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REVIEW

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Toxicological implications of mitochondrial localization of CYP2E1

Jessica H. Hartman, *[a] Grover P. Miller[b] and Joel N. Meyer[a]

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Cytochrome P450 2E1 (CYP2E1) metabolizes an extensive array of pollutants, drugs, and other small molecules, often resulting in bioactivation to reactive metabolites. Therefore, it is unsurprising that it has been the subject of decades of research publications and reviews. However, while CYP2E1 has historically been studied in the endoplasmic reticulum (erCYP2E1), active CYP2E1 is also present in mitochondria (mtCYP2E1). Relatively few studies have specifically focused on mtCYP2E1, but there is growing interest in this form of the enzyme as a driver in toxicological mechanisms given its activity and location. Many previous studies have linked total CYP2E1 to conditions that involve mitochondrial dysfunction (fasting, diabetes, non-alcoholic steatohepatitis, and obesity). Furthermore, a large number of reactive metabolites that are formed by CYP2E1 through metabolism of drugs and pollutants have been demonstrated to cause mitochondrial dysfunction. Finally, there appears to be significant inter-individual variability in targeting to the mitochondria, which could constitute a source of variability in individual response to exposures. This review discusses those outcomes, the biochemical properties and toxicological consequences of mtCYP2E1, and highlights important knowledge gaps and future directions. Overall, we feel that this exciting area of research is rich with new and important questions about the relationship between mtCYP2E1, mitochondrial dysfunction, and pathology.

Introduction

Cytochrome P450 2E1 (CYP2E1) is a cytochrome P450 monooxygenase enzyme that is best known for its role in hepatic metabolism of ethanol in the microsomal ethanol oxidizing system. However, this versatile enzyme is also responsible for the metabolism of a broad range of substrates, including endogenous molecules such as ketones and fatty acids, and low molecular weight drugs, pollutants, and dietary compounds. Furthermore, CYP2E1 is expressed in high levels in the liver and is expressed and inducible in many extrahepatic tissues including the brain, kidney, nasal mucosa, skin, heart, lungs, ovary, testes, skeletal muscle, and bone marrow. Within cells, active CYP2E1 localizes to both the endoplasmic reticulum (erCYP2E1) and mitochondria (mtCYP2E1). The CYP2E1 oxidation reaction utilizes electrons from NADPH and molecular oxygen to introduce one oxygen atom into the substrate, resulting in a metabolite that is more water-soluble and easily excreted. However, in many cases, oxidation of the molecule by CYP2E1 results in the formation of a reactive metabolite, such as an epoxide or aldehyde. Furthermore, in addition to the oxidation of substrates, CYP2E1 undergoes "uncoupling" of its catalytic cycle wherein electrons are consumed to generate reactive oxygen species (ROS). These features make CYP2E1 an important enzyme for toxicology.

The importance of CYP2E1 in toxicology is well-known and has been reviewed extensively elsewhere.[1–5] This review aims to highlight the potential importance of mitochondria-localized CYP2E1 in driving mitochondrial dysfunction: while mitochondrial CYP2E1 was discovered decades ago, the vast majority of what is known about CYP2E1 derives from studies which utilized the liver microsomal fraction (membrane fraction of cells containing mostly endoplasmic reticulum) or did not distinguish between endoplasmic reticulum- or mitochondria-localized CYP2E1. Therefore, known endogenous and exogenous roles of total cellular CYP2E1 will be discussed in the context of mitochondrial dysfunction, followed by an account of what is known about mitochondrial CYP2E1.

Endogenous role of CYP2E1

CYP2E1 is generally considered a xenobiotic-metabolizing enzyme; however, CYP2E1 also metabolizes endogenous compounds, including ketones, fatty acids, and hormones that could play a role in mitochondrial dysfunction in multiple pathophysiological conditions. CYP2E1 is regulated by

[a]*Nicholas School of the Environment, Duke University, Durham, NC, USA. E-mail: jessica.h.hartman@duke.edu; Fax: +1 919 668 1799; Tel: +1 919 613 8027*
[b]*Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR, USA*

changes in pituitary and sex hormones, and undergoes induction during fasting/starvation, diabetes, nonalcoholic steatohepatitis (NASH), and obesity. An important common feature among all of these conditions is mitochondrial dysfunction and oxidative stress (see Fig. 1). Mitochondrial dysfunction can take many forms, depending on the nature of the damage. Hallmarks of mitochondrial dysfunction may include insufficient production of ATP, inappropriate (too much, or too little) production of reactive oxygen species, or alterations in other critical mitochondrial functions including iron and calcium homeostasis, steroid synthesis, and apoptosis. Dysfunction occurs when there is a more damage to mitochondrial DNA, proteins, and lipids than can be repaired through homeostatic pathways including proteases, chaperones, antioxidant enzymes and molecules, lipases, DNA repair and biogenesis, and removal of damaged mitochondria *via* mitophagy (mitochondrial clearance), mitochondrial-derived vesicles, and apoptosis. Mitochondria and mitochondrial components such as DNA are particularly vulnerable to such damage for a variety of reasons, including the absence of some DNA repair pathways.[6] Therefore, mitochondrial-localized CYP2E1 may plausibly contribute to such dysfunction by producing reactive metabolites in close proximity to vulnerable mitochondrial nucleophiles. The experimental links between CYP2E1 and these conditions will be detailed below.

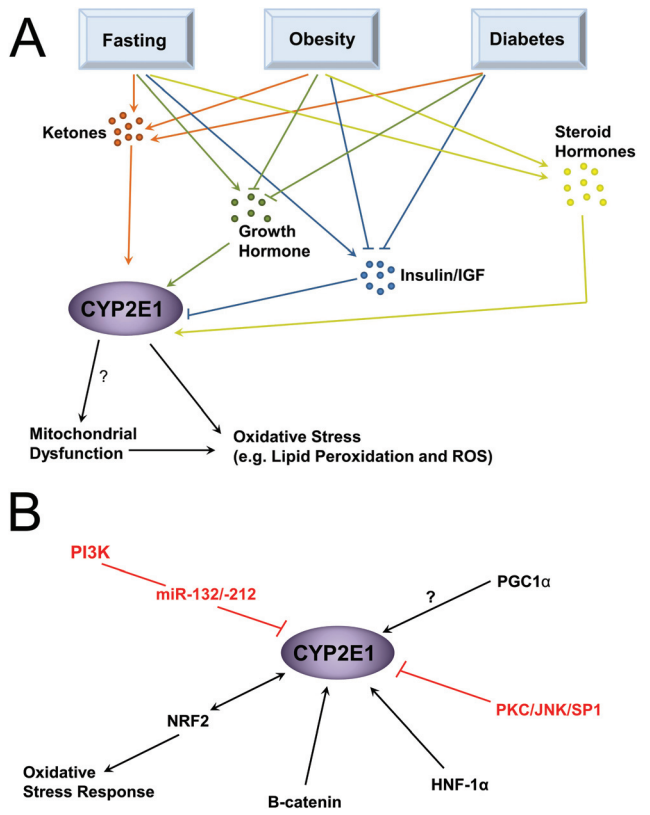

Fig. 1 Factors influencing CYP2E1 expression and potential downstream consequences. Panel A shows the impact of fasting, obesity, and diabetes on CYP2E1. These conditions modulate ketone, growth hormone, and steroid hormone levels as well as impact the effect of insulin and insulin-like growth factor (IGF) on CYP2E1. These changes in CYP2E1 levels are then reflected in increased oxidative stress and perhaps mitochondrial dysfunction. Panel B illustrates CYP2E1 interactions with major cellular signaling pathways known to impact mitochondrial function.

