The Role of Hypoxia-Inducible Factor-1 α in Acetaminophen Hepatotoxicity

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ABSTRACT

Hypoxia-inducible factor-1 α (HIF-1 α) is a critical transcription factor that controls oxygen homeostasis in response to hypoxia, inflammation, and oxidative stress. HIF has been implicated in the pathogenesis of liver injury in which these events play a role, including acetaminophen (APAP) overdose, which is the leading cause of acute liver failure in the United States. APAP overdose has been reported to activate HIF-1 α in mouse livers and isolated hepatocytes downstream of oxidative stress. HIF-1 α signaling controls many factors that contribute to APAP hepatotoxicity, including mitochondrial cell death, inflammation, and hemostasis. Therefore, we tested the hypothesis that HIF-1 α contributes to APAP hepatotoxicity. Conditional HIF-1 α deletion was generated in mice using an inducible Cre-lox system. Control (HIF-1 α -sufficient) mice developed severe liver injury 6 and 24 h after APAP overdose (400 mg/kg). HIF-1 α -

deficient mice were protected from APAP hepatotoxicity at 6 h, but developed severe liver injury by 24 h, suggesting that HIF-1 α is involved in the early stage of APAP toxicity. In further studies, HIF-1 α -deficient mice had attenuated thrombin generation and reduced plasminogen activator inhibitor-1 production compared with control mice, indicating that HIF-1 α signaling contributes to hemostasis in APAP hepatotoxicity. Finally, HIF-1 α -deficient animals had decreased hepatic neutrophil accumulation and plasma concentrations of interleukin-6, keratinocyte chemoattractant, and regulated upon activation normal T cell expressed and secreted compared with control mice, suggesting an altered inflammatory response. HIF-1 α contributes to hemostasis, sterile inflammation, and early hepatocellular necrosis during the pathogenesis of APAP toxicity.

Introduction

Hypoxia-inducible factor (HIF) is the master regulator of oxygen homeostasis. It regulates the expression of a large

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battery of genes involved in angiogenesis, erythropoiesis, glycolysis, inflammation, and cell death (Lee et al., 2007). HIF comprises two constitutively expressed subunits: HIF-1 α and HIF-1 β . HIF-1 α is regulated primarily at the level of protein stability: at normal oxygen tension, oxygen-dependent proline hydroxylation of HIF-1 α targets it for rapid proteasomal degradation. In hypoxia, decreased proline hydroxylation causes HIF-1 α to accumulate and translocate to the nucleus, where it binds to HIF-1 β , forming the transcriptionally competent HIF-1 that binds hypoxia response elements in DNA. HIF-regulated genes include plasminogen activator inhibitor-1 (PAI-1), vascular endothelial growth factor (VEGF), tumor necrosis factor α (TNF α), interleukin-1 β (IL-1 β), and cell death proteins such as BNIP3 and

ABBREVIATIONS: HIF, hypoxia-inducible factor; ALT, alanine aminotransferase; APAP, *N*-acetyl-*p*-aminophenol; IL, interleukin; KC, keratinocyte chemoattractant; PA, plasminogen activator; PAI-1, PA inhibitor-1; PMN, polymorphonuclear neutrophil; RANTES, regulated upon activation normal T cell expressed and secreted; TAM, tamoxifen; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; PCR, polymerase chain reaction; SAL, saline; OIL, corn oil; BB, blocking buffer; Cox IV, cytochrome c oxidase subunit IV; MIP, macrophage inflammatory protein; TAT, thrombin-antithrombin; HPRT, hypoxanthine guanine phosphoribosyl transferase; BNIP3, BCL2/adenovirus E1B 19-kDa protein-interacting protein 3.

Nix (Murdoch et al., 2005; Lee et al., 2007). HIF-1 α expression and activation are also regulated by oxidative stress (Klimova and Chandel, 2008), inflammatory cytokines (Walmsley et al., 2005a), and thrombin (Görlach et al., 2001). Because of the many factors that can modulate HIF induction and the variety of downstream signaling targets, HIF has been identified as a key regulator of a generalized stress response (James et al., 2006).

HIF-1 α has been implicated in hepatocyte death in models of liver injury that have an inflammatory or oxidative stress component, such as sepsis (Peyssonnaud et al., 2007), ischemia/reperfusion (Cursio et al., 2008), alcoholic liver disease (Li et al., 2006), and fibrosis (Copple et al., 2009). Oxidative stress and mitochondrial dysfunction play a key role in acetaminophen [*N*-acetyl-*p*-aminophenol (APAP)]-induced liver injury (James et al., 2003). APAP overdose is the leading cause of drug-induced liver failure in the United States (Lee, 2007). At toxic doses, APAP is bioactivated by cytochrome P450 enzymes to *n*-acetyl-*p*-benzoquinone imine, which is reactive, depletes GSH, and binds covalently to intracellular proteins, leading to mitochondrial dysfunction, production of reactive oxygen species, and hepatocellular necrosis (Jollow et al., 1973).

A report by James et al. (2006) indicated that APAP overdose causes nuclear accumulation of HIF-1 α in mouse livers as early as 1 h after treatment, which is before the onset of liver hypoxia and hepatocellular injury. Furthermore, *N*-acetyl cysteine, which inactivates *n*-acetyl-*p*-benzoquinone imine (James et al., 2006) or cyclosporin A, which prevents mitochondrial permeability transition, prevented HIF-1 α accumulation (Chaudhuri et al., 2011). Taken together, these data suggest that mitochondrial dysfunction and reactive oxygen species are important contributors to early HIF-1 α stabilization in APAP overdose.

In addition to cellular necrosis caused by oxidative stress and mitochondrial dysfunction, APAP hepatotoxicity is associated with disturbances to the hemostatic system in humans (James et al., 2002) and experimental animals (Ganey et al., 2007). APAP overdose caused tissue factor-dependent activation of the coagulation system in mice, elevated circulating concentration of PAI-1, and fibrin deposition in liver (Ganey et al., 2007). Inhibition of coagulation system activation through genetic or pharmacologic methods attenuated APAP-induced liver injury, suggesting a role for thrombin and the coagulation system in the pathogenesis. During injury progression, fibrin deposition can contribute to tissue ischemia and hypoxia, which might enhance HIF-1 α accumulation above that caused by oxidative stress alone.

