

Nonalcoholic Steatosis and Steatohepatitis II. Cytochrome *P*-450 enzymes and oxidative stress

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Robertson, Graham, Isabelle Leclercq, and Geoffrey C. Farrell. Nonalcoholic Steatosis and Steatohepatitis. II. Cytochrome *P*-450 enzymes and oxidative stress. *Am J Physiol Gastrointest Liver Physiol* 281: G1135–G1139, 2001.—Oxidative stress is present in the liver of humans with steatosis and nonalcoholic steatohepatitis (NASH) and is a plausible mediator of cellular injury, inflammatory recruitment, and fibrogenesis. CYPs 2E1 and 4A are the microsomal oxidases involved with fatty acid oxidation. Both enzymes can reduce molecular oxygen to produce prooxidant species, which, if not countered efficiently by antioxidants, create oxidative stress. In this theme article, we present the evidence that, in the context of hepatic steatosis, CYPs 2E1 and 4A could generate the “second hit” of cellular injury, particularly when antioxidant reserves are depleted, and propose ways in which this could contribute to the pathogenesis of NASH.

nonalcoholic steatohepatitis; CYP2E1; CYP4A; microsomal lipid peroxidation; antioxidants

HEPATIC STEATOSIS IS THE SETTING for nonalcoholic steatohepatitis (NASH), but the pathogenesis of liver cell injury, inflammation, and hepatic fibrosis are unclear. One potential biochemical mechanism is oxidative stress (7, 9), defined as an imbalance between prooxidant and antioxidant chemical species with evidence of oxidative damage to cellular macromolecules; the mere demonstration of an increase in prooxidant pathways does not itself prove the operation of oxidative stress. Evidence of oxidative stress has been found in human livers showing steatosis or NASH (25); it is also a cardinal feature of alcoholic steatohepatitis (20) and experimental models of NASH (18, 28). The increased levels of free fatty acids present in the fatty liver provide a perpetuating and propagating mechanism for oxidative stress via the process of lipid peroxidation, with secondary damage to cellular membranes and key organelles such as the mitochondria.

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A present focus of research into the pathogenesis of NASH is whether biochemical processes that generate oxidative stress can initiate hepatocellular injury and secondary recruitment of inflammation. The alternative proposal is that inflammation could be the primary mediator of liver cell injury, in which case oxidative stress may be secondary to release of inflammatory mediators such as reactive oxygen species (ROS) and nitroradicals. Within hepatocytes, there are three potential sites at which prooxidant ROS can be generated: mitochondria, peroxisomes, and the smooth endoplasmic reticulum, which, on cell fractionation, forms the microsomes. The narrow focus of the present theme article is on microsomal lipid-oxidizing CYPs as prooxidants. However, it will be seen that the relationship of these enzymes and the generation of oxidative stress in NASH are highly dependent on additional factors including the antioxidant reserves of the cell and on other pathways of lipid oxidation in mitochondria and peroxisomes.

REGULATION OF CYP2E1 AND CYP4A

CYP2E1 and CYP4A are inducible hepatic microsomal cytochromes *P*-450 involved with hydroxylation of fatty acids, and both can initiate the autopropagative process of lipid peroxidation. Besides being highly inducible by ethanol, CYP2E1 is upregulated in the aberrant nutritional states of fasting, diabetes, and obesity as well as by a high-fat/low-carbohydrate diet. The increased circulating levels of ketone bodies and fatty acids observed in these seemingly disparate conditions may be directly involved in CYP2E1 induction.

Hormonal regulation of CYP2E1 is complex. Insulin has a repressive effect (30), whereas in leptin-deficient *ob/ob* mice, CYP2E1 expression is reduced (12, 19, 27). Depending on the inducing agent or physiological state, regulation of CYP2E1 operates at a variety of levels from transcriptional activation to posttranslational enzyme stability. Whatever the molecular mechanisms by which it is accomplished, high hepatic activity of CYP2E1 is associated with each of the factors commonly associated with NASH: obesity, diabetes, and hyperlipidemia. Most importantly, insulin resis-

tance, which seems to be the universal feature of clinical NASH (7, 25), leads directly to increased CYP2E1 by loss of the repressive effect of insulin.

