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Preliminary characterization of a murine model for 1-bromopropane neurotoxicity: Role of cytochrome P450

Cai Zong[a,b], C. Edwin Garner[c], Chinyen Huang[a], Xiao Zhang[a,b], Lingyi Zhang[b], Jie Chang[a,d], Shinya Toyokuni[e], Hidenori Ito[f], Masashi Kato[a], Toshihiro Sakurai[b], Sahoko Ichihara[g], Gaku Ichihara[b,*]

[a] Department of Occupational and Environmental Health, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[b] Department of Occupational and Environmental Health, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda 278-8510, Japan
[c] Lovelace Respiratory Research Institute, Albuquerque, NM 87108, USA
[d] School of Public Health, Medical College of Soochow University, Suzhou 215123, China
[e] Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan
[f] Institute for Developmental Research, Aichi Human Service Center, Kasugai 480-0392, Japan
[g] Graduate School of Regional Innovation Studies, Mie University, Tsu 514-8507, Japan

HIGHLIGHTS

- Inhibition of P450s enabled mice to survive after 1-BP exposure at a lethal level.
- Hepatotoxicity of 1-BP was significantly reduced by inhibition of P450s.
- The present model is the first to show 1-BP-induced decrease in brain weight in mice.
- The present model is the first to show 1-BP-induced increase in brain GRP78 in mice.
- The present model is the first to show 1-BP-induced increase in brain Ran in mice.

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ABSTRACT

Neurotoxicity of 1-bromopropane (1-BP) has been reported in both human cases and animal studies. To date, neurotoxicity of 1-BP has been induced in rats but not in mice due to the lethal hepatotoxicity of 1-BP. Oxidization by cytochromes P450 and conjugation with glutathione (GSH) are two critical metabolism pathways of 1-BP and play important roles in toxicity of 1-BP. The aim of the present study was to establish a murine model of 1-BP neurotoxicity, by reducing the hepatotoxicity of 1-BP with 1-aminobenzotriazole (1-ABT); a commonly used nonspecific P450s inhibitor. The results showed that subcutaneous or intraperitoneal injection of 1-ABT at 50 mg/kg body weight BID (100 mg/kg BW/day) for 3 days, inhibited about 92–96% of hepatic microsomal CYP2E1 activity, but only inhibited about 62–64% of CYP2E1 activity in brain microsomes. Mice treated with 1-ABT survived even after exposure to 1200 ppm 1-BP for 4 weeks and histopathological studies showed that treatment with 1-ABT protected mice from 1-BP-induced hepatic necrosis, hepatocyte degeneration, and hemorrhage. After 4-week exposure to 1-BP, the brain weight of 1-ABT(+)/1200 ppm 1-BP group was decreased significantly. In 1-ABT-treated groups, expression of hippocampal Ran protein and cerebral cortical GRP78 was dose-dependently increased by exposure to 1-BP. We conclude that the control of hepatic P450 activity allows the observation of effects of 1-BP on the murine brain at a higher concentration by reduction of hepatotoxicity. The study suggests that further experiments with liver-specific control of P450 activity using gene technology might provide better murine models for 1-bromopropane-induced neurotoxicity.

* Corresponding author at: Department of Occupational and Environmental Health, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Building No. 15, 2641 Yamazaki, Noda 278-8510, Japan.
E-mail address: gak@rs.tus.ac.jp (G. Ichihara).

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1. Introduction

1-Bromopropane (1-BP, CAS No. 106-94-5) was introduced as an organic solvent and metal cleaner based on its less harmful ozone layer-depletion property (Ichihara et al., 2000; Ichihara, 2005). It is widely used in electronic parts cleaning, dry cleaning, as well as in the synthesis of pharmaceuticals and pesticides. Occupational exposure to 1-BP through spraying glue or cleaning metal parts in poorly ventilated workplace caused serious disorders of the central nervous system or peripheral nerve (Sclar, 1999; Ichihara et al., 2002; Majersik et al., 2007; Samukawa et al., 2012). US EPA has decided 1-BP as an acceptable substitute to ozone-depleting solvents as a cleaning agent, but not as a solvent for glue because many sprayers were suffered from 1-BP induced neurologic disorders (EPA, 2007). USA imports 10.3 million of 1-BP in 2011 (National Toxicology Program, 2013). There is a concern on not only exposure to workers but also exposure to general population, as recently 1-BP has come to be used also as a solvent for dry cleaning (Blando et al., 2010). Workers intoxicated with 1-BP complained of numbness, decrease in vibration sense in the lower extremities, headache, and dysphagia (Ichihara et al., 2012). Higher central nervous system function disorders including depression, anxiety and memory deficits in humans have also been reported (Ichihara et al., 2012, 2004, 2002; Majersik et al., 2007). Animal studies indicated that 1-BP also damages the liver (Lee et al., 2005; Liu et al., 2009, 2010) and reproductive system (Ichihara et al., 2000; Banu et al., 2007; Garner et al., 2007), and can induce carcinogenesis (National Toxicology Program, 2013, 2003) in mice and rats. Neurotoxicity of 1-BP in animal studies was reported in rats (Ichihara et al., 2000, 2012; Mohideen et al., 2009, 2011; Huang et al., 2011, 2012; Zhang et al., 2013), but very little is known about such neurotoxicity in mice. It was reported previously that mice are more susceptible to 1-BP than rats; mice died after exposure to 300–400 ppm 1-BP for 3–7 days due to severe hepatotoxicity, before the appearance of any signs of neurotoxicity (Liu et al., 2009).