APAP hepatotoxicity is accompanied by a sterile inflammatory response (Williams et al., 2010), and concurrent inflammation can sensitize mice to APAP-induced liver injury (Maddox et al., 2010). Mediators released from necrotic hepatocytes activate Kupffer cells, recruit and activate polymorphonuclear neutrophils (PMNs), and consequently produce cytokines that influence APAP-induced hepatocellular injury (James et al., 2005; Cover et al., 2006). The role of PMNs in APAP hepatotoxicity remains controversial, with evidence both for and against a contribution of PMNs to injury progression (Jaeschke, 2008). HIF-1 α plays a critical role in PMN function; it influences phagocytosis, motility, invasiveness, and apoptosis (Cramer et al., 2003; Peyssonnaud et al., 2005; Walmsley et al., 2005a,b). HIF-1 α also contributes to

inflammatory cytokine production (Zinkernagel et al., 2007). Therefore, HIF-1 α might participate in the inflammatory response that accompanies APAP-induced liver injury.

In addition to the many factors mentioned above that associate APAP-induced liver injury with hypoxia signaling, HIF-1 α can contribute directly to the cell death of hepatocytes by up-regulation of cell death genes. Nonetheless, it is currently unknown whether HIF-1 α is involved causally in APAP-induced liver injury. To test the hypothesis that HIF-1 α contributes to the pathogenesis of APAP-induced liver injury, conditional HIF-1 α -deficient animals were generated, and the role of HIF-1 α in APAP-induced hepatotoxicity, disruption of hemostasis, and inflammation was evaluated.

Materials and Methods

Materials. Unless otherwise stated, all reagents were purchased from Sigma-Aldrich (St. Louis, MO).

Generation of Conditional HIF-1 α -Deficient Animals. HIF-1 $\alpha^{\text{lox/lox}}$ mice (Ryan et al., 1998) were a gift from Randall Johnson (University of California, San Diego, CA), and UBC-Cre-ERT2(+/-) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The Cre-ERT2 is regulated by the ubiquitin C promoter and is expressed in virtually all cell types. Cre-ERT2 is a fusion protein composed of Cre recombinase and a mutated estrogen receptor that is selectively activated and targeted to the nucleus by (Z)-1-(*p*-dimethylaminoethoxyphenyl)-1,2-diphenyl-1-butene, *trans*-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine [tamoxifen (TAM)] but not estrogen (Ruzankina et al., 2007). C57BL/6 HIF-1 $\alpha^{\text{lox/lox}}$ and UBC-Cre-ERT2(+/-) transgenic mice were mated to generate UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice capable of conditional recombination in the floxed HIF-1 α gene when treated with TAM. Male UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice (4–5 weeks old) were treated once a day for 5 days with 200 μ g/g body weight TAM in corn oil (OIL) vehicle by oral gavage (Ruzankina et al., 2007). TAM-treated UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice were HIF-1 α -deficient (denoted as HIF-1 $\alpha^{\Delta/\Delta}$), and OIL-treated animals were HIF-1 α -sufficient (denoted as HIF-1 $\alpha^{+/+}$) (Fig. 1A). UBC-Cre-ERT2(-/-)/HIF-1 $\alpha^{\text{lox/lox}}$ littermate controls were treated with OIL or TAM to evaluate the potential effects of TAM on APAP metabolism. Animals kept in a 12-h light/dark cycle were fed a standard rodent chow/Tek 8640 (Harlan Teklad, Madison, WI) and allowed access to water ad libitum. All procedures were performed according to the guidelines of the American Association for Laboratory Animal Science and the University Laboratory Animal Research Unit at Michigan State University.

Experimental Protocol. Eleven or 21 days after OIL or TAM administration, mice were fasted overnight then given 400 mg/kg APAP or saline (SAL) vehicle via intraperitoneal injection, and food was returned. Mice were anesthetized 2, 6, or 24 h after APAP with sodium pentobarbital (50 mg/kg i.p.), and blood was collected from the vena cava into a syringe containing sodium citrate (final concentration 0.76%) for preparation of plasma. The left lateral liver lobe was fixed in 10% formalin and paraffin-blocked for evaluation of histopathology. The left medial lobe was snap-frozen in liquid nitrogen for protein, DNA, and RNA analysis. The right medial lobe was embedded in Tissue-Tek O.C.T. compound and frozen in liquid nitrogen-cooled isopentane for immunohistochemical analyses.

Genotyping and Real-Time PCR. Genotyping of mice was performed for the Cre transgene, HIF-1 α , and HIF-1 $\alpha^{\text{lox/lox}}$ mice using previously published primer sequences (Saini et al., 2008) (Table 1). Genomic DNA was extracted from tail clippings using the Direct PCR extraction system (Viagen Biotech, Los Angeles, CA) and used to quantify the Cre transgene. Genotyping of livers from HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice was performed to determine the recombination efficiency. Genomic DNA was extracted using the Extract-N-Amp system (Sigma-Aldrich) according to the manufacturer's instructions. PCR con-

Fig. 1. Conditional HIF-1 α deletion in mice. **A**, 5-week-old CRE-ERT2(+/-)/HIF-1 $\alpha^{lox/lox}$ mice were treated with OIL or 200 μ g/g TAM for 5 days to generate HIF-1 $\alpha^{+/fl}$ or HIF-1 $\alpha^{\Delta/\Delta}$ mice. Twenty one days later, mice were treated with 400 mg/kg APAP or SAL intraperitoneally. Liver samples were taken 2 and 6 h after APAP administration. **B**, HIF-1 α mRNA was measured in liver tissue and is expressed as an average of the ratios of HIF-1 α /HPRT copy number normalized to SAL controls. **C**, formalin-fixed livers were stained for HIF-1 α protein, which appears as dark brown stain. Arrow indicates positive staining.

ditions were standardized for all alleles: denaturation at 94°C for 3 min, 38 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and polymerization at 72°C for 60 s followed by a 7-min extension at 72°C.

