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On the General Mechanism of Selective Induction of Cytochrome P450 Enzymes by Chemicals: Some Theoretical Considerations

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Abstract

Importance of the field—The cytochrome P450 (CYP) isoforms that are selectively induced following exposure to structurally-diverse chemicals often are the ones capable of metabolizing these chemicals. However, the molecular mechanism underlying this apparent functional coupling is not understood at present.

Areas covered in this review—Three hypotheses are developed to explain the complex process of selective chemical induction of CYPs: (i) each inducible CYP may have a corresponding intracellular receptor that interacts with the inducer chemical and mediates the selective induction of this CYP, (ii) each inducible CYP and its corresponding receptor may share a highly similar steric structure for their substrate/inducer binding sites, and (iii) each chemically-inducible CYP gene may have distinct genomic response element(s) that interact selectively with the corresponding receptor.

What the reader will gain—The readers are introduced to a novel theoretical framework that offers a plausible mechanistic explanation at the molecular levels concerning the complex process of how an organism selectively activates the biosynthesis of certain CYP isoform(s) that can effectively metabolize a chemical to which the organism is exposed.

Take home message—The theoretical framework developed herein seeks to ignite additional critical thinking on this important research subject as well as to promote experimental testing of the proposed theories in the future. Undoubtedly, these studies will enhance the understanding of the molecular mechanisms for the selective induction of CYP enzymes by chemicals.

Keywords

Cytochrome P450 isoform; chemical inducers; selective enzyme induction; Ah receptor; nuclear receptors; mechanism of enzyme induction

1. INTRODUCTION

It is estimated that at least several dozens of cytochrome P450 (CYP¹) isoforms exist in animals and humans [1,2]. These structurally-related hemeprotein monooxygenases fulfill many important biological functions, which include, but are not limited to, synthesis of bioactive compounds (such as steroid hormones) and biotransformation of a vast array of endogenous and exogenous chemicals [3-9]. One of the most well-known features of this enzyme system perhaps is the selective induction (*i.e.*, induced *de novo* biosynthesis) of certain CYP isoforms in animal or human cells following exposure to chemicals such as phenobarbital, tetrachlorodibenzo-*p*-dioxin (TCDD), or polycyclic aromatic hydrocarbons [8-13]. The purpose of the induction of drug-metabolizing enzymes is primarily for accelerated metabolism of the chemicals being exposed to, which often inactivates and/or detoxifies these chemicals.

Of all the CYPs, the mechanism of CYP1A1 induction by chemicals has been most extensively studied over the past two decades and is perhaps best understood at present [8-15]. Inducer chemicals, such as TCDD, first bind the intracellular aryl hydrocarbon receptor (AhR), and then the liganded AhR is translocated into the nucleus in a heterodimeric complex with the AhR translocator protein. This nuclear AhR complex, acting as a ligand-dependent transcription factor, binds specifically to the aryl hydrocarbon responsive element (AhRE) in the *CYP1A1* gene and subsequently activates its expression. In recent years, similar mechanisms were also observed for some of the other inducible CYP isoforms [16-26].

Notably, there are many unique features that are generally associated with the inducer-stimulated biosynthesis of CYP isoforms: (*i*) the CYP isoform that is induced after exposure to a chemical very often is the one capable of effectively metabolizing this chemical; (*ii*) usually more than one CYP isoform are induced to varying degrees; (*iii*) although exposure to an inducer chemical stimulates the biosynthesis of some CYP isoforms, often the biosynthesis of some other isoform(s) is simultaneously inhibited; and (*iv*) the inducibility and the magnitude of induction of a CYP isoform often differ dramatically in different tissues/cells following exposure to the same inducer. Although great advances have been made in the past decade in the study of nuclear receptors and their roles in mediating CYP isozyme induction, the molecular mechanisms for these as well as other characteristics associated with chemical induction of various CYP isoforms are still not fully understood at present. In this paper, some theoretical aspects concerning the underlying molecular mechanisms are discussed alongside a discussion of some of the available supporting evidence. The theoretical framework developed in this paper seeks to ignite more critical thinking on this research subject as well as to promote experimental testing of the proposed theoretical elements in the future. Undoubtedly, these additional studies will enhance the understanding of the molecular mechanisms for the selective induction of CYP enzymes by chemicals.