The induction of CYP2E1 has long been associated with fasting or starvation conditions.[7,8] The fasting phenotype is characterized by high circulating ketone bodies, including acetone, acetoacetate, and beta-hydroxybutyrate, which would require a clearance mechanism to avoid toxicity. Acetone and acetoacetate administration alone induce CYP2E1,[9] and CYP2E1-null fasted mice accumulate acetone to levels >20 times greater than wild-type mice.[10] Those observations suggest an endogenous role for CYP2E1 in the clearance of ketones. Furthermore, the metabolites that CYP2E1 produces from acetone metabolism, *i.e.* acetol and methylglyoxyl, are precursors to an alternative entry point for gluconeogenesis.[11] CYP2E1 is expressed in the brain as well as liver, and thus ketone bodies generated during starvation may provide energy to the brain; ketones are generated in the brain but also cross the blood–brain barrier.

Moreover, elevated ketones occur during diabetic conditions, as well as hyperglycemia and hypercholesterolemia. In particular, diabetes is associated with increased CYP2E1 expression,[12,13] both in mitochondria and ER,[14] that may be mechanistically linked to pathogenesis due to impairment of GLUT4 through a NRF2-mediated pathway.[15] Specifically, overexpression of CYP2E1 in cell culture suppressed GLUT4 gene expression, while suppression of CYP2E1 had the opposite effect; these effects could be blocked by co-expression of a dominant negative form of NRF2 or by administration of antioxidants, suggesting that the role of CYP2E1 is both NRF2- and ROS-dependent. Aside from regulating glucose metabolism, insulin signaling is closely tied with CYP2E1 expression;[16] higher insulin levels decrease CYP2E1 expression, which may be mediated through miR-132/-212[17] and phosphatidylinositol 3′ kinase (PI3K).[17,18] On the other hand, overexpression of CYP2E1 leads to impaired hepatic insulin signaling in cell culture[19] and in mice[20] and knockout of CYP2E1 in mice confers protection against high-fat diet-induced obesity and insulin resistance.[21] Taken together, signaling pathways involved in diabetic conditions regulate the expression of CYP2E1 and hence suggest its function in responding to pathophysiological conditions.

Similarly, CYP2E1 expression plays an important role in non-alcoholic steatohepatitis (NASH), which is a common liver disease characterized by fatty liver (steatosis), inflammation, and eventually fibrosis of the liver. The cause of NASH has not been identified; however, release of inflammatory cytokines and oxidative stress are thought to be important for NASH development. In humans many hallmark features of NASH including degree of steatosis, hyperketonemia, and hyperlipidemia[22–27] positively associate with CYP2E1 activity. At the molecular level, CYP2E1 may be an important mechanistic driver in this pathology:[28,29] CYP2E1 is the primary catalyst driving a 100-fold increase in lipid peroxidation in a

murine dietary model of NASH. However, there are compensatory mechanisms when CYP2E1 expression is low, because the absence of CYP2E1 (CYP2E1-null mice) leads to up-regulation of CYP4A enzymes that drive the pathogenesis.[30] Finally, another consequence of NASH is oxidative stress, which may be exacerbated by CYP2E1.[29]

Higher expression of CYP2E1 is also associated with obese individuals, who have CYP2E1 activities around three-fold greater than those with normal body weight.[22] When obese individuals undergo weight-loss surgery, they exhibit a decrease in CYP2E1 activity concomitant with decreased body weight. The relationship between obesity and CYP2E1 may impact toxicology, as has been shown for the common drug acetaminophen in cultured cells treated with fatty acids[31] and in obese and non-obese humans.[32] In those cases, higher body fat and/or treatment of cells with free fatty acids resulted in higher CYP2E1 levels and higher production of the toxic quinoneimine metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), resulting in more hepatotoxicity and mitochondrial dysfunction. Therefore, obesity may interplay with CYP2E1-related toxicities through chronic induction of the enzyme, resulting in increased mitochondrial dysfunction, which may in turn further drive obesity-related pathologies.[33]

A unifying theme among all of these conditions (fasting, diabetes, NASH, and obesity) is altered bioenergetics and redox status (see Fig. 1). However, it is unclear whether changes in bioenergetics are due to endogenous CYP2E1 processes or represent an interplay between endogenous and exogenous regulation and activity of the enzyme. It is also largely unknown whether the pathophysiological conditions described herein that regulate total or erCYP2E1 also impact mtCYP2E1 by similar or distinct magnitudes and mechanisms. It has been increasingly evident, however, that many pathologies arise from the sum of exposures, diet, stresses, and genetics; therefore, understanding individual processes will eventually lead to better predictions and treatments for those pathologies.

Exogenous substrates of CYP2E1 that damage mitochondria

The broad specificity of CYP2E1 includes a wide array of low molecular weight hydrophobic xenobiotic substrates, including drugs, pollutants, and dietary compounds. The promiscuous nature of CYP2E1 has been extensively discussed elsewhere;[1–5] however, those reviews are limited to discussing the toxicological role of CYP2E1 found in microsomal fractions or lack altogether references to the impact of its subcellular localization. This portion of the review provides a focused update highlighting toxicologically important substrates of CYP2E1 that specifically target mitochondria and result in mitochondrial dysfunction. In many cases, specific roles have not yet been explored for mtCYP2E1 in activation of toxicants and mitochondrial dysfunction; however, there is direct evidence linking mtCYP2E1 activity and mitochondrial dysfunction in limited cases, which we mention here and detail later in the review. Other cases lack direct evidence, yet the possibility is likely given the ability of mtCYP2E1 to generate reactive metabolites during metabolism and reported mitochondrial dysfunction resulting from exposure to those CYP2E1 substrates, although contributions from erCYP2E1 cannot be ruled out. Further mechanistic studies on mitochondrial dysfunction are clearly necessary to distinguish between CYP2E1-generated reactive metabolites generated *in situ* and those migrating from the endoplasmic reticulum.

CYP2E1 does not play a major role in metabolic clearance of drugs; however, there are notable exceptions such as acetaminophen, cisplatin, and isoniazid. CYP2E1 is primarily responsible for converting acetaminophen to a reactive metabolite that damages mitochondria and ultimately leads to hepatocellular necrosis.[34] In cultured cells, expression of mitochondrial CYP2E1 alone was sufficient to drive mild mitochondrial dysfunction and increased cytotoxicity,[35] despite lower overall cellular CYP2E1 activity compared to cells that expressed both mtCYP2E1 and erCYP2E1.

