BIOCHEMICAL PHARMACOLOGY 75 (2008) 1230–1239

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Expression of cytochrome P450 2A5 in a glucose-6-phosphate dehydrogenase-deficient mouse model of oxidative stress

Kathleen D. Nichols, Gordon M. Kirby *

Department of Biomedical Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

ARTICLE INFO

Article history:
Received 25 September 2007
Accepted 30 October 2007

Keywords:
CYP2A5
Oxidative stress
G6PD-deficiency
Gene regulation
Hepatotoxicity
ER stress

ABSTRACT

Murine hepatic cytochrome P450 2A5 (CYP2A5), unlike most CYP enzymes, is upregulated during hepatitis and hepatotoxic conditions, but the common stimulus for its induction remains unknown. We investigated the involvement of oxidative stress in the regulation of CYP2A5 expression using an oxidative stress-sensitive glucose-6-phosphate dehydrogenase (G6PD)-deficient mouse model. Treatment of deficient and wild-type mice with the prototypical CYP2A5-inducer pyrazole for 72 h led to a significantly greater degree of induction of CYP2A5 mRNA, protein and activity in deficient mice, with the greatest increase observed in animals homozygous for the deficiency. However, markers of oxidative stress including protein carbonyl, 8-hydroxydeoxyguanosine, malondiadehyde and 4-hydroxyalkenal levels were unaltered with pyrazole treatment. Furthermore, CYP2A5 expression was not altered in G6PD-deficient mice treated with the pro-oxidant menadione whereas DNA, lipid, and protein markers of oxidative stress were significantly increased. The antioxidant polyethylene glycol-conjugated catalase, while decreasing oxidative stress in menadione-treated mice, did not prevent the induction of CYP2A5 by pyrazole. Finally, the ER stress marker protein, GRP78, was increased following pyrazole treatment in G6PD-deficient compared to wild-type mice. These findings do not support a central role for generalized cellular oxidative stress in the regulation of CYP2A5 and suggest that additional factors related to G6PD-deficiency, such as ER stress, may be involved.

1. Introduction

Cytochrome P450 (CYP) enzymes play a central role in the activation and detoxification of endogenous and xenobiotic substrates. Cytochrome P450 2A5 (CYP2A5) is the major catalyst of coumarin 7-hydroxylation in murine liver and 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]. However, the common mechanism responsible for this induction remains unclear.

Evidence from models of hepatitis and chemically induced liver damage indicates that CYP2A5 overexpression

* *Corresponding author*. Tel.: +1 519 824 4120x54948; fax: +1 519 767 1450.
E-mail address: gkirby@uoguelph.ca (G.M. Kirby).

Abbreviations: CYP, cytochrome P450; G6PD, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Gclc, glutamate-cysteine ligase catalytic subunit; Gpx, glutathione-peroxidase; Gsr, glutathione reductase; GST, glutathione-S-transferase; 4-HAE, 4-hydroxyalkenal; MEN, menadione; MDA, malondialdehyde; Nqo1, NADPH:quinone oxidoreductase; PC, polyethylene glycol-conjugated catalase; PYR, pyrazole; Srxn1, sulfiredoxin; TCPOBOP, 1,4-bis-[2-(3,5,-dichloropyridyloxy)] benzene.

0006-2952/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2007.10.032

may be due to altered cellular redox status. In *H. hepaticus* (bacterial hepatitis)-infected livers CYP2A5 overexpression occurs concurrently with the presence of 8-hydroxydeoxyguanosine adducts, a sensitive marker of oxidative DNA damage [8]. CYP2A5 overexpression has also been localized with high levels of reactive oxygen species (ROS) following exposure to CYP2A5 inducers [5]. Moreover, treatment with the antioxidant vitamin E prior to pyrazole exposure has been shown to significantly reduce the induction of CYP2A5 both *in vitro* and *in vivo* [9], suggesting the involvement of oxidative stress. Recently, the oxidative stress-responsive transcription factor nuclear erythroid 2 p45-related factor 2 (Nrf2) has also been implicated in the regulation of CYP2A5 expression [10].

Increased susceptibility to oxidative stress has been demonstrated in a mouse model of glucose-6-phosphate dehydrogenase (G6PD)-deficiency [11,12]. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the rate-limiting step in the pentose phosphate pathway, which synthesizes ribose for nucleic acid production and is the principle intracellular source of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) [13]. NADPH is an essential reductant in many biosynthetic reactions, and has a protective role against hydrogen peroxide and superoxide free radical-induced oxidative damage in cells, by maintaining reduced glutathione reserves and the stability of catalase [13]. Cells overexpressing G6PD have decreased intracellular ROS accumulation and greater preservation of glutathione (GSH) stores leading to protection against exogenous and endogenous oxidative stress [14], while G6PD-deficient fibroblasts manifest accelerated cell death and generate increased levels of ROS following hydrogen peroxide exposure [15].

For this study, glucose-6-phosphate dehydrogenase (G6PD)-deficient mice were utilized to investigate the role of oxidative stress in the regulation of CYP2A5 expression. Since G6PD-deficient organisms are more susceptible to oxidative stress, we hypothesized that constitutive and inducible levels of CYP2A5 would be higher in G6PD-deficient mice than wild-type mice. Thus, deficient and wild-type mice were treated with the known CYP2A5-inducer pyrazole or the pro-oxidant menadione, with or without the antioxidants polyethylene glycol (PEG)-conjugated catalase or vitamin E. We found that G6PD-deficiency increased the inducibility of CYP2A5 by pyrazole but that catalase did not abrogate this induction. While menadione treatment caused a significant increase in markers of oxidative stress, CYP2A5 expression was not altered. Our results suggest that a factor other than generalized cellular oxidative stress is responsible for the increased induction of CYP2A5 during G6PD-deficiency.

2. Methods

2.1. Chemicals and antibodies

All reagents were obtained from Sigma–Aldrich Canada Ltd. (Oakville, Ont.) unless otherwise indicated. Polyclonal chicken CYP2A5 antiserum was a generous gift from Dr R. Juvonen (University of Kuopio, Kuopio, Finland).

2.2. Mice

G6PD-deficient breeding pairs (± females, −/y males) on a C3H background were obtained from the MRC Mammalian Genetics Unit (Oxford, England) and C3H mice were purchased from Charles River Canada (St. Constant, QC). These deficient mice carry a mutation in the 5' untranslated sequence of the X-linked G6PD gene and, through a splicing defect, exhibit decreased G6PD protein expression and enzyme activity [16]. All mice were given food and water *ad libitum*, and were housed at 23 °C with a 12-h photoperiod. Following weaning, DNA was isolated from tail clippings, and mice were genotyped as described by Nicol et al. [11]. 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.

