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Induction of cytochrome P450 2E1 by 1-bromopropane in male ICR mice

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ICR 마우스 수컷에서 1-bromopropane에 의한 cytochrome P450 2E1의 유도

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ABSTRACT

1-bromopropane (1-BP) has been used in numerous purposes such as an intermediate in the synthesis of pharmaceuticals, a solvent for fats, waxes, or resins and a substitute for chlorofluorocarbons that destroy the ozone layer. However, the studies related to the modulation of activities of hepatic cytochrome P450s (CYPs) are not reported yet. This study was the first study to investigate the potential effect for the activities of hepatic CYPs by the treatment of 1-BP *in vivo*. When 1-BP was treated to male ICR mice by dose-dependently at the dose levels of 200, 500 and 1,000 mg/kg of body weight once, the activity of CYP2E1 was selectively increased for 24 h. The inductive potency for the activity of CYP2E1 by 1-BP was equal to induction by acetone a well-known selective CYP2E1 inducer. The present results indicated that 1-BP would affect the metabolism of 1-BP itself and/or other xenobiotics.

Key words : 1-bromopropane, CYP 2E1, inducer, drug-drug interaction

INTRODUCTION

Cytochrome P450s (CYP)-mediated interaction is one of the major concerns in clinical practice and toxicology research (Lin, 2006; Guengerich, 2008). There are two major issues associated with CYP-mediated interaction: the one is the physiological factors like species, gender, age, disease and genetic mutation and the other is the environmental factors such as dietary components, environmental ligands and xenobiotic inducers and inhibitors (Lin, 2006). The most potential factor would be the induction of metabolic enzymes by drugs, dietary components and/or exposure to certain chemicals.

1-bromopropane (1-BP) has been used in numerous purposes including as an intermediate in the synthesis of pharmaceuticals, insecticides, quaternary ammonium compounds, flavors, fragrances, and a solvent for

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fats, waxes, or resins (Boekelheide *et al.*, 2004). Recently, 1-BP is widely used as a substitute for chlorofluorocarbons that destroy the ozone layer because of their lower ozone-depleting potency, high volatility and non-flammability (Låg *et al.*, 1991; Lee *et al.*, 2005). Regarding the toxicity of some halopropanes, in July 1995, an outbreak of reproductive and hematopoietic disorders occurred in male and female workers exposed to the cleansing solvent containing 2-bromopropane (2-BP) and 1,2-dibromopropane (1,2-DBP) as 1-BP analogs in an electronic factory in Korea (Kim *et al.*, 1996; Park *et al.*, 1997).

Since then, many studies on the halopropanes-induced toxicity have been conducted. Meanwhile, although 1-BP might be easily exposed in our living environment, the studies related to the modulation of hepatic CYP activities by 1-BP have not been performed yet. Hence, the effects of 1-BP on the activities of some monooxygenases and a hydroxylase associated with major isozymes of CYPs were investigated for the first time *in vivo*.

MATERIALS AND METHODS

1. Materials

1-BP was obtained from Aldrich Chemical Co. (Milwaukee, USA). Bovine serum albumin, benzyloxyresorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, p-nitrophenol, erythromycin, glucose 6-phosphate, NADPH and glucose 6-phosphate dehydrogenase were purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were of analytical grade and used as received.

2. Animal treatment

Specific pathogen-free male ICR mice (28 to 33 g) were obtained from the Orient (Seoul, Korea). The animals received at 4 weeks of age were acclimated for at least 2 weeks. Upon arrival, animals were randomized and housed five per cage. The animal quarters were strictly maintained at 23±3°C and 50±10% relative humidity. A 12 h light and dark cycle was used with an intensity of 150~300 Lux. All animal procedures were followed based on a guideline recommended by the Society of Toxicology (USA) in 1989.

1-BP in corn oil was treated orally to animals at 200, 500 and 1,000 mg/kg once (Lee *et al.*, 2007). One day after the dose, the animals were euthanized. For time course studies, 1-BP at 1,000 mg/kg was treated orally. Animals were subjected to necropsy at 6, 12 or 24 hr after the treatment. To compare the inductive effect of 1-BP with specific CYP2E1 inducer, animals were pretreated with acetone (5 mL/kg, p.o., once) at 48 hr before the treatment of 1-BP (500 and 1,000 mg/kg). The livers were removed and homogenized with 4 volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4. Aliquots of liver homogenates were stored at −80°C until use.