CYP4A genes are partly controlled by peroxisome proliferator-activated receptor- α (PPAR α), a transcription factor that governs genes involved with fatty acid β -oxidation and transport. Microsomal oxidation is usually a relatively minor pathway of fatty acid disposal, but CYP4A proteins may be key intermediaries in an adaptive response to the perturbations of hepatic lipid metabolism that accompany fasting, diabetes, and overnutrition.

It is now emerging that the metabolic roles of CYP2E1 and CYP4A in lipid oxidation may be complementary and that this may lead to interactions in the regulation of the individual enzymes. Thus, although CYP2E1 appears to play some physiological role in lipid metabolism, it is not indispensable because *Cyp2e1* null mice do not display an obvious phenotype. However, they are more prone to develop hepatic steatosis, especially when challenged with a high-fat diet or ethanol (18, 26). They also have increased constitutive expression (26) and heightened inducibility of *Cyp4a* genes (18). In addition, the upregulation of PPAR α and PPAR α -responsive genes involved in lipid metabolism in *Cyp2e1* null mice indicates an interplay between CYP2E1 and PPAR α -mediated fatty acid homeostasis (26). The concept of overlap and/or redundancy in the role of CYP2E1 and CYP4As as microsomal lipoxygenases is supported by the increases in specific CYP4A genes observed in obese diabetic *ob/ob* mice and *fal/fa* Zucker rats in which CYP2E1 is downregulated (12). It appears that in situations of reduced CYP2E1 activity, there is a compensatory increase in CYP4As.

CYPS AS A SOURCE OF PROOXIDANTS IN LIVER CELLS

Microsomal cytochrome P-450 enzymes are capable of undergoing "futile cycling" in the absence of substrate to produce active oxygen species: superoxide anions, hydroxyl radicals, and hydrogen peroxides. CYP2E1 is particularly prone to do this. Because of the observation of loose coupling of the CYP redox cycle, CYP2E1 has been described as a "leaky" enzyme. The production of oxyradicals is the basis for the greater capacity for CYP2E1 to initiate NADPH-dependent lipid peroxidation (3, 14). Using CYP2E1-specific antibodies, Ekström and Engelman-Sundberg (11) demonstrated that CYP2E1 is a major microsomal source of hydrogen peroxide and NADPH-dependent lipid peroxidation. CYP4A enzymes are also capable of reducing oxygen to superoxide and H₂O₂ due to such uncoupled turnover (10).

Although this work establishes the biochemical capacity of CYP2E1 and CYP4A as leaky enzymes to generate prooxidants, it does not demonstrate that this process is operative in damaging intact cells. Unequivocal evidence for the involvement of microsomal CYP-derived prooxidants in causing cellular damage has

come from work in isolated liver cells. Primary hepatocytes isolated from animals treated with 4-methylpyrazole to induce CYP2E1 displayed heightened sensitivity to the toxicities of ethanol and the polyunsaturated fatty acid arachidonic acid. The mechanism involved CYP2E1-derived oxidative stress because either diallylsulfide, a CYP2E1 inhibitor, or the antioxidant vitamin E analog Trolox abrogated the toxicity (31).

Creation of liver cell lines that overexpress CYP2E1 confirm the direct link between the generation of prooxidants by microsomal pathways and cellular injury. Whereas much of the focus has been to define the role of CYP2E1 in ethanol hepatotoxicity, recent work has explored aspects of oxidative stress of relevance to NASH. Thus, in HepG2 cells overexpressing CYP2E1, but not in control cells, addition of arachidonic acid caused toxicity and cell death predominantly by apoptosis (6). The essential role of CYP2E1 in generation of ROS and lipoperoxides was demonstrated with specific CYP2E1 inhibitors that blocked oxidative stress and provided protection against cytotoxicity.

CELLULAR PROTECTION BY ANTIOXIDANTS

Experiments with CYP2E1-overexpressing cells also illustrate the critical relationship between prooxidants and antioxidants in production of oxidative stress and resultant cell injury. Thus, when levels of the key cellular antioxidant, reduced glutathione (GSH), were lowered by inhibiting GSH synthesis with buthionine sulfoximine (BSO), arachidonic acid toxicity was enhanced. Conversely, supplementing cellular defenses with a range of antioxidants, including vitamin E, conferred protection.

