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# Differential Expression of Xenobiotic-Metabolizing Enzymes by Benzylisothiazole in Association with Hepatotoxicity: Effects on Rat Hepatic Epoxide Hydrolase, Glutathione S-Transferases and Cytochrome P450s

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***ABSTRACT*** *: Previous studies have shown that the heterocycles including thiazoles are efficacious in inducing phase II metabolizing enzymes as well as certain cytochrome P450s and that the induction of these metabolizing enzymes by the heterocyclic agents is highly associated with their hepatotoxicity. In the present study, the effects of benzylisothiazole (BIT), which has a isothiazole moiety, on the expression of microsomal epoxide hydrolase (mEH), major glutathione S-transferases and cytochrome P450s were studied in the rat liver in association with its hepatotoxicity. Treatment of rats with BIT (1.17 mmol/kg, 1~3 d) resulted in substantial increases in the mEH, rGSTA2, rGSTM1 and rGSTM2 mRNA levels, whereas rGSTA3 and rGSTA5 mRNA levels were increased to much lesser extents. A time-course study showed that the mRNA levels of mEH and rGSTs were greater at 24 hr after treatment than those after 3 days of consecutive treatment. Relative changes in mEH and rGST mRNA levels were consistent with those in the proteins, as assessed by Western immunoblot analysis. Hepatic cytochrome P450 levels were monitored after BIT treatment under the assumption that metabolic activation of BIT may affect expression of the enzymes in conjunction with hepatotoxicity. Immunoblot analysis revealed that cytochrome P450 2B1/2 were 3- to 4-fold induced in rats treated with BIT (1.17 mmol/kg/day, 3 days), whereas P450 1A2, 2C11 and 3A1/2 levels were decreased to 20~30% of those in untreated rats. P450 2E1 was only slightly decreased by BIT. Thus, the levels of several cytochrome P450s were suppressed by BIT treatment. Rats treated with BIT at the dose of 1.17 mmol/kg for 3 days exhibited extensive multifocal nodular necrosis with moderate to extensive diffuse liver cell degeneration. No notable toxicity was observed in the kidney. These results showed that BIT induces mEH and rGSTs in the liver with increases in the mRNA levels, whereas the agent significantly decreased major cytochrome P450s. The changes in the detoxifying enzymes might be associated with the necrotic liver toxicity after consecutive treatment.*

***Key Words*** *: Glutathione S-transferase, Microsomal epoxide hydrolase, Cytochrome P450, Benzylisothiazole*

## I. INTRODUCTION

Previous studies have shown that thiazoles are very effective in inducing detoxifying enzymes including microsomal epoxide hydrolase (mEH) and major glutathione S-transferase (GST) enzymes in the liver. Mechanistic molecular studies in this laboratory revealed that thiazoles are prone to be under ring cleavage probably through cytochrome P450-mediated oxidation and certain methylthiazoles serve as the inducers for phase II detoxification enzymes (Kim and Cho, 1996). The production of prooxidants from thiazoles appeared to be responsible for generation of oxidative stress *in vivo*, which would be attributable to induction of mEH and GST (Kim and Cho, 1996). Carbon atoms adjacent to certain specific heterocyclic sites seemed to be easily oxidized to products that result in ring cleavage when the agents undergo attack either at and/or adjacent to the heteroatoms (Wilson, 1989; Mizutani *et al.*, 1994).

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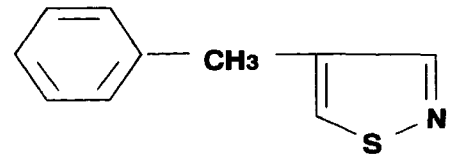
ABBREVIATIONS: ARE, antioxidant responsive element; BIT, benzylisothiazole; P450 1A1/2, cytochrome P450 1A1/2; P450 2B1/2, cytochrome P450 2B1/2; P450 2C11, cytochrome P450 2C11; P450 2E1, cytochrome P450 2E1; P450 3A1/2, cytochrome P450 3A1/2; EDTA, ethylenediamine tetraacetic acid; GST, glutathione S-transferase; mEH, microsomal epoxide hydrolase; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; SSC, standard saline citrate;

The ring cleavage site of heterocycles may involve the presence of certain chemical moieties including -NCS- or free -SH groups present in heterocycles. Ring cleavage products may also be associated with xenobiotic-inducible hepatotoxicity and nephrotoxicity in animals. It is highly likely that ring cleavage products from thiazole and thiazole derivatives produce the reactive oxygen species including drug free radicals and hydroxyl free radicals. Studies in this laboratory have shown that reactive oxygen species from heterocycles are ultimately responsible for the transcriptional activation of mEH and certain GSTs. Changes in the cellular redox state greatly affect the expression of detoxifying enzymes. Expression of major GST subunits were increased to similar extents in response to the organic inducers with N or S atoms, as was observed in the mEH mRNA level.

