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Microarray analysis of hepatic gene expression in pyrazole-mediated hepatotoxicity: Identification of potential stimuli of *Cyp2a5* induction

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ABSTRACT

Cytochrome P450 2a5 (*Cyp2a5*) expression is induced during liver damage caused by hepatotoxins such as pyrazole, however, the mechanism underlying this overexpression is unclear. In order to identify pathophysiological and cellular responses to pyrazole that might alter *Cyp2a5* expression, we examined the effect of pyrazole on mouse hepatic gene expression in C57BL/6 mice using Affymetrix 430 2.0 microarrays. Over 3000 differentially expressed genes were identified 24-h after pyrazole treatment that were associated with a variety of cellular pathways. Upregulated genes were primarily involved in the splicing and processing of RNA and the unfolded protein response pathway, while downregulated genes were associated with amino acid and lipid metabolism, and generation of precursor metabolites for energy production. We also examined the effects of pyrazole on cellular pathways linked to metabolic and histopathological changes observed with pyrazole toxicity. Increased mRNA levels were observed for genes involved in bilirubin production, whereas the major genes of the urea cycle were strongly decreased. Changes in genes involved in carbohydrate metabolism were also observed which could explain pyrazole-induced glycogen depletion and decreased serum glucose. In addition, over 100 genes involved in the cellular stress response were upregulated by pyrazole treatment, including genes involved in the unfolded protein response and redox status. Based on these results and previous evidence concerning the regulation of *Cyp2a5*, we have identified several pathophysiological changes including altered energy homeostasis, hyperbilirubinemia, ER stress, and altered redox status that are associated with CYP2A5 overexpression and may represent potential stimuli for the induction of *Cyp2a5*.

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Abbreviations: AMPK, AMP-activated protein kinase; CYP, cytochrome P450; Gclc, glutamate-cysteine ligase catalytic subunit; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GK, glucokinase; Gpx, glutathione peroxidase; Gsr, glutathione reductase; GST, glutathione S-transferase; Gys2, glycogen synthase 2; Hmox-1, heme oxygenase; HnRNPA1, heterogeneous nuclear ribonucleoprotein A1; mOGG1, murine oxyguanine glycosylase; Nqo1, NAD(P)H quinone oxidoreductase; Pck1, phosphoenolpyruvate carboxykinase 1; Nrf2, NF-E2-related factor-2; PYR, pyrazole; Sod2, superoxide dismutase; Srxn1, sulfiredoxin.

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1. Introduction

Cytochrome P450 2A5 (CYP2A5), the mouse ortholog of human CYP2A6, is the major catalyst of coumarin 7-hydroxylation in the liver. High levels of the enzyme have been correlated with increased metabolism of nicotine, *N*-nitrosodiethylamine (NDEA), the tobacco-specific carcinogen NNK, and aflatoxin B1 [1–4]. Increased expression of CYP2A5 occurs during conditions typically associated with CYP downregulation including viral, fulminant, and bacterial hepatitis [5], certain tumors [6], and following treatment with a variety of structurally unrelated chemicals including hepatotoxins, heavy metals, and porphyrinogenic agents [7]. Despite this unusual expression, a common mechanism responsible for this induction has not yet been identified.

Pyrazole is a nitrogen heterocycle that strongly upregulates CYP2A5 expression at the mRNA, protein and activity levels, primarily by mRNA stabilization [8]. Pyrazole was originally investigated as a treatment for alcohol abuse due to its ability to inhibit alcohol dehydrogenase, but its usefulness has been limited by its toxicity. Acute administration of pyrazole has been shown to reduce the activity of the microsomal ethanol-oxidizing system, and to inhibit catalase activity up to 90% [9]. Acute exposure has also been shown to decrease serum glucose and urea [9], to increase bilirubin content [9], and to cause glycogen depletion in pericentral hepatocytes [10]. Ultrastructural changes including increased smooth endoplasmic reticulum, reduced rough endoplasmic reticulum, swelling of the mitochondria, and lipid accumulation have also been observed [9,10]. Although pyrazole is currently used in mechanistic studies of the regulation of CYP2A5 and CYP2E1, the cellular effects of pyrazole and its mechanism of hepatotoxicity remain poorly understood.

