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Cytochrome P450 2E1 and Its Roles in Disease

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Abstract

Cytochrome P450 (P450) 2E1 is the major P450 enzyme involved in ethanol metabolism. That role is shared with two other enzymes that oxidize ethanol, alcohol dehydrogenase and catalase. P450 2E1 is also involved in the bioactivation of a number of low molecular weight cancer suspects, as validated *in vivo* in mouse models where cancers could be attenuated by deletion of *Cyp2e1*. P450 2E1 does not have a role in global production of reactive oxygen species but localized roles are possible, e.g. in mitochondria. The structures, conformations, and catalytic mechanisms of P450 2E1 have some unusual features among P450s. The concentration of hepatic P450 varies ≥ 10-fold among humans, possibly in part due to single nucleotide variants. The level of P450 2E1 may have relevance in the rates of oxidation of drugs, particularly acetaminophen and anesthetics.

Keywords

Cytochrome P450; P450 2E1; CYP2E1; ethanol; nitrosamines; kinetic isotope effects; SNP; SNV

1. Introduction to cytochrome P450 enzymes

Cytochrome P450 (P450, CYP) enzymes are responsible for ~ 95% of the oxidation and reduction of chemicals, based on all literature citations [1]. These enzymes were first studied because they are the main catalysts involved in the metabolism of drugs, steroids, fat-soluble vitamins, chemical carcinogens, industrial chemicals, and other entities [2]. In humans there are 57 P450, or *CYP*, genes, but the more than 380,000 P450 genes are found throughout nature and are responsible for many biosynthetic reactions [3, 4].

A major area of interest regarding human P450s is the relationship to disease. One major issue is single nucleotide variants in the population that link to lack of function and lead to

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Declaration of interests

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maladies. Classic examples are seen in endocrinology due to defects in steroid metabolism [5]. Another area of interest is nearly the opposite, in a sense, where drugs are used to inhibit a P450 in certain medical conditions, e.g. blocking P450 19A1 (steroid aromatase) to lower estrogen levels in breast cancer [6, 7].

What has been more difficult is to define the contributions of human P450s in cases where the roles may be less obvious, e.g. chemical carcinogenesis [8]. Although studies with experimental animals were quite definitive in associating cancer with P450 expression [9] and these findings have been extended in very elegant matter with transgenic animals [10, 11], application to problems in human medicine has been less straightforward. It is in this latter category that P450 2E1 fits.

2. Background on P450 and ethanol oxidation

Historically most of the interest in ethanol oxidation involved alcohol and aldehyde dehydrogenases [12-14]. Catalase can also oxidize ethanol [15].

In 1965 Orme-Johnson and Ziegler [16] demonstrated the NADPH-dependent oxidation of ethanol and methanol in rat, rabbit, and hog microsomes. Interestingly, the system was not inhibited by carbon monoxide and no activity was seen with longer-chain alcohols. Similar activity was also reported by Lieber and DeCarli [17], who also found that the activity was enhanced by feeding ethanol to rats. The work in the initial report was expanded in 1970 [18] and the activity was found to be inhibited by carbon monoxide.

A role for P450 in oxidation was difficult to accept, in light of the general nature of the known larger and more hydrophobic substrates. However, Mezey *et al.* [19] reported that a partially purified rat liver P450 preparation could oxidize ethanol to acetaldehyde. In 1978 Miwa *et al.* [20] could demonstrate ethanol oxidation by highly purified rat and rabbit liver P450 preparations, demonstrating a lack of both alcohol dehydrogenase and catalase in their systems.

Coon’s laboratory isolated a P450 enzyme from ethanol-treated rabbits (3a, now known as 2E1) that oxidized ethanol [21]. P450 2E1 (then termed P450j) was also purified and characterized from rat and human liver [22]. In 1986 Gonzalez’s group [23] cloned the rat and human cDNAs for P450 2E1 and later characterized the gene [24].