Gene Expression Analysis. Liver tissue (50 mg) was homogenized in 1 ml of TRI reagent (Sigma-Aldrich) using a Precellys 24 Tissue Homogenizer (Cayman Chemical, Ann Arbor, MI), and RNA was extracted. Total RNA was quantified spectrophotometrically using the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA). Total RNA (1 μ g) was reverse-transcribed using an iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). The expression of PAI-1, BNIP3, and HIF-1 α was analyzed by quantitative real-time PCR using SYBR green (Applied Biosystems, Foster City, CA). Copy number was determined by comparison with standard curves of the respective genes. Expression level was normalized to the hypoxan-

thine guanine phosphoribosyl transferase (HPRT) gene. Gene-specific primers are listed in Table 1.

Assessment of Hepatocellular Injury and Liver GSH Concentration. Hepatocellular injury was estimated from increases in plasma alanine aminotransferase (ALT) activity and histopathologic evaluation of fixed tissue. ALT activity was determined spectrophotometrically using Infinity ALT Liquid Stable Reagent (Thermo Fisher Scientific). Paraffin sections of liver (5 μ m) were stained with hematoxylin-eosin and examined for evidence of hepatocellular necrosis. To measure GSH concentration, frozen liver samples (100 mg) were homogenized in 1 ml of cold buffer (0.2 M 2-*N*-morpholino ethanesulfonic acid, 50 mM phosphate, and 1 mM EDTA, pH 6.0). Homogenates were spun at 10,000g for 15 min then deproteinized with metaphosphoric acid. Total hepatic GSH concentration was determined spectrophotometrically using a commercially available kit (Cayman Chemical).

Immunohistochemistry. Liver sections were stained immunohistochemically for HIF-1 α as described previously (Saini et al., 2008). Paraffin was removed from formalin-fixed liver sections (5 μ m), and endogenous peroxidase activity was quenched with 6% H₂O₂. Sections were probed with HIF-1 α antibody (1:500 dilution; NB100-479, Novus Biologicals, Inc., Littleton, CO), which was visualized with a Rabbit Vector Elite ABC kit (Vector Laboratories, Burlingame, CA), and sections were counterstained with Nuclear Fast Red. The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay was used to analyze DNA fragmentation. Liver sections were stained with an in situ cell death detection kit (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions. Hepatic fibrin staining was performed as described previously (Copple et al., 2002). Dakocytomation rabbit anti-human/mouse fibrinogen (Dako North America, Inc., Carpinteria, CA) was the primary antibody, and a donkey anti-rabbit IgG conjugated to Alexa Fluor 488 (Invitrogen, Carlsbad, CA) was used as the secondary antibody. Fibrin images were obtained with an Olympus IX71 inverted fluorescent microscope (Olympus America Inc., Center Valley, PA), and positive staining was quantified using Image J software (National Institutes of Health, Bethesda, MD). Background staining in livers from SAL-treated mice was set as the threshold, and the percentage of pixels above threshold is presented. PMNs were stained as described previously (Maddox et al., 2010), and PMN accumulation was quantified by counting the average number of PMNs in 20 randomly selected high-power fields ($\times 400$).

Detection of Bax. Frozen liver sections (5 μ m) were fixed in 4% paraformaldehyde for 30 min at room temperature. Fixed sections were washed 3 $\times$ 7 min in phosphate-buffered saline (PBS) and blocked in 10% donkey serum + 0.1% Triton X-100 (blocking buffer; BB) for 1 h. Sections were incubated overnight at 4°C with the following primary antibodies (and their dilutions): rabbit anti-Bax (1:200) (Cell Signaling Technology, Danvers, MA) and goat anti-cytochrome *c* oxidase subunit IV (Cox IV) (1:100) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) diluted in BB. After incubation, sections were washed 3 $\times$ 7 min in PBS, blocked in BB for 1 h, and washed 3 $\times$ 7 min again. Sections were incubated with donkey anti-rabbit Alexa Fluor 568 (1:1000) and donkey anti-goat Alexa Fluor 488 (1:1000) (Invitrogen) in BB and washed, then mounted with VectaShield Mounting Medium with 4,6-diamidino-2-phenylindole (Vector Laboratories). Slides were stored at -20°C before imaging.

TABLE 1
Primer sequences

Allele	Forward (5'–3')	Reverse (5'–3')
BNIP3	TGCAGGCACCTTTATCACTCTGCT	CGCCCGATTTAAGCAGCTTTGGAT
Cre transgene	TGCCACGACCAAGTGACAGCAATG	AGAGACGGAAATCCATCGCTCG
HIF-1 α	CGTGTGAGAAACTTCTGGATG	CATGTCGCCGTCATCTGTGA
HIF-1 α (floxed)	TTGGGGATGAAAACATCTGC	CATGTCGCCGTCATCTGTGA
HPRT	AGGAGTCCTGTTGATGTTGCCAGT	GGGACGACGAACTGACATTTCTA

Fig. 2. Effect of HIF-1 α deletion on APAP hepatotoxicity. Five-week-old CRE-ERT2(-/-)/HIF-1 α ^{fllox/fllox} mice [labeled Cre (-)/HIF-1 α ^{fl/fl}] or CRE-ERT2(+/-)/HIF-1 α ^{fllox/fllox} mice [labeled Cre (+)/HIF-1 α ^{fl/fl}] were treated with OIL or 200 μ g/g TAM daily for 5 days. Twenty one days later, mice were treated with APAP or SAL intraperitoneally. Plasma ALT activity was measured 6 h after APAP administration. Data represent means $\pm$ S.E.M. of $n = 3-8$ animals per group. a indicates significantly different from respective SAL-treated mice; b indicates significantly different from OIL-treated mice; c indicates significantly different from HIF-1 α ^{+/+/fl} mice.

Fluorescent slides were viewed with the Olympus FluoView 1000 confocal laser scanning microscope. Images were collected with Olympus FluoView 1000 software, version 2.0. Alexa Fluor 568 was

detected with a 543-nm HeNeG laser with a BA 560-620 emission filter, and Alexa Fluor 488 was detected with a 488-nm Ar laser with a BA 505-525 emission filter. Images were scanned with sequential scan setting for the two lasers. A 60 $\times$ oil Plan/APO objective (numerical aperture 1.42) was used to acquire images. Five fields of view from each liver section were selected at random. Colocalization of Bax and Cox IV pixels was analyzed with Image J software (National Institutes of Health), and data are represented as the percentage of colocalized pixels in an image area.