The chemotherapeutic agent cisplatin has long been used for the treatment of solid tumors. While generally effective, it has a common adverse effect of causing nephrotoxicity, specifically in the proximal tubule. Reactive oxygen species (ROS) have been implicated in the mechanism of renal toxicity, and CYP2E1 has been identified as a potential source of ROS in this mechanism, although it is not known exactly how CYP2E1 plays a role in cisplatin-induced toxicity. CYP2E1 localizes to the proximal tubule, and CYP2E1-null mice are protected from cisplatin-induced nephrotoxicity.[36] CYP2E1 expression is necessary for the cisplatin-induced generation of oxidative stress in cell culture and in kidney slices, and may ultimately drive apoptosis in the proximal tubule.[37–40] Glutathione status also impacts the observed cytotoxicity, which further implicates a role for CYP2E1-derived ROS in the toxicological mechanism.[38,39] Mitochondrial dysfunction has also been identified as a key step in the mechanism of cisplatin nephrotoxicity based on decreased respiration, respiratory complex activities, ATP synthesis, and membrane potential and the amelioration of those effects by co-administration of antioxidants.[41–43] Cisplatin has been reported to cause slightly less or much more mitochondrial than nuclear DNA damage,[6] and there is evidence that in aerobic cells, it is this damage, resulting in impairment of mitochondrial protein synthesis and mitochondrial ROS generation, that causes cytotoxicity.[44]

The anti-tuberculosis drug isoniazid causes idiosyncratic hepatotoxicity and neurotoxicity in a mechanism that has not been fully elucidated; nevertheless, CYP2E1 activity has been identified as playing a role,[45–47] possibly through the generation of free radicals.[48] Importantly, isoniazid hepatotoxicity is triggered by mitochondrial dysfunction[49–51] and specific impairment of mitochondrial respiratory complexes I and II.[49]

Several industrial chemicals are activated by CYP2E1 to mitochondrial toxicant metabolites. Thioacetamide is an organosulfur compound that was at one time widely used as an industrial solvent; its use has been greatly diminished since its classification as a human carcinogen. Toxic activation

of thioacetamide involves two oxidative steps to its *S*-oxide and *S*,*S*-dioxide that are primarily catalyzed by CYP2E1.[52,53] This process leads ultimately to liver necrosis. Protection against thioacetamide toxicity is conferred by genetic knockdown of CYP2E1[54] or administration of CYP2E1 inhibitors.[52,53] Interestingly, protection also resulted from pharmacological or genetic knockdown of superoxide dismutase, which also appeared to decrease CYP2E1 activity.[55] This observation could suggest a direct role for hydrogen peroxide signaling in CYP2E1 regulation and hence its role in toxicity. Furthermore, fasting and diabetic conditions that induced cellular CYP2E1 potentiated thioacetamide-induced liver injury in rats.[56] Thioacetamide administration impairs mitochondrial complex I activity in primary hepatocytes in culture[53] and in brain mitochondria isolated from thioacetamide-treated rats.[57] Alterations in overall mitochondrial respiration and in phospholipid content of mitochondria have also been reported.[58–60]

Carbon tetrachloride was initially produced on a large scale for consumer and industrial uses, yet only persists today for industrial applications. Exposure to carbon tetrachloride has been linked to liver and kidney damage, central nervous system toxicity, and carcinogenicity.[61] The U.S. Environmental Protection Agency has recently placed carbon tetrachloride on the priority list of ten chemical substances that it will evaluate for potential risks to human health and the environment under the amended Toxic Substances Control Act.[62] Mechanistic studies on the mode of action of carbon tetrachloride identified formation of the trichloromethyl radical by CYP2E1 and subsequent formation of trichloromethyl peroxy radical as key events in the toxicity pathway.[63–66] Carbon tetrachloride toxicity is potentiated by the induction of CYP2E1 by ethanol,[67–69] and CYP2E1-null mice are protected from carbon tetrachloride hepatotoxicity.[63] Carbon tetrachloride toxicity includes mitochondrial dysfunction, with exposures resulting in impaired liver mitochondrial respiration, reduced mitochondrial DNA integrity, and impairment of individual mitochondrial respiratory complex activities.[70–72]

Benzene is a ubiquitous industrial chemical and environmental pollutant as well as a confirmed human carcinogen. CYP2E1 is primarily responsible for the bioactivation of benzene to its toxicologically active hydroquinone metabolites.[73] CYP2E1 is induced in peripheral lymphocytes and liver to a similar extent by benzene and classical CYP2E1 inducers such as pyrazole and acetone.[74] Knockout of CYP2E1 protects mice from benzene toxicity by reducing DNA damage in bone marrow, thymus, lymphocytes, and spleen. A higher risk of benzene poisoning correlates with either ethanol consumption or genetic polymorphisms in the noncoding region of the CYP2E1 gene that result in induction of CYP2E1 expression.[75] Incubation of isolated mitochondria from bone marrow with benzene oxidative metabolites resulted in covalent binding to mitochondrial DNA and decreased mitochondrial RNA synthesis.[76] In occupationally exposed workers, benzene exposure resulted in increased mitochondrial DNA copy number in circulating leukocytes, which the authors speculate is a compensatory response to mitochondrial damage.[77]

Furan is a widely used industrial chemical as well as a component of cigarette smoke and diesel exhaust. This common pollutant is classified as a liver toxicant and cholangiocarcinogen.[78] Furan toxicity results from CYP2E1 bioactivation to a reactive dialdehyde, *cis*-2-butene-1,4-dial.[79] The mechanism of furan toxicity involves oxidative stress, DNA damage, and inflammation.[80] An early step in furan toxicity is uncoupling of hepatic oxidative phosphorylation, evidenced through lower ATP levels in rat hepatocytes and impaired respiration which preceded cell death in culture.[81] These effects were eliminated by administration of garlic oil,[82] which contains diallyl sulfide components that irreversibly inhibit CYP2E1.[83]

Trichloroethylene (TCE) is a pervasive environmental pollutant in which exposure leads to carcinogenicity and neurotoxicity through CYP2E1 activity. In fact, there is an association of Parkinson's Disease in humans with TCE exposure[84–88] with up to a six-fold increased risk in one study.[89] TCE exposure studies using animal models provide further evidence for a toxicological role for CYP2E1 activtiy.[87,90] In those experiments, TCE exposure resulted in mitochondrial dysfunction (impairment of mitochondrial complex I activity) through an unknown mechanism.

1,3-Butadiene is a cigarette smoke constituent and human carcinogen that is activated by CYP2E1 to reactive epoxide metabolites.[91] The metabolites have been shown to modify nuclear DNA and result in genotoxicity; however, the possibility of mitochondrial dysfunction had not been explored. We have recently shown that genetically diverse mice exposed to a high dose of inhaled butadiene exhibit impaired mitochondrial complex activities in complexes I, II, and IV and that impairment is correlated with increased CYP2E1 activity in mitochondria but not in the endoplasmic reticulum.[92] This finding demonstrates a link between mtCYP2E1 activation of butadiene and exposure-induced mitochondrial dysfunction, although its relevance in toxicity of environmental exposures to butadiene remains to be explored.