Subsequent work by Gonzalez and others revealed the complexity of mechanisms involved in the regulation of P450 2E1 [25]. The *in vivo* activity is difficult to study because ethanol is both an inducer and an inhibitor of the enzyme. P450 2E1 is quite different in its regulatory mechanisms from many other major P450 enzymes involved in the oxidation of xenobiotics, which are dominated largely by nuclear receptor activation mechanisms. Transcriptional regulation is involved, at least in part [26]. There is also a role of ethanol in stabilization of the protein and a role of the proteasome. The difficulties in studying regulation are exemplified by the induction with placental lactogen and through the phosphoinositol 3-kinase pathway [27]: *CYP2E1*-humanized mice did not exhibit enhanced P450 2E1 expression during pregnancy due to interspecies differences in placental lactogen physiology.

A significant development in the field was the generation of *Cyp2e1*$^{-/-}$ knockout mice by the Gonzalez laboratory, which have proven to very useful in evaluation of *in vivo* roles of the enzyme, at least in mice. These have proven to be valuable in the assessment of the role of mouse P450 2e1 and human P450 2E1 in the metabolism of drugs and potentially toxic chemicals [11, 28-31]. Further, "humanized" P450 2E1 mice (devoid of mouse P450 2e1, but expressing human P450 2E1) have been very useful [32, 33]. A biomarker of P450 2e1 (2-piperidone) has been identified using these animals (although it is not known if this is useful in humans) [34]. There are still some caveats in interpretation of results with these animals though, e.g. P450 2E1-humanized mice showed acute proximal renal tubule injury (as did wild-type control mice) but did not show hepatic lipid accumulation with high doses of perchloroethylene [33].

3. Metabolism of drugs by P450 2E1

Probably because of its preference for small molecules, P450 2E1 does not contribute to the metabolism of many drugs. One group of drugs where P450 2E1 is a major factor is anesthetics (Figure 1). P450 2E1 is a major factor in the disposition of halothane, isoflurane, sevoflurane, enflurane, and desflurane [35-39].

Another low molecular weight drug that P450 2E1 is involved in the metabolism of is acetaminophen. In mice, P450 2e1 is a major factor in toxicity, as shown with transgenic animals [29, 30]. Of interest is a report in which very obese humans were shown to have elevated levels of acetaminophen-derived cysteine conjugates, ascribed to P450 2E1 oxidation, as a result of elevated P450 2E1 levels [40].

Another issue with anesthetics is with halothane, a substrate for P450 2E1 (Figure 1). A large fraction of patients with halothane-induced hepatitis have autoantibodies that recognize P450 2E1 [41, 42]. These antibodies are also found in anesthesiologists, presumably due to exposure (to halothane?), but even in the absence of injury [43]. The role of the antibodies in the etiology remains unclear, as has been the case with other drugs whose use is associated with autoantibodies [44].

4. P450 2E1 and carcinogen metabolism

Early work by Wrighton *et al.* [22] and in Yang's laboratory [45] showed the involvement of what is now known as P450 2E1 in the metabolic activation of *N*,*N*-dimethylnitrosamine and other carcinogenic nitrosamines, the oxidation of which had been difficult to identify catalysts for [46]. The toxicology literature had numerous reports of the effects of ethanol and disulfiram (Antabuse®) on the metabolism and carcinogenicity of various chemicals (e.g., ethylene dibromide [47]), which had been difficult to explain. P450 2E1 was demonstrated to be involved in the metabolism of a variety of low molecular weight chemicals, many of which are cancer suspects, including not only the smaller alkyl nitrosamines but also vinyl monomers, alkyl halides, and others (Figure 2) [48-50].

An interesting case is urethane, or ethyl carbamate. Work in the Millers' laboratory had suggested that vinyl carbamate might be formed and then converted to an epoxide, which could modify DNA [51], but it was difficult to detect vinyl carbamate. Subsequently our

laboratory was able to demonstrate that P450 2E1 could slowly desaturate ethyl carbamate and then the resulting vinyl carbamate was rapidly oxidized by the same enzyme to the epoxide, which reacts with DNA to form etheno adducts (Figure 3) [52]. In mice the expression of P450 2e1 is involved in urethane-induced lung tumors [53].

Ethanol treatment has also been shown to increase the levels of etheno adducts ~ 2-fold in mice treated with high chronic doses of ethanol, presumably due to P450 2e1 induction [54-56].

