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Thyrotoxic rubber antioxidants, 2-mercaptobenzimidazole and its methyl derivatives, cause both inhibition and induction of drug-metabolizing activity in rat liver microsomes after repeated oral administration

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ABSTRACT

We examined the effects of thyrotoxic rubber antioxidants, 2-mercaptobenzimidazole (MBI, 0.3 mmol/kg/day) and its methyl derivatives, methyl-MBIs [4-methyl-MBI (4-MeMBI, 0.6 mmol/kg/day), 5-methyl-MBI (5-MeMBI, 0.6 mmol/kg/day), and 4(or 5)-methyl-MBI (4(5)-MeMBI, 0.6 or 1.2 mmol/kg/day)], on the drug-metabolizing activity in male rat liver microsomes by 8-day repeated oral administration. The weight of liver and thyroid were increased by all the test chemicals; MBI was most potent, and there was no additive or synergistic effect between 4-MeMBI and 5-MeMBI. MBI decreased the cytochrome P450 (CYP) content, NADPH-cytochrome P450 reductase (POR) activity, 7-ethoxycoumarin *O*-deethylation (ECOD) activity, and flavin-containing monooxygenase (FMO) activity, but increased the 7-pentoxyresorufin *O*-dependentylation (PROD) activity, suggesting inhibition of the drug-metabolizing activity on the whole but induce some activities such as the CYP2B activity. On the contrary, all the methyl-MBIs increased the CYP content, CYB5 content, ECOD activity, 7-ethoxyresorufin *O*-deethylation (EROD) activity, and PROD activity, indicating that they are mostly inducible of the CYP activity. However, the methyl-MBIs decreased the FMO activity, and 5-MeMBI and 4(5)-MeMBI appeared inhibitory for CYPs 2C11 and 2C13. Between 4-MeMBI and 5-MeMBI, there was no additive or synergistic effect on the drug-metabolizing activity, but was counteraction. It was concluded that MBI and methyl-MBIs had both inhibitory and inducible effects on the drug-metabolizing activity in rat liver microsomes at thyrotoxic doses. The effects of 4(5)-MeMBI indicated that the increased liver weight alone can be a hepatotoxic sign but not an adaptive no-adverse response in toxicity studies. The present results were related to the toxicokinetic profiles of MBI and 4(5)-MeMBI in the repeated toxicity studies.

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1. Introduction

2-Mercaptobenzimidazole (MBI) has been widely used as industrial materials, such as rubber antioxidant, corrosion inhibitor, and copper-plating brightener [1]. Consequently, unintentional human exposures to MBI are occurring through rubber materials and environmental water wastes [2,3]. In addition, a methyl derivative of MBI, 4(or 5)-methyl-2-mercaptobenzimidazole (4(5)-MeMBI), has been supplied for the same industrial use as a 1:1 mixture of 4-methyl-2-mercaptobenzimidazole (4-MeMBI) and 5-methyl-2-mercaptobenzimidazole (5-MeMBI) [4].

Abbreviations: MBI, 2-mercaptobenzimidazole; 4-MeMBI, 4-methyl-2-mercaptobenzimidazole; 5-MeMBI, 5-methyl-2-mercaptobenzimidazole; 4(5)-MeMBI, 4(or 5)-methyl-2-mercaptobenzimidazole; CYP, cytochrome P450; CYB5, cytochrome *b*5; POR, NADPH-cytochrome P450 reductase; ECOD, 7-ethoxycoumarin *O*-deethylation; EROD, 7-ethoxyresorufin *O*-deethylation; PROD, 7-pentoxyresorufin *O*-dependentylation; FMO, flavin-containing monooxygenase.

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MBI has strong thyrotoxicity due to the thioureylene structure as shown in repeated oral administration toxicity studies. In the 28-day repeated oral administration study, MBI caused marked enlargement of thyroid with diffuse follicular hyperplasia histologically characterized by tall columnar epithelial cells and decreased colloid, as a result from decreased thyroid hormones and increased thyroid-stimulating hormone [5]. The methyl-MBIs (4-MeMBI, 5-MeMBI, and 4(5)-MeMBI) also caused thyrotoxicity but to a lesser degree with smaller or no changes in thyroid hormones [6,7].

It has been shown that the differences in the thyrotoxicity between MBI and the methyl-MBIs are largely due to their toxicokinetic profiles. The area under the curve (0-24 h or 0-10 h) was about 10-fold larger for MBI than for the methyl-MBIs after the single or repeated oral administration [6,8]. As for inhibitory effects on thyroid hormone biosynthesis, MBI was stronger at most 2.2-fold than the methyl-MBIs when their 50% inhibitory concentrations for lactoperoxidase, a substitute of thyroid peroxidase, were compared [6].