Evaluation of Plasma and Intrahepatic Cytokine Concentrations. The plasma concentration of active PAI-1 was measured with a commercially available enzyme-linked immunosorbent assay kit (Molecular Innovations, Southfield, MI), following the manufacturer's instructions. The plasma concentrations of interferon- γ , IL-1 β , IL-2, IL-4, IL-6, IL-10, IL-12 (p70), keratinocyte chemoattractant (KC), macrophage inflammatory protein (MIP)-1 α , regulated upon activation normal T cell expressed and secreted (RANTES), TNF- α , and VEGF were measured with a custom Milliplex MAP kit for mouse cytokines (Millipore Corporation, Billerica, MA) using the Bio-Plex 200 System (Bio-Rad Laboratories). For determination of hepatic cytokine concentrations, livers were homogenized in 0.1% Triton X-100 in PBS containing Halt Protease and Phosphatase inhibitors (Thermo Fisher Scientific), and proteins were quantified by the bicinchoninic acid assay. Concentrations of KC and RANTES were determined by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) and normalized to protein concentrations of the samples.

Statistical Analyses. All data are represented as mean $\pm$ S.E.M. Data that were not normally distributed were transformed via Box-cox power transformation. Two- or three-way analysis of variance was used as appropriate, and multiple comparisons were evaluated

Fig. 3. Time course of APAP-induced liver injury in HIF-1 α -deficient mice. HIF-1 α ^{+/+/fl} or HIF-1 α ^{Δ/Δ} mice were treated with APAP (400 mg/kg) or SAL, and plasma and liver samples were taken 2, 6, and 24 h later. A, liver injury was assessed from plasma ALT activity; data represent means $\pm$ S.E.M. of $n = 3-8$ animals per group. a indicates significantly different from SAL-treated mice; b indicates significantly different from APAP-treated HIF-1 α ^{+/+/fl} mice; c indicates significantly different from the same treatment at 2 h. B, livers were processed for histology and stained with hematoxylin and eosin. Sections are shown from mice with ALT values near the median.

TABLE 2
Hepatic BNIP3 mRNA expression after APAP overdose
HIF-1α^{+fl/+fl} and HIF-1α^{Δ/Δ} mice were treated with SAL or APAP then killed at 2, 6, or 24 h. Real-time PCR was used to analyze liver tissue for expression of BNIP3 mRNA. For each sample, the copy number of BNIP3 was normalized to that of HPRT, then further normalized to SAL-treated HIF-1α^{+fl/+fl} mice. Data represent mean BNIP3/HPRT/SAL ratio ± S.E.M. of n = 3–6 animals.

Mouse	Real-Time PCR (BNIP3/HPRT versus SAL in HIF-1α ^{+fl/+fl})					
	SAL			APAP		
	2 h	6 h	24 h	2 h	6 h	24 h
HIF-1α ^{+fl/+fl}	1.0 ± 0.02	1.0 ± 0.02	1.0 ± 0.1	0.72 ± 0.13	0.33 ± 0.04	0.4 ± 0.7 ^a
HIF-1α ^{Δ/Δ}	0.78 ± 0.1	0.77 ± 0.1	1.2 ± 0.24	2.1 ± 0.5 ^{a,b}	0.77 ± 0.26	0.32 ± 0.1 ^a

^a Significantly different from SAL-treated mice of the same genotype.
^b Significantly different from corresponding HIF-1α^{+fl/+fl} mice.

statistically with appropriate post hoc tests. *P* < 0.05 was the criterion for significance.

Results

Effect of Conditional Deletion of HIF-1α Gene on Liver HIF-1α Expression. Conditional HIF-1α knockout mice were generated by mating HIF-1α^{fllox/fllox} mice (Ryan et

al., 1998) with transgenic mice that express the Cre-recombinase transgene (Ruzankina et al., 2007) under the control of the ubiquitin C promoter, and the HIF-1α gene was inactivated upon TAM treatment (Fig. 1A). Mice treated with TAM (HIF-1α^{Δ/Δ} mice) displayed no obvious phenotypic differences compared with control animals. There was significantly less expression of HIF-1α mRNA in HIF-1α^{Δ/Δ} mice compared with HIF-1α^{+fl/+fl} controls (Fig. 1B), demonstrating effective HIF-1α deletion. At 24 h, APAP overdose increased expression of HIF-1α mRNA at 24 h by 437-fold in HIF-1α^{+fl/+fl}. In HIF-1α^{Δ/Δ} mice, there was a modest increase in HIF-1α mRNA expression (by 26-fold) at 24 h. Expression of HIF-1α protein in livers was evaluated immunohistochemically 2 h after SAL or APAP treatment. SAL-treated HIF-1α^{+fl/+fl} mice had minimal staining, whereas HIF-1α staining was not observed in HIF-1α^{Δ/Δ} mice (Fig. 1C). APAP overdose increased hepatocellular HIF-1α stain-

Fig. 4. Mitochondrial Bax translocation. Mice were treated with SAL or 400 mg/kg APAP, and 6 h later liver samples were taken. A, quantification of Bax: Cox IV colocalization was performed as described under Materials and Methods. a indicates significantly different from SAL-treated mice. There were no significant differences between SAL-treated HIF-1α^{+fl/+fl} and HIF-1α^{Δ/Δ} mice, so they were combined for statistical purposes. B, representative ×60 confocal fluorescent micrographs of frozen liver sections from two to three animals per group. DAPI, 4,6-diamidino-2-phenylindole.

Fig. 5. DNA fragmentation after APAP treatment. DNA fragmentation was evaluated by the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay in HIF-1α^{+fl/+fl} and HIF-1α^{Δ/Δ} mice treated with SAL or 400 mg/kg APAP 6 or 24 h earlier. Magnification: ×200.

ing in HIF-1 $\alpha^{+/n/+n}$ mice; the staining seemed to be cytoplasmic in most cells, with some nuclear staining. This supports previous evidence that APAP overdose caused HIF-1 α accumulation in mouse liver (James et al., 2006; Chaudhuri et al., 2011). There was markedly less HIF-1 α staining in HIF-1 $\alpha^{\Delta/\Delta}$ mice treated with APAP, confirming successful deletion of HIF-1 α .

