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Research Report

Involvement of decreased neuroglobin protein level in cognitive dysfunction induced by 1-bromopropane in rats

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ARTICLE INFO

Article history:
Accepted 10 December 2014
Available online 31 December 2014

Keywords:
1-Bromopropane
Neurotoxicity
Oxidative stress
Neuroglobin

ABSTRACT

1-Bromopropane (1-BP) is used as a substitute for ozone-depleting solvents (ODS) in industrial applications. 1-BP could display central nervous system (CNS) neurotoxicity manifested by cognitive dysfunction. Neuroglobin (Ngb) is an endogenous neuroprotectant and is predominantly expressed in the nervous system. The present study aimed to investigate Ngb involvement in CNS neurotoxicity induced by 1-BP in rats. Male Wistar rats were randomly divided into 5 groups ($n=14$) and treated with 0, 100, 200, 400 and 800 mg/kg bw 1-BP, respectively, by gavage for consecutive 12 days. Rats displayed cognitive dysfunction dose-dependently through Morris water maze (MWM) test. Significant neuron loss in layer 5 of the prelimbic cortex (PL) was observed. Moreover, 1-BP decreased Ngb protein level in cerebral cortex and Ngb decrease was significantly positively correlated with cognitive dysfunction. Glutathione (GSH) content, GSH/oxidized glutathione (GSSG) ratio and glutamate cysteine ligase (GCL) activity decreased in cerebral cortex, coupled with the increase in GSSG content. GSH and GSH/GSSG ratio decrease were significantly positively correlated with cortical Ngb decrease. Additionally, levels of N-epsilon-hexanoyl-lysine (HEL) and 4-hydroxy-2-nonenal (4-HNE) modified proteins in cerebral cortex of 1-BP-treated rats increased significantly. In conclusion, it was suggested that 1-BP resulted in decreased endogenous neuroprotectant Ngb in cerebral cortex, which might play an important role in CNS neurotoxicity induced by 1-BP and that 1-BP-induced oxidative stress in cerebral cortex might partly be responsible for Ngb decrease.

Abbreviations: 1-BP, 1-bromopropane; 4-HNE, 4-hydroxy-2-nonenal; AD, Alzheimer's disease; CFCs, chlorofluorocarbons; HCFCs, hydrochlorofluorocarbons; HEL, N-epsilon-hexanoyl-lysine; ODP, ozone depleting potential; ODS, ozone-depleting solvents; CNS, central nervous system; PNS, peripheral nervous system; PL, prelimbic cortex; PUFA, polyunsaturated fatty acid; Mrps, multidrug resistance proteins; MWM, Morris water maze; GCL, glutamate cysteine ligase; GR, glutathione reductase; GS, glutathione synthetase; GSH, glutathione; GSSG, oxidized glutathione; ROS, reactive oxygen species; Ngb, neuroglobin; PFA, paraformaldehyde; PBS, phosphate buffer saline; BSA, Bovine Serum Albumin; DAB, diaminobenzidine

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http://dx.doi.org/10.1016/j.brainres.2014.12.046
0006-8993/

1. Introduction

1-Bromopropane (1-BP), regarded as a highly volatile organic compound, has a transient existence with a half-life of approximately 15 days in the ambient environment (Boublík et al., 1984; Nelson et al., 1997). Additionally, compared to chlorofluorocarbons (CFCs) and hydrochlorofluorocarbons (HCFCs), 1-BP has a lower ozone depleting potential (ODP) of 0.013–0.018 at middle latitude (USEPA, 2007). Therefore, as an alternative to ozone-depleting solvents (ODS), 1-BP is widely applied in industrial uses, such as cleaning metals and electronic components (OSHA/NIOSH, 2014) and manufacturing of pesticides, pharmaceuticals, flavors and fragrances (NTP, 2014). However, 1-BP could induce sensory and motor dysfunction, and central nervous system (CNS) disadvantages including reduced short-term memory in humans (Ichihara et al., 2002, 2004; Majersik et al., 2007; Sclar, 1999). In animal experiments, rats began to show peripheral nervous system (PNS) deficits after 4 weeks of exposure to 800 ppm 1-BP; whereas abnormal CNS signs were observed in rats exposed to 50–200 ppm 1-BP for only 3 weeks (Honma et al., 2003; Ichihara et al., 2000). Based on previous studies, 1-BP was concluded to induce CNS and PNS neurotoxicity, the former occurring earlier than the latter. Therefore, researches on the dose-dependent severity of 1-BP-induced CNS neurotoxicity and its possible mechanisms might be beneficial to early identification, prevention and treatment of 1-BP intoxication. Furthermore, cognitive dysfunction, contained in chief complaints of most human cases and abnormal CNS signs of intoxicated animals, could be regarded as an indicator of CNS neurotoxicity of 1-BP and reliably quantified by Morris Water Maze (MWM) test in animal researches (Vorhees and Williams, 2006).

