Chapter 1
Cytochrome P450 2E1: Its Clinical Aspects and a Brief Perspective on the Current Research Scenario

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Abstract Research on Cytochrome P450 2E1 (CYP2E1), a key enzyme in alcohol metabolism has been very well documented in literature. Besides the involvement of CYP2E1 in alcohol metabolism as illustrated through the studies discussed in the chapter, recent studies have thrown light on several other aspects of CYP2E1 i.e. its extrahepatic expression, its involvement in several diseases and pathophysiological conditions; and CYP2E1 mediated carcinogenesis and modulation of drug efficacy. Studies involving these interesting facets of CYP2E1 have been discussed in the chapter focusing on the recent observations or ongoing studies illustrating the crucial role of CYP2E1 in disease development and drug metabolism.

Keywords Cytochrome P450 2E1 • Drugs • Reactive oxygen species • Diseases • Injury

1.1 Introduction

Cytochrome P450 2E1 (CYP2E1) is implicated in several diseases and is a key player in alcohol metabolism and oxidative stress (Gonzalez 2005; Brzezinski et al. 1999; Wu and Cederbaum 2005). CYP2E1 which is induced due to alcohol consumption plays a major role in human health due to its ability to bioactivate numerous hepatotoxins and metabolize alcohol (Koop 1992; Cederbaum 2010). The abundance of expression of CYP2E1 in liver and extrahepatic tissues holds importance

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keeping in view its role in generating oxidative stress (Joshi and Tyndale 2006a). Further, due to its ability to modulate the effects of drugs, CYP2E1 plays a crucial role in drug metabolism (Lieber 2004; Joshi and Tyndale 2006a).

1.1.1 Purification and Characterization of CYP2E1

The initial purification of CYP2E1 from rabbits led to its discovery in several other animal species and humans (Koop et al. 1982). CYP2E1 is primarily an endoplasmic reticulum resident protein (Lieber 2004; Konishi and Ishii 2007), but recent studies have also shown that CYP2E1 is also present in mitochondria (Bhagwat et al. 1995; Neve and Ingelman-Sundberg 1999; Robin et al. 2001). The localization of hepatic CYP2E1 is predominantly restricted to petrivenous region in liver (Bühler et al. 1991; Lieber 1993). Regulation of CYP2E1 occurs through transcriptional activation, mRNA stabilization, increased mRNA translatability and decreased protein degradation and the principal mechanism which controls the induction process depends on several factors such as the chemical nature of the inducer, the age, and the nutritional and hormonal status of the animal (Koop and Tierney 1990).

1.1.2 The Role of CYP2E1 as a Potent Source for Oxidative Stress

CYP2E1 catalyzes several oxidative biochemical reactions for which it requires NADPH and the incomplete reduction of oxygen in CYP2E1 catalyzed reactions leads to generation of free radical species (Wu and Cederbaum 2003). As a poor coupling link exists between NADPH-cytochrome P450 reductase and CYP2E1, CYP2E1 has been shown to exhibit increased NADPH oxidase activity which leads to generation of reactive oxygen species (ROS) (Ekström and Ingelman-Sundberg 1989; Gorsky et al. 1984; Cederbaum 2010). The ability of CYP2E1 to generate ROS such as the superoxide anion radical and hydrogen peroxide is enhanced in the presence of iron catalysts, and powerful oxidants such as the hydroxyl radical are generated (Lu and Cederbaum 2008). CYP2E1 dependent toxicity is closely linked to oxidative stress injury along with peroxynitrite, tumor necrosis factor alpha (TNF alpha), protein adducts and several other mechanisms which provide the complete picture (Reed 2004).

1.1.3 CYP2E1 and Ethanol-Mediated Oxidative Stress

Ethanol-mediated oxidative stress plays a crucial role in the development of liver injury due to alcohol consumption (Cederbaum 1991). Among several pathways which have been suggested to contribute to the ability of ethanol to induce a state of

oxidative stress, one central pathway seems to be the induction of CYP2E1 by ethanol (Lu and Cederbaum 2008).

Generation of ROS by CYP2E1 is a sequential process, as elegantly described by Koop, 2006 and illustrated in the following section (Koop 2006). The use of oxygen by CYP2E1 to metabolize alcohol, leads to generation of ROS by the following chain of events:

- Ethanol binds to the enzyme.
- As the first electron is passed to the heme of CYP2E1 and oxygen is bound, the electron can move and exist on the oxygen, essentially generating superoxide bound to the heme of CYP2E1. Occasionally, the superoxide will break down, releasing free superoxide and generating the starting enzyme.
- If the second electron is added to the enzyme, then a second form of reduced oxygen is produced that is identical to a heme-bound form of the two electron–reduced oxygen (i.e., peroxide).
- When this product breaks down, it picks up two hydrogens to generate hydrogen peroxide.
- The production of these ROS by CYP2E1 is referred to as an "uncoupled reaction" because the oxygen does not end up in the substrate.
- If the ROS remains bound, then the enzyme will transfer one oxygen atom to the substrate and the other atom becomes water, producing an unstable intermediate (i.e., a gem-diol) product that decomposes to acetaldehyde.

1.1.4 Importance of CYP2E1 in Health and Disease

Several pathophysiological conditions such as alcoholism, diabetes etc. and drug administration lead to the induction of CYP2E1 as illustrated by the studies in the following sections. Numerous targets exist for CYP2E1 mediated injury-DNA, protein, mitochondria, etc., thus disrupting the essential structural and functional integrity of the cell and the organism as a whole. CYP2E1 also modulates the actions of several drugs, thus altering their therapeutic efficacy, and it also activates xenobiotics to their carcinogenic forms, besides its multifarious toxic functions. Some of the drugs exerting their hepatotoxic effects through the involvement of CYP2E1 and discussed in the chapter have been summarized in Table 1.1. All the above stated factors underlie the role of CYP2E1 as an emerging player in health and disease.

Interindividual variability in the expression and functional activity of CYP2E1 has been observed (Neafsey et al. 2009) and genetic polymorphisms in CYP2E1 have been linked to altered susceptibility to several diseases (Trafalis et al. 2010). Also, chronic exposure to CYP2E1 inducers, such as ethanol, isoniazid, various solvents and chemicals, also increase the probability of developing malignancy, especially for carriers of certain CYP2E1 alleles (Trafalis et al. 2010).

Table 1.1 Drugs exerting hepatotoxic effects modulated through CYP2E1

Drug	Clinical usage	References
Geldanamycin	Anti-tumour	Dey and Cederbaum (2006)
Acetaminophen	Analgesic	Dai and Cederbaum (1995), Jones et al. (2002), Bae et al. (2001), Knockaert et al. (2011), Cheung et al. (2005), Chen et al. (2008, 2009), Bai and Cederbaum (2004), Lee et al. (2006a), and Abdelmegeed et al. (2010)
Cisplatin	Anti-cancer	Lu and Cederbaum (2006)
Sodium salicylate	Anti-inflammatory	Wu and Cederbaum (2001)
Chlorzoxazone	Muscle relaxant	Dupont et al. (1998), Cheung et al. (2005), Cummings et al. (1999, 2001), Carpenter et al. (1997), Raucy et al. (1997), Upadhya et al. (2000), Orellana et al. (2006), Tindberg and Ingelman-Sundberg (1996), Gebhardt et al. (1997), Lee et al. (2006c), Khemawoot et al. (2007a, b), Howard et al. (2001), Chalasani et al. (2003), Huan and Koop (1999), Varela et al. (2008), Zhukov and Ingelman-Sundberg (1999), Nedelcheva et al. (1999), Lerche et al. (1996), Lejus et al. (2002), Khalighi et al. (1999), Ramaiah et al. (2001), and Fairbrother et al. (1998)
Ciprofibrate	Peroxisome proliferator and anti-hyperlipidemic	Zangar et al. (1995)
Clofibrate	Lipid lowering agent	Cummings et al. (2001), Raucy et al. (2004), and Carpenter et al. (1996)
Isoniazid	Anti-tuberculosis	Park et al. (1993)
Sevoflurane	Anaesthetic	Hase et al. (2000)
Phenobarbital	Anti-convulsant	Lee et al. (2006c)
Pyridine	Precursor to pharmaceuticals	Cummings et al. (2001) and Lash et al. (2007)
Hydrazine	Precursor to pharmaceuticals	Runge-Morris et al. (1996)
Phenelzine	Anti-depressant and anxiolytic	Runge-Morris et al. (1996)

1.2 Research Studies Elucidating the Role of CYP2E1 in Disease and Drug Metabolism

The following sections deal with the studies related to the physiological, pharmacological and toxicological aspects of CYP2E1 which have been performed in laboratories of eminent scientists globally, thus stressing upon the importance of CYP2E1 in health and disease. Although the list of the studies discussed here is not complete and the studies which have not been represented do not account for lesser importance of the

findings but the emphasis is more on ongoing studies with CYP2E1 or studies dealing with the indispensable role of CYP2E1 in drug metabolism and disease development.

I

C. Lieber (1931–2009)

CYP2E1 as an Integral Component of the Microsomal Ethanol Oxidizing System and Its Physiologic and Pathologic Roles

Discovery of the Microsomal Ethanol Oxidizing System

The pioneering work of Dr. Charles Lieber and his group led to the discovery of CYP2E1 as an integral component of the microsomal ethanol oxidizing system (MEOS). The discovery of the proliferation of the smooth endoplasmic reticulum after chronic alcohol consumption, suggested the existence of an additional pathway for ethanol metabolism apart from alcohol dehydrogenase (ADH) which was described by Lieber and DeCarli, as the microsomal ethanol oxidizing system, involving cytochrome P450 (CYP) (Lieber and DeCarli 1968). The MEOS was distinct from the alcohol metabolizing enzymes- ADH and catalase and was a CYP dependent reaction (Teschke et al. 1972, 1974). Further, after chronic ethanol consumption, the activity of the MEOS increased with an associated rise in cytochrome P450 in rodents and humans (Ohnishi and Lieber 1977; Wrighton et al. 1986; Song et al. 1986) and the ethanol inducible cytochrome P450 was designated as CYP2E1 (Nelson et al. 1993).

Contributions of Other CYPs to MEOS

Using HepG2 cells heterologously expressing human CYP2E1, CYP1A2, and CYP3A4 and livers isolated from alcoholic patients and assessing their ethanol oxidation using selective inhibitors-4-methylpyrazole (CYP2E1), furafylline (CYP1A2), and troleandomycin (CYP3A4), it has been observed that the specific activities for ethanol oxidation in human liver microsomes follows the pattern: CYP2E1 > CYP1A2 > CYP3A4 (Salmela et al. 1998). Thus, in human liver microsomes, CYP2E1 plays the major role in the pathogenesis of alcoholic liver disease (Salmela et al. 1998). However, CYP1A2 and CYP3A4 contribute significantly to microsomal ethanol oxidation and may, therefore, also be involved in the pathogenesis of alcoholic liver disease (Salmela et al. 1998). The diseases or pathophysiological conditions associated with induction of CYP2E1 have been summarized in Table 1.2.

Development of Assay for the Measurement of Catalytic Activity of CYP2E1

A highly sensitive, simple assay for the determination of 4-nitrocatechol formed during the CYP2E1-dependent hydroxylation of p-nitrophenol utilizing high-performance liquid chromatography with electrochemical detection has been developed (Mishin et al. 1996).

Ethanol Inducible Hepatic CYP2E1: Evidences from Rodent and Human Models

In human subjects comprised of recently drinking alcoholics (<36 h), acinar regions of liver show elevated CYP2E1 transcripts with mRNA increase occurring mainly in perivenular cells (zone 3) and marked elevations in CYP2E1 protein

Table 1.2 Diseases or pathophysiological conditions associated with induction of CYP2E1 in animal and human models

Disease	References
Alcohol induced liver injury/alcohol induced liver cell toxicity	Salmela et al. (1998), Takahashi et al. (1993), Tsutsumi et al. (1993), Koivisto et al. (1996), Ma et al. (1991), Aleynik et al. (1999), Mi et al. (2000), Aleynik and Lieber (2001), Xu et al. (2005), Lieber et al. (2007b), Dai et al. (1993), Carroccio et al. (1994), Wu and Cederbaum (1993b, 1996, 2000), Kukielka and Cederbaum (1994), Lu et al. (2008, 2010), Bai and Cederbaum (2006), Nieto et al. (2000), Osna et al. (2003, 2005, 2007, 2008, 2009, 2010), Castillo et al. (1992), Gebhardt et al. (1997), Oneta et al. (2002), Wang et al. (2009), Curry-McCoy et al. (2010), Wan et al. (1995), Morimoto et al. (1993, 1994, 1995a, b), Gouillon et al. (1999, 2000), Bardag-Gorce et al. (2000, 2002, 2006), Donohue et al. (2006), Garige et al. (2005), Albano et al. (1996), Dupont et al. (1998), Clot et al. (1997), Vidali et al. (2007), Liu et al. (2001), Veeramachaneni et al. (2008), Nanji et al. (1993, 1994), Roberts et al. (1994, 1995), Jeong et al. (2000), Kim et al. (2006), Baumgardner et al. (2007), Otani et al. (2005), Raucy et al. (2004), Kunitoh et al. (1997), Niemelä et al. (1998, 1999), Esfandiari et al. (2005), Korourian et al. (1999), Rowlands et al. (2003), Ronis et al. (2010), Demeilliers et al. (2002), Robin et al. (2005), Knockaert et al. (2011), Howard et al. (2001, 2003a), Ferguson et al. (2011), Yue et al. (2009), Bailey et al. (2009), Carpenter et al. (1996, 1997), Sampey et al. (2003), Roede et al. (2008), Bradford et al. (2005), Bühler et al. (1991, 1992), Takahashi et al. (1992), Hu et al. (1994), Butura et al. (2009), Simi and Ingelman-Sundberg (1999), Clot et al. (1996), Lytton et al. (1999), Fang et al. (1998), French et al. (1993), and Chandrasekaran et al. (2011, 2012b)
Alcohol mediated neurotoxicity	Vasiliou et al. (2006), Howard et al. (2003a, b), Joshi and Tyndale (2006a, b), Zimatkin et al. (2006), Brzezinski et al. (1999), Yadav et al. (2006), Kapoor et al. (2006), Anandatheerthavarada et al. (1993), Bhagwat et al. (1995), Upadhya et al. (2000), and Tindberg and Ingelman-Sundberg (1996)
Alcohol mediated renal injury	Shankar et al. (2008)
NASH	Lieber et al. (2004a, b), Baumgardner et al. (2008), Leclercq et al. (2000b), Chalasani et al. (2003), Wang et al. (2008), Abdelmegeed et al. (2011), Varela et al. (2008), and Mantena et al. (2009)

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Table 1.2 (continued)

Disease	References
Diabetes	Wu and Cederbaum (1993a), Arinç et al. (2005), Wang et al. (2000), Sindhu et al. (2006), Zaluzny et al. (1990), Song et al. (1990), Raza et al. (2004), Woodcroft and Novak (1997), Arinç et al. (2005), Leclercq et al. (2000a), and Martínez-Chantar et al. (2002)
Chronic hepatitis C	Vidali et al. (2007), Rigamonti et al. (2009), Osna et al. (2008), and Otani et al. (2005)
NAFLD	Videla et al. (2004), Orellana et al. (2006), and Kathirvel et al. (2009)
Carcinogenesis	Wang et al. (2009), Millonig et al. (2011), Ghanayem et al. (2005a, b, c), Ghanayem (2007), Wang et al. (2002a, b), Hoffler et al. (2003, 2005), Garner et al. (2007), Garro et al. (1981), Lerche et al. (1996), Howard et al. (2001), Ma et al. (1991), Huan and Koop (1999), Roberts et al. (1995), Arinç et al. (2007), Zaluzny et al. (1990), Dey et al. (2002, 2005), Kapoor et al. (2006), Khan et al. (2011), Anandatheerthavarada et al. (1993), and Bhagwat et al. 1995
Methionine deficiency induced steatohepatitis	Martínez-Chantar et al. (2002) and Schattenberg et al. (2005)
Cigarette smoking	Micu et al. (2003), Lee et al. (2006b), Yue et al. (2009), Ferguson et al. (2011), and Joshi and Tyndale (2006a, b)
Maturity onset diabetes of the young (type 3 diabetes)	Cheung et al. (2003)
Hyperglycemia	Chandrasekaran et al. (2012a)
Hyperglycemia and alcoholism	Chandrasekaran et al. (2012b)
Parkinson's disease	Singh et al. (2008)
Alcoholic liver cirrhosis	Khan et al. (2009, 2010, 2011)
Head and neck squamous cell carcinoma	Ruwali et al. (2009)

content in both perivenular and midzonal (zone 2) hepatocytes (Takahashi et al. 1993). The tissue and organ specific expression of CYP2E1 has been summarized in Table 1.3.

Further, as observed in rats fed liquid diets containing 36% of total calories as ethanol, the *in vivo* induction of hepatic CYP2E1 protein by ethanol involves increased enzyme synthesis rather than decreased enzyme degradation (half life of 27–28 h) (Tsutsumi et al. 1993). This enhancement of *de novo* CYP2E1 synthesis most likely entails the ethanol-mediated increase of steady-state levels of CYP2E1 mRNA and/or the stimulation of its translational efficiency (Tsutsumi et al. 1993).

Table 1.3 Tissue and organ specific expression of CYP2E1 including transfected cell lines

Tissue/organ	References
Liver	Lieber and DeCarli (1968), Teschke et al. (1972, 1974), Ohnishi and Lieber (1977), Wrighton et al. (1986), Song et al. (1986), Salmela et al. (1998), Takahashi et al. (1993), Tsutsumi et al. (1993), Kessova et al. (1998, 2001), Ma et al. (1991), Aleynik et al. (1999), Aleynik and Lieber (2001), Lieber et al. (2004a, b, 2007a, b), Kukielka and Cederbaum (1994), Kessova and Cederbaum (2007), Wu and Cederbaum (1993a), Dey and Cederbaum (2007), Lu et al. (2005, 2008, 2010), Lu and Cederbaum (2006), Garro et al. (1981), Gebhardt et al. (1997), Wang et al. (2009), Morimoto et al. (1993, 1994), Morimoto et al. (1995a, b), Wan et al. (1995), Bardag-Gorce et al. (2000, 2002, 2005), Gouillon et al. (1999, 2000), Garige et al. (2005), Curry-McCoy et al. (2010), Vasiliou et al. (2006), Castillo et al. (1992), Albano et al. (1996), Dupont et al. (1998), Liu et al. (2001), Veeramachaneni et al. (2008), Wang et al. (2008), Nanji et al. (1993, 1994), Chang et al. (2006), Tierney et al. (1992), Runge-Morris et al. (1996), Videla et al. (2004), Fernández et al. (2003), Orellana et al. (2006), Varela et al. (2008), Asai et al. (1996), Kunitoh et al. (1997), Jeong et al. (2000), Roberts et al. (1994, 1995), Abdelmegeed et al. (2010, 2011), Khemawoot et al. (2007a), Park et al. (1993), Baumgardner et al. (2007, 2008), Lejus et al. (2002), Arinç et al. (2005, 2007), Wang et al. (2002a), Morgan et al. (2002), Kathirvel et al. (2009, 2010), Niemelä et al. (1999), Esfandiari et al. (2005), Sindhu et al. (2006), Cheng et al. (2003), Leclercq et al. (2000a, b), Korourian et al. (1999), Morgan (1993), Sewer et al. (1998), Sewer and Morgan (1998), Chen et al. (1999), Ronis et al. (2010), Robin et al. (2005), Martínez-Chantar et al. (2002), Raza et al. (2004), Howard et al. (2001), Micu et al. (2003), Lee et al. (2006b), Ferguson et al. (2011), Starkel et al. (2000), Vasiliou et al. (2006), Zaluzny et al. (1990), Bradford et al. (2005), Mantena et al. (2009), Bailey et al. (2009), Cheung et al. (2003, 2005), Chalasani et al. (2003), Andersen et al. (1998), Sampey et al. (2003), Roede et al. (2008), Carpenter et al. (1996), Wang et al. (2000), Ramaiah et al. (2001), Chilakapati et al. (2007), Lee et al. (2006c), Bühler et al. (1991, 1992), Takahashi et al. (1992), Hu et al. (1994), Johansson et al. (1990), Ronis et al. (1991), Neve and Ingelman-Sundberg (2001), Terelius et al. (1991), Albano et al. (1995), Gut et al. (1996), Nedelcheva et al. (1999), Clot et al. (1996), Lytton et al. (1999), Fang et al. (1998), Eliasson et al. (1992), Zhukov et al. (1993), French et al. (1993), Qu et al. (2009), Niemelä et al. (1998), and Zaluzny et al. (1990)
Prenatal liver	Khalighi et al. (1999) and Brzezinski et al. (1999)
Fetal liver	Carpenter et al. (1997) and Wu and Cederbaum (1993b)
Liver mitochondria	Lieber et al. (2007b), Demeilliers et al. (2002), Robin et al. (2001), and Mantena et al. (2009)

(continued)

Table 1.3 (continued)

Tissue/organ	References
Hepatocytes	Mi et al. (2000), Wu and Cederbaum (2000, 2001), Caro and Cederbaum (2001), Wang et al. (2009), Clot et al. (1997), Zangar et al. (1995), Osna et al. (2009), Kraner et al. (1993), Raucy et al. (2004), Woodcroft and Novak (1997, 1999), Woodcroft et al. (2002), Abdelmegeed et al. (2005), Abdel-Razzak et al. (1993), Morgan et al. (1994), Robin et al. (2005), Lash et al. (2007), Sidhu et al. (2001), Ronis et al. (1991), Butura et al. (2009), Johansson et al. (1991), and Eliasson et al. (1992)
Rat hepatocyte cell line RALA255-10G	Liu et al. (2002), Jones et al. (2002), Singh et al. (2009), and Schattenberg et al. (2004, 2005)
Co-culture of rat liver epithelial cells (RLECs) and hepatocytes	Lerche et al. (1996)
HepG2 cells	Salmela et al. (1998), Xu et al. (2003a, b, 2005), Dai et al. (1993), Carroccio et al. (1994), Wu and Cederbaum (1996, 2001), Chen and Cederbaum (1998), Dey and Cederbaum (2006), Dey et al. (2006), Caro et al. (2009), Chen et al. (1997), Caro and Cederbaum (2001, 2002a, b, 2003), Lu and Cederbaum (2006), Dai and Cederbaum (1995), Bai and Cederbaum (2004), Dey et al. (2006), Bardag-Gorce et al. (2006), Garige et al. (2005), Donohue et al. (2006), Osna et al. (2003, 2005, 2007, 2008, 2009), Lagadic-Gossmann et al. (2000), Kim et al. (2006), Qu et al. (2009), and Chandrasekaran et al. (2011, 2012a, b)
HepG2 mitochondria	Bai and Cederbaum (2006) and Kim et al. (2006)
Kupffer cells	Koivisto et al. (1996) and Koop et al. (1991)
Hepatic stellate cells	Nieto et al. (1999, 2000)
Co-culture of HepG2 cells and hepatic stellate cells	Nieto et al. (2002a, b)
HeLa cells	Huan and Koop (1999) and Huan et al. (2004)
Huh 7 cells	Otani et al. (2005) and Osna et al. (2010)
FGC-4 hepatoma cells	Rowlands et al. (2003)
Fao rat hepatoma cells	Simi and Ingelman-Sundberg (1999) and Zhukov and Ingelman-Sundberg (1997)
RAW 264.7 macrophages	Cao et al. (2005)
Kidney	Wu and Cederbaum (1994), Runge-Morris et al. (1996), Chen et al. (1999), Cummings et al. (1999, 2001), Roberts et al. (1994), Arinç et al. (2007), and Zaluzny et al. (1990)
Monkey kidney cell line COS-7	Knockaert et al. (2011)
Renal proximal tubule cells (RPTCs)	Shankar et al. (2008)
Blood	Oneta et al. (2002), Sutti et al. (2010a, b), Daly et al. (2006), Fairbrother et al. (1998), Vidali et al. (2007), Rigamonti et al. (2009), and Khemawoot et al. (2007b)

(continued)

Table 1.3 (continued)

Tissue/organ	References
Lymphocytes	Soh et al. (1996), Song et al. (1990), Chalasani et al. (2003), Raucy et al. (1997), and Dey et al. (2002, 2005)
B-lymphoblastoid cells	Asai et al. (1996)
Mononuclear cells	Hase et al. (2000) and Yano et al. (2001)
Pancreas	Kessova et al. (1998)
Lung	Arinç et al. (2007) and Wang et al. (2002a)
Nasal tissue including olfactory epithelial cells	Wang et al. (2002a)
Brain	Vasiliou et al. (2006), Vaglini et al. (2004), Farin and Omiecinski (1993), Yadav et al. (2006), Johri et al. (2007), Roberts et al. (1994), Pardini et al. (2008), Viaggi et al. (2009), Correa et al. (2009), and Zimatkin et al. (2006)
Neuronal and glial cells	Kapoor et al. (2006)
C6 glioma cells	Bae et al. (2001) and Lee et al. (2006a)
Brain (Cortical glial cells)	Tindberg et al. (1996)
Brain (frontal, cortical and pyramidal neurons, cerebellar Purkinje cells)	Lee et al. (2006c)
Brain (olfactory bulbs, frontal cortex, hippocampus, cerebellum, olfactory tubercle, brain stem)	Howard et al. (2003a)
Brain (frontal cortex and cerebellum)	Joshi and Tyndale (2006a)
Brain (frontal cortex, hippocampus, cerebellum)	Joshi and Tyndale (2006b)
Brain (cortex, hippocampus, hypothalamic nuclei, basal ganglia, reticular nucleus and brain stem)	Anandatheerthavarada et al. (1993)
Brain (cortex, hippocampus)	Upadhya et al. (2000)
Brain mitochondria	Bhagwat et al. (1995)
Brain (hippocampus)	Tindberg and Ingelman-Sundberg (1996)
Maternal brain	Carpenter et al. (1997)
Prenatal brain	Boutelet-Bochan et al. (1997) and Brzezinski et al. (1999)
Reticulocytes	Kocarek et al. (2000)
Human umbilical vein endothelial cells (HUVEC)	Farin et al. (1994)
Placenta	Carpenter et al. (1997)
Primary and human papillomavirus immortalized oral and cervical epithelial cells	Farin et al. (1995)

(continued)

Table 1.3 (continued)

Tissue/organ	References
Reticulocytes	Kocarek et al. (2000)
Squamous epithelial cells of the cheek mucosa, tongue, esophagus, forestomach	Shimizu et al. (1990)
Esophageal mucosa	Millonig et al. (2011)
Alimentary tract (duodenal and jejunal villous cells, surface epithelium of proximal colon)	Shimizu et al. (1990)
Upper gastrointestinal tract	Roberts et al. (1994)

Presence of CYP2E1 in Kupffer Cells

The content of CYP2E1 in Kupffer cells is several times lower than in hepatocytes and is located in the endoplasmic reticulum of Kupffer cells *in vivo* suggesting that it is possibly the major pathway for ethanol metabolism in Kupffer cells (Koivisto et al. 1996). The induction of CYP2E1 by ethanol in Kupffer cells isolated from rats fed ethanol-containing Lieber-DeCarli diets for 3 weeks suggests its role in causing significant changes in intracellular acetaldehyde concentrations which, together with increased lipid peroxidation, may contribute to the development of alcoholic liver injury (Koivisto et al. 1996). The mechanisms associated with the toxic actions of CYP2E1 have been summarized in Table 1.4.

Oxidant generation after CYP2E1 overexpression in RAW 264.7 macrophages transfected with CYP2E1 and possessing stable increased CYP2E1 expression (E2) appears to be central to macrophage priming and their sensitization to lipopolysaccharide (LPS) stimuli (Cao et al. 2005). Accordingly, CYP2E1 priming could explain the sensitization of Kupffer cells to LPS activation by ethanol, a crucial early step in alcoholic liver disease (Cao et al. 2005).

Presence of CYP2E1 in Extrahepatic Tissues

Immunoreactive CYP2E1 is detectable only in duodenal and jejunal villous cells in rats fed a control diet consisting of carbohydrate for 3 weeks (Shimizu et al. 1990). The content of CYP2E1 increases in duodenal and jejunal villi, and the enzyme is also detectable in squamous epithelial cells of the cheek mucosa, tongue, esophagus, and forestomach, and in surface epithelium of the proximal colon in rats pair-fed liquid diets containing 36% of total calories as ethanol (Shimizu et al. 1990). Thus, the presence of CYP2E1 in the alimentary tract, when considered together with the xenobiotic activation properties of CYP2E1, may partly explain why alcohol abuse is a risk factor for cellular damage or cancer or both in those alimentary tract tissues in which CYP2E1 is inducible by chronic ethanol intake (Shimizu et al. 1990).

In pancreatic and hepatic microsomes isolated from rats administered ethanol, ethanol induces CYP2E1 protein and p-nitrophenol hydroxylase activity, which implicates its role in the pathogenesis of pancreatitis and/or pancreatic cancer (Kessova et al. 1998).

Table 1.4 Mechanisms associated with CYP2E1 mediated injury

Integral component of the microsomal ethanol oxidizing system (Lieber and DeCarli 1968)
Acetaldehyde generation (Correa et al. 2009; Vasiliou et al. 2006; Zimatkin et al. 2006; Niemelä et al. 1998; Koivisto et al. 1996; Garige et al. 2005; Donohue et al. 2006; Kunitoh et al. 1997; Niemelä et al. 1999; Jeong et al. 2000; Carpenter et al. 1996)
ROS generation (Lu et al. 2008, 2010; Xu et al. 2005; Lieber et al. 2004b, 2007a; Chen and Cederbaum 1998; Caro and Cederbaum 2002a; Bardag-Gorce et al. 2006; Roede et al. 2008; Kathirvel et al. 2009, 2010; Garige et al. 2005; Osna et al. 2003; Zhukov and Ingelman-Sundberg 1999; Jones et al. 2002; Chen et al. 1997; Xu et al. 2003b; Lu and Cederbaum 2006; Nieto et al. 1999, 2002a, b; Chen et al. 2008, 2009; Liu et al. 2002; Schattenberg et al. 2004; Chandrasekaran et al. 2011, 2012a, b; Dai et al. 1993; Nieto et al. 2002a; Bailey et al. 2009)
Lipid peroxidation (Morimoto et al. 1995a, b; Leclercq et al. 2000b; Martínez-Chantar et al. 2002; Khalighi et al. 1999; Niemelä et al. 1998; Liu et al. 2002; Koivisto et al. 1996; Wang et al. 2008; Xu et al. 2003a; Lieber et al. 2007b; Dai et al. 1993; Chen and Cederbaum 1998; Chen et al. 1997; Caro and Cederbaum 2001, 2002a, b, 2003; Wu and Cederbaum 2001; Nieto et al. 2002a, b; Wang et al. 2009; Garige et al. 2005; Castillo et al. 1992; Albano et al. 1996; Wang et al. 2008; Nanji et al. 1994; Hu et al. 1994; Ronis et al. 1991; French et al. 1993; Sampey et al. 2003; Singh et al. 2009; Dey et al. 2002; Chandrasekaran et al. 2011, 2012a)
Mitochondrial oxidative stress and damage (Bansal et al. 2010; Xu et al. 2005; Caro and Cederbaum 2002b; Bai and Cederbaum 2004, 2006; Kim et al. 2006; Otani et al. 2005; Demeilliers et al. 2002; Knockaert et al. 2011; Raza et al. 2004; Robin et al. 2005)
Protein adduct formation (Bai and Cederbaum 2004; Sampey et al. 2003; Roede et al. 2008; Niemelä et al. 1998, 1999; Jeong et al. 2000; French et al. 1993)
Involved in priming of macrophages and their sensitization to lipopolysaccharide stimuli (Cao et al. 2005)
Oxidative damage and inactivation of microsomal Ca2+-ATPase resulting in elevated calcium level (Caro et al. 2009)
Increased influx of intracellular Ca2+ and activation of Ca2+ dependent proteases (Caro and Cederbaum 2002a)
Increase in collagen expression (Nieto et al. 1999, 2000, 2002a, b; Lu et al. 2010)
Upregulation of COX-2 and prostaglandin E2 (Nieto et al. 2000)
Fibrogenesis (Castillo et al. 1992; Nieto et al. 2002a; Rigamonti et al. 2009; Niemelä et al. 1999; French et al. 1993)
DNA adduct formation (Wang et al. 2009; Millonig et al. 2011; Ghanayem et al. 2005c; Gut et al. 1996)
DNA damage (Demeilliers et al. 2002; Bailey et al. 2009; Bradford et al. 2005; Kukielka and Cederbaum 1994; Ghanayem et al. 2005b; Bansal et al. 2010; Bae et al. 2001; Tumer et al. 2010)
Depletion of glutathione (Xu et al. 2005; Robin et al. 2005; Bansal et al. 2010; Chen et al. 2008; Otani et al. 2005; Martínez-Chantar et al. 2002; Roede et al. 2008; Curry-McCoy et al. 2010; Lieber et al. 2007b)
Downregulation of regulator for fatty acid oxidation-PPAR alpha (Lu et al. 2008; Abdelmegeed et al. 2011)
Significantly greater 18:1/18:0 fatty acids (Morimoto et al. 1995a)
Nitrosative stress (Dey and Cederbaum 2007; Lu et al. 2005; Kathirvel et al. 2010; Mantena et al. 2009; Bailey et al. 2009; Osna et al. 2003)
Decreased levels of ubiquitin pathway proteins or genes (Gouillon et al. 1999; Bardag-Gorce et al. 2006; Tierney et al. 1992)

(continued)

Table 1.4 (continued)

Inhibition of proteasome activity (Osna et al. 2007, 2008, 2009, 2010; Gouillon et al. 2000; Bardag-Gorce et al. 2000, 2006; Donohue et al. 2006)
Accumulation of oxidized proteins (Bardag-Gorce et al. 2000)
Protein carbonyl formation (Bardag-Gorce et al. 2006; Roede et al. 2008)
4-hydroxy-2-nonenal adduct formation (Bardag-Gorce et al. 2006; Niemelä et al. 1998, 1999; Wang et al. 2009; French et al. 1993; Sampey et al. 2003; Chandrasekaran et al. 2011, 2012a; Clot et al. 1996)
Induction of cytokeratin 8 and cytokeratin 18 (Bardag-Gorce et al. 2006; Butura et al. 2009)
Formation of cytokeratin aggresomes (Bardag-Gorce et al. 2005, 2006)
In vitro Mallory body like inclusion formation (Bardag-Gorce et al. 2006)
Marked decreases in Gal beta 1,4 GlcNAc alpha 2,6-sialyl transferase (2,6-ST) levels (Garige et al. 2005)
Suppression of IFN gamma signal transduction (Osna et al. 2005)
Reduction of STAT1 phosphorylation (Osna et al. 2003, 2005)
Triglyceride accumulation (Martínez-Chantar et al. 2002; Ronis et al. 2010; Chalasani et al. 2003; Roede et al. 2008; Lu et al. 2008; Morimoto et al. 1995a)
Induction of TNF alpha (Lieber et al. 2004a, b; Ronis et al. 2010; Abdelmegeed et al. 2011; Fang et al. 1998)
Increased CCl4 mediated toxicity (Martínez-Chantar et al. 2002; Tierney et al. 1992)
Inhibition of fatty acid oxidation (Chen et al. 2009; Lu et al. 2008)
Inhibition of PPAR alpha activity (Chen et al. 2009; Abdelmegeed et al. 2011)
Down regulation of insulin signalling leading to insulin resistance (Chalasani et al. 2003; Schattenberg et al. 2005; Lieber et al. 2004b; Kathirvel et al. 2009)
Impaired protein methylation (Osna et al. 2010)
Behavioural changes (Vasiliou et al. 2006; Correa et al. 2009)
Hydroxyethyl radical generation (Albano et al. 1996; Dupont et al. 1998; Clot et al. 1996, 1997)
Increased IgG complex formation with hydroxyethyl radical formation (Dupont et al. 1998)
Development of auto-antibodies against CYP2E1 (Albano et al. 1996; Vidali et al. 2007; Rigamonti et al. 2009; Sutti et al. 2010a, b; Lytton et al. 1999)
Necroinflammation (Rigamonti et al. 2009; Sutti et al. 2010b)
Enhanced retinoic acid catabolism (Liu et al. 2001)
JNK activation (Liu et al. 2002; Wang et al. 2008; Bae et al. 2001; Singh et al. 2009)
Decrease in arachidonic acid content (Nanji et al. 1993)
Decrease in phospholipase A and C activities (Nanji et al. 1993)
Decreased Akt phosphorylation (Schattenberg et al. 2005)
Reduction in glycogen storage (Kathirvel et al. 2009)
Increased glucose synthesis (Kathirvel et al. 2009)
Nitrosylation of catalase and superoxide dismutase (Kathirvel et al. 2010)
Inflammation (Morimoto et al. 1994; Abdelmegeed et al. 2011; Niemelä et al. 1999; Tindberg et al. 1996; Sampey et al. 2003)
Fat accumulation (Lu et al. 2008; Kathirvel et al. 2009)
Steatosis (Esfandiari et al. 2005; Bailey et al. 2009; Sampey et al. 2003; Lieber et al. 2004a; Lu et al. 2010; Curry-McCoy et al. 2010; Wang et al. 2008; Abdelmegeed et al. 2011; Baumgardner et al. 2008; Videla et al. 2004; Orellana et al. 2006)
Endoplasmic reticulum stress (Esfandiari et al. 2005; Dey et al. 2006; Ronis et al. 2010)
Oxidative or nitrosative modification of mitochondrial proteins (Kim et al. 2006; Mantena et al. 2009; Sampey et al. 2003)

(continued)

Table 1.4 (continued)

Ubiquitin mediated protein degradation (Abdelmegeed et al. 2010)
Decreased circulating 1, 25-dihydroxycholecalciferol (1,25 (OH) 2 D3) (Shankar et al. 2008)
Diminished antioxidant capacity (Videla et al. 2004; Abdelmegeed et al. 2010; Fernández et al. 2003; Otani et al. 2005; Roede et al. 2008)
Cytokine activation (Fang et al. 1998; Starkel et al. 2000)
Inhibition of t-retinoic acid synthesis (Khalighi et al. 1999)

Aggravation of Ethanol Induced Hepatotoxicity due to Other Hepatotoxins

Co-administration of ethanol and the hepatocarcinogen N-nitrosodimethyl amine (NDMA) to rats results in much greater hepatotoxicity than either agent alone (Ma et al. 1991). The addition of ethanol inhibits CYP-dependent demethylation and denitrosation of NDMA in liver microsomes, whereas both activities are enhanced markedly by chronic ethanol administration (Ma et al. 1991). Further, the study suggests the involvement of alcohol-inducible CYP2E1 in both NDMA bioactivation (demethylation and denitrosation) reactions (Ma et al. 1991). Thus, bioactivation plays a crucial role in the hepatotoxicity of NDMA and its aggravation by chronic alcohol consumption (Ma et al. 1991).