2.3. Treatments

Female mice were utilized for all experiments to allow the study of three levels of G6PD activity (+/+, +/−, −/−). Heterozygous and recessive G6PD-deficient and wild-type C3H mice between 20 and 27 weeks of age were age-matched and treated by daily intraperitoneal injection of pyrazole (50 mg/kg or 200 mg/kg), menadione (20 mg/kg), or saline, for 24–72 h. Doses of pyrazole and menadione were selected based on previous studies in our lab [9] and others [17] that have shown that these doses significantly elevate aspartate aminotransferase and alanine aminotransferase levels in wild-type mice. A low dose of menadione was selected to prevent toxicity in G6PD-deficient mice since G6PD-deficient organisms are more susceptible to the drug [18]. Similarly, pyrazole experiments with deficient mice were performed using 50 mg/kg which maximizes CYP2A5 induction and minimizes hepatic damage, although time course experiments in wild-type mice were performed at a higher dose of 200 mg/kg. This increased dose was to permit any changes in oxidative damage to be detected since markers of oxidative stress such as protein carbonyl levels have not been observed at lower pyrazole doses in wild-type mice [9]. Unfortunately insufficient numbers of deficient animals were available to allow time course experiments to be completed in deficient mice.

To further investigate the role of oxidative stress in regulation of CYP2A5, mice were also pre-treated by intraperitoneal injection of the antioxidants polyethylene glycol (PEG)-catalase (20 kU/kg) or vitamin E (100 mg/kg). PEG-catalase and vitamin E treatments were administered 24 h and 1 h prior to pyrazole or menadione injection. The dose and timing of PEG-catalase treatment was slightly modified from [19] in order to match the timing of injection of Vitamin E as performed by Gilmore et al. [22].

2.4. Preparation of microsomal and cytosolic fractions

Approximately one-half of each liver was homogenized and microsomal fractions were prepared by differential centrifugation at 10 000/g for 30 min followed by 105 000/g for 1 h, as previously described [20]. Cytosolic and microsomal fractions were separated and stored at −80 °C until use. Protein content

was determined by the Bradford method using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories, Mississauga, ON).

2.5. Coumarin 7-hydroxylase activity

Microsomal CYP2A5 activity was determined using the protocol described in [21], modified for 96-well plates. In brief, coumarin 7-hydroxylase activity was assessed using 0.1 mg of microsomal protein incubated with coumarin (100 μM) and NADPH for 15 min. The amount of hydroxylated coumarin was determined by fluorescence with an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a FLUOstar Optima spectrophotometer (Fisher Scientific, Nepean, Ontario).

2.6. RNA isolation and comparative real-time RT-PCR analysis

Fifty to 100 mg of liver tissue from each mouse was homogenized in 1 mL of TRIzol reagent (Invitrogen, Burlington, ON). Total RNA was isolated according to the manufacturer's protocol, stored at −80 °C, and RNA quality was confirmed by denaturing gel electrophoresis. Messenger RNA analysis was conducted by relative real-time reverse transcription-polymerase chain reaction (RT-PCR) using a Roche Molecular Biochemicals (Indianapolis, IN) Lightcycler instrument and the DNA Master SYBR Green I kit (Roche Diagnostics, Mississauga, ON). One microgram of RNA was treated with 1 unit of DNase (RQ1 RNase-Free DNase; Promega, Madison, WI), and was reverse-transcribed using 0.1 μg of random primers, 20 units of RNase inhibitor (RNasin; Promega, Madison, WI), and 200 units of Murine-Moloney Leukemia Virus reverse transcriptase (M-MLV RT). PCR was performed in a 10 μL volume containing 1 μL of SYBR Green I, 2 mM Mg^+, and 5 μM of each primer. The PCR parameters were as follows: denaturation (95 °C for 10 min), followed by 45 cycles of PCR (95 °C, 15 s; 70 °C, 5 s; 72 °C, 15 s). The threshold cycle at which the fluorescent signal reached an arbitrarily set threshold near the middle of the log-linear phase of amplification for each reaction was calculated and relative quantities of mRNA for *Cyp2a5* were determined, as well as the oxidative stress-associated transcripts for the catalytic subunit of glutamate-cysteine ligase (Gclc), glutathione peroxidase (Gpx2), glutathione reductase (Gsr), glutathione-S-transferases mu, pi, and alpha 1 (GSTμ1, π, α1), NAD(P)H:quinone oxidoreductase (Nqo1), and sulfiredoxin (Srxn1). Gene specific primers were designed to cross introns in order prevent amplification of genomic DNA contamination, and melting curve analysis was performed to confirm the presence of a single product for each primer set. All mRNA levels were normalized against mRNA levels of the housekeeping gene GAPDH. Primer set sequences and the location of the primers on the cDNA are as follows:

Cyp2a5
Forward 5′-GGACAAAGAGTTCCTGTCACTGCTTC-3′ (606–631)
Reverse 5′-GTGTTCCACTTTCTTGGTTATGAAGTCC-3′ (759–786)
GAPDH
Forward 5′-ACAGTCCATGCCATCACTGCC-3′ (581–601)
Reverse 5′-GCCTGCTTCACCACCTTCTTG-3′ (826–846)
Gclc
Forward 5′-GCATCAGGCTGTCTGCACCA-3′ (411–430)
Reverse 5′-TCCGATGCCGGATGTTTCTT-3′ (570–589)
GPx2
Forward 5′-GAGGCAGGGCTGTGCTGATT-3′ (211–230)
Reverse 5′-CACCCCCAGGTCGGACATAC-3′ (392–411)
Gsr
Forward 5′-CGACTGCCTTTACCCCGATG-3′ (1567–1586)
Reverse 5′-CCCCCATTTTCACCGCTACA-3′ (1712–1731)
GSTα1
Forward 5′-AAGATGGGAATTTGATGTTTGACC-3′ (204–227)
Reverse 5′-TTCTCTTTGGTCTGGGGGACA-3′ (401–421)
GSTμ1
Forward 5′-TGGTTTGCAGGGGACAAGGT-3′ (637–656)
Reverse 5′-TCCAGTGGGCCATCTTTGAA-3′ (825–844)
GSTπ
Forward 5′-GCCGCTCTTTGGGGCTTTAT-3′ (270–289)
Reverse 5′-CCCTGGTTCTGGGACAGCAG-3′ (445–465)
Nqo1
Forward 5′-GGCCGATTCAGAGTGGCATC-3′ (716–736)
Reverse 5′-AACAGGCTGCTTGGAGCAAAA-3′ (873–893)
Srxn1
Forward 5′-AACGTACCAATCGCCGTGCT-3′ (138–157)
Reverse 5′-GGCAGCCCCCAAAGGAATAG-3′ (290–309)

2.7. Western blot analysis

Hepatic CYP2A5 and GRP78 proteins were identified by Western blot analysis as described previously [9]. In brief, 10 or 15 μg of microsomal protein were separated by SDS-PAGE on a 10% gel and transferred to Immobilon-P membrane (Millipore Corporation, Bedford, MA). Membranes were blocked in 5% (w/v) powdered low fat skim milk in Tris-buffered saline with 0.5% Tween-20 (T-TBS) overnight at 4 °C. Membranes were then incubated with chicken anti-mouse Cyp2a5 (1:20 000) or rabbit anti-GRP78 (1:2000, StressGen Biotechnologies Corporation, San Diego, CA) polyclonal antibodies in T-TBS and 5% milk, followed by rabbit anti-chicken (1:10 000) or goat anti-rabbit (1:2000) peroxidase secondary antibody (Vector Laboratories, Burlington, Ont.) diluted in T-TBS with 2% normal goat serum (Sigma–Aldrich Canada Ltd.). Chemiluminescence detection was performed by the ECL + -Plus™ Western blotting method (Amersham Biosciences Corp., Baie d'Urfe, QC) using a Typhoon 9410 scanner (GE Health Sciences, Piscataway, NJ). Equal protein loading was determined using actin as a control and by amido black staining. The relative concentrations of CYP2A5 or GRP78 protein were determined by densitometry using Image J® (NIH) software.

