主論文の要旨

Effects of sub-acute and sub-chronic inhalation of 1-bromopropane on neurogenesis in adult rats

（1-ブロモプロムパンによるラット脳内神経伝導物質レベル及び
BDNF（脳由来神経栄養因子）発現量の変化）

名古屋大学大学院医学系研究科　機能構築医学専攻
病理病態学講座　腫瘍病理学分野
（指導：髙橋 雅英　教授）
张 灵逸

Introduction

1-Bromopropane (1-BP) was introduced as an alternative to ozone-depleting solvents, such as chlorofluorocarbons and 1,1,1-trichloroethane, and is mainly used as a cleaning agent for metal parts of precision instruments and as a solvent in spray adhesives. However, previous studies have shown that 1-BP has neurotoxicity in humans and rats. Disorders of the higher cerebral function including depression, anxiety and memory deficits in humans were also reported. These were also supported by experimental studies which showed reduced noradrenalin axons in prefrontal cortex and amygdala, and neurobehavioral abnormalities in rats after exposure to 1-BP.

However, the mechanism of how exposure to 1-BP induces disorder of the higher cerebral function remains elusive. On the other hand, neurogenesis has been reported to be closely related to higher cerebral function. Based on the understanding of the relations among disorder of the higher cerebral function, neurogenesis, GR, BDNF and neurotransmitters, the present study was designed to investigate the effect of exposure to 1-BP on neurogenesis, the expression of BDNF and GR in hippocampus, and monoamine levels in different brain regions, including hippocampus in adult rats, in order to understand the mechanism underlying disorders of the higher cerebral function induced by exposure to 1-BP.

Material and Methods

The present study consisted of three parts. Four groups of 12 male Wistar rats were exposed to 1-BP at 0, 400, 800 and 1000 ppm, 8 hrs/day for 7 days. Other four groups of six rats were exposed to 1-BP at 0, 400, 800 and 1000 ppm for two weeks and 0, 200, 400 and 800 ppm for another 2 weeks. The other four groups of six rats were exposed to 1-BP at 0, 200, 400 and 800ppm for 4 weeks.

To determine the effect of 1-BP exposure on neurogenesis, after the last exposure, 12 rats of each group were each injected with BrdU (Sigma, St. Louis, MO) at 24 mg/100 g body weight in 0.9% saline, i.p. every 2 hours for three times. Rats injected with BrdU were transcardially perfused with 4% paraformaldehyde (PFA) 12 hours after the last injection. The whole brain was dissected out and embedded in optimal cutting temperature (OCT) compound to make frozen blocks.

Coronal brain sections of 30 μm were cut on cryostat and mounted on slides. Every three sections were collected covering the whole dentate gyrus (Bregma -2.56 to -6.04 mm). BrdU-positive cells were labeled using BrdU labeling & detection kit II (Roche Diagnostics, Mannheim, Germany) according to the protocol supplied by the manufacturer. Sections were examined under an optical microscope and the number of BrdU-positive cells was counted by an examiner blinded to the exposure

group. Every three sections covering the entire dentate gyrus (total 15 sections per rat) were examined. BrdU-positive cells in the dentate gyrus were counted and recorded in an area including the granule cell layer and subgranule zone (SGZ).

On the other hand, 12 rats of each group that were not injected with BrdU were decapitated and the whole brain was dissected out within 5 minutes. The hippocampus, striatum and prefrontal cortex were separated and frozen on dry ice immediately.

The hippocampal mRNA expression levels of BDNF (brain-derived neurotrophic factor) and glucocorticoid receptor (GR) were measured by quantitative RT-PCR.

The levels of monoamines, including noradrenalin, dopamine, serotonin, and their metabolites, were measured in the hippocampus, striatum and pre-front cortex (PFC), respectively, using high performance liquid chromatography (HPLC) with an electrochemical detector (ENO-10, Eicom Co., Kyoto, Japan).