Further, the pro-vitamin A carotenoid beta-carotene potentiates the induction of CYP2E1 protein and catalytic activity by ethanol in rat liver and also increases CYP4A1, which may, at least in part, explain the associated hepatotoxicity (Kessova et al. 2001). The agents acting as co-inducers of CYP2E1 have been summarized in Table 1.5.

Ethanol Induced Hepatotoxicity and Protective Agents

Ethanol mediated increases in CYP2E1 content and MEOS is significantly reduced with the addition of carbonyl iron in livers of rats fed ethanol (Aleynik et al. 1999). This iron-induced decrease is corrected by Polyenylphosphatidylcholine (PPC), a 94–96% pure mixture of linoleate-rich polyunsaturated phosphatidylcholines that protects against alcohol-induced liver injury (Aleynik et al. 1999). Further, in the absence of iron, the ethanol-mediated induction of CYP2E1 and its corresponding enzyme activities and oxidative stress are significantly less with PPC (Aleynik et al. 1999). In addition, PPC attenuates alcohol-induced apoptosis of rat hepatocytes; this effect may provide a mechanism for PPC's protection against liver injury, possibly in association with its antioxidative action via the down-regulation of ethanol-mediated CYP2E1 induction (Mi et al. 2000).

Dilinoleoylphosphatidylcholine (DLPC) is the major component of PPC, and DLPC significantly decreases CYP2E1 content and its corresponding activities in rats fed ethanol diet and thus could serve a therapeutic target for the prevention of alcoholic liver disease (Aleynik and Lieber 2001). Further, DLPC decreases the cytotoxicity (apoptosis) induced by alcohol in HepG2 cells expressing CYP2E1, a protective action due, at least in part, to an attenuation of the alcohol-induced oxidative stress (diminished hydrogen peroxide production), the alteration in the

Table 1.5 Agents acting as co-inducers of CYP2E1

Agent	References
Ethanol and N-nitrosodimethylamine	Ma et al. (1991)
Ethanol and beta-carotene	Kessova et al. (2001)
Ethanol and extremely low carbohydrate diet	Rowlands et al. (2003)
Ethanol and polyunsaturated fatty acids	Morimoto et al. (1993, 1994) and Nanji et al. (1993)
Ethanol plus (Fe-nitrilotriacetic acid (Fe-NTA)) plus arachidonic acid	Bardag-Gorce et al. (2006)
Ethanol and lycopene	Veeramachaneni et al. (2008)
Ethanol and undernutrition	Baumgardner et al. (2007)
Ethanol and hepatitis C virus core protein	Otani et al. (2005)
Ethanol and castration	Niemelä et al. (1999)
Ethanol and folate deficiency	Esfandiari et al. (2005)
Ethanol and carbohydrate deficiency	Korourian et al. (1999)
Ethanol and nicotine	Ferguson et al. (2011), Yue et al. (2009), and Howard et al. (2001, 2003a)
Ethanol and environmental tobacco smoke and hypercholesterol (Apoprotein E deficiency)	Bailey et al. (2009)
Ethanol and high glucose	Chandrasekaran et al. (2012b)
Streptozocin and 4-methyl pyrazole	Wu and Cederbaum (1993a)
Streptozocin and thioacetamide	Wang et al. (2000)
Pyrazole and lipopolysaccharide	Lu et al. (2005)
Pyrazole and obesity	Dey and Cederbaum (2007)
Arachidonic acid and iron (Fe-NTA)	Caro and Cederbaum (2001)
Acetone and obesity	Dey and Cederbaum (2007) and Leclercq et al. (2000a)
Fasting and obesity	Leclercq et al. (2000a)
4-methyl pyrazole and obesity	Leclercq et al. (2000a)
Pyridine plus Thioacetamide and diet restriction	Ramaiah et al. (2001)

mitochondrial membrane potential and partial restoration of mitochondrial glutathione (GSH) (Xu et al. 2005). Moreover, in a high-fat diet (HF) rat model, the combination of S-adenosylmethionine plus DLPC decreases liver triacylglycerols and CYP2E1 mRNA and CYP2E1 protein, accompanied by a reduction of hepatic 4-HNE, reflecting control of oxidative stress (Lieber et al. 2007a). The agents conferring protection against CYP2E1 mediated toxicity have been summarized in Table 1.6.

Lycopene, a carotenoid with high anti-oxidant capacity, protects HepG2 cells expressing CYP2E1 (HepG2 cells transfected with pCI-neo/2E1 (2E1)) against arachidonic acid (AA) toxicity. This is due, at least in part, to inhibition of hydrogen peroxide production and of the resulting lipid peroxidation, confirming the potent anti-oxidant properties of lycopene and its suitability for clinical studies (Xu et al. 2003a). Further, lycopene opposes the ethanol-induced oxidative stress and apoptosis in 2E1 cells (Xu et al. 2003b).

Table 1.6 Agents conferring protection against CYP2E1 mediated injury

Agent	References
Polyenylphosphatidylcholine	Aleynik et al. (1999)
Dilinoleoylphosphatidylcholine	Aleynik and Lieber (2001)
S-adenosyl methionine	Lieber et al. (2007a), Martínez-Chantar et al. (2002), Osna et al. (2010), and Esfandiari et al. (2005)
Lycopene	Xu et al. (2003a, b)
Medium chain triglycerides	Lieber et al. (2007b)
Acarbose	Lieber et al. (2004a)
Chlormethiazole	Gebhardt et al. (1997), Gouillon et al. (2000), Hu et al. (1994), Simi and Ingelman-Sundberg (1999), Tindberg and Ingelman-Sundberg (1996), Tindberg et al. (1996), Lytton et al. (1999), Fang et al. (1998), and Wang et al. (2009)
cAMP	Gouillon et al. (1999)
Propofol	Lejus et al. (2002)
Insulin	Sidhu et al. (2001, 2006) and Woodcroft and Novak (1997, 1999)
Endotoxin	Morgan (1993), Sewer et al. (1998), Sewer and Morgan (1998), and Cheng et al. (2003)
Interleukins 1&6	Morgan et al. (1994)
Diallyl sulfide	Morimoto et al. (1995a, b), Albano et al. (1996), Ronis et al. (2010), Martínez-Chantar et al. (2002), Zimatkin et al. (2006), Ramaiah et al. (2001), Bardag-Gorce et al. (2006), Osna et al. (2005, 2007, 2008), and Albano et al. (1996)
Phenethyl isothiocyanate	Morimoto et al. (1995a, b), Albano et al. (1996), and Zimatkin et al. (2006)
YH439	Jeong et al. (2000) and Bae et al. (2001)
Isoniazid	French et al. (1993)
SKF-525A	Cummings et al. (2001)
4-methyl pyrazole	Ronis et al. (2010), Donohue et al. (2006), Osna et al. (2007), and Huan and Koop (1999)

Further, the alpha-glucosidase inhibitor acarbose which is beneficial in the prevention of type 2 diabetes has been found to decrease steatosis and inflammation, accompanied by decreases in protein and mRNA expression of the hepatic inflammatory cytokine TNF-alpha, CYP2E1, and collagen in a rat model of non-alcoholic steatohepatitis (NASH) (Lieber et al. 2004a).

In rats fed either 32% of calories as dietary long-chain triglycerides (LCT) (alcohol), or 16% as LCT + 16% as medium-chain triglycerides (MCT) (alcohol-MCT 16%), or 32% as MCT only (alcohol-MCT 32%), both alcohol and alcohol-MCT 16% groups have a significant increase in mitochondrial and microsomal CYP2E1 (Lieber et al. 2007b). When MCT replaces all the fat, like in the alcohol-MCT 32% group, CYP2E1 is significantly reduced both in mitochondria and microsomes (Lieber et al. 2007b). Thus, mitochondria participate in the induction of CYP2E1 by alcohol and contribute to lipid peroxidation and GSH depletion and a diet rich in MCT is beneficial in ameliorating injury.

CYP2E1 and Rodent NASH Model

Rats fed high fat diet reproduce the key features of human NASH which is frequently associated with obesity and diabetes and exhibit insulin resistance and increased hepatic TNF alpha, collagen type 1, alpha1(I) procollagen and CYP2E1 mRNA. In addition, these rats show CYP2E1 induction and oxidative stress with increased 4-hydroxynonenal formation (Lieber et al. 2004b). Thus, NASH in a rodent model is associated with upregulation of CYP2E1.

Arthur I. Cederbaum

Characterization of Biochemical & Toxicolological Actions of CYP2E1

Studies in Dr. Cederbaum's laboratory are mainly directed towards characterization of biochemical and toxicological properties of CYP2E1.

Establishment of CYP2E1 Over-Expressing HepG2 Cell Lines

A human-hepatoma-derived cell line clone MV2E1-9, stably and constitutively expressing the coding sequence of the human CYP2E1 in HepG2, was established by recombinant retroviral expression (Dai et al. 1993). MV2E1-9 metabolized p-nitrophenol, dimethylnitrosamine, aniline, and ethanol and exhibited several fold higher rates of superoxide and H2O2 production and lipid peroxidation when compared to control clones (Dai et al. 1993). Ethanol increases the content of CYP2E1 and catalytic oxidation of CYP2E1 substrates in MV2E1-9 cells, possibly through protein stabilization (Carroccio et al. 1994). The list of potent carcinogens such as dimethylnitrosamine which are metabolized by CYP2E1 have been summarized in Table 1.7.

Ethanol and other substrates such as dimethyl sulfoxide, carbon tetrachloride, isoniazid, and N,N-dimethylnitrosamine exhibit cytotoxic effects in another model for transduced HepG2 cells- HepG2 E9 cells, which express CYP2E1 (Wu and Cederbaum 1996). Further, other transduced HepG2 subclonal cells that overexpress CYP2E1- Hep G2-CI2E1-43 and -47 (E47) cells exhibited slower growth rate than parental HepG2 cells or control subclones that do not express CYP2E1, but remained fully viable (Chen and Cederbaum 1998). Low lipid peroxidation levels are observed in E47 cells, reflective of the ability of CYP2E1 to generate ROS even in the absence of added metabolic substrate (Chen and Cederbaum 1998).

CYP2E1 Mediated Hepatotoxicity: Underlying Mechanisms

CYP2E1 exerts its hepatoxic actions through several mechanisms and some of these mechanisms which have been studied in Dr. Cederbaum's laboratory have been discussed in this section. DNA strand cleavage occurs due to the production of hydroxyl radicals by rat liver microsomes and is further increased after chronic ethanol treatment (Kukielka and Cederbaum 1994). Further, this increased microsomal DNA cleavage in the presence of NADPH and NADH is partially due to induction of CYP2E1, as observed due to inhibition of the process in the presence of anti-(CYP2E1) IgG and inhibitors of CYP2E1, such as diethyl dithiocarbamate and tryptamine (Kukielka and Cederbaum 1994). Further, incubation of hepatocytes isolated from rats treated with pyrazole, with ethanol or arachidonic acid results in the release of cytochrome c and activation of caspase 3,

Table 1.7 CYP2E1 and carcinogenesis

Potent carcinogens	References
Thioacetamide	Wang et al. (2000), Ramaiah et al. (2001), and Chilakapati et al. (2007)
Ethanol	Wang et al. (2009) and Millonig et al. (2011)
Acrylamide	Ghanayem et al. (2005a, b, c) and Ghanayem (2007)
Methacrylonitrile	Wang et al. (2002a)
Acrylonitrile	Wang et al. (2002b)
Urethane	Hoffler et al. (2003, 2005)
1-bromopropane	Garner et al. (2007)
Dimethylnitrosamine	Garro et al. (1981), Arinç et al. (2007), Ma et al. (1991), Dey et al. (2002, 2005), Kapoor et al. (2006), Khan et al. (2011), Anandatheerthavarada et al. (1993), Bhagwat et al. (1995), Huan and Koop (1999), Roberts et al. (1995), and Zaluzny et al. (1990)
N-methyl formamide	Lerche et al. (1996)
Diethylnitrosamine	Lerche et al. (1996)
Nicotine	Howard et al. (2001)

which contributes towards the apoptotic effects of CYP2E1 in the liver cells (Wu and Cederbaum 2000). Heat shock proteins (Hsps) are crucial for the stability and function of numerous proteins and geldanamycin, an inhibitor of Hsp90, causes pronounced oxidative stress and apoptosis in E47 cells suggesting that the inhibition of the molecular chaperone Hsp90 promotes CYP2E1 mediated oxidative stress in liver cells (Dey and Cederbaum 2006). CYP2E1 oxidatively damages and inactivates the microsomal Ca2+-ATPase in CYP2E1 over-expressing E47 cells accounting for the elevated calcium level during CYP2E1 toxicity, suggesting that this may contribute to elevated cytosolic calcium and CYP2E1-potentiated injury (Caro et al. 2009). Studies showing CYP2E1 mediated apoptotis or necrosis have been summarized in Table 1.8.

Antioxidant Depletion Promotes CYP2E1 Mediated Liver Injury: Crucial Role for Oxidative Stress as a Major Mechanism for the Deleterious Effects of CYP2E1

Inhibition of GSH synthesis by treatment with buthionine sulfoximine, results in rapid decline of GSH levels in E47 cells and elevated lipid peroxidation which are not observed in control cells, which is most likely a reflection of CYP2E1-catalyzed formation of ROS (Chen and Cederbaum 1998). Thus, under conditions of CYP2E1 overexpression, two modes of CYP2E1-dependent toxicity can be observed in HepG2 cells: a slower growth rate when cellular GSH levels are maintained and a loss of cellular viability when cellular GSH levels are depleted (Chen and Cederbaum 1998). Further, chronic alcohol consumption induces liver injury in Cu, Zn-superoxide dismutase-deficient mice (Sod1−/−), with extensive centrilobular necrosis, inflammation and mitochondrial dysfunction (Kessova and Cederbaum 2007).

Table 1.8 Modes of cell death associated with CYP2E1 mediated injury

Mode of cell death	References
Apoptosis	Aleynik and Lieber (2001), Wang et al. (2008), Donohue et al. (2006), Ronis et al. (2010), Esfandiari et al. (2005), Bae et al. (2001), Abdelmegeed et al. (2011), Jones et al. (2002), Xu et al. (2003b), Dey and Cederbaum (2006), Lu et al. (2005), Chandrasekaran et al. (2011, 2012a, b), Chen et al. (1997), Chen and Cederbaum (1998), and Mi et al. (2000)
Necrosis	Kessova and Cederbaum (2007), Lu et al. (2005), Niemelä et al. (1999), Korourian et al. (1999), Ronis et al. (2010), French et al. (1993), Sampey et al. (2003), and Jones et al. (2002)

Regulation of Hepatic CYP2E1 Mediated by Pathophysiological Conditions Such as Obesity, Diabetes and Chemical Inducers

Treatment of rats with the chemical inducer-4-methylpyrazole and streptozotocin which is commonly used to induce diabetes, increases CYP2E1 protein and catalytic activity and the values are additive for each inducer alone suggesting that diabetes may increase the susceptibility to toxins which are activated by CYP2E1, more so if pre-exposure to chemical inducers similar to 4-methylpyrazole, e.g., ethanol, isoniazid occurs (Wu and Cederbaum 1993a).

Pyrazole and 4-methylpyrazole, inducers for hepatic CYP2E1 induce renal CYP2E1, through a post-transcriptional mechanism-possibly involving increased protein stabilization (Wu and Cederbaum 1994). Further, acetone- or pyrazole-mediated induction of CYP2E1 potentiates liver injury in obesity (Dey and Cederbaum 2007). Acetone- or pyrazole-treated obese mice liver exhibit elevated CYP2E1 levels, increased oxidative stress parameters, and greater liver injury (Dey and Cederbaum 2007). Thus, obesity contributes to oxidative stress and liver injury which is potentiated due to the induction of CYP2E1 (Dey and Cederbaum 2007).

Ethanol Mediated Induction of Neonatal CYP2E1

Fetal rat liver is characterized by absence of CYP2E1 because activation of the gene occurs shortly after birth and ethanol induces CYP2E1 in adult rats (Wu and Cederbaum 1993b). Further, consumption of an ethanol-containing liquid diet in pregnant rats starting on the 9th day of gestation induces CYP2E1 content and catalytic activities with no elevations in CYP2E1 mRNA in hepatic microsomes from neonates of mothers compared with controls (Wu and Cederbaum 1993b).

Ethanol Mediated Inducibility of CYP2E1 and Its Hepatotoxic Actions: Effects in CYP2E1 Knock 'out' and Knock 'in' Models

CYP2E1 has been shown to play a role in experimental alcoholic fatty liver in an oral ethanol-feeding model (Lu et al. 2008). In wild type mice administered ethanol, macrovesicular fat accumulation and accumulation of triglyceride, induced CYP2E1 in liver, higher oxidative stress, downregulation of a target gene of the fatty acid oxidation regulator-Peroxisome proliferator-activated receptor alpha (PPARalpha), acyl CoA oxidase are observed but not in CYP2E1-knockout mice (Lu et al. 2008). Further,

it suggests that CYP2E1-derived oxidative stress may inhibit oxidation of fatty acids by preventing up-regulation of PPAR alpha by ethanol, resulting in fatty liver.

In a study using CYP2E1 knock out (KO) mice, and humanized CYP2E1 knock-in (KI) mice (in which the human 2E1 has been added back to mice deficient in the mouse CYP2E1) fed a high-fat Lieber-DeCarli ethanol liquid diet, ethanol-induced steatosis and oxidant stress are blunted in the KO mice (no liver injury) but restored in the KI mice (Lu et al. 2010). Significant liver injury is produced in the ethanol-fed KI mice, accompanied by elevated levels of the human CYP2E1 compared to levels of the mouse CYP2E1 in wild type mice and increased levels of collagen type 1 and smooth muscle actin (Lu et al. 2010). Thus, CYP2E1 plays a major role in ethanol-induced fatty liver and oxidant stress and it is the human CYP2E1 that restores the injurious effects of ethanol, suggesting that results for fatty liver and oxidant stress from rodent models of ethanol intake and mouse CYP2E1 can be extrapolated to human models of ethanol intake and to human CYP2E1 (Lu et al. 2010).

CYP2E1 Potentiates Injury Mediated by Several Hepatotoxins

Arachidonic acid (AA), a representative polyunsaturated fatty acid, causes a concentration- and time-dependent toxicity (apoptosis) to HepG2-MV2E1-9 cells which could be mediated by lipid peroxidation occurring in the cells over-expressing CYP2E1 due to elevated production of ROS (Chen et al. 1997). Further, pyrazole administration to rats increases lipopolysaccharide (LPS)-induced necrosis in liver which appears to involve an increase in oxidative and nitrosative stress generated by the combination of LPS plus elevated CYP2E1 levels (Lu et al. 2005).

The combination of Fe-nitrilotriacetic acid (Fe-NTA) and AA produce synergistic injury, increased lipid peroxidation, and damaged mitochondria in E47 cells (Caro and Cederbaum 2001). Similarly, hepatocytes isolated from pyrazole-treated rats exhibit more Fe + AA toxicity suggesting that low concentrations of Fe and AA can act as priming or sensitizing factors for CYP2E1-induced injury in HepG2 cells, and such interactions may play a role in alcohol-induced liver injury (Caro and Cederbaum 2001).

CYP2E1- and oxidative stress-dependent toxicity with iron and arachidonic acid combination in E47 cells has been shown to be mediated by the activation of lipid peroxidation, followed by an increased influx of extracellular Ca2+ and activation of Ca2+-dependent proteases (Caro and Cederbaum 2002a). Further, CYP2E1-dependent mitochondrial damage, in the presence of extracellular calcium as observed in E47 cells preloaded with AA and then incubated with Fe-NTA reveals CYP2E1-dependent, necrotic, and lipid peroxidation-dependent toxicity which is preceded by oxidative damage to mitochondria and the permeability transition (Caro and Cederbaum 2002b). Further, Ca(2+) mobilization and the activation of calpain (calcium dependent cysteine protease) contributes to the more rapid onset of mitochondrial damage, while oxidative damage and lipid peroxidation are involved in the Ca(2+)-independent later onset of mitochondrial damage (Caro and Cederbaum 2002b).

The release of stored calcium by AA+Fe-NTA, induced by lipid peroxidation, initially activates calpain and phospholipase A2 (PLA2) activity, PLA2 activation is

crucial for a subsequent increased influx of extracellular Ca2+, and the combination of increased PLA2 and calpain activity, increased calcium and oxidative stress cause mitochondrial damage, that ultimately produces the rapid toxicity of AA+Fe in E47 cells (Caro and Cederbaum 2003).

Role of CYP2E1 in Modulating the Hepatotoxicity of Drugs

The analgesic and anti-pyretic drug acetaminophen at high concentrations (above 5 mM) and in the presence of depleted intracellular GSH, causes severe cytotoxicity in MVh2E1-9 cells due to the ability of human CYP2E1 to activate acetaminophen to reactive metabolites which form covalent protein adduct, suggesting the ability of CYP2E1 to modulate the therapeutic index of drugs and cause drug toxicity (Dai and Cederbaum 1995). Further, adenovirus-mediated overexpression of human CYP2E1 in HepG2 cells activates acetaminophen to reactive metabolites which damage mitochondria, form protein adducts, and result in toxicity to HepG2 cells (Bai and Cederbaum 2004).

Sodium salicylate, an anti-inflammatory drug serves as a substrate of CYP2E1, increases CYP2E1 protein and potentiates AA induced lipid peroxidation and toxicity in CYP2E1 over-expressing E47 cells and hepatocytes isolated from pyrazole treated rats, thus suggesting the possible limitations in the use of salicylate and salicylate precursors such as acetylsalicylic acid with certain other drugs (Wu and Cederbaum 2001). In E47 cells and mice subjected to acetone administration, elevated CYP2E1 enhances the anti-cancer drug cisplatin-induced hepatotoxicity, possibly through increased ROS generation and subsequent oxidative stress (Lu and Cederbaum 2006).

CYP2E1 Mediated Oxidative Stress and Endoplasmic Reticulum Dysfunction

CYP2E1 mediated oxidative stress has been shown to downregulate the expression of endoplasmic reticulum resident proteins GRP78 and GRP94 proteins in HepG2 cells and is an important mechanism in causing ER dysfunction in these cells as E47 cells exhibit decreased mRNA and protein expression of GRP78 and GRP94 along with the accumulation of ubiquinated and aggregated proteins (Dey et al. 2006).

Establishment of HepG2 Cells Over-Expressing Mitochondrial CYP2E1: An Insight into the Mechanisms of Liver Injury due to Mitochondrial CYP2E1

HepG2 lines overexpressing CYP2E1 in mitochondria (mE10 and mE27 cells) were established by transfecting a plasmid containing human CYP2E1 cDNA lacking the hydrophobic endoplasmic reticulum targeting signal sequence into HepG2 cells followed by G418 selection (Bai and Cederbaum 2006). A 40-kDa catalytically active NH2-terminally truncated form of CYP2E1 (mtCYP2E1) detected in the mitochondrial compartment is induced due to chronic alcohol consumption and induces oxidative stress in the mitochondria, damaged mitochondria membrane potential, and causes a loss of cell viability (Bai and Cederbaum 2006).

CYP2E1 and Liver Fibrosis: Use of Hepatic Stellate Cells as an Important Tool to Understand the Mechanisms Involved

In a rat hepatic stellate cell line (HSC-T6) expressing CYP2E1, elevated production of ROS occurs which is associated with both elevation and stabilization of alpha 2 collagen type I (COL1A2) messenger RNA levels thus elucidating signaling pathways responsible for oxidant stress-mediated collagen gene induction (Nieto et al. 1999).

In a hepatic stellate cell line over-expressing the ethanol-inducible CYP2E1 (E5 cells), addition of AA elevates cyclooxygenase-2 (COX-2) levels and production of prostaglandin E(2); and ethanol and AA up-regulate alpha 2 collagen type I (COL1A2) gene expression (Nieto et al. 2000). Further, inhibition of COX-2 blocks the effect of AA, but not of ethanol, on COL1A2 expression suggesting that CYP2E1 activates COX-2 expression, and the oxidation of AA by COX-2 is responsible for the increase in COL1A2 (Nieto et al. 2000).

The fibrogenic effects of CYP2E1-dependent generation of ROS are observed in a co-culture of E47 cells with hepatic stellate cells (Nieto et al. 2002a). An increase in H(2)O(2), lipid peroxidation, and collagen type I protein in stellate cells co-cultured with E47 cells occurs (Nieto et al. 2002a). Similar results are observed in co-cultures of hepatocytes isolated from pyrazole-treated rats and primary stellate cells (Nieto et al. 2002a).

The activation of hepatic stellate cells in the presence of CYP2E1-derived ROS as apparent through co-incubation of primary stellate cells with E47 cells is characterized by morphologic changes and loss of lipid droplets (Nieto et al. 2002b). A more pronounced increase in alpha-smooth muscle actin (alpha-sma), intracellular and secreted collagen type I protein, and intra- and extracellular H(2)O(2) and lipid peroxidation products in the cells under co-culture occurs (Nieto et al. 2002b). Thus, hepatocytes containing CYP2E1 release diffusible mediators including ROS, which can activate HSC and besides perturbing the homeostasis of hepatocytes, CYP2E1-derived diffusible oxidants may also interact with stellate cells and contribute to hepatic fibrosis (Nieto et al. 2002b).

The above studies are amongst several other ongoing studies involving in vitro and in vivo models elucidating the role of CYP2E1 as a key player in alcohol induced liver injury from Dr. Cederbaum's laboratory.

Helmut K. Seitz

CYP2E1 and Ethanol Mediated Carcinogenesis

Chronic ethanol ingestion in rats results in an increase in hepatic microsomal dimethylnitrosamine (DMN) demethylase activity and in an increase in hepatic microsomal activation of DMN to a mutagen (Garro et al. 1981). These effects of ethanol on DMN metabolism are detectable *in vitro* at DMN concentrations as low as 0.3–1 mM and as high as 100 mM (Garro et al. 1981). This ability of ethanol to increase the rate of DMN metabolism over such a broad range of DMN concentrations is in marked contrast to the effects of other microsomal enzyme inducers, such as phenobarbital and 3-methylcholanthrene, which increase the rate of DMN metabolism only at relatively high DMN concentrations and repress its metabolism at low DMN concentrations (Garro et al. 1981). The cytochrome P-450 content of hepatic microsomes from rats chronically fed the ethanol-containing diet generally is increased by 25–50% relative to microsomes from control rats and DMN demethylase activity is likely to be related to cytochrome P-450 levels rather than to microsomal protein levels (Garro et al. 1981). Thus, nitrosamine metabolism is catalyzed by CYP2E1 and that chronic alcohol consumption increases the activation of nitrosamines which is important in carcinogenesis (Garro et al. 1981).

Significantly increased levels of carcinogenic etheno-DNA adducts are formed by the reaction of the major lipid peroxidation product, 4-hydroxynonenal (4-HNE) with nucleobases in hepatocytes of alcoholic liver disease (ALD) patients and in the liver of alcohol-fed lean (Fa/?) and obese (fa/fa) Zucker rats (Wang et al. 2009). The protein-bound 4-HNE strongly correlates with CYP2E1 expression in patients with ALD (Wang et al. 2009). The increased level of etheno-DNA adducts detected in ALD patients and the rodent models correlates significantly with CYP2E1 expression (Wang et al. 2009). Further, the role of CYP2E1 in the formation of etheno-DNA adducts is explicitly proved as ethanol increases etheno-DNA adducts in the nuclei of HepG2 cells stably transfected with human CYP2E1 (E47 cells) in a concentration-dependent and time-dependent manner which is significantly blocked with chlormethiazole (Wang et al. 2009). Thus, ethanol-mediated induction of hepatic CYP2E1 leading to the formation of highly miscoding lipid peroxidation-derived DNA lesions may play a central role in hepatocarcinogenesis in patients with ALD (Wang et al. 2009).

In non-tumorous esophageal biopsies of patients with upper aerodigestive tract cancer, chronic alcohol ingestion results in a significant induction of CYP2E1 which correlates with the amount of alcohol consumed (Millonig et al. 2011). Furthermore, a significant correlation between CYP2E1 and the generation of the carcinogenic exocyclic etheno-DNA adducts 1,N(6)-ethenodeoxyadenosine and 3,N(4)-ethenodeoxycytidine is observed (Millonig et al. 2011). Non-smokers and non-drinkers have the lowest rate of cell proliferation, CYP2E1 expression and DNA lesions (Millonig et al. 2011). Thus, ethanol mediated induction of CYP2E1 in the esophageal mucosa in a dose dependent manner occurs in human beings and explains, at least in part, the generation of carcinogenic DNA lesions in this target organ (Millonig et al. 2011).

Drug Mediated Inhibition of Ethanol Inducible CYP2E1

Chlormethiazole, a sedative and anticonvulsive drug used in the treatment of alcohol withdrawal and a potent inhibitor of alcohol-inducible rat hepatic CYP2E1, has been shown to be an effective inhibitor of the metabolism of CYP2E1 probe chlorzoxazone and thus of CYP2E1 activity in alcoholic and control patients; and human liver microsomes (Gebhardt et al. 1997). Therefore, detoxification treatment of alcohol with chlormethiazole may prove to be beneficial in alleviating the detrimental effects of CYP2E1 induction after chronic ethanol consumption (Gebhardt et al. 1997).

Ethanol Mediated Regulation of Human CYP2E1: Dynamics Involved

A significant CYP2E1 induction occurs 1 week following the ingestion of alcohol in human beings and is increased further after 4 weeks (Oneta et al. 2002). The disappearance of CYP2E1 is found to be significant 3 days following ethanol withdrawal in alcoholics and further decreases up to day 8 (Oneta et al. 2002). Thereafter, no significant changes occur and CYP2E1 activities are comparable with those in patients with non-alcoholic liver disease (Oneta et al. 2002). Therefore, a significant and quick induction of CYP2E1 activity occurs at moderate alcohol consumption, which may be of importance in the pathogenesis of alcoholic liver disease, of ethanol, drug and vitamin A interactions and in alcohol associated carcinogenesis (Oneta et al. 2002).

