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Myeloperoxidase-mediated bioactivation of 5-hydroxythiabendazole: A possible mechanism of thiabendazole toxicity

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ABSTRACT

Thiabendazole (TBZ), an antihelminthic and antifungal agent, is associated with a host of adverse effects including nephrotoxicity, hepatotoxicity, and teratogenicity. Bioactivation of the primary metabolite of TBZ, 5-hydroxythiabendazole, has been proposed to yield a reactive intermediate. Here we show that this reactive intermediate can be catalyzed by myeloperoxidase (MPO), a neutrophil-bourne peroxidase. Using a cell viability endpoint, we examined the toxicity of TBZ, 5OH-TBZ, and MPO-generated metabolites in cell-based models including primary rat proximal tubule epithelial cells, NRK-52E rat proximal tubule cells, and H9C2 rat myocardial cells. Timecourse experiments with MPO showed complete turnover of 5OH-TBZ within 15 min and a dramatic leftward shift in dose–response curves after 12 h. After a 24 h exposure *in vitro*, the LC_{50} of this reactive intermediate was 23.3 ± 0.2 μM reduced from greater than 200 μM from 5OH-TBZ alone, an approximately 10-fold decrease. LC_{50} values were equal in all cell types used. Comparison of lactate dehydrogenase leakage and caspase 3/7 activity revealed that cell death caused by the reactive intermediate is primarily associated with necrosis rather than apoptosis. This toxicity can be completely rescued via incubation with rutin, an inhibitor of MPO. These results suggest that MPO-mediated biotransformation of 5OH-TBZ yields a reactive intermediate which may play a role in TBZ-induced toxicity.

1. Introduction

Thiabendazole [2-(4-thiazolyl)benzimidazole (TBZ)] is widely used as an antihelminthic in humans and animals (Ames et al., 1963; Cuckler, 1961; Satou et al., 2001). Additionally, TBZ is used as an agricultural antifungal and food preservative (Walton et al., 1999). Radiometric assays in humans, conducted by Tocco et al. (1966), have shown that oral dosage of TBZ yields a peak plasma level after 2 h. After 24 h, 85% of the radio-labeled drug has been excreted in urine (61%) or feces (24%). Only 1% of the drug is excreted as TBZ parent compound; the majority is excreted as 5-hydroxythiabendazole (5-hydroxy-2-(4-thiazolyl)benzimidazole, 5OH-TBZ). Complete excretion of TBZ and its metabolites occurs within 5 days (Cochran, 2001). 5OH-TBZ is generated by the 5-hydroxylation of TBZ by cytochrome P450 1A2 (Fig. 1; Coulet et al., 1998; Li et al., 2003; Rey-Grobellet et al., 1996). While TBZ is generally well tolerated by humans and animals at therapeutic concentrations, it is associated with a host of adverse effects including nephrotoxicity, hepatotoxicity, and teratogenicity.

Nephrotoxicity has been associated with TBZ *in vivo*. In CD-1 mice, these effects include tubular necrosis or atrophy, peritubular fibrosis, inflammatory cell infiltration, glomerular deformation, hydronephrosis, and appearance of proteinaceous calculi in the renal pelvis (Tada et al., 1992, 2001). It has been postulated that this renal toxicity is caused by the formation of thioformamide via decomposition of the thiazole moiety in TBZ (Fig. 1; Mizutani et al., 1993). This hypothesis is substantiated by the finding that, when mice are treated with DL-buthionine sulfoximine to deplete glutathione, exposure to thioformamide equally reproduces the tubular necrosis associated with TBZ (Mizutani et al., 1996).

The majority of TBZ-associated hepatic injury reports involve intrahepatic cholestasis (Davidson et al., 1988) or bile duct injury (Manivel et al., 1987), while, micronodular cirrhosis (Rex et al., 1983; Roy et al., 1989) has been reported. Transcriptome analysis in Sprague–Dawley rat liver samples has shown significant perturbations in the expression of five genes associated with tumor suppression, cell cycling, and repair of oxidative DNA damage (Stierum et al., 2008).

Teratogenicity and carcinogenicity, possibly linked to the changes in gene expression mentioned above, have been associated with TBZ therapy. TBZ is a known teratogen to ICR mice and mouse embryos are capable of metabolizing TBZ to 5OH-TBZ (Fujitani et al., 1991). TBZ has been linked with alterations in cell cycle regulation following spindle disruption (Holden et al., 1980). TBZ

Abbreviations: ATP, adenosine triphosphate; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; LDH, lactate dehydrogenase; MPO, myeloperoxidase; TBZ, thiabendazole; 5OH-TBZ, 5-hydroxythiabendazole.

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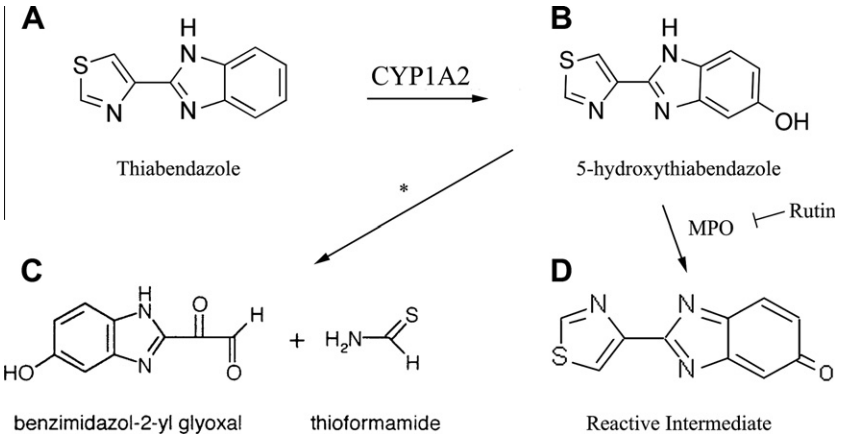