Certain isothiazole derivatives have antiproliferative, antiviral and antimicrobial activities (Swayze *et al.*, 1997; Cocco *et al.*, 1994). Some of the agents have cytotoxic effects as well (Swayze *et al.*, 1997). Given the structural similarity of thiazole with isothiazole, we were interested in assessing the effects of benzylisothiazole (BIT), a derivative of isothiazole on the expression of xenobiotic-metabolizing enzymes in the liver. Because thiazoles induce mEH and GST via P450-mediated metabolic activation, it is highly likely that common molecular basis may exist in the expression of the genes which is mediated with the activation of antioxidant responsive element(s) following the production of reactive metabolic intermediates.

In the present study, we determined whether BIT was capable of inducing phase II enzymes including mEH, rGSTA1/2, rGST3/5 and rGSTM1/2 levels. The levels of cytochrome P450 1A2, 2B1/2, 2C11, 2E1 and 3A1/2 were additionally monitored in the liver. Production of ring cleavage products may result in hepatotoxicity and nephrotoxicity in animals. It has been shown that inhibitors of P450 prevent the nephrotoxicity of certain thiazole given in combination with a glutathione depleting agent (Mizutani *et al.*, 1992). In a view that ring cleavage products from certain heterocycle derivatives may produce the reactive metabolites, the possible hepatotoxic effect of BIT was monitored toward the end of study.




Benzyl isothiazole (BIT)

**Fig. 1.** Chemical structure of BIT.

## II. MATERIALS AND METHODS

### 1. Materials

[$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol) and [$\gamma$-$^{32}$P]ATP (3000 Ci/mmol) were purchased from New England Nuclear Research Products (Boston, MA). The chemical structure of BIT was shown in Fig. 1. BIT was purchased from Aldrich Chemical Co. (Milwaukee, WI). Biotinylated donkey anti-goat IgG, streptavidin-conjugated horseradish peroxidase, biotinylated goat anti-rabbit IgG and BCIP/NBT were supplied from GIBCO BRL (Gaithersburg, MD). Random prime-labeling and 5'-end-labeling kits were purchased from Promega (Madison, WI). Form-specific polyclonal rabbit anti-rat rGSTA1/2 antibody was purchased from Biotrin International (Dublin, Ireland). Polyclonal mouse anti-rat cytochrome P450 1A1/2, P450 2B1/2 and P450 2C11 antibodies were obtained from Oxford Biomedical Research (Oxford, MI). Most of the reagents in the molecular studies were purchased from Sigma Chemical Co. (St. Louis, MO).

### 2. Animal treatment

Male Sprague-Dawley rats (180~220 g) were obtained from Korea Food and Drug Administration (Seoul, Korea) and maintained at a temperature between 20~23°C with a relative humidity of 50%. Animals were caged under the supply of filtered pathogen-free air, given food (Cheiljedang rodent chow, Seoul, Korea) and water *ad libitum*. Rats were fasted for 16 hr before sacrifice. Rats were intraperitoneally injected with BIT in corn oil solution as a vehicle at a single dose of 1.17 mmol/kg and were killed at 24 hr. For time course studies,

BIT was administered at the dose of 1.17 mmol/kg per day for 1 to 3 day(s) to determine mEH and GST mRNA levels. P450 expression was assessed after three consecutive daily treatment with BIT. Four animals were used for each treatment group.

### 3. Isolation of microsomal and cytosolic proteins

Hepatic microsomal and cytosolic fractions prepared by differential centrifugation were washed in pyrophosphate buffer and stored in 50 mM Tris acetate buffer (pH 7.4) containing 1 mM EDTA and 20% glycerol. Microsomal and cytosolic preparations were stored at -70°C until use. Protein content was determined by the method of Lowry *et al.* (1951).