3. Preparation of S-9 fraction

The S-9 fractions were prepared form the livers for determining CYP-associated monooxygenase activities. Livers were homogenized with four volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4, using a homogenizer, then centrifuged at 9,000×g for 20 min at 4°C. Aliquots of S-9 fractions were store at −80°C until use. The content of protein was determined using bovine serum albumin as a standard (Lowry *et al.*, 1951).

4. Monooxygenase assays

The ethoxyresorufin *O*-deethylase (EROD) activity was determined as described by Blank *et al.* (1987) with a slight modification using FLUOstar OPTIMA, a high-performance multidetection plate reader (BMG LABTECH GmbH, Offenburg, Germany). The reaction mixture (0.2 mL) consisted of 0.1 M potassium phosphate buffer, pH 7.4, containing 2 mg/mL of bovine serum albumin, 5 mM glucose 6-phosphate, 1 U of glucose 6-phosphate dehydrogenase, 5 μM NADPH and 2.5 μM 7-ethoxyresorufin. The formation of resorufin was monitored fluorometrically at an excitation maximum of 550 nm and an emission maximum of

Table 1. Effects of 1-bromopropane on CYP-associated monooxygenase activities in male ICR mice: dose-response studies

Dose (mg/kg)	Protein (mg/mL)	Monooxygenase activity				
		EROD	MROD	PROD	BROD	ERDM
VH	45.1±1.6	4.15±0.59	2.58±0.09	672±88	108±5	657±65
200	50.0±2.8	4.01±0.63	3.00±0.54	516±48	125±11	638±97
500	43.2±1.8	4.96±0.65	3.46±0.40	546±30	130±10	686±43
1000	43.6±1.7	4.86±0.73	3.95±0.73	551±69	158±18*	542±56

Animals were treated orally with 200, 500 and 1,000 mg/kg of 1-bromopropane in corn oil once. All animals were subjected to necropsy 24 hr after the treatment. Each value represents the mean±S.E. of five animals. The value significantly different from the vehicle control (VH) at $P<0.05$ was indicated as an asterisk. EROD, ethoxyresorufin *O*-deethylase, pmole resorufin/min per mg protein; MROD, methoxyresorufin *O*-demethlyase, pmole resorufin/min per mg protein; PROD, pentoxyresorufin *O*-depentylase, pmole resorufin/min per mg protein; BROD, benzyloxyresorufin *O*-debenzylase, pmole resorufin/min per mg protein; ERDM, erythromycin *N*-demethylase, pmole formaldehyde/min per mg protein.

Table 2. Effects of 1-bromopropane on CYP-associated monooxygenase activities in male ICR mice: time-course studies

Time (hr)	Protein (mg/mL)	Monooxygenase activities				
		EROD	MROD	PROD	BROD	ERDM
0	45.1±1.6	4.15±0.59	2.58±0.09	672±88	108±5	657±65
6	44.6±1.8	2.95±0.43	2.87±0.40	1101±139*	202±13*	537±39
12	38.6±1.8	1.92±0.31*	1.64±0.44	517±91	143±18	527±45
24	43.2±1.8	4.86±0.73	3.95±0.73	551±69	158±18*	542±56

Animals were treated orally with 1,000 mg/kg of 1-bromopropane in corn oil once. Animals were subjected to necropsy 0, 6, 12 and 24 hr after the treatment. Each value represents the mean±S.E. of five animals. The values significantly different from the 0-hr control at $P<0.05$ were indicated as an asterisk. EROD, ethoxyresorufin *O*-deethylase, pmole resorufin/min per mg protein; MROD, methoxyresorufin *O*-demethlyase, pmole resorufin/min per mg protein; PROD, pentoxyresorufin *O*-depentylase, pmole resorufin/min per mg protein; BROD, benzyloxyresorufin *O*-debenzylase, pmole resorufin/min per mg protein; ERDM, erythromycin *N*-demethylase, pmole formaldehyde/min per mg protein.

585 nm. Methoxyresorufin *O*-demethylase (MROD), pentoxyresorufin *O*-depentylase (PROD) and benzyloxyresorufin *O*-debenzylase (BROD) activities were determined by a literature (Lubet *et al.*, 1985) with a slight modification as mentioned above. All reaction components and assay procedures were exactly the same as the EROD assay, except that the substrates were 2.0 μM. The *p*-nitrophenol hydroxylase (PNPH) activity was determined as described by Koop (Koop, 1986). The reaction mixture (1.0 mL) was composed of 0.1 M potassium phosphate buffer, pH 7.4, containing 100 μM *p*-nitrophenol, 1 mM NADPH and an enzyme source. The amount of 4-nitrocatechol formed was measured spectrophotometrically at 512 nm. Erythromycin *N*-demethylase (ERDM) activity was determined by measuring the amount of formaldehyde formed, as described previously (Nash, 1953). Erythromycin at 400 μM was used as a substrate for assaying ERDM.