Food and beverage constituents similarly undergo bioactivation into reactive metabolites by CYP2E1 action. Ethanol is one of the most well studied dietary substrates for CYP2E1. CYP2E1 activity is induced by ethanol through a ROS-mediated mechanism in a feedback loop, as evidenced from its blockage due to the co-administration of antioxidants such as vitamin C. The inhibition of PKCζ, JNK, or SP1 also blocks ethanol induction of CYP2E1, which implicates this pathway in the regulation of CYP2E1 levels.[93] Ethanol metabolism enhances carcinogenesis through CYP2E1 production of reactive oxygen species (ROS) and the reactive metabolite acetaldehyde.[94] At the cellular level, ethanol and likely CYP2E1 activity induce oxidative stress, lipid peroxidation, and mitochondrial dysfunction, which has been reviewed extensively elsewhere.[95–97] Ethanol toxicity is similar in cultured cells expressing only mtCYP2E1 compared to those which express CYP2E1 in both mitochondria and ER, suggesting that the mitochondrial form may be more important for toxicity.[35]

Acrylamide has industrial uses but is also a constituent in the human diet, forming in many starchy foods when cooked

at high temperatures. Acrylamide is carcinogenic and neurotoxic, and has also been linked to reproductive toxicity. The toxicity of acrylamide depends on the formation of the metabolite glycidamide by CYP2E1[98–101] and involves mitochondrial dysfunction.[102,103]

Together, these compounds comprise only a portion of the many chemicals activated by CYP2E1 that may target mitochondria. Although there is a clear link between CYP2E1 activation of the compounds and mitochondrial dysfunction, it is not clear in most cases if erCYP2E1, mtCYP2E1, or both forms of the enzyme drive the dysfunction. Their respective contributions may depend on substrate transport into mitochondria to undergo activation, the ability of the reactive metabolites to diffuse between the organelles, and the availability of NADPH and/or glutathione levels in the proximity of the enzyme. All of these intriguing mechanistic possibilities remain to be explored for delineating the roles of mt- and erCYP2E1 in toxicant bioactivation and resulting mitochondrial dysfunction.

Reactive oxygen species production by CYP2E1

CYP2E1 activity not only generates reactive metabolites but also reactive oxygen species (ROS). ROS results from uncoupling of the CYP2E1 catalytic cycle and contribute to toxicity, as demonstrated for erCYP2E1 and reviewed elsewhere.[104–107] Recent attention is shifting to understanding the effects of subcellular localization of CYP2E1 on its role in toxicity, for example by using cell lines expressing CYP2E1 targeted solely to the endoplasmic reticulum or mitochondria. Human hepatoma HepG2 cell lines lacking CYP2E1 expression were stably transfected by endoplasmic reticulum-localized (E47) or mitochondria-localized (mE10) CYP2E1. HepG2 cells expressing CYP2E1 in either subcellular compartment have higher basal levels of ROS and higher Nrf2 levels; those conditions led to increased sensitivity to toxicity induced by ethanol, polyunsaturated fatty acids, and iron.[104] Furthermore, induction of CYP2E1 *in vivo* and in primary tissue culture leads to lipid peroxidation, oxidative stress, and oxidative damage to DNA.[108–111] However, despite this indirect evidence that mtCYP2E1 may be driving oxidative stress through production of ROS, no detailed studies have yet tested whether mtCYP2E1 is "uncoupled" in its catalytic cycle similarly to erCYP2E1.

Genetic polymorphisms in CYP2E1

To date, it is unclear if there exist any functionally important polymorphic variants of CYP2E1. No coding region variants have been identified that impact CYP2E1 activity,[112] but there is evidence for polymorphisms that affect transcriptional regulation. Many studies report polymorphisms in the 5′ flanking region upstream of the CYP2E1 gene which may impact gene expression. The most common polymorphisms identified to date include the CYP2E1*5B (RsaI/PstI; rs2031920/rs3813867) variant, which has increased transcription and higher enzyme activity,[113] and the DraI variant, which is considered to only increase transcription.[114] This discrepancy is suggestive of further posttranscriptional regulation, although the exact mechanisms of regulation in these variants have not been extensively characterized. There are ethnic differences in the allelic frequency of these polymorphisms, with Asian populations having a higher frequency of both.[115] There is much conflicting information in the literature about the importance of these polymorphisms. For example, the PstI polymorphism was reported to be associated with a reduced risk of gastric cancer[116] in some studies, an increased risk in others,[117,118] while a recent meta-analysis revealed no associations between CYP2E1 polymorphisms and gastric cancer risk.[119] Similarly, while most studies report association of the RsaI/PstI and DraI polymorphisms with decreased lung cancer risk, this finding remains controversial.[120–123] The RsaI/PstI variant was associated with increased colorectal cancer risk, while there was no association with the DraI variant.[124] CYP2E1 RsaI/PstI polymorphisms may reduce the risk of respiratory cancer and liver cancer.[125] One potential reason for the lack of consistent findings with genetic polymorphisms is the complex regulation and inducibility of CYP2E1 by many endogenous and exogenous factors. For this reason, it has been suggested that CYP2E1 activity rather than genotype be used as for assessment of risk.[112]

The discovery of mitochondrial CYP2E1

Despite relatively few published studies focused on mtCYP2E1, the presence of mitochondria-localized CYP2E1 has been known for nearly two decades, with the first reports emerging in 1997. Research labs led by Narayan Avadhani (in 1997) and Magnus Ingelman-Sundberg (in 1999) independently discovered the mitochondrial enzyme initially labeled P450 MT5, and sought to validate the protein as truly mitochondrial and not an artifact of subcellular fractionation. Most studies used a subcellular fractionation approach with fresh cells or liver with western blot detection of the protein in each fraction.[126–129] In all cases, the authors included marker proteins from each fraction to show that the preparations were not detectably contaminated with the other fraction. Perhaps the most convincing evidence was immunoelectron microscopic localization experiments in isolated mitochondria,[126] which showed clear signal inside mitochondria for CYP2E1.