Besides their effects on the thyroid, MBI and the methyl-MBIs increased liver weight in different ways. In the 28-day repeated oral administration study, MBI increased liver weight with changes in blood biochemistry, such as ALP and AST, indicating liver injury [5]. On the other hand, 4(5)-MeMBI increased liver weight and caused hepatocyte swelling without changes in blood biochemistry, suggesting an adaptive no-adverse response inducible of detoxification enzymes [7]. On the bases of these findings, in the present study we examined and compared the effects of MBI and the methyl-MBIs on the drug-metabolizing activity in rat liver microsomes.

2. Materials and methods

2.1. Test chemicals

The structures of the test chemicals are shown in Fig. 1. MBI (CAS No. 583-39-1) was purchased from Wako Pure Chemicals Industries (Osaka, Japan). 4(5)-MeMBI (CAS No. 53988-10-6) was supplied from Ohuchi Shinko Chemical Ind., Ltd. (Tokyo, Japan). 5-MeMBI (CAS No. 27231-36-3) was purchased from Aldrich Japan Inc. (Tokyo, Japan). 4-MeMBI (CAS No. 27231-33-0) was isolated from 4(5)-MeMBI by repeated fractional recrystallization [4].

2.2. Experimental animals

Male Wistar rats (4-weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and acclimatized for one-week prior to use. The basal pellet diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were given ad libitum. The animal room was maintained at a temperature of 24 ± 1 °C and 55 ± 5% humidity with a 12-hr light/dark cycle. All animal experiments were carried out according to the guidelines for animal use of the National Institute of Health Sciences.

2.3. Administration and autopsy

MBI (0.3 mmol/kg/day), 4-MeMBI (0.6 mmol/kg/day), 5-MeMBI (0.6 mmol/kg/day), and 4(5)-MeMBI (0.6 and 1.2 mmol/kg/day) were dissolved in corn oil to make the dose volume at 0.5 ml/kg/day, and were given to 5 rats per dose group by gavage for consecutive 8 days. The dose and administration period were selected based on the previous studies where MBI (0.3 mmol/kg/day) increased the liver and thyroid weight in rats [6]. One day after the final administration, the animals were sacrificed by decapitation, and were autopsied. For each of the animals, the liver and thyroid were removed and weighed.

2.4. Preparation of liver microsomes

Microsomal pellets were obtained by sequential centrifugations (10,000 × g for 20 min at 4 °C, and 105,000 × g for 60 min at 4 °C) of the supernatants of rat liver homogenate, and were used after resuspension in 1.15% (w/v) KCl [9].

2.5. Determination of content and activity of drug-metabolizing enzyme system

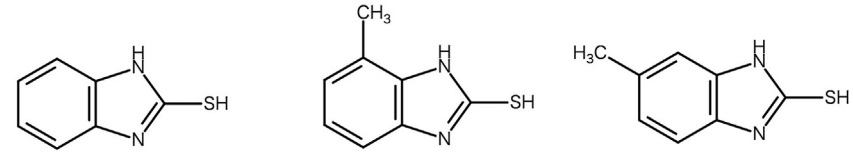
The contents of cytochrome P450 (CYP), and cytochrome *b*5 (CYB5) [10], and the activity of NADPH-cytochrome P450 reductase (POR) [11], 7-ethoxycoumarin *O*-deethylation (ECOD) [12], 7-ethoxyresorufin *O*-deethylation (EROD), 7-pentoxyresorufin *O*-depentylation (PROD) [13], and flavin-containing monooxygenase (FMO) [14] were determined as reported previously.

2.6. Western blot analysis

Microsomal CYPs and FMO3 were semi-quantified by the western blot analysis of the liver microsomes pooled at an equal proportion from all the animals in each dose group. The samples were separated by SDS-polyacrylamide gel electrophoresis with 12.5%T gels and were blotted on nitrocellulose membranes [15]. Goat antibodies specific for anti-rat CYP1A1/2, 2B1/2, 2C6, 2C11, 2C13, 2E1, 3A2, 4A1, and FMO3 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) and a HRP-conjugated rabbit anti-goat IgG (Medical &Biological Laboratories Co., Ltd., Nagoya, Japan) were used. The protein bands were visualized with 3, 3′-diaminobenzidine (Sigma, St. Louis, MO, USA), and were quantified with the Quantity One 1-D Analysis Software (Bio-Rad, Hercules, CA, USA) after scanning with the GS-800 image densitometer (Bio-Rad).

2.7. Statistical analysis

Statistical significance of the differences between the experimental groups was examined by the one-way analysis of variance



2-Mercaptobenzimidazole (MBI) 4-Methyl-2-mercaptobenzimidazole (4-MeMBI) 5-Methyl-2-mercaptobenzimidazole (5-MeMBI)

Fig. 1. Structures of 2-mercaptobenzimidazole (MBI), 4-methyl-MBI (4-MeMBI), and 5-methyl-MBI (5-MeMBI). 4(or 5)-Methyl-MBI (4(5)-MeMBI) is not shown because it is a 1:1 mixture of 4-MeMBI and 5-MeMBI.

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