HIF-1 α Inactivation Protects from Early APAP Hepatotoxicity. To determine whether TAM treatment could affect APAP hepatotoxicity, UBC-Cre-ERT2(–/–)/HIF-1 $\alpha^{\text{lox/lox}}$ mice, which do not express a functional Cre recombinase and cannot remove HIF-1 α , were treated with OIL or TAM for 5 days, and 3 weeks later were treated with SAL or 400 mg/kg APAP. Both OIL- and TAM-treated mice developed severe liver injury 6 h after treatment, indicating that TAM alone did not

affect APAP hepatotoxicity (Fig. 2). In contrast, when UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice, which are capable of TAM-induced Cre recombination, underwent the same treatments OIL-treated UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice (HIF-1 α -sufficient) developed severe liver injury 6 h after treatment, but injury was essentially absent in TAM-treated UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice (HIF-1 α -deficient) (Fig. 2), indicating that the acute liver injury depended on HIF-1 α signaling. All subsequent experiments were performed in UBC-Cre-ERT2(+/-)/HIF-1 $\alpha^{\text{lox/lox}}$ mice.

Time Course of APAP Hepatotoxicity. HIF-1 $\alpha^{+/n/+n}$ mice treated with APAP had significantly greater plasma ALT activity at 2 h compared with SAL-treated animals. The former developed severe liver injury by 6 h, which continued to increase through 24 h (Fig. 3A). APAP-treated HIF-1 $\alpha^{\Delta/\Delta}$

Fig. 6. Effect of HIF-1 α deletion on thrombin production and fibrin deposition. SAL or 400 mg/kg APAP was administered to HIF-1 $\alpha^{+/n/+n}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice, and plasma and liver samples were taken 2, 6, and 24 h later. A, plasma TAT dimer was measured as a marker of thrombin generation. Frozen liver samples were stained immunohistochemically for fibrin. B, quantification of fibrin. a indicates significantly different from SAL-treated mice; b indicates significantly different from APAP-treated HIF-1 $\alpha^{+/n/+n}$ mice; c indicates significantly different from the same group at 2 and 6 h. C, representative liver sections from HIF-1 $\alpha^{+/n/+n}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice treated with APAP.

TABLE 3

Hepatic PAI-1 mRNA expression after APAP overdose in HIF-1 $\alpha^{+/n/+n}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice

HIF-1 $\alpha^{+/n/+n}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice were treated with SAL or APAP then killed after 2, 6, or 24 h. Real-time PCR was used to analyze liver tissue for the expression of PAI-1 mRNA. For each sample, the copy number of PAI-1 was normalized to that of HPRT. Data represent mean of the PAI-1/HPRT ratio $\pm$ S.E.M. of $n = 3-6$.

Mouse	Real-Time PCR (PAI-1/HPRT ratio) $\times 100$					
	SAL			APAP		
	2 h	6 h	24 h	2 h	6 h	24 h
HIF-1 $\alpha^{+/n/+n}$	1 $\pm$ 0.5	1.0 $\pm$ 0.5	0.03 $\pm$ 0.1	16 $\pm$ 7 ^a	14 $\pm$ 7 ^a	12 $\pm$ 4 ^a
HIF-1 $\alpha^{\Delta/\Delta}$	0.1 $\pm$ 0.03 ^b	0.2 $\pm$ 0.03 ^b	0.6 $\pm$ 0.2	26 $\pm$ 20 ^a	2.9 $\pm$ 1 ^b	11 $\pm$ 5 ^a

^a Significantly different from SAL-treated mice of the same genotype.

^b Significantly different from corresponding HIF-1 $\alpha^{+/n/+n}$ mice.

Fig. 7. Effect of HIF-1 α deletion on PAI-1 production. APAP or SAL (400 mg/kg) was administered to HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice, and plasma samples were taken after 2, 6, or 24 h. Active PAI-1 protein was measured in plasma, and data represent means $\pm$ S.E.M. of $n = 3-8$ animals per group. a indicates significantly different from SAL-treated mice; b indicates significantly different from APAP-treated HIF-1 $\alpha^{+/+}$ mice.

mice had complete attenuation of liver injury at 2 and 6 h; however, plasma ALT activity was the same as in HIF-1 $\alpha^{+/+}$ mice at 24 h. Histological analysis confirmed centrilobular hepatocellular necrosis in HIF-1 $\alpha^{+/+}$ mice at 6 and 24 h after APAP overdose, and HIF-1 $\alpha^{\Delta/\Delta}$ mice had no necrosis at 6 h but significant lesions at 24 h (Fig. 3B). Hepatic GSH depletion was used as an indication of APAP bioactivation. HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice treated with SAL had 4.4 ± 0.5 and 5.3 ± 0.6 μ mol GSH/g liver, respectively. After APAP administration, GSH concentration was reduced in HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice to 0.4 ± 0.02 and 1.52 ± 1.1 μ mol/g liver, respectively; these values were not significantly different from one another.

Expression of Cell Death Proteins. The contribution of HIF-1 α to production of cell death proteins in APAP overdose was evaluated. APAP overdose did not affect the expression of BNIP3 mRNA in liver (Table 2), nor did it alter hepatic expression of BNIP3 protein (data not shown). Bax is a proapoptotic protein that translocates to the mitochondria upon activation and contributes to APAP-induced hepatocellular necrosis (Bajt et al., 2008a). In the livers of HIF-1 $\alpha^{+/+}$ mice, APAP overdose increased the colocalization of Bax with Cox IV, a mitochondrial marker (Fig. 4). In contrast this effect was not observed in APAP-treated HIF-1 $\alpha^{\Delta/\Delta}$ mice. APAP overdose caused DNA fragmentation in centrilobular hepatocytes in HIF-1 $\alpha^{+/+}$ mice at 6 and 24 h; this effect was attenuated upon HIF-1 α deletion (Fig. 5).