Both labs presented solid evidence for the localization of CYP2E1 within mitochondria, but a major discrepancy was the reported molecular weight of the mitochondrial enzyme. The Avadhani group described a full length ~52 kDa protein purified from rat liver.[126] By contrast, the Ingelman-Sundberg group reported a N-terminally truncated form of the enzyme (40 kDa fragment) that was sent to mitochondria in a transfected mouse hepatocellular cell line (H2.35 cells).[127] Moreover, those authors reported a CYP2E1-immunoreactive band at 40 kDa isolated mitochondria from control rat liver, and further characterized the truncated mitochondrial CYP2E1[128,129] by assessing its subcellular localization dependence on the lengths of the N-terminus. Unfortunately, those studies did not address the discrepancies between their find-

ings and those by the Avadhani group. Meanwhile, the Avadhani group published several more studies characterizing full-length mitochondrial CYP2E1[130,131] including the presence of both full-length and truncated (40 kDa) mitochondria-targeted CYP2E1 in stably transfected COS-7 cells.[132] Interestingly, the truncated version was only observed after integrating the transgene; transient transfection resulted in a full-length mtCYP2E1 protein. The authors attributed the difference in reported molecular weights to a cell culture artifact, but did not provide evidence for how or under what conditions the truncated mtCYP2E1 formed. It remains unclear why mtCYP2E1 expression includes full-length and truncated versions in rat liver and transfected cells, and which of the forms is more biologically relevant; perhaps both versions could be present under certain conditions. Regardless, these studies have effectively demonstrated that mitochondrial targeting can be increased by either truncation to a 40 kDa protein or mutations that enhance the mitochondrial targeting sequence (described below).

Irrespective of truncation, mtCYP2E1 is catalytically active. Initial reports demonstrated common reactions of mtCYP2E1 that were known to be catalyzed by erCYP2E1 including *p*-nitrophenol hydroxylation,[126,130] chlorzoxazone hydroxylation,[127–129] and dimethylnitrosamine demethylation.[130] These specific activities were increased with induction of CYP2E1 by pyrazole and blocked by CYP2E1 inhibitors. Nevertheless, these studies did not explore the mechanisms and efficiencies of the reactions to assess their relative significance to current understanding of CYP2E1 metabolism. Therefore, we purified rat liver mitochondria and microsomes and determined the kinetic parameters for mtCYP2E1 and erCYP2E1 oxidation of three well-studied substrates, *viz* the probe substrate 4-nitrophenol and pollutants aniline and styrene.[133] Importantly, all three of these substrates are metabolized through cooperative mechanisms by erCYP2E1, specifically substrate inhibition (4-nitrophenol),[134,135] positive cooperativity (styrene),[136] and negative cooperativity (aniline).[137] When compared to erCYP2E1, mtCYP2E1 shows similar affinity for all three substrates at the active site. Unlike erCYP2E1, mtCYP2E1 displays simple, non-cooperative Michaelis–Menten kinetics for oxidation of 4-nitrophenol, aniline, and styrene. Based on these mechanisms, we estimated the impact of CYP2E1 subcellular localization on the metabolic flux of pollutants to show that erCYP2E1 dominates aniline metabolism at all concentrations of aniline, but the initial importance of mtCYP2E1 in metabolism of styrene shifts to erCYP2E1 at higher concentrations. Together, this work showed that subcellular localization of CYP2E1 results in distinctly different enzyme activities that could impact metabolism and bioactivation in a substrate-dependent manner.

The distinct catalytic properties for CYP2E1 based on localization may be due to the formation of different functional complexes. CYP2E1 activity requires electrons from redox partners to activate oxygen for metabolic reactions. At the endoplasmic reticulum, cytochrome P450 reductase (CPR) fulfills this role while the complex adrenodoxin and adrenodoxin reductase (ADX-ADR) are responsible for supporting CYP2E1 reactions in mitochondria. Nevertheless, it is not accessibility to these specific redox partners that determines their catalytic activities because erCYP2E1 will only form a functional complex with CPR and mtCYP2E1 with the ADX-ADR complex.[130] The specificity of those interactions suggests that the contact regions for erCYP2E1 and mtCYP2E1 differ toward their respective redox partners. As evidence for distinct CYP2E1 conformations, circular dichroism experiments with the purified enzymes revealed differences in secondary structure between the proteins localized in each compartment. Together, these data suggest that when mtCYP2E1 is imported into mitochondria and folded by mitochondrial chaperones, its final structure differs from that in the endoplasmic reticulum.

While functional, the question remains as to how and why CYP2E1 is targeted to mitochondria. An early clue was an observed higher percentage of phosphorylated mtCYP2E1 when compared to the microsomal enzyme.[130] The target site is Ser-129, which can undergo phosphorylation by Protein Kinase A to reveal a cryptic mitochondrial targeting signal at amino acids 21–31. This modification results in increased mtCYP2E1 (but no increase in erCYP2E1) expression with cAMP signaling.[131] According to a model proposed by the authors, newly synthesized CYP2E1 polypeptide is immediately phosphorylated before it associates with the signal recognition particle, which would retain the protein in the endoplasmic reticulum. Phosphorylation exposes the cryptic mitochondrial targeting sequence to traffic the enzyme to mitochondria and once imported, the polypeptide is packaged with heme and deposited on the inner membrane of mitochondria. This attractive mechanism fits well with the data supporting a role for Protein Kinase A and cAMP signaling and avoids the need to traffic a folded CYP2E1 protein into mitochondria by having it be folded there *in situ*. Interestingly, phosphorylation of mtCYP2E1 within mitochondria is only 20–25%, which may reflect a dynamic equilibrium of the process in mitochondria. By contrast, phosphorylation at the same site when the protein is present in the endoplasmic reticulum results in rapid proteasomal degradation. This mechanism has some experimental support, but further work is needed to determine if phosphorylation is the only targeting cue to send CYP2E1 to mitochondria.

Together, these early characterizations provided insight into the molecular determinants of mitochondrial targeting of CYP2E1, and critical details about the similarity (and dissimilarity) of mtCYP2E1 to its much more well-studied counterpart in the endoplasmic reticulum. More work is needed to fully establish the specificity and efficiency of mitochondrial CYP2E1 and to determine its role in both endogenous and exogenous biochemical processes.

Toxicological consequences of mitochondrial CYP2E1 localization

In vitro evidence for mtCYP2E1-related toxicity

Early characterization of truncated and full-length mitochondrial CYP2E1 led to the generation of *in vitro* cell line reagents to study the biological consequences of mitochondrial CYP2E1

localization. The truncated form identified by the Ingelman-Sundberg group lacks amino acids 2–34 and was transfected into HepG2 human hepatoma cell lines to generate new cell lines, *i.e.* mE10 and mE24 cells, expressing the 40 kDa construct only in mitochondria,[138] although subsequent studies used only mE10 cells. The Avadhani group introduced mutations into the wild-type sequence to drive full-length CYP2E1 expression mainly into mitochondria in the COS-7 monkey kidney cell line (called MT+ and MT++, with medium and high basal (but not further inducible by ethanol) mtCYP2E1 expression levels, respectively).[132] While it remains unclear which cell model more accurately reflects *in vivo* mtCYP2E1 activity, both approaches, importantly, generated cell lines expressing catalytically active mtCYP2E1 and resulted in increased oxidative stress, as discussed herein.