Previous animal studies demonstrated two critical metabolic pathways for 1-BP; oxidization by cytochromes P450 (P450s, mainly CYP2E1), and conjugation with glutathione (GSH) (Garner et al., 2006; Garner et al., 2007; Garner and Yu, 2014; Lee et al., 2010a, 2010b). The relative flux through these two metabolic pathways may modulate 1-BP-induced hepatotoxicity (Garner et al., 2015; Garner and Yu, 2014; Liu et al., 2009; Lee et al., 2005, 2010a, 2010b). Prior studies have shown that modulation of CYP activity *in vivo* in rodents using the potent general CYP inhibitor 1-Aminobenzotriazole (1-ABT) results in a nearly complete elimination of oxidative metabolism (Garner et al., 2006) and that reproductive toxicity is eliminated in CYP2E1 knockout mice (Garner et al., 2007). 1-ABT acts as a suicide substrate of hepatic, pulmonary, and renal P450s and is particularly well suited for use *in vivo* because 1-ABT is well tolerated and produces little overt toxicity (Ortiz de Montellano and Mathews, 1981; Balani et al., 2004; Mugford et al., 1992).

The aim of the present study was to establish a murine model of 1-BP neurotoxicity. A murine model of 1-BP neurotoxicity could allow the study of the molecular and cellular mechanisms of such toxicity, because mice provide far much variety of transgenic models than rats. In the past, we aimed to study the involvement of Nrf-2 in neurotoxicity of 1-BP, but failed to do that as mice developed hepatic necrosis after exposure to 1-BP before the development of overt neurotoxicity (Liu et al., 2010). For the purpose of establishing the murine model, we took advantage of the effects of 1-ABT in suppressing hepatic P450 activity and to reduce hepatotoxicity caused by 1-BP in mice in order to reduce lethality and prolong survival, in order to provide chance for the appearance of neurotoxicity. Preliminary studies showed a nominal inhibitory effect for 1-ABT on brain P450 activity, in other words, the use of 1-ABT could prolong survival even after exposure to higher 1-BP concentrations, which could cause overt neurotoxicity.

2. Materials and methods

2.1. Chemicals

1-BP (99.81% purity, LOT No. 3Y203) used in this study was supplied by Tosoh Co. (Tokyo, Japan). 1-ABT (>98.0% purity, LOT No. A1464) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). All other chemicals were purchased from commercial sources and were of the highest purity available.

2.2. Animals

This study was conducted according to the Japanese act on the protection and control of animals and the Animal Experimental Guidelines of Nagoya University. C57BL/6J mice (male, 8-week-old) were purchased from Clea, Inc. (Tokyo, Japan). All mice were housed and acclimated to the new environment for one week in a temperature (23–25 °C)- and humidity (55–60%)-controlled room under a 12:12 h light: dark cycle. Food and water were provided *ad libitum*.

2.3. Effects of 1-ABT on CYP2E1 activity in liver and brain

While intraperitoneal injection of 1-ABT inhibits CYP2E1 activity in the liver (Mugford et al., 1992), there is no information on whether subcutaneous injection produces the same effect. Therefore, we first compared the effects of subcutaneous and intraperitoneal injections of 1-ABT on hepatic CYP2E1.

Twelve male C57BL/6J mice were divided into three groups of four each. Mice of the first group were injected 1-ABT subcutaneously at 50 mg/kg BID (100 mg/kg body weight/day) for three days. Mice of the second group received the same treatment but 1-ABT was injected intraperitoneally (Balani et al., 2004), while those of the third control group were subcutaneously injected with saline at a similar dose. On day 4, the liver and brain were dissected out carefully and rinsed with saline to remove blood cells, and stored in fresh tubes at −80 °C until use.

Liver microsomes were prepared using the method described previously (Gandhi et al., 2012) with minor modifications. Briefly, one gram of liver was homogenized using a motorized homogenizer in 9 ml of ice-cold homogenization buffer containing 50 mM potassium phosphate buffer (pH 7.4), 250 mM sucrose and 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenized mixture was centrifuged at 18,500 × *g*, at 4 °C for 15 min. The supernatant was then centrifuged at 86,000 × *g*, at 4 °C for 60 min. After removing the supernatant, the precipitate was re-suspended in 250 mM sucrose. Protein concentration was determined using a protein assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), using bovine serum albumin as a standard. Brain microsomes were prepared using a similar procedure.

CYP2E1 enzyme activity was measured as described previously (Lee, 2006) with minor modifications. Briefly, microsomes (0.3 mg) and chlorzoxazone (CZX, 20 μg) were incubated in the presence of 3 mg/ml NADPH, 0.1 M Mg/Cl_2, 0.1 M Tris-HCl buffer (pH 7.6) in a total volume of 1 ml and allowed to react for 20 min at 37 °C. The reaction was stopped by adding 200 μl of acetonitrile containing 50 ng/μl phenacetin as the internal standard. The sample was then vortexed for 30 s and placed on ice. After centrifugation at 10,000 × *g* for 4 min, the supernatant was transferred to a fresh tube, mixed with 2 ml diethyl ether, vortexed for 30 s and then centrifuged at 2000 × *g* for 1 min to separate the layers. The diethyl