### 4. Immunoblot analysis

SDS-PAGE analysis was performed according to Laemmli (1970) using a Hoefer gel apparatus. Immunoblot analysis was performed according to previously published procedures (Kim, 1992; Kim and Kim, 1992). Microsomal and cytosolic proteins were separated by 8% and 12% SDS-PAGE, respectively, and electrophoretically transferred to nitrocellulose paper. The nitrocellulose paper was incubated with rabbit anti-rat GST or mEH antibody, followed by incubation with biotinylated secondary antibodies. Immunoreactive proteins were visualized by incubation with streptavidin-horseradish peroxidase, followed by addition of 4-chloro-1-naphthol and hydrogen peroxide. Filters were allowed to react with polyclonal mouse anti-rat cytochrome P450 antibodies, incubated with alkaline phosphatase-conjugated goat anti-mouse IgG as the secondary antibody and developed using BCIP/NBT. P450 2E1 immunoblotting was carried out with goat anti-rat P450 2E1 antibody and biotinylated donkey anti-goat IgG, as described previously (Kim and Novak, 1993).

### 5. Isolation of total RNA

Total RNA was isolated using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction according to the method of Puissant and Houdebine (1990).

### 6. Preparation of cDNA probes for GST and mEH

A cDNA for mEH was prepared as described previously (Kim, 1992). Specific cDNA probes for GST genes including *rGSTA2* (287-684), *rGSTA3* (122-488) and *rGSTM1* (643-963) were amplified by reverse transcriptase-polymerase chain reaction using the selective primers for each gene, as described previously (Pickett *et al.*, 1984; Ding *et al.*, 1985; Ding *et al.*, 1986; Hayes *et al.*, 1994). cDNA derived from hepatic poly(A)$^+$ RNA obtained from rats treated with pyrazine was cloned in a pGEM+T vector (Promega, Madison, WI).

### 7. Northern blot hybridization

Northern blot was carried out according to the procedures described previously (Kim, 1992; Kim *et al.*, 1996). Briefly, total RNA isolated from rat livers was resolved by electrophoresis in an 1% agarose gel containing 2.2 M formaldehyde and transferred to supported nitrocellulose paper. The nitrocellulose paper was baked in a vacuum oven at 80°C for 2 hr. The blot was incubated with hybridization buffer containing 50% deionized formamide, 5x Denhardt's solution [0.1% Ficoll, 0.1% polyvinylpyrrolidine and 0.1% bovine serum albumin (Pentex Fraction V)], 0.1% SDS, 200 μg/ml of sonicated salmon sperm DNA and 5x SSPE (1x SSPE: 0.15 M NaCl, 10 mM $NaH_2PO_4$, and 1 mM $Na_2EDTA$, pH 7.4) at 42°C for 1 hr without probe. Hybridization was performed at 42°C for 18 hr with a heat-denatured cDNA probe, which was random prime-labeled with [α-$^{32}$P]dCTP. Filters were washed twice in 2x SSC and 0.1% SDS for 10 min at room temperature and twice in 0.1x SSC and 0.1% SDS for 10 min at room temperature as well. The filters were finally washed in the solution containing 0.1x SSC and 0.1% SDS for 60 min at 55°C. After quantitation of mRNA levels, the membranes were stripped and rehybridized with $^{32}$P-end-labeled poly(dT)$_{16}$ to quantify the amount

of RNA loaded onto the membranes.

### 8. Scanning densitometry

Scanning densitometry was performed with a Microcomputer Imaging Device, Model M1 (Imaging Research, St. Catharines, Ontario, Canada). The area of each lane was integrated using MCID software version 4.20, rev 1.0, followed by background subtraction.

### 9. Data analysis

Data were analyzed using computer programs for pharmacological calculations (Tallarida and Murray, 1987). One-way analysis of variance procedures were used to assess significant differences among treatment groups. For each significant effect of treatment, the Newmann-Keuls test was used for comparison of multiple group means. The criterion for statistical significance was set at $\alpha$= 0.05 or $\alpha$=0.01.

### 10. Evaluation of hepatic toxicity

The effect of BIT on hepatic morphology *in vivo* were assessed by light microscopy. Livers were removed and fixed in 10% buffered formalin, processed and embedded in paraffin. Sections, 5 μm in thickness, were stained with hematoxylin and eosin prior to examination.

## III. RESULTS

### 1. mEH expression

The effect of BIT on the expression of hepatic mEH gene was assessed in rats by Northern blot analysis (Fig. 2A and 2B). Exposure of animals to a single dose of BIT (1.17 mmol/kg) resulted in a substantial 30-fold increase in the mEH mRNA level at 24 hr. Rats were treated with BIT for 3 days to confirm the increases of mEH mRNA level after consecutive treatment. The mEH mRNA level after 3 days of treatment was 50% reduced from the increased level observed at day 1 post-treatment, resulting in a 15-fold increase relative to that in