To gain insight into the molecular mechanisms underlying pyrazole toxicity and the upregulation of *Cyp2a5*, we utilized a microarray approach which allowed us to observe the global effects of pyrazole on hepatic gene expression. The first goal of this study was to examine the effects of pyrazole on hepatic gene expression to identify expression changes that might explain metabolic and histological changes that are observed with pyrazole treatment. The second goal was to utilize the gene expression patterns to identify potential stimuli for the induction of CYP2A5. Our results indicate that pyrazole affects the expression of over 3000 genes in diverse cellular pathways, and that many of the cellular effects of pyrazole may result from altered metabolic gene expression at the mRNA level. In addition, based on the major pathways affected by pyrazole treatment, several stimuli have been identified that should be investigated for their role in the regulation of CYP2A5 including impaired energy homeostasis, hyperbilirubinemia, ER stress, and redox imbalance.

2. Methods

2.1. Mouse treatment

C57BL/6 mice were purchased from Charles River Canada (St. Constant, QC). All mice were given food and water *ad libitum*, and were housed at 23 °C with a 12-h photoperiod. All mouse treatments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and the Animal Care Committee of the University of Guelph. Eight-week-old mice were treated with a single intraperitoneal injection of either pyrazole (200 mg/kg) or saline, with four mice in each treatment group. Twenty-four hours later, mice were euthanized by CO_2 gas and serum and liver samples were collected. Serum samples were analyzed for alanine aminotransferase (ALT), cholesterol, glucose, total bilirubin, and urea (BUN) levels by the Clinical Pathology lab at the University of Guelph using an automated Hitachi 911 Bioanalyzer (Roche, Indianapolis, IN). Hepatic glycogen content was also examined in paraffin-embedded tissue sections using periodic acid-Schiff (PAS) staining according to the manufacturer's instructions (Sigma–Aldrich, St. Louis, MO). Specificity of the PAS stain for glycogen was confirmed by digesting glycogen in serial tissue sections with salivary diastase for 20 min prior to PAS staining.

2.2. Microarray assay

Gene expression profiles for liver tissue isolated from control and pyrazole-treated mice were compared by microarray analysis using Affymetrix Mouse Genome 430 2.0 arrays (Affymetrix, Santa Clara, CA). Total RNA was isolated using TRIzol reagent (Invitrogen, Burlington, ON) according to the manufacturer's protocol, and stored at −80 °C. Total RNA was then submitted to the University Health Network Microarray Centre (Toronto, Canada), where RNA quality was analyzed using an Agilent 2100 Bioanalyzer, and cRNA was generated and labelled using the one-cycle target labelling method. cRNA from each mouse was hybridized to a single array according to standard Affymetrix protocols, for a total of eight arrays. Initial image analysis of the microarray chips was performed with Affymetrix GCOS 1.4 software.

2.3. Microarray data analysis and statistics

The GeneSifter® microarray data analysis system (VizX Labs LLC, Seattle, WA, USA; http://www.genesifter.net) was used to analyze data generated in this study. This program identifies differentially expressed genes and establishes the biological significance based on Gene Ontology (GO) Consortium (http://www.geneontology.org/GO.doc.html) and KEGG public pathway resource (http://www.systems-biology.org/001/001.html). The CEL files for each array were uploaded into GeneSifter® and the data was normalized and log-transformed using the GC-RMA algorithm [11]. Differentially expressed genes were identified using Student's *t*-test (two-tailed, unpaired) and a threshold of 2.0 was used to limit the data set to genes upregulated or downregulated 2-fold or greater. Correction for multiple testing was then performed using the Benjamini–Hochberg method [12] to derive a false discovery rate estimate from the raw *p*-values. A false discovery rate of 5% was used as a cutoff for statistical significance.

The biological process ontologies and KEGG pathway terms associated with the differentially expressed genes were examined using a z-score report. The z-score was derived by subtracting the expected number of genes in a GO term meeting the criterion from the observed number of genes, and dividing by the standard deviation of the observed number of