# Cytochrome P450 2E1: Its Role in Disease and Drug Metabolism

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Editor

# Cytochrome P450 2E1: Its Role in Disease and Drug Metabolism

Springer

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*To the memory of my parents*
*and*
*To Prof. Arthur I. Cederbaum,*
*A mentor* par excellence

# Preface

This book deals with various clinical aspects of cytochrome P4502E1 (CYP2E1) which is a potent source for oxidative stress. Cytochrome P-450 (CYP) enzymes are proteins that essentially contain a heme moiety and are involved in diverse oxidative metabolism of a wide spectrum of endogenous compounds as well as xenobiotics. Further, they are induced by several stimuli which include pathophysiological conditions, thus emphasizing their critical role in human physiology and diseases.

Ethanol-inducible CYP2E1 which forms the key enzyme in the microsomal ethanol-oxidizing system, besides metabolizing ethanol to acetaldehyde, also catalyzes oxidative metabolism of substrates primarily through acting as a monooxygenase and generating reactive oxygen species in the process. Oxidative stress is critical for pathogenesis of diseases and CYP2E1 is a major contributor for oxidative stress. Several clinical disorders are associated with changes in regulation of CYP2E1 and the consequent abnormalities which include alcoholic liver disease, alcoholic pancreatitis, carcinogenesis, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, obesity, hepatitis C virus infection, reproductive organ toxicity, hepatocellular and cholestatic injury.

The list for the involvement of clinical and metabolic disorders associated with changes in regulation of CYP2E1 is extensive which includes bone loss, cross-tolerance in smokers and people treated with nicotine (e.g., smokers, patients with Alzheimer's disease, ulcerative colitis, neuropsychiatric motor disorders), disorders of central nervous system due to exposure to certain environmental chemicals and changes in the metabolism of protoxicants in the circulatory system.

Changes in regulation of CYP2E1 may occur due to endotoxemia, inflammatory stimuli, complex endocrine regulation by pituitary and testicular hormones, expression of methionine adenosyltransferase genes, nicotine or environmental tobacco smoke exposure, polymorphic gene expression, transcription factor hepatocyte nuclear factor 1 alpha, calmodulin dependent protein kinase, protein kinase C and cAMP dependent protein kinase, drugs such as isoniazid and clofibrate, starvation, insulin, diabetes or alcohol consumption.

The several mechanisms through which CYP2E1 exerts its damaging effects include increased oxidative stress, acetaldehyde formation and accumulation, increased hepatotoxicity of carcinogens like nitrosodimethylamine, urethane and acrylamide, oxidative DNA damage, augmentation of iron induced hepatotoxicity, priming of Kupffer cells to lipopolysaccharide-induced toxicity, affecting therapeutic index of drugs, i.e. potentiating acetaminophen mediated toxicity, increasing polyunsaturated fat mediated injury, depletion of the levels of the major cellular antioxidant glutathione and increase in collagen expression. Other mechanisms for clinical abnormalities associated with CYP2E1 include mitochondrial dysfunction, apoptotic cell death, potentiation of lipopolysaccharide or cisplatin mediated injury, inhibition of microsomal Ca2+−ATPase, formation of carcinogenic etheno-DNA adducts, modulation of the immune response, increases in proinflammatory cytokines, polymorphic gene expression and increased hydroxyethyl radical formation.

Further, CYP2E1 causes metabolic abnormalities through formation of autoantibodies against CYP2E1, necroinflammation, increased degradation of retinoic acid and vitamin A, JNK activation, decrease in proteasome activity with subsequent accumulation of oxidized proteins, formation of cytokeratin aggresomes and Mallory body-like inclusions. Several other mechanisms through which CYP2E1 exerts toxicity include increased formation of Kupffer cell-generated metabolites, which may contribute to Kupffer cell toxicity; elevated c-fos mRNA; oxidative modifications of heat shock protein 60; protein disulfide isomerase; mitochondrial aldehyde dehydrogenases, prohibitin, and other proteins; formation of 3-nitrotyrosine adducts and high molecular weight microsomal ubiquitin conjugates; increased levels of endoplasmic reticulum stress marker tribbles-related protein 3 and chemokine CXCL-2; impairment of insulin signaling; formation of protein adducts of aldehydes such as acetaldehyde, malondialdehyde and 4-hydroxy-nonenal; suppression of activities of antigen-trimming enzymes, thereby decreasing the cleavage of C-extended and N-extended peptides which may potentially result in decreased MHC class I-restricted antigen presentation on virally infected liver cells; impairment of interferon gamma signaling; irreversible inhibition of fatty acid oxidation, potentially through suppression of PPARalpha-regulated pathways; and potentiation of thioacetamide mediated hepatotoxicity.