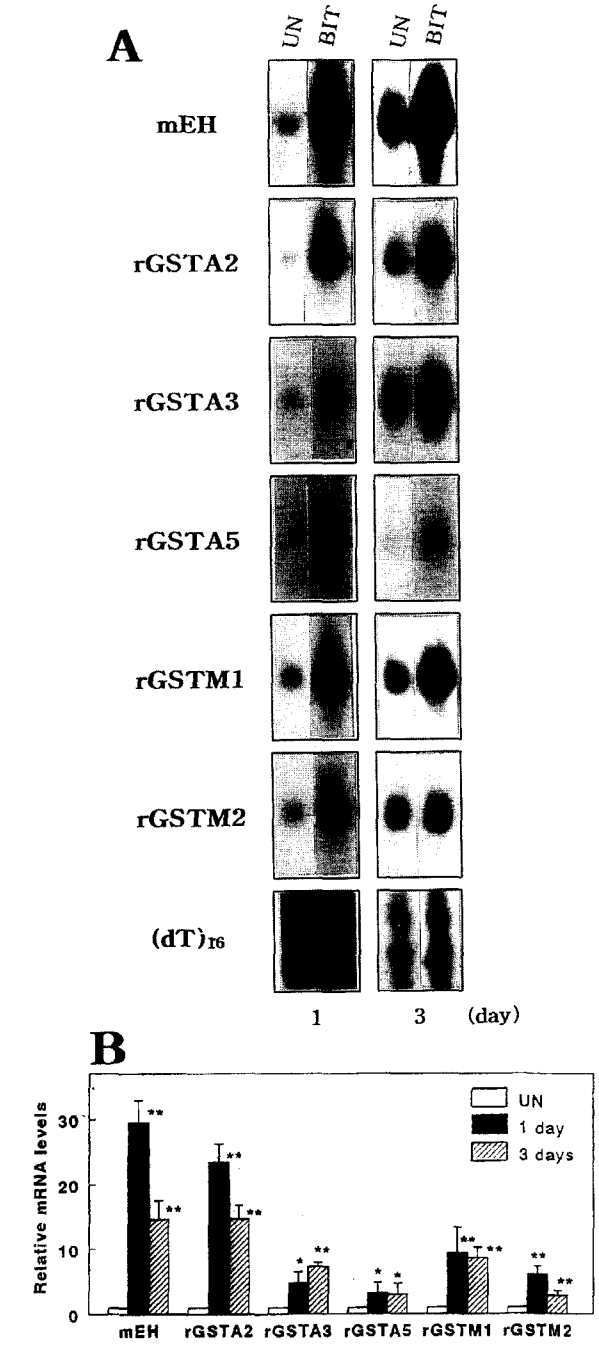



**Fig. 2.** Effect of BIT on the levels of hepatic mEH and rGST mRNA levels. (A) Northern blot analyses of mEH, rGSTA2, rGSTA3, rGSTA5, rGSTM1 and rGSTM2 mRNA levels in total RNA fractions isolated from rats treated with BIT. Hepatic total RNA samples were isolated at day 1 or 3 after treatment with BIT at the daily dose of 1.17 mmol/kg/day. The amount of RNA loaded in each lane (20 μg each) was assessed by hybridization of the stripped membrane with $^{32}$P-labeled poly(dT)$_{16}$. UN, vehicle-treated rats. (B) Changes in mEH and rGST mRNA levels relative to those in the vehicle-treated rats for the respective days were depicted following scanning densitometry of the Northern blots. Significantly different from control (**p<0.01, *p<0.05).

untreated animals.

### 2. rGST expression

Previous studies have shown that mEH induction is accompanied by that of rGSTA2 in response to a number of structurally-different chemical inducers. The expression of hepatic rGSTA2 gene in response to BIT was also quantified by Northern blot analysis (Fig. 2A and 2B). rGSTA2 mRNA level was elevated to the similar extent, as observed with mEH at 24 hr after treatment. The effects of BIT on the mRNA levels of other GSTs including rGSTA3, rGSTA5, rGSTM1 and rGSTM2 were also monitored in the rat liver. rGSTA3 mRNA levels were elevated 5- to 7-fold at 1 to 3 day(s) after BIT treatment, whereas rGSTA5 was minimally altered. BIT also increased the mRNA levels for class mu GSTs with rGSTM1 mRNA being increased ~9-fold, as compared to control. The rGSTM2 mRNA level was affected to a lesser extent.