Ann K. Daly

Genetic Polymorphisms in CYP2E1

To investigate whether interindividual variation in CYP2E1 levels can be explained by genetic polymorphism, DNA samples from 40 healthy individuals were analyzed for polymorphisms in the CYP2E1 coding sequence and promoter region (Fairbrother et al. 1998). Polymorphisms were detected at positions −316 (A to G), −297 (T to A), −35 (G to T), 1107 (G to C; intron 1), 4804 (G to A Val179Ile; exon 4) and 10157 (C to T; exon 8) (Fairbrother et al. 1998). All individuals positive for either A(−316)G, G(−35)T, G(4804)A or the previously described RsaI polymorphism at −1,019 were also positive for T(−297)A, which had the highest allele frequency of the observed polymorphisms (0.20). A(−316)G, G(−35)T and G(4804)A were detected at allele frequencies of 0.022, 0.052 and 0.013, respectively (Fairbrother et al. 1998). The functional significance of the upstream polymorphisms was examined by preparing constructs of positions −549 to +3 of CYP2E1 containing the observed combinations of the polymorphisms fused to luciferase reporter genes and transfecting HepG2 cells (Fairbrother et al. 1998). For the G(−35)T/T(−297)A construct, a 1.8-fold increase in luciferase activity compared with the wild-type sequence ($P = 0.06$) and 2.5-fold compared with T(−297)A only ($P = 0.025$) was observed (Fairbrother et al. 1998). No significant difference in activity was observed between the other constructs. The significance of the predicted Val179Ile base change from G(4804)A was determined by expression of the wild-type and mutated full length cDNAs in lymphoblastoid cells (Fairbrother et al. 1998). No significant difference in kinetic constants for chlorzoxazone hydroxylation between mutant and wild-type was observed (Fairbrother et al. 1998). The study demonstrates six novel CYP2E1 polymorphisms, including three upstream of the promoter, but with the possible exception of G(−35)T, none appeared to be of functional significance (Fairbrother et al. 1998).

Methods for Detecting CYP2E1 Polymorphisms

Protocols for the extraction of DNA from human blood and for genotyping for a number of common cytochrome P450 polymorphisms using either polymerase chain reaction (PCR)-restriction fragment length polymorphism or PCR-single-strand conformational polymorphism (SSCP) analysis have been established (Daly et al. 2006). General guidelines for performing amplification using PCR have been established with electrophoresis protocols for analysis of restriction digests of PCR products with agarose and polyacrylamide gels including the use of polyacrylamide-based gels for SSCP analysis (Daly et al. 2006). Protocols for the following specific isoforms and alleles are also discussed: CYP1A1 (*2B and *4 alleles), CYP2C8 (*3 and *4 alleles), CYP2C9 (*2, *3, and *11 alleles), CYP2C19 (*2 and *3 alleles), CYP2D6 (*3, *4, *5, and *6 alleles), CYP2E1 (*5A, *5B, and *6 alleles), and CYP3A5 (*3 allele) (Daly et al. 2006).

Samuel W. French

Polyunsaturated Fats Aggravate Alcohol Mediated Liver Injury: CYP2E1, a Key Player

Increasing the contents of linoleic acid or polyunsaturated fatty acids (PUFAs) in the diet with intragastric tube feeding model worsens the pathology where CYP2E1

is increased posttranslationally by high blood alcohol level (Morimoto et al. 1993). Thus, CYP2E1 induction plays a central role in the pathogenesis of ALD which is worsened by increase in dietary PUFA content (Morimoto et al. 1993). Further, fish oil diet, like corn oil, potentiates ethanol-induced liver injury which includes severe inflammation and focal fibrosis, fatty liver, and increased microsomal NADPH peroxidation and these pathological changes are related to CYP2E1 induction and the presence of polyunsaturated fatty acids in the diet (i.e., either n-6 or n-3) (Morimoto et al. 1994).

Fatty Acid Metabolism and Hepatic CYP2E1

Peroxisome proliferator-activated receptor (PPAR) and retinoid x receptor (RXR) play important roles in fatty acid metabolism (Wan et al. 1995). In contrast to the unchanged levels of RAR and RXR isoforms and catalase, the levels of PPAR and CYP2E1 mRNAs are down- and up-regulated by chronic dosage of ethanol in rat liver, respectively (Wan et al. 1995). The levels of CYP2E1 mRNAs correlate positively with blood alcohol levels (BAL) (Wan et al. 1995). The level of PPAR mRNA and the content of PUFA decreases in ethanol-fed rat livers and the authors conclude that decreased PPAR gene expression in ethanol-fed rats might result from a decrease in the content of polyunsaturated fatty acid in the liver (Wan et al. 1995).

The CYP2E1 inhibitors, diallyl sulfide (DAS) and phenethyl isothiocyanate (PIC) ameliorate both the ethanol-induced changes in fatty acids and the shift in succinic dehydrogenase in livers in rats fed ethanol intragastrically (Morimoto et al. 1995a). Rats fed ethanol without the CYP2E1 inhibitors have significantly greater hepatic total fatty acids and triglyceride fractions, significantlylower ratio of fatty acids with 20:4/18:2 composition and greater 18:1/18:0 fatty acids and many of these effects are inhibited with CYP2E1 inhibitors (Morimoto et al. 1995a). Thus, the changes in the fatty acid composition due to ethanol ingestion are due to CYP2E1-dependent lipid peroxidation and fatty acid metabolism.

Alcohol Mediated CYP2E1 Induction and Lipoperoxidative Liver Injury

In rats fed ethanol intragastrically, ingestion of the CYP2E1 inhibitor PIC decreases ethanol mediated increases in microsomal CYP2E1 protein levels and catalytic activity, and microsomal reduced form of nicotinamide-adenine dinucleotide (NADPH)-dependent lipid peroxidation (Morimoto et al. 1995b). Both DAS and PIC decrease CYP2E1 mRNA (Morimoto et al. 1995b). The lobular distribution of CYP2E1 in liver changes from the centrilobular to a diffuse pattern, with an increase in the periportal region when the CYP2E1 inhibitors are co-administered with ethanol, and this change correlates with the change in the distribution of fat in the lobule (Morimoto et al. 1995b). Thus, a link exists between CYP2E1 induction by ethanol, consequent lipid peroxidation and the early phase of ethanol-induced liver injury in this rat model.

CYP2E1 and Mallory Body Formation

The formation of Mallory body (MB), aggregates of proteins, principally cytokeratin is a complex phenomenon seen in chronic liver disease and CYP2E1 may

play a role in preventing MB formation since it is involved in the elimination of toxic drugs and chemicals (Bardag-Gorce et al. 2005). When mice are fed with diethyl-1,4-dihydro-2,4,6-trimethyl-3,5-pyridinedicarboxylate (DDC), a suicide inhibitor of CYP2E1 for 10 weeks, Mallory bodies (MBs) develop in the liver at the end of this period (Bardag-Gorce et al. 2005). When DDC feeding is combined with CMZ (an efficient *in vivo* CYP2E1 inhibitor), more MBs form associated with decreased levels of hepatic CYP2E1 protein compared to DDC feeding alone (Bardag-Gorce et al. 2005). When CYP2E1 knockout mice and CYP2E1 over-expressed mice are fed with DDC or DDC and CMZ for 10 weeks, MB formation increases markedly in the liver of CYP2E1 knockout mice when fed with DDC only (Bardag-Gorce et al. 2005). CYP2E1 over-expressed mice show an increase in MB formation when the mice are fed with the combination of DDC and CMZ where the amount of CYP2E1 is reduced to levels seen in wild type mice (Bardag-Gorce et al. 2005). Thus, CYP2E1 inhibits MB formation by increasing the rate of elimination of DDC and/or its toxic intermediates (Bardag-Gorce et al. 2005).

cAMP: Its Protective Effects on Ethanol Mediated Liver Injury Through Decrease in CYP2E1 Synthesis, with Ubiquitin and Proteosomal Enzymes as Co-Players

The factors which govern CYP2E1 degradation and turnover include cAMP, ubiquitin, proteasomal enzymes and CYP2E1 mRNA (Gouillon et al. 1999). The cAMP treatment of rats fed ethanol ameliorates the increased liver fat storage and changes in the hepatic fatty acid composition (Gouillon et al. 1999). The amounts of ubiquitin and ubiquitin conjugates and level of ubiquitin mRNA are markedly reduced by ethanol treatment (Gouillon et al. 1999). cAMP ameliorates the inhibition of the proteasomal enzyme proteolysis caused by ethanol feeding (Gouillon et al. 1999). The ethanol-induced increase in the CYP2E1 protein is partially inhibited by cAMP treatment (Gouillon et al. 1999). cAMP treatment decreases CYP2E1 mRNA levels in both ethanol-fed and pair fed control rats (Gouillon et al. 1999). Thus, cAMP treatment partially protects the liver from ethanol-induced fatty liver by reducing CYP2E1 induction through cAMP's effects on CYP2E1 synthesis (Gouillon et al. 1999).

Chlormethiazole: An Effective *In Vivo* Inhibitor of Alcohol Inducible Hepatic CYP2E1 Activity

Administration of the sedative and hypnotic drug chlormethiazole to rats fed ethanol intragastrically for 2 months have significantly less liver injury (Gouillon et al. 2000). Chlormethiazole inhibits the increase in the ethanol-induced CYP2E1 activity *in vivo*, but does not affect the level of increased CYP2E1 apoprotein (Gouillon et al. 2000). Likewise, the reduction in proteasome proteolytic enzyme activity produced by ethanol feeding is blunted in chlormethiazole-fed rats (Gouillon et al. 2000). Thus, chlormethiazole treatment partially protects the liver from injury by inhibiting CYP2E1 activity *in vivo* (Gouillon et al. 2000).

Oxidative Protein Damage and Alcohol Induced Liver Injury: A Consequence of Inhibition of Proteasome Activity by CYP2E1

The proteasomal chymotrypsin-like activity is not reduced in ethanol-fed CYP2E1 knockout mice liver (Bardag-Gorce et al. 2000). The 26S proteasomal activity is decreased more by ethanol feeding than the 20S proteasomal fraction in ethanol-fed wild-type mice liver (Bardag-Gorce et al. 2000). Thus, CYP2E1 induction is responsible for the decrease in proteasome activity seen in the wild-type mice which leads to the accumulation of oxidized proteins in the ethanol treated wild type but not in the knockout mice which are increased as the result of free radicals generated by CYP2E1 metabolism of ethanol (Bardag-Gorce et al. 2000).

CYP2E1 and Proteasome: A Reciprocal Relationship

CYP2E1 degradation *in vivo* using a potent proteasome inhibitor PS-341 has been characterized (Bardag-Gorce et al. 2002). Ethanol is withdrawn at the same time that PS-341 is injected, 24 h before the rats are sacrificed (Bardag-Gorce et al. 2002). Ethanol treatment induces a threefold increase in CYP2E1 and inhibition of liver proteasomal chymotrypsin-like activity (ChT-L) (Bardag-Gorce et al. 2002). When ethanol is withdrawn, CYP2E1 decreases to control levels and the proteasomal ChT-L activity returns to control levels (Bardag-Gorce et al. 2002). In ethanol-withdrawn rats injected with PS-341, CYP2E1 remains at the induced level and the ChT-L activity is significantly inhibited before withdrawal (Bardag-Gorce et al. 2002). Thus, the proteasome is responsible for ethanol-induced CYP2E1 degradation *in vivo* (Bardag-Gorce et al. 2002).

Inhibition of Proteasome Activity by Alcohol Inducible CYP2E1: Involvement of HNE Adducts

In the arachidonic acid plus Fe-NTA plus ethanol treated E47 cells over expressing CYP2E1, CYP2E1 is induced accompanied by a higher level of ROS and carbonyl protein formation, and decreased proteasome activity (Bardag-Gorce et al. 2006). The decrease in proteasome activity in E47 cells is prevented by CYP2E1 inhibition with DAS (Bardag-Gorce et al. 2006). Down regulation of the proteasome subunit, as well as ubiquitin pathway proteins occurs in the ethanol-treated E47 cells (Bardag-Gorce et al. 2006). Increased 4-HNE adducts are observed in the E47 cells treated with ethanol (Bardag-Gorce et al. 2006). Furthermore, the immunoprecipitated 4-HNE modified proteins from these cells stain positive with antibodies to the proteasome subunit alpha 6 (Bardag-Gorce et al. 2006). Thus, ethanol mediated induction of CYP2E1 generates oxidative stress that is responsible for the decrease in proteasome activity (Bardag-Gorce et al. 2006).

Cytokeratin 8 and 18 are induced by ethanol treatment of E47 cells and polyubiquitinated forms of these proteins are found in the polyubiquitin smear (Bardag-Gorce et al. 2006). Cytokeratin aggresomes and Mallory body-like inclusions form in the ethanol-treated E47 cells, indicating that the ubiquitinated cytokeratins accumulate as a result of the inhibition of the proteasome by ethanol treatment when oxidation due to ethanol induced oxidative stress occurs (Bardag-Gorce et al. 2006).

Thus, ethanol causes Mallory body-like cytokeratin inclusions in transformed human liver cells over-expressing CYP2E1 *in vitro* (Bardag-Gorce et al. 2006).

II

M. Raj Lakshman

Ethanol Mediated Decrease in Gal Beta l, 4GlcNAc Alpha 2,6-Sialyltransferase Activity; Role of CYP2E1 Mediated Acetaldehyde Generation and Lipid Peroxidation

Gal beta l, 4GlcNAc alpha 2,6-sialyltransferase (2,6-ST) mediates the addition of alpha 2,6-linked sialic acid to glycoproteins in the Golgi compartment (Garige et al. 2005). Down-regulation of its gene and consequent impaired activity of 2,6-ST seems to be the major cause for the appearance of asialoconjugates in the blood of long-term alcoholics (Garige et al. 2005). Long-term ethanol feeding in rats causes marked decreases of 2,6-ST activity and mRNA level in liver that are due to the decreased stability of its mRNA (Garige et al. 2005). Similar actions of ethanol on 2,6-ST mRNA levels are observed in human liver HepG2 cells stably transfected with ethanol-inducible human CYP2E1 (CYP2E1 cells), or with high alcohol dehydrogenase (HAD cells) but not in wild-type HepG2 cells lacking CYP2E1 expression (Garige et al. 2005). However, incubation of wild-type cells with acetaldehyde causes a dramatic decrease in the 2,6-ST mRNA levels (Garige et al. 2005). Furthermore, exposure of CYP2E1 cells to 4-HNE strongly decreases 2,6-ST mRNA level (Garige et al. 2005). Thus, 2,6-ST gene is highly sensitive to ethanol action in human liver cells either *via* its oxidation product, acetaldehyde, or *via* ROS leading to the generation of a more reactive aldehyde such as 4-HNE (Garige et al. 2005). It is interesting to note that ethanol-inducible CYP2E1 is involved in all the above stated processes, thus suggesting the CYP2E1 mediated regulation of sialyl transferases and hence dysregulated sialyl transferase machinery in alcoholic condition.

Terrence M. Donohue

In Vitro CYP2E1 Mediated Metabolism of Ethanol Leads to Inhibition of Proteasome in Liver Cells

HepG2 cells are transfected with recombinant plasmids, one carrying the murine ADH gene and the other containing the gene encoding human CYP2E1 (Donohue et al. 2006). One of recombinant clones called VL-17A exhibits ADH and CYP2E1 specific activities comparable to those in isolated rat hepatocytes (Donohue et al. 2006). VL-17A cells oxidize ethanol and generate acetaldehyde, the levels of which depend upon the initial ethanol concentration (Donohue et al. 2006). Compared with unexposed VL-17A cells, ethanol exposure increases the cellular redox and causes cell toxicity (Donohue et al. 2006). Exposure of VL-17A cells to 100 mM ethanol significantly elevates caspase 3 activity, an indicator of apoptosis, but not in parental HepG2 cells (Donohue et al. 2006). Because ethanol consumption causes a decline in hepatic protein catabolism, the influence of ethanol exposure on proteasome activity in HepG2, VL-17A (CYP2E1(+)) and (ADH(+)), E-47 (CYP2E1(+)) and VA-13 (ADH(+)) cells has been investigated (Donohue et al. 2006). Exposure to 100 mM ethanol causes a 25% decline in the chymotrypsin-like activity of the

proteasome in VL-17A cells (Donohue et al. 2006). This inhibitory effect on the proteasome is blocked when ethanol metabolism is blocked by 4-MP (Donohue et al. 2006). Thus, recombinant VL-17A cells, which express both ADH and CYP2E1, exhibit hepatocyte-like characteristics in response to ethanol (Donohue et al. 2006). Furthermore, the metabolism of ethanol by these cells *via* ADH and CYP2E1 is sufficient to bring about an inhibition of proteasome activity that may lead to apoptotic cell death (Donohue et al. 2006).

Deficiency of SOD Is Associated with Decreased Ethanol Metabolism and Lack of Alcohol Mediated Inducibility of CYP2E1

Ethanol-fed SOD(−/−) mice exhibit lower ADH activity and lack of CYP2E1 inducibility, thereby causing decreased ethanol metabolism compared with wild-type mice (Curry-McCoy et al. 2010). Further, the other atypical responses to ethanol, including the absence of ethanol-induced steatosis and enhanced GSH levels, appear to be linked to enhanced oxidative stress due to lack of antioxidant enzyme capacity.

Natalia A. Osna/Terrence M. Donohue

Regulation of CYP2E1 and Proteasome Activity by IFNgamma

Interferon gamma (IFNgamma) initiates signal transduction, which alters the activities of CYP2E1 and iNOS, thereby producing ROS in VL-17A (ADH(+), CYP2E1(+)) and E-47 (CYP2E1(+)) cells (Osna et al. 2003). One of these oxidants, possibly peroxynitrite, may be directly involved in proteasome activation (Osna et al. 2003). Ethanol metabolism by VL-17A cells suppresses IFNgamma-mediated induction of proteasome activity, in part, by preventing STAT1 phosphorylation (Osna et al. 2003).

CYP2E1 and ADH Mediated Metabolism of Ethanol Leads to Reduction in STAT1 Phosphorylation

IFNgamma signal transduction is suppressed by ethanol in VL-17A cells (Osna et al. 2005). The mechanisms by which STAT1 phosphorylation is blocked by ethanol treatment in VL-17A cells have been evaluated (Osna et al. 2005). Reduction of STAT1 phosphorylation by 100 mM ethanol is prevented in the presence of 4MP, DAS, or uric acid, indicating that the oxidative products from ethanol metabolism are partly responsible for suppression of STAT1 phosphorylation (Osna et al. 2005). Ethanol exposure decreases STAT1 tyrosine phosphorylation, whereas serine phosphorylation on the protein is unchanged (Osna et al. 2005). These effects of ethanol are mimicked by the peroxynitrite (PN) donor, SIN-1, which also blocks tyrosine, but not serine phosphorylation, on STAT1 (Osna et al. 2005). Further, under conditions of ethanol-elicited oxidative stress, peroxynitrite (PN) prevents STAT1 phosphorylation by stabilization of SOCS1, and possibly by nitration of tyrosine residues in STAT1 protein cells in expressing either ADH (VA-13 cells) or CYP2E1 (E-47 cells) (Osna et al. 2005).

CYP2E1 Mediated Metabolism of Ethanol Leads to Oxidative Stress Which Suppresses HCV Mediated Low Oxidative Stress and Inhibits Proteasome Activity

Proteasome activity in inducible Hepatitis C core-positive 191–20 cells is 20% higher than that in core-negative cells and is enhanced threefold in CYP2E1-expressing L14

cells (Osna et al. 2008). Exposure of core-positive cells to glutathione ethyl ester, catalase, or the CYP2E1 inhibitor DAS partially reverses the elevation of proteasome activity in core-positive cells, whereas ethanol exposure suppresses proteasome activity (Osna et al. 2008). Thus, proteasome activity is up-regulated by low levels of HCV core-induced oxidative stress but down-regulated by high levels of ethanol-elicited stress (Osna et al. 2008).

Suppression of Activities of Antigen-Trimming Enzymes Through Ethanol Metabolism: Involvement of CYP2E1

Processing of peptides for antigen presentation is catalyzed by antigen-trimming enzymes, including the proteasome and leucine aminopeptidase (Osna et al. 2007). Oxidative stress suppresses proteasome function (Osna et al. 2007). Ethanol exposure to VL-17A cells increases CYP2E1 and decreases proteasome peptidase activities. The latter effect is prevented by treatment of cells with inhibitors, 4-MP and DAS. Ethanol metabolism suppresses activities of antigen-trimming enzymes, thereby decreasing the cleavage of C-extended and N-extended peptides in ethanol-metabolizing VL-17A cells or WIF-B cells over-expressing CYP2E1 (Osna et al. 2007). This defect may potentially result in decreased MHC class I-restricted antigen presentation on virally infected liver cells (Osna et al. 2007).

The proteasome is a major enzyme that cleaves proteins for antigen presentation (Osna et al. 2009). Cleaved peptides traffic to the cell surface, where they are presented in the context of major histocompatibility complex (MHC) class I (Osna et al. 2009). Recognition of these complexes by cytotoxic T lymphocytes is crucial for elimination of cells bearing "nonself" proteins (Osna et al. 2009). In primary mouse hepatocytes, even in the absence of IFNgamma, a similar decline in proteasome activity and antigen presentation after ethanol exposure has been observed (Osna et al. 2009). Proteasome function is directly suppressed by ethanol metabolism and indirectly by preventing the activating effects of IFNgamma (Osna et al. 2009). Ethanol-elicited reduction in proteasome activity contributes to the suppression of the ovalbumin peptide SIINFEKL-H2Kb presentation on the surface of mouse hepatocyte cell line (CYP2E1/ADH-transfected HepB5 cells) (Osna et al. 2009).

Suppression of Proteasome Activity by Impaired Methylation of Proteasome Subunits in CYP2E1 Expressing Liver Cells

The chymotrypsin-like proteasome activity in Huh7 cells stably transfected with CYP2E1 plasmid, hepatocytes isolated from mice or prepared liver cytosols and nuclear extracts or purified 20S proteasome under conditions that maintain or prevent protein methylation has been determined (Osna et al. 2010). Reduction of proteasome activity of hepatoma cell and hepatocytes by ethanol or tubercidin is prevented by simultaneous treatment with S-adenosylmethionine (SAM) (Osna et al. 2010). Moreover, the tubercidin-induced decline in proteasome activity occurs in both nuclear and cytosolic fractions (Osna et al. 2010). *In vitro* exposure of cell cytosolic fractions or highly purified 20S proteasome to low SAM:S-adenosylhomocysteine (SAH) ratios in the buffer also suppresses proteasome function, indicating that one or more methyltransferase(s) may be associated with

proteasomal subunits (Osna et al. 2010). Impaired methylation of proteasome subunits suppresses proteasome activity in liver cells indicating an additional, yet novel mechanism of proteasome activity regulation by ethanol (Osna et al. 2010).

Vasilis K. Vasiliou
Brain CYP2E1 and Ethanol Sensitivity
The role of CYP2E1 and catalase in ethanol metabolism and sensitivity, using transgenic knockout Cyp2e1(−/−) mice, acatalasemic (Cs/Cs) mice, and double mutant Cyp2e1(−/−)/Cs/Cs mice has been investigated (Vasiliou et al. 2006). Cs/Cs, Cyp2e1(−/−) and Cyp2e1(−/−)/Cs/Cs mice exhibit longer ethanol-induced sleep times, especially at higher ethanol doses (Vasiliou et al. 2006). This infers that there is less acetaldehyde produced in the brains of these animals and is in opposition to the idea that increased acetaldehyde increases the actions of ethanol (Vasiliou et al. 2006). The Cyp2e1(−/−) animals produce lower whole blood levels of acetaldehyde than wild-type controls; however, this difference is seen only at higher doses of ethanol (Vasiliou et al. 2006). The amount of acetaldehyde produced following the incubation of ethanol with liver and brain microsomes is greater in tissues derived from wild type mice with normal CYP2E1 expression than in those from Cyp2e1(−/−) mice (Vasiliou et al. 2006). Although the contribution of CYP2E1 and catalase in ethanol oxidation may be of little significance, these enzymes appear to play a significant role in ethanol sensitivity in the brain (Vasiliou et al. 2006).

H. Tsukamoto
Development of Intragastric Model for Alcohol Consumption and Liver Injury: Role of CYP2E1
Adult male Wistar rats intragastrically infused with a high-fat diet and ethanol or glucose for 16 weeks exhibit hepatic lipid peroxidation and fibrogenesis (Castillo et al. 1992). A 52% higher basal lipid peroxidation is observed in microsomes from alcohol-fed rats compared to the controls. Enhancement of lipid peroxidation induced by CC14 or iron is also accentuated twofold in the microsomes from the former rats (Castillo et al. 1992). All these increases in basal, CC14- induced, and iron-catalyzed lipid peroxidation in microsomes from alcohol-fed rats are completely blocked by addition of anti-CYP2E1 IgG but not by nonimmune control IgG (Castillo et al. 1992). These results are also complemented by a severalfold increase in the level of CYP2EI protein and catalytic activity (Castillo et al. 1992). Thus, CYP2E1 plays an important role in the enhanced microsomal lipid peroxidation in experimental alcoholic liver disease and to the increased vulnerability of the microsomes from alcohol-fed rats to CC14 and iron-induced oxidative stress.

E. Albano
CYP2E1 Mediated Ethanol Metabolism Leads to Generation of Hydroxyethyl Radicals and Formation of Auto-Antibodies
Treatment of rats with DAS and PIC significantly decreases the trapping of hydroxyethyl free radicals in liver microsomes incubated *in vitro* with ethanol (Albano et al. 1996). Furthermore, these inhibitors also greatly reduce the production of hydroxyethyl radical-derived epitopes detectable *in vivo* in the liver of ethanol-fed rats

(Albano et al. 1996). The actions of DAS and PIC on the formation of hydroxyethyl radicals parallel their inhibitory effect on lipid peroxidation (Albano et al. 1996). Thus, these results indicate a link between the induction of CYP2E1 by ethanol, the formation of hydroxyethyl radicals and the stimulation of lipid peroxidation (Albano et al. 1996). The pathological scores in the livers of rats fed with ethanol plus or minus DAS and PIC also correlate with levels of hydroxyethyl radical-derived epitopes (Albano et al. 1996). Rats fed intragastrically with ethanol develop antibodies and the formation of these antibodies is greatly reduced by DAS and PIC (Albano et al. 1996). Thus, CYP2E1 plays an important role in the generation of hydroxyethyl radicals during chronic alcohol feeding and that ethanol-derived free radicals might play a role in the onset of liver injury in this model of alcohol administration (Albano et al. 1996).

In alcoholic patients, oxidation of chlorzoxazone, a CYP2E1 probe increases consistently with CYP2E1 induction by ethanol (Dupont et al. 1998). IgG reacting with hydroxyethyl free radical-protein adducts significantly increases in alcoholics with induced CYP2E1 activity (Dupont et al. 1998). Further, chlorzoxazone oxidation is significantly lowered in alcoholics without clinical and biochemical signs of liver disease as compared to patients with alcoholic liver disease (Dupont et al. 1998). Thus, CYP2E1 activity greatly influences the formation of hydroxyethyl radicals in humans and plays a possible role in the development of alcoholic liver disease (Dupont et al. 1998).

CYP2E1-hydroxyethyl radical adducts are present in the plasma membranes of isolated rat hepatocytes incubated *in vitro* with ethanol or obtained from ethanol-treated animals (Clot et al. 1997). Further, cytotoxicity is observed in ethanol-treated hepatocytes incubated with immunoglobulin G from patients with ALD and normal human blood mononuclear cells (Clot et al. 1997). The observed cytotoxicity is blocked by preabsorbing the sera with human albumin complexed with hydroxyethyl radicals, which also eliminates the antibody reaction with the plasma membranes (Clot et al. 1997). Thus, hydroxyethyl radicals bound to CYP2E1 on hepatocyte plasma membranes can target immune reactions triggered by alcohol abuse (Clot et al. 1997).

Formation of CYP2E1 Autoantibodies in Hepatitis C Patients

Auto-antibodies against CYP2E1 are significantly increased in chronic hepatitis C (CHC) patients with and without alcohol consumption (Vidali et al. 2007). Further, anti-CYP2E1 auto-reactivity is significantly associated with the severity of periportal/periseptal interface hepatitis (Vidali et al. 2007). Further, anti-CYP2E1 IgG associated with CHC recognizes CYP2E1 exposed on the outer side of hepatocyte plasma membranes (Vidali et al. 2007). Thus, hepatitis C virus (HCV) infection favours the breaking of self-tolerance against CYP2E1 that might contribute to hepatocyte injury (Vidali et al. 2007).

The antigen specificity and the possible origin of circulating auto-antibodies targeting conformational antigens on CYP2E1 detectable in CHC patients have been characterized (Sutti et al. 2010a, b). In CHC patients, cross-reactivity between CYP2E1 and specific sequences in the NS5b protein of HCV promotes the

development of auto-antibodies targeting conformational epitopes on the CYP2E1 surface that might contribute to hepatic injury (Sutti et al. 2010a). The development of anti-CYP2E1 auto-antibodies targeting conformational CYP2E1 epitopes is associated with more severe liver damage-necro-inflammatory and fibrosis in CHC (Sutti et al. 2010b).

Post-orthotropic Liver Transplantation Recurrent Hepatitis C and CYP2E1

As autoimmune reactions are increasingly detected after orthotopic liver transplantation (OLT), the prevalence and significance of anti-CYP2E1 autoantibodies in patients with post-OLT recurrent hepatitis C has been studied (Rigamonti et al. 2009). IgG against recombinant human CYP2E1 above the control threshold is detected in sera collected immediately before and after OLT (Rigamonti et al. 2009). Although anti-CYP2E1 reactivity is not modified by OLT, the patients with persistently elevated anti-CYP2E1 IgG show significantly higher prevalence of recurrent hepatitis with severe necroinflammation and fibrosis than those persistently negative or positive only either before or after OLT (Rigamonti et al. 2009). Moreover, the probability of developing severe necroinflammation is significantly higher in persistently anti-CYP2E1-positive subjects (Rigamonti et al. 2009). The persistence of anti-CYP2E1 IgG, together with a history of acute cellular rejection and donor age >50 years, is an independent risk factor for developing recurrent hepatitis C with severe necroinflammation (Rigamonti et al. 2009). Thus, autoimmune reactions involving CYP2E1 might contribute to hepatic damage in a subgroup of transplanted patients with recurrent hepatitis C (Rigamonti et al. 2009).

Xiang-Dong Wang

CYP2E1 and Retinoic Acid Metabolism

Investigations into the role of CYP2E1 in the metabolism of retinoic acid (RA) show that the incubation of the liver microsomal fraction isolated from ethanol-treated rats with RA results in greater disappearance of RA and increased appearance of 18-hydroxy-RA and 4-oxo-RA compared with control rat liver microsomal fractions (Liu et al. 2001). The enhancement of RA catabolism by ethanol is inhibited by CYP2E1 inhibition in a dose-dependent fashion (Liu et al. 2001). Thus, ethanol-induced CYP2E1 plays a major role in the degradation of RA, which may provide a possible biochemical mechanism for chronic and excessive ethanol intake as a risk for both hepatic and extrahepatic cell proliferation and carcinogenesis (Liu et al. 2001).

The Antioxidant Lycopene Mediated CYP2E1 Induction

Lycopene supplementation at the higher dose significantly induces hepatic CYP2E1 protein, TNFalpha mRNA, and the incidence of inflammatory foci in the alcohol-fed rats (Veeramachaneni et al. 2008). Therefore, an interaction between chronic alcohol ingestion and lycopene supplementation occurs and suggests a need for caution among individuals consuming high amounts of both alcohol and lycopene (Veeramachaneni et al. 2008).

High Fat Diet Induced NASH and CYP2E1 Induction

The key histological features of NASH, including steatosis, inflammatory cell infiltration, and ballooning degeneration of hepatocytes, are induced in rats fed high-fat diet (HFD), with increased hepatic TNFalpha mRNA expression (Wang et al. 2008). HFD-fed rats have elevated lipid peroxidation products and CYP2E1 protein in the liver (Wang et al. 2008). HFD feeding increases both hepatic phosphorylated JNK and apoptotic parameters (Wang et al. 2008). Thus, the increased oxidative stress and its associated JNK activation as well as an imbalance of pro- and anti-apoptotic proteins in the Bcl-2 family all contribute to high hepatocyte apoptosis that may play an important role in the pathogenesis of NASH in this model, which is associated with induction of CYP2E1 (Wang et al. 2008).

A.A. Nanji

A Close Relationship Between CYP2E1 and Conjugated Dienes: *In Vivo* and *In Vitro* Studies

A significant correlation is observed between the level of CYP2E1 and the decrease in arachidonic acid (AA) in rats fed ethanol and corn oil intragastrically (Nanji et al. 1993). The decrease in AA also correlates with the increase in conjugated dienes (Nanji et al. 1993). Phospholipase C (PLC) and phospholipase A (PLA) activities are both significantly increased with correlated decline in AA in the corn oil plus dextrsose and ethanol plus corn oil fed rats (Nanji et al. 1993). The correlations observed between the decrease in microsomal AA and CYP2E1 induction and conjugated diene formation suggest that these processes may be interlinked especially in regard to generation of lipid peroxides that may play a role in alcoholic liver injury (Nanji et al. 1993).

The role of changes in CYP2E1 and lipid peroxidation in relation to development of severe liver injury in fish oil-ethanol-fed rats has been studied (Nanji et al. 1994). Greater pathological changes and significantly higher CYP2E1 protein levels are evident in the rats fed fish oil and ethanol than in the corn oil and ethanol group (Nanji et al. 1994). Higher levels of eicosapentaenoic and docosahexaenoic acids and lower levels of AA are detected in liver microsomes from rats fed fish oil (fish oil and ethanol/fish oil and dextrose) compared with corn oil and ethanol group (Nanji et al. 1994). A significant correlation is obtained between CYP2E1 protein and conjugated diene levels (Nanji et al. 1994). The markedly increased CYP2E1 induction and lipid peroxidation in the fish oil and ethanol group provides one possible explanation for the greater severity of liver injury in this group (Nanji et al. 1994).

