Effects of acute di-n-butyl phthalate administration on oxidative stress parameters

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Abstract

Di-n-butyl phthalate (DBP) is used extensively in the plastic industry and has been known as an environmental hormone (endocrine disruptor). Present study was undertaken to examine whether DBP can induce oxidative stress in mice. In this study, oxidative stress was measured in terms of the modification of lipid peroxidation and gamma-glutamyltranspeptidase (γ-GT) activity. The activity of γ-GT, the level of lipid peroxidation and serum toxicity index were measured in male ICR mice after treatment with DBP (5 g/kg, po). Administration of DBP was found to significantly increase the level of lipid peroxidation approximately 2 fold in liver. The activity of γ-GT in the liver of DBP-exposed animals was also increased approximately 2.5 fold. However, DBP did not alter the parameters for hepatotoxicity and nephrotoxicity such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine. These results indicate that DBP can induce oxidative stress in mice. The γ-GT activity is considered to be increased as one of the adaptive defense mechanisms to oxidative stress induced by DBP.

Introduction

Dialkyl phthalate esters are used as plasticizers in polyvinyl chloride (PVC) to give the plastic characteristics such as flexibility, softness, and workability. The phthalate esters have possible effects on the endocrine system. Di-n-butyl phthalate (DBP) is one of the most commonly used phthalic acid esters. It is extensively used as a plasticizer in elastomers, as a solvent for printing inks and resins, and as a textile lubricating agent. The major use of DBP is a coalescing aid in latex adhesive. It is also present in the formulations of various cosmetic products. DBP is produced in massive amounts worldwide. 49,000 tons of DBP was produced in Western Europe in 1994 and 11,400 tons in United States in 1987. Up to 50 % of the plastic may consist of phthalate esters, and since the compounds are dissolved in the plastic, they may contaminate the environment and foodstuffs. DBP has been known as an environmental hormone (endocrine disruptor). Several studies using mice and rats demonstrated that DBP is

embryolethal and capable of producing various reproductive defects.

A key enzyme in the metabolism of glutathione (GSH), its mercaptides and S-conjugates is gamma-glutamyltranspeptidase (γ-GT). γ-GT is a glycoprotein attached to the external surface of various cell types and whose physiological function in the GSH metabolism is to recover cysteine from extracellular GSH and GSH derivatives. Thus cells expressing γ-GT use external GSH as a permanent supply of cysteine to maintain their thiol redox and detoxification capacity involving GSH conjugates and peroxides. Induction of this enzyme in the response to an oxidative stress is an essential element of the cell response to oxidative injury. The control of GSH level by the expression of γ-GT and other GSH related enzymes is probably one of the key switches in the antioxidant response

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Methods and Materials

Adult male ICR mice, weighing 30-40 g, were used throughout the study. They were housed in environmentally controlled rooms (temperature 22 ± 2 □, humidity 55 ± 5 %) under a 12 hr dark:light cycle. Animals were provided with regular lab feed and tap water ad libitum until experimentation. 24 hr following the DBP (5 g/kg, po) treatment blood samples were collected by cardiac puncture on mice under light ether anaesthesia. The blood samples were allowed to clot at room temperature followed by centrifugation to separate the serum. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were determined by using the method of Reitman and Frankel. Serum creatinine level was measured using Sigma diagnostic Kits 555-A. γ-GT activity was measured using 6 mM L-gamma-glutamy-p-nitroanilide as a donner substrate in the presence of 150 mM glycylglycine as an accceptor in 0.1 M Tris-HCl buffer, pH 8.6. The content of lipid peroxides was determined by the classical method of measuring thiobarbituric acid-reactive substance (TBARS).

Results and Discussion

A single dose of DBP alone did not alter the activities of serum ALT, AST or creatinine measured 24 hr following the treatment (Table 1), indicating that this treatment is not hepatotoxic and nephrotoxic at the dose used in this study.

Table 1. Effects of di-n-butyl phthalate (DBP) on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine level in mice. Values are the mean ± standard deviation of three individual determinations.

	Control	DBP
ALT (units/ml)	46 ± 9	52 ± 9
AST (units/ml)	105 ± 9	115 ± 12
Creatinine (mg/dl)	1.12 ± 0.05	1.09 ± 0.06

The hepatic level of malondialdehyde (MDA) was significantly increased in mice following the DBP treatment (Fig. 1). This result indicates that DBP can induce oxidative stress in mice.

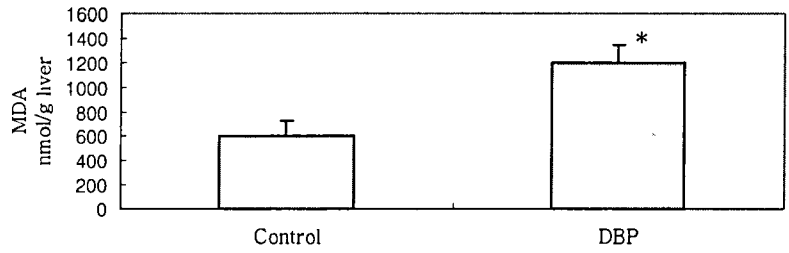

Fig. 1. Effect of di-n-butyl phthalate (DBP) on hepatic malondialdehyde (MDA) values. Values are the mean ± standard deviation of three individual determinations. * indicates a significant difference from control (Student's *t*-test, $p < 0.05$).

To evaluate whether the increased oxidative stress modifies GSH-related antioxidant enzyme, the modification of γ-GT activity was investigated. The γ-GT activity was increased about 2.5-fold 24 hr after the treatment of DBP (Fig. 2).

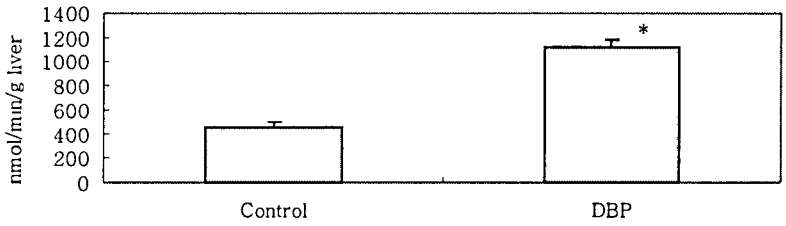

Fig. 2. Effect of di-n-butyl phthalate (DBP) on hepatic gamma-glutamyl transpeptidase (γ-GT) activity. Values are the mean ± standard deviation of three individual determinations. * indicates a significant difference from control (Student's *t*-test, $p < 0.05$).

Adaptation to oxidant stimuli is critical for short- and long-term survival of cells exposed to oxidative stress. Although much less documented than glutathione-S-transferase, γ-GT activity has been shown to be increased as an adaptive mechanism against different kinds of insults leading to oxidative stress such as quinones and radiation. Increased γ-GT activity provided increased resistance against oxidative insults in several cell lines. Hepatocytes with elevated levels of γ-GT have a selective advantage over γ-GT-negative hepatocytes when the animal is treated with carcinogens or promoting agents that deplete intracellular GSH. Because γ-GT generally favors the reconstitution of intracellular GSH, the increased activity of γ-GT in DBP-treated mice could be considered as a member of the antioxidant defense against DBP-induced oxidative stress.

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