5. Statistics

The results were expressed as the mean±S.E. and the statistical differences between the different dose groups and the vehicle control were determined by one-way analysis of variance followed by the Dunnett's t-test (SPSS program, ver 10.0). The significant values at either $P<0.05$ (*) or $P<0.01$ (**) were represented as asterisks.

RESULTS

1. Acute effects of 1-BP on hepatic CYP activities

In Table 1, the activities of EROD, MROD, BROD, BROD and ERDM were determined in livers of male ICR mice treated with the oral 1-BP at the dose levels of 200, 500 and 1,000 mg/kg of body weight once.

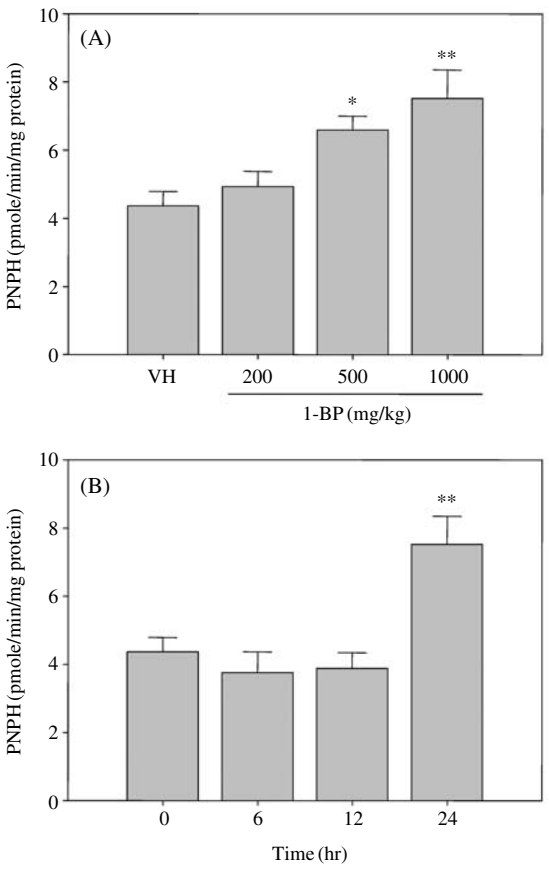

Fig. 1. Effects of 1-bromopropane on the hepatic activities of *p*-nitrophenol hydroxylase (PNPH) in male ICR mice. (A) A dose-response study. (B) A time-course study. The experimental conditions were the same as Tables 1 and 2. Each value represents the mean ± S.E. of five animals. The bars significantly different from either the vehicle control (VH) or the 0-hr control at either $P < 0.05$ (*) or $P < 0.01$ (**) were indicated as asterisks.

The amounts of protein in liver were not significantly changed by 1-BP (Table 1). The measured CYP activities except BROD were not changed by the treatment of 1-BP. The hepatic BROD activity was significantly increased at 1,000 mg/kg of 1-BP (Table 1). Meanwhile, the activity of PNPH was significantly increased dose-dependently from 500 mg/kg of 1-BP (Fig. 1A).

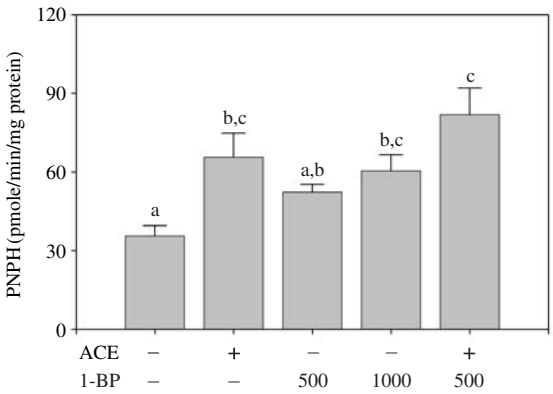

Fig. 2. Effects of 1-bromopropane and acetone on the hepatic activities of *p*-nitrophenol hydroxylase (PNPH) in male ICR mice. Animals were treated orally with 1-bromopropane in corn oil once for 24 hr or acetone at 5 mL/kg once for 72 hr. For combination studies, animals were pretreated with acetone at 48 hr before the treatment of 1-BP. All animals were subjected to necropsy 24 hr after the last treatment. Each value represents the mean ± S.E. of five animals. The values significantly different from the each other at $P < 0.05$ were indicated as different alphabets.