2. THEORETICAL CONSIDERATIONS

It is hypothesized that the biological process of selective induction of a CYP isoform by an inducer chemical is primarily determined by the following three core elements (schematically illustrated in Fig. 1):

- Each chemically-inducible CYP isoform may have a corresponding intracellular receptor. This receptor interacts with the inducer chemicals and mediates the selective induction of this CYP isoform.
- Each chemically-inducible CYP isoform and its corresponding receptor may share a highly similar steric structure for their substrate/inducer binding sites.
- Each inducible *CYP* gene may have a distinct genomic response element that interacts selectively with the corresponding receptor.

In this paper, the term *induction* refers only to the commonly-known biological process involving chemical-stimulated transcription of a *CYP* gene followed by translation of its mRNA, which results in increased *de novo* biosynthesis and elevated intracellular levels of the CYP protein. Although a chemical may also increase the steady-state concentrations of a CYP isoform in a cell by affecting the degradation of a CYP protein or its mRNA [27-29], the proposed theory is not intended to explain these particular phenomena.

Here it should also be noted that the term “selective induction” was used on the basis of the following two main reasons: (*i*) Exposure of experimental animals or human subjects to most of the chemical inducers does not result in an equal or similar induction of the various CYP enzymes, rather it almost always predominantly induces one or a few CYP enzymes when most of the other inducible CYP enzymes in the same tissue or cells are only very mildly affected.

(ii) The CYP enzyme that is induced after exposure to an inducer chemical very often is the one capable of effectively metabolizing this chemical. While the use of the “selective induction” terminology may help more readily understand some of the biological purposes behind the proposed theories as outlined above, recall that generally the selectivity of the CYP enzyme induction by various inducer chemicals is rather low (when compared to many other biological systems such as hormone and neurotransmitter receptors or antibodies), and thus this term should be applied only in relative terms.

3. LITERATURE REVIEW AND DISCUSSION

As discussed below, the proposed theory is in accordance with many experimental findings. Despite the fact that some of the evidence discussed below appears to be rather unsystematic and scattered in unrelated scientific literature, these fragments of evidence, when viewed collectively, lend considerable support to each of the theoretical elements proposed.

3.1. Hypothesis 1: Each chemically-inducible CYP isoform has a corresponding intracellular receptor

A widely-accepted alternative to this premise is that one receptor system may mediate the induction of *multiple* CYP isoforms. For instance, it is generally accepted that the AhR system mediates the induction of several CYP1 family isozymes (CYP1A1, 1A2, and 1B1). If this is correct, then we should expect to see a highly consistent induction pattern for each of the *co-inducible* CYP isoforms in a given tissue or cell following exposure to a series of inducers (*i.e.*, different agonists for the same mediating receptor). However, previous studies with almost any co-inducible CYP isoforms have found little or no support for this prediction.

Let us use the chemical induction of CYP1A1 and CYP1A2 as an example. These two CYP isoforms are highly homologous in their amino acid sequences, similar in their catalytic activities, and often co-induced after exposure to a variety of AhR agonists (*e.g.*, TCDD and 3-methylcholanthrene). For years, most investigators believed that the same AhR system mediated the induction of both CYP isoforms [10,30]. However, studies have shown that while TCDD induced both CYP1A1 and CYP1A2 in the liver, it only induced CYP1A1 (but not CYP1A2) in the kidney and lung [31]. Moreover, the induction ratios of CYP1A1 to CYP1A2 in the same tissue or cell line often differed markedly with different inducers. For instance, inducers (such as 2-methoxy-4-nitroaniline, piperonyl butoxide, isosafrole, 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole, and several methylenedioxyphenyl compounds) could induce CYP1A2 mRNA and/or protein without inducing CYP1A1 [32-35]. Additional studies also showed that piperonyl butoxide, isosafrole, and 2,2-dimethyl-5-*t*-butyl-1,3-benzodioxole (inducers of CYP1A2) were unable to displace TCDD and 3-methylcholanthrene from the AhR *in vitro* [36,37], and the induction of CYP1A2 by methylenedioxyphenyl compounds was not correlated with their activity at the aromatic hydrocarbon-responsive locus [38,39].

Earlier studies using the AhR knock-out mice further showed that some of the chemical inducers, such as piperonyl butoxide and acenaphthylene, could still induce CYP1A2 expression, independent of the AhR [40]. Similarly, it was shown that while phenobarbital failed to induce CYP1A1 in the AhR knock-out mice, it induced CYP1A2 [41]. These data appear to provide some support for the hypothesis that the AhR does not mediate the induction of CYP1A2.