HIF-1 α Deletion Attenuates Coagulation System Activation. Thrombin-antithrombin (TAT) concentration reflects the activation of thrombin in plasma. In HIF-1 $\alpha^{+/+}$ mice, plasma TAT concentration was significantly increased by APAP overdose 2, 6, and 24 h after treatment, although it was somewhat less at 24 h. In HIF-1 $\alpha^{\Delta/\Delta}$ mice, plasma TAT was elevated 2 h after APAP, returned to baseline at 6 h, then increased significantly by 24 h to a concentration greater than the value in HIF-1 $\alpha^{+/+}$ mice (Fig. 6A). A consequence of thrombin activation in liver is deposition of fibrin, which was assessed by immunohistochemical staining. In HIF-1 $\alpha^{+/+}$ mice, fibrin deposition was detected at 6 h after overdose and increased sig-

nificantly by 24 h. In contrast, there was no fibrin deposition in HIF-1 $\alpha^{\Delta/\Delta}$ mice detected until 24 h after APAP (Fig. 6B). In HIF-1 $\alpha^{+/+}$ mice, fibrin deposition at 6 h seemed to be centrilobular and sinusoidal after APAP overdose (Fig. 6C).

The fibrinolytic system consists of plasminogen and the plasminogen activators tPA and uPA, which cleave plasminogen to plasmin to dissolve fibrin clots. PAI-1 is the endogenous inhibitor of PAs, and elevation of active PAI-1 in plasma suggests inhibition of fibrinolysis. Hepatic PAI-1 mRNA was measured 2, 6, and 24 h after treatment. In SAL-treated mice, basal PAI-1 mRNA expression was small (Table 3). In HIF-1 $\alpha^{+/+}$ mice treated with APAP, hepatic PAI-1 mRNA was elevated by more than 10-fold as early as 2 h after APAP and remained so through 24 h. In HIF-1 $\alpha^{\Delta/\Delta}$ mice, PAI-1 mRNA increased to the same level as HIF-1 $\alpha^{+/+}$ mice 2 h after APAP, then decreased to baseline at 6 h only to increase again by 24 h. The circulating concentration of active PAI-1 was also evaluated at 6 and 24 h. In HIF-1 $\alpha^{+/+}$ mice, APAP overdose increased the appearance of active PAI-1 in plasma at 6 h, and PAI-1 concentration increased further by 24 h (Fig. 7). This increase in PAI-1 was attenuated in HIF-1 $\alpha^{\Delta/\Delta}$ mice at 6 h but was similar to that seen in HIF-1 $\alpha^{+/+}$ mice by 24 h.

The Role of HIF-1 α in the Inflammatory Response to APAP. Plasma concentrations of cytokines were measured 6 h after APAP exposure. Neither HIF-1 α deletion nor APAP overdose affected the plasma concentrations of IL-1 β , IL-2, IL-4, TNF α , MIP-1 α , or VEGF at this time (Table 4). APAP overdose increased plasma concentrations of IL-6, RANTES, and KC in HIF-1 $\alpha^{+/+}$ mice, and these increases were significantly attenuated upon HIF-1 α deletion (Table 4). Plasma concentrations of IL-6, KC, and RANTES were evaluated 24 h after APAP treatment. APAP overdose increased IL-6 (Fig. 8A) and KC (Fig. 8B) in both HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice at 24 h; however, there were no changes in RANTES (data not shown). Intrahepatic concentrations of KC and RANTES were also determined. Hepatic RANTES was not altered by APAP (Fig. 8C), but hepatic KC was significantly increased at 6 and 24 h after

TABLE 4

Cytokine concentrations in plasma of APAP-treated HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice

HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice were treated with SAL or APAP, and plasma was collected 6 h after administration and analyzed for cytokine concentrations using bead array.

Cytokine	Plasma Cytokine			
	SAL		APAP	
	HIF-1 $\alpha^{+/+}$	HIF-1 $\alpha^{\Delta/\Delta}$	HIF-1 $\alpha^{+/+}$	HIF-1 $\alpha^{\Delta/\Delta}$
	pg/ml			
Interferon- γ	15.9 $\pm$ 1.0	17.3 $\pm$ 1.4	17.4 $\pm$ 1.5	17.6 $\pm$ 0.5
IL-1 β	6.1 $\pm$ 0.5	6.2 $\pm$ 0.3	6.9 $\pm$ 0.6	6.5 $\pm$ 0.5
IL-2	4.0 $\pm$ 0.4	3.8 $\pm$ 0.2	5.8 $\pm$ 0.9	4.4 $\pm$ 0.3
IL-4	4.8 $\pm$ 0.3	5.0 $\pm$ 0.5	4.5 $\pm$ 0.4	4.2 $\pm$ 0.3
IL-6	23.5 $\pm$ 3.0	27.8 $\pm$ 3.7	231 $\pm$ 45.1 ^a	38.2 $\pm$ 5.6 ^b
IL-10	10.9 $\pm$ 0.4	11.6 $\pm$ 0.4	14.0 $\pm$ 1.4	11.5 $\pm$ 0.2
IL-12 (p70)	14.8 $\pm$ 0.6	15.4 $\pm$ 0.2	14.6 $\pm$ 0.5	14.4 $\pm$ 0.3
KC (mIL-8)	49.8 $\pm$ 7.4	40.3 $\pm$ 8.0	573 $\pm$ 372 ^a	110 $\pm$ 25.5 ^b
MIP-1 α	11.5 $\pm$ 0.5	12.1 $\pm$ 0.3	12.4 $\pm$ 0.7	12.0 $\pm$ 0.3
RANTES	28.5 $\pm$ 4.0	27.3 $\pm$ 1.5	52.9 $\pm$ 10.9 ^a	33.2 $\pm$ 3.2 ^b
TNF α	17.9 $\pm$ 0.4	18.4 $\pm$ 0.5	20.0 $\pm$ 1.0	18.3 $\pm$ 0.4
VEGF	19 $\pm$ 0.4	20.5 $\pm$ 0.6	19.1 $\pm$ 0.6	19.5 $\pm$ 0.6

^a Significantly different from SAL-treated HIF-1 $\alpha^{+/+}$ mice.

^b Significantly different from APAP-treated HIF-1 $\alpha^{+/+}$ mice.