### 3. mEH and GST protein expression

Western immunoblot analysis confirmed that BIT induced mEH protein after 3 days of treatment (Fig. 3). Immunoblot analyses were also carried out with rabbit anti-rat rGSTA1/2, rGSTA3/5, rGSTM1 and rGSTM2 antibodies to assess whether the changes in the mRNAs were consistent with those in proteins after BIT treatment for 3 days (1.17 mmol/kg per day, ip) (Fig. 3). BIT treatment resulted in ~3-fold induction of most GST subunits in the liver. The rGSTA1/2 induction by the agent was comparable to that of mEH (Fig. 3).

### 4. Cytochrome P450 expression

The expression of major hepatic cytochromes P450 was immunochemically monitored in rats treated with the BIT for 3 consecutive days (Fig. 4). BIT caused 3- to 4-fold induction of P450 2B1/2, relative to that untreated. It was noteworthy that cytochrome P450 1A2, 2C11 and 3A1/2 levels were reduced to 20% to 30% of those in untreated animals. P450 2E1 level was also slightly decreased, as compared to control. These results demonstrated

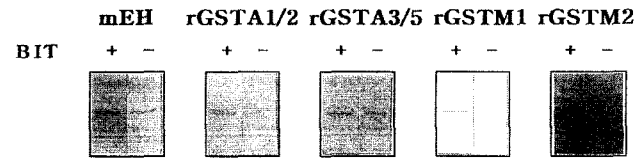



**Fig. 3.** Hepatic mEH and rGST protein expression after 3 consecutive daily treatment with BIT. Immunoblot analyses were carried out with rabbit anti-rat mEH, rGSTA1/2, rGSTA3/5, rGSTM1 and rGSTM2 antibodies. Each lane was loaded with 5 and 3 μg of microsomal and cytosolic proteins for mEH and GST immunoblottings, respectively.

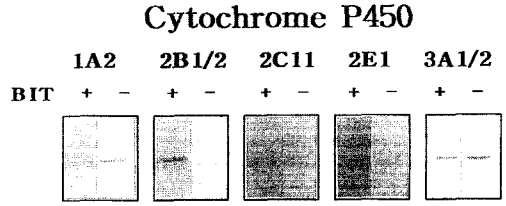



**Fig. 4.** Cytochrome P450 expression in the liver after treatment with BIT. These blots show immunochemically-detectable P450 1A1/2, P450 2B1/2, P450 2C11, P450 2E1 and P450 3A1/2 levels in the hepatic microsomes produced after treatment of rats with BIT at the dose of 1.17 mmol/kg/day (ip) for 3 days. Each lane was loaded with 10~15 μg of rat hepatic microsomal proteins for P450 immunoblottings. The relative changes were determined from multiple immunoblottings using different groups of animals (N=4).

that BIT significantly decreased the levels of major cytochrome P450s except P450 2B1/2.

### 5. Histopathology of hepatotoxicity

Liver morphology was monitored in rats after treatment with BIT to assess the possible relationship of metabolizing enzyme expression with metabolic activation of the agent (Fig. 5A and 5B). Liver tissues excised from rats at 24 hr after consecutive 3 day treatment with BIT were microscopically examined after hematoxylin and eosin-staining. BIT caused multifocal nodular necrosis with moderate to extensive diffuse liver cell degeneration. Fig. 5A and 5B show the liver cell damage at different magnifications. However, no notable injury was observed in the kidney except cloudy swelling of tubular epithelial cells (Fig. 5C), supporting the possibility that BIT is primarily metabolized in the liver.

These results demonstrated that BIT induces mEH and major GSTs in the liver with increases in