Overexpression of catalase also counteracted the effects of ROS generated by CYP2E1-mediated metabolism of arachidonic acid in the presence of iron (2). Targeting catalase to either the mitochondria or the cytosol was equally protective, suggesting that hydroperoxide causing mitochondrial injury during arachidonic acid oxidation in CYP2E1 overexpressing cells diffuses into the mitochondria rather than being locally produced within mitochondria. The critical role of antioxidants in protecting against CYP2E1-mediated toxicity is further demonstrated by the cellular damage that results from GSH depletion in the absence of other agents. Thus BSO treatment of CYP2E1-overexpressing cells impaired mitochondrial function and resulted in apoptosis (32). The disruption of mitochondrial membrane potential and resultant decline in cellular ATP levels reinforce the idea that mitochondria are a potential target for damage by CYP2E1-generated ROS.

CYP-MEDIATED UPREGULATION OF ANTIOXIDANT ENZYMES

As part of the adaptive response to overproduction of prooxidants, cells increase the expression of genes involved in antioxidant defenses. Hepatocyte-derived cell lines with high levels of CYP2E1, which exhibit in-

creased ROS in the absence of added toxins, also exhibited higher levels of GSH. It was shown that this compensatory increase in antioxidant pathways was due to upregulation of γ -glutamylcysteine synthetase (21), the rate-limiting step in GSH synthesis. Other antioxidant pathways were also induced, including GSH S-transferases and catalase (22). One consequence of this compensatory induction of antioxidants in CYP2E1-expressing cells is that they are actually more resistant to a range of oxidative insults (16, 22). On this basis, before cellular injury can occur in a liver that chronically expresses high levels of CYP2E1, there needs to be either a major increase in production of ROS, such as might accompany ethanol exposure or increased hepatic levels of free fatty acids, and/or an accompanying depletion of GSH or other antioxidant pathways.

EVIDENCE LINKING HEPATIC CYP2E1 AND/OR CYP4A PROTEINS TO OXIDATIVE STRESS IN NASH

In vitro studies conducted in CYP2E1-overexpressing hepatocyte cell lines indicate potential links between CYP2E1-dependent oxidative stress, GSH homeostasis, and mitochondrial damage leading to cell death. It is therefore of interest that hepatic CYP2E1 is consistently upregulated in both clinical and experimental NASH and that evidence of both oxidative stress and mitochondrial injury can be found (25).

Several of the factors that regulate hepatic CYP2E1 and CYP4A expression have been linked to the pathogenesis of hepatic steatosis and NASH (1, 7). Thus levels of hepatic CYP2E1 increase with type 2 diabetes, insulin resistance, central obesity, and fasting. However, unlike alcoholic liver disease in which hepatic CYP2E1 activity subsides within days of discontinuing toxic alcohol intake, CYP2E1 is persistently increased in the livers of patients with NASH (29). Furthermore, the distribution of CYP2E1 protein is in the perivascular (acinar zone 3) regions, corresponding to the site of maximal hepatocellular injury in NASH (4).

Hepatic CYP2E1 levels and activity are also strikingly increased in both rats and mice after intake of a lipid-rich diet deficient in methionine and choline (18, 28). There are corresponding reductions in other CYPs, particularly the most abundant form, CYP3A4. As in human liver, the distribution of CYP2E1 in experimental NASH corresponds to that of liver injury. Oxidative stress is profound in the rodent dietary model, with a 100-fold increase in hepatic levels of lipoperoxides and a 25% reduction in GSH levels. In key experiments with anti-CYP2E1 antiserum or chemical inhibitors of CYP2E1 activity, we demonstrated that CYP2E1 is the catalyst of microsomal lipid peroxidation in wild-type mice with methionine/choline-deficient (MCD) diet-induced NASH (18).