2. Time course effects of 1-BP on hepatic CYP activities

When mice were treated orally with 1,000 mg/kg of 1-BP once for 6, 12 and 24 hr, the amount of protein in liver and the activities of MROD and ERDM were not significantly changed (Table 2). PROD and BROD activities were temporarily induced by 1-BP 6 hr after the treatment. The activities of PNPH were significantly increased to 172% when compared with control groups (Fig. 1B).

3. Inductive effects of 1-BP on hepatic CYP2E1 activity

Following the independent treatment with acetone as selective CYP2E1 inducer and either 500 or 1,000 mg/kg of 1-BP once, the activities of CYP2E1 by either acetone (5 mL/kg) or 1-BP (1,000 mg/kg) were significantly increased by 184% and 169%, respectively, when compared with the control group (Fig. 2). Following the co-treatment with acetone and 500 mg/kg

of 1-BP, CYP2E1 activity was also significantly increased to 230% of control group (Fig. 2).

DISCUSSION

CYPs are the major enzymes involved in drug metabolism accounting for ~75% oxidation of a wide variety of substrates such as therapeutic agents, environmental toxicants, mutagens, and carcinogens (Ghanayem and Hoffler, 2007; Guengerich, 2008). In particular, CYP2E1 has a role in the bioactivation of a variety of small hydrophobic chemicals including urethane, acrylamide, acrylonitrile, benzene, vinyl chloride, styrene, and 1-BP (Raucy *et al.*, 1993; Garner *et al.*, 2007).

Moreover, CYP2E1 is effectively induced in the liver by a diverse set of chemicals having various structures such as ethanol, acetone, pyridine and isoniazid mediated through enzyme stabilization and increased rate of gene transcription (Hétu and Joly, 1988; Ueng and Ueng, 1991; Ueng *et al.*, 1991; Ingelman-Sundberg *et al.*, 1993; Chen and Ueng, 1997). The induction of CYP2E1 can not only increase the toxicity of certain xenobiotics (Kenyon *et al.*, 1998; Guengerich FP, 2008; Pardini *et al.*, 2008), but also cause adverse drug-drug interactions (Lin, 2006; Gómez-Moreno *et al.*, 2008).

Although 1-BP is widely used as a substitute for chlorofluorocarbons, industrial applications for cleaning metals, optical instruments and electronics and as a component in cleaning agents or adhesive solvent in workplaces, the modulatory effects of 1-BP on hepatic CYP activities have not been studied (Boekelheide *et al.*, 2004; Lee *et al.*, 2007). In this study, the activity of CYP2E1 was selectively increased by 1-BP (Fig. 1). The induction potential by 1-BP was quite comparable to the induction by acetone, a well-known selective CYP2E1 inducer (Fig. 2). Meanwhile, CYP2E1 activity in animals co-treated with both acetone and 1,000 mg/kg of 1-BP was not induced, probably because the induced CYP2E1 by acetone might increase the hepatotoxicity of 1-BP (Data not shown). In other words, the induction of CYP2E1 by acetone would produce more hepatotoxic metabolite(s) from 1-BP, consequently the CYP2E1 expression would be reduced in damaged hepatocytes. In our unpublished data, the hydroxylation of 1-BP by CYP2E1 would be the main cause of 1-BP-induced depletion of glutathione, followed by oxidative stress to hepatotoxicity (data not shown).

A temporal increase in PROD and BROD activities by 1-BP would be another interesting finding in the present study (Table 2). A further study must be followed to characterize whether the result was from induction of CYP2B proteins or stimulation of pre-existing enzyme activities by 1-BP.

Based on these results, it was revealed that 1-BP might be a specific inducer for CYP2E1 in male ICR mice. Therefore, it is possible that the activity of hepatic CYP2E1 would be increased in workers chronically exposed to 1-BP. The induction of CYP activities by occupational or habitual exposure to xenobiotics would not be a rare case. For examples, the induction of CYP1A2 in smoking person was involved with pharmacokinetic interactions with drugs that are CYP1A2 substrates, such as caffeine, olanzapine, tacrine, and theophylline (Kroon, 2007). Daily consumption of at least three cups of coffee significantly increased CYP1A2 enzyme activity in both Serbs and Swedes (Djordjevic *et al.*, 2008). Likewise, the people exposed to 1-BP might possess increased hepatic CYP2E1 activity and the increased activity would cause the pharmacokinetic interaction and/or toxicant bioactivation.

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