The first chapter gives an overview of the research on different aspects of CYP2E1 and the aim of the chapter is to acquaint the readers with a general picture regarding CYP2E1 before they delve deeper into further chapters which are specialized research findings discussed in detail by the different experts with respect to the studies being performed in their own respective laboratories. The subsequent chapters deal with some of the research activities dealing with CYP2E1 in major laboratories around the world.

Dr. Arthur I. Cederbaum discusses about the role of the transcription factor Nrf2, the key regulator of cytoprotective enzymes as a protective mechanism against CYP2E1 mediated oxidative stress in a human hepatoma cell line transfected with CYP2E1. Dr. Helmut K. Seitz discusses about the important role of ethanol inducible CYP2E1 in promoting alcohol mediated carcinogenesis. Dr. Samuel W. French deals with CYP2E1 mediated drug metabolism and the consequent drug mediated

hepatitis due to co-administration of ethanol and drugs. Also, epigenetic effects due to induction of CYP2E1 are discussed.

Dr. Ann K. Daly discusses about the role of CYP2E1 as a genetic risk factor for non-alcoholic fatty liver disease- evidences in favour and against it, as documented in studies involving genetic analyses. Drs. Terence M. Donohue and Natalia A. Osna discuss about the role of CYP2E1 in regulating cytokine signaling, antigen presentation, and macromolecular degradation leading to liver injury. Dr. M. Raj Lakshman discusses about the role of CYP2E1 and ethanol mediated oxidative stress in downregulating the hepatic expression of paraoxonase 1, a multifunctional antioxidant enzyme that prevents LDL oxidation and detoxifies the homocysteine metabolite, homocysteinethiolactone. Dr. Vasilis K. Vasiliou discusses about the role of CYP2E1 in ethanol metabolism in the central nervous system, including its regulation and expression and its influence on sensitivity to ethanol in the brain.

Thus, CYP2E1 is implicated in several clinical disorders through diverse mechanisms of injury. It is interesting to explore some of these pathways which shed light on the several other aspects linked with this enzyme. The different biochemical, toxicological and clinical aspects of CYP2E1 and the underlying mechanisms through which CYP2E1 plays a critical and indispensible role in modulating the therapeutic effects of drugs, and in development and pathogenesis of clinical disorders, form the core of the book.

# Acknowledgements

First and foremost, I would like to thank Dr. Meran Owen, Senior Editor, Springer London Cell Biology, Molecular Biology for giving me this opportunity to put together a book on 'Cytochrome P4502E1: Its Role in Disease and Drug Metabolism'. This provided me a wonderful chance to review and acquaint myself with the valuable contributions of different experts in the field of CYP2E1 who have carried on studies regarding the different aspects of the enzyme.

I would also like to thank the contributing authors for most graciously accepting my invitation, taking the time out of their busy schedules and putting their painstaking efforts to write the different chapters. In this regard, I would especially thank my postdoctoral mentor Prof. Arthur I. Cederbaum who in a way introduced me to the field of CYP2E1 in a deeper sense and helped me to develop my understanding of the subject. An excellent mentor and a far better human being; he has always been a source of inspiration for me.

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I would also like to thank my brothers Abhishek and Abhijit and their families for their unfailing help and support through all these years which formed the foundation for all my professional endeavors. My special thanks to my nephews Aditya, Aniruddha and my niece Aishwarya for their smiles and laughter which always made my days bright and sunny.

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Chennai, India
21 September 2012

Aparajita Dey

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

**Aparajita Dey**

**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) |

(continued)

**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,