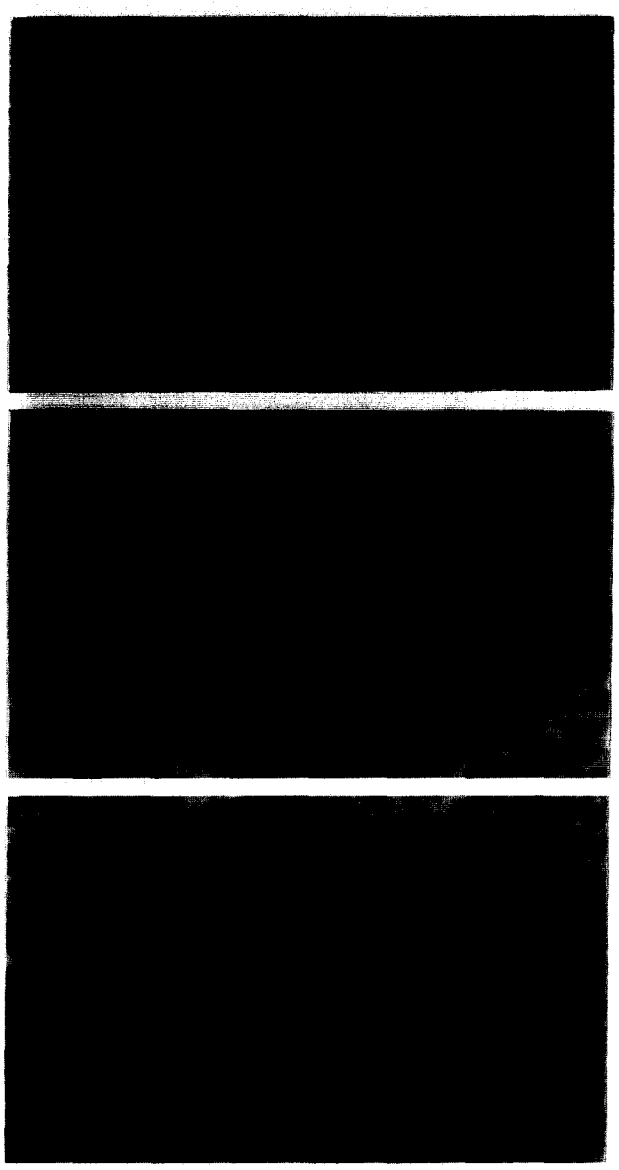


**Fig. 5.** Effects of BIT on the morphology of the liver and kidney. Liver and kindey tissues derived from rats at 24 hr after 3 consecutive days of BIT treatment were examined microscopically. Hematoxylin and eosin-stained sections were photographed; A, liver from a rat treated with BIT (1.17 mmol/kg/day, ip, 3d). Severe necrosis around a central vein and degenearation of hepatocytes was apparent (×100). BIT treatment of rats caused multifocal nodular necrosis with moderate to extensive diffuse liver cell degeneration. B, Diffuse nodular necrosis and degenerated parenchymal tissue was noted in the liver (×450). C, No notable injury was observed in the kidney except cloudy swelling of tubular epithelial cells (×100).

the mRNA levels whereas the agent significantly decreased major cytochrome P450s and that the changes in the detoxifying enzymes by BIT might be associated with its liver toxicity.

## IV. DISCUSSION

Previous studies have shown that levels of mEH mRNA were substantially increased after treatment with certain heterocycles (Kim *et al.*, 1993; Kim and Cho, 1996). mEH and GSTs catalyze the detoxication of reactive epoxide intermediates. Given the observations that both mEH and GST mRNA levels are elevated after treatment with thiazoles (Kim and Cho, 1996), this study was additionally carried out to examine the changes in mEH and major GST gene expression in association with the hepatotoxicity induced by a derivative of isothiazole. BIT stimulated the mEH and GST genes, whereas this agent suppressed the expression of certain cytochrome P450s. P450 suppression by BIT may be associated with production of reactive metabolic intermediate(s) from BIT by cytochrome P450 and subsequent formation of metabolite intermediate complex in the liver, although a further study is necessary to confirm this. This was further supported by the observation that suppression of cytochrome P450s was accompanied by the induction of mEH and GST, the expression of which is considered to be mediated with reactive oxygen species (Hayes and Pulford, 1995). The present study also showed that BIT differentially affected P450 2E1 and P450 2B1/2 levels. In particular, P450 2B1/2 was induced by BIT to the greatest extent among the major forms of P450 examined in this study. Thus, BIT may serve as a phenobarbital-like inducer. The differential P450 expression by BIT raised the possibility that substrate specificity of BIT for these P450s is different and that the P450 expression is mediated with their distinct induction mechanism. The present study corroborates the hypothesis that BIT might generate prooxidant species through *in vivo* metabolism of the agent and that the oxidative stress from the heterocycle would subsequently lead to the transcriptional activation of mEH and certain GST genes such as rGSTA2, as was observed with thiazole and thiazole derivatives.[1]

Studies have shown that the antioxidant responsive element (ARE) on the rGSTA2 gene is responsive to hydrogen peroxide, reactive oxygen species and

phenolic antioxidants that undergo redox cycling (Rushmore *et al.*, 1991). Thus, the induction of GST gene expression seems to be mediated by oxidative stress derived from a variety of chemicals. Differential induction and limitation of maximal induction by thiazolic compounds may involve ARE, which have been identified in the genes of some phase II metabolizing enzymes (Hayes and Pulford, 1995; Jaiswal, 1994). The rGSTA2 gene is transcriptionally regulated by xenobiotics through the expression of ARE BP-1 and ARE BP-2 as transcriptional regulatory proteins (Nguyen *et al.*, 1996; Fahl and Wasserman, 1996). The complexation of these nuclear binding proteins to ARE sites in the 5'-flanking region of the rGSTA2 and mEH genes are considered to be responsible for basal and inducible expression by certain xenobiotics (Jaiswal, 1994; Daniel, 1993).