For the HepG2 cell lines expressing CYP2E1, it became possible to evaluate the impact of mtCYP2E1 *vs* erCYP2E1 expression after the creation of HepG2-derived E47 cells expressing full-length ER-localized CYP2E1. For unknown reasons, CYP2E1 expressed in E47 cells was only present at the endoplasmic reticulum based on a lack of co-localization with mitochondrial SOD in immunofluorescence experiments. Both E47 and mE10 cells showed a similar increase in ROS upon depletion of glutathione with buthionine sulfoximine (BSO) that was greater than the control (non-CYP2E1 expressing cells), indicating that CYP2E1 can cause similar ROS generation from different subcellular compartments. The increased ROS in mtCYP2E1-expressing BSO-treated cells was accompanied by increased cell death, decreased mitochondrial membrane potential, increased mitochondrial protein oxidation and lipid peroxidation adducts, and impaired mitochondrial aconitase activity when compared to BSO-treated cells lacking CYP2E1. The mitochondrial membrane potential could be rescued by the addition of antioxidants like glutathione ester or Trolox or by the mitochondrial membrane stabilizer cyclosporine A. Taken together, these findings suggest that mtCYP2E1 localization could have deleterious effects; however, the study did not assess and compare those effects for erCYP2E1-expressing cells, making it impossible to understand the individual contributions of mtCYP2E1 and erCYP2E1 to toxicity.

Similarly, mutations to the N-terminal targeting sequence generated Mt+ and Mt++ COS-7 cell lines along with an ER+ mutant expressing full-length CYP2E1 localized primarily to the endoplasmic reticulum as a foundation for comparative studies on the importance of CYP2E1 subcellular localization. In untreated cells, wild-type and ER+ cells showed a mild depletion (15–20%) of cellular and mitochondrial glutathione, while the Mt+ and Mt++ cells showed a dramatic decrease in both glutathione pools (40–50%). Ethanol treatment further reduced GSH levels, with greater effects observed in Mt+ and Mt++ compared to wild-type and ER+ cells. Notably, these transgenic cells express high or very high levels of CYP2E1 and are not induced by ethanol exposure in contrast to typical endogenous expression of CYP2E1. Mt+ and Mt++ cells showed increased basal levels of ROS when compared to wild-type or ER+ cells, as measured by dichlorofluorescein (ROS-indicator) fluorescence. This effect could be attenuated by the addition of the CYP2E1 suicide inhibitor diallyl sulfide or the antioxidant *N*-acetyl cysteine. Treatment with ethanol increased ROS in Mt+ and Mt++ cells but not ER+ or wild-type cells, and the effect was blocked by addition of diallyl sulfide or *N*-acetylcysteine. This work provided the first direct comparison of the consequences of mtCYP2E1 and erCYP2E1 localization, suggesting that mitochondrial localization could result in greater cellular toxicity.

The level of mtCYP2E1 expression compared to erCYP2E1 could play a major role in determining its relative toxicological importance. A critical publication from 2013 showed that there is significant inter-person variability in the amount of CYP2E1 localized in the mitochondria and microsomes isolated from human liver.[139] For this study, human liver mitochondria and microsomes from 16 individual donors showed wide variability with some samples having mostly erCYP2E1 and others having mostly mtCYP2E1. This effect could be partially attributed to genetic polymorphisms in the N-terminal targeting sequence because when those mutations were introduced into the wild-type sequence, COS cells transfected with the mutated sequence showed the same pattern of localization as that observed in the human liver. Similar to the findings in other studies, increased mtCYP2E1 localization resulted in higher ROS, reduced mitochondrial membrane potential, altered mitochondrial respiration, and lower glutathione, particularly upon treatment with ethanol; those effects could be blocked by addition of diallyl sulfide or antioxidants.

In vivo studies linking mtCYP2E1 to toxicity

A study in streptozotocin (STZ)-induced diabetic rats showed that mitochondria and microsomes from pancreas, kidney, liver, and brain showed increased ROS production and lipid peroxidation compared to the control rats.[14] All of these tissues had measurable CYP2E1 protein (measured with western blot) and activity (dimethylnitrosamine *N*-demethylation activity) in mitochondria and microsomes from control animals, and elevated levels in diabetic animals. Similarly, there were higher Hsp70 protein and GST A4-4 protein/activity in mitochondria and cytosol, indicating increased oxidative stress response in the diabetic rat model. Under *in vitro* conditions, COS cells transiently transfected with CYP2E1 expressed both mtCYP2E1 and erCYP2E1, demonstrated by western blot. Those transfected cells showed similar increases in Hsp70 and GSTA4-4 compared to untransfected cells and suggest that CYP2E1 (particularly in mitochondria) may be playing some role in oxidative stress in diabetic conditions. For both the rat and cell culture experiments, wild-type CYP2E1 was present in mitochondria and microsomes, so that it is not possible to delineate the specific roles of mtCYP2E1 and erCYP2E1 in these stress responses.

Similar to the diabetic condition, ethanol has been shown to induce CYP2E1 in both mitochondria and microsomes. This induction correlates with increased oxidative stress in rats; however, the increased oxidative stress was attenuated by the addition of medium-chain triglycerides.[140] The mitochon-

drial-targeted antioxidant demethyleneberberine (DMB) from the Chinese herb *Cortex Phellodendri chinensis* also has a protective effect in acutely and chronically ethanol-treated mice.[141] DMB suppressed the 2–3-fold ethanol induction of mtCYP2E1 in liver mitochondria for both 52 kDa and 40 kDa enzyme forms (as shown by Western blot of purified mitochondrial fractions), as well as attenuated the 1.5-fold ethanol induction of overall total liver CYP2E1 (mt + erCYP2E1). The decreased mtCYP2E1 levels accompanied a decrease in oxidative stress, mitochondrial dysfunction, and liver injury. Because the authors did not investigate erCYP2E1 directly, it is not clear whether mtCYP2E1 decreased with a concomitant change in erCYP2E1. Furthermore, it is unclear whether decreases in mtCYP2E1 are protective, or simply accompanied a direct protection by medium-chain triglycerides or DMB. However, these studies do demonstrate that the regulation of mtCYP2E1 is somehow correlated with ethanol-induced liver toxicity.

In an *ex vivo* model, we recently showed that mitochondrial CYP2E1 activity corresponded with greater impairment of mitochondrial function in mice after exposure to 1,3-butadiene.[92] For this study, sixty individual strains of mice were used from the genetically diverse Collaborative Cross model to assess the impact of genetic variability in mtCYP2E1 expression on exposure-induced toxicity to mitochondria. Two ten-day exposure groups were compared: air (control) and 200 ppm inhaled butadiene. The butadiene-exposed mice showed impaired activity of mitochondrial respiratory complexes I, II, and IV compared to the control mice (about 30% reduction in the population mean), and that reduction was inversely correlated with mtCYP2E1 in butadiene-exposed mice. In other words, higher mtCYP2E1 activity corresponded to greater impairment of mitochondrial respiratory complex activity. By contrast, erCYP2E1 did not correlate with the impaired activity of any of the complexes. This finding is the first evidence that mtCYP2E1 and not erCYP2E1 in an *in vivo* model can drive mitochondrial damage caused by exposure to CYP2E1-activated substrates.