Data are expressed as mean±SD. One-way analysis of variance (ANOVA) was used for comparison of group data, followed by Dunnett's multiple comparison. The significance level was set at $p < 0.05$. Data were analyzed using the JMP 8 software (SAS Institute Inc., Cary, NC).

Results

Quantitative RT-PCR. Quantitative RT-PCR showed no changes in BDNF, GR after 1-week exposure to different concentrations of 1-BP (0, 400, 800 and 1000 ppm). The expression of BDNF mRNA relative to β-actin was lower in both the 400 ppm and 800 ppm groups but not in 200 ppm group, compared to the control group (Fig. 1a). The GR mRNA expression levels after exposure to 200 ppm, 400 ppm, and 800 ppm were also significantly lower than the control group (Fig. 1b).

Changes in neurotransmitter levels. Exposure to 800 and 1000 ppm of 1-BP for 1 week reduced NE levels, but not those of serotonin or 5-HIAA, in the striatum (Fig.2). Furthermore, the ratio of DOPAC/DA decreased in the striatum, and the decrease varied proportionately with the 1-BP concentration. The ratio of HVA/DA after 1-week exposure to 400 ppm of 1-BP was low in pre-frontal cortex). Exposure to 1-BP for 4 weeks resulted in more pronounced changes in hippocampus neurotransmitter levels compared with those observed after 1-week exposure: hippocampal NE was lower at 800 ppm (Fig.3), and HVA, DOPAC/DA, HVA/DA, and the (DOPAC+HVA)/DA ratio were higher in the 800 ppm group. However, 1-BP did not affect hippocampal serotonin levels, irrespective of the dose and duration of exposure, whereas it reduced hippocampal DA in the 200 and 800 ppm groups. Exposure to 800 ppm decreased the concentrations of NE and 5-HIAA levels, and

decreased HVA and HVA/DA ratio in the pre-frontal cortex, as well as reduced NE but had no effect on other neurotransmitters in the striatum.

BrdU-positive cell count. Exposure to 1-BP for 1 week did not result in any change to neurogenesis, whereas exposure to 800/1000 ppm for 4 weeks reduced the number of BrdU-positive cells (Fig. 4, Fig. 5).

Discussion

The present study showed that 4-week exposure to 1-BP decreased neurogenesis in the dentate gyrus. To the best of our knowledge, this is the first study to demonstrate the effect of 1-BP on neurogenesis in animals. Decrease in hippocampal neurogenesis might play an important role in 1-BP-induced disorders of the higher cerebral function, including depressive mood, impairment of cognitive function and memory loss. With regard to the monoamine levels, 1-week exposure to 1-BP resulted in early-onset fall in NE level in the striatum and 4-week exposure decreased to a greater extent NE level in the hippocampus, prefrontal cortex and striatum, but no changes in serotonin level. Taken together with our previous study showing that 4-week exposure to 1-BP decreased the number of noradrenergic axons, but not serotonin axons, in F344 rats, it seems that exposure to 1-BP may target the noradrenalin system in the central nervous system (CNS).

In the present study, exposure levels of 200 to 1000 ppm were selected to match those found in the work environment of cases identified with 1-BP neurotoxicity. A recent case report of human 1-BP intoxication showed that estimated exposure level was 553 ppm (mean of time-weighted averages, range 353-663 ppm) and the ambient concentration of 1-BP at the height of worker's nose in front of the washing tank containing 1-BP was around 1,500 ppm (data not shown). Thus, the concentrations of 1-BP used in the present study of 200-1,000 ppm are representative from those found in poorly controlled workplaces associated with the reported human cases of neurotoxicity. Admittedly, however, it is difficult to directly compare the effects of different exposure levels on humans and animals at this stage, because of possible species difference in susceptibility.

Conclusion

The present results in rats suggest that the disorders of the higher cerebral function noted in workers exposed to 1-BP may be explained by suppression of neurogenesis in the hippocampus, and that this effect might be, at least in part, due to the negative effect of 1-BP on GR and BDNF mRNAs and hippocampal NE level.