Other work has shown that CYP2E1 is not unique as the microsomal lipid peroxidase in generating lipid peroxides in NASH. Thus, in *Cyp2e1* nullizygous mice, there is a compensatory increase in expression of CYP4As, referred to earlier. Feeding the lipid-rich

MCD diet led to a further major increase in activity of CYP4A enzymes, which, by analogous experiments with CYP4A antibodies, were shown to participate as the major catalysts of lipid peroxidation. Therefore, CYP4A enzymes may contribute to microsomal oxidative stress in the absence of CYP2E1. A recent report of persistence of alcohol-induced liver injury in *Cyp2e1* null mice may be consistent with a similar replacement of CYP2E1 function (17).

Activation of PPAR α in other situations has also been implicated as leading to NASH. Mice lacking peroxisomal fatty acyl CoA oxidase (AOX), the rate-limiting enzyme in the peroxisomal β -oxidation spiral, develop florid NASH and mitochondrial injury (13). In the absence of AOX, long-chain fatty acids accumulate, causing induction of CYP4A via PPAR α -activation. Increased production of hydrogen peroxide generated by CYP4A-mediated oxidation of long-chain fatty acids causes cell injury and steatohepatitis. The involvement of CYP4A enzymes in this process is further supported by the demonstration that mice nullizygous for both PPAR α and AOX do not develop steatohepatitis or mitochondrial injury (15). Together, these findings are consistent with a role for PPAR α -induced genes, especially members of the CYP4A family, in determining severity of steatosis and necroinflammatory injury in livers with defective peroxisomal β -oxidation.

RELATIONSHIPS AMONG OXIDATIVE STRESS PRODUCED BY MICROSOMAL LIPID OXIDASES, LIVER CELL INJURY, INFLAMMATORY RECRUITMENT, AND HEPATIC FIBROGENESIS IN NASH

The nexus between CYP-induced oxidative stress and cellular injury in the face of fatty acid excess provides a plausible explanation for hepatocellular damage in NASH. However, oxidative stress also activates a suite of proinflammatory pathways including secretion of proinflammatory cytokines, such as tumor necrosis factor; chemokines, such as interleukin-8; and adhesion molecules ICAM-1, E-selectin, or P-selectin. The end products of lipid peroxidation are also potent chemoattractants for inflammatory cells (8). Clearly, this potential pathway toward incitement and perpetuation of hepatic inflammation in NASH should now be explored experimentally.

Although the inflammatory activity in NASH, with its obvious implications for cytokine-mediated activation of stellate cells and profibrotic pathways, is important in hepatic fibrogenesis, oxidative stress may also play a role. ROS are increasingly recognized as potential mediators of stellate cell activation (5). This has been partially explored by studies in CYP2E1-overexpressing hepatic stellate cell lines. In the absence of exogenous substrate, CYP2E1-dependent oxidative stress was associated with upregulation of collagen I; this could be further enhanced by GSH depletion and was reversed by antioxidants (23). Addition of CYP2E1 substrates such as ethanol or arachidonic acid to CYP2E1 expressing stellate cells activated collagen I gene expression to an even greater extent than oc-

curred spontaneously (24). However, whereas ethanol-derived prooxidants directly stimulated collagen I transcription, arachidonic acid required metabolism by the inducible cyclooxygenase, COX-2, before activating collagen I. This work shows that intracellular generation of ROS can be a signal for stellate cell activation and also confirms the potential for CYP2E1-derived oxidants to participate in this process.

CONCLUDING REMARKS AND DIRECTIONS FOR FUTURE STUDIES

Experimental NASH is strongly associated with hepatic microsomal lipid peroxidation and accumulation of lipid peroxides, which, at least in the *ob/ob* mouse, are not features of uncomplicated hepatic steatosis. CYP2E1 appears to be the inducible microsomal cytochrome P-450 usually involved with generating microsomal lipid peroxidation in humans, rats, and mice. However, it is not a unique catalyst for peroxidation of endogenous lipids, and in special circumstances, CYP4A enzymes can be an alternative pathway. Because factors that favor PPAR α -activation are usually operative in patients with NASH, it is now important to determine whether CYP4A is also expressed at increased levels in the liver of these patients.