The significance of P450 2E1 in carcinogen metabolism has been demonstrated with knockout mouse models. For instance, *Cyp2e1*$^{-/-}$ mice were not susceptible to benzene toxicity or genotoxicity [28]. It is also possible to interpret the effects of P450 2E1 inhibitors. For instance, disulfiram increased the carcinogenicity of ethylene dibromide by blocking the detoxication and thus making more of the compound available for bioactivation by a glutathione conjugation pathway [57]. In a similar way, knocking out *Cyp2e1* in mice diverted the pathway for trichloroethylene from oxidation to conjugation [33]. Dietary ethanol enhanced the levels for O^6-methyldeoxyguanosine adducts in rats treated with the carcinogen *N*,*N*-dimethylnitrosamine [54], presumably due to P450 2E1 induction.

5. Reactive oxygen species (ROS)

The literature is replete with discussion of ROS production due to P450 2E1. The vast majority of this has been developed *in vitro* with liver microsomes [58, 59] and cultured cells [60]. Many of the commonly used ROS assays are not validated, e.g. *in vivo* malondialdehye assays [61] and dichlorofluorescein fluorescence [62]. In the ROS field, the most appropriate “gold standard” for ROS is F_2-isoprostane production, which can be measured both *in vitro* and *in vivo* [63, 64].

We questioned whether P450 2E1 (2e1 in mice) was really so highly uncoupled and could produce large scale levels of ROS. Treatment of animals with ethanol is complicated in that it can be both an inducer and inhibitor (*vide supra*). Accordingly, we treated rats with the P450 2E1 inducer isoniazid and did not see an increase in F_2-isoprostane levels [65]. Subsequent work showed that *Cyp2e1*$^{-/-}$ mice had very similar levels of liver, brain, and urinary isoprostanes as the wild-type animals [66]. P450 2E1 does not appear to increase global levels of ROS, and any increases due to ethanol treatment are not related to P450 2E1 induction. Although purified P450 2E1 is not well-coupled to NADPH consumption, neither are several other P450s that we and others have examined [67-69]. Others have suggested that ROS production may be coupled to the production of $CH_3CHO\cdot$ radicals [70, 71].

Although P450 2E1 does not appear to be involved in large changes in global ROS, the results do not rule out the possibility of localized ROS production. In this regard, mutation of the N-terminal sequence of rat P450 2E1 or protein kinase A-mediated phosphorylation of Ser-129 enhanced mitochondrial translocation of the protein due to enhanced affinity for binding to the HSP70 chaperone protein [72]. Hepatic mitochondria isolated from ethanol-treated rats showed enhanced isoprostane levels after eight weeks, but microsomes did not [73]. The relevance of the enhanced mitochondrial ROS production may be seen in the

distribution of mitochondrial versus microsomal localization of P450 2E1 in human liver samples, i.e. in some individuals a large fraction of P450 2E1 was localized in mitochondria [74]. The ROS may be the result of poor (mitochondrial) P450 2E1 coupling with the alternate electron transfer accessory protein adrenodoxin.

6. Structures of P450 2E1

Some X-ray crystal structures of P450 2E1 have been published by Scott and associates [75-77]. These include both a small ligand and a larger one, a fatty acid.

The existence of multiple conformations of the enzyme raises questions about the origin of these. Two general models can explain the results. In a conformational selection model an equilibrium exists between different conformations, in the absence of ligands, and one of these binds the ligand [78]. Alternatively, in a true induced fit model there is a single conformation of the unbound enzyme, and the initial binding of the ligand to the enzyme induces a conformation change that changes the enzyme into a more efficient catalytic state (Figure 4) [79, 80]. Several human P450s have now been shown to operate primarily through conformational selection modes [81, 82], but kinetic studies with P450 2E1 and its substrate hexyl isonicotinate could not distinguish between the conformational selection and induced fit models for P450 2E1 [82].

7. Catalytic mechanism

The catalytic mechanism involves the usual steps of substrate binding, reduction of ferric iron to ferrous, O_2 binding, introduction of a second electron into the iron-oxygen complex, transformation of the iron-oxygen complex to Compound I (FeO^{3+}), abstraction of a hydrogen atom, oxygen rebound, and product release (Figure 5) [83, 84].