2.8. Immunohistochemistry

CYP2A5 protein expression was confirmed and localized in paraffin-embedded mouse liver samples as previously described [22]. To visualize CYP2A5 protein, slides were

incubated for 1 h at room temperature with chicken anti-mouse CYP2A5 (1:800) polyclonal antibody, followed by 1 h at room temperature with biotinylated goat antiserum to chicken immunoglobulin (Vectastain Elite ABC Kit, Vector Laboratories, Mississauga, ON). Sections were then incubated with biotinylated avidin horse-radish peroxidase, followed by final color development with hydrogen peroxide-activated 0.1% diaminobenzidine tetrahydrochloride (Sigma–Aldrich Canada Ltd., Oakville, ON) in 0.1 M Tris-buffer pH 7.2 for 1 min. Sections were counterstained with Mayer's hematoxylin (Sigma–Aldrich Canada Ltd., Oakville, ON) diluted 1:1 in phosphate-buffered saline.

2.9. Protein carbonyl assay

Protein carbonyl levels were determined in 10 μg samples of cytosolic protein using the method described by Robinson et al. [23]. A Bio-Dot®SF slot blot apparatus (BioRad Laboratories Inc., Mississauga, ON), was utilized to transfer the protein to polyvinyl difluoride membrane (PVDF; Millipore Corporation, Bedford, MA) which was then incubated in 100 μg/ml dinitrophenyl hydrazone (DNPH) solution. Derivatized protein carbonyl groups were identified using rabbit anti-DNP antiserum (Sigma–Aldrich Canada Ltd., Oakville, ON-Aldrich) and goat anti-rabbit horseradish peroxidase secondary antibody (Sigma–Aldrich, Oakville, ON). Chemiluminescence detection was performed as described for the Western blot analysis. Equal loading of protein was assessed by Amido black staining.

2.10. 8-hydroxy-2′-deoxyguanosine assay

DNA was extracted from 15 to 25 mg of liver tissue using Qiagen's DNeasy kit (Qiagen, Mississauga, ON). At least 30 μg of DNA eluted in Buffer TE was incubated with 2 μL of 1 N HCl and 40 U of deoxyribonuclease at 37 °C for 30 min. The DNA was then hydrolyzed through incubation with 2 μL of 3 M sodium acetate (pH 5.2) and 10 U of nuclease S1 (VWR, Mississauga, ON) in 300 mM sodium acetate, 1 mM $ZnSO_4$, DTPA, pH 5.3) for 1 h at 37 °C. Each sample was then dephosphorylated with 1 U of alkaline phosphatase (in 5 mM Tris–HCl, 0.2 $MgCl_2$, pH 8.0) for 30 min at 37 °C, followed by incubation with 0.02 units of PDEI (Amersham, Baie d'Urfe, QC), and PDEII for 30 min at 37 °C. Samples were spin filtered using Ultrafree-MC (30 kDa) tubes (Millipore Corp., Bedford, MA) and dried under vacuum for 3 h at 45 °C. Samples were resuspended in 25–30 μL of mobile phase 30 min prior to use. 8OHdG and 2-deoxyguanonsine (2dG) were resolved by HPLC with a YMCbasic C18 column (4.6 mm × 150 mm; particle size 3 μm) (Waters Corp., Milford, MA) and quantified using a CoulArray electrochemical detection system (ESA Inc., Chelmsford, MA). A mobile phase of 100 mM sodium acetate, pH 5.2, with 5% methanol was utilized to elute the nucleosides from the column at a flow rate of 1 mL/min. The mobile phase was filtered using a 0.2 μm filter before use with the HPLC. Potentials of the four coulometric analytical cells of the CoulArray System were 800, 450, 200 and −50 mV. Calibration curves were generated from standards ranging from 2.5 to 150 μM for 2dG and 10 nM to 300 nM for 8OHdG. Data were recorded, analyzed and stored using CoulArray for Windows data analysis software (ESA Inc., Chelmsford, MA).

2.11. Lipid peroxidatin assay

Fifty milligrams of liver tissue that had been stored at −80 °C was homogenized in 20 mM phosphate buffer supplemented with 5 mM butylated hydroxytoluene (BHT) to prevent sample oxidation. Homogenates were centrifuged at 13 000 × *g* for 10 min at 4 °C and the protein in the supernatant was quantified by the Bradford method using the Bio-Rad Protein Assay Reagent (Bio-Rad Laboratories, Mississauga, ON). Lipid peroxidation was then determined by measuring malondialdehyde and 4-hydroxyalkenal (MDA + HAE) levels with the Colorimetric Microplate Assay for Lipid Peroxidation (Oxford Biochemical Research, Oxford, MI) according to the manufacturer's instructions.

2.12. Statistical analysis

Data was analyzed by one-way analysis of variance and Tukey's multiple comparison test. Results were considered to be significant at $p < 0.05$.

3. Results

3.1. G6PD deficiency increases CYP2A5 responsiveness to pyrazole-mediated induction

In order to determine whether increased susceptibility to oxidative stress alters basal and inducible levels of CYP2A5, wild-type, heterozygous, and homozygous female mice were treated with saline or pyrazole (50 mg/kg) for 3 consecutive days. Immunostaining for CYP2A5 in liver sections from saline-treated mice revealed that there were no alterations in constitutive CYP2A5 levels according to genotype (Fig. 1). Pyrazole treatment resulted in hepatocellular injury to the pericentral hepatocytes, characterized by eosinophilic cytoplasm (H&E), and increased CYP2A5 expression, with greater overexpression occurring in the G6PD-deficient mice than the wild-type mice (Fig. 1).

Differences in CYP2A5 expression were confirmed quantitatively at the mRNA, protein, and activity levels. G6PD-deficiency alone had no significant effect on constitutive levels of mRNA transcripts, protein, or the ability to hydroxylate coumarin. However, following three consecutive days of pyrazole treatment, up to 3, 1.7, and 5.2 times greater expression ($p < 0.05$) of CYP2A5 was observed in −/− deficient mice compared to wild-type mice at the mRNA, protein and activity level, respectively (Fig. 2).