Together, these *in vitro* cell culture-based and *ex vivo* rodent model experiments have demonstrated that mtCYP2E1 localization could be a factor in driving cellular toxicity and especially mitochondrial dysfunction. This toxicological mechanism could be mediated through the generation of reactive metabolites from CYP2E1 substrates, both endogenous (*i.e.* lipid oxidation) and exogenous (see Fig. 2). Alternatively, the toxicity could mainly be driven by the production of reactive oxygen species by mtCYP2E1, or could be a combination of these two possibilities. Either way, mitochondrial targeting of CYP2E1 (Fig. 3) may prove to be a risk factor for pathogenesis, particularly etiologies that involve mitochondrial dysfunction.

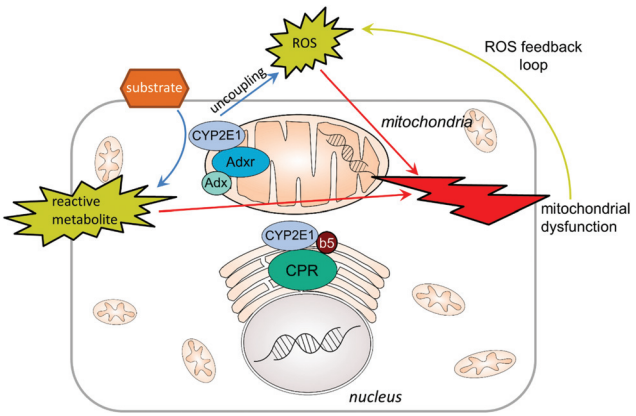

Fig. 2 Proposed mechanism of CYP2E1-driven mitochondrial dysfunction. As described in the text, CYP2E1 localized on the inner mitochondrial membrane can produce reactive metabolites through substrate oxidation and/or produce ROS when its catalytic cycle is uncoupled, damaging mitochondrial DNA, proteins, and lipids. In turn, this damage can cause increase ROS production from the mitochondrial electron transport chain, which can further damage mitochondria ultimately leading to mitochondrial dysfunction in the cell.

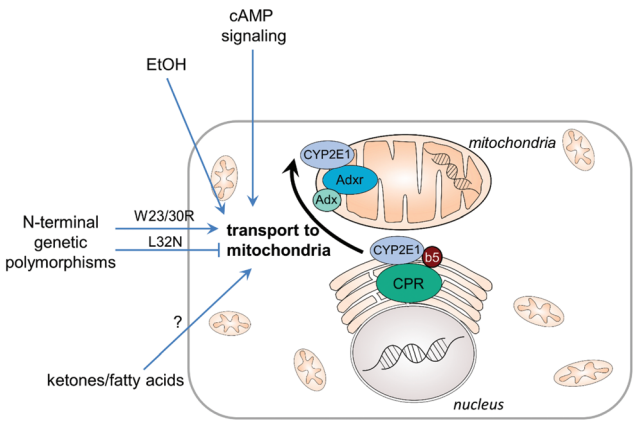

Fig. 3 Factors influencing transport of CYP2E1 to the mitochondria. The details of the cellular regulatory mechanisms governing CYP2E1 trafficking to the inner mitochondrial membrane are still not well understood; however, certain polymorphisms, ethanol, cyclic adenosine monophosphate (cAMP) signaling, and potentially ketones and fatty acids may impact the levels found in the mitochondrial compartment.

Unresolved questions about mtCYP2E1

Early characterization provided critical insights into the localization of CYP2E1 to mitochondria and proved that the enzyme could be active there for oxidation of CYP2E1 substrates. However, many questions still remain unresolved in the field to predict metabolic flux and physiological consequences of mitochondrial and endoplasmic reticulum-localized CYP2E1. Answers to questions in this section will help advance an understanding of the conditions that regulate CYP2E1 subcellular localization, its catalytic activity, and overall contribution to toxicological mechanisms.

What is the true molecular weight of mtCYP2E1?

Under what conditions is it truncated to 40 kDa, and what is the mechanism of CYP2E1 processing to the truncated form?

Does the 40 kDa form share the same secondary structure as the full-length form studied by Avadhani *et al.*, and do the full-length and truncated mtCYP2E1 enzymes have the same affinity for substrates and efficiency? It is possible that some antibodies may not recognize the truncated version so it could have been missed in Western blots if the authors were using different anti-CYP2E1 antibodies. These differences must be addressed in the future by gaining a mechanistic understanding of *how* and *why* a truncated 40 kDa CYP2E1 enzyme forms, rather than showing a presence or absence of truncation.

What are the physiological conditions that drive mtCYP2E1 localization?

Given its ability to metabolize ketones and fatty acids, it is conceivable that mitochondrial localization is driven by the need to oxidize these molecules. For example, it would be interesting to know if high ketone and fatty acid conditions change the localization of CYP2E1 (higher ratio of mitochondria/microsomal) rather than simply inducing the enzyme in both fractions. Do conditions known to induce CYP2E1 also alter mitochondrial localization? What is the endogenous role of CYP2E1 in mitochondria?

How is mitochondrial CYP2E1 regulated?

Many detailed and laborious studies have been carried out to determine the complex and extensive transcriptional, translational, and post-translational regulation of CYP2E1 localized in the endoplasic reticulum. However, very little is known about the regulation of mitochondrial CYP2E1. For example, we have recently observed that mitochondrial CYP2E1 has a very long half-life (>48 h) in cultured cells using a cycloheximide chase experiment (unpublished data, manuscript in preparation), compared to the bimodal half-life of erCYP2E1 consisting of a very rapid phase (~6–12 h half-life) and a longer phase (36–48 h). It is also not clear if the same mechanisms apply to induction of mitochondrial CYP2E1. For example, is the protein stabilized by bound substrates? Furthermore, what is the mechanism of mtCYP2E1 degradation? Is it degraded by the proteasome as erCYP2E1 is, or does it follow the same degradation pathways as many other mitochondrial proteins (*e.g.*, proteases or autophagy)? Finally, how are levels of CYP2E1 in individual mitochondria regulated? Is the distribution of CYP2E1 in the cell impacted by fission and fusion dynamics of mitochondria? And how could this impact mitochondrial dysfunction and cellular toxicity?

What is the coupling efficiency of mtCYP2E1?

Many studies detailing the stoichiometry of NADPH consumption, oxygen uptake, substrate oxidation, and the formation of reactive oxygen species have shown that the erCYP2E1 catalytic cycle is loosely coupled and leads to the formation of ROS in cells. However, such studies have not been done for mtCYP2E1, and therefore it is unknown if mtCYP2E1 produces ROS within mitochondria. Direct evidence must be acquired to answer this question; especially considering that mtCYP2E1 has a distinct structure compared to erCYP2E1 and associates with a different set of redox partners for its catalytic activity, it is unlikely that it will have the same coupling efficiency as erCYP2E1. This question could have broad implications given the importance of ROS in mitochondrial signaling and should be prioritized in future experiments on mtCYP2E1.

What determines CYP2E1 accessibility to substrates within the mitochondria?

