at 405 nm. The rate of chromophore production is proportional to the concentration of total-SH within the sample. The values were expressed as μmol/mg protein.