3.2. Pyrazole does not alter markers of DNA, protein, or lipid oxidation

To further investigate the putative relationship between oxidative stress and pyrazole-mediated induction of CYP2A5 in G6PD-deficient mice, markers of oxidative stress were assessed in wild-type and recessive mice after 3 consecutive days of pyrazole treatment. Protein oxidation was determined

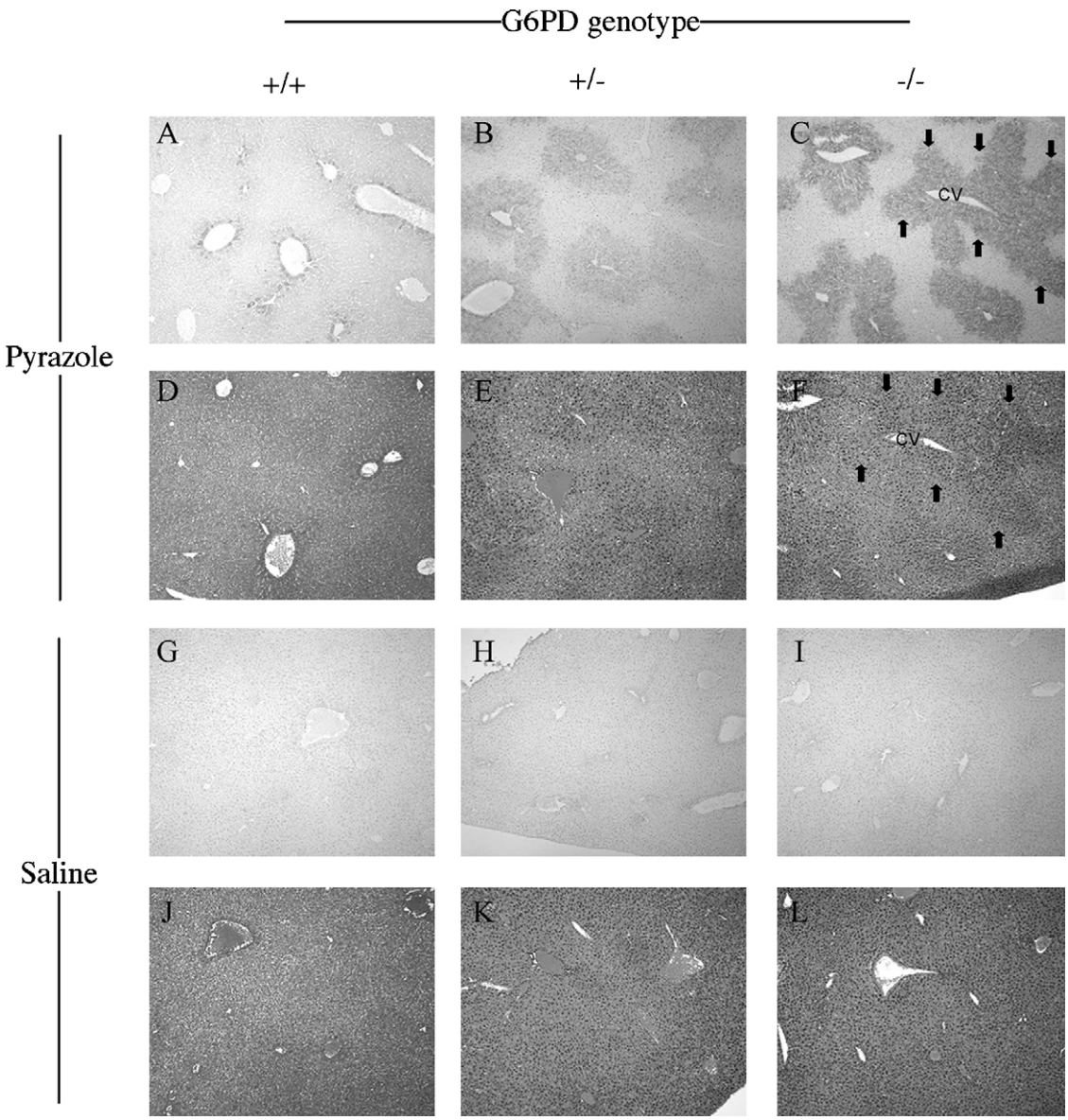

Fig. 1 – Immunoreactive CYP2A5 expression is greater in G6PD-deficient genotypes than wild-type mice following pyrazole treatment. Wild-type (panels A, D, G, J), heterozygous (panels B, E, H, K), or recessive (panels C, F, I, L) mice were treated with pyrazole (50 mg/kg, panels A–F) or saline (panels G–L) and immunostained for CYP2A5 (panels A–C, G–I) or stained with hematoxylin and eosin (panels D–F, J–L). Arrows delineate a region of CYP2A5 induction (panel C) corresponding to an area of liver damage (panel F) in hepatocytes surrounding a central vein (CV). Original magnification 100×.

in cytosolic samples using the protein carbonyl assay, DNA oxidation by HPLC analysis of 8-hydroxy-2′-deoxyguanosine (8OHdG) adducts, and lipid peroxidation by colorimetric analysis of MDA and HAE levels. Table 1 demonstrates that regardless of genotype, pyrazole did not elevate protein carbonyl, 8OHdG, or lipid peroxide levels compared to respective saline controls.

3.3. *Menadione increases markers of oxidative stress but does not alter CYP2A5 levels in G6PD-deficient mice*

To further test the hypothesis that oxidative stress leads to induction of CYP2A5, G6PD-deficient mice were treated daily with the known pro-oxidant menadione (vitamin K_3, 20 mg/kg) and CYP2A5 expression was assessed after 72 h. CYP2A5 expression was not altered by menadione at the mRNA, protein, or activity levels, in either +/+ or −/− G6PD genotypes (Table 2). However, the presence of menadione-induced oxidative stress in the livers of G6PD-deficient mice was confirmed by significant increases in the levels of protein carbonyls (1.5-fold, $p < 0.05$), 8OHdG adducts (1.6-fold, $p < 0.01$) and lipid peroxidation (2.1-fold, $p < 0.05$).

3.4. *Menadione, but not pyrazole, increases oxidative stress markers at time points earlier than 24 h*

Since pyrazole and menadione administration in the G6PD-deficient mice occurred over 3 consecutive days and samples