It should also be noted that animal models of steatohepatitis are associated with reduced levels of GSH compared with appropriate controls. This could reflect either consumption of GSH in combating the chronically increased production of prooxidants or a pathogenic role of lowered GSH levels in predisposing to oxidative stress. The failure of GSH levels to increase in the presence of increased activity of CYP2E1 could be the result of dietary deficiency of sulfhydryl amino acids in the dietary model of NASH. Whereas dietary factors leading to GSH depletion have been proposed in alcoholic steatohepatitis and in steatohepatitis after jejunoileal bypass surgery, to date, there are no informative studies of hepatic antioxidant levels in patients with NASH. If lipid-oxidizing cytochrome P-450s are responsible for creating hepatic oxidative stress in NASH, the way in which this causes hepatocellular injury and mitochondrial damage requires further investigation. Finally, the proposal that the oxidative stress is also responsible for hepatic inflammation should be subject to critical analysis.

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REFERENCES

1. **Angulo P and Lindor KD.** Insulin resistance and mitochondrial abnormalities in NASH: a cool look into a burning issue. *Gastroenterology* 120: 1281–1285, 2001.
2. **Bai J and Cederbaum AI.** Adenovirus-mediated overexpression of catalase in the cytosolic or mitochondrial compartment protects against cytochrome P450 2E1-dependent toxicity in HepG2 cells. *J Biol Chem* 276: 4315–4321, 2001.
3. **Bell LC and Guengerich FP.** Oxidation kinetics of ethanol by human cytochrome P450 2E1. Rate-limiting product release accounts for effects of isotopic hydrogen substitution and cytochrome b5 on steady-state kinetics. *J Biol Chem* 272: 29643–29651, 1997.
4. **Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, and Bacon BR.** Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 94: 2467–2474, 1999.
5. **Casini A, Ceni E, Salzano R, Biondi P, Parola M, Galli A, Foschi M, Caligiuri A, Pinzani M, and Surrenti C.** Neutrophil-derived superoxide anion induces lipid peroxidation and stimulates collagen synthesis in human hepatic stellate cells: role of nitric oxide. *Hepatology* 25: 361–367, 1997.
6. **Chen Q, Galleano M, and Cederbaum AI.** Cytotoxicity and apoptosis produced by arachidonic acid in Hep G2 cells overexpressing human cytochrome P4502E1. *J Biol Chem* 272: 14532–14541, 1997.
7. **Chitturi S and Farrell GC.** Etiopathogenesis of nonalcoholic steatohepatitis. *Semin Liver Dis* 21: 27–41, 2001.
8. **Curzio M, Esterbauer H, Poli G, Biasi F, Cecchini G, Di Mauro C, Cappello N, and Dianzani MU.** Possible role of aldehydic lipid peroxidation products as chemoattractants. *Int J Tissue React* 9: 295–306, 1987.
9. **Day CP and James OF.** Steatohepatitis: a tale of two “hits”? *Gastroenterology* 114: 842–845, 1998.
10. **Dierks EA, Davis SC, and Ortiz de Montellano PR.** Glu-320 and Asp-323 are determinants of the CYP4A1 hydroxylation regioselectivity and resistance to inactivation by 1-aminobenzotriazole. *Biochemistry* 37: 1839–1844, 1999.
11. **Ekström G and Ingelman-Sundberg M.** Rat-liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450 IIE1). *Biochem Pharmacol* 38: 1313–1319, 1989.
12. **Enriquez A, Leclercq I, Farrell GC, and Robertson G.** Altered expression of hepatic CYP2E1 and CYP4A in obese, diabetic *ob/ob* mice, and *fa/fa* Zucker rats. *Biochem Biophys Res Commun* 255: 300–306, 1999.
13. **Fan CY, Pan J, Usuda N, Yeldandi AV, Rao MS, and Reddy JK.** Steatohepatitis, spontaneous peroxisomal proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisomal proliferator-activated receptor α natural ligand metabolism. *J Biol Chem* 273: 15639–15645, 1998.
14. **Gorsky LD, Koop DR, and Coon MJ.** On the stoichiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome P-450. Products of oxygen reduction. *J Biol Chem* 259: 6812–6817, 1984.
15. **Hashimoto T, Fujita T, Usuda N, Cook W, Qi C, Peters JM, Gonzalez FJ, Yeldandi AV, Rao MS, and Reddy JK.** Peroxisomal and mitochondrial fatty acid beta-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. *J Biol Chem* 274: 19228–19236, 1999.
16. **Huan JY and Koop DR.** Tightly regulated and inducible expression of rabbit CYP2E1 using a tetracycline-controlled expression system. *Drug Metab Dispos* 27: 549–554, 1999.
17. **Kono H, Bradford BU, Yin M, Sulik KK, Koop DR, Peters JM, Gonzalez FJ, McDonald T, Dikalova A, Kadiiska MB, Mason RP, and Thurman RG.** CYP2E1 is not involved in early alcohol-induced liver injury. *Am J Physiol Gastrointest Liver Physiol* 277: G1259–G1267, 1999.
18. **Leclercq IA, Farrell GC, Field J, Bell D, Gonzales FJ, and Robertson GR.** CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine non-alcoholic steatohepatitis. *J Clin Invest* 105: 1067–1075, 2000.
19. **Leclercq I, Field J, Enriquez A, Farrell GC, and Robertson G.** Constitutive and inducible expression of hepatic CYP2E1 and CYP4A in leptin-deficient *ob/ob* mice. *Biochem Biophys Res Commun* 268: 337–344, 2000.