In various inducible human cell lines in culture, similar observations have also been made. For instance, the extent of CYP1A1 and CYP1A2 induction by polycyclic aromatic hydrocarbons (PAHs) or by various nitro-PAHs was highly CYP isoform-dependent, and the induction of each isoform was not related to the levels of AhR present [42].

In the same context, it is of note that most researchers consider that the AhR also mediates the chemical induction of CYP1B1. However, the induction pattern of CYP1B1 is very different from that of CYP1A1 or 1A2 in different human tissues or cells examined [43-47]. For instance, CYP1A1 was induced by TCDD to a much higher level (45-fold) than CYP1B1 in human HepG₂ cells, but CYP1A1 was not induced by TCDD in ACHN cells where only CYP1B1 was selectively induced by TCDD [48]. In JEG-3 cells, while the CYP1A1 mRNA was induced by up to 9000-fold, the expression of CYP1B1 was unaffected [49]. In animal studies, low levels of CYP1B1 expression could still be induced in the liver of AhR^{-/-} mice after treatment with some of the chemical inducers [50]. These observations, along with many others, likely suggest that the inducibility of CYP1B1 is regulated independently of CYP1A1.

The proposed hypothesis speculates that each *inducible* CYP isoform may have a corresponding intracellular receptor that mediates its induction by chemicals. This premise logically leads to several deducible principles pertaining to the receptor-mediated chemical induction of CYP isoforms: (i) The presence of the mediating receptor in a cell would be a major determinant for the chemical *inducibility* of the respective CYP isoform. (ii) The density of the mediating receptor in a cell would be another important determinant of the magnitude of the maximum induction of the respective CYP isoform in that cell, although it is not the sole determinant of the absolute magnitude of CYP enzyme induction following exposure to a chemical inducer (discussed in a later section). (iii) The affinity with which a chemical binds to and activates the mediating receptor would be an important determinant of the chemical's potency for inducing a CYP isoform. Stated differently, the induction potency of a chemical for a given CYP isoform should largely parallel its binding affinity for the corresponding intracellular receptor. (iv) The rank order of potency for the induction of a CYP isoform by a series of inducers (*i.e.*, different agonists for the same mediating receptor) would be highly similar in different responsive cells. (v) Certain chemicals may function as selective inhibitors for the induction of a given CYP isoform if they can function as antagonists for the mediating receptor.

Notably, almost each of the above predictions is strongly supported by experimental evidence in the case of CYP1A1 induction by chemical inducers, a process that is known to be mediated by the AhR. For instance, many previous studies have shown that the binding affinity of AhR agonists correlated closely with their ability to induce CYP1A1. TCDD had a very high binding affinity for the AhR (~ 1 to 10 nM) and correspondingly it had a very high potency for inducing CYP1A1 [51,52]. In comparison, 3-methylcholanthrene had a much lower affinity than TCDD for the AhR and, correspondingly, it had a weaker inducing potency for CYP1A1 [53]. Similarly, studies with various peroxisome proliferators have shown that their binding affinity for the peroxisome proliferator receptors correlated very closely with their ability to stimulate liver peroxisome proliferation and particularly the induction of a CYP4A isoform [23-25].

Although the ratio of CYP1A2 to CYP1A1 induction or the ratio of CYP1B1 to CYP1A1 induction in different tissues or cells may vary, considerable experimental evidence scattered in the literature consistently shows that the induction of CYP1A2 or CYP1B1 individually by different chemical inducers agrees well with the principles predicted above. This would be in agreement with the suggestion that the induction of CYP1A2 or CYP1B1 is predominantly mediated by their respective specific receptors, although there may be certain degrees of coordination in the induction of functionally-similar metabolizing CYP enzymes (discussed later).

Most of the above-predicted features for the receptor-mediated induction process have also been observed with the chemical induction of other CYP isoforms, in particular the CYP3A and CYP4A family isoforms. For instance, induction of CYP3A isoforms by various chemicals and induction of CYP4A by various peroxisome proliferators each showed highly consistent

rank order of induction potency in different responsive tissues or cells. Moreover, their induction by different chemicals showed a high degree of tissue or cell selectivity. Studies in the past decade have shown that the nuclear receptor PXR selectively mediates the chemical induction of a CYP3A family isoform [18-22,25,26] and the peroxisome proliferator-activated receptors (PPARs) may selectively mediate the induction of CYP4A family isoforms [54]. There are many recent review articles describing these developments. These new findings with CYP enzymes of the 3A and 4A families provide further support for the general concept that specific receptors may mediate the induction of each chemically-inducible CYP isoform.