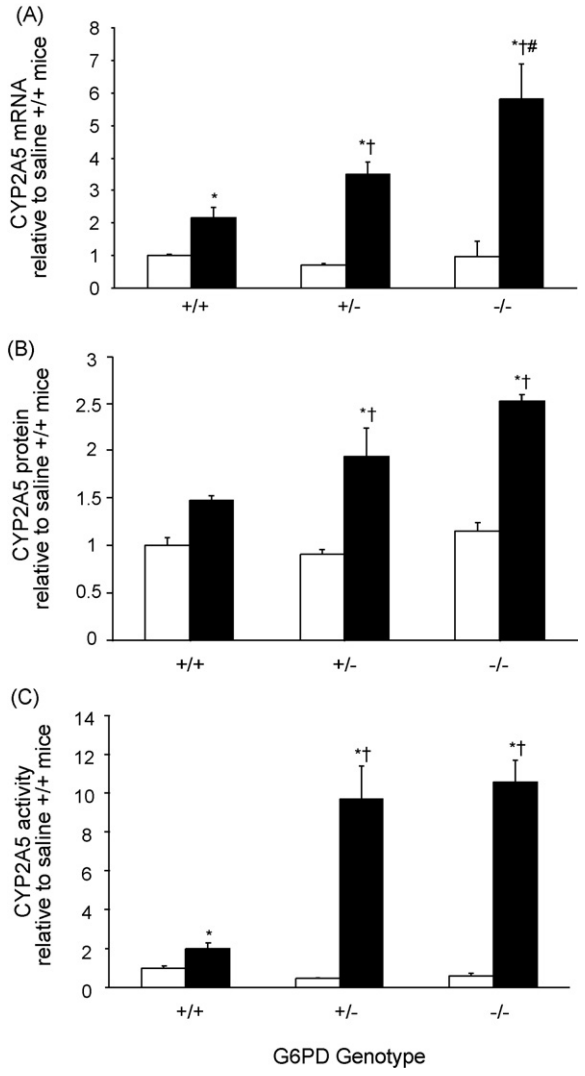

Fig. 2 – Pyrazole-mediated increases in CYP2A5 correspond to G6PD genotype. Mice were treated with saline (open bars) or 50 mg/kg pyrazole (closed bars) for 72 h and CYP2A5 mRNA (A), protein (B), and activity (C) levels were determined by comparative real-time RT-PCR, Western blot analysis, or COH assay, respectively. Results represent the average for three independent experiments, for a total of nine mice per treatment/genotype combination. For all panels, values are significantly different from (*) saline treated +/+ mice, (†) pyrazole-treated +/+ mice, (#) pyrazole-treated +/− mice, $p < 0.05$. Note the progressive increase in staining for CYP2A5 (arrows) around the central veins (CV) in G6PD-deficient mice and the evidence of cellular injury in this region as indicated by homogeneous eosinophilic cytoplasm (arrows).

were not collected until 24 h after the final treatment, it was possible that markers of oxidative stress were elevated earlier during the progression of hepatotoxicity and in the case of pyrazole had returned to normal prior to sample collection. To address this concern, we assessed oxidative damage and CYP2A5 expression in wild-type mice 0, 6, 12, and 24 h after treatment with 200 mg/kg pyrazole or 20 mg/kg menadione. Table 3 demonstrates that CYP2A5 protein was increased ($p < 0.05$) by pyrazole but was not altered by menadione. CYP2A5 mRNA and activity levels showed a similar pattern of induction to that observed at the protein level (data not shown). Both lipid peroxidation and protein carbonyl markers were elevated 6 h after menadione treatment and remained elevated, however, pyrazole had no effect (Table 3). 8OHdG adducts levels also remained unaltered in pyrazole-treated mice throughout the 24-h time period (data not shown).

3.5. *Influence of antioxidants on oxidative stress and CYP2A5 expression*

We have previously shown that Vitamin E prevents the induction of CYP2A5 by pyrazole, therefore we determined if administration of the antioxidant enzyme catalase also prevents the upregulation of CYP2A5. Wild-type mice were treated with PEG-catalase 1 h prior to administration of pyrazole or menadione each day for three consecutive days, and 24 h before the first treatment with pyrazole or menadione. PEG-catalase did not abrogate the response of CYP2A5 mRNA, protein, or activity to pyrazole treatment (Table 4) but did reduce levels of 8OHdG adducts and lipid peroxidation in menadione-treated mice by 20 and 26%, respectively ($P < 0.05$).

3.6. *Pyrazole, but not menadione, increases GRP78 protein levels inG6PD-deficient mice*

In order to reconcile the finding that induction of CYP2A5 by pyrazole is higher in G6PD-deficient mice than wild-type mice and yet does not result in elevated markers of oxidative stress, we tested the alternate hypothesis that the increased responsiveness in G6PD-deficient mice was due to elevated levels of ER stress rather than oxidative stress. Previous evidence in our laboratory [9] has demonstrated that a specific type of ER stress induced by the oxidized form of DTT is involved in the regulation of *Cyp2a5*. Thus, we assessed protein levels of the ER-stress marker glucose-regulated protein 78 (GRP78) in pyrazole and menadione-treated G6PD-deficient mice, and the effect of vitamin E on GRP78 expression in wild-type mice. Fig. 3 demonstrates that pyrazole causes a greater increase in GRP78 protein in G6PD-deficient mice than wild-type mice but that menadione treatment does not alter GRP78 levels in either genotype (Fig. 3a). Vitamin E completely prevents the increase in GRP78 protein by pyrazole (Fig. 3b).

4. Discussion

This study investigated the hypothesis that oxidative stress is involved in the regulation of CYP2A5 expression, utilizing an oxidative stress-sensitive G6PD-deficient mouse model. This hypothesis was based on previous evidence of increased CYP2A5 expression in regions of the liver with high levels of reactive oxygen species (ROS) [5], and strong induction of CYP2A5 during bacterial hepatitis with increased 8OHdG levels [8]. However, our results suggest that although G6PD-deficient mice have increased response

Table 1 – Pyrazole does not increase DNA, protein, or lipid markers of oxidative stress

Marker[a]	G6PD +/+		G6PD −/−	
	Saline	Pyrazole	Saline	Pyrazole
8OHdG	1.00 ± 0.08	1.04 ± 0.08	1.11 ± 0.05	0.94 ± 0.05
Protein carbonyl	1.00 ± 0.23	1.08 ± 0.15	0.96 ± 0.08	0.94 ± 0.12
MDA + HAE	1.00 ± 0.37	0.77 ± 0.16	0.75 ± 0.12	0.93 ± 0.32

[a] 8-Hydroxydeoxyguanosine (8OHdG), protein carbonyl, and malondialdehyde + 4-hydroxyalkenal (MDA + HAE) levels were measured as described in Section 2 to assess oxidative stress-dependent DNA, protein, and lipid damage. Mice were treated with 50 mg/kg pyrazole or saline for 72 h. Results are representative of three independent experiments, utilizing three mice per treatment/genotype combination for each experiment, and all values expressed as mean ± standard relative to saline-treated +/+ mice ($p > 0.05$).

Table 2 – Menadione increases markers of oxidative stress but not CYP2A5 expression in G6PD-deficient mice

Measure[a]	G6PD +/+		G6PD −/−	
	Saline	Menadione	Saline	Menadione
CYP2A5 mRNA	1.00 ± 0.03	0.72 ± 0.28	0.98 ± 0.34	0.75 ± 0.15
CYP2A5 protein	1.00 ± 0.08	0.67 ± 0.07	1.15 ± 0.09	0.77 ± 0.17
CYP2A5 activity	1.00 ± 0.11	0.67 ± 0.05	1.33 ± 0.35	0.63 ± 0.10
8OHdG	1.00 ± 0.08	1.35 ± 0.15	1.11 ± 0.05	1.64 ± 0.08*†
Protein carbonyl	1.00 ± 0.13	0.95 ± 0.05	0.96 ± 0.08	1.50 ± 0.01*†#
MDA + HAE	1.00 ± 0.05	1.38 ± 0.19	1.17 ± 0.23	2.14 ± 0.26*†

[a] CYP2A5 transcript, protein, and activity levels were determined by comparative real-time RT-PCR, Western blot, and COH assay, respectively, as described in Section 2. Oxidative stress markers were determined as described in Table 2. Mice were treated with 20 mg/kg menadione or saline for 72 h. All values are the mean ± standard error for nine mice per treatment/genotype combination and are expressed relative to saline-treated +/+ mice. Values are significantly different from (*) saline treated +/+ mice, (†) saline-treated −/− mice, (#) pyrazole-treated +/+ mice, $p < 0.05$.

to pyrazole, generalized cellular oxidative stress is not the stimulus for upregulation of CYP2A5.