There are two anomalies regarding catalytic mechanisms of P450 2E1. The first is a role for cytochrome b_5 (b_5), which seems to be the case in most reactions [85-89]. In contrast to several other P450s, apo-b_5 (devoid of heme) is ineffective in stimulating (chlorzoxazone 6-hydroxylation catalyzed by) P450 2E1 [89] and the conclusion is that electron transfer to the $Fe^{2+}O_2$ complex is done by b_5.

Another anomaly is the kinetic deuterium isotope effect on C-H bond-breaking. We were lead to pursue this research area because of microsomal results on the oxidation of *N,N*-dimethylnitrosamine showing a kinetic deuterium isotope effect on K_m and not V_{max} [90], plus the newer knowledge that P450 2E1 was a major P450 enzyme involved in that reaction [45]. We initiated studies with human P450 2E1 and the oxidation of ethanol, finding the same pattern of a strong kinetic isotope effect on K_m but not k_{cat} [87]. The basis of this effect was attributed to the “burst kinetics,” i.e. a rate-limiting step following product formation, which results in expression of the kinetic deuterium isotope effect in the K_m [87]. Pre-steady-state kinetic measurements showed the isotope effect on C-H bond-breaking [87]. Accordingly, consideration of the relevant expression shows that K_m is not an independent parameter [91].

The kinetics of P450 2E1, with a rate-limiting step, after product formation, produce some kinetics that can be considered unusual. With a simplified system

$$\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\leftrightarrows}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\leftrightarrows}} \mathrm{EP} \underset{k_{-3}}{\overset{k_3}{\leftrightarrows}} \mathrm{E + P}$$

where S is the substrate and P the product,

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3}$$

[87, 92] and

$$K_m = \frac{k_3(k_{-1} + k_2)}{k_1(k_2 + k_3)}$$

If $k_3 > k_5$ then these reduce to

$$k_{cat} \cong k_3$$

and

$$K_m \cong \frac{k_3 k_{-1}}{k_1 k_2}$$

As discussed elsewhere [87, 93], this analysis has a number of implications for kinetic deuterium isotope effects.

The kinetics is also relevant to inhibition. For instance, consider a model reaction (Figure 6), simulated in KinTek software [94]. A system was set up with the following rate constants

$$\mathrm{E + S} \underset{10^2\ \mathrm{s^{-1}}}{\overset{10^7\ \mathrm{M^{-1}\,s^{-1}}}{\leftrightarrows}} \mathrm{ES} \xrightarrow{50\ \mathrm{s^{-1}}} \mathrm{EP} \xrightarrow{1\ \mathrm{s^{-1}}} \mathrm{E + P}$$

and

$$\mathrm{E + I} \underset{5\ \mathrm{s^{-1}}}{\overset{10^7\ \mathrm{M^{-1}\,s^{-1}}}{\leftrightarrows}} \mathrm{EI}$$

where E is P450 2E1, S is the substrate, P is the product, and I is an inhibitor so that the designed K_d for S is 10 μM and the K_i (K_d for I) is 5 μM. The fits (Figure 6) give k_{cat} = 0.02 s^{-1}. As shown in Figure 6 the actual K_d values for S and I are 10 μM and 0.5 μM, respectively, as set up in the model. The K_m value for S is 0.41 μM (Figure 6C) and the value of K_i calculated using a classic competitive inhibition model is 0.056 μM. Thus, due to

the kinetic nature of the system, the K_m and K_i values are much lower than the actual affinities (i.e., dissociation constants) for S and I.

8. Single nucleotide variants (SNVs)

The number of reported SNVs for the *CYP2E1* gene is relatively small, with only 19 reported in www.pharmvar.org/gene/CYP2E1 (Table 1). (Note: the term SNV is used instead of single nucleotide polymorphism, which by definition is a variant found at a frequency of ≥1% in a population.) Of these, there are only four with distinct amino acid sequence changes (CYP2E1.1 and R76H, V389I, and V179I). Of the SNVs, the specific catalytic activity (chlorzoxazone 6-hydroxylation) was similar to CYP2E1.1 but the level of expression in COS-7 cells was 40% that for CYP2E1.1, which is not a large change [95].