The previous other studies demonstrated that methylthiazoles differentially stimulate mEH and GST genes. Bioactivation of thiazoles with methyl group(s) at the positions of 2, 4 and/or 5 was comparatively evaluated in association with the correlation of mEH and GST expression by the agents. Suppression of P450 1A2 and 3A1/2 after treatment with thiazoles and isothiazole appeared to parallel with the increases in the mEH or rGSTA2 mRNA levels. This study also showed that P450 1A2, 3A1/2 and 2C11 levels were decreased by BIT, which was consistent with those observed with thiazole derivatives including 4-methylthiazole. Thus, both thiazole and isothiazole derivatives appeared to have common substrate specificity for cytochome P450s. Further studies support the possibility that P450 2E1 was responsible for the oxidative metabolism of thiazoles presumably including BIT, which leads to increases in the mEH and rGSTA2 mRNA levels.

Studies have shown that ring cleavage sites probably involve position 5 of thiazole (Mizutani *et al.*, 1993, 1994). Thus, ring cleavage products from 4-methylthiazole through P450 oxidation would produce drug free radicals and reactive oxygen species including hydroxyl free radicals, which would lead to tissue injury. The alkyl substituted position of BIT is in accordance with that of 4-methylthiazole. Previously, we showed that P450-mediated metabolic activation of the agents is related with the tissue injury caused by thiazole derivatives, as shown by the experiment performed with isosafrole in rats, which inhibits the metabolic activity of many cytochrome P450s in rats (Zhao and O' Brien, 1996). Liver toxicity of BIT was comparable to that of 4-methylthiazole, the toxicity of which was much greater than that of thiazole alone at the same dose. This study demonstrated that BIT may act like other thiazole derivatives in terms of suppression of cytochrome P450 as well as the extent of tissue injury.

[1]**Footnote**: Differential expression of epoxide hydrolase and glutathione S-transferase genes by methylthiazoles: The role of cytochrome P450 2E1-mediated metabolic activation, Cho, M. K. and Kim, S. G. manuscript in preparation.

## ACKNOWLEDGMENTS

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## REFERENCES

Cocco, M.T., Congiu, C., Maccioni, A., Onnis, V., Schivo, M.L. and De Logu, A. (1994): Synthesis and antimicrobial activity of new 3,5-diaminoisothiazole derivatives, *Farmaco*, **49**(2), 137-140.

Daniel, V. (1993): Glutathione S-transferases: Gene structure and regulation of expression, *Crit. Rev. Biochem. Mol. Biol.*, **28**, 173-207.

Ding, G.J.-F., Lu, A.Y.H. and Pickett, C.B. (1985): Rat liver glutathione S-transferases: Nucleotide sequence analysis of a Yb1 cDNA clone and prediction of the complete amino acid sequence of the Yb1 subunit, *J. Biol. Chem.*, **260**, 13268-13271.

Ding, G.J.-F., Ding, V.D.-H., Rodkey, J.A., Bennett, C. D., Lu, A.Y.H. and Pickett, C.B., (1986): Rat liver glutathione S-transferases: DNA sequence analysis of a Yb2 cDNA clone and regulation of the Yb1 and Yb2 mRNAs by phenobarbital, *J. Biol. Chem.*, **261**, 7952-7957.

Fahl, W.E. and Wasserman, W.W. (1996): Functional antioxidant responsive elements and the mediating protein ARE-BP-1, Proceedings of XIth International Symposium on Microsomes and Drug Oxidations, pp. 160.

Hayes, J.D., Nguyen, T., Judah, D.J., Petersson, D.G. and Neal, G.E. (1994): Cloning of cDNAs from fetal rat liver encoding glutathione S-transferase Yc polypeptides, *J. Biol. Chem.*, **269**, 20707-20717.

Hayes, J.D. and Pulford, D.J. (1995): The glutathione S-transferase supergene family: Regulation of GST and the contribution of the isoenzymes to chemoprotection and drug resistance, *Crit. Rev. Biochem. Mol. Biol.*, **30**, 445-600.

Jaiswal, A.K. (1994): Antioxidant response element, *Biochem. Pharmacol.*, **48**, 439-444.