D.J. Waxman

Metabolism of Organic Solvents and Development of Assay for the Catalytic Activity of CYP2E1

The ability of CYP2E1 to catalyze the oxidative metabolism of many solvents and other small organic molecules has been utilized in developing a spectrophotometric method for determination of CYP2E1 activity by monitoring the formation of p-nitrocatechol from p-nitrophenol by cDNA-expressed CYP2E1 or isolated liver microsomes (Chang et al. 2006). The mehod holds importance in assessing the content of catalytically active CYP2E1 through an enzymatic assay.

D.R. Koop

CYP2E1 and Kupffer Cells

CYP2E1 is immunochemically detectable at low levels in Kupffer cell homogenates from untreated rats and is greatly induced by acetone-treatment (Koop et al. 1991). The presence of CYP2E1 in Kupffer cells from untreated rats is confirmed by inhibition of benzene hydroxylation with anti-CYP2E1 immunoglobulin G (Koop et al. 1991). Benzene hydroxylase activity is induced to a similar extent and similar specific activity is observed in Kupffer cells and hepatocytes isolated from acetone-treated rats (Koop et al. 1991). Further, the induction in benzene hydroxylation in Kupffer cells is inhibited by anti-P4502E1 antibody (Koop et al. 1991). The presence and inducibility of CYP2E1 in Kupffer cells suggests that, under conditions where CYP2E1 is induced, Kupffer cell-generated metabolites may contribute to Kupffer cell toxicity, as well as general hepatic injury (Koop et al. 1991).

Differential Degradation of Hepatic CYP2E1 Protein and the Central Role of Ubiquitin

Different modes of inactivation of hepatic CYP2E1 by different agents has been investigated (Tierney et al. 1992). Carbon tetrachloride (CCl4) is metabolized by CYP2E1 to trichloromethyl free radicals which exert hepatotoxic effects. Treatment of mice with CCl4 at the point of maximal induction of CYP2E1 after a single oral dose of acetone results in the complete loss of CYP2E1-dependent p-nitrophenol hydroxylation and a 75% loss of immunochemically detectable protein within 1 h of administration (Tierney et al. 1992).

Further, treatment with the CYP inhibitor 1-aminobenzotriazole at the point of maximal induction causes a complete loss of CYP2E1-dependent p-nitrophenol hydroxylation but only a 12% loss of immunochemically detectable protein 1 h after administration (Tierney et al. 1992).

Treatment of mice with CYP2E1 inhibitor 3-amino-1,2,4-triazole causes a rapid loss of both catalytic activity and microsomal p-nitrophenol hydroxylase activity (Tierney et al. 1992). However, unlike CCl4 treatment, the activity and enzyme level rebounds 5 and 9 h after treatment (Tierney et al. 1992).

The CYP2E1 ligand, 4-MP, administered at the point of maximal induction maintains the acetone-induced catalytic and immunochemical level of CYP2E1 (Tierney et al. 1992). Thus, differentially modified forms of CYP2E1 show a characteristic susceptibility to degradation (Tierney et al. 1992). While there are many potential pathways for protein degradation, the loss of CYP2E1 is associated with increased formation of high molecular weight microsomal ubiquitin conjugates (Tierney et al. 1992). The formation of ubiquitin-conjugated microsomal protein which correlates with CYP2E1 loss suggests that ubiquitination may represent a proteolytic signal for the rapid and selective proteolysis of certain labilized conformations of CYP2E1 from the endoplasmic reticulum (Tierney et al. 1992).

Regulation of CYP2E1 by the Broad-Spectrum Antibiotic Tetracycline: Induction, Stability and Degradation

A tetracycline (Tc)-controlled gene expression system comprised of subcloned rabbit CYP2E1 cDNA that quantitatively controls gene expression in eukaryotic

cells has been used to express CYP2E1 in HeLa cells in culture (Huan and Koop 1999). A time-dependent induction and more than 100-fold induction of CYP2E1 is observed after removal of Tc (Huan and Koop 1999). At maximal levels of expression, the enzyme catalyzes the formation of much higher amounts of 6-hydroxychlorzoxazone (Huan and Koop 1999). In addition, the level of the enzyme could be modulated by the concentration of Tc in the media (Huan and Koop 1999). In the absence of Tc, exposure of cells to NDMA causes a significant dose-dependent decrease in cell viability (Huan and Koop 1999). A rapid turnover of CYP2E1 with a half-life of 3.9 h is observed 72 h after removal of Tc (Huan and Koop 1999). Addition of the ligand, 4-MP, and the suicide substrate, 1-aminobenzotrizole, decreases the degradation of CYP2E1 (Huan and Koop 1999). This cell line offers a useful system to examine the role of CYP2E1 in the cytotoxicity of xenobiotics and to investigate post-translational regulation of the enzyme (Huan and Koop 1999).

The degradation of ethanol-inducible CYP2E1 expressed in tetracycline (Tc)-inducible HeLa cell linehas been characterized (Huan et al. 2004). A half-life of 3.8 h is observed for CYP2E1 (Huan et al. 2004). Lactacystin and other selective proteasome inhibitors including N-benzyloxycarbonyl-leucyl-leucyl-leucinal (MG132) and N-benzyloxycarbonyl-L-leucyl-L-leucyl-L-norvalinal (MG115) significantly inhibit CYP2E1 degradation (Huan et al. 2004). Further, the turnover of CYP2E1 is slightly inhibited by calpain inhibitors (Huan et al. 2004).

R.F. Novak

Peroxisome Proliferation and Induction of CYP2E1: The Dynamics Involved

Treatment of primary cultured rat hepatocytes with the peroxisome proliferator and anti-hyperlipidemic drug ciprofibrate (CIPRO) for the time period of 24 h causes a significant increase in CYP2E1 mRNA at a wide range of dosage (30–300 μM) of CIPRO (Zangar et al. 1995). CYP2E1 mRNA levels are maximally elevated between two and threefold at both 24 and 48 h and return to basal levels by 72 h with 30 μM CIPRO (Zangar et al. 1995).

Insulin Mediated Regulation of CYP2E1

Insulin itself, in the absence of other diabetes-induced metabolic or hormonal alterations, affects CYP2E1 expression in primary cultured rat hepatocytes (Woodcroft and Novak 1997). Decreasing the concentration of insulin in the culture medium provides a method by which CYP2E1 levels can be increased in primary cultured hepatocytes to facilitate mechanistic studies on the regulation of CYP2E1 expression (Woodcroft and Novak 1997).

The signaling pathways involved in insulin and glucagon regulation of CYP2E1 expression are examined in primary cultured rat hepatocytes (Woodcroft and Novak 1999). Glucagon (100 nM) opposes the effects of insulin (1 nM) on CYP2E1 mRNA expression and conversely, insulin blocks the effects of glucagon (Woodcroft and Novak 1999). Thus, the regulation of CYP2E1 expression occurs via mutually antagonistic signaling pathways involving insulin and glucagon (Woodcroft and Novak 1999).

The ketone body and insulin mediated regulation of CYP2E1 expression has been studied in primary cultured rat hepatocytes (Woodcroft et al. 2002). The ketone bodies-hydroxybutyrate and acetoacetate (AC), alone or in combination, either fail to affect or decrease CYP2E1 mRNA levels by up to 90% relative to untreated hepatocytes (Woodcroft et al. 2002). Insulin produces a concentration-dependent decrease in CYP2E1 mRNA levels, and transcriptional and posttranscriptional mechanisms are involved in the insulin-mediated regulation of CYP2E1 and implicate phosphatidylinositol 3-kinase, p70 S6 kinase, and Src kinase in mediating these effects (Woodcroft et al. 2002).

Regulation of CYP2E1 by Ketone Bodies

The ketone body acetoacetate decreases CYP2E1 mRNA expression through inhibition of gene transcription while simultaneously elevating CYP2E1 protein levels through increased translation and decreased protein degradation in primary cultured rat hepatocytes (Abdelmegeed et al. 2005).

Structural Elucidation of CYP2E1 mRNA for Understanding Its Translational Efficiency

Regulation of hepatic CYP2E1 by xenobiotic or physiological stimuli is largely mediated through post-transcriptional mechanisms that may include altered CYP2E1 mRNA translation and/or protein degradation (Kocarek et al. 2000). Approximately 30–40% of CYP2E1 mRNA is not associated with polysomes and therefore not actively engaged in protein synthesis (Kocarek et al. 2000). To examine the CYP2E1 mRNA molecule for sequences that might affect its translational efficiency, a series of CYP2E1 recombinant RNAs (rcRNAs) with modified 5′ or 3′ untranslated regions (UTRs) is translated *in vitro* using the rabbit reticulocyte lysate system (Kocarek et al. 2000). It is concluded that the secondary structure in the 5′ UTR of CYP2E1 mRNA is at least partially responsible for the inefficient translation of this mRNA (Kocarek et al. 2000). The poly(A) tail and sequences contained within the 3′ UTR appear to be important for protecting CYP2E1 mRNA from RNase activity associated with the translation machinery (Kocarek et al. 2000).

Renal and Hepatic Injury Mediated by Hydrazine Derived Antidepressants: Role of CYP2E1

The hepatic and renal toxicity associated with hydrazine treatment has been linked to free radical damage resulting from oxidative metabolism by CYP2E1 (Runge-Morris et al. 1996). Treatment with hydrazine or the therapeutic hydrazine phenelzine significantly increases the expression of rat renal CYP2E1 protein through a posttranscriptional mechanism (Runge-Morris et al. 1996).

K. Miyamoto

Obesity and CYP2E1

The induction of CYP2E1 in obese Zucker rats and its effect on the disposition kinetics of chlorzoxazone (CZX) has been investigated (Khemawoot et al. 2007a). In Zucker rats fed a normal diet (ND), normal Zucker rats fed a high-fat diet (HF), and genetically obese Zucker rats fed a normal diet (OB) and administered CZX, the values of the area under the plasma concentration-time curve from 0 to infinity

(AUC(infinity)) of CZX are in the order of ND > HF > OB rats (Khemawoot et al. 2007a). The AUC (infinity) values of total 6-hydroxychlorzoxazone (6OHCZX-T), which is considered to be a CYP2E1 metabolic marker, are in the opposite order (Khemawoot et al. 2007a). The values of the AUC (infinity) ratio (6OHCZX-T/CZX) in ND, HF and OB rats are approximately 0.2, 0.3 and 0.4, respectively (Khemawoot et al. 2007a). Induction of CYP2E1 protein is greater in both liver and fat of OB rats than in those of HF rats (Khemawoot et al. 2007a). Microsomal activity of CYP2E1 in liver and fat is also in the order of OB > HF > NM rats (Khemawoot et al. 2007a). Thus,CYP2E1 may be induced in liver and fat of obese patients, thereby potentially altering the disposition kinetics of not only CZX, but also other lipophilic drugs metabolized by CYP2E1 (Khemawoot et al. 2007a).

Circadian Rhythm of CYP2E1 and Its Effects on Drug Metabolism

Microsomal CYP2E1 shows a substantial circadian variation in rats, and this is associated with a decrease of chlorzoxazone half life, and an increase of 6-hydroxychlorzoxazone production (Khemawoot et al. 2007b). Therefore, the temporal variations of therapeutic response and toxicological effects may have to be taken into consideration for other xenobiotics that are predominantly metabolized by CYP2E1, particularly those with a short half-life (Khemawoot et al. 2007b).

B.J. Song

Translational Induction of CYP2E1 by the Anti-tuberculosis Drug Isoniazid

The induction of CYP2E1 by isoniazid is not accompanied by an increased level of CYP2E1 mRNA, and is completely blocked by pretreatment with cycloheximide or sodium fluoride, inhibitors of mRNA translation (Park et al. 1993). CYP2E1 induction by isoniazid is due to activation of CYP2E1 mRNA translation and the hydrazide group on the pyridine ring of isoniazid is important both in the selective induction of CYP2E1 and for magnitude of effect (Park et al. 1993).

Ethanol Mediated Protein Stabilization of CYP2E1

Chronic ethanol administration to rats induces hepatic and extra-hepatic CYP2E1 protein, which reverts to normal levels 12 h after ethanol withdrawal, unaccompanied by changes in CYP2E1 mRNA, suggesting ethanol mediated post-translational regulation of CYP2E1 (Roberts et al. 1994). CYP2E1 possesses a half-life of 6 h or less in the liver and is rapidly degraded following the removal of ethanol (Roberts et al. 1994). Similar patterns of CYP2E1 turnover are also observed in other tissues such as kidney, brain and upper gastro-intestinal tract, suggestive of a similar mode of regulation (Roberts et al. 1994). Thus, ethanol mediated induction of CYP2E1 is through a post translational mechanism involving protein stabilization.

Acetaldehyde-Protein Adduct Formation by Ethanol Inducible Hepatic CYP2E1: A Mechanism for Alcohol Induced Hepatotoxicity

Immunoblot analysis from livers isolated from rats fed an isocaloric control or alcohol liquid diet with and without cotreatment of YH439, an inhibitor of CYP2E1 gene transcription reveals that the acetaldehyde-protein adduct, absent in the pair-fed control, is evident in alcohol-fed rats but is markedly reduced by YH439 treatment (Jeong et al. 2000). The 37-kDa adduct is predominantly localized in the pericentral

region of the liver where CYP2E1 protein is mainly expressed (Jeong et al. 2000). This staining disappears in the pericentral region after YH439 treatment (Jeong et al. 2000). However, the level of the 37-kDa protein adduct positively correlates with the hepatic content of CYP2E1 (Jeong et al. 2000). The 37-kDa adduct could be produced by CYP2E1-mediated ethanol metabolism in addition to ADH-dependent formation (Jeong et al. 2000).

CYP2E1 Degradation: A Rapid Process

Ethanol-inducible CYP2E1 activity is measured using the enzyme markers N-nitrosodimethylamine demethylase (NDMAd), p-nitrophenol hydroxylase (PNPH) and aniline hydroxylase (AH) in rat liver microsomes (Roberts et al. 1995). Activities are found to be induced significantly after chronic ethanol feeding using all three assays (Roberts et al. 1995). Upon ethanol withdrawal, all three activities drop markedly, with NDMAd and PNPH at control values at 24 h and all subsequent time points (Roberts et al. 1995). AH activity remains threefold higher than controls at 24–72 h (Roberts et al. 1995). Immunoreactive CYP2E1 returns to control at 24 h, consonant with NDMAd and PNPH activities (Roberts et al. 1995). The prolonged induction of AH activity following ethanol withdrawal indicates that it is not a specific marker of CYP2E1-catalyzed reactions (Roberts et al. 1995). Collectively, these data are suggestive of a rapid mechanism of CYP2E1 degradation in the rat liver (Roberts et al. 1995).

Alcohol Inducible CYP2E1 Mediated Oxidative Protein Damage: A Proteomics Approach

A targeted proteomics approach utilizing biotin-N-maleimide (biotin-NM) as a specific probe to label oxidized cysteinyl residues has been employed to investigate which mitochondrial proteins are modified during and after alcohol exposure (Kim et al. 2006). Human hepatoma HepG2 cells with transduced CYP2E1 (E47 cells) are used as a model to generate ROS through CYP2E1-mediated ethanol metabolism (Kim et al. 2006). Heat shock protein 60, protein disulfide isomerase, mitochondrial aldehyde dehydrogenases, prohibitin, and other proteins are oxidized after alcohol exposure (Kim et al. 2006). This method is also used to identify oxidized mitochondrial proteins in the alcohol-fed mouse liver (Kim et al. 2006). Exposure to ethanol causes oxidation of various mitochondrial proteins that may negatively affect their function and contribute to alcohol-induced mitochondrial dysfunction and cellular injury (Kim et al. 2006).

Fasting Mediated Pretranslational Induction of CYP2E1 in Blood Lymphocytes: Its Implications for Development of Biomarkers for Pathophysiological Conditions

CYP2E1 in fresh lymphocytes is elevated about fivefold by fasting, comparable to the induction observed in cultured lymphocytes (Soh et al. 1996). This induction is accompanied by increased level of CYP2E1 mRNA (Soh et al. 1996). CYP2E1 in fresh lymphocytes is pretranslationally induced by fasting, in parallel to the hepatic enzyme, and thus the measurement of CYP2E1 in the lymphocyte homogenate may be useful to estimate the hepatic CYP2E1 level in a relatively non-invasive manner (Soh et al. 1996).

Acetaminophen Induced Cytotoxicity: Mechanisms Involved and the Role of CYP2E1

Neurotoxic Actions of the Drug Acetaminophen: Involvement of CYP2E1 Mediated JNK Activation

Acetaminophen (AAP, 4-hydroxyacetanilide), a widely used analgesic drug, causes time- and concentration-dependent apoptosis and DNA fragmentation of C6 glioma cells through activation of c-Jun N-terminal protein kinase (JNK) cell signaling pathway (Bae et al. 2001). Pretreatment with YH439, an inhibitor of CYP2E1 gene transcription, markedly reduces CYP2E1 mRNA, protein content, and activity, as well as the rate of AAP-induced JNK activation and cell death (Bae et al. 2001), suggesting the crucial role of CYP2E1 in acetaminophen mediated cytotoxicity.

Acetaminophen Induced Neurotoxicity and Decreased Pro-apoptotic Proteins p53 and p21: Role of CYP2E1

Acetaminophen or its reactive metabolite(s) can directly reduce the pro-apoptotic p53 content through mdm2-mediated ubiquitin conjugation, despite phosphorylation of p53 at its N terminus (Lee et al. 2006a). The inhibition of CYP2E1 significantly lowers the CYP2E1 enzyme activity and the rate of APAP-induced cell death while it prevents the reduction of p53 and p21 in C6 glioma cells (Lee et al. 2006a).

The Essential Role of CYP2E1 in 3-NT Adduct Formation and Protein Degradation, Independent of NOSin Acetaminophen Mediated Cytotoxicity

CYP2E1 plays a key role in nitrotyrosine protein adducts formation, ubiquitin-mediated protein degradation, and liver damage, which is independent of NOS, and decreased levels of many proteins such as cytosolic superoxide dismutase (SOD1), in the wild-type mice when compared with Cyp2e1-null mice which likely contribute to APAP-related toxicity (Abdelmegeed et al. 2010).

Induction of CYP2E1 in PPAR Alpha Null Mice: A Possible Role in NASH Development

Increased steatosis, oxidative stress, inflammation, hepatocyte apoptosis accompanied by elevated levels of ethanol-inducible CYP2E1 and TNFα observed in PPAR alpha-null mice fed a HFD, suggests that inhibition of PPARα functions may increase susceptibility to high fat-induced NASH and CYP2E1 may be associated with the process (Abdelmegeed et al. 2011).

III

M.J. Ronis

Increased CYP2E1 Mediated Oxidative Stress due to Synergistic Hepatotoxicity of Alcohol and Undernutrition

A combination of undernutrition and intragastric administration of ethanol to rats increases the induction of hepatic CYP2E1 and CYP4A1 mRNA, apoprotein, and activities. This is accompanied by increased oxidative stress (Baumgardner et al. 2007). The development of alcohol-induced liver pathology at 154 kcal·kg(−3/4)·day(−1) is accompanied by decreased expression of fatty acid synthesis genes and increased expression of PPAR-alpha-regulated fatty acid degradation pathways and greater

hepatocyte proliferation (Baumgardner et al. 2007). Undernutrition does not exacerbate alcoholic steatohepatitis despite additional oxidative stress produced by an increased induction of CYP2E1 and CYP4A1 (Baumgardner et al. 2007). However, enhanced ethanol-induced cellular proliferation, perhaps as a result of enhanced PPAR-alpha signaling, may contribute to an increased risk of hepatocellular carcinoma in undernourished alcoholics (Baumgardner et al. 2007). Thus, undernutrition and ethanol consumption both can aggravate CYP2E1 mediated oxidative stress.

Induction of CYP2E1 in Total Enteral Nutrition Based NASH Model
Total enteral nutrition (TEN) has been used to moderately over-feed rats with high-polyunsaturated fat diets to develop a model NASH (Baumgardner et al. 2008). The development of steatosis as indicated through increased expression of CD36 and l-fabp mRNA may be associated with increased fatty acid transport and the intragastric infusion of a high-polyunsaturated fat diet at a caloric level of 17% excess total calories results in pathology similar to clinical NASH which is accompanied by induction in hepatic CYP2E1 (Baumgardner et al. 2008).

Ethanol Mediated Dysregulation of Vitamin D Homeostasis: A Key Role of Ethanol Inducible CYP2E1 Mediated Oxidative Stress
Bone loss resulting from chronic ethanol abuse is frequently accompanied by altered vitamin D3 homeostasis (Shankar et al. 2008). Intragastric administration of ethanol to rats and ethanol exposure of *in vitro* in primary cultures of rat renal proximal tubule cells (RPTCs) and in NRK-52E cells reduces circulating 1,25-dihydroxycholecalciferol (1,25 (OH)2 D3) concentrations as the result of CYP24A1(1,25 (OH)2 D3-24-hydroxylase) induction (Shankar et al. 2008). CYP24A1 induction is mediated *via* mitogen activated protein kinase (MAPK) activation resulting from renal oxidative stress produced by local metabolism of EtOH *via* CYP2E1 and antidiuretic hormone-1 (Shankar et al. 2008).

L.A. Videla
NAFLD and CYP2E1: A Close Association
Oxidative stress is developed in the liver of NAFLD patients with steatosis and is exacerbated further in patients with steatohepatitis, which is associated with CYP2E1 induction (Videla et al. 2004). Oxidative stress leads to substantial protein oxidation followed by proteolysis of the modified proteins, which may explain the co-existence of a diminished antioxidant capacity and protein oxidation in the liver of patients with steatohepatitis (Videla et al. 2004). Thus, hepatic CYP2E1 induction is associated with oxidative stress in NAFLD patients.

Pesticide or Hormone Mediated Regulation of CYP2E1
The pesticide gamma-hexachlorocyclohexane (HCCH) or the hormone L-3,3,5-triiodothyronine (T3) enhance the expression and activity of CYP2E1 and that of NADPH-cytochrome P450 reductase in rat liver, regardless of the changes in total cytochrome P450 content, representing major contributory mechanisms to microsomal NADPH-dependent O2.-generation (Fernández et al. 2003). Further, the hepatic activity of the antioxidant enzyme SOD is decreased, reflecting the increased oxidative stress in the cellular environment (Fernández et al. 2003).

Induction of CYP2E1 in NAFLD Patients

The hepatic CYP2E1 content and the CLZ hydroxylation of obese patients with steatosis and particularly, with steatohepatitis are significantly higher than controls and correlates positively with both the severity of the liver damage (Orellana et al. 2006). Thus, CYP2E1 is involved in the mechanism of liver injury found in obese NAFLD patients (Orellana et al. 2006). Also, the correlation between liver CYP2E1 content and *in vivo* CLZ hydroxylation would validate the latter as a reliable indicator of liver injury in NAFLD, thus providing a simple and non-invasive method to study these patients (Orellana et al. 2006).

Higher Incidence of RsallPstl Polymorphisms in NASH Patients: Genetic Aspects of CYP2E1 Linked to Liver Injury

The c2 rare allele of RsallPstl polymorphisms but no C allele of Dral polymorphism is positively associated with chlorzoxazone hydroxylation, which in turn is correlated with liver CYP2E1 content in female obese NASH patients (Varela et al. 2008). Further, c2 allele is positively associated with liver injury in NASH (Varela et al. 2008). This allele may determine a higher transcriptional activity of the gene, with consequent enhancement in pro-oxidant activity of CYP2E1 thus leading to liver toxicity (Varela et al. 2008).

J.L. Raucy

Acetone Mediated Increased *De Novo* Synthesis of Hepatic CYP2E1 Protein: A Mechanism for Xenobiotic Mediated Regulation of CYP2E1

Acetone increases CYP2E1 protein levels in cultured rabbit hepatocytes by stimulating its rate of *de novo* synthesis (Kraner et al. 1993). Since this increase in CYP2E1 synthesis stems, at least in part, from the acetone-mediated enhancement of hepatocyte CYP2E1 mRNA content and is inhibited by the transcriptional inhibitor, alpha-amanitin, transcriptional activation of the rabbit CYP2E1 gene is apparently involved in the induction of CYP2E1 protein by acetone (Kraner et al. 1993).

Presence of CYP2E1 in Fetal Liver and Its Inducibility by Ethanol and Clofibrate: Its Implications for the Deleterious Effects of Xenobiotics on the Development of Foetus

CYP2E1 protein which exhibits a slightly lower molecular weight than that found in adult liver samples has been detected in liver samples from fetuses ranging in gestational age from 16 to 24 weeks (Carpenter et al. 1996). Embryonic CYP2E1 expression is further confirmed by the reverse transcriptase reaction with RNA from a 19-week gestational fetal liver used as template (Carpenter et al. 1996). The rate of ethanol oxidation to acetaldehyde, in human fetal microsomes is 12–27% of those exhibited by adult liver microsomes (Carpenter et al. 1996). Immunoinhibition studies with CYP2E1 antibodies reveal that the corresponding antigen is the major catalyst of this reaction in both fetal and adult tissues (Carpenter et al. 1996). Treatment of primary fetal hepatocyte cultures with either ethanol or clofibrate demonstrates a twofold increase in CYP2E1 levels compared with untreated cells (Carpenter et al. 1996). Thus, CYP2E1 is present in human fetal liver, that the enzyme is functionally similar to CYP2E1 from adults, and that fetal hepatocyte CYP2E1 is inducible in culture by xenobiotics, including ethanol (Carpenter et al. 1996).

Fetal Hepatic CYP2E1: Analogies with Adult Liver CYP2E1

The presence of CYP2E1 protein and gene in fetal liver, placenta and maternal brainof rat dams fed a liquid diet throughout gestation containing 5% ethanol has been shown (Carpenter et al. 1997). Maternal and fetal liver of dams fed ethanol display a 1.4- and 2.4-fold increase in CYP2E1 protein, respectively, compared with microsomes from pair-fed controls (Carpenter et al. 1997). The rate of chlorzoxazone metabolism by maternal hepatic microsomes from ethanol fed dams is 2.6-fold greater than that of controls (Carpenter et al. 1997). Conversely, a negligible increase is observed in the rate of metabolism by hepatic microsomes from ethanol-exposed fetuses compared with pair-fed animals (Carpenter et al. 1997). These same fetal samples exhibit greater rates of nitrosodimethylamine demethylation activity (1.5-fold) compared with microsomes from control animals (Carpenter et al. 1997). However, this increase is not as great as expected considering the 2.4-fold increase in CYP2E1 protein. Collectively, fetuses exposed to a 5% ethanol diet throughout gestation exhibit transplacental induction of a hepatic CYP2E1 that may possess different catalytic properties from the analogous adult enzyme (Carpenter et al. 1997).

Ethanol Mediated Induction of Human Lymphocyte CYP2E1

The *in vivo* chlorzoxazone metabolism and pharmacokinetic parameters with CYP2E1 expression in blood has been investigated (Raucy et al. 1997). Human subjects, who consume alcohol more frequently, exhibit higher rates of chlorzoxazone metabolism (Raucy et al. 1997). Indeed, a correlation is obtained when scores are compared with the pharmacokinetic parameter AUC for chlorzoxazone (Raucy et al. 1997). Lymphocyte microsomes reveal the presence of CYP2E1 mRNA (Raucy et al. 1997). CYP2E1 protein is 2.3-fold higher in alcoholics than in control subjects (Raucy et al. 1997). This increase in lymphocyte CYP2E1 content in alcoholic subjects coincides with a 2.1-fold increase in chlorzoxazone clearance and a twofold decrease in the AUC for chlorzoxazone (Raucy et al. 1997). Importantly, a correlation is observed between CYP2E1 content in lymphocytes and chlorzoxazone clearance rates (Raucy et al. 1997). Thus, monitoring lymphocyte CYP2E1 expression may provide a substitute for estimating hepatic activity of this P450 (Raucy et al. 1997).

Transcriptional Regulation of CYP2E1 in Human Liver by Xenobiotics (Ethanol), Drugs (Clofibrate) and Fatty Acids (Palmitate)

Primary cultures of human hepatocytes have been used to determine if certain xenochemicals could regulate CYP2E1 and CYP4A11 (Raucy et al. 2004). Ethanol significantly increases expression of CYP2E1 mRNA, but does not alter CYP4A11 mRNA accumulation (Raucy et al. 2004). In contrast, hepatocytes exposed to ethanol exhibit only a slight elevation in CYP2E1 protein and a negligible effect on CYP4A11 protein (Raucy et al. 2004). Clofibrate significantly enhances both CYP4A11 mRNA and protein, but does not increase CYP2E1 (Raucy et al. 2004). Palmitic acid significantly increases CYP2E1 mRNA (Raucy et al. 2004). The agents that enhance CYP2E1 and CYP4A11 at the transcription level have been identified and suggest that fatty acids may represent a similar mode of regulation for these P450 enzymes (Raucy et al. 2004). The lack of induction of CYP2E1 protein by ethanol in human hepatocytes indicates that for certain CYP enzymes, isolated hepatocytes may not be an adequate tool for predicting *in vivo* responses (Raucy et al. 2004).

L. Corcos

Presence and Xenobiotic Mediated Inducibility of CYP2E1 in Rat Liver Epithelial Cells

Rat liver epithelial cells (RLECs) isolated from the livers of normal 10 days old rats are largely used in co-culture with primary hepatocytes (Lerche et al. 1996). All of the different preparations of RLECs express a high level of CYP2E1 mRNA (Lerche et al. 1996). The presence of the CYP2E1 apoprotein in microsomes of RLECs, together with chlorzoxazone 6-hydroxylation, has been observed (Lerche et al. 1996). In addition, acetone treatment of these cells results in an increase in both CYP2E1 apoprotein and chlorzoxazone 6-hydroxylation activity (Lerche et al. 1996). Finally, RLECs are susceptible to N-methyl formamide- and diethylnitrosamine-induced toxicity, suggesting metabolic activation by CYP2E1 (Lerche et al. 1996). Thus, RLECs may cooperate with hepatocytes to CYP2E1-mediated metabolism in the co-culture model (Lerche et al. 1996). In addition, transfection experiments with a CYP2E1 promoter construct, in which the proximal 539 bp containing the binding site for hepatocyte nuclear factor 1 (HNF1) alpha are inserted upstream of the chloramphenicol acetyl transferase gene, demonstrates a strong induction upon co-transfection with an HNF1alpha expression plasmid (Lerche et al. 1996). Thus, RLECs provide a useful tool for studying metabolism and cytotoxicity of CYP2E1 substrates in the absence of other expressed CYPs, and for analyzing CYP2E1 promoter function (Lerche et al. 1996).

Regulation of Human Liver CYP2E1 by Interleukin 4: Crosstalk Between CYP2E1 and Immune System

Interleukin 4 (IL-4) coordinately induces CYP2E1 transcription, mRNA and apoprotein levels in human hepatoma B16A2 cellsin a PKC-dependent manner, potentially through the activity of the PKCzeta isoform as PKC inhibitors (H7 and calphostin C) strongly block any induction of the gene, as well as the IL-4-dependent translocation of PKCS (Lagadic-Gossmann et al. 2000). The study suggests a close relationship between CYP2E1 and interleukins.

Y. Funae

CYP1A2, a Co-player with CYP2E1 in Human MEOS

The ethanol oxidization activity in human hepatic microsomes and multiple forms of human hepatic CYPs expressed in B-lymphoblastoid cells has been examined to assess the contribution of CYP to the MEOS in humans (Asai et al. 1996). Although CYP2E1 is a major contributor to the MEOS in humans; CYP1A2 also plays an important role in the MEOS (Asai et al. 1996).

Acetaldehyde Oxidation Activity of CYP2E1 in Liver Microsomes: Complementary Roles for CYP2E1 and Acetaldehyde Dehydrogenase in Alcohol Metabolism

Acetaldehyde is oxidized by rat and human hepatic microsomes in the presence of NADPH, and a NADPH-dependent oxidation system MAOS (microsomal acetaldehyde-oxidizing system) has been developed to distinguish it from the

NAD-dependent acetaldehyde oxidation system of acetaldehyde dehydrogenase in mitochondria and cytosol (Kunitoh et al. 1997). Hepatic CYP2E1 mainly contributes to MAOS in rats and humans, the pathway of which may play an alternative role against acetaldehyde in the liver after alcohol consumption together with acetaldehyde dehydrogenase in the metabolism of acetaldehyde (Kunitoh et al. 1997).

Metabolism of the Anaesthetic Sevoflurane in Human Blood by CYP2E1
Since the amount of inorganic fluoride released after anesthesia with sevoflurane depends on the dose of administered sevoflurane and CYP2E1 activity in the liver, a reliable and noninvasive probe for CYP2E1 for predicting plasma inorganic fluoride levels after anesthesia has been developed (Hase et al. 2000). In patients (American Society of Anesthesiologists physical status I), aged 20–68 year undergoing body surface surgery with general anesthesia with sevoflurane, there is a significant correlation between level of CYP2E1 mRNA in mononuclear cells and the area under the plasma concentration-time curve of plasma inorganic fluoride from the beginning of sevoflurane administration to infinity in uninduced and uninhibited patients (Hase et al. 2000).

Presence of CYP2E1 mRNA in Blood Mononuclear Cells of ALD Patients: Role as a Biomarker of Liver Injury
The presence of CYP2E1 mRNA levels of mononuclear cells obtained from healthy individuals who did and did not drink habitually and patients with ALD has been investigated (Yano et al. 2001). The CYP2E1 mRNA level in mononuclear cells increases during drinking and decreases in abstinence for a short period of 3–4 days (Yano et al. 2001). Thus, CYP2E1 mRNA level may be used as an effective marker for alcoholic intake (Yano et al. 2001).

IV

A. Guillouzo
Cytokine Mediated Regulation of Hepatic CYP2E1
The cytokine IFN-gamma suppresses CYP2E1 mRNA levels in primary human hepatocyte cultures (Abdel-Razzak et al. 1993). In addition, IL-4 has the opposite effect, compared with other cytokines-IL-1 beta, IL-6 and TNF alpha, on CYP2E1 mRNA, which is increased up to fivefold (Abdel-Razzak et al. 1993). Thus, various cytokines act directly on human hepatocytes to affect expression of CYP2E1 gene (Abdel-Razzak et al. 1993).