Fig. 1. TBZ-relevant structures and reactions. Cytochrome P450 1A2 metabolism of TBZ (A) yields 5OH-TBZ (B). The asterisk indicates the series of reactions proposed by Mizutani et al. (1993), which yield nephrotoxic thioformamide (C). Exposure of 5OH-TBZ to myeloperoxidase yields a reactive product (D); this reaction can be inhibited by rutin.

inhibited chondrogenesis in a mouse limb bud cell culture model (Tsuchiya et al., 1987). In CD-1 mouse bladder, TBZ also been associated with squamous metaplasia and nodular hyperplasia of the transitional epithelium. The presence of crystalline calculi in the bladder of these mice suggests that mechanical irritation of the bladder's epithelium may cause these lesions (Tada et al., 2001). The etiological factors behind these toxicities remain ambiguous.

TBZ-associated toxicity may be a result of 5OH-TBZ bioactivation by endogenous enzymes. Dalvie et al. (2006) described the generation of 5OH-TBZ dimers and glutathione conjugates following incubation with peroxidases. In 2010, an internal compound structurally similar to 5-hydroxythiabendazole was shown to yield a reactive intermediate following incubation with myeloperoxidase (MPO). In this paper, we investigated toxicity of the reaction products of 5OH-TBZ and MPO. Neutrophils are the primary carriers of MPO, which is the most abundant protein in the azurophilic granules (5%) and comprises 1% of total monocyte protein (Gross et al., 2009). MPO catalyzes reactions forming bactericidal hypochlorous acid, tyrosol radicals, aldehydes, and nitrite (Gross et al., 2009; Malle et al., 2003). The activity of MPO can be chemically inhibited by exposure to rutin or quercetin (Pincemail et al., 1988). In order to better understand the toxicity associated with TBZ exposure we used MPO to generate a reactive intermediate from 5OH-TBZ and compare its toxicity in *in vitro* cell models.

2. Materials and methods

2.1. Materials

Unless otherwise noted, all chemicals were purchased from Sigma–Aldrich (St. Louis, MO). Collagenase Type I and Hank's Balanced Salt Solution were purchased from Invitrogen (Carlsbad, CA). Cell-Titer Glo Luminescent Cell Viability, CytoTox-ONE Homogeneous Membrane Integrity, and Caspase Glo 3/7 assay kits were purchased from Promega (Madison, WI) and used according to manufacturer's instructions. Uncoated, 96-well, white with clear-bottom, plates were purchased from Corning (Lowell, MA). 96-well, white with clear-bottom, plates coated with rat tail collagen were purchased from Thermo Scientific (Rochester, NY). Cell strainers were purchased from BD Biosciences (San Jose, CA).

2.2. Isolation of primary rat proximal tubule cells

Rat proximal tubule cells (rPTC) were isolated as described by Sikka and McMartin (1996) with minor modifications. Briefly, three rats were euthanized and six kidneys were collected in ice-cold Hank's Balanced Salt Solution (HBSS). Each kidney was decapsulated and the cortex was separated from the medulla with a scalpel. Cortical fragments were chopped into small pieces and placed into kidney digestion buffer (322 U/ml Collagenase Type I, 552 Kunitz Units/ml Deoxyribonuclease, 33 μg/ml Soybean Trypsin Inhibitor in HBSS) at a ratio of 14 ml per kidney and incubated at 37 °C while shaking at 170 rpm for 1 h. The solution was filtered through a 100 μm strainer and pelleted by centrifugation at 210 rcf for 10 min at room temperature (all subsequent centrifugation steps used these conditions). The pellet was washed via resuspension in rPTC medium consisting of DMEM with 25 mM glucose, 500 μg/ml Pen/Strep, 1× Insulin-Transferin-Selenium (Invitrogen, Carlsbad, CA), 5 mM HEPES, with 10% FBS. Cells were once again pelleted and subsequently resuspended in 10% FBS rPTC medium, filtered through a 40 μm cell strainer and pre-plated for 1 h on a 15 cm dish at 37 °C, 5% CO_2, and 95% relative humidity. After pre-plating, the cell suspension was collected and gently triturated. The suspension was again filtered through a 40 μm cell strainer and pelleted by centrifugation. Final cell pellet was resuspended in 10% FBS rPTC medium and seeded into a collagen-coated 150 cm^2 cell culture flask and incubated overnight. 24 h post-isolation, FBS concentration in the rPTC medium was changed from 10% to 1%, and placed in the incubator on a plate shaker set to 60 rpm. Media was changed daily thereafter. Cells reached ≈70% confluence within 4 days. For experiments, cells were removed from the flasks with 0.05% Trypsin–EDTA and transferred to experiment-appropriate, collagen-coated, plates.

2.3. Subculture of cell lines

NRK-52E and H9C2 cell lines were obtained from ATCC and cultured in phenol-red free, high-glucose, Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) containing 25 mM glucose and 1 mM sodium pyruvate and supplemented with 5 mM N-2-hydroxyethylpiperazine-N#-2-ethanesulfonic acid (HEPES), 10% FBS, and penicillin–streptomycin (pen–strep; 500 μg/ml final concentration).

2.4. Myeloperoxidase-mediated biotransformation of 5OH-TBZ

Myeloperoxidase (MPO) from human leukocytes was purchased from Sigma–Aldrich (St. Louis, MO). MPO was added to cell-culture media at 2.4 units/ml. This concentration was determined by titrating the MPO between 0.3 and 38.4 units/ml and selecting a