Hepatotoxic and Immunotoxic Effects produced by 1,3-Dibromopropane and Its Conjugation with Glutathione in Female BALB/c Mice

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To determine a possible role of glutathione (GSH) conjugation in 1,3-dibromopropane (1,3-DBP)-induced hepatotoxicity and immunotoxicity, female BALB/c mice were treated orally with 1,3-DBP. Based on the liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS) analyses, two forms of S-bromomethylpropyl GSH were observed at m/z 427.9 and 429.9 in the positive ESI spectrum with a retention time of 5.29 and 5.23 min, respectively. Following single treatment of mice with 150, 300 or 600 mg/kg 1,3-DBP for 12 hr, the amount of S-bromomethylpropyl GSH was detected maximally in liver homogenates at 600 mg/kg 1,3-DBP. Hepatic GSH levels were significantly decreased by treatment with 1,3-DBP. In a time course study, production of S-bromomethylpropyl GSH rose maximally 6 hr after treatment and decreased gradually thereafter. The liver weights were significantly increased by treatment with 600 mg/kg 1,3-DBP. When mice were treated orally with 600 mg/kg 1,3-DBP for 12 hr, the activities of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were increased by 365- and 83-fold, respectively. In addition, oral 1,3-DBP significantly suppressed the antibody response to a T-dependent antigen at 600 mg/kg 1,3-DBP. 1,3-DBP elevated hepatic levels of malondialdehyde and suppressed the activities of some hepatic enzymes involved in anti-oxidation. Taken together, the formation of GSH conjugate with 1,3-DBP may deplete cellular GSH and, subsequently, produce hepatotoxicity and immunotoxicity via damage to the cellular anti-oxidative system.

INTRODUCTION

1,3-Dibromopropane (1,3-DBP), a clear, colorless to yellow liquid, has been used in industry in chemical syntheses and employed for stabilization of wool by cross-linking to render the compound less susceptible to attack by moths (James et al., 1981). Many halide compounds, such as 1-bromopropane (1-BP), 1,2-dibromopropane (1,2-DBP), 2,3-dibromopropano (2,3-DBP) and 1,2-dibromo-3-chloropropane (1,2-DBCP) are metabolized by conjugation with glutathione (GSH) (Barnsley, 1966; Burinon et al., 1982; Zoetemeil et al., 1986; Tomorovelez et al., 2004; Lee et al., 2005a).

It has been implicated that toxicities of some halocarbons capable of forming GSH conjugates are related directly or indirectly with GSH conjugation. For example, the conjugation of 2,3-DBCP and 1,2-dibromomethane with GSH formed DNA adducts thus increasing toxicity and genotoxicity (Kim and Guengerich, 1990; Kim et al., 1990; Humphreys et al., 1991). Mice treated orally with 1-BP showed enhanced hepatotoxicity and immunotoxicity following depletion of GSH in liver and spleen (Lee et al., 2005b). In addition, the hepatotoxicity and immunotoxicity produced by 2-BP and 1,2-DBP were also increased by the formation of conjugates with GSH (Kim et al., 2003; Lee et al., 2005a).

1,3-DBP has also been investigated with respect to oxidative biotransformation and conjugation with GSH (James et al., 1981; Jones and Wells, 1981; Onkenhout et al., 1986; Tomorovelez et al., 2004). Two conjugated metabolites were isolated from urine and identified as S-(3-hydroxypropyl)cysteine and N-acetyl-S-(3-hydroxypropyl)cysteine (Jones and Wells, 1981). When rats were orally administered with 1,3-DBP, the level of hepatic GSH was markedly decreased and several metabolites were excreted in urine (James et al., 1981). A major metabolite was N-acetyl-S-(1-bromo-3-propyl)-cysteine. Moreover, 3 metabolites such as N-acetyl-S-3-chloropropyl-cysteine, N-acetyl-S-2-carboxyethyl cysteine and N-acetyl-S-3-hydroxypropyl-cysteine were identified in rats treated with 1,3-DBP (Onkenhout et al., 1986).

Recently, studies were conducted with the oral administration of 1,3-DBP to newborn rats from postnatal days 4 to 21 to determine the no observed adverse effect levels and unequivocally toxic level (Hirata-Kozumi et al., 2005). In this study,

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hypertrophy of hepatocytes with alterations in biochemical parameters was observed. Serum biochemical parameters, including total protein, albumin, total cholesterol, triglycerides, phospholipids and total bilirubin, were increased with an upward trend for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. However, a possible role of GSH conjugation was not determined in their study, although formation of GSH conjugates with 1,3-DBP might be a critical factor in 1,3-DBP-induced toxicity.