G6PD deficiency increased the induction of CYP2A5 by pyrazole without affecting the basal expression of CYP2A5. This indicates that G6PD-deficiency alone is insufficient to alter CYP2A5 expression and that additional stimuli or stress factors are required to increase CYP2A5 expression. This is not surprising since G6PD-deficient and wild-type organisms display similar phenotypes in the absence of a stimulus to cause the utilization and depletion of NADPH in G6PD-deficiency. Furthermore, the ability of an underlying condition to potentiate the induction of CYP2A5 has previously been observed during restraint stress. Physical stress has no effect on basal CYP2A5 activity, but increases the response to TCPOBOP by 2-fold after stress exposure [24]. Collectively, these findings suggest that an external stress or toxin, oxidative or otherwise, is required to create cellular conditions that upregulate CYP2A5, although an underlying condition may potentiate this induction.

The heterozygous and recessive G6PD-deficient mice utilized in this study have previously been shown to have reduced red blood cell and hepatic G6PD activity increasing their susceptibility to oxidative stress [11,25]. Our results confirm that G6PD-deficient mice are more sensitive to oxidative stress caused by menadione treatment. Menadione led to a significant increase in 8OHdG, protein carbonyl, and lipid peroxide levels in the hepatic tissue of deficient but not wild-type mice. However, despite this increase in oxidative damage, menadione treatment did not increase levels of CYP2A5 mRNA, protein or activity, in wild-type or G6PD-deficient mice.

Table 3 – Menadione, but not pyrazole, increases markers of oxidative stress at time points earlier than 24 h

Time	CYP2A5 protein		Protein carbonyls		Lipid peroxidation	
	PYR	MEN	PYR	MEN	PYR	MEN
0	1.00 ± 0.14	1.00 ± 0.01	1.00 ± 0.19	1.00 ± 0.27	1.00 ± 0.10	1.00 ± 0.12
6	1.66 ± 0.33	1.07 ± 0.07	0.87 ± 0.12	1.65 ± 0.13	1.08 ± 0.08	2.59 ± 0.40*
12	2.09 ± 0.10*	0.98 ± 0.06	0.91 ± 0.10	2.45 ± 0.30*	0.62 ± 0.27	2.48 ± 0.15*
24	4.14 ± 0.29*	1.18 ± 0.11	1.09 ± 0.22	2.56 ± 0.27*	1.13 ± 0.01	2.58 ± 0.57*

Wildtype mice were treated with a single i.p. injection of saline, 200 mg/kg pyrazole (PYR) or 20 mg/kg menadione (MEN) and three mice were sacrificed 6, 12 or 24 h after treatment. CYP2A5 protein levels were determined by Western blot analysis using 10 μg of protein, while protein carbonyl levels and lipid peroxidation were determined as described in Methods. All values are relative to time 0 and represent the mean ± standard error for 3 treated mice normalized against saline-treated controls. *Values are significantly different from control mice (time 0), $p < 0.05$.

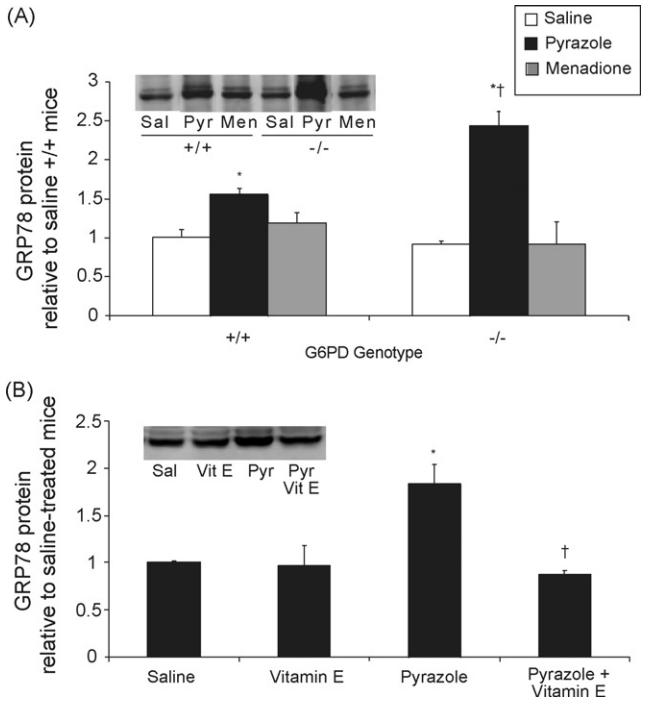

Fig. 3 – GRP78 protein expression is altered by pyrazole and vitamin E but not menadione. For panel A, G6PD +/+ and −/− mice were treated every 24 h with saline (Sal), pyrazole (Pyr, 50 mg/kg), or menadione (Men, 20 mg/kg) for a total of three treatments, as described in Section 2. For panel B, mice were treated with saline or vitamin E (Vit E, 100 mg/kg) 24 h and 1 h prior to administration of pyrazole (200 mg/kg). GRP78 protein levels were determined by Western blot analysis (representative blots are shown). Results represent the average of three mice per treatment/genotype combination. For all panels, values are significantly different from (*) saline treated +/+ mice, (†) pyrazole-treated +/+ mice, (#) pyrazole-treated +/− mice, $p < 0.05$.

The lack induction of CYP2A5 by menadione was unexpected since menadione increases CYP2A5 expression in mouse hepatocytes in primary culture [9]. However, previous studies have identified other CYP2A5 inducers that act differently *in vitro* and *in vivo*. Salonpaa et al. demonstrated that several efficient *in vivo* inducers, such as cobalt, cerium, thioacetamide, and aminotriazole, do not increase CYP2A5 expression in primary hepatocytes [33]. This suggests that the regulatory pathways involved in induction of CYP2A5 *in vivo* differ relative to primary hepatocytes. For menadione, the lack of induction may be related to (1) the selected dose, (2) sequestration of the drug in tissues other than the liver, (3) metabolism and inactivation of the drug *in vivo* preventing CYP2A5 induction, or (4) extrahepatic effects of menadione that prevent CYP2A5 upregulation.

The fact that pyrazole is a nitrogen heterocycle suggests that it may undergo redox cycling resulting in oxidative stress as has been observed with other nitrogen heterocycles [26]. However, our results demonstrate that this is unlikely since pyrazole did not increase 8OHdG, protein carbonyl, or MDA + 4-HAE markers of oxidative stress at 6, 12, 24, or 72 h following treatment of wild-type or deficient mice. While it cannot be ruled out that oxidative damage may have occurred at a level below the detection limit of the 8OHdG, protein carbonyl, or lipid peroxidation assays, this is unlikely since a high dose of pyrazole (200 mg/kg) was utilized for time course experiments. This dose of pyrazole is sufficient to cause significant hepatotoxicity, as evidenced by a 19-fold increase in serum ALT [9], and it would be expected that oxidative damage would be observed if this mechanism was involved. Furthermore, menadione, which does undergo redox cycling, caused increases in all three biomarkers suggesting that the methods were sufficiently sensitive to detect oxidative damage.