Whether the non-coding region changes have effects on expression levels or not is unknown. In a review in 2015, Daly [8] considered a *5 (*rs2031920*) variant ("RasI") and its relationship to lung cancer, although the results seem to be equivocal [96-98]. A decreased risk was seen with the SNV in a meta analysis of 15 studies but only in Asians [98], and no mechanism is proposed. Hakenewereth et al. [99] identified two minor alleles (*rs38138675, rs8192772*) that had odds ratios of 1.6-2.0 for decreased head and neck cancer survival, but these are also non-coding region differences. These were not among the eight *CYP2E1* loci identified in a more recent genome-wide association study (for oral/pharyngeal cancer) by the same author [100].

A PubMed search for CYP2E1 polymorphisms and diseases yielded 493 hits. Included among the study topics were alcoholism, systolic disfunction, coronary artery lesions, ischemic stroke, liver function, pre-term birth, semen quality, hepatitis, gout, oral leukoplakia, lupus, pancreatitis, Parkinson's disease, non-alcoholic fatty liver disease, hypertension, oral fibrosis, leprosy, tuberculosis, schizophrenia, endometriosis, chronic obstructive pulmonary disease, oral cleft, cirrhosis, gastritis, amyotrophic lateral sclerosis, and various cancers including bladder, lung, gastric, Hodgkin's and non-Hodgkin's lymphoma, cervical, leukemia, head and neck, stomach, prostate, ovarian, breast, liver, and pancreatic. As pointed out by Daly [8], there may prove to be some associations with alcoholic liver disease and nasopharyngeal and lung cancer, but overall the associations are still not very striking. It is possible that stronger associations may be seen in future studies.

9. Conclusions and Future Directions

More than fifty years after the first evidence that a microsomal oxidation system might be involved in ethanol metabolism, what have we learned? We definitely know that some P450s can oxidize ethanol to acetaldehyde, the major one being P450 2E1. P450 2E1 appears to be one of the more invariant P450s across species [101], but it does not have a critical role in physiology as judged by the mouse *Cyp2e1*$^{-/-}$ phenotype [29]. P450 2E1 can contribute to *in vivo* ethanol metabolism, although this role is shared with alcohol dehydrogenase and possibly other systems. We know that the structure of P450 2E1 is such that it can explain the preference of this P450 in oxidizing small molecules [48, 75], but the enzyme can also change conformations and expand its active site to accommodate larger molecules [76, 82].

P450 2E1 can oxidize acetaldehyde to acetic acid [88, 102, 103], and the kinetics of this process are also unusual [88]. In our experience, the appearance of a rate-limiting step after product formation [87, 88] is still unusual among P450s and is not explained by product affinity in the case of acetaldehyde [104].

Although localized production of ROS can be attributed to P450 2E1, its contribution to systemic ROS cannot, at least in rodents [66]. P450 2E1 clearly contributes to the metabolism of many cancer suspects, including vinyl monomers, halogenated hydrocarbons, and dialkylnitrosamines [45, 48, 105]. Epidemiological studies have not revealed strong associations between SNVs and cancer or any other diseases to date, but it is possible that analysis of subsets of the population exposed to known pro-toxicants (e.g., vinyl chloride) might be more revealing if enough individuals could be identified. One issue is that the number of known SNVs is small and knowledge of their effects on enzyme function is limited. The regulation of expression—and inhibition—are complex in animal models [25]. The concentration of human P450 2E1 does vary considerably (≥ 10-fold) [106-108]. It is possible that a phenotypic analysis of P450 2E1 (function) (e.g., chlorzoxazone phenotyping) [109] might be more revealing in the analysis of disease states with P450 2E1.

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Abbreviations used:

P450 (or CYP)	cytochrome P450
ROS	reactive oxygen species

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Highlights:

- P450 2E1 contributes to ethanol metabolism.
- P450 2E1 also has a role in the oxidation of some drugs and chemical carcinogens.
- P450 2E1 does not cause global oxidative stress but it can be local.
- The kinetic mechanism is unusual and leads to some apparent anomalies.
- Single nucleotide variations exist but have not been implicated in diseases much.