20. **Lieber CS.** Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. *Adv Pharmacol* 38: 601–628, 1997.
21. **Mari M and Cederbaum AI.** CYP2E1 overexpression in HepG2 cells induces glutathione synthesis by transcriptional activation of gamma-glutamylcysteine synthetase. *J Biol Chem* 275: 15563–15571, 2000.
22. **Mari M and Cederbaum AI.** Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpressing HepG2 cells and protection against short-term oxidative stress. *Hepatology* 33: 652–661, 2001.
23. **Nieto N, Friedman SL, Greenwel P, and Cederbaum AI.** CYP2E1-mediated oxidative stress induces collagen type I expression in rat hepatic stellate cells. *Hepatology* 30: 987–996, 1999.
24. **Nieto N, Greenwel P, Friedman SL, Zhang F, Dannenberg AJ, and Cederbaum AI.** Ethanol and arachidonic acid increase alpha 2(I) collagen expression in rat hepatic stellate cells overexpressing cytochrome P450 2E1. Role of H₂O₂ and cyclooxygenase-2. *J Biol Chem* 275: 20136–20145, 2000.
25. **Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, and Clore JN.** Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120: 1183–1192, 2001.
26. **Wan YY, Cai Y, Li J, Yuan Q, French B, Gonzalez FJ, and French SW.** Regulation of peroxisome proliferator activated receptor alpha-mediated pathways in alcohol fed cytochrome P450 2E1 deficient mice. *Hepatology Res* 19: 117–130, 2001.
27. **Watson AM, Poloyac SM, Howard G, and Blouin RA.** Effect of leptin on cytochrome P-450, conjugation, and antioxidant enzymes in the ob/ob mouse. *Drug Metab Dispos* 6: 695–700, 1999.
28. **Weltman MD, Farrell GC, and Liddle C.** Increased hepatocyte CYP 2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* 111: 1645–1653, 1996.
29. **Weltman MD, Farrell GC, Hall P, Ingelman-Sundberg M, and Liddle C.** Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 27: 128–133, 1998.
30. **Woodcroft KJ and Novak RF.** Insulin effects on CYP2E1, 2B, 3A, and 4A expression in primary cultured rat hepatocytes. *Chem Biol Interact* 107: 75–91, 1997.
31. **Wu D and Cederbaum AI.** Ethanol and arachidonic acid produce toxicity in hepatocytes from pyrazole-treated rats with high levels of CYP2E1. *Mol Cell Biochem* 204: 157–167, 2000.
32. **Wu D and Cederbaum AI.** Removal of glutathione produces apoptosis and necrosis in HepG2 cells overexpressing CYP2E1. *Alcohol Clin Exp Res* 25: 619–628, 2001.