Kim, S.G. and Cho, M.K. (1996): Expression of glutathione S-transferases Ya, Yb1, Yb2, Yc1 and Yc2 and microsomal epoxide hydrolase gene by thiazole, benzothiazole and benzothiadiazole, *Biochem. Pharmacol.*, **52**, 1831-1841.

Kim, S.G., and Kim Y.H. (1992): Gender-related expression of rat microsomal epoxide hydrolase during maturation: Post-transcriptional regulation, *Mol. Pharmacol.*, **42**, 75-81.

Kim, S.G. and Novak, R.F. (1993): The induction of cytochrome P450 2E1 by nitrogen- and sulfur-containing heterocycles: Expression and molecular regulation, *Toxicol. Appl. Pharmacol.*, **120**, 257-265.

Kim, S.G. (1992): Transcriptional regulation of rat microsomal epoxide hydrolase gene by imidazole antimycotic agents, *Mol. Pharmacol.*, **42**, 273-279.

Kim, S.G., Chung, H.C. and Cho, J.Y. (1996): Molecular mechanism for alkyl sulfide-modulated carbon tetrachloride-induced hepatotoxicity: The role of P450 2E1, P450 2B and glutathione S-transferase expression, *J. Pharmacol. Exp. Therap.*, **277**, 1058-1066.

Kim, S.G., Kedderis, G.L., Batra, R. and Novak, R.F. (1993): Thiazole and pyrazine induction of microsomal epoxide hydrolase in rats: 2-Cyanoethylene oxide hydrolysis, *Carcinogenesis*, **14**, 1665-1670.

Laemmli, U.K. (1970): Cleavage of structural proteins during assembly of the head of the bacteriophage T4, *Nature*, **227**, 680-685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, F.J. (1951): Protein measurement with the folin phenol reagent, *J. Biol. Chem.*, **193**, 265-275.

Mizutani, T., Yoshida, K. and Kawazoe, S. (1994): Formation of toxic metabolites from thiabendazole and other thiazoles in mice: Identification of thioamides as ring cleavage products, *Drug Metab. Dispos.*, **22**, 750-755.

Mizutani, T., Yoshida, K. and Kawazoe, S. (1993): Possible role of thioformamide as a proximate toxicant in the nephrotoxicity of thiabendazole and related thiazoles in glutathione-depleted mice: Structure-toxicity and metabolite studies, *Chem. Res. Toxicol.*, **6**, 174-179.

Mizutani, T., Yoshida, K. and Ito, K. (1992): Nephrotoxicity of thiazoles structurally related to thiabendazole in mice depleted of glutathione by treatment with buthionine sulfoximine, *Res. Commun. Chem. Pathol. Pharmacol.*, **75**, 29-38.

Nguyen, T., Favreau, L. and Pickett, C.B. (1996): Regulation of glutathione S-transferase gene expression, Proceedings of XIth International Symposium on Microsomes and Drug Oxidations, pp 99.

Pickett, C.B., Telakowski-Hopkins, C.A., Ding, G.J.-F., Argenbright, L. and Lu, A.Y.H. (1984): Rat liver glutathione S-transferases: Complete nucleotide sequence of a glutathione S-transferases mRNA and the regulation of the Ya, Yb and Yc mRNAs by 3-methylcholanthrene and phenobarbital, *J. Biol. Chem.*, **259**, 5182-5188.

Puissant, C. and Houdebine, L.-M. (1990): An improvement of the single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, *Biotechniques*, **8**, 148-149.

Rushmore, T.H., Morton, M.R. and Pickett, C.B. (1991): The antioxidant responsive element, *J. Biol. Chem.*, **266**, 11632-11639.

Swayze, E.E., Drach, J.C., Wotring, L.L. and Townsend, L.B. (1997): Synthesis, antiproliferative and antiviral activity of imidazo [4,5-d] isothiazole nucleosides as 5:5 fused analogs of nebularine and 6-methylpurine ribonucleoside, *J. Med. Chem.*, **40**, 771-784.

Tallarida, R.J. and Murray, R.B. (1987): Manual of pharmacologic calculations with computer programs, Springer-Verlag, New York.

Wilson, K. (1989): Chlormethiazole: Anticonvulsant, hypnotic and anxiolytic. In: Sulfur-containing drugs and related organic compounds (Damani L. A., Eds.). Ellis Horwood Limited, Chichester, West Sussex, England, pp 143-163.

Zhao, Z.S. and O' Brien, P.J. (1996): The prevention of $CCl_4$-induced liver necrosis in mice by naturally occurring methylenedioxybenzenes, *Toxicol. Appl. Pharmacol.*, **140**, 411-421.