Interactions Between the Anaesthetic Propofol and CYP2E1
While almost anesthetics are metabolized by the CYP3A4, some major volatile ones such as halothane and sevoflurane are metabolized by CYP2E1 in humans (Lejus et al. 2002). A widely used intravenous anesthetic agent, 2,6-diisopropylphenol (propofol), known to inhibit CYP3A4 and CYP1A2, inhibits CYP2E1 as detected through 6-OH hydroxylation of chlorzoxazone in human and porcine microsomes (Lejus et al. 2002). Thus, propofol could have a protective effect on toxic metabolite activation of compounds catalyzed by CYP2E1.

E. Arinc

Increased Incidence of Childhood Acute Lymphoblastic Leukemia (ALL) Is Associated with Polymorphisms in CYP2E1

The CYP2E1 gene possesses several polymorphisms in humans, and among them, CYP2E1*5B and *6 have been shown to be associated with increased risks of several chemical-induced diseases (Ulusoy et al. 2007). The possible association of CYP2E1*5B, *6 and *7B alleles, alone or in combination, with the risk of incidence of childhood acute lymphoblastic leukemia (ALL) in a Turkish population has been investigated (Ulusoy et al. 2007). When both CYP2E1*5B and *6 alleles are considered together, the risk of childhood ALL increases significantly (2.9-fold) (Ulusoy et al. 2007). Moreover, the presence of at least two variant alleles of any combination increases the risk significantly 3.9 times, suggesting a combined effect (Ulusoy et al. 2007). Individuals carrying combinations of CYP2E1*5B, *6 and *7B variants together are likely associated with the risk of developing childhood ALL (Ulusoy et al. 2007).

Association Between CYP2E1 Variant Alleles and Impaired DNA Repair Capacity: Increased Incidence of Childhood ALL

The co-presence of X-ray repair cross-complimenting group 1 (XRCC1) Arg399Gln variant and CYP2E1*5B and *6 polymorphisms in the same individuals considerably increases the risk for childhood ALL to 3.7-fold with borderline significance in a segment of Turkish population under study (Tumer et al. 2010). The observed combined effect is considerably more prominent among females (Tumer et al. 2010). Thus, the combined associations of XRCC1 399Gln, CYP2E1*5B and *6 alleles is associated with the risk of development of childhood ALL (Tumer et al. 2010).

O. Adali

Diabetes Mediated Induction of Hepatic and Extrahepatic CYP2E1

A significant induction of liver CYP2E1 protein and catalytic activities is observed in alloxan-induced rabbits and the CYP2E1 content of diabetic microsomes is highly correlated with the activities of liver aniline 4-hydroxylase and p-nitrophenol hydroxylase (Arinç et al. 2005). Similarly, induction of CYP2E1 protein and markedly increased aniline 4-hydroxylase and p-nitrophenol hydroxylase activities are also observed in alloxan induced diabetic rabbit lung and kidney (Arinç et al. 2007). Further, the procarcinogen and food contaminant, NDMA is converted to its carcinogenic form after it is activated with NDMA N-demethylase and a significant increase of liver, kidney and lung NDMA N-demethylase activity associated with CYP2E1 is shown in diabetic rabbit, suggesting the risk of nitrosamine induced carcinogenesis will be greater in liver, kidney and lung of the diabetic subjects (Arinç et al. 2007).

B.I. Ghanayem

CYP2E1 and Chemical Carcinogenesis

Methacrylonitrile

Methacrylonitrile (MAN) is a widely used aliphatic nitrile and is structurally similar to the known rat carcinogen and suspected human carcinogen acrylonitrile (AN)

(Ghanayem et al. 1999). In rats, MAN is metabolized by CYP2E1 to acetone, which is eliminated along with parent MAN in breath (Wang et al. 2002a). Gavage administration of MAN to rats causes olfactory epithelial damage and liver enlargement (Wang et al. 2002a). Further, administration of MAN to rats causes increased expression of CYP2E1 in lung, liver, and nasal tissues (Wang et al. 2002a). Also, acetone induces the expression of CYP2E1 at both the mRNA and protein levels in rat nasal and lung tissues (Wang et al. 2002a). MAN increases the expression of CYP2E1, and this effect varies as a function of time, length of exposure, and tissue examined (Wang et al. 2002a). Thus, treatment of rats with MAN results in differential expression of CYP2E1 and possibly other CYPs in tissues leading to tissue-specific toxicity via increased *in situ* formation of cytotoxic MAN metabolites (Wang et al. 2002a).

MAN and AN are metabolized *via* GSH conjugation or epoxide formation. CYP2E1 is essential for AN epoxidation and subsequent cyanide liberation (El Hadri et al. 2005). While significant reduction in blood cyanide levels occurs in MAN-treated CYP2E1-null *vs.* WT mice, AN metabolism to cyanide is largely abolished in CYP2E1-null mice (El Hadri et al. 2005). Pretreatment of mice with 1-aminobenzotriazole (ABT, CYP inhibitor) demonstrates that CYPs other than CYP2E1 also contribute to MAN metabolism to cyanide (El Hadri et al. 2005). Thus, while CYP2E1 is the only enzyme responsible for AN metabolism to cyanide, other CYPs also contribute to MAN metabolism (El Hadri et al. 2005).

Acrylonitrile and Acrylamide

Acrylonitrile (AN) and acrylamide (AM) are commonly used in the synthesis of plastics and polymers (Sumner et al. 1999). In rodents, AM and AN are metabolized to epoxides (Sumner et al. 1999). Wild-type (WT) mice excrete metabolites derived from the epoxides and from direct GSH conjugation with AM or AN. Only metabolites derived from direct GSH conjugation with AM or AN are observed in the urine from ABT-pretreated WT mice and mice devoid of CYP2E1 (P450 2E1-null). The evaluation of urinary metabolites at these doses, suggests that CYP2E1 is possibly the only CYP enzyme involved in the metabolism of AM and AN in mice, that inhibiting total CYP activity does not result in new pathways of non-P450 metabolism of AM, and that mice devoid of CYP2E1 (P450 2E1-null mice) do not excrete metabolites of AM or AN that would be produced by oxidation by other cytochrome P450s (Sumner et al. 1999).

Using CYP2E1-null mice treated with AN, it has been shown that CYP2E1-mediated oxidation is a prerequisite for AN metabolism to cyanide (Wang et al. 2002b). Since earlier studies have shown that CYP2E1 is the only enzyme responsible for AN epoxidation, it is concluded that AN metabolism to cyanoethylene oxide (CEO) is a prerequisite for cyanide formation which plays an essential role in the causation of the acute toxicity/mortality of AN, and this pathway is exclusively catalyzed by CYP2E1 (Wang et al. 2002b).

A key oxidative metabolite of acrylamide is the epoxide glycidamide, generated by CYP2E1 (Ghanayem et al. 2005a). Dose-related increases in resorption moles (chromosomally aberrant embryos) and decreases in the numbers of pregnant females and the proportion of living fetuses are seen in females mated to acrylamide-treated

wild-type mice (Ghanayem et al. 2005a). No changes in any fertility parameters are seen in females mated to acrylamide-treated CYP2E1-null mice (Ghanayem et al. 2005a). Thus, acrylamide-induced germ cell mutations in male mice require CYP2E1-mediated epoxidation of acrylamide (Ghanayem et al. 2005a).

In male germ cells, significant dose-related increases in micronucleated erythrocytes and DNA damage in somatic cells are induced in acrylamide-treated wild-type but not in the CYP2E1-null mice (Ghanayem et al. 2005b). Thus, genetic damage in somatic and germ cells of mice-treated with acrylamide is dependent upon metabolism of the parent compound by CYP2E1 (Ghanayem et al. 2005b). This dependency on metabolism has implications for the assessment of human risks resulting from occupational or dietary exposure to acrylamide (Ghanayem et al. 2005b).

Acrylamide, besides being an animal carcinogen is also, a neurotoxin, and reproductive toxin (Ghanayem et al. 2005c). The role of CYP2E1 in the epoxidation of acrylamide to glycidamide (GA) and the formation of DNA and hemoglobin (HGB) adducts has been assessed (Ghanayem et al. 2005c). Administration of acrylamide to wild-type mice causes a large increase in N7-GA-Gua and N3-GA-Ade adducts in the liver, lung, and testes (Ghanayem et al. 2005c). While traces of N7-GA-Gua adducts are measured in the tissues of acrylamide-treated CYP2E1-null mice, these levels are several fold lower than in wild-type mice (Ghanayem et al. 2005c). Significant elevation of both acrylamide- and GA-HGB adducts is detected in acrylamide-treated wild-type mice (Ghanayem et al. 2005c). In acrylamide-treated CYP2E1-null mice, levels of acrylamide-HGB adducts are roughly twice as high as those in wild-type mice (Ghanayem et al. 2005c). Thus, CYP2E1 is the primary enzyme responsible for the epoxidation of acrylamide to GA, which leads to the formation of GA-DNA and HGB adducts (Ghanayem et al. 2005c).

Urethane

Urethane ([carbonyl-(14)C]ethyl carbamate) is a fermentation by-product in alcoholic beverages and foods and is classified as reasonably anticipated to be a human carcinogen (Hoffler et al. 2003). CO(2) has been confirmed as the main metabolite of urethane (Hoffler et al. 2003). Using CYP2E1-null (KO) mice, CYP2E1's contribution to urethane metabolism has been elucidated (Hoffler et al. 2003) and it has been observed that CYP2E1, not esterase, is the principal enzyme responsible for urethane metabolism (Hoffler et al. 2003).

The relationships between CYP2E1-mediated metabolism and urethane-induced genotoxicity and cell proliferation as determined by induction of micronucleated erythrocytes (MN) and expression of Ki-67, respectively, using CYP2E1-null and wild-type mice have been investigated (Hoffler et al. 2005). Thus, CYP2E1-mediated metabolism of urethane, presumably *via* epoxide formation, is necessary for the induction of genotoxicity, and cell proliferation in the liver and lung of wild-type mice (Hoffler et al. 2005).

Multiple dosing (14)C-ethyl-labeled urethane leads to considerable bioaccumulation of urethane in CYP2E1−/− and CYP2E1+/+ mice; however, greater retention occurs in CYP2E1−/− versus CYP2E1+/+ mice (Hoffler and Ghanayem 2005). Furthermore, greater bioaccumulation of (14)C-ethyl-labeled than [(14)C]carbonyl-labeled urethane

is observed in mice (Hoffler and Ghanayem 2005). Comparison of the metabolism of ethyl-versus carbonyl-labeled urethane is necessary for tracing the source of CO(2) and therefore C-hydroxylation is a likely pathway of urethane metabolism (Hoffler and Ghanayem 2005).

Using Cyp2e1−/− mice, the relationships between urethane metabolism and carcinogenicity has been assessed (Ghanayem 2007). A significant reduction in the incidences of liver hemangiomas and hemangiosarcomas occurs in Cyp2e1−/− compared to Cyp2e+/+ mice (Ghanayem 2007). Lung nodules increase in a dose-dependent manner and are less prevalent in Cyp2e1−/− compared to Cyp2e+/+ mice (Ghanayem 2007). Bronchoalveolar adenomas are observed, and in one Cyp2e1+/+ mouse treated with 100 mg/kg urethane, a bronchoalveolar carcinoma is diagnosed (Ghanayem 2007). Significant reduction in the incidence of adenomas and the number of adenomas/lung are observed in Cyp2e1−/− compared to Cyp2e1+/+ mice (Ghanayem 2007). In the Harderian gland, the incidences of hyperplasia and adenomas are significantly lower in Cyp2e1−/− compared to Cyp2e+/+ mice at the 10 mg/kg dose, with no significant differences observed at the high or low doses (Ghanayem 2007). Thus, a significant reduction of urethane-induced carcinogenicity occurs in Cyp2e1−/− compared to Cyp2e1+/+ mice and CYP2E1-mediated oxidation plays an essential role in urethane-induced carcinogenicity (Ghanayem 2007).

1-Bromopropane

1-bromopropane (1-BrP) induces dose- and time-dependent reproductive organ toxicity and reduced sperm motility in rodents (Garner et al. 2007). Metabolites produced through CYP2E1-mediated oxidation could lead to 1-BrP-induced sperm toxicity (Garner et al. 2007). Both 1-BrP and 2OHBrP inhibit the motility of sperm obtained from WT mice *in vitro* (Garner et al. 2007). However, only 2OHBrP reduces the motility of sperm obtained from Cyp2e1−/− mice in vitro, suggesting that conversion of parent compound to 2OHBrP within the spermatozoa may contribute, at least in part, to reduced motility (Garner et al. 2007). Thus, metabolism of 1-BrP is mediated in part by CYP2E1, and activation of 1BrP via this enzyme may contribute to the male reproductive toxicity of this chemical (Garner et al. 2007).

S.A. Weinman

Synergistic Interactions Between Hepatitis C Virus Core Protein and CYP2E1: Its Implications for Enhanced Alcohol Mediated Liver Injury in Chronic Hepatitis

Huh-7 cells expressing Hepatitis C virus core protein, CYP2E1, or both are exposed to tertiary butyl hydroperoxide, TNF alpha, and/or ethanol (Otani et al. 2005). Expression of core/CYP2E1 synergistically enhances cell death induced by either tertiary butyl hydroperoxide or TNF alpha (Otani et al. 2005). After tertiary butyl hydroperoxide treatment, total ROS production increases more than threefold compared with cells that do not express core and CYP2E1 (Otani et al. 2005). Mitochondrial depolarization and reduced GSH depletion occurs as well, and cell death is prevented by inhibition of mitochondrial permeability transition or caspase activity (Otani et al. 2005). Confocal microscopy shows

that the mitochondria themselves are the origin of the ROS. In the absence of core/CYP2E1 expression, mitochondrial changes and cell death does not occur (Otani et al. 2005). Ethanol treatment further decreases mitochondrial reduced GSH content and exacerbates mitochondrial ROS production, depolarization, and cell death (Otani et al. 2005). Thus, mitochondrial ROS production is induced by hepatitis C virus core and CYP2E1, resulting in a reduction of mitochondrial antioxidant capacity and sensitivity to oxidants and TNF alpha and alcohol further depletes mitochondrial reduced GSH, which exacerbates depolarization and cell death (Otani et al. 2005).

T.R. Morgan

Development of CYP2E1 Over-Expressing Transgenic Mice and Increased Alcohol Mediated Liver Injury

Transgenic mice that express human CYP2E1 cDNA under the control of mouse albumin enhancer-promoter in the liver, when fed a nutritionally complete alcohol diet, develop more liver damage which includes higher serum ALT levels, higher histologic scores and ballooning hepatocytes than nontransgenic mice (Morgan et al. 2002).

Downregultion of Insulin Signaling due to CYP2E1 Mediated Oxidative Stress in a Rodent NAFLD Model

Hepatocyte-specific overexpression of CYP2E1 in transgenic mice increases hepatic oxidative stress in the liver, fasting insulin, and histological liver damage (Kathirvel et al. 2009). CYP2E1 over-expression reduces hepatic insulin signaling and glycogen storage and increases glucose synthesis (Kathirvel et al. 2009). Thus, an association exists between hepatic CYP2E1 and increased oxidative stress, increased systemic insulin resistance, decreased insulin signaling in the liver and increased hepatic fat accumulation (Kathirvel et al. 2009).

CYP2E1 and Nitrosative Stress: A Close Association in NAFL Model

Hepatocyte-specific CYP2E1 over-expression in transgenic mice exhibiting greater histological liver injury results in increased oxidative stress and nitrosative stress (Kathirvel et al. 2010). Gene expression of antioxidant enzymes Nrf2, CAT, GPx, HO-1 are significantly upregulated (Kathirvel et al. 2010). iNOS activity and nitrosylation of CAT and SOD is greater in liver of CYP2E1 over-expressing transgenic mice (Kathirvel et al. 2010). Failure of corresponding increase in total protein and activity of anti-oxidant enzymes suggests modification/degradation, possibly by nitrosylation, due to increased iNOS activity in a CYP2E1 overexpressing NAFL mouse model (Kathirvel et al. 2010).

C.H. Halsted

Regulation of CYP2E1 by Sex Steroids

To assess possible links between ethanol-induced oxidant stress, expression of hepatic CYP enzymes, and sex steroid status, the generation of protein adducts of acetaldehyde (AA), malondialdehyde (MDA), and 4-HNE with the amounts of CYP2E1, CYP2A, and CYP3A in the livers of castrated and noncastrated male micropigs fed ethanol for 12 months have been compared (Niemelä et al. 1999).

In castrated micropigs, ethanol feeding results in accumulation of fat, hepatocellular necrosis, inflammation, and centrilobular fibrosis, whereas only minimal histopathology is observed in their noncastrated counterparts (Niemelä et al. 1999). Ethanol feeding increases the hepatic content of CYP2E1 and CYP3A in the noncastrated animals and in CYP2E1 and CYP2A in the castrated animals most significantly (Niemelä et al. 1999). Ethanol-fed castrated animals also show the greatest abundance of perivenular adducts of AA, MDA, and HNE (Niemelä et al. 1999). In the noncastrated ethanol-fed micropigs, a low expression of each CYP form is associated with scant evidence of aldehyde-protein adducts (Niemelä et al. 1999). Significant correlations emerge between the levels of different CYP forms, protein adducts, and plasma levels of sex steroids (Niemelä et al. 1999). Thus, the generation of protein-aldehyde adducts is associated with the induction of several cytochrome enzymes including CYP2E1 in a sex steroid-dependent manner (Niemelä et al. 1999). It appears that the premature, juvenile, metabolic phenotype, as induced by castration, favors liver damage (Niemelä et al. 1999). It implicates the role of the gender differences on the adverse effects of ethanol in the liver (Niemelä et al. 1999).

Folate Deficiency Exacerbates Alcohol Induced Liver Injury: Its Association with CYP2E1

Feeding micropigs ethanol with a folate-deficient diet promotes the development of hepatic injury while increasing hepatic levels of homocysteine and S-adenosylhomocysteine (SAH) and reducing the level of S-adenosylmethionine (SAM) and the SAM-to-SAH ratio (Esfandiari et al. 2005). The induction of abnormal hepatic methionine metabolism through the combination of ethanol feeding with folate deficiency is associated with the activation of CYP2E1 and enhances endoplasmic reticulum stress signals that promote steatosis and apoptosis (Esfandiari et al. 2005).

C.K. Roberts

Insulin Mediated Downregulation of CYP2E1

The expression of major CYP isozymes in STZ-induced diabetes with concomitant insulin therapy has been studied (Sindhu et al. 2006). CYP2E1 protein is markedly induced in the STZ-induced diabetic group (Sindhu et al. 2006). Insulin therapy results in complete amelioration of CYP2E1 whereas CYP2B1 protein is partially ameliorated (Sindhu et al. 2006). By contrast, CYP2C11 protein is decreased over 99% in the diabetic group and is partially ameliorated by insulin therapy (Sindhu et al. 2006). Thus, widespread alterations exist in the expression of CYP isozymes in diabetic rats that are ameliorated by insulin therapy (Sindhu et al. 2006).

E.T. Morgan

Endotoxin Mediated Regulation of Hepatic CYP2E1

Growth hormone (GH) is an important regulator of CYP gene expression in the rat and the effects of bacterial endotoxin injection in hypophysectormized rats are compared to those in normal animals (Morgan 1993). In intact females, endotoxin suppresses total CYP and hepatic expression of CYP2C6, CYP2C7, CYP2C12, and

CYP2E1 mRNAs, as well as CYP2C12 and CYP2E1 proteins with the greatest decreases being observed with CYP2C7 and CYP2E1 mRNAs (Morgan 1993). Endotoxin treatment also induces the mRNA for the hepatic acute phase protein, haptoglobin in female rats (Morgan 1993). In hypophysectomized females supplemented with GH infusion, endotoxin causes the same or greater effects on expression of the CYP and haptoglobin gene products than are observed in the intact animals (Morgan 1993). It is concluded that the CYP suppression observed after endotoxin administration can occur independently of an effect on pituitary hormone secretion (Morgan 1993). Thus, endotoxin suppresses hepatic CYP2E1 protein and mRNA levels (Morgan 1993).

Interleukin Mediated Downregulation of Hepatic CYP2E1

CYP2E1 mRNA and protein are significantly suppressed only by the combination of IL1 and dexamethasone in rat hepatocytes cultured on Matrigel in the presence of growth hormone (Morgan et al. 1994). Further, IL6 treatment of male rats downregulates CYP2E1 mRNA at a dose of 4.5 μg/kg, which is lower than that required to induce haptoglobin mRNA, a prototype acute phase gene product (Morgan et al. 1994). Thus, hepatic CYP2E1 is regulated by cytokines, implicatimg a cross talk between immune system and CYPs.

Endotoxin Mediated Downregulation of Hepatic CYP2E1 Occurs Independently of NO

The role of NO in regulating the decreases in both gene expression and activity of hepatic CYP2E1 in endotoxemic parental and inducible nitric oxide synthase (NOS2) knockout mice has been examined (Sewer et al. 1998). Microsomal CYP2E1 protein level is decreased in both strains of mouse (Sewer et al. 1998). Similar results are obtained in parental strain endotoxemic mice co-administered the NOS inhibitor aminoguanidine (Sewer et al. 1998). Thus, the down-regulation of CYP2E1 protein and mRNA in the endotoxemic mouse can occur independently of NO production (Sewer et al. 1998).

Endotoxin (LPS) treatment suppresses both mRNA and protein expression of CYP2E1 in rats (Sewer and Morgan 1998). Coadministration of the NOS inhibitor aminoguanidine to bacterial endotoxin lipopolysaccharide (LPS)-treated rats completely inhibits the release of NO into the plasma but does not reverse the down-regulation of expression of CYP2E1 (Sewer and Morgan 1998). Thus, NO is not required for endotoxin-evoked down-regulation of CYP2E1 mRNA or protein expression as observed in rats and NOS2 knock out mice.

Endotoxin Mediated Downregulation of CYP2E1 Involves Transcriptional Suppression

The rate of transcription of the CYP2E1 gene is reduced to 10% of control levels, respectively, in rat liver within 1–2 h of injection of LPS (1 mg/kg) and injection of curcumin significantly inhibits the rapid transcriptional suppression of CYP2E1 (Cheng et al. 2003). The magnitude and rapidity of these effects indicate that transcriptional suppression is a primary reason for the decline in CYP2E1 mRNA (Cheng et al. 2003).

V

G. Robertson

Leptin Mediated Regulation of CYP2E1

In a genetic model of obesity and non-insulin dependent (type II) diabetes, the leptin-deficient ob/ob mouse, hepatic CYP2E1 levels are decreased compared to lean littermates (Leclercq et al. 2000a). Treatment with leptin increases hepatic CYP2E1 in obese mice to the levels observed in lean animals, but fails to alter CYP2E1 expression in lean animals (Leclercq et al. 2000a). Leptin also reduces food intake in treated mice compared to saline-treated controls (Leclercq et al. 2000a). In obese mice pair-fed the reduced amount of food, there is a significant increase in CYP2E1 mRNA unaccompanied by increases in CYP2E1 protein or enzyme activity (Leclercq et al. 2000a). Fasting and administration of acetone and 4-MP increases CYP2E1 mRNA as well as protein and activity in both obese and lean mice (Leclercq et al. 2000a). While CYP2E1 is still inducible in obese mice by xenobiotics and fasting, full constitutive expression of CYP2E1 requires leptin to be present (Leclercq et al. 2000a). This effect of leptin appears to be at least partly independent of the hypothalamic control of food intake (Leclercq et al. 2000a).

CYP2E1 Mediated Oxidative Stress in a NASH Model

In a dietary model of NASH-mice fed a methionine- and choline-deficient (MCD) diet, liver injury is associated with both induction of CYP2E1 and a 100-fold increase in hepatic content of lipid peroxides (Leclercq et al. 2000b). Further, microsomal NADPH-dependent lipid oxidases contribute to the formation of these lipid peroxides, and *in vitro* inhibition studies demonstrate that CYP2E1 is the major catalyst (Leclercq et al. 2000b). Thus, the induction of CYP2E1 and subsequent formation of lipid peroxides by CYP2E1 may be one of the major mechanisms for promoting NASH mediated liver injury.

T.M. Badger

Carbohydrate Deficiency Exacerbates CYP2E1 Mediated Alcohol Induced Liver Injury

In rats fed with the carbohydrate-deficient diet and ethanol, a strong positive association between low dietary carbohydrate, enhanced hepatic microsomal CYP2E1 apoprotein induction and hepatic necrosis occurs (Korourian et al. 1999). Thus, in the presence of low carbohydrate intake, ethanol induction of CYP2E1 is enhanced to levels sufficient to cause necrosis, possibly through ROS and other free radicals generated by CYP2E1 mediated metabolism of ethanol and unsaturated fatty acids (Korourian et al. 1999).

Hormonal Regulation of CYP2E1

Depletion of pituitary hormones by hypophysectomy (Hx) results in 12–14-fold increases in renal CYP2E1 compared with sixfold increases in CYP2E1 apoprotein in the liver (Chen et al. 1999). The increase in hepatic CYP2E1 is associated with increased gene transcription (Chen et al. 1999). Restoration of renal CYP2E1 to control levels by hormone treatment requires both growth hormone and an

intact testis, whereas partial restoration of CYP2E1 apoprotein levels in liver is accomplished by growth hormone, but not testosterone (Chen et al. 1999). Thus, CYP2E1 appears to be under complex endocrine regulation by pituitary and testicular hormones in a tissue specific manner (Chen et al. 1999).

Acute Alcohol Plus Glucose Mediated Downregulation of CYP2E1

FGC-4 rat hepatoma cells have been used to test the hypothesis that carbohydrates could down-regulate ethanol-induced CYP2E1 induction *in vitro* (Rowlands et al. 2003). FGC-4 cells grown in a glucose-free media and treated with 1–100 mM ethanol for 24 h exhibit a dose-dependent increase in CYP2E1, with maximum mRNA steady-state or protein levels measured at 30 or 100 mM ethanol, respectively (Rowlands et al. 2003). In cells treated with 30 mM ethanol, a glucose concentration-dependent inhibition of CYP2E1 mRNA is observed between 2.5 and 10 mM glucose (Rowlands et al. 2003). Induction by 30 mM ethanol of CYP2E1 protein is reduced in cells co-treated with 1 mM or greater glucose concentration and complete inhibition is measured with 5 mM glucose co-treatment (Rowlands et al. 2003). Under culture conditions of extremely low carbohydrate concentrations, ethanol treatment of FGC-4 cells results in elevated steady-state levels of CYP2E1 mRNA and protein; and glucose inhibits this increase (Rowlands et al. 2003).

M. J.Ronis/T. M.Badger

CYP2E1 Mediated Ethanol Metabolism: Triglyceride Accumulation, Induction of TNF-Alpha, and Chemokine Production

The effects of selective inhibition of CYP2E1 have been compared with the inhibition of overall ethanol metabolism on the development of alcoholic steatohepatitis (Ronis et al. 2010). Liver pathology scores and levels of apoptosis are elevated by total ethanol through enteral nutrition but do not differ significantly on co-treatment with CYP2E1 inhibitor DAS or ADH inhibitor 4-MP (Ronis et al. 2010). However, liver triglycerides are lower when ethanol-fed rats are treated with DAS or 4-MP (Ronis et al. 2010). Serum ALT values are significantly lower in ethanol-fed 4-MP-treated rats indicating reduced necrosis (Ronis et al. 2010). Hepatic oxidative stress and the ER stress marker tribbles-related protein 3 are increased after ethanol; further increased by DAS but partly attenuated by 4-MP. Both DAS and 4-MP reverse ethanol increases in the cytokine, TNF-alpha, and the chemokine CXCL-2 (Ronis et al. 2010). Ethanol and DAS additively induce hepatic hyperplasia (Ronis et al. 2010). Thus a significant proportion of hepatic injury after ethanol exposure is independent of alcohol metabolism (Ronis et al. 2010). Ethanol metabolism by CYP2E1 may be linked in part to triglyceride accumulation, to induction of TNF-alpha, and to chemokine production (Ronis et al. 2010). Ethanol metabolism by ADH may be linked in part to oxidative and ER stress and necrotic injury (Ronis et al. 2010).

C.P. Day

Presence of CYP2E1 c2 Allele and ADH3 Genotype Increases Risk for Alcoholic Liver Damage

Since most of the deleterious effects of alcohol are caused by its metabolism, attention has focused upon genes encoding ethanol metabolizing enzymes (Grove et al.

1998). Caucasians are polymorphic at only two of the gene loci – CYP2E1 and ADH3 (Grove et al. 1998). Although rare in Caucasians, possession of the mutant c2 allele of CYP2E1 increases the risk of alcoholic liver disease at a given level of cumulative alcohol consumption (Grove et al. 1998). This risk appears to be particularly manifest in individuals carrying the ADH3*2 allele, presumably reflecting increased metabolism of ethanol by CYP2E1 (Grove et al. 1998). In the absence of the c2 allele of CYP2E1, ADH3 genotype does not influence the risk of advanced alcoholic liver disease but, in males at least, may increase the susceptibility to alcoholism (Grove et al. 1998).

B. Fromenty

Induction of Mitochondrial Hepatic CYP2E1 due to Binge Alcohol

The effect of repeated alcohol binges on hepatic mtDNA in mice has been studied (Demeilliers et al. 2002). Levels of mtDNA are decreased for 48 h after the last dose in mice administered ethanol (Demeilliers et al. 2002). Two and 24 h after the fourth dose, DNA lesions are observed that block the progress of the polymerases and organellomtDNA synthesis is decreased (Demeilliers et al. 2002). Mitochondria exhibit ultrastructural abnormalities, and respiration is impaired 2 and 24 h after the fourth binge. CYP2E1, mitochondrial generation of peroxides, thiobarbituric acid reactants, and ethane exhalation are increased (Demeilliers et al. 2002). After repeated doses of ethanol, the accumulation of unrepaired mtDNA lesions (possibly involving lipid peroxidation-induced adducts) blocks the progress of polymerase gamma on mtDNA and prevents adaptive mtDNA resynthesis, causing prolonged hepatic mtDNA depletion (Demeilliers et al. 2002). Thus, mitochondrial structural and functional abnormalities occur along with induction of CYP2E1 due to repeated binge alcohol drinking.

Alcohol Mediated Mitochondrial CYP2E1 Induction

Ethanol increases microsomal and mitochondrial CYP2E1 in cultured rat hepatocytes and in the liver of lean mice (Robin et al. 2005). This is associated with decreased levels of GSH, possibly reflecting increased oxidative stress (Robin et al. 2005). In contrast, in leptin-deficient obese mice, ethanol administration does not increase mitochondrial CYP2E1, nor it depletes mitochondrial GSH, suggesting that leptin deficiency hampers mitochondrial targeting of CYP2E1 (Robin et al. 2005). Thus, ethanol intoxication increases CYP2E1 not only in the endoplasmic reticulum but also in mitochondria, thus favouring oxidative stress in these compartments (Robin et al. 2005).

M.A. Robin

Mitochondrial CYP2E1 and Ethanol or Acetaminophen Mediated Toxicity

When acetaminophen or ethanol are used as CYP2E1 substrates, the exclusive localization of CYP2E1 within mitochondria is sufficient to induce ROS overproduction, depletion of GSH, increased expression of mitochondrial Hsp70, mitochondrial dysfunction and cytotoxicity (Knockaert et al. 2011). Importantly, these harmful events occur despite lower cellular level and activity of CYP2E1 when compared to monkey kidney cell line COS-7 expressing CYP2E1 in both endoplasmic

reticulum and mitochondria, and this is particularly obvious with acetaminophen (Knockaert et al. 2011). Thus, mitochondrial CYP2E1 could play a major role in drug-induced oxidative stress and cell demise (Knockaert et al. 2011).

S.C. Lu

Regulation of CYP2E1 by Chronic Hepatic S-Adenosylmethionine Deficiency

In mammals, methionine metabolism occurs mainly in the liver via methionine adenosyltransferase-catalyzed conversion to SAM (Martínez-Chantar et al. 2002). Of the two genes that encode methionine adenosyltransferase (MAT1A and MAT2A), MAT1A is mainly expressed in adult liver whereas MAT2A is expressed in all extrahepatic tissues (Martínez-Chantar et al. 2002). Mice lacking MAT1A have reduced hepatic SAM content and hyperplasia and spontaneously develop nonalcoholic steatohepatitis (Martínez-Chantar et al. 2002). An abnormal expression of genes involved in the metabolism of lipids and carbohydrates occurs in MAT1A knockout mice, a situation that is reminiscent of that found in diabetes, obesity, and other conditions associated with nonalcoholic steatohepatitis (Martínez-Chantar et al. 2002). This aberrant expression of metabolic genes in the knockout mice is associated with hyperglycemia, increased hepatic CYP2E1 and UCP2 expression and triglyceride levels, and reduced hepatic GSH content (Martínez-Chantar et al. 2002). The knockout animals have increased lipid peroxidation and enhanced sensitivity to CCl4 (a substrate of CYP2E1)-induced liver damage, which is largely due to increased CYP2E1 expression because DAS, an inhibitor of CYP2E1, prevents CCl4-induced liver injury (Martínez-Chantar et al. 2002). Hepatocellular carcinoma develops in more than half of the knockout mice (Martínez-Chantar et al. 2002). Thus, SAM plays a crucial role in maintaining normal hepatic function and tumorigenesis of the liver and SAM knock out mice are more predisposed to CCl4-induced liver injury due to induction of CYP2E1 in the absence of SAM (Martínez-Chantar et al. 2002).

N.G. Avadhani

Biochemical Characterization of Mitochondrial CYP2E1

Hepatic mitochondria contain an inducible cytochrome P450, referred to as P450 MT5, which cross-reacts with antibodies to microsomal CYP2E1 and it has been purified, partially sequenced, and the enzymatic properties of the rat liver mitochondrial form have been determined (Robin et al. 2001). The N terminus of purified mitochondrial CYP2E1 protein is identical to that of the microsomal CYP2E1 (Robin et al. 2001). The mitochondrial CYP2E1 displays the same catalytic activity as the microsomal counterpart, although the activity of the mitochondrial enzyme is supported exclusively by adrenodoxin and adrenodoxin reductase which may be due to a different conformational state of the mitochondrial targeted CYP2E1 (Robin et al. 2001). Further, the mitochondrial CYP2E1 is phosphorylated at a higher level compared with the microsomal counterpart (Robin et al. 2001). Thus, hepatic mitochondrial CYP2E1 possesses distinct biochemical characteristics (Robin et al. 2001).