Fig. 8. Hepatic and plasma concentration of cytokines. Plasma concentrations of IL-6 and KC were measured at 24 h, liver lysates were prepared, and the concentrations of KC and RANTES were determined 6 and 24 h after SAL or APAP. Data represent means $\pm$ S.E.M. of $n = 3-8$ animals per group. A, plasma IL-6 concentration at 24 h. B, plasma KC concentration at 24 h. C, hepatic RANTES concentration. D, hepatic KC concentration. a indicates significantly different from SAL-treated mice; b indicates significantly different from APAP-treated HIF-1 $\alpha^{+/+}$ mice; c indicates significantly different from the same treatment at 6 h.

APAP overdose in HIF-1 $\alpha^{+/+}$ mice and by 24 h in HIF-1 $\alpha^{\Delta/\Delta}$ mice (Fig. 8D). KC is a chemokine important for PMN infiltration, so hepatic PMNs were quantified. There were significantly fewer PMNs in the livers of HIF-1 $\alpha^{\Delta/\Delta}$ mice 6 and 24 h after APAP administration compared with HIF-1 $\alpha^{+/+}$ mice (Fig. 9).

Discussion

HIF-1 α deletion protected mice from early APAP-induced liver injury, but it did not prevent the development of severe liver injury 24 h after overdose (Fig. 3). The protection from toxicity at 2 and 6 h was not caused by decreased bioactivation, because the depletion of GSH was similar in HIF-1 $\alpha^{+/+}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice. These data suggest that HIF-1 α has dual roles in the pathogenesis of APAP-induced liver injury. HIF-1 α seems to have a damaging role in early progression of injury, possibly through its contribution to insertion of Bax into the mitochondria (Fig. 4), hemostasis (Figs. 6 and 7), and/or the inflammatory response (Table 4 and Figs. 8 and 9). The loss of protection at 24 h suggests that HIF-1 α has a protective role at later times, or that its absence delays the onset of liver injury. The former suggestion is consistent with a recently published report indicating that hepatocytes exposed to moderate hypoxia were protected from APAP-induced cell death (Yan et al., 2010). The protective effect of hypoxia was attributed to hypoxic preconditioning, because HIF-1 α can induce the transcription of protective factors, such as heme oxygenase-1 or erythropoietin (Bernhardt et al., 2007). Furthermore, Kato et al. (2011) found that the HIF-1 α -regulated gene VEGF is important in liver repair from APAP overdose.

APAP overdose increased hepatic HIF-1 α protein at 2 h in HIF-1 $\alpha^{+/+}$ mice (Fig. 1C), an effect that was attenuated in

HIF-1 $\alpha^{\Delta/\Delta}$ mice. This is consistent with published reports that APAP overdose caused nuclear accumulation of HIF-1 α in liver extracts and isolated mouse hepatocytes 1 h after treatment, an effect that was maintained through 12 h (James et al., 2006; Chaudhuri et al., 2011). HIF-1 α accumulation occurred before the development of hypoxia in the liver (Chaudhuri et al., 2011), suggesting that the initial mechanism by which HIF-1 α is stabilized is independent of hypoxia. However, the coagulation system is activated and fibrin deposits appear in the liver beginning 2 h after administration of APAP (Ganey et al., 2007), and tissue hypoxia becomes apparent between 2 and 4 h (Chaudhuri et al., 2011); accordingly, coagulation-dependent hypoxia could contribute to prolonged stabilization of HIF-1 α during the progression of liver injury.

APAP overdose caused HIF-1 α -dependent translocation of Bax to the mitochondrial membrane (Fig. 4). APAP overdose causes c-Jun NH₂-terminal kinase-dependent Bax insertion into the mitochondrial membrane beginning 1 h after treatment (Saito et al., 2010), and Bax(-/-) mice were protected from APAP hepatotoxicity at 6 h, but not 12 h (Bajt et al., 2008a). Bajt et al. hypothesized that Bax contributes to early mitochondrial permeability transition formation and release of mitochondrial intermembrane proteins that initiate DNA fragmentation and hepatocellular necrosis, but continuous oxidative stress supplants this mechanism to cause cell damage at later times. Our observation that HIF-1 $\alpha^{\Delta/\Delta}$ mice had reduced Bax translocation (Fig. 4) at 6 h after APAP is consistent with this hypothesis. In addition, APAP-induced DNA fragmentation was attenuated in HIF-1 $\alpha^{\Delta/\Delta}$ mice compared with HIF-1 $\alpha^{+/+}$ animals (Fig. 5). These data suggest that HIF-1 α is necessary for early Bax translocation and DNA

Fig. 9. Hepatic PMN accumulation after APAP treatment in HIF-1 $\alpha^{+/+/fl}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice. **A**, PMNs were quantified in 20 randomly selected high-power fields (HPF; $\times 400$). Data represent means $\pm$ S.E.M. of $n = 3$ –8 animals per group. **a** indicates significantly different from SAL; **b** indicates significantly different from APAP-treated HIF-1 $\alpha^{+/+/fl}$ mice; **c** indicates significantly different from the same group at 6 h. **B**, representative sections from HIF-1 $\alpha^{+/+/fl}$ and HIF-1 $\alpha^{\Delta/\Delta}$ mice.

fragmentation; however, other APAP-induced signaling overcomes this protection by 24 h.

In addition to its role in cell death signaling, HIF-1 α might contribute to APAP-induced liver injury by modulating the hemostatic system. APAP overdose activates the coagulation system and results in sinusoidal fibrin deposition in mice (Ganey et al., 2007), and it is associated with alterations in plasma hemostatic factors in humans (James et al., 2002). Furthermore, reduction in coagulation attenuated liver injury 6 h, but not 24 h, after APAP overdose in mice (Ganey et al., 2007), similar to our current finding in HIF-1 $\alpha^{\Delta/\Delta}$ mice (Fig. 3). In the absence of HIF-1 α expression, there was significant attenuation of thrombin generation (Fig. 6A) and fibrin deposition (Fig. 6B) at 6 h. By 24 h, thrombin generation in HIF-1 $\alpha^{\Delta/\Delta}$ mice had exceeded that seen in HIF-1 $\alpha^{+/+/fl}$ mice at the same time, and there was significant sinusoidal fibrin (Fig. 6B). This raises the possibility that the protection afforded by HIF-1 α deletion is mediated by its ability to delay thrombin generation and fibrin deposition, thereby delaying the development of liver injury. However, it is also possible that in the absence of liver injury in HIF-1 $\alpha^{\Delta/\Delta}$ mice at 2 and 6 h there is not a stimulus for activation of thrombin.