The localization within mitochondria necessarily provides a different environment and specificity. Based on our general understanding of CYP2E1 specificity, there is a preference for binding of hydrophobic substrates and inhibitors. These properties make it likely that substrates/inhibitors pass through membranes, but how efficient is the process? How much reaches the inner membrane to create an accessible level for metabolism by CYP2E1?

What is the actual level of NADPH available to drive mtCYP2E1 metabolism?

Most activity assays performed with mitochondrial CYP2E1 have required addition of NADPH and often exogenous cofactors including ADX and ADR. Therefore, the measured activities may not reflect the true values within cells. The mitochondrial pool of NADPH is distinct from the cellular pool and is highly regulated. The major source of mitochondrial NADPH is from the conversion of NADH and NADP+ to NADPH by nucleotide transhydrogenase (NNT), which is highly efficient in mitochondria with an intact membrane potential. When there is a loss of proton gradient, this process is inhibited. Under these conditions, would the NADPH pool limit CYP2E1 activity?

How do differences in antioxidant capacity between subcellular compartments contribute to differential toxicity?

The anti-oxidant capacity differs between the cytosol and mitochondria and may play a role in the biological significance of mt- and erCYP2E1 activity. For example, glutathione, one of the most important antioxidant mechanisms in cells, is maintained in separate pools within the cell. The cytosol contains 85–90% of total glutathione cellular content;[142] however, the nucleus, endoplasmic reticulum, peroxisomes, and mitochondria also have stores of glutathione.[143] Although the mitochondrial portion of the cellular glutathione is only 10–15% of the total cellular pool, the concentrations are equal or greater than the cytoplasmic concentrations.[144] Furthermore, the selective depletion of the mitochondrial, not cytosolic, glutathione pool results in oxidative stress and cell death.[145] Glutathione status, in addition to the differential expression of antioxidant enzymes in different cellular compartments, is inexorably linked to CYP2E1 toxicity through Nrf2.[104] Subcellular compartment-specific antioxidants could be a key factor in determining the propensity of mtCYP2E1-derived reactive metabolites to cause damage to mitochondria. This intriguing possibility should be explored in future research.

What role does mtCYP2E1 play in liver diseases?

As described herein, the majority of studies involving mtCYP2E1 have focused on the liver. There is still relatively little known about its role in hepatic disease despite major advances in our understanding of the molecular basis for CYP2E1 localization and characterization of the enzyme. mtCYP2E1 has been shown to increase ROS and oxidative stress, which are known factors in the pathogenesis of fatty liver disease including alcoholic and non-alcoholic forms of the disease. Furthermore, it has been increasingly recognized that mitochondrial dysfunction is a hallmark of cancer, and particularly important in hepatocellular carcinoma.[146–148] However, a potential specific role of mtCYP2E1 in contributing to this mitochondrial dysfunction in hepatic cancer or fatty liver disease has not been identified.

What is the role of mtCYP2E1 in heart disease?

Even less is known about the role of mtCYP2E1 in extrahepatic tissues. In the heart, CYP2E1 is strongly up-regulated in murine models of dilated cardiomyopathy.[149] Furthermore, overexpression of CYP2E1 in heart tissue exacerbates cardiomyopathy, drives mitochondrial dysfunction, and increases ROS and lipid peroxidation. Similarly, inhibition of CYP2E1 decreased the myocardial dysfunction induced by chronic alcohol intake in mice.[150] Finally, in contrast to the laboratory studies, the high activity/transcription CYP2E1 DraI and PstI/RsaI polymorphisms in human studies in a Lebanese population were associated with a significant decrease in coronary artery disease.[151] All of this evidence supports some role for CYP2E1 in heart disease; however, to our knowledge no one has investigated the localization of CYP2E1 in these tissues. It is conceivable that mitochondrial expression of CYP2E1 in the mitochondria-rich cardiomyocytes could be significantly contributing to these pathologies. An *in vivo* model lacking CYP2E1 with transgenic expression of mitochondrial CYP2E1 in the heart would be ideal to study these effects.

How does mitochondrial CYP2E1 contribute to cisplatin-induced kidney injury?

As described previously in the review, CYP2E1 has been clearly implicated as a key player in cisplatin-induced nephrotoxicity, through a mechanism that involves mitochondrial dysfunction associated with a high burden of cellular and mitochondrial ROS.[37–40] Importantly, CYP2E1-null mice are protected from this nephrotoxicity.[36] It is therefore a logical and interesting question to ask if it is mtCYP2E1 specifically that is driving this toxicity, and if inhibition of mtCYP2E1 could be protective. An important related question should be, however, if mtCYP2E1 also contributes to the cytotoxicity in cancer cells. Sensitization to cisplatin has been observed for sulforaphene, mitaplatin, and resveratrol through induction of mitochondrial dysfunction.[152–154] Therefore, if cancer cells are expressing mtCYP2E1, they may be more sensitive to cisplatin-induced mitochondrial dysfunction and cell death. However, most cancer cells downregulate xenobiotic-metabolizing enzymes, so if the cancer cells lack mtCYP2E1 then inhibition of mtCYP2E1 may protect against nephrotoxicity without sacrificing chemotherapeutic efficacy. Careful future research is needed to establish the relevance of this mechanism.

What are the implications for mtCYP2E1 in the brain?

CYP2E1 is expressed throughout the brain and is highly inducible by chemical inducers of CYP2E1,[155,156] pathophysiological conditions known to induce CYP2E1,[157] and even by status epilepticus (severe epileptic seizures).[158] CYP2E1 has also been detected in brain mitochondria,[159,160] although the specific role of mtCYP2E1 in neurotoxicity has not been explored. Nevertheless, as discussed in this review, many CYP2E1 substrates have been linked to both mitochondrial dysfunction and neurotoxicity/neurodegeneration; therefore, it is a critical future area of concern to determine the specific role of mtCYP2E1 in driving mitochondrial dysfunction in the brain.

Concluding remarks

Together, the data summarized herein provides a foundation for the role of mitochondria-localized CYP2E1 in driving mitochondrial dysfunction. Additionally, it highlights significant gaps in our understanding of mtCYP2E1 that must be addressed, from basic biological questions to the relationship between mtCYP2E1, mitochondrial dysfunction, and many pathologies. Furthermore, investigation of mtCYP2E1 will potentially provide some new and interesting opportunities for intervention. Because there is significant inter-individual variation in the localization of CYP2E1, if mtCYP2E1 is specifically driving pathogenesis it could be important to stratify the population to estimate risk. Alternatively, it may be possible to specifically target mitochondrial CYP2E1 pharmacologically: first, by targeting specific inhibitors of CYP2E1 to mitochondria, or second, by driving CYP2E1 localization toward the ER. Overall, it will be important to consider both mtCYP2E1 and erCYP2E1 for prediction of toxicological outcomes and pharmacological interventions, as the field has clearly demonstrated a distinct role for CYP2E1 in each compartment.

Conflict of interest

The authors have no conflicts of interest to declare.

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