Diabetes Mediated Induction of Mitochondrial CYP2E1

A five- to eightfold increase of CYP2E1 and glutathione S-transferase (GST) A4-4 levels occurs in mitochondria from STZ-treated rat tissues compared with those in

nondiabetic rat tissues, suggesting their possible roles in the disease process (Raza et al. 2004). Transient transfection of COS cells with CYP2E1 cDNA causes a similar accumulation of CYP2E1 and GST A4-4 in mitochondria and increased production of mitochondrial ROS (Raza et al. 2004). Thus, a marked increase in mitochondrial oxidative stress in target tissues of STZ-treated rats and implicates a direct role for mitochondrial CYP2E1 in the generation of intramitochondrial ROS.

Alcohol Inducible Mitochondrial CYP2E1: A Potential Source for Oxidative Stress

CYP2E1 is bimodally targeted to both the endoplasmic reticulum (microsomes) (mc CYP2E1) and mitochondria (mt CYP2E1) (Bansal et al. 2010). The role of mtCYP2E1 in ethanol-mediated oxidative stress in stable cell lines expressing predominantly mt CYP2E1 or mc CYP2E1 has been studied (Bansal et al. 2010). The ER+ mutation (A2L, A9L), which increases the affinity of the nascent protein for binding to the signal recognition particle, preferentially targets CYP2E1 to the endoplasmic reticulum (Bansal et al. 2010). The Mt+ (L17G) and Mt++ (I8R, L11R, L17R) mutant proteins, showing progressively lower affinity for signal recognition particle binding are targeted to mitochondria at correspondingly higher levels (Bansal et al. 2010). The rate of GSH depletion, used as a measure of oxidative stress, is higher in cells expressing Mt++ and Mt+ proteins as compared with cells expressing ER+ protein (Bansal et al. 2010). In addition, the cellular level of F(2)-isoprostanes, a direct indicator of oxidative stress, is increased markedly in Mt++ cells after ethanol treatment (Bansal et al. 2010). Notably, expression of Mt++ CYP2E1 protein in yeast cells causes more severe mitochondrial DNA damage and respiratory deficiency than the wild type or ER+ proteins as tested by the inability of cells to grow on glycerol or ethanol (Bansal et al. 2010). Additionally, liver mitochondria from ethanol-fed rats containing high mt CYP2E1 show higher levels of F(2)-isoprostane production (Bansal et al. 2010). Thus, mt CYP2E1 induces oxidative stress and augments alcohol-mediated cell/tissue injury.

G.U. Corsini

A Protective Role of CYP2E1 Against MPTP Induced Neurotoxicity

Elucidation of the biochemical steps leading to the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced degeneration of the nigrostriatal dopamine (DA) pathway has provided new clues to the pathophysiology of Parkinson's disease (Vaglini et al. 2004). In line with the enhancement of MPTP toxicity by diethyldithiocarbamate (DDC), the potentiation of the selective DA neuronal degeneration in mice by CYP2E1 inhibitors, such as DAS and PIC has been investigated (Vaglini et al. 2004). In addition, CYP2E1 mRNA and protein are present in the brain and in the basal ganglia of C57/bl mice (Vaglini et al. 2004). A kinetic analysis of MPTP and its metabolites indicates that no detoxification metabolic pathway is affected by any of these inhibitors (Vaglini et al. 2004). This does not rule out, however, that an undetected detoxification pathway involving CYP2E1 is operating (Vaglini et al. 2004). In order to provide direct evidence for this isozyme involvement, CYP2E1 knockout mice are challenged with MPTP or the combined treatment (Vaglini et al. 2004). These transgenic mice have a low sensitivity to MPTP alone, similar to their

wild-type counterparts, suggesting that it is likely that transgenic mice compensate for the missing enzyme (Vaglini et al. 2004). However, DDC pretreatment completely fails to enhance MPTP toxicity in CYP2E1 knockout mice, whereas this enhancement is regularly present in wild-type animals (Vaglini et al. 2004). Thus, the occurrence of CYP2E1 in C57/bl mouse brain is relevant to MPTP toxicity, and suggests that this isozyme may have a detoxificant role related to the efflux transporter of the toxin (Vaglini et al. 2004).

It has been shown that DDC potentiates MPTP toxicity in mice as a result of increased levels of 1-methyl-4-phenylpyridinium ion (MPP(+)) in the striatum (Pardini et al. 2008). Brain CYP2E1 inhibition by DDC in C57Bl mice is responsible for increased toxicity and striatal MPP(+) accumulation (Pardini et al. 2008). However, CYP2E1-null mice do not show any enhanced sensitivity to MPTP or any MPP(+) accumulation (Pardini et al. 2008). This unexpected finding suggests that the CYP2E1-null mice compensate with other isozymes as already described for acetaminophen-induced liver damage (Pardini et al. 2008). MPP(+) intoxication of mesencephalic cell cultures from CYP2E1-null mice indicates a reduced sensitivity of dopaminergic (DA) neurons from knockout animals (Pardini et al. 2008). Surprisingly, MPP(+) cell distribution under these conditions indicates that the toxin accumulates more intracellularly in knockout cultures, suggesting further that CYP2E1 has a role in MPP(+) storage and efflux (Pardini et al. 2008).

The sensitivity of Cyp2e1(–/–) mice to the acute administration of MPTP in comparison with their wild-type counterparts has been investigated (Viaggi et al. 2009). In Cyp2e1(–/–) mice, the reduction of striatal DA content is less pronounced 7 days after MPTP treatment compared to treated wild-type mice (Viaggi et al. 2009). Similarly, tyrosine hydroxylase immunoreactivity analysis of the substantia nigra of Cyp2e1(–/–) mice does not show any neuronal lesions after MPTP treatment (Viaggi et al. 2009). In contrast to this, wild-type animals show a minimal but significant lesioning by the toxin in this brain area (Viaggi et al. 2009). Striatal levels of DA metabolites after 7 days are variably affected by the toxin, but consistent differences between the two animal strains are not observed (Viaggi et al. 2009). Striatal MPP(+) is cleared more rapidly in Cyp2e1(–/–) mice than in wild-type animals and, consistently, striatal DA content decreases faster in Cyp2e1(–/–) mice than in wild-type animals, and 3-methoxytyramine and homovanillic acid levels show an early and sharp rise (Viaggi et al. 2009). Cyp2e1(–/–) mice are weakly sensitive to MPTP-induced brain lesions, markedly in contrast with a protective role of the enzyme as suggested previously (Viaggi et al. 2009). The differences observed between the knockout mice and their wild-type counterparts are modest and may be due to an efficient compensatory mechanism or genetic drift in the colonies (Viaggi et al. 2009).

Involvement of CYP2E1 in Acute and Chronic Behavioral Effects of Ethanol

It has been shown that acetaldehyde is an active metabolite of ethanol with central actions that modulate behavior (Correa et al. 2009). Catalase has been proposed as the main enzyme responsible for the synthesis of acetaldehyde from ethanol in the brain (Correa et al. 2009). Recent studies, however, suggest that CYP2E1 can also contribute

to the central metabolism of ethanol (Correa et al. 2009). Spontaneous locomotion in CYP2E1 knockout (KO) mice is lower than that seen in the wild type (WT) mice (Correa et al. 2009). Acute administration of ethanol increases locomotion to a similar extent in both strains of mice (Correa et al. 2009). Repeated intermittent administration of ethanol produces sensitization in both strains, but it is very subtle in the KO mice compared with the effect in the WT mice (Correa et al. 2009). KO mice show a reduction in preference for ethanol intake at low concentrations (4–8% v/v) (Correa et al. 2009). Interestingly, KO mice have higher levels of catalase protein expression in the brain and liver compared with WT mice (Correa et al. 2009). Thus, some impact of the mutation on ethanol-induced sensitization and on voluntary ethanol preference is observed (Correa et al. 2009). The lack of a substantial impact of the mutation can be explained by the fact that the CYP2E1 KO animals have a compensatory increase in catalase expression compared with WT mice, therefore possibly showing alterations in the formation of acetaldehyde after ethanol administration (Correa et al. 2009).

R.F. Tyndale

Synergistic Induction of CYP2E1 by Ethanol and Nicotine

The use of ethanol and nicotine is strongly linked; 80–95% of heavy alcohol users are also smokers (Howard et al. 2001). In humans, cigarette smoking significantly enhances CYP2E1 activity, as measured by increased metabolism of chlorzoxazone *in vivo* (Howard et al. 2001). CYP2E1 also bioactivates tobacco smoke and other procarcinogens and several hepatotoxins (Howard et al. 2001). It has been investigated whether nicotine increases CYP2E1 activity like ethanol (Howard et al. 2001). After ethanol or nicotine administration, immunostaining for CYP2E1 is increased in the centrilobular regions of rat liver (Howard et al. 2001). Hepatic CYP2E1 protein levels are increased by ethanol and nicotine (Howard et al. 2001). *In vitro* chlorzoxazone 6-hydroxylation analyses demonstrate elevated Vmax values by using hepatic microsomes from high-dose ethanol or nicotine-treated rats, with no change in affinity (Howard et al. 2001). The magnitude of enhanced chlorzoxazone metabolism by microsomes from drug-treated animals is consistent with the observed increase in CYP2E1 protein (Howard et al. 2001). Thus, nicotine may increase CYP2E1-induced toxicity and contribute to cross-tolerance in smokers and people treated with nicotine (e.g., smokers, patients with Alzheimer's disease, ulcerative colitis, neuropsychiatric motor disorders) (Howard et al. 2001).

Nicotine increases CYP2E1 protein and activity in the rat liver (Micu et al. 2003). The induction peaks at 4 h postnicotine treatment and recovers within 24 h (Micu et al. 2003). No induction is observed after a single injection, and 18 days of treatment does not increase the levels beyond that found at 7 days (Micu et al. 2003). CYP2E1 is induced by very low doses of chronic (×7 days) nicotine with an ED50 value of 0.01 mg/kg s.c.; 0.01 mg/kg in a rat model results in peak cotinine levels (nicotine metabolite) similar to those found in people exposed to environmental tobacco smoke (passive smokers; 2–7 ng/ml) (Micu et al. 2003). Thus, nicotine does not regulate CYP2E1 expression by protein stabilization (Micu et al. 2003). Nicotine increases CYP2E1 at very low doses and may enhance CYP2E1-related toxicity in smokers, passive smokers, and people treated with nicotine (Micu et al. 2003).

In monkeys, nicotine induction of *in vivo* CZN disposition is related to the rates of *in vitro* CZN metabolism and hepatic microsomal CYP2E1 protein levels (Lee et al. 2006b). Nicotine is one component in cigarette smoke that can increase *in vivo* CZN metabolism *via* induction of hepatic CYP2E1 levels (Lee et al. 2006b). Thus, nicotine exposure may affect the metabolism of CYP2E1 substrates such as acetaminophen, ethanol, and benzene (Lee et al. 2006b).

Ethanol self-administration and nicotine treatment, alone and in combination to monkeys, significantly increases *in vivo* CZN disposition compared to control animals (Ferguson et al. 2011). The effect of ethanol is only observed at higher levels of intake (Ferguson et al. 2011). Ethanol and nicotine increase CYP2E1 protein levels and *in vitro* CZN metabolism, with combined exposure to both drugs resulting in the greatest increase (Ferguson et al. 2011). Chronic exposure to ethanol and nicotine induces hepatic CYP2E1 activity and protein levels, particularly when both drugs are used in combination and when ethanol intake is high (Ferguson et al. 2011).

Nicotine pretreatment of rats increases voluntary ethanol intake on day 10 compared to saline pretreatment (Yue et al. 2009). CYP2E1 is increased 1.7, 1.8, and 1.4 fold by the three doses of nicotine alone; CYP2E1 levels are increased by voluntary ethanol intake alone and a further 2.4, 2.2, and 1.8 fold by 0.4, 0.8, and 1.2 mg/kg nicotine respectively versus saline pretreatment (Yue et al. 2009). CYP2E1 level correlates with alcohol consumption on day 10 (Yue et al. 2009). Chronic nicotine increases voluntary ethanol intake thereby enhancing CYP2E1 level (Yue et al. 2009). Thus CYP2E1 is regulated not only directly by nicotine and ethanol, but also indirectly *via* an increase in the ethanol consumption in the presence of nicotine pretreatment (Yue et al. 2009). Together this may contribute to the co-abuse of these drugs and alter the metabolism of clinical drugs and endogenous substrates (Yue et al. 2009).

Induction of Brain CYP2E1 by Nicotine and Its Underlying Mechanisms

Ethanol treatment significantly increases CYP2E1 in olfactory bulbs, frontal cortex, hippocampus and cerebellum, while nicotine induces CYP2E1 in olfactory bulbs, frontal cortex, olfactory tubercle, cerebellum and brainstem and the induction is cell-type specific as assessed through immunocytochemical analysis (Howard et al. 2003a). Consistent with the increased CYP2E1 found in rat brain following drug treatments, brains from alcoholics and alcoholic smokers show greater staining of granular cells of the dentate gyrus and the pyramidal cells of CA2 and CA3 hippocampal regions as well as of cerebellar Purkinje cells compared to nonalcoholic nonsmokers (Howard et al. 2003a). Moreover, greater CYP2E1 immunoreactivity is observed in the frontal cortices in the alcoholic smokers in comparison to nonalcoholic nonsmokers and alcoholic nonsmokers (Howard et al. 2003a). Significantly higher CYP2E1 immunostaining is observed in nicotine-treated human neuroblastoma IMR-32 cells in culture (0.1–10 nM), suggesting that nicotine could contribute to the increased CYP2E1 observed in alcoholic smokers (Howard et al. 2003a). CYP2E1 induction in the brain, by ethanol or nicotine, may influence the central effects of ethanol and the development of nervous tissue pathologies observed in alcoholics and smokers (Howard et al. 2003a).

CYP2E1 levels varies among saline-treated African green monkeybrain regions and expression is cell-type specific (Joshi and Tyndale 2006a). Chronic nicotine treatment induces CYP2E1 expression in the frontal cortex and cerebellum, specifically in cortical pyramidal neurons and cerebellar Purkinje cells but no change is seen in temporal cortex, hippocampus, putamen and thalamus (Joshi and Tyndale 2006a). CYP2E1 expression pattern in monkey brain following chronic nicotine treatment is similar to that in smokers, suggesting that nicotine may be the primary component in cigarette smoke that induces CYP2E1(Joshi and Tyndale 2006a). Increased CYP2E1 in brain may contribute to oxidative stress and alter localized metabolism, and resulting pharmacology, of centrally acting drugs metabolized by CYP2E1 (Joshi and Tyndale 2006a).

Chronic nicotine treatment increases CYP2E1 in rat liver and brain (Joshi and Tyndale 2006b). Chronic 7-day nicotine treatment shows the highest levels of CYP2E1 12 h after the last injection in frontal cortex (1.4-fold) versus 8 h in hippocampus (1.8-fold) and cerebellum (1.4-fold), returning to basal levels by 24 h (Joshi and Tyndale 2006b). In contrast, acute nicotine treatment does not induce CYP2E1 in frontal cortex and hippocampus but increases CYP2E1 in cerebellum 8 h after treatment (1.6-fold) (Joshi and Tyndale 2006b). Brain CYP2E1 mRNA levels does not increase after chronic nicotine treatment, suggesting nontranscriptional regulation (Joshi and Tyndale 2006b). Thus, humans exposed to nicotine may have altered CYP2E1-mediated metabolism of centrally acting drugs and toxins as well as altered toxicity because of oxidative stress caused by CYP2E1 (Joshi and Tyndale 2006b). Those affected may include current and passive smokers and people that may be treated with nicotine such as smokers and, potentially, patients with Alzheimer's, Parkinson's disease, or ulcerative colitis (Joshi and Tyndale 2006b).

Association of CYP2E1 Variant Allele with Alcohol and Nicotine Dependence

The CYP2E1*1D polymorphism has been associated with greater CYP2E1 inducibility (Howard et al. 2003b). The frequency of the variant allele in eight ethnic groups has been determined (Howard et al. 2003b). Further, the Canadian Native Indian, South-east Asian Canadian and Caucasian Canadian groups are stratified by alcohol and nicotine dependence (as measured by DSM-IV criteria) to examine the potential association of CYP2E1*1D with drug dependence (Howard et al. 2003b). A significantly greater frequency of the CYP2E1*1D allele occurs among Indo-Asian Canadians (0.31), Chinese Canadians (0.19), Taiwanese (0.20), Japanese Canadians (0.18), African Americans (0.13), African Canadians (0.10) and Canadian Native Indians (0.09) compared to Caucasian Canadians (0.02)(Howard et al. 2003b). Although the power of the association study is low among some subgroups, the CYP2E1*1D genotype (subjects with at least one variant allele) is associated with alcohol as well as nicotine dependence (Howard et al. 2003b). Specifically, Canadian Native Indians dependent on nicotine alone or alcohol alone exhibit significantly greater CYP2E1*1D frequencies compared to non-drug dependent controls, while the variant frequency among Southeast Asians dependent on nicotine is greater than their non-drug dependent counterparts (Howard et al. 2003b). CYP2E1*1D genotype is associated with significantly greater 3-hydroxycotinine per cigarette in

African Americans (Howard et al. 2003b). The variable frequency of CYP2E1*1D among ethnic groups suggests a greater risk for diseases putatively related to CYP2E1 in some non-Caucasian ethnic groups (Howard et al. 2003b). The association of CYP2E1*1D with alcohol and nicotine dependence suggests that CYP2E1 may contribute to the development of these dependencies (Howard et al. 2003b).

Phenobarbital Mediated Brain CYP2E1 Induction

The effect of chronic phenobarbital treatment on *in vivo* chlorzoxazone disposition, *in vitro* chlorzoxazone metabolism, and hepatic and brain CYP2E1 protein levels in African Green monkeys has been examined (Lee et al. 2006c). Phenobarbital does not induce *in vivo* chlorzoxazone disposition, *in vitro* chlorzoxazone metabolism or hepatic CYP2E1 protein levels in monkeys (Lee et al. 2006c). However, phenobarbital induces brain CYP2E1 protein levels by 1.26-fold in the cerebellum and 1.46-fold in the putamen (Lee et al. 2006c). Phenobarbital also increases cell-specific CYP2E1 expression, for example in the frontal cortical pyramidal neurons and cerebellar Purkinje cells (Lee et al. 2006c). Phenobarbital does not alter hepatic metabolism, but may alter metabolism of CYP2E1 substrates within the brain (Lee et al. 2006c).

J.M. Maher/C.D. Klaasen

Regulation of CYP2E1 by HNF 1 Alpha – A Key Regulator of Glucose Metabolism

The transcription factor HNF1alpha is involved in regulation of glucose metabolism and transport, and in the expression of several drug and bile acid metabolizing enzymes (Maher et al. 2006). Targeted disruption of the HNF1alpha gene results in decreased Cyp1a2, and Cyp2e1 expression, and increased Cyp4a1 and Cyp7a1 expression, suggesting these enzymes are HNF1alpha target genes (Maher et al. 2006).

Y. Horsmans

Down-Regulation of CYP2E1 During the Early Phase of Liver Regeneration

The modification of cytochrome P450 expression in the regenerating liver has been studied (Starkel et al. 2000). Ligation of branches of the portal vein (PBL) perfusing 70% of the liver parenchyma, which produces regeneration and atrophy within the same liver, constitutes an ideal model to study the relative specificity of the early events in the regenerating liver and their relationship to the loss of liver mass (Starkel et al. 2000). In this PBL model and in sham models, the expression and the metabolic activity of CYP2E1 have been studied (Starkel et al. 2000). The metabolic activity of CYP2E1 is transiently and simultaneously down-regulated in the regenerating and atrophying lobes during the first 2–5 h after PBL (Starkel et al. 2000). No significant modification is observed at the protein level. In contrast, iNOS protein is significantly induced in both lobes (Starkel et al. 2000). Similar results are observed after sham operation (Starkel et al. 2000). The reduction of these CYP activities in both lobes after PBL and in sham livers suggests that other mechanisms than the regenerating process itself or the reduction of the liver mass might account for such down-regulation during the early phase of liver regeneration (Starkel et al. 2000). The activation of nitric oxide (NO) and/or pro-inflammatory cytokine

production provides clues to pathways liable to affect the CYP activities in the regenerating liver (Starkel et al. 2000).

R.A. Deitrich

Regulation of CYP2E1: Role of Kinases

Phosphorylation of pure CYP2E1 is achieved *in vitro* using Ca2+/calmodulin-dependent protein kinase II (CaM kinase II), protein kinase C (PKC) and cAMP-dependent protein kinase (PKA) (Menez et al. 1993). CaM kinase II is the most efficient enzyme capable of catalyzing this phosphorylation reaction: the maximum incorporation of 32PO4 is 0.8 mol/mol CYP2E1 in 20 min. PKA phosphorylates a maximum of 0.7 mol of 32PO4/mol of cytochrome within 60 min (Menez et al. 1993). The phosphorylation by PKC reaches a maximum of 0.19 mol of 32PO4/mol of cytochrome and this occurs within a few minutes of incubation (Menez et al. 1993). Limited digestion by *S. aureus* V8 protease (SAP) of CYP2E1, which has been phosphorylated by either PKA and PKC, yields a single major phosphopeptide with an M(r) of approximately 18,000 (Menez et al. 1993). Limited digestion of CYP2E1 that has been phosphorylated by CaM kinase II, yields phosphorylated polypeptides with M(r) of approximately 18,000 and 15,000 (Menez et al. 1993). Thus, the three kinases may be involved in the regulation of CYP2E1 (Menez et al. 1993).

Increased Ethanol Sensitivity in Brain: CYP2E1, A Key Player

The contribution of the different enzymatic pathways to ethanol oxidation in brain homogenates from mice and rats has been investigated (Zimatkin et al. 2006). The catalase inhibitors sodium azide and aminotriazole as well as CYP2E1 inhibitors DAS and beta-PIC significantly lower the accumulation of the ethanol-derived acetaldehyde (AC) and acetate in brain homogenates (Zimatkin et al. 2006). The ADH inhibitor 4-MP significantly decreases the acetate but not the AC accumulation (Zimatkin et al. 2006). Ethanol-derived AC accumulation in brain homogenates of acatalasemic mice is 47% of the control value, 91% in CYP2E1-null mice, and 24% in double mutants (with deficiency of both catalase and CYP2E1) (Zimatkin et al. 2006). The highest levels of ethanol oxidation are found in microsomal and peroxisomal subcellular brain fractions, where CYP2E1 and catalase are located, respectively (Zimatkin et al. 2006).

VI

M. Ingelman-Sundberg

Region Specific Expression of Liver CYP2E1

The acinar distribution of ethanol-inducible cytochrome P450 has been studied by immunohistochemistry using anti-rat CYP2E1 serum (Bühler et al. 1991). In all 17 human liver specimens staining is confined to perivenous hepatocytes (Bühler et al. 1991). Staining is stronger in livers of alcoholics than in non-alcoholics (Bühler et al. 1991). A similar selective perivenous staining is observed in rat liver (Bühler et al. 1991). This pattern is exacerbated by chronic ethanol treatment, with staining appearing especially intense in hepatocytes surrounding the terminal hepatic veins (Bühler et al. 1991). Thus, chronic ethanol intake causes induction of CYP2E1 in

the perivenous region and this regiospecific expression and induction may contribute to the perivenous damage caused by alcohol and several other hepatotoxins known to be metabolized by this enzyme (Bühler et al. 1991).

The regional expression of six different CYP forms in rat liver under constitutive and induced conditions has been compared (Bühler et al. 1992). Immunostaining of consecutive thin sections from control liver reveal that the same hepatocytes, forming a 6–8 cells thick layer surrounding the terminal hepatic venules, are stained for CYP2B1/2, CYP2E1 and CYP3A1 (Bühler et al. 1992). Staining of CYP2A1 extends further into the midzonal region, whereas all cells of the acinus stain for CYPEtOH2 (Bühler et al. 1992). CYPEtOH2 denotes an ethanol-inducible P450 with regulation distinct from CYP2E1 and sequence determination indicates that the protein belongs to the CYP2C family (Bühler et al. 1992). Three distinct patterns of constitutive P450 protein expression: perivenous-restricted (CYP2B1/2, CYP2E1 and CYP3A1); perivenous-dominated (CYP2A1) and panacinar (CYPEtOH2) exist (Bühler et al. 1992). Chronic exposure to ethanol causes induction of CYP2E1 in the same cells already being constitutively expressed, whereas CYPEtOH2 is more induced in the periportal area. Acetone induces CYP2E1, CYP2C11 and CYP3A1 selectively in the perivenous area (Bühler et al. 1992). A particular P450 isozyme is generally induced in the same cells where it is constitutively expressed, and that this regional selectivity is independent of the kind of inducer (Bühler et al. 1992). Thus, during maturation, the hepatocytes acquire various phenotypes in the periportal and perivenous region, to respond differently to endogenous and exogenous signals in the control of P450 expression (Bühler et al. 1992).

Dietary Fat Plus Ethanol Mediated Induction of CYP2E1

Ethanol-induction of apoCYP2B1 (2×) and paranitrophenol hydroxylase (6–8×) is the same for rats fed corn oil and rats fed tallow as the fat component (2×); but not for apoCYP2E1 (21 and 8×, respectively) and MEOS activity (8 and 2.6×, respectively) (Takahashi et al. 1992).

Chlormethiazole: An Effective and Specific Inhibitor of CYP2E1

The effects of a drug used for treatment of ethanol withdrawal states; CMZ on CYP2E1 expression in rat liver has been evaluated (Hu et al. 1994). A 4-fold induction of CYP2E1 is observed after 3 days of starvation, accompanied by a similar increase in the level of the corresponding mRNA (Hu et al. 1994). CMZ specifically inhibits the elevation of CYP2E1 mRNA and protein, but does not prevent CYP2B1 and CYP3A1 or CYP1A1 induction caused by treatment with phenobarbital or beta-naphthoflavone, respectively (Hu et al. 1994). The rate of the CYP2E1 gene transcription is inhibited greatly by CMZ treatment (Hu et al. 1994). Rats treated with ethanol in a total enteral nutrition model have higher CYP2E1-dependent hepatic microsomal activities of p-nitrophenol hydroxylase and carbon tetrachloride-induced lipid peroxidation than controls, and simultaneous CMZ treatment abolishes the ethanol-dependent induction (Hu et al. 1994). *In vitro* experiments with rat liver microsomes show that CMZ does not act as an inhibitor of CYP2E1-dependent catalytic activities or as an inhibitor of microsomal

NADPH and CYP2E1-dependent lipid peroxidation (Hu et al. 1994). Thus, CMZ might constitute an efficient and specific inhibitor of CYP2E1 expression suitable for *in vivo* experiments (Hu et al. 1994).

Modes of Regulation of CYP2E1

CYP2E1 gene has been shown to be transcriptionally activated after birth, but the expression of ethanol-inducible CYP2E1 protein, hereafter, is regulated by post-transcriptional mechanisms (Johansson et al. 1990). The constitutive expression of CYP2E1 protein is restricted to the perivenous region of the liver lobule (Johansson et al. 1990). Studies with in situ hybridization and run off experiments indicate that-this regioselectivity is caused by a higher rate of gene transcription in the perivenous hepatocytes (Johansson et al. 1990). The transcription of the CYP2E1 gene is activated by starvation, indicating that also this P450 gene is under transcriptional control under certain physiological conditions (Johansson et al. 1990).

The regulation of CYP2E1 has been studied by following mRNA levels, catalytic activities and the subcellular distribution of the apoprotein in rat liver at different time points after a single intragastric dose of acetone (Ronis et al. 1991). No changes are observed in hepatic CYP2E1 mRNA levels at any time after acetone treatment, whereas rapid rises are observed in the microsomal amount of CYP2E1 protein and CYP2E1-catalyzed 4-nitrophenol hydroxylase and carbon-tetrachloride-initiated lipid-peroxidation activities (Ronis et al. 1991). However, CYP2E1-dependent catalytic activities decline much faster than the immunodetectable CYP2E1 protein, suggesting that this cytochrome P-450 is inactivated prior to degradation (Ronis et al. 1991). Similar results are seen in primary hepatocyte cultures (Ronis et al. 1991). CYP2E1 is acutely inactivated in the endoplasmic reticulum and that degradation of this isozyme occurs, at least in part, by the lysosomal route (Ronis et al. 1991).

CYP2E1 Overexpression Upregulates Stress Related Genes in Alcohol Induced Liver Injury

Transgenic mice expressing human CYP2E1 treated with ethanol exhibit increased liver injury (Butura et al. 2009). Increased expression of glutathione transferases, monooxidases (several cytochrome P450's included, including Cyp2b9 and Cyp2c55), hydrolases, membrane proteins such as receptors (e.g. prolactin receptor), proteins involved in DNA processing, oxido-reductases and apoptosis-related genes occurs and the expression of structural genes, particularly cytokeratin 8 and 18, is highly related to pathology, suggesting that CYP2E1 overexpression aggravates injury and aids the progression of alcoholic liver disease (Butura et al. 2009).

Mitochondrial Targeting of CYP2E1

The role of the NH(2)-terminus of CYP2E1 in intracellular targeting has been investigated (Neve and Ingelman-Sundberg 1999). In the absence of the hydrophobic NH(2)-terminal sequence, a putative mitochondrial import signal is exposed which targets CYP2E1 to this organelle where it is further processed (Neve and Ingelman-Sundberg 1999).

CYP2E1 lacking the hydrophobic NH(2)-terminal hydrophobic transmembrane domain is specifically targeted to mitochondria, where it is processed to a soluble and catalytically active form (Delta2E1) with a mass of about 40 kDa (Neve and Ingelman-Sundberg 2001). Small amounts of Delta2E1 have been also observed in mitochondria isolated from rat liver, indicating that this form of CYP2E1 is also present *in vivo* (Neve and Ingelman-Sundberg 2001). The mitochondrial targeting signal has been identified and characterized by the use of several NH(2)-terminally truncated and mutated forms of CYP2E1 that are expressed in the mouse H2.35 hepatoma cell line (Neve and Ingelman-Sundberg 2001). Mitochondrial targeting of CYP2E1 is mediated through a sequence located between amino acid residues 74 and 95 and that positively charged residues as well as a hydrophobic stretch present in the beginning of this sequence are essential for this process (Neve and Ingelman-Sundberg 2001).

Transport of CYP2E1 to the Plasma Membrane

The molecular basis for the transport of rat ethanol-inducible CYP2E1 to the plasma membrane has been investigated by transfection of several different mutant cDNAs into mouse H2.35 hepatoma cells (Neve and Ingelman-Sundberg 2000). The NH(2)-terminal transmembrane domain of CYP2E1 plays a crucial role in directing the protein to the cell surface and that topological inversion of a small fraction of CYP2E1 in the endoplasmic reticulum directs the protein to the plasma membrane (Neve and Ingelman-Sundberg 2000).

Polymorphisms in CYP2E1 Gene

In almost 200 individuals belonging to either a Chinese, an Italian, or a Swedish population, two new CYP2E1 gene variants are found with functional mutations: one (CYP2E1*2) in which a G1168A point mutation in exon 2 caused an R76H amino acid substitution, and the other (CYP2E1*3) in which a G10059A base substitution in exon 8 yielded a V3891 amino acid exchange (Hu et al. 1997). The corresponding CYP2E1 cDNAs are constructed, subcloned into the pCMV4 expression vector, and expressed in COS-1 cells (Hu et al. 1997). The CYP2E1*3 cDNA variant is indistinguishable from the wild-type cDNA on all variables investigated, whereas CYP2E1*2 cDNA, although yielding similar amounts of mRNA, only causes 37% of the protein expression and 36% of the catalytic activity compared with the wild-type cDNA (Hu et al. 1997). Complete screening by single-stranded conformation polymorphism of the three populations studied reveals that these variant alleles are rare (Hu et al. 1997). Thus, the human CYP2E1 gene is functionally surprisingly well conserved compared with other cytochrome P450 enzymes active in drug metabolism, which suggests an important endogenous function in humans (Hu et al. 1997).

The 5′-flanking region of the human and rat CYP2E1 genes have been sequenced and characterized (Hu et al. 1999). The identity between the human and rat sequences (−3.8 to +1 kb) is generally between 35 and 60%, and the most similar regions are found in the proximal part of the sequence (Hu et al. 1999). Two more distant regions at −1.6 to −2.0 kb and −2.5 to −2. 8 kb in the human sequence are also found to have high identity to the rat sequence (Hu et al. 1999). A polymorphic repeat

sequence in the human gene is found between −2,178 and −1,945 bp (Hu et al. 1999). The common allele (CYP2E1*1C) contains 6 repeats (each 42–60 bp long) and the rare allele (CYP2E1*1D) have 8 repeats with an allele frequency of 1% among Caucasians and 23% among Chinese (Hu et al. 1999). The CYP2E1 5′-flanking regions of the human (−3,712 to +10 bp) and rat (−3,685 to +28 bp) genes are ligated in front of a luciferase reporter gene and transfected into rat hepatoma Fao and human hepatoma B16A2 cells (Hu et al. 1999). Important species specificity is noted in the control of gene expression and regions of negative and positive cis-acting elements are localized (Hu et al. 1999). No difference is seen in the constitutive expression between the two polymorphic forms (Hu et al. 1999).

Short Half Life of CYP2E1: Mechanism Involved

Ethanol-inducible CYP2E1 involved in the metabolism of gluconeogenetic precursors and some cytotoxins is distinguished from other cytochrome P450 enzymes by its rapid turnover (*in vivo* half-life of 4–7 h), with ligands to the haem iron, both substrates and inhibitors, stabilizing the protein (Zhukov and Ingelman-Sundberg 1999). CYP2E1 is also known to have a high oxidase activity in the absence of substrate, resulting in the production of ROS (Zhukov and Ingelman-Sundberg 1999). The rapid intracellular turnover of the enzyme may be partly due to covalent modifications by such radicals or to other changes during catalytic cycling, in which case the inhibition of electron supply from NADPH-cytochrome P450 reductase would be expected to stabilize the protein (Zhukov and Ingelman-Sundberg 1999). Fao hepatoma cells, where CYP2E1 shows a half-life of 4 h upon serum withdrawal, are treated for 1 h with 0.3 μM diphenylene iodonium (DPI), a suicide inhibitor of flavoenzymes, which results in approximately 90% inhibition of the microsomal NADPH-cytochrome P450 reductase and CYP2E1-dependent chlorzoxazone hydroxylase activities (Zhukov and Ingelman-Sundberg 1999). Subsequent cycloheximide chase reveals that the CYP2E1 half-life increases to 26 h (Zhukov and Ingelman-Sundberg 1999). The short half-life of CYP2E1 *in vivo* may be largely due to the rapid destabilization of the enzyme during catalytic cycling rather than to the intrinsic instability of the protein molecule (Zhukov and Ingelman-Sundberg 1999).