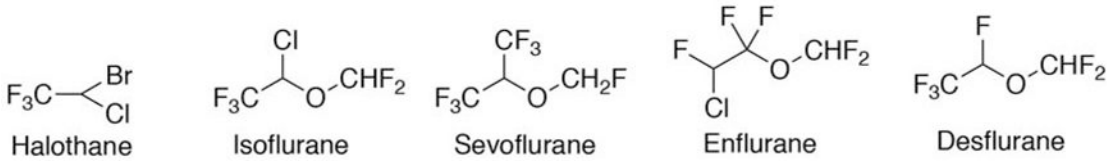

Figure 1.
Anesthetic substrates for P450 2E1.

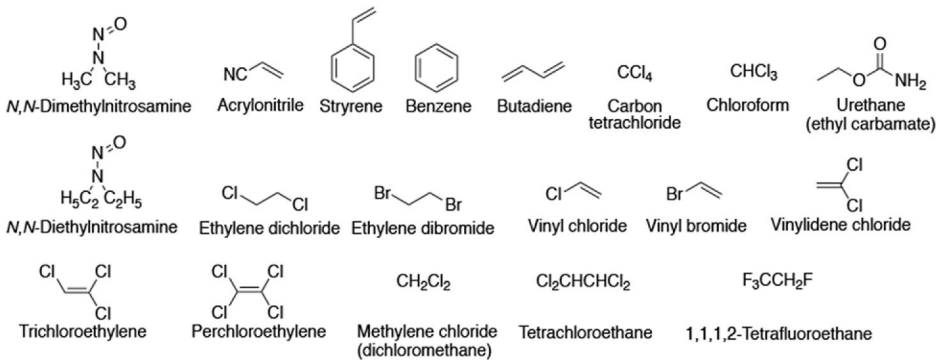

Figure 2.
Chemical cancer suspect substrates for P450 2E1 [48, 50].

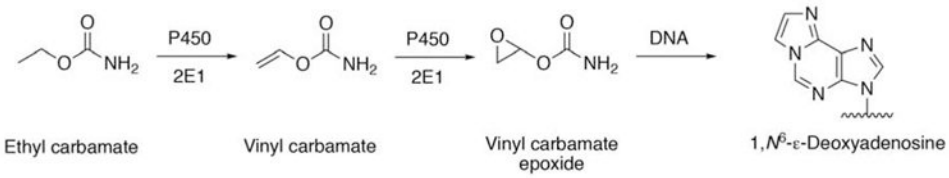

Figure 3.
Activation of ethyl carbamate (urethane) by P450 2E1 [51-53].

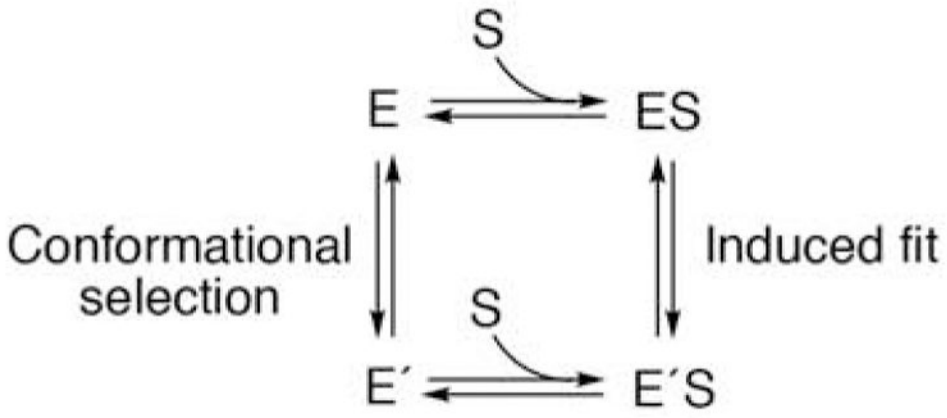

Figure 4.
Thermodynamic box for complex substrate binding possibilities. The free energy for the conversion of E to E'S is identical for the conformational selection and induced fit routes [81].

