主論文の要約

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

（1-ブロモプロパンの神経毒性のためのマウスモデルの予備的キャラクタリゼーション：シトクロム P450 の役割）

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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. It is widely used in electronic parts cleaning, dry cleaning, as well as in the synthesis of pharmaceuticals and pesticides. However, neurotoxicity of 1-BP has been reported in many workplace cases. Animal studies indicated that 1-BP also damages the liver and reproductive system, and can induce carcinogenesis in mice and rats. Neurotoxicity of 1-BP in animal studies was reported in rats, 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.

Previous animal studies demonstrated two critical metabolic pathways for 1-BP, including oxidization by cytochromes P450 (P450s, mainly CYP2E1) and conjugation with glutathione (GSH). The relative flux through these two metabolic pathways plays important roles in 1-BP-induced hepatotoxicity.

The aim of the present study was to establish a murine model for study of 1-BP neurotoxicity. We took advantage of the effects of 1-ABT, which is a non-specific P450s inhibitor, 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.

Materials and methods

To measure effects of 1-ABT on CYP2E1 activity in liver and brain, two groups of mice were injected with 1-ABT subcutaneously or intraperitoneally, and one group was subcutaneously injected with saline as control, for 3 days. After dissection, liver and brain microsomes were prepared. Microsomes were then incubated with chlorzoxazone (CZX), which is a substrate of CYP2E1. The amount of produced metabolite OH-CZX was measured by HPLC.

Preliminary exposure experiments were performed. 1200 ppm was determined as the maximum tolerated concentration of 1-BP for 1-ABT treated mice, and 250 ppm for 1-ABT-untreated mice.

In 1-BP exposure experiment, 4 groups of 1-ABT-pretreated mice were exposed to 1-BP at 0, 50, 250 and 1200 ppm, 8 hrs/d, for 4 weeks. Another 3 groups of saline-injected mice were exposed to 1-BP at 0, 50, and 250 ppm (Table 1). Body weight was measured and recorded every day.

After 4-week exposure, mice were dissected and brain was dissected on ice cold plates into different parts, including the cerebral cortex and hippocampus. Parts of the left lobes of livers were rinsed with saline and fixed in 4% PFA. Pathological analysis of livers was performed. To evaluate effects of 1-BP on the central nervous system,

western blot was performed to measure the expressions of Ran, γ-enolase, c-Fos, and GRP78 in the hippocampus or cortex of mice brain.

For statistical analysis, one-way ANOVA was used to compare differences between different groups. The Dunnett's test was used to compare differences between different exposure levels. A p value <0.05 denoted the presence of a statistically significant difference.

Results

CYP2E1 inhibitory effect of 1-ABT in the liver and brain. Administration of 1-ABT for 3 days resulted in 92-96% inhibition of hepatic microsomal CYP2E1 activity in male C57BL/6J mice but only inhibited 62-64% CYP2E1 activity in the brain (Table 2).

Maximum tolerated 1-BP concentration after pretreatment with 1-ABT. Preliminary exposure experiments determined 1200 ppm as as the maximum tolerated 1-BP concentration which is permissible for 4-week 1-BP exposure experiment for 1-ABT-pretreated mice. And 250 ppm was determined as the highest 1-BP exposure level for 1-ABT untreated mice, according to previous study.

Effects of 4-week exposure to 1-BP on body weight. No changes in body weight were noted during the entire 4-week inhalation exposure, irrespective of 1-BP concentration and use or no-use of 1-ABT. However, at day 28, mice pretreated with 1-ABT and exposed to 1200 ppm 1-BP, showed significant body weight loss (FIG. 1).

Effects of 4-week exposure to 1-BP on liver pathology. Exposure of 1-ABT-untreated mice to 250 ppm 1-BP induced severe liver pathological changes. Severe and large areas of necrosis, hemorrhage and hepatocyte degeneration were observed (FIG. 2). However, no such necrotic areas were found in the 1-ABT-pretreated mice, even in 1200 ppm 1-BP exposed mice (FIG. 3).

Effects of 4-week exposure to 1-BP on the central nervous system. The brain weight was significantly lower at the end of 4-week exposure in the 1-ABT (+) /1200 ppm group, compared with the control, while no such change was noted in the other exposure groups (Table 3). The result of western blot analysis showed that Ran protein expression in the hippocampus was increased in 1-ABT treated groups, and that such increase was 1-BP dose-dependent; but in 1-ABT untreated groups Ran protein expression was not changed (FIG. 4). On the other hand, in the cerebral cortex, no change in Ran protein expression was noted, irrespective of 1-BP exposure level. GRP78 expression was increased dose-dependently with significant change at 1200 ppm of 1-BP in mice pre-treated with 1-ABT, but was not changed in 1-ABT-untreated mice (FIG. 5). In contrast to Ran protein, no change was observed in GRP78 expression level in the hippocampus. The expression levels of γ-enolase and c-Fos proteins showed no changes, neither in the hippocampus, nor in the cortex.

Discussion

In the present study, inhibition of CYP2E1 reduced hepatotoxicity of 1-BP. It suggests that oxidation of 1-BP in the liver may play important roles in 1-BP-induced hepatotoxicity. In this murine model, brain CYP2E1 activity was preserved relative to that in the liver. Therefore, this mouse model might be a promising tool for studying effects of CNS P450s mediated metabolism to the neurotoxicity of 1-BP. Also, our present study implies rationality for developing a murine model with liver-specific inhibition of CYP2E1 using gene technology in the future.

Exposure to 1-BP for 4 weeks resulted in a significant decrease in brain weight in 1-ABT (+)/1200 ppm 1-BP group. The decrease in brain weight may be due to brain injury. Furthermore, hippocampal Ran protein expression level showed a dose-dependent increase in 1-ABT pretreated groups. Hippocampal Ran expression level was thought to be a promising biomarker to be associated with 1-BP neurotoxicity. Cerebral cortical GRP78 expression was increased in 1-BP dose-dependent manner in mice pre-treated with 1-ABT. The up-regulation of GRP78 in the cortex of mice exposed to 1-BP probably suggests damages related to ER stress caused by 1-BP exposure happened in the cortex.

We used 1-ABT to develop the murine model of 1-BP neurotoxicity, but did not use CYPIIE1-null mice. Firstly, 1-ABT can inhibit not only CYPIIE1 but also other isoforms of CYP non-specifically, enabling us to inhibit almost fully oxidative metabolism of 1-BP. Secondly, 1-ABT can be injected to any transgenic mice to study the role of target gene in neurotoxicity of 1-BP without development of double-transgenic mice, which may need longer time if the backgrounds of the strains are different.

Conclusion

The present study established a preliminary murine model of neurotoxicity of 1-BP, in which administration of general P450s inhibitor reduced the incidence and severity of 1-BP hepatotoxicity, thus allowing the survival of mice to ascertain neurological effect of 1-BP. Considering the availability of mice and convenient utilization of transgenic technology in mice, this murine model should be helpful in studying the cellular and molecular mechanisms of neurotoxicity of 1-BP and perhaps other chemicals.