CYP2E1 Mediated Acetaldehyde Metabolism

Liver microsomes from starved and acetone-treated rats catalyze NADPH-support metabolism of acetaldehyde at a rate eightfold higher than corresponding control microsomes (Terelius et al. 1991). CYP2E1 is an aldehyde oxidase and thus metabolizes both ethanol and its primary oxidation product and this might have implications *in vivo* for acetaldehyde metabolism in liver and brain (Terelius et al. 1991).

CYP2E1 Mediated Alkyl Hydrazine Oxidation in Rat Liver Microsomes

CYP2E1, as compared to other rat liver cytochromes P-450-CYP2B1, CYP1A2, CYP2B4 and CYP2C4 is an efficient catalyst of NADPH-dependent oxidation of alkylhydrazines-1-methyl-,1-ethyl-, and 1-isopropylhydrazine to free radical intermediates, in rat liver microsomes and in reconstituted enzyme systems, a finding

that might be of importance in the development of the toxicity of these compounds (Albano et al. 1995).

CYP2E1 Mediated Benzene Oxidation in Rat Liver Microsomes

CYP2B1 and 2E1 oxidize toluene, aniline and monochlorobenzene (MCB) to water-soluble metabolites and to products covalently binding to microsomal proteins from rats at high efficiency (Gut et al. 1996). Oxidation of benzene to covalently binding metabolites is catalysed by CYP2B1 and 2E1 more effectively than the formation of water-soluble metabolites, especially at low benzene levels (Gut et al. 1996). CYP2B1 and 2E1 in rats appears essential for metabolic activation of benzene derivatives to potentially genotoxic products; 1,4-Benzoquinone dominates the covalent binding of benzene to proteins, whereas DNA adducts are largely due to benzene oxide (Gut et al. 1996).

Benzene Oxidation and CYP2E1 Levels in Human Liver Microsomes

In human liver microsomes, the oxidation of benzene, chlorzoxazone, aniline, dimethylformamide, and 4-nitrophenol are significantly correlated with each other and with the level of CYP2E1 protein (Nedelcheva et al. 1999). Moreover, benzene oxidation to water-soluble metabolites is suppressed by 0.1 mM diethyldithiocarbamate, supposedly a specific inhibitor of CYP2E1 at this level (Nedelcheva et al. 1999). None of these metabolic rates correlate with immunochemically determined levels of CYP1A2, 2C9, and 3A4 nor oxidation of 7-ethoxyresorufin, tolbutamide, and nifedipine (Nedelcheva et al. 1999). Benzene oxidation to water-soluble metabolites is characterized by typical Michaelis-Menten kinetics (Nedelcheva et al. 1999). The different benzene K(m) values seen in individual human microsomal samples are not correlated with the level or activity of CYP1A2, 2C9, 2E1, and 3A4 but could be due to CYP2E1 microheterogeneity (Nedelcheva et al. 1999). The lowest K(m) for benzene oxidation could be related to C/D and/or c1/c2 polymorphism of CYP2E1 gene (Nedelcheva et al. 1999). Covalent binding of benzene reactive metabolites to microsomal proteins is also correlated with the CYP2E1 metabolic rates and immunochemical levels (Nedelcheva et al. 1999).

CMZ Mediated Inhibition of CYP2E1: Mechanisms

Ethanol treatment of the Fao rat hepatoma cells causes a twofold induction of CYP2E1 protein levels, which is inhibited by CMZ (Simi and Ingelman-Sundberg 1999). Change of medium unexpectedly causes an increase in CYP2E1 gene transcription (CYP2E1 hnRNA) 4 h later (Simi and Ingelman-Sundberg 1999). However, CMZ fails to influence the expression of CYP2E1 hnRNA or mRNA both constitutively and after medium change, indicating no effect on gene transcription or mRNA synthesis/stability (Simi and Ingelman-Sundberg 1999). Cycloheximide treatment of the cells does not abolish the inhibitory action of CMZ, further indicating an action at the post-translational level (Simi and Ingelman-Sundberg 1999). In addition, CMZ inhibits CYP2E1 expression in V79 cells with stably expressed CYP2E1 under the control of the SV40 promoter (Simi and Ingelman-Sundberg 1999). The CYP2E1 gene is transcriptionally activated in response to medium

change and that CMZ, apart from being a transcriptional inhibitor of CYP2E1 expression, acts in addition as an efficient high-affinity post-translational inhibitor of CYP2E1, probably due to an allosteric destabilization of the enzyme (Simi and Ingelman-Sundberg 1999). This indicates a very rapid and effective CMZ-mediated inhibition of CYP2E1 *in vivo* (Simi and Ingelman-Sundberg 1999).

Presence of Hydroxyethyl Radical-CYP2E1 Adducts in Liver due to Alcohol Consumption

Antibodies from alcoholic cirrhotics specifically recognize hydroxyethyl radical-CYP2E1 adducts, suggesting the possible implication of these antigens in the development of autoimmune reactions in alcoholic liver disease (Clot et al. 1996).

Presence of CYP2E1 Autoantibodies in Liver due to Alcohol Consumption

Among alcoholics, antibodies against ethanol-derived radical protein adducts are observed that are dependent on CYP2E1 for their formation (Lytton et al. 1999). A time-dependent appearance of IgG against rat CYP3A1 and CYP2E1 is evident during chronic ethanol feeding of rats (Lytton et al. 1999). Anti-CYP2E1 reactivity shows positive correlation with the levels of hepatic CYP2E1 and is inhibited by the CYP2E1 transcriptional inhibitor chlormethiazole (Lytton et al. 1999). Screening of the human sera by enzyme-linked immunosorbent assay reveals reactivity against CYP3A4 and CYP2E1 in about 20–30% and 10–20% of the alcoholic sera, respectively (Lytton et al. 1999). Anti-human CYP2E1 reactivity in 8 of 85 alcoholic sera and 3 of 58 control sera is observed (Lytton et al. 1999). Therefore, alcoholics develop autoantibodies against CYP2E1 (Lytton et al. 1999).

Ethanol Mediated Induction of CYP2E1 Is Correlated with Increased Cytokine Expression in Liver

The zonal differences of cytokine expression in rat liver and how these are affected by alcohol exposure and by CMZ, which is a transcriptional and posttranslational inhibitor of hepatic CYP2E1 has been investigated (Fang et al. 1998). Chronic ethanol treatment significantly increases the expression of CYP2E1, microsomal p-nitrophenol hydroxylase activity (indicative for CYP2E1 enzyme activity), and the expression of TGF-beta1, TNF-alpha, and interleukin (IL)-1beta (Fang et al. 1998). CMZ treatment causes a reduction in hepatic CYP2E1 expression and in the ethanol-induced cytokine expression by 40–60% (Fang et al. 1998). Expression of IL-6, IL-2, and IL-4 mRNA occurs preferentially in the periportal region, whereas ethanol causes a pronounced increase in the perivenous expression of TGF-beta1, which is inhibited by CMZ as monitored both on the mRNA and protein levels (Fang et al. 1998). Thus, a link exists between increased CYP2E1 expression and enhanced cytokine expression as important events in the development of ALD.

Degradation of CYP2E1: Mechanisms Involved

Addition of adrenalin to primary rat hepatocytes causes a threefold increase in [32P]-incorporation into CYP2E1 (Johansson et al. 1991). Adrenalin also increases the rate of CYP2E1 degradation at similar concentrations as needed for phosphorylation of the protein ($r = 0.93$) (Johansson et al. 1991). Ethanol (75 mM) completely protects from adrenalin dependent phosphorylation and degradation of CYP2E1

(Johansson et al. 1991). Examination of para-nitrophenol hydroxylase reveals that ethanol stabilizes the catalytically active form of CYP2E1 (Johansson et al. 1991). Insulin treatment causes a stabilization of CYP2E1 (Johansson et al. 1991). Thus, degradation of CYP2E1 is the subject of hormonal control (Johansson et al. 1991).

The differences in post-translational regulation between rat liver ethanol-inducible CYP2E1 and phenobarbital-inducible CYP2B1 using hepatocyte cultures and subcellular fractions, prepared from starved and acetone-treated rats have been investigated (Eliasson et al. 1992). The intracellular degradation of CYP2E1 is rapid (approximate t1/2 = 9 h) and increased by glucagon treatment of the cells in an isozyme-specific manner, whereas CYP2B1 degradation in the same cells, is slower (t1/2 = 21 h) (Eliasson et al. 1992). The glucagon effect on CYP2E1 degradation is abolished by either cycloheximide treatment of cells, indicating the involvement of protein components with rapid turnover, or by lowering of the culture temperature to 23°C (Eliasson et al. 1992). The rapid phase of CYP2E1 degradation is not influenced by inhibitors of the autophagosomal/lysosomal pathway (Eliasson et al. 1992). *In vitro* experiments with isolated liver microsomes reveal the presence of a Mg(2+)-ATP-activated proteolytic system active on CYP2E1, previously modified by phosphorylation on Ser-129 or denatured by reactive metabolites formed from carbon tetrachloride (Eliasson et al. 1992). Imidazole, a CYP2E1 substrate, specifically inhibits the rapid intracellular degradation of CYP2E1 and also prevents phosphorylation and subsequent proteolysis in isolated microsomes (Eliasson et al. 1992). In contrast, no proteolysis of CYP2B1 occurs under the conditions used (Eliasson et al. 1992). The microsomal Mg(2+)-ATP-dependent CYP2E1 proteolysis could not be solubilized with high salt and 0.05% sodium cholate, indicating the action of membrane-integrated protease(s) (Eliasson et al. 1992). The Mg(2+)-ATP-dependent proteolytic system active on CYP2E1 is present in both rough and smooth endoplasmic reticulum (Eliasson et al. 1992). Thus, hepatic cytochromes P450 may be degraded both in a bulk process, according to the autophagosomal/lysosomal pathway and more rapidly, in a hormone- and substrate-regulated fashion, by a specific proteolytic system in the endoplasmic reticulum, active on physiologically or exogenously modified molecules (Eliasson et al. 1992).

Two serine proteinases capable of digesting CYP2E1 have been purified from sodium cholate solubilized rat liver microsomal membranes (Zhukov et al. 1993). The CYP2E1-degrading activity is illustrated through two peaks, and the two proteinases purified have a M(r) of 32,000 on SDS-PAGE, are optimally active at pH 8, and show a susceptibility to inhibitors typical of serine proteinases (Zhukov et al. 1993). CYP2E1 degradation patterns exhibited by the proteinases are identical to each other and similar to that observed during the proteolysis of endogenous CYP2E1 in the microsomal membranes, which indicates that the proteinases can degrade CYP2E1 in its native environment (Zhukov et al. 1993). Thus, a role of these proteinases in the rapid phase of cytochrome P450 degradation in the endoplasmic reticulum has been suggested (Zhukov et al. 1993).

In the Fao hepatoma cell line, CYP2E1 is found to be fairly stable (half-life of 26 h), but serum withdrawal results in its rapid disappearance from the microsomal fraction (half-life of about 7 h) as evaluated using cycloheximide

chase (Zhukov and Ingelman-Sundberg 1997). The effect of serum withdrawal could be partially reversed by the addition of albumin to the culture medium, whereas insulin and the insulin-like growth factor IGF-I have no additional effect (Zhukov and Ingelman-Sundberg 1997). The effect of serum withdrawal is specific for CYP2E1 since (a) no concomitant fast degradation of CYP2B1 and NADPH-cytochrome P-450 reductase is observed and (b) the CYP2E1 ligands ethanol and imidazole prevent the fast degradation of the enzyme (Zhukov and Ingelman-Sundberg 1997). The lysosomotropic agent ammonium chloride and the inhibitor of autophagocytosis 3-methyladenine slows down CYP2E1 degradation by about 30%, while leupeptin has no effect (Zhukov and Ingelman-Sundberg 1997). Under the same conditions, the degradation of total long-lived cell protein shows the same sensitivity to ammonium chloride, but is significantly less sensitive to 3-methyladenine and serum and not sensitive to ethanol and imidazole (Zhukov and Ingelman-Sundberg 1997). CYP2E1 degradation is inhibited by combined treatment with brefeldin A and nocodazole, which blocks both anterograde and retrograde vesicular transport between endoplasmic reticulum and the Golgi apparatus (Zhukov and Ingelman-Sundberg 1997). Thus, a selective mechanism for the degradation of membrane proteins in serum-deprived cells exists in addition to non selective autophagocytosis (Zhukov and Ingelman-Sundberg 1997). The selective degradation of CYP2E1 may be attained by means of its selective vesicular transport to an acidic post-endoplasmic reticulum compartment (Zhukov and Ingelman-Sundberg 1997).

CYP2E1 Expression in Central Nervous System

CYP2E1 and its mRNA are found to be expressed in the rat hippocampus, where the enzyme is localized mainly to the microsomal fraction (Tindberg and Ingelman-Sundberg 1996). Chlorzoxazone (CZN) is 6-hydroxylated in hippocampal homogenates (Tindberg and Ingelman-Sundberg 1996). CYP2E1 is also expressed *in vitro* in cortical glial cultures where CYP2E1 mRNA levels are found to be 1,000-fold lower than in rat liver (Tindberg and Ingelman-Sundberg 1996). Exposure of cortical glial cultures to 25 or 100 mM ethanol for 24 h causes a fourfold and sixfold increase, respectively, in the rate of CYP2E1-dependent 6-hydroxylation of CZN (Tindberg and Ingelman-Sundberg 1996). After a continuous exposure to 100 mM ethanol for 48 or 72 h, however, the hydroxylation rate is down-regulated (Tindberg and Ingelman-Sundberg 1996). CMZ inhibits the ethanol-dependent induction of CYP2E1 by 50% (Tindberg and Ingelman-Sundberg 1996). *In vivo*, acute ethanol treatment of rats results in a 1.8-fold increase in the rate of CZN 6-hydroxylation in hippocampal homogenates (Tindberg and Ingelman-Sundberg 1996). Thus, CYP2E1 is expressed and catalytically active in the rat CNS, and that CYP2E1 can be induced by a relatively low concentration of ethanol in cortical glial cultures (Tindberg and Ingelman-Sundberg 1996).

Inflammatory Factors Upregulate Brain CYP2E1

Lipopolysaccharide and interleukin-1 beta stimulate the expression of catalytically active CYP2E1 (but not CYP1A1 or CYP2B) up tosevenfold in rat brain primary cortical glial cultures (Tindberg et al. 1996). The induction reaches a maximum after

24 h and is accompanied by an increase in CYP2E1 mRNA (Tindberg et al. 1996). CMZ completely inhibits the induction of CYP2E1 at the mRNA and enzyme levels (Tindberg et al. 1996). Immunofluorescence studies show CYP2E1 to be expressed in a subset of astrocytes in the lipopolysaccharide-stimulated cortical glial cultures (Tindberg et al. 1996). In a model of global ischemic injury in the gerbil, CYP2E1 is induced *in vivo* in astrocytes in the inflammatory phase, 1–3 weeks after the lesion (Tindberg et al. 1996). Likewise, CYP2E1 is induced in the rat cortex 1 week after a focal ischemic injury (Tindberg et al. 1996). Thus, tissue-specific regulation of CYP2E1 by inflammatory factors occurs and that CYP2E1 may play a role in astrocytes during inflammation in the brain (Tindberg et al. 1996).

Alcohol Induced Liver Fibrosis Is Associated with the Induction of CYP2E1
A fatty liver with focal necrosis and fibrosis is observed in rats fed ethanol and a high fat diet by continuous intragastric tube feeding for 72 days, maintaining the blood alcohol levels above 200 mg/dl (French et al. 1993). This pathology is associated with an increased total cytochrome P450, an increased CYP2E1 isoenzyme, a decrease in the NADPH-cytochrome P450 reductase activity, an increased rate of NADPH oxidation and an increased NADPH-dependent lipid peroxidation in liver microsomes compared to controls (French et al. 1993). Serum protein adducts with MDA and 4-HNE are significantly increased. Thus, the alcohol-induced liver pathology is associated with the induction of CYP2EI, lipid peroxidation, and protein adduct formation (French et al. 1993). When isoniazid (INH) in therapeutic doses is fed to rats with ethanol, these parameters are changed in that central-central bridging fibrosis is increased, as is lipid peroxidation, whereas INH reduces the ethanol-induced decrease in the reductase, the increase in total P450 and CYP2EI, as well as the NADPH oxidation rate and the elevation of serum transaminase levels (French et al. 1993). Thus a link may exist between central-central bridging fibrosis with increased lipid peroxidation and aldehyde-protein adduct formation caused by ethanol (French et al. 1993).

VII

S.M. Bailey
Induction of CYP2E1 in a NASH Rodent Model
Feeding a HFD to mice for 16 weeks induces NASH-like pathology accompanied by elevated triacylglycerols, increased CYP2E1 and iNOS protein, and significantly enhances hypoxia in the pericentral region of the liver (Mantena et al. 2009). Chronic exposure to a HFD negatively affects the bioenergetics of liver mitochondria and this probably contributes to hypoxic stress and deleterious NO-dependent modification of mitochondrial proteins (Mantena et al. 2009).

Regulation of CYP2E1 in a Hypercholesterolemic Condition Through Combined Exposure to Alcohol and Environmental Tobacco Smoke
It has been investigated whether combined exposure to alcohol and environmental tobacco smoke (ETS) on a hypercholesterolemic background increases liver injury through oxidative/nitrative stress, hypoxia, and mitochondrial damage (Bailey et al. 2009).

Exposure to ethanol+ETS induces the largest increase in CYP2E1 and iNOS protein, as well as increased 3-nitrotyrosine, mtDNA damage, and decreased cytochrome c oxidase protein in male apoprotein E(−/−) mice compared to mice subjected to an ethanol-containing diet or ETS alone (Bailey et al. 2009). Similarly, the largest increase in HIF1alpha expression is observed in the ethanol+ETS group, indicating enhanced hypoxia (Bailey et al. 2009). Thus, ETS increases alcohol-dependent steatosis and hypoxic stress (Bailey et al. 2009). Therefore, ETS may be a key environmental "hit" that accelerates and exacerbates alcoholic liver disease in hypercholesterolemic apoprotein E(−/−) mice (Bailey et al. 2009).

F.J. Gonzalez

Regulation of CYP2E1 by HNFalpha – A Key Player in the Development of Type 3 Diabetes

HNF1alpha is a liver enriched homeodomain-containing transcription factor that has been shown to transactivate the promoters of several CYP genes, including CYP2E1, CYP1A2, CYP7A1, and CYP27, *in vitro* (Cheung et al. 2003). In humans, mutations in HNF1alpha are linked to the occurrence of maturity onset diabetes of the young type 3, an autosomal dominant form of non-insulin-dependent diabetes mellitus in which afflicted subjects generally develop hyperglycemia before 25 years of age (Cheung et al. 2003). Mice lacking HNF1alpha also develop similar phenotypes reminiscent of non-insulin-dependent diabetes mellitus (Cheung et al. 2003). A marked reduction in expression of CYP2E1 gene is observed in the livers of mice lacking HNF1alpha, suggesting a crucial role for HNF1alpha in the regulation of CYP2E1 *in vivo* (Cheung et al. 2003).

Development of CYP2E1-humanized Mouse and Increased Acetaminophen Mediated Hepatotoxicity

A transgenic mouse line expressing the human CYP2E1 gene has been established (Cheung et al. 2005). Human CYP2E1 protein expression and enzymatic activity have been observed in the liver of CYP2E1-humanized mice (Cheung et al. 2005). Treatment of mice with the CYP2E1 inducer acetone demonstrates that human CYP2E1 is inducible in this transgenic model (Cheung et al. 2005). Hepatotoxicity resulting from the CYP2E1-mediated activation of the CYP2E1 substrate acetaminophen has been observed in the livers of CYP2E1-humanized mice (Cheung et al. 2005). Thus, in this humanized mouse model, human CYP2E1 is functional and can metabolize and activate different CYP2E1 substrates such as chlorzoxazone, p-nitrophenol, acetaminophen, and acetone (Cheung et al. 2005).

Development of CYP2E1 Null Mice and Feedback Regulation of CYP2E1 by Acetaminophen

The resistance of Cyp2e1-null mice to APAP treatment has been studied in wild-type and Cyp2e1-null mice (Chen et al. 2008). However, unexpectedly, profiling of major known APAP metabolites in urine and serum reveals that the contribution of CYP2E1 to APAP metabolism decreases with increasing APAP doses administered (Chen et al. 2008). Measurement of hepatic GSH and hydrogen peroxide levels exposes the importance of oxidative stress in determining the consequence of APAP

overdose (Chen et al. 2008). Urinary ions high in wild-type mice treated with 400 mg/kg APAP have been elucidated as 3-methoxy-APAP glucuronide (VII) and three novel APAP metabolites, including S-(5-acetylamino-2-hydroxyphenyl)mercaptopyruvic acid (VI), 3,3′-biacetaminophen (VIII), and a benzothiazine compound (IX) (Chen et al. 2008). Dose-, time-, and genotype-dependent appearance of these minor APAP metabolites implicate their association with the APAP-induced toxicity and potential biomarker application (Chen et al. 2008). Overall, the oxidative stress elicited by CYP2E1-mediated APAP metabolism might significantly contribute to APAP-induced toxicity (Chen et al. 2008).

Acetaminophen: Both a Substrate and Inducer for CYP2E1
Serum metabolomics of APAP-induced hepatotoxicity in control and APAP-treated wild-type and Cyp2e1-null mice reveals that the CYP2E1-mediated metabolic activation and oxidative stress following APAP treatment can cause irreversible inhibition of fatty acid oxidation, potentially through suppression of PPARalpha-regulated pathways (Chen et al. 2009).

M.P. Waalkes
The Nitric Oxide Donor Pro Drug V-PROLI/NO Protects Against Arsenic Induced Hepatotoxicity as an Inducer and Substrate of CYP2E1
O(2)-Vinyl 1-[2-(Carboxylato)pyrrolidin-1-yl]diazen-1-ium-1,2-diolate (V-PROLI/NO) is a nitric oxide (NO) donor prodrug that is metabolized by liver cytochromes P450 to release NO (Qu et al. 2009). V-PROLI/NO increases CYP2E1 transcriptional expression in a dose-dependent manner and CYP2E1 expression is directly related to the level of NO produced and the reduction in arsenic cytotoxicity in HepG2 cells (Qu et al. 2009). The prodrug, V-PROLI/NO, protects against arsenic toxicity in cultured human liver cells, reducing cytolethality, apoptosis and dysregulation of mitogen-activated protein kinases, through generation of NO formed after metabolism by liver cell enzymes, possibly including CYP2E1 (Qu et al. 2009).

N. Chalasani
CYP2E1 Is Induced and Is Associated with Several Metabolic Disarrangements in Non-diabetic Patients with NASH
The hepatic CYP2E1 activity and its correlates in a cohort of nondiabetic patients with NASH (NDN) and controls have been determined to explore its role in the pathogenesis of human NASH (Chalasani et al. 2003). Hepatic CYP2E1 activity assessed through the oral clearance (CL(PO)) of chlorzoxazone (CHZ) in 20 NDN and 17 age, gender, and body mass index (BMI)-matched controls reveals that the CL(PO) of CHZ is significantly greater in NDN compared with controls (Chalasani et al. 2003). Lymphocyte CYP2E1 mRNA is significantly higher in NDN compared with controls (Chalasani et al. 2003). The BMI, respiratory quotient, high-density lipoprotein, triglycerides, insulin, insulin resistance, hypoxemia, and beta-OH butyrate significantly correlate with hepatic CYP2E1 activity (Chalasani et al. 2003). In conclusion, hepatic CYP2E1 activity and lymphocyte CYP2E1 expression are enhanced in NDN (Chalasani et al. 2003). The significant correlations noted between CYP2E1 and hypoxemia and beta-OH butyrate suggest that these factors

play a role in increased CYP2E1 activity that is seen in patients with NASH (Chalasani et al. 2003).

C.J. Omiecinski

Region-Specific CYP2E1 Expression in Brain

The regional and cellular expression patterns of CYP2E1 in human brain tissue have been identified (Farin and Omiecinski 1993). The mRNA expression pattern of CYP2E1 although detected in all brain regions examined, the red nucleus (RN), and substantia nigra (SN) exhibit lower levels of CYP2E1 mRNA expression compared to other regions (Farin and Omiecinski 1993). Thus, localized biotransformation events within the certain central nervous system may account for toxicities initiated by exposure to certain environmental chemicals (Farin and Omiecinski 1993).

Presence of CYP2E1 in Human Umbilical Vein Endothelial Cells

In view of the potential role of CYP2E1 – a biotransformation enzyme in the metabolism of protoxicants in the circulatory system, the CYP2E1 expression has been determined in several primary cultures of human umbilical vein endothelial cells (HUVEC), each established from a different individual (Farin et al. 1994). Constitutive level of CYP2E1 gene expression in HUVEC cultures is evident (Farin et al. 1994). Constitutive CYP2E1 transcript levels are present in all HUVEC cultures examined and vary among individuals (Farin et al. 1994). Thus, human endothelial cells express CYP2E1 gene products and CYP2E1 may play important roles in determining metabolic fates for circulating protoxicants (Farin et al. 1994).

Presence of CYP2E1 in HPV-Expressing Oral and Cervical Epithelial Cells and Its Inducibility by Polycyclic Aromatic Hydrocarbons: *In Vitro* Indicators for Exposure to Tobacco Smoke

Established primary and human papillomaviruses (HPV)-immortalized oral and cervical epithelial cell lines have been analyzed for morphology, mRNA and protein expression patterns of CYP2E1 to study the oral and cervical epithelial-specific expression of CYP2E1 (Farin et al. 1995). Primary human oral and cervical epithelial cells have been immortalized using retroviral infection with HPV-16 E6/E7 genes (Farin et al. 1995). Primary human keratinocyte cells have been immortalized by transfection of HPV-18 and made tumorigenic with nitrosomethylurea treatment (Farin et al. 1995). Expression profile for CYP2E1 has been evaluated in these cultures in the presence or absence of a polycyclic aromatic hydrocarbons (PAH) inducer. CYP2E1 mRNA expression is greatest in the oral epithelial cultures and detectable in all other epithelial cultures except for the HPV-18 immortalized keratinocyte cell line (Farin et al. 1995). CYP2E1 protein is detectable in primary and HPV-immortalized oral and cervical epithelial cultures (Farin et al. 1995). Thus, both primary and HPV immortalized cells appear to express CYP2E1 necessary for the activation of tobacco-specific nitrosamines and PAHs and an *in vitro* system has been characterized which should prove useful in examining interactive mechanisms of HPV with xenobiotic activation in the etiology of squamous cell carcinomas (Farin et al. 1995).

Development of a Quantitative Competitive Reverse Transcriptase-Polymerase Chain Reaction Assay to Determine Hepatic CYP2E1 mRNA

A quantitative competitive RT-PCR (QC RT-PCR) assay has been developed to measure mRNA levels of seven human CYP genes and microsomal epoxide hydrolase (EH) simultaneously (Andersen et al. 1998). This assay employs an exogenous recombinant RNA (rcRNA) molecule as an internal standard that shares PCR primer and hybridization probe sequences with CYP1A1, CYP1A2, CYP2A6/7, CYP2D6, CYP2E1, CYP2F1, CYP3A4/5/7, and EH mRNA (Andersen et al. 1998). Because each rcRNA molecule contains several primer sequences, an entire battery of genes that exhibit differential responsiveness to various classes of xenobiotics may be measured simultaneously from one population of cDNA molecules (Andersen et al. 1998). The quantitative profiles of CYP and EH mRNA abundance in eight human livers has been demonstrated. CYP2E1 mRNA maintains the highest abundance and least variation in all livers examined (Andersen et al. 1998). CYP1A2, CYP2A6/7, CYP2D6, CYP3A4/5, and EH mRNAs are approximately one order of magnitude less abundant than CYP2E1 transcripts, with CYP2D6 levels exhibiting the greatest variation between individuals (Andersen et al. 1998). This QC RT-PCR assay may prove valuable for measuring basal and induced mRNAs in different cell types in vitro, as well as in biomonitoring applications where individuals are exposed or hypersusceptible to certain xenobiotic-initiated toxicities (Andersen et al. 1998).

Phosphatidylinositol 3′ Kinase and Hepatic CYP2E1

Insulin-associated signaling pathways are crucial in the regulation of hepatic physiology (Sidhu et al. 2001). Recent inhibitor-based studies have implicated a mechanistic role for phosphatidylinositol 3′ kinase (PI3K) in the insulin-mediated suppression of CYP2E1 mRNA levels in hepatocytes (Sidhu et al. 2001). The dose dependence for this response and for the effects of insulin and extracellular matrix on PI3K signaling and CYP2E1 mRNA expression levels has been determined in a highly defined rat primary hepatocyte culture system (Sidhu et al. 2001). Although PI3K inhibitors wortmannin and LY294002 reverse the suppressive effects of insulin on CYP2E1 expression, these effects only occur at concentrations well in excess of those required to achieve complete inhibition of protein kinase B (PKB) phosphorylation (Sidhu et al. 2001). These same concentrations produce cytotoxic responses as evidenced by perturbed cellular morphology and elevated release of lactate dehydrogenase (Sidhu et al. 2001). Thus, the suppression of CYP2E1 mRNA expression by insulin is not directly associated with PI3K-dependent pathway activation, but rather is linked to a cytotoxic response stemming from acute challenge with PI3K inhibitors (Sidhu et al. 2001).

D.J. Petersen

Ethanol Mediated Aldehyde Protein Adduct Formation Is Associated with Induction of CYP2E1 in Liver

The hepatocellular formation of aldehyde-protein adducts during early stages of alcohol-induced liver injury has been characterized (Sampey et al. 2003). After 36 days of treatment, rats receiving the intragastric administration of a total enteral nutrition diet containing ethanol display hepatic histopathologies characterized by marked micro- and

macrosteatosis associated with only minor inflammation and necrosis (Sampey et al. 2003). Alcohol administration results in a threefold elevation of plasma alanine aminotransferase activity and threefold increases in hepatic CYP2E1 apoprotein and activity (Sampey et al. 2003). Significant fivefold increases in MDA- and 4-HNE modified proteins are detected in liver sections prepared from rats treated with alcohol (Sampey et al. 2003). The MDA- or 4-HNE modified proteins are contained in hepatocytes displaying intact morphology and are colocalized primarily with microvesicular deposits of lipid (Sampey et al. 2003). Alcohol-induced lipid peroxidation is an early event during alcohol-mediated liver injury and may be a sensitizing event resulting in the production of bioactive aldehydes that have the potential to initiate or propagate ensuing proinflammatory or profibrogenic cellular events (Sampey et al. 2003).

Induction of Hepatic CYP2E1 by Ethanol Is Associated with Decreased Peroxiredoxin 6 Expression

Periredoxins constitute a family of antioxidant enzymes. The effect of chronic ethanol treatment on inducing hepatic oxidative stress and peroxiredoxin 6 (PRX6) expression has been investigated (Roede et al. 2008). After 9 weeks of treatment with an ethanol-containing diet, significant increases in serum ALT activity, liver to body weight ratio, liver triglycerides, CYP2E1 protein expression, and CYP2E1 activity are observed (Roede et al. 2008). Chronic ethanol feeding results in oxidative stress as evidenced by decreases in hepatic GSH content and increased deposition of 4-hydroxynonenal and 4-oxononenal protein adducts (Roede et al. 2008). In addition, novel findings of decreased PRX6 protein and mRNA and increased levels of carbonylated PRX6 protein are observed in the ethanol-treated animals compared to the pair-fed controls (Roede et al. 2008). Lastly, NF-kappaB activity is found to be significantly increased in the ethanol-treated animals (Roede et al. 2008). Chronic ethanol treatment results in oxidative stress, implicating NF-kappaB activation as an integral mechanism in the negative regulation of PRX6 gene expression in the mouse liver (Roede et al. 2008). Thus, ethanol mediated hepatic CYP2E1 induction may be accompanied by downregulation of PRX6 gene expression.

M.R. Juchau

Presence of CYP2E1 in Prenatal Human Cephalic Tissues

RT-PCR with oligonucleotide primers designed to target cDNA nucleotides 1,241–1,357 corresponding to exons 8 (3′ end) and 9 (5′ end) in human genomic CYP2E1 detect consistently strong signals in 9 of 10 prenatal human brains (Boutelet-Bochan et al. 1997). Cephalic tissues analyzed are between 54 and 78 days of gestation (Boutelet-Bochan et al. 1997). RT-PCR signals for expression of CYP2E1 in corresponding human hepatic or adrenal tissues are weaker or, with only two exceptions, undetectable (Boutelet-Bochan et al. 1997). CYP2E1 mRNA levels in human prenatal whole brain tissues tend to increase as a function of gestational age but, at the early stages investigated, are far lower than the constitutive levels in hepatic tissues of adult humans or male rats (Boutelet-Bochan et al. 1997). Localized CYP2E1-dependent cephalic bioactivation of ethanol, with associated generation of several reactive chemical species, could contribute significantly to the etiology of neuroembryotoxic effects of prenatal ethanol exposure (Boutelet-Bochan et al. 1997).