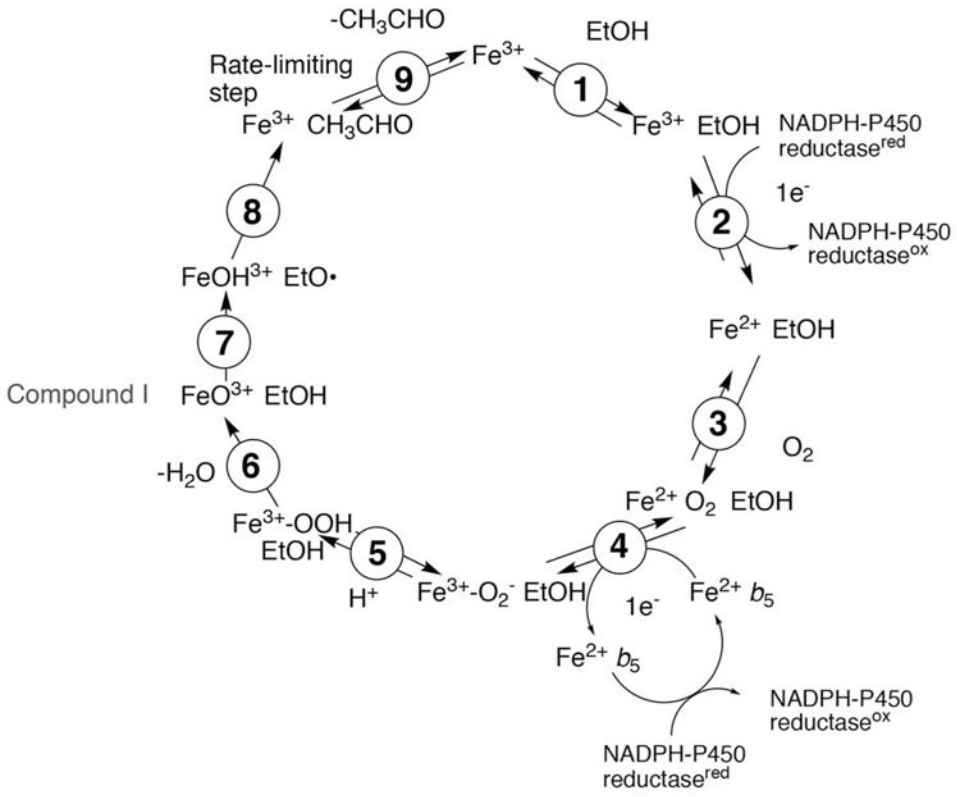

Figure 5.
P450 catalytic cycle with P450 2E1 features.