Previous evidence generated in our laboratory demonstrated that vitamin E blocks induction of CYP2A5 by pyrazole *in vitro* and *in vivo* [9]. In view of the ability of vitamin E to act as a chain-breaking antioxidant, to directly scavenge peroxyl radicals, and to modulate glutathione levels [27], we hypothesized that *Cyp2a5* is regulated by the generation of reactive oxygen species. However, the results from the current study demonstrate that administration of PEG-catalase prior to pyrazole treatment does not prevent the induction of CYP2A5. Since PEG-catalase only catalyzes the decomposition of hydrogen peroxide and does not interact with other ROS, it is possible that CYP2A5 could be regulated by oxidative stress through a mechanism not involving hydrogen peroxide. However, due to the lack of evidence of oxidative damage, and the fact that G6PD-deficient mice are more susceptible to oxidative stress, in part, because of decreased catalase stability, the inability of PEG-catalase to block CYP2A5 induction supports the notion that factors other than oxidative stress are involved in CYP2A5 regulation. This also suggests that vitamin E may attenuate CYP2A5 induction by means other than protecting against ROS. In addition to its antioxidant properties, vitamin E modulates the expression of genes involved in lipid uptake, control of extracellular proteins, inflammation, cell signaling, and cell cycle control (reviewed in [27]). While the specific mechanism by which vitamin E blocks induction of CYP2A5 by pyrazole is currently unclear, our findings suggest that it may be related to an additional role such as the prevention of ER stress as discussed below.

The increased responsiveness of CYP2A5 to pyrazole in G6PD-deficient mice in the absence of oxidative damage suggests that differences other than susceptibility to oxidative stress exist between deficient and wild-type mice. For

Table 4 – Treatment with PEG-catalase does not inhibit the induction of CYP2A5 by pyrazole

Treatment[a]	CYP2A5 protein	CYP2A5 activity
Saline	1.00 ± 0.12	1.00 ± 0.13
PEG-catalase	1.60 ± 0.05	1.38 ± 0.05
Pyrazole	2.00 ± 0.09*	1.91 ± 0.05*
Pyrazole/PEG-catalase	2.08 ± 0.03*	2.43 ± 0.15**
Menadione	0.94 ± 0.16	1.40 ± 0.37
Menadione/PEG-catalase	1.04 ± 0.05	1.02 ± 0.03

[a] Wild-type mice were treated with 50 mg/kg pyrazole, 20 mg/kg menadione and/or 20 KU PEG-catalase. Protein and activity levels were determined by Western blot analysis and COH assay, respectively. Values represent the mean ± standard error for three mice and are significantly different from saline-treated mice *$p < 0.05$, **$p < 0.01$.

instance, we have demonstrated for the first time that GRP78 levels are higher in G6PD-deficient mice than wild-type mice following pyrazole treatment. GRP78 is a well-characterized ER chaperone protein, and a classical marker of ER stress, that has roles in protein folding, ER calcium homeostasis, targeting of misfolded proteins for degradation, and sensing ER stress by regulating the ER stress sensors PERK, Ire1p and ATF6 [28]. Increased expression of GRP78 following pyrazole treatment in G6PD-deficient relative to wild-type mice suggests that the response to ER-stress stimuli may be increased by G6PD deficiency. Furthermore, the ability of Vitamin E to block the induction of CYP2A5 and GRP78 in our model, and the inability of menadione to induce either gene suggest that ER stress may be necessary to upregulate CYP2A5. While it is unclear at this time whether a direct causal relationship exists between elevated ER stress and CYP2A5 in G6PD-deficient mice, other inducers of CYP2A5 including viral hepatitis and DTTox have been previously associated with ER stress [9,29]. We have also previously shown that pyrazole increases mRNA levels of the ER-stress-associated transcription factors ATF4 and ATF6, as well as many other ER-stress genes [34]. In addition, the transcription factor Nrf2, which has recently been shown to regulate *Cyp2a5* [10], can be activated by an oxidative stress-independent pathway involving ER stress [30], further supporting a possible role for ER stress in the regulation of *Cyp2a5*. The specific ER stress stimulus responsible for upregulation of CYP2A5 remains unknown, however, we have previously ruled out altered ER calcium homeostasis and glycosylation of newly synthesized proteins. While generalized cellular oxidative stress is not likely involved in regulation of *Cyp2a5*, we cannot rule out the possibility that ER stress is occurring in response to localized, transient, low level oxidative stress in the ER, and that this is responsible for upregulation of CYP2A5. It is also possible that ER stress is a consequence of CYP2A5 overexpression. Overexpression of other CYP enzymes, including CYP2C2 and CYP2E1, has been shown to induce significant ER protein damage and to upregulate GRP78 [31,32]. Elucidating the function of CYP2A5 during cellular injury may clarify whether CYP2A5 induction is the cause or consequence of ER stress, and the mechanism responsible for the increased responsiveness of CYP2A5 in G6PD-deficient mice.

In conclusion, our findings demonstrate a novel association between G6PD-status and inducibility of CYP2A5 and indicate that oxidative stress is not likely the key regulator of CYP2A5 expression. The lack of a central role of generalized cellular oxidative stress in the regulation of *Cyp2a5* is supported by several pieces of evidence: (1) pyrazole does not increase 8OHdG, lipid peroxide, or protein carbonyl levels that are common markers of oxidative stress, (2) menadione upregulates markers of oxidative stress *in vivo*, however, CYP2A5 expression is unaltered, and (3) PEG-catalase administration does not block the induction of CYP2A5 by pyrazole. Ongoing studies in our lab are investigating the mechanism responsible for increased responsiveness of G6PD-deficient mice to pyrazole-mediated CYP2A5 induction. The link between G6PD-deficiency and induction of CYP2A5 may have important implications in humans. G6PD-deficiency is the most common enzymopathy in humans [11] and *CYP2A6*, the human orthologous gene of *Cyp2a5*, is the principal enzyme involved in nicotine metabolism and elimination, smoking behavior and carcinogen activation. Further studies may clarify the relationship between G6PD-deficiency and the regulation of *CYP2A6* and the associated impact on human health.

Acknowledgements

We would like to thank Ann Maslen for her assistance with mouse genotyping. We would also like to thank Drs. Joanne Wan and Louise Winn (Queen's University, Kingston, Ontario) for their assistance with the 8OHdG assay. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). Kathleen Nichols is a recipient of an Ontario Graduate Scholarship from the Ontario Ministry of Training, Colleges and Universities, and a Postgraduate Scholarship from NSERC.