Cognitive performances in MWM is associated with several brain regions such as cerebral cortex, hippocampus, basal forebrain, striatum and cerebellum (D'Hooge and De Deyn, 2001). Previous studies proved that lesions in cerebral cortex might be relevant to cognitive dysfunction and that MWM could be considered as one of the measures of cortical function (Brown et al., 2000; D'Hooge and De Deyn, 2001; Rinwa and Kumar, 2012; Scheff et al., 1997). Hence, this study discussed cortical changes induced by 1-BP.

Based on many previous researches on cognitive disorders, oxidative stress was proposed to have a possible causal relationship with cognitive dysfunction (Andersen, 2004; Chang et al., 2011; Yan et al., 2013). In CNS, glutathione (GSH) is the primary intracellular antioxidant and plays an important role in detoxification of reactive oxygen species (ROS) and xenobiotics. Based on previous studies, it was indicated that stable GSH content was essential for maintaining normal cognitive function and that GSH depletion might partly contribute to cognitive dysfunction, although the causation was often suggested (An et al., 2012; Ballatori et al., 2009; Dean et al., 2009; Liu et al., 2004; Martin and Teismann, 2009; Shukitt-Hale et al., 1998; Yabuki and Fukunaga, 2013). Recent studies assumed that 1-BP-induced CNS neurotoxicity was associated with oxidative stress and GSH depletion (Huang et al., 2011, 2012; Subramanian et al., 2012). However, the mechanism of oxidative stress and GSH depletion induced by 1-BP has not been fully illustrated yet.

In addition to GSH, there are other important antioxidants present in the nervous system among which neuroglobin is a novel antioxidant and plays neuroprotective roles. Ngb, firstly discovered in 2000, is expressed in neurons, astrocytes, retina and some endocrine tissues (Burmester et al., 2000; Burmester and Hankeln, 2004; De Marinis et al., 2013a). Brunori and Vallone (2007) demonstrated its highly conserved structure and function. As the firstly discovered hexacoordinate hemoglobin in vertebrates, Ngb has a heme iron, either in ferrous (reduced) or ferric (oxidized) form. Ngb was generally accepted to have neuroprotective effects, due to the evidences that overexpression of Ngb protected neural cells from hypoxic-ischemia injury (Khan et al., 2006; Sun et al., 2001), oxidative stress-induced cell death (Fordel et al., 2006; Liu et al., 2009) and beta-amyloid neurotoxicity (Khan et al., 2007; Li et al., 2008b) *in vitro*, and protected animals from experimental stroke (Sun et al., 2003) and Alzheimer's disease (AD) (Khan et al., 2007) *in vivo*. Although not fully elucidated, there are several possible mechanisms of the neuroprotective roles of Ngb, involving reactive species scavenging (Yu et al., 2009) and anti-apoptosis (Brittain, 2012) in the processes of which Ngb works in a ferrous state and then turns to ferric Ngb which could be converted to ferrous Ngb again by utilization of the intracellular antioxidants (Hota et al., 2012). Cognitive dysfunction is associated with ROS-induced oxidative damage and unexpected apoptosis of neural cells in the cerebral cortex the severity of which, to some degree, is probably connected with the level of Ngb expression and the conversion of ferric Ngb to ferrous Ngb.

On account of the neuroprotective effects of Ngb, this study aimed to investigate Ngb involvement in CNS neurotoxicity induced by 1-BP in rats. The exposure levels of 1-BP were selected based on our preliminary experiment as well as our previous experiment estimating 1-BP PNS toxicity (Wang et al., 2012), aiming to mimic actual clinical manifestations of human 1-BP-intoxicated cases. In our previous experiment, exposure to 400 and 800 mg/kg bw 1-BP for 16 weeks could induce significant decrease in hindlimb grip strength and paralysis which were similar to clinical signs of human cases. In the early weeks in that experiment, we also observed 1-BP-induced CNS toxic effects, such as emotional changes. Furthermore, in our preliminary experiment, it was indicated that 200, 400 and 800 mg/kg bw 1-BP could induce CNS neurotoxicity manifested by cognitive dysfunction which was similar to clinical symptoms of human cases. Therefore, 100, 200, 400 and 800 mg/kg bw 1-BP were used as intoxication doses. Neuron loss in prelimbic cortex (PL) was estimated and Ngb protein level in cerebral cortex was measured. Since the redox state was critical to the conversion of ferric Ngb to ferrous Ngb and the protein level of Ngb, GSH content and lipid peroxidation condition in cerebral cortex were determined. Besides, glutamate cysteine ligase (GCL) activity was measured to investigate the possible mechanism of GSH alteration. Ngb involvement in CNS neurotoxicity induced by 1-BP has not been discussed by previous studies. Therefore the data in the current study might provide further