HIF-1 α also plays a role in fibrinolysis through the regulation of PAI-1 expression (Coppole et al., 2009). In the present

study, hepatic PAI-1 mRNA and plasma protein were elevated at all times measured in APAP-treated HIF-1 $\alpha^{+/+/fl}$ mice (Table 3), consistent with previous findings (Ganey et al., 2007; Bajt et al., 2008b). In contrast, in HIF-1 $\alpha^{\Delta/\Delta}$ mice PAI-1 mRNA was elevated at 2 h, returned to baseline by 6 h, then increased to the same level as HIF-1 $\alpha^{+/+/fl}$ mice by 24 h (Table 3). The appearance of active PAI-1 in the plasma followed a similar pattern (Fig. 7). This result raises the possibility that during APAP overdose PAI-1 expression is a consequence of hepatocellular death and hemostasis, rather than caused by a direct regulatory role by HIF-1 α ; indeed other transcription factors such as *egr-1* and HIF-2 α contribute to PAI-1 expression (Coppole et al., 2009). PAI-1(–/–) mice had enhanced liver necrosis and increased mortality after administration of 200 mg/kg APAP compared with control animals, suggesting a protective role for PAI-1; the enhanced liver injury in PAI-1(–/–) mice was associated with decreased expression of proliferating cell nuclear antigen and was therefore attributed to delayed tissue repair (Bajt et al., 2008b).

Appropriate tissue repair is necessary for recovery from liver injury (Mehendale, 2005), and the HIF-1 α -regulated gene VEGF has been identified as an important mediator of tissue repair after APAP hepatotoxicity (Donahower et al., 2006; Kato et al., 2011). We found no increase in plasma VEGF at 6 h (Table 4), which is in contrast with previously published reports in which hepatic VEGF protein was increased starting 8 h after APAP overdose (Donahower et al., 2006; Kato et al., 2011). VEGF is produced by hepatocytes and acts locally on sinusoidal endothelial cells; therefore, it might not have reached detectable concentrations in plasma. VEGF plays an important role in hepatocyte regeneration and restoration of liver microvasculature, through activation of repair pathways (Donahower et al., 2006) and angiogenesis (Kato et al., 2011). Because VEGF is regulated by HIF-1 α , it is possible that the progression of liver injury between 6 and 24 h in HIF-1 $\alpha^{\Delta/\Delta}$ mice occurs because of the loss of regeneration and other repair mechanisms that are initiated by VEGF.

APAP overdose is associated with increases in inflammatory cytokines, and the role of HIF-1 α in the production of cytokines is well documented in other conditions. Mice with HIF-1 α -deficient monocytes produced less TNF α , IL-6, IL-12, IL-1 α , and IL-1 β in response to lipopolysaccharide compared with wild-type animals (Peyssonnaud et al., 2007). In human patients, large plasma concentrations of IL-6, IL-8, and monocyte chemoattractant protein-1 correlated with the severity of liver injury caused by APAP overdose (James et al., 2005). Furthermore, APAP overdose increased plasma concentrations of IL-1 β , IL-6, KC, monocyte chemoattractant protein-1, MIP-2, and TNF α in mice (Ishida et al., 2002, 2004; Masubuchi et al., 2003). In our study, APAP overdose caused an increase in plasma concentrations of IL-6, KC, and RANTES at 6 h in HIF-1 $\alpha^{+/+/fl}$ mice, which was attenuated by deletion of HIF-1 α (Table 4). In addition, APAP overdose increased hepatic concentration of KC 6 and 24 h after treatment in HIF-1 $\alpha^{+/+/fl}$ mice and 24 h after treatment in HIF-1 $\alpha^{\Delta/\Delta}$ mice (Fig. 7). KC is a chemokine important for the recruitment of PMNs to the liver. HIF-1 $\alpha^{\Delta/\Delta}$ mice had smaller plasma concentrations of KC and RANTES at 6 h (Table 4), which

was associated with fewer hepatic PMNs 6 and 24 h after APAP compared with HIF-1 α ^{+fl/+fl} mice (Fig. 6).

The role of PMNs in APAP-induced liver injury remains controversial (Jaeschke, 2008). There is evidence that PMNs promote liver injury (Liu et al., 2006; Jaeschke, 2008) in APAP overdose; however, more recent evidence suggests that they accompany the sterile inflammatory response but do not contribute to injury (Jaeschke, 2008; Williams et al., 2010). PMNs are necessary for the phagocytosis of necrotic hepatocytes in APAP hepatotoxicity (Lawson et al., 2000). HIF-1 α -deficient monocytes have reduced phagocytic capacity and reduced release of antimicrobial proteins and granule proteases such as elastase and cathepsin G (Cramer et al., 2003; Zinkernagel et al., 2007), raising the possibility that HIF-1 α ^{$\Delta\Delta$} mice might have reduced ability to phagocytose necrotic hepatocytes and thus reduced tissue repair capacity. This might explain why hepatocellular injury seems to return by 24 h.

In summary, deletion of HIF-1 α protects mice from the early progression of APAP-induced liver injury but does not afford lasting protection. At early times, HIF-1 α regulates Bax translocation to the mitochondria and consequent DNA fragmentation that results in hepatocellular necrosis. It also contributes to regulation of the coagulation and fibrinolytic systems, as well as the production of inflammatory mediators that can influence the pathogenesis of APAP-induced liver injury. At later times, HIF-1 α deletion does not protect from severe liver injury, possibly through regulation of factors that support tissue repair and regeneration, such as PMN infiltration, VEGF, and PAI-1. Our results suggest that HIF-1 α has dual roles in APAP-induced liver injury, promoting damage early and conferring protection later during the pathogenesis.

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Authorship Contributions

Participated in research design: Sparkenbaugh, Saini, LaPres, Luyendyk, Copple, Maddox, Ganey, and Roth.

Conducted experiments: Sparkenbaugh, Luyendyk, and Maddox.

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Performed data analysis: Sparkenbaugh.

Wrote or contributed to the writing of the manuscript: Sparkenbaugh, LaPres, Luyendyk, Copple, Ganey, and Roth.

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