Inhibition of Human Prenatal Hepatic All-Trans-Retinoic Acid Formation by Ethanol Mediated Lipid Peroxidation Products Could Be Possibly Linked to Increased Generation of Lipid Peroxides by Ethanol-Inducible CYP2E1

Biotransformation of all-trans-retinol (t-ROH) and all-trans-retinal (t-RAL) to all-trans-retinoic acid (t-RA) in human prenatal hepatic tissues (53–84 gestational days) has been investigated using human adult hepatic tissues as positive controls (Khalighi et al. 1999). Catalysis of the biotransformation of t-ROH by prenatal human cytosolic fractions results in accumulation of t-RAL with minimal t-RA (Khalighi et al. 1999). Oxidations of t-ROH catalyzed by prenatal cytosol are supported by both NAD+ and NADP+, although NAD+ is a much better cofactor (Khalighi et al. 1999). In contrast, catalysis of the oxidation of t-RAL to t-RA appears to be solely NAD+ dependent (Khalighi et al. 1999). Substrate Km values for conversions of t-ROH to t-RAL and of t-RAL to t-RA are 82.4 and 65.8 μM, respectively (Khalighi et al. 1999). At concentrations of 10 and 90 mM, ethanol inhibits the conversion of t-ROH to t-RAL by 25 and 43%, respectively, but does not inhibit the conversion of t-RAL to t-RA significantly (Khalighi et al. 1999). In contrast, acetaldehyde reduces the conversion of t-RAL to t-RA by 25 and 87% at 0.1 and 10 mM respective concentrations (Khalighi et al. 1999). Several alcohols and aldehydes known to be generated from lipid peroxides also exhibit significant inhibition of t-RA biosynthesis in human prenatal hepatic tissues (Khalighi et al. 1999). Among the compounds tested, 4-HNE is highly effective in inhibiting the conversion of t-RAL to t-RA (Khalighi et al. 1999). A 20% inhibition is observed at a concentration of only 0.001 mM, and nearly complete inhibition is produced at 0.1 mM (Khalighi et al. 1999). Human fetal and embryonic hepatic tissues each exhibit significant CYP2E1 protein and mRNA expression and chlorzoxazone 6-hydroxylation suggesting that lipid peroxidation can be initiated via CYP2E1-catalyzed ethanol oxidation in human embryonic hepatic tissues (Khalighi et al. 1999). Thus, ethanol may affect the biosynthesis of t-RA in human prenatal hepatic tissues directly and indirectly (Khalighi et al. 1999). Ethanol and its major oxidative metabolite, acetaldehyde, both inhibit the generation of t-RA (Khalighi et al. 1999). Concurrently, the CYP2E1-catalyzed oxidation of ethanol can initiate lipid peroxidation via generation of a variety of free radicals (Khalighi et al. 1999). The lipid peroxides thereby generated could then be further converted *via* CYP2E1-catalyzed reactions to alcohols and aldehydes, including 4-HNE, that act as potent inhibitors of t-RA synthesis (Khalighi et al. 1999).

Quantitative Assessment of CYP2E1 in Human Cephalic Tissues

The proposed role of CYP2E1 in the etiology of alcohol teratogenesis ie. its significant contribution to ethanol metabolism and the formation of the highly reactive metabolite acetaldehyde and the leaky property of this enzyme which results in the generation of ROS that can induce oxidative stress and cytotoxic conditions deleterious to development has been investigated (Brzezinski et al. 1999). CYP2E1 has been quantified in prenatal human brain, a tissue that is highly vulnerable to the damaging effects of ethanol throughout gestation (Brzezinski et al. 1999). In microsomal samples prepared from pools of brain tissues, both immunoreactive and functional proteins have been detected (Brzezinski et al. 1999). CYP2E1 transcript

is consistently detected in RNA samples prepared from individual brain tissues (Brzezinski et al. 1999). There is a dramatic increase in human brain CYP2E1 content around gestational day 50 and a fairly constant level is maintained throughout the early fetal period, until at least day 113 (Brzezinski et al. 1999). The relatively low levels of the CYP isoform present in conceptal brain may be sufficient to generate reactive intermediates that elicit neuroembryotoxicity following maternal alcohol consumption (Brzezinski et al. 1999).

R.G. Thurman
Formation of Aldehyde Protein Adducts Is Associated with Induction of Hepatic CYP2E1
Expression of both CYP2E1 and CYP3A correlates with the amount of acetaldehyde, MDA, and 4-HNE adducts in the liver of rats fed alcohol with a high-fat diet for 2–4 weeks according to the Tsukamoto-French procedure (Niemelä et al. 1998). Distinct CYP2E1-positive immunohistochemistry is seen in 3 of 7 of the ethanol-fed animals. In 5 of 7 of the ethanol-fed animals, the staining intensities for CYP3A markedly exceed those obtained from the controls (Niemelä et al. 1998). Thus, acetaldehyde and lipid peroxidation-derived adducts are generated in the early phase of alcohol-induced liver disease (Niemelä et al. 1998). The formation of protein adducts appears to be accompanied by induction of both CYP2E1 and CYP3A (Niemelä et al. 1998).

M. Murray
Differential Regulation of CYP2E1 in Obesity and Diabetes
In obese male Zucker rats, a significant decrease in the catalytic activities of hepatic NDMA demethylase (CYP2E1/P-450j) and aniline p-hydroxylase occurs (Zaluzny et al. 1990). In streptozotocin-induced diabetic male Wistar rats, significant increases in the rates of hepatic N-nitrosodimethylamine demethylase and aniline p-hydroxylase occur (Zaluzny et al. 1990). Further, significant correlation is found between serum concentrations of insulin and catalytic activity of P-450j (Zaluzny et al. 1990).

L.H. Lash
Renal CYP2E1 Expression
CYP2E1 protein is expressed in rat kidney microsomes at approximately 10% of hepatic levels (Cummings et al. 1999). Microsomes from renal cortical, proximal tubular (PT) and distal tubular (DT) cells all express CYP2E1, with DT microsomes expressing slightly higher levels than PT microsomes (Cummings et al. 1999). In contrast, chlorzoxazone hydroxylation activity is markedly higher in microsomes from PT cells than in those from DT cells (Cummings et al. 1999). A pattern of CYP2E1 mRNA distribution similar to that of CYP2E1 protein is observed in PT and DT cells (Cummings et al. 1999).

Renal and Hepatic CYP Mediated Metabolism of Trichloroethylene
Pretreatment of rats with pyridine increases trichloroethylene (Tri) metabolite chloral hydrate (CH) formation in both liver and kidney microsomes, whereas pretreatment of rats with clofibrate increases CH formation only in kidney microsomes (Cummings et al. 2001). Pyridine increases CYP2E1 expression in both rat liver and

kidney microsomes, whereas clofibrate has no effect on hepatic but increases renal CYP2E1 protein level (Cummings et al. 2001). Studies with the general P450 inhibitor SKF-525A and the CYP2E1 competitive substrate chlorzoxazone provide additional support for the role of CYP2E1 in both tissues (Cummings et al. 2001). However, pretreatment of rats with either pyridine or clofibrate has no effect on CYP2E1 or CYP2C11 protein levels or on CH formation in isolated cells (Cummings et al. 2001). Thus, Tri can be metabolized to at least one of its CYP metabolites in the kidneys (Cummings et al. 2001).

CYP2E1 Mediated Tri- and Perchloroethylene Metabolism

Modulation of P450 status in hepatocytes produces larger changes in Tri- and perchloroethylene (Perc)-induced cytotoxicity than in kidney cells, with non-selective P450 inhibitors increasing toxicity (Lash et al. 2007). Induction of CYP2E1 with pyridine also markedly increases sensitivity of hepatocytes to Tri but has little effect on Perc-induced cytotoxicity (Lash et al. 2007).

I. Rusyn

Crucial Role of Hepatic CYP2E1 in Oxidative DNA Damage

In rats and wild-type mice, Tsukamoto-French model of intragastric ethanol infusion treatment for 4 weeks leads to an increase in oxidative DNA damage and induction of expression of the base excision DNA repair genes that are known to remove oxidative DNA lesions (Bradford et al. 2005). No increase in either of the endpoints is observed in ethanol-treated Cyp2e1-null mice, whereas the magnitude of response in p47(phox)-null mice and transgenic hCyp2e1 is identical to that in wild types (Bradford et al. 2005). The increase in expression of DNA repair genes is completely abolished by treatment with the P450 inhibitor 1-aminobenzotriazole (Bradford et al. 2005). In conclusion, oxidative stress to DNA is induced in liver by ethanol (Bradford et al. 2005). Furthermore, although it has been shown that nicotinamide adenine dinucleotide phosphate oxidase-derived oxidants are crucial for the development of ethanol-induced liver injury, CYP2E1 is required for the induction of oxidative stress to DNA, and thus may play a key role in ethanol-associated hepatocarcinogenesis (Bradford et al. 2005).

M.A. Correia

Ubiquitination of CYP2E1 – Mechanisms Involved

CYP2E1 substrate complexation converts it into a stable slow-turnover species degraded largely *via* autophagic lysosomal degradation (Wang et al. 2011). Substrate decomplexation/withdrawal results in a fast turnover CYP2E1 species, putatively generated through its futile oxidative cycling, that incurs endoplasmic reticulum-associated ubiquitin-dependent proteasomal degradation (UPD). CYP2E1 thus exhibits biphasic turnover in the mammalian liver (Wang et al. 2011). The heterologous expression of human CYP2E1 in *Saccharomyces cerevisiae* shows that its autophagic lysosomal degradation and UPD pathways are evolutionarily conserved, even though its potential for futile catalytic cycling is low due to its sluggish catalytic activity in yeast (Wang et al. 2011). This suggests that other factors (i.e. post-translational modifications or "degrons") contribute to its UPD (Wang et al. 2011).

Indeed, in cultured human hepatocytes, CYP2E1 is detectably ubiquitinated, and this is enhanced on its mechanism-based inactivation (Wang et al. 2011). Studies in Ubc7p and Ubc5p genetically deficient yeast strains versus corresponding isogenic wild types identify these ubiquitin-conjugating E2 enzymes as relevant to CYP2E1 UPD (Wang et al. 2011). Consistent with this, *in vitro* functional reconstitution analyses reveal that mammalian UBC7/gp78 and UbcH5a/CHIP E2-E3 ubiquitin ligases are capable of ubiquitinating CYP2E1, a process enhanced by protein kinase (PK) A and/or PKC inclusion (Wang et al. 2011). Inhibition of PKA or PKC blocks intracellular CYP2E1 ubiquitination and turnover (Wang et al. 2011). Through mass spectrometric analyses, some CYP2E1 phosphorylation/ubiquitination sites in spatially associated clusters have been identified (Wang et al. 2011). Thus, these CYP2E1 phosphorylation clusters may serve to engage each E2-E3 ubiquitination complex *in vitro* and intracellularly (Wang et al. 2011).

P. Saenger

Type 1 Diabetes Mediated Regulation of Lymphocyte CYP2E1

CYP2E1 is imeasured in peripheral lymphocytes of 14 patients with uncontrolled insulin-dependent diabetes mellitus (Song et al. 1990). Only one major form (mol wt, 48,000 Da) of CYP2E1 is detected with a specific polyclonal antibody against CYP2E1 (Song et al. 1990). Levels of CYP2E1 are very low to undetectable in human lymphocytes from seven normal subjects (Song et al. 1990). However, levels of CYP2E1 are elevated in lymphocytes from patients with insulin-dependent diabetes mellitus (Song et al. 1990). Elevated levels of CYP2E1 protein correlate positively with the levels of hemoglobin A1, a metabolic indicator in diabetic subjects (Song et al. 1990). In one study subject in whom diabetic control is improved, the drop in hemoglobin A1C levels is accompanied by normalization of CYP2E1 levels (Song et al. 1990).

M.J. Czaja

TNF Alpha and CYP2E1 Mediated Hepatotoxicity: Close Partners

TNF-alpha-treatment of rat hepatocyte cell line RALA255–10G transfected with pCI-neo expression vectors (containing the human CYP2E1 cDNA in either a sense or antisense orientation resulting in differential CYP2E1 expression) demonstrate that overexpression of CYP2E1 converts the hepatocyte TNF-alpha response from proliferation to apoptotic and necrotic cell death (Liu et al. 2002). Death occurs despite the presence of increased levels of NF-kappaB transcriptional activity and is associated with increased lipid peroxidation and GSH depletion (Liu et al. 2002). CYP2E1-overexpressing hepatocytes have increased basal and TNF-alpha-induced levels of c-Jun NH(2)-terminal kinase (JNK) activity, as well as prolonged JNK activation after TNF-alpha stimulation (Liu et al. 2002). Sensitization to TNF-alpha-induced cell death by CYP2E1 overexpression is inhibited by antioxidants or adenoviral expression of a dominant-negative c-Jun. Increased CYP2E1 expression sensitizes hepatocytes to TNF-alpha toxicity mediated by c-Jun and overwhelming oxidative stress (Liu et al. 2002). Thus, the chronic increase in intracellular oxidant stress created by CYP2E1 overexpression may serve as a mechanism by which hepatocytes are sensitized to TNF-alpha toxicity in liver disease (Liu et al. 2002).

Hepatic CYP2E1 and Oxidative Stress Mediated Injury: *In Vitro* Evidence

To determine the effect of CYP2E1 expression on the hepatocellular response to injury, stably transfected hepatocytes expressing increased (S-CYP15) and decreased (AN-CYP10) levels of CYP2E1 are generated from the rat hepatocyte line RALA255-10G (Jones et al. 2002). S-CYP15 cells have increased levels of CYP2E1 protein and mRNA, catalytic activity, and increased cell sensitivity to death from acetaminophen (Jones et al. 2002). Death in S-CYP15 cells is significantly decreased relative to that in AN-CYP10 cells following treatment with hydrogen peroxide and the superoxide generator menadione (Jones et al. 2002). S-CYP15 cells undergo apoptosis in response to these ROS, whereas AN-CYP10 cells die by necrosis (Jones et al. 2002). This differential sensitivity to ROS-induced cell death is partly explained by markedly decreased levels of GSH in AN-CYP10 cells (Jones et al. 2002). However, chemically induced GSH depletion triggers cell death in S-CYP15 but not AN-CYP10 cells (Jones et al. 2002). Increased expression of CYP2E1 confers hepatocyte resistance to ROS-induced cytotoxicity, which was mediated in part by GSH (Jones et al. 2002). However, CYP2E1 over-expression leaves cells vulnerable to death from GSH depletion (Jones et al. 2002).

Induced Hepatic CYP2E1 and ERK Activation

Chronic CYP2E1 overexpression leads to sustained extracellular signal-regulated kinase 1/2 (ERK1/2) activation mediated by epidermal growth factor receptor (EGFR)/c-Raf signaling in CYP2E1 over-expressing hepatocytes (Schattenberg et al. 2004). This adaptive response in hepatocytes exposed to chronic oxidative stress confers differential effects on cellular survival, protecting against menadione-induced apoptosis, but sensitizing to necrotic death from PUFA (Schattenberg et al. 2004).

Induction of Hepatic CYP2E1 and Downregulation of Insulin Signaling

The effects of *in vitro* and *in vivo* CYP2E1 overexpression on hepatocyte insulin signaling have been examined (Schattenberg et al. 2005). CYP2E1 overexpression in a hepatocyte cell line decreases tyrosine phosphorylation of insulin receptor substrate IRS-1 and IRS-2 in response to insulin (Schattenberg et al. 2005). CYP2E1 overexpression is also associated with increased inhibitory serine 307 and 636/639 IRS-1 phosphorylation (Schattenberg et al. 2005). In parallel, the effects of insulin on Akt activation, glycogen synthase kinase 3, and FoxO1a phosphorylation, and glucose secretion are all significantly decreased in CYP2E1 overexpressing cells (Schattenberg et al. 2005). This inhibition of insulin signaling by CYP2E1 overexpression is partially c-Jun N-terminal kinase dependent (Schattenberg et al. 2005). In the methionine- and choline-deficient diet mouse model of steatohepatitis with CYP2E1 overexpression, insulin-induced IRS-1, IRS-2, and Akt phosphorylation are similarly decreased (Schattenberg et al. 2005). Thus increased hepatocyte CYP2E1 expression and the presence of steatohepatitis result in the down-regulation of insulin signaling, potentially contributing to the insulin resistance associated with NAFLD (Schattenberg et al. 2005).

Hydroxynonenal Mediated Hepatic Toxicity and CYP2E1

The effect of HNE on hepatocyte injury and JNK activation has been examined in cells under chronic oxidant stress from overexpression of the prooxidant enzyme

CYP2E1, which occurs in NAFLD (Singh et al. 2009). CYP2E1-generated oxidant stress sensitizes a rat hepatocyte cell line to death from normally nontoxic concentrations of HNE (Singh et al. 2009). CYP2E1-overexpressing cells undergo a more profound depletion of GSH in response to HNE secondary to decreased gamma-glutamylcysteine synthetase activity (Singh et al. 2009). GSH depletion leads to overactivation of JNK/c-Jun signaling at the level of mitogen-activated protein kinase kinase 4 that induces cell death (Singh et al. 2009). Oxidant stress and the lipid peroxidation product HNE cause synergistic overactivation of the JNK/c-Jun signaling pathway in hepatocytes, demonstrating that HNE may not be just a passive biomarker of hepatic oxidant stress but rather an active mediator of hepatocellular injury through effects on JNK signaling (Singh et al. 2009).

H.M. Mehendale

Thioacetamide Mediated Hepatotoxicity and Diabetes: Crucial Role of CYP2E1

Thioacetamide (TA)-induced hepatotoxicity is potentiated in STZ-induced diabetic rats (Wang et al. 2000). Hepatic CYP2E1 appears to be primarily involved in bioactivation of TA (Wang et al. 2000). In the STZ-induced diabetic rat, diabetes-induced CYP2E1 appears to be responsible for the potentiated liver injury; even though hepatic flavin-containing monooxygenase (FMO1) is induced in the diabetic rat, it is unlikely to mediate the potentiated TA hepatotoxicity (Wang et al. 2000).

Thioacetamide Mediated Hepatotoxicity and Diet Restriction: Crucial Role of CYP2E1

A 4.6-fold increase in CYP2E1 protein, which corresponds with a threefold increase in CYP2E1 activity as measured by chlorzoxazone hydroxylation is observed in rats maintained on dietrestriction (DR, 35% of ad libitum fed rats, 21 days) (Ramaiah et al. 2001). A single administration of 50 mg of TA/kg is given to rats 24 and 18 h after pretreatment with pyridine (PYR) and isoniazid (INZ), specific inducers of CYP2E1 (Ramaiah et al. 2001). TA liver injury is >2.5- and >3-fold higher at 24 h in PYR + TA and INZ + TA groups, respectively, compared with the rats receiving TA alone (Ramaiah et al. 2001). Pyridine pretreatment results in significantly increased total CYP450 content accompanied by a 2.2-fold increase in CYP2E1 protein and twofold increase in enzyme activity concordant with increased liver injury of TA, suggesting mechanism-based bioactivation of TA by CYP2E1 (Ramaiah et al. 2001). Hepatic injury of TA in DR rats pretreated with DAS is significantly decreased (60%) at 24 h. CCl(4) (4 ml/kg i.p.), a known substrate of CYP2E1, causes lower liver injury and higher animal survival confirming inhibition of CYP2E1 by DAS pretreatment (Ramaiah et al. 2001). Thus, induction of CYP2E1 may account as the primary mechanism of increased bioactivation-based liver injury of TA in DR rats (Ramaiah et al. 2001).

Thioacetamide Bioactivation and CYP2E1: Conclusive Evidences

The role of CYP2E1 using cyp2e1 knockout mice (KO) in TA mediated liver injury has also been investigated (Chilakapati et al. 2007). Injury assessed over time (0–48 h) in wild type (WT) and KO mice after LD(100) dose (500 mg/kg) in WT

reveals that while WT mice exhibit robust injury which progress to death, KO mice exhibit neither initiation nor progression of injury (Chilakapati et al. 2007). Thus, CYP2E1 is responsible for TA bioactivation (Chilakapati et al. 2007).

VIII

P.K. Seth

Lymphocyte CYP2E1 Mediated Lipid Peroxidation

Rat blood lymphocytes catalyse NADPH dependent (basal) lipid peroxidation and demethylation of NDMA (Dey et al. 2002). Treatment of rats with ethanol or pyrazole or acetone results in significant increase in the NADPH dependent lipid peroxidation and the activity of NDMA-d in blood lymphocytes (Dey et al. 2002). *In vitro* addition of CCl(4) to the blood lymphocytes isolated from control or ethanol pretreated rats results in an increase in the NADPH dependent lipid peroxidation (Dey et al. 2002). Significant inhibition of the basal and CCl(4) supported NADPH dependent lipid peroxidation and NDMA-d activity in blood lymphocytes isolated from control or ethanol pretreated rats by dimethyl formamide or dimethyl sulfoxide or hexane, solvents known to inhibit CYP2E1 catalysed reactions in liver and anti-P450 2E1, indicate the role of CYP2E1 in the NADPH dependent lipid peroxidation in rat blood lymphocytes (Dey et al. 2002). Similarities in the NADPH dependent lipid peroxidation and NDMA-d activity in blood lymphocyte with the liver microsomes suggest that blood lymphocyte CYP 2E1 could be used as a surrogate to monitor and predict hepatic levels of the enzyme (Dey et al. 2002).

D. Parmar/P.K. Seth

Lymphocyte CYP2E1: A Suitable Marker for the Hepatic Enzyme

Freshly isolated rat blood lymphocytes are characterized by the presence of significant mRNA of CYP2E1 in control rats (Dey et al. 2005). Lymphocyte CYP2E1 demonstrates significant immunoreactivity, comigrating with the liver isoenzyme, in freshly isolated control rat blood lymphocytes (Dey et al. 2005). Similar to that observed in liver, ethanol treated rat blood lymphocytes exhibit increased CYP2E1 isoenzyme (Dey et al. 2005). Blood lymphocytes are also found to catalyze the CYP2E1 dependent N-demethylation of NDMA which increases with known CYP2E1 inducers (Dey et al. 2005). Significant increase in the apparent Vmax and the affinity towards the substrate in rat blood lymphocytes occurs indicating that as observed in liver, the increase in mRNA and protein expression following exposure to CYP2E1 inducers is associated with the increased catalytic activity of CYP2E1 in freshly isolated rat blood lymphocytes (Dey et al. 2005). Thus, similarities of the blood lymphocyte CYP2E1 with the liver enzyme suggest that lymphocyte CYP2E1 levels in freshly isolated rat blood lymphocytes could be used to monitor tissue enzyme levels (Dey et al. 2005).

D. Parmar

Brain CYP2E1 Expression: Region Specific Phenomenon

Rat olfactory lobes exhibit the highest CYP2E1 expression and catalytic activity in control rats (Yadav et al. 2006). Furthermore, several fold increase in the mRNA expression and activity of CYP2E1 in cerebellum and hippocampus while

a relatively small increase in the olfactory lobes and no significant change in other brain regions following ethanol pretreatment indicate that CYP2E1 induction may be involved in selective sensitivity of these brain areas to ethanol induced free radical damage and neuronal degeneration (Yadav et al. 2006).

***In Vitro* Evidence for Brain CYP2E1**

The expression and catalytic activity of the constitutive and inducible forms of CYP2E1 in cultured rat brain neuronal and glial cells has been characterized (Kapoor et al. 2006). These cells exhibit relatively twofold higher activity of NDMA-d when compared with the liver enzyme (Kapoor et al. 2006). Pretreatment with ethanol reveals a significant time and concentration dependent induction in NDMA-d activity in both cell types (Kapoor et al. 2006). Significant induction of CYP2E1 protein and mRNA occurs in the cultured brain cells (Kapoor et al. 2006). Interestingly, the neuronal cells exhibit greater magnitude of induction than the glial cells (Kapoor et al. 2006). The relatively higher degree of induction in cultures of neurons indicates enhanced sensitivity of neurons to the inductive effects of ethanol (Kapoor et al. 2006). This enhanced induction of CYP2E1 in neuronal cells indicates that like regional specificity, cell specificity also exists in the induction of CYP2E1 (Kapoor et al. 2006).

Induction of Fetal Brain CYP2E1 with the Pesticide Lindane

Low dose prenatal exposure to the pesticide lindane has the potential to produce overexpression of xenobiotic metabolizing CYP1A, 2B and 2E1 isoenzymes in brain and liver of the rat offsprings which may account for the behavioral changes observed in the rat offsprings (Johri et al. 2007).

Parkinson's Disease Is Associated with CYP2E1*5B Allele

The presence of four combinations of glutathione S-transferase T1 (GSTT1) null and manganese-superoxide dismutase MnSOD(−9Val)/GST null and monoamine oxidase-B(MAO-B) variant allele G (MAOB-G)/CYP2E1*5B (RsaI) and MAO-B-AG/CYP2E1*5B and dopamine receptor-D2 (DRD2) (Taq1A-het) genotypes in the patients exhibit several fold higher and significant association with risk to Parkinson's disease (PD) (Singh et al. 2008). Polymorphism in the genes involved in detoxification and dopamine regulation may modulate the susceptibility to PD and could be important risk factors in the pathogenesis of PD (Singh et al. 2008).

Head and Neck Squamous Cell Carcinoma and CYP2E1 Polymorphism

A significant increase in Head and Neck Squamous Cell Carcinoma (HNSCC) risk occurs in cases with variant genotypes of CYP2E1*5B (RsaI) and CYP2E1*6 (DraI) (Ruwali et al. 2009). Haplotype T-A is associated with a greater than tenfold increase in risk for HNSCC (Ruwali et al. 2009). A several fold increase in HNSCC risk in cases carrying a combination of variant genotypes of CYP2E1 with the null genotype of glutathione-S-Transferase M1 (GSTM1) or X-Ray Repair Cross Complementing Group I (XRCC1) variant genotypes occurs (Ruwali et al. 2009). Alcohol or tobacco use (both smoking and chewing) are also found to interact with variant genotypes of CYP2E1 in significantly enhancing HNSCC risk (Ruwali et al. 2009). This increase in risk associated with an interaction of CYP2E1

genotypes with GSTM1 or XRCC1 or with tobacco and alcohol use demonstrates the importance of gene-gene and gene-environment interactions in the development of HNSCC (Ruwali et al. 2009).

Alcoholic Cirrhosis and CYP2E1 Polymorphism

The variant genotypes of CYP2E1 *5B exhibit significant association with the alcoholic liver cirrhosis when compared to non-alcoholic controls or non-alcoholic cirrhosis patients or alcoholic controls (Khan et al. 2009). Haplotype T-A-T is found to be associated with more than fivefold increase in risk for alcoholic cirrhosis (Khan et al. 2009). Likewise, combination of variant genotype of CYP2E1 *5B with null genotype of GSTM1, a phase II detoxification enzyme, results in several fold increase in risk in alcoholic cirrhotic patients when compared with non-alcoholic controls or non-alcoholic cirrhotic patients (Khan et al. 2009). Further, the combination of variant genotype of CYP2E1 *5B with gamma-aminobutyric acid receptor gamma2 (GABRG2), significantly increases the risk upto 6.5-fold in alcoholic cirrhotic patients when compared with non-alcoholic controls thereby suggesting the role of gene-gene interaction in alcoholic cirrhosis (Khan et al. 2009).

A much higher risk to alcoholic liver cirrhosis is observed in patients carrying a combination of wild genotypes of alcohol dehydrogenase ADH1C (ADH1C*1/*1) and variant genotype of ADH1B (ADH1B*2/*2) or CYP2E1 (CYP2E1*5B) or null genotype of GSTM1 (Khan et al. 2010). Thus, an interaction occurs amongst the genes involved in metabolizing alcohol and in generating and detoxifying free radicals with susceptibility to alcoholic liver cirrhosis (Khan et al. 2010).

Induction of Lymphocyte CYP2E1 in Alcoholic Liver Cirrhosis

Significant increases in CYP2E1 mRNA and protein expression are observed in freshly prepared blood lymphocytes isolated from alcoholic controls (ACs) and alcoholic liver cirrhotic (ACP) patients as compared with respective nonalcoholic controls (NACs) or nonalcoholic cirrhotic patients (NACP) patients (Khan et al. 2011). A concomitant increase in NDMA demethylase activity is evident in the blood lymphocytes of ACs and ACP patients (Khan et al. 2011). Interestingly, the comparative increase observed in CYP2E1 expression is of greater magnitude in the blood lymphocytes isolated from ACP patients, although they abstained from alcohol drinking (Khan et al. 2011). Thus, significant increase in the CYP2E1 mRNA and protein expression in the blood lymphocytes, isolated from early stage ACP patients, can be used to predict alcohol-induced toxicity (Khan et al. 2011).

V. Ravindranath

Ethanol Mediated Induction of Brain CYP2E1

The presence of CYP2E1 and associated mono-oxygenase activities in brain and the effect of chronic ethanol treatment on brain CYP has been studied (Anandatheerthavarada et al. 1993). Aniline hydroxylase, NDMA N-demethylase and p-nitrophenol hydroxylase activities are detectable in brain microsomes from untreated rats and are about 5, 125 and 8.3%, respectively, of the corresponding hepatic levels (Anandatheerthavarada et al. 1993). Chronic ethanol treatment results in induction of the above enzyme activities in brain microsomes by 243, 496 and 155%, respectively (Anandatheerthavarada

et al. 1993). Addition of the antisera raised against rat liver CYP2E1 markedly inhibits brain microsomal p-nitrophenol hydroxylase activity (Anandatheerthavarada et al. 1993). The induction of brain CYP2E1 occurs following chronic ethanol administration (Anandatheerthavarada et al. 1993). The preferential localization of the enzyme occurs in the neuronal cell bodies in the cortex, hippocampus, basal ganglia, hypothalamic nuclei and reticular nuclei in the brainstem of rats treated chronically with ethanol (Anandatheerthavarada et al. 1993). Thus, chronic alcohol ingestion could enhance the sensitivity of certain regions of the brain to environmental chemicals that are metabolized to more toxic derivatives by the CYP system (Anandatheerthavarada et al. 1993).

Characterization of Rat Brain Mitochondrial CYP2E1

The ability of brain mitochondria to metabolize the potent carcinogen NDMA is more than twofold that of the corresponding microsomal activity, while the 7-ethoxycoumarin-O-deethylase activity is significantly lower in mitochondria (Bhagwat et al. 1995). The immunoreactive bands for CYP (2B1/2B2), CYP1A1, and CYP2E1 are present in isolated brain mitochondria (Bhagwat et al. 1995). Various antibodies to CYP (2B1/2B2), CYP1A1, and CYP2E1 inhibit the brain mitochondrial monooxygenase activities to significant, though varying extent (Bhagwat et al. 1995). Chronic ethanol administration results in twofold induction of total CYP content and the monooxygenase activities known to be mediated by CYP2E1, such as NDMA-N-demethylase and p-nitrophenol hydroxylase in brain mitochondria (Bhagwat et al. 1995). The study demonstrates the presence of multiple forms of P450 in the rat brain mitochondria, their inducibility, and their capability to metabolize xenobiotics (Bhagwat et al. 1995).

Region Specific Induction of Brain CYP2E1

Chlorzoxazone hydroxylation in brains from the chronic ethanol treated rats is induced in hippocampus and cortex, downregulated in brainstem, and unchanged in cerebellum, striatum, and thalamus (Upadhya et al. 2000). The presence of functionally active CYP2E1 is also seen in human brain regions obtained at autopsy from traffic accident victims (Upadhya et al. 2000). The constitutive presence of a corresponding CYP2E1 transcript in rat and human brain is observed (Upadhya et al. 2000). The constitutive expression of CYP2E1 preferentially in the neuronal cells in rat and human brain is observed (Upadhya et al. 2000). CYP2E1 expression is seen in neurons within the cerebral cortex, Purkinje and granule cell layers of cerebellum, granule cell layer of dentate gyrus, and pyramidal neurons of CA1, CA2, and CA3 subfields of hippocampus in both rat and human brain (Upadhya et al. 2000). Thus, the constitutive expression of CYP2E1 in brain, its differential induction in rat brain regions by chronic ethanol treatment, and its topographic distribution in rat and human brain has been demonstrated (Upadhya et al. 2000).

Aparajita Dey

Ethanol/High Glucose Inducibility of CYP2E1: *In Vitro* Evidence and Potential Implications for Liver Injury due to Alcohol Consumption in Diabetics

Using the recombinant human hepatoma cell line VL-17A that over-expresses the alcohol metabolizing enzymes – alcohol dehydrogenase (ADH) and CYP2E1,

the mechanism and mode of cell death due to chronic ethanol exposure have been studied (Chandrasekaran et al. 2011). Chronic alcohol exposure causes a significant decrease in viability in VL-17A cells (Chandrasekaran et al. 2011). Chronic ethanol mediated cell death in VL-17A cells is predominantly apoptotic, with increased oxidative stress as the underlying mechanism (Chandrasekaran et al. 2011). Interestingly, the level of the antioxidant GSH is found to be upregulated in VL-17A cells treated with ethanol, which may be a metabolic adaptation to the persistent and overwhelming oxidative stress (Chandrasekaran et al. 2011).

Oxidative stress parameters are greatly increased and apoptotic cell death is observed in high glucose exposed VL-17A cells (Chandrasekaran et al. 2012a). Inhibition of CYP2E1 or caspase 3 or addition of the antioxidant trolox leads to significant decreases in high glucose mediated oxidative stress and toxicity (Chandrasekaran et al. 2012a). Thus, the over-expression of ADH and CYP2E1 in liver cells is associated with increased high glucose mediated oxidative stress and toxicity (Chandrasekaran et al. 2012a).

The toxicity due to chronic alcohol plus high glucose has been studied in VL-17A cells (Chandrasekaran et al. 2012b). When present together, ethanol plus high glucose treated VL-17A cells exhibit greater oxidative stress and toxicity than other groups (Chandrasekaran et al. 2012b). Apoptosis is observed in the ethanol plus high glucose treated VL-17A cells accompanied by increased CYP2E1 protein expression (Chandrasekaran et al. 2012b). The combined oxidative insult due to alcohol plus high glucose leads to greater liver injury, which may prove to be a timely warning for the injurious effects of alcohol consumption in diabetics (Chandrasekaran et al. 2012b).

1.3 Conclusions

As illustrated through the above sections, CYP2E1 appears to play a crucial role in drug metabolism and disease development. CYP2E1 exerts its multifarious activities through several mechanisms, oxidative stress being the major one. The occurrence of CYP2E1 in the body is apparently ubiquitous i.e. it exhibits a wide tissue specific expression. CYP2E1, apart from its role as a xenobiotic metabolizing enzyme, is also involved in modulating several physiological processes. Several drugs acts as inducers and substrates for CYP2E1, thus the therapeutic indices for the drugs can be altered due to the actions of CYP2E1. CYP2E1 is induced in several pathophysiological conditions and CYP2E1 is actively involved or associated with progression of diseases or chemical induced carcinogenesis. Certain pathophysiological conditions can exacerbate alcohol mediated cellular injury due to synergistic induction of CYP2E1, while some agents can act as co-inducers for CYP2E1. Besides, polymorphisms in CYP2E1 are linked with incidences of several diseases. Thus, the role of CYP2E1 as a key player in health and disease cannot be understated.

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