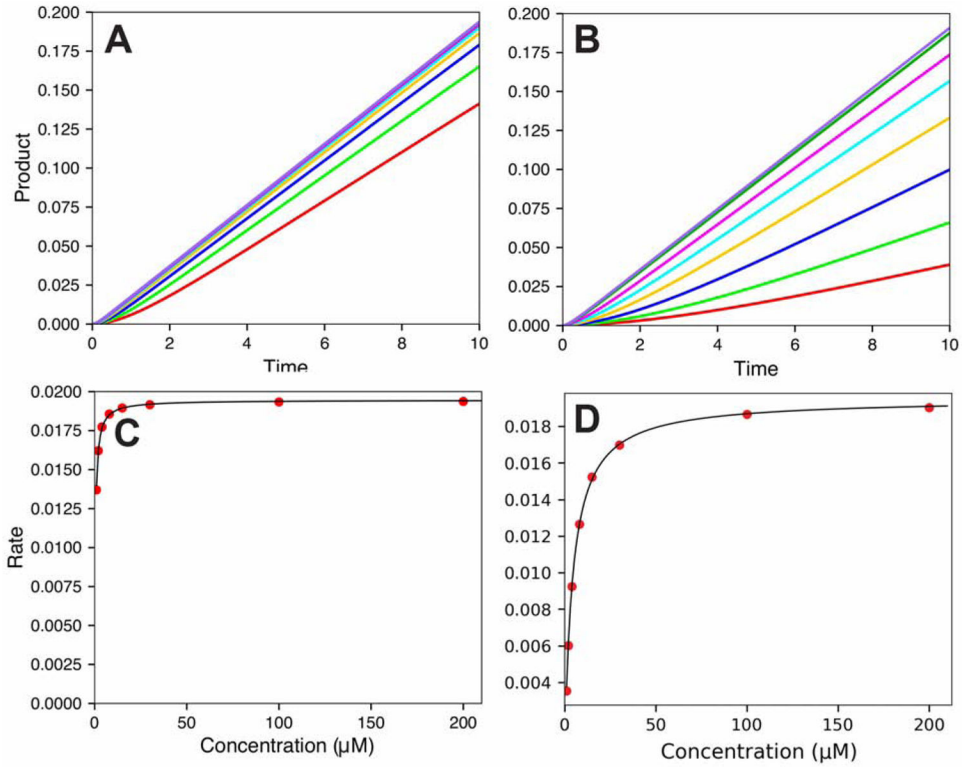

Figure 6.

Simulated plots for a reaction with a rate-limiting step following product formation. The model used in KinTek Explorer 8.0 was

$$\mathrm{E + S} \underset{10^2\,\mathrm{s^{-1}}}{\overset{10^7\,\mathrm{M^{-1}\,s^{-1}}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{50\,\mathrm{s^{-1}}} \mathrm{EP} \xrightarrow{1\,\mathrm{s^{-1}}} \mathrm{E + P}$$

$$\mathrm{E + I} \underset{5\,\mathrm{s^{-1}}}{\overset{10^7\,\mathrm{M^{-1}\,s^{-1}}}{\rightleftharpoons}} \mathrm{EI}$$

and the S concentrations used were 1, 2, 4, 8, 15, 30, 100, and 200 μM, with E = 0.1 μM and the time indicated in seconds. Rates of product formation increased with the substrate concentration. *A*, traces of P formation versus time without I. *B*, traces of product formation versus time in the presence of 5 μM I. *C*, rates of product formation in the absence of I. D, rates of product formation in the presence of 5 μM I. The K_m in the absence of I was 0.41 μM (Part C) and the K_m, apparent in the presence of 5 μM I was 4.4 μM, and using the standard equation for competitive inhibition

$$v = \frac{k_{cat}[S]}{Km\left(1 + \frac{[I]}{Ki}\right) + [S]}$$

the K_i was calculated to be 0.056 μM. These values may be compared to the actual K_d values for S (10 μM) and I ((0.5 μM) from the model.

Table 1.

CYP2E1 alleles (www.pharmvar.org/gene/CYP2E1) (accessed 11 December 2019)

Allele	**Protein**	**Nucleotide changes, Gene**	**Effect**	**Enzyme activity**	
*CYP2E1*1A*	CYP2E1.1	None		*In vivo*	*In vitro*
*CYP2E1*1B*	CYP2E1.1	9896C>G		Normal	Normal
*CYP2E1*1C*	CYP2E1.1	6 repeats in the 5' flanking region			
*CYP2E1*1Cx2*	CYP2E1.1				
*CYP2E1*1D*	CYP2E1.1	8 repeats in the 5' flanking region		Increased activity after alcohol exposure and in obese subjects	
*CYP2E1*2*	CYP2E1.2	1132G>A	R76H		Reduced
*CYP2E1*3*	CYP2E1.3	10023G>A	V389I		Normal
*CYP2E1*4*	CYP2E1.4	4768G>A	V179I		Normal
*CYP2E1*5A*	CYP2E1.1	−1293G>C; −1053C>T (c1>c2); 7632T>A			
*CYP2E1*5B*	CYP2E1.1	−1293G>C; −1053C>T (c1>c2)			
*CYP2E1*6*	CYP2E1.1	7632T>A			
*CYP2E1*7A*	CYP2E1.1	−333T>A			
*CYP2E1*7B*	CYP2E1.1	−71G>T; −eeeT>A			
*CYP2E1*7C*	CYP2E1.1	−333T>A; −352A>G			
Additional SNVs, where the haplotype has not yet been determined					
		6431C>A			
		9630T>G			
		1031C>T; 1199G>A; 1316C>T; 4451C>G; 4486G>T; 4529C>T; 4696G>A; 4845T>C; 4904T>C; 5625G>A; 6317C>T; 9745C>T; 9987C>G; 11024C>G; 11276T>C; 11356A>C			
		11112A>T	H457L		