REFERENCES

[1] Camus AM, Geneste O, Honkakoski P, Bereziat JC, Henderson CJ, Wolf CR, et al. High variability of nitrosamine metabolism among individuals: role of cytochromes P450 2A6 and 2E1 in the dealkylation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in mice and humans. Mol Carcinog 1993;7:268–75.
[2] Felicia ND, Rekha GK, Murphy SE. Characterization of cytochrome P450 2A4 and 2A5-catalyzed 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolism. Arch Biochem Biophys 2000;384:418–24.
[3] Pelkonen P, Lang MA, Negishi M, Wild CP, Juvonen RO. Interaction of aflatoxin B1 with cytochrome P450 2A5 and its mutants: correlation with metabolic activation and toxicity. Chem Res Toxicol 1997;10:85–90.
[4] Siu EC, Wildenauer DB, Tyndale RF. Nicotine self-administration in mice is associated with rates of nicotine inactivation by CYP2A5. Psychopharmacology (Berl) 2006;184:401–8.
[5] Sipowicz MA, Chomarat P, Diwan BA, Anver MA, Awasthi YC, Ward JM, et al. Increased oxidative DNA damage and hepatocyte overexpression of specific cytochrome P450 isoforms in hepatitis of mice infected with *Helicobacter hepaticus*. Am J Pathol 1997;151:933–41.
[6] Kobliakov V, Kulikova L, Samoilov D, Lang MA. High expression of cytochrome P450 2a-5 (coumarin 7-hydroxylase) in mouse hepatomas. Mol Carcinog 1993;7:276–80.
[7] Kojo A, Viitala P, Pasanen M, Pelkonen O, Raunio H, Juvonen R. Induction of CYP2A5 by pyrazole and its derivatives in mouse primary hepatocytes. Arch Toxicol 1998;72:336–41.
[8] Chomarat P, Sipowicz MA, Diwan BA, Fornwald LW, Awasthi YC, Anver MR, et al. Distinct time courses of increase in cytochromes P450 1A2, 2A5 and glutathione *S*-transferases during the progressive hepatitis associated with *Helicobacter hepaticus*. Carcinogenesis 1997;18:2179–90.
[9] Gilmore WJ, Kirby GM. Endoplasmic reticulum stress due to altered cellular redox status positively regulates murine hepatic CYP2A5 expression. J Pharmacol Exp Ther 2004;308:600–8.
[10] Abu-Bakar A, Lamsa V, Arpiainen S, Moore MR, Lang MA, Hakkola J. Regulation of CYP2A5 gene by the transcription factor nuclear factor (erythroid-derived 2)-like 2. Drug Metab Dispos 2007;35:787–94.
[11] Nicol CJ, Zielenski J, Tsui LC, Wells PG. An embryoprotective role for glucose-6-phosphate

dehydrogenase in developmental oxidative stress and chemical teratogenesis. FASEB J 2000;14:111–27.
[12] Jain M, Cui L, Brenner DA, Wang B, Handy DE, Leopold JA, et al. Increased myocardial dysfunction after ischemia-reperfusion in mice lacking glucose-6-phosphate dehydrogenase. Circulation 2004;109:898–903.
[13] Nelson DL, Cox MM. Glycolysis and the catabolism of hexoses. In: Nelson DL, Cox MM, editors. Lehninger Principles of Biochemistry. New York: Worth Publishers; 2000. p. 527–66.
[14] Leopold JA, Zhang YY, Scribner AW, Stanton RC, Loscalzo J. Glucose-6-phosphate dehydrogenase overexpression decreases endothelial cell oxidant stress and increases bioavailable nitric oxide. Arterioscler Thromb Vasc Biol 2003;23:411–7.
[15] Ho HY, Cheng ML, Lu FJ, Chou YH, Stern A, Liang CM, et al. Enhanced oxidative stress and accelerated cellular senescence in glucose-6-phosphate dehydrogenase (G6PD)-deficient human fibroblasts. Free Radic Biol Med 2000;29:156–69.
[16] Sanders S, Smith DP, Thomas GA, Williams ED. A glucose-6-phosphate dehydrogenase (G6PD) splice site consensus sequence mutation associated with G6PD enzyme deficiency. Mutat Res 1997;374:79–87.
[17] Radjendirane V, Joseph P, Lee YH, Kimura S, Klein-Szanto AJ, Gonzalez FJ, et al. Disruption of the DT diaphorase (NQO1) gene in mice leads to increased menadione toxicity. J Biol Chem 1998;273:7382–9.
[18] Scott MD, Zuo L, Lubin BH, Chiu DT. NADPH, not glutathione, status modulates oxidant sensitivity in normal and glucose-6-phosphate dehydrogenase-deficient erythrocytes. Blood 1991;77:2059–64.
[19] de Winter BY, van Nassauw L, de Man JG, de Jonge F, Bredenoord AJ, Seerden TC, et al. Role of oxidative stress in the pathogenesis of septic ileus in mice. Neurogastroenterol Motil 2005;17:251–61.
[20] Kirby GM, Wolf CR, Neal GE, Judah DJ, Henderson CJ, Srivatanakul P, et al. In vitro metabolism of aflatoxin B1 by normal and tumorous liver tissue from Thailand. Carcinogenesis 1993;14:2613–20.
[21] Aitio A. A simple and sensitive assay of 7-ethoxycoumarin deethylation. Anal Biochem 1978;85:488–91.
[22] Gilmore WJ, Hartmann G, Piquette-Miller M, Marriott J, Kirby GM. Effects of lipopolysaccharide-stimulated inflammation and pyrazole-mediated hepatocellular injury on mouse hepatic Cyp2a5 expression. Toxicology 2003;184:211–26.
[23] Robinson CE, Keshavarzian A, Pasco DS, Frommel TO, Winship DH, Holmes EW. Determination of protein carbonyl groups by immunoblotting. Anal Biochem 1999;266:48–57.
[24] Konstandi M, Marselos M, Radon-Camus AM, Johnson E, Lang MA. The role of stress in the regulation of drug metabolizing enzymes in mice. Eur J Drug Metab Pharmacokinet 1998;23:483–90.
[25] Pretsch W, Charles DJ, Merkle S. X-linked glucose-6-phosphate dehydrogenase deficiency in *Mus musculus*. Biochem Genet 1988;26:89–103.
[26] Biaglow JE, Varnes ME, Roizen-Towle L, Clark EP, Epp ER, Astor MB, et al. Biochemistry of reduction of nitro heterocycles. Biochem Pharmacol 1986;35:77–90.
[27] Azzi A, Gysin R, Kempna P, Munteanu A, Negis Y, Villacorta L, et al. Vitamin E mediates cell signaling and regulation of gene expression. Ann N Y Acad Sci 2004;1031:86–95.
[28] Kaufman RJ. Orchestrating the unfolded protein response in health and disease. J Clin Investig 2002;110:1389–98.
[29] Waris G, Siddiqui A. Regulatory mechanisms of viral hepatitis B and C. J Biosci 2003;28:311–21.
[30] Ho HK, White CC, Fernandez C, Fausto N, Kavanagh TJ, Nelson SD, et al. Nrf2 activation involves an oxidative-stress independent pathway in tetrafluoroethylcysteine-induced cytotoxicity. Toxicol Sci 2005;86:354–64.
[31] Lewis MD, Roberts BJ. Role of CYP2E1 activity in endoplasmic reticulum ubiquitination, proteasome association, and the unfolded protein response. Arch Biochem Biophys 2005;436:237–45.
[32] Szczesna-Skorupa E, Chen C-D, Liu H, Kemper B. Gene expression changes associated with the endoplasmic reticulum stress response induced by microsomal cytochrome P450 overproduction. J Biol Chem 2004;279:13953–61.
[33] Salonpaa P, Kottari S, Pelkonen O, Raunio H. Regulation of CYP 2 A 5 induction by porphyrinogenic agents in mouse primary hepatocytes. Naunyn Schmiedebergs Arch Pharmacol 1997;355:8–13.
[34] Nichols KD, Kirby GM. Microarray analysis of hepatic gene expression in pyrazole-mediated hepatotoxicity: Identification of potential stimuli of Cyp2a5 induction. Mol Pharmacol, in press.