In the present study, formation of GSH conjugates was investigated following treatment of mice with 1,3-DBP by using liquid chromatography/electrospray ionization-tandem mass spectrometry (LC/ESI-MS). In addition, dose response and time course effects of 1,3-DBP on hepatotoxicity and immunotoxicity parameters were determined to characterize whether or not GSH conjugation is an important factor in 1,3-DBP-induced toxicity.

MATERIALS AND METHODS

Materials

1,3-DBP (purity, > 99%) was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA). 5,5'-Dithio-bis(2-nitrobenzoic acid) and 5-sulfosalicylic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). The kits for ALT and AST assays were purchased from Asan Pharm. Co. (Hwasung, Korea). Acetonitrile (ACN) was HPLC-grade from Merck Ltd. (Poole, UK). All other chemicals were of analytical grade and used as received.

Animal Treatment

Specific pathogen-free female BALB/c mice were obtained from Orient (Seoul, Korea). The animals received at 4 weeks of age were acclimatized for at least 2 weeks. Upon arrival, animals were randomized and housed 5 per cage. The animals 19 to 21 g of body weights were used in this study. The animal quarters were strictly maintained at 23 ± 3°C and 50 ± 10% relative humidity. A 12 hr 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 (Reston, VA, USA) in 1989.

1,3-DBP in corn oil was given once orally to animals at 150, 300 or 600 mg/kg. The dose was selected according to literature and a dose range finding study. Twelve hr after the dose, the animals were euthanized with ether. For time course studies, 1,3-DBP at 600 mg/kg was given orally. Animals were subjected to necropsy at 0, 6, 12, 24 or 48 hr after oral administration. For antibody response study, 1,3-DBP was given orally with a single dose of 150, 300 or 600 mg/kg in corn oil, followed by a sensitization with 5x10^6 SRBCs per mouse in 0.5 ml of Earle’s balanced salt solution (EBSS) ip 30 min after the treatment with 1,3-DBP. Four days later, the numbers of antibody-forming cells (AFCs) were enumerated. Following the blood collection, the livers were removed and homogenized with 4 volumes of ice-cold 0.1 M potassium phosphate buffer, pH 7.4. Aliquots of tissue homogenates and sera were stored at −80°C until use. In all studies, corn oil at 10 ml/kg was used for the vehicle control.

Sample Analysis by LC/ESI-MS

The conjugates in liver homogenates were extracted by an addition of 4 volumes of ACN. After a vortex mixing for 20 min and a centrifugation at 15°C to remove the proteins, the resulting supernatant was evaporated under a stream of nitrogen in a water bath maintained at 60°C. The resulting residue was reconstituted in a mixture of 50/50 of 0.1% aqueous formic acid/ACN by vortexing and a subsequent centrifugation at 15,000g for 10 min at 15°C. Then, a 100 µl aliquot was used for the LC/ESI-MS analysis. The HPLC consisted of a surveyor system (Thermo Finnigan, San Jose, CA, USA) with the LCQ advantage trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) equipped with an electrospray ionization source. The column used for the separation was an Xterra C18 (2.1 x 50 mm, 5 µm). The HPLC mobile phases consisted of 0.1% aqueous formic acid, pH 4 (A), and 50% ACN in 0.1% formic acid (B). A gradient program was used for the HPLC separation with a flow rate of 230 µl/min. The initial composition was 10% B and programmed linearly to 100% B after 6 min. Nitrogen was used both as the sheath gas at 70 l/min and as the auxiliary gas at 6.6 l/min with a capillary temperature of 215°C and the spray voltage set to 4 kV. The mass spectrometer was operated in the positive ion mode in m/z range 100 – 450. Helium was used as the collision gas for the tandem mass spectrometric experiments, followed by the isolation of ions over a selected mass window of 1 Da.

Parameters for oxidative stress

Liver GSH levels were determined by the Ellman’s method (1959). The activities of catalase (CAT), superoxide dismutase (SOD), GSH-peroxidase (GSHPx), oxidized glutathione reductase (GSSGR), and content of malondialdehyde (MDA) were determined according to the methods of Horecker and Kornberg (1948), Aebi (1969), Marklund and Marklund (1974), Carlberg and Mannervik (1975) and Ohkawa et al. (1979), respectively. The content of liver homogenate protein was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Hepatotoxicity Parameters

For assaying the activities of ALT and AST, the serum was prepared by a centrifugation of the blood at 3,000xg for 15 min at 4°C. The activities of the enzymes were determined according to an instruction manual prepared by the manufacturer.