Similarly, studies have shown that the nuclear receptor CAR mediates the induction of certain CYP2B family isoform. The discovery of the CAR receptor as a mediator of the induction of a CYP2B family isoform provides further support for the general concept that each inducible CYP isoform may have its own specific receptor. It is of interest to review some of the earlier indirect evidence that suggested a receptor-based mechanism for the induction of CYP2B1 by phenobarbital and related chemicals: (i) The induction of this CYP isoform by inducer chemicals exhibited a high degree of tissue and cell specificity. For instance, CYP2B1 was readily inducible in small intestine and liver by a number of inducers, but little or no induction was seen in some other tissues (such as lung and testis) with the same inducers [55-57]. (ii) The induction of CYP2B1 by phenobarbital showed saturable sigmoidal dose-response curves both *in vivo* and in cultured hepatocytes [58,59], which are characteristic of a receptor-mediated process. (iii) Although CYP2B1 inducers include chemicals from many different classes, there is a clear structure-activity relationship for inducers within each individual class (*e.g.*, phenobarbital and phenobarbital-like chemicals). (iv) Several studies showed a highly consistent rank order of potency for CYP2B1 induction in liver and intestine by different inducers of the same class. (v) There is evidence for the selective antagonism of CYP2B1 induction by certain chemicals [59]. Taken together, all of these earlier observations were highly indicative of a receptor-mediated mechanism for the induction of CYP2B1 by the inducer chemicals.

In addition, there is also evidence that supports the notion that the induction of CYP2B1 and 2B2 may be mediated by different receptors. For instance, the magnitude of induction of CYP2B1 and 2B2 isoforms often varies with different chemical inducers and in different tissues, similar to the differential induction of CYP1A family isoforms (CYP1A1, CYP1A2, and CYP1B1) as discussed above. Many chemicals induce CYP2B1 to a much greater extent than they induce CYP2B2 [57,60,61], but 4-n-alkyl-methylenedioxybenzene-type inducers induced CYP2B2 more than CYP2B1 [62]. Interestingly, although the responsiveness of CYP2B2 to phenobarbital in the QdJ:SD strain of the Sprague-Dawley rats was selectively impaired, the responsiveness of CYP2B1 to phenobarbital was not impaired [63]. These observations are consistent with the hypothesis that each CYP2B isoform likely has its own mediating receptor.

Lastly, it should be noted that the hypothesis that each inducible CYP isoform may have a mediating receptor also provides an explanation for a commonly observed, yet poorly understood, phenomenon that while many chemicals function as selective inducers of CYP isoform biosynthesis, some other chemicals may function as selective inhibitors of the same process. Typically, an agonist interacts specifically with the receptor resulting in its activation, and an antagonist often also interacts specifically with the same receptor, but its binding does not activate the receptor, instead it blocks the receptor from interacting with the available agonist. If each inducible CYP isoform has a mediating receptor, then it can be understood that, in accordance with the general principals, some chemicals that function as inducers would activate the mediating receptor and stimulate the biosynthesis of a CYP isoform, while some others that function as inhibitors may block the mediating receptor from interacting with the existing inducer and thereby inhibit the biosynthesis of this CYP isoform.

In summary, many of the experimental observations discussed above are in agreement with the premise that selective chemical induction of the CYP1A1, CYP1A2, CYP1B1, CYP2B1, CYP2B2, CYP3A4, CYP3A5, and CYP4A isoforms is mediated by specific receptors, in a mechanism likely analogous to the known AhR-mediated induction of CYP1A1.

3.2. Hypothesis 2: Each inducible CYP isoform and its mediating intracellular receptor may have highly similar steric structures in their substrate/ligand binding sites

This hypothesis predicts that the mediating receptor for a given CYP isoform will recognize the same steric structure of a chemical at which this CYP isoform catalyzes the metabolic reaction. Consequently, the mediating receptor that is effectively activated by the chemical will lead to the induction of a CYP isoform that usually can effectively metabolize it (as depicted in Fig. 1).

There are many examples of CYP enzymes that can be selectively induced by their substrates. Using CYP3A4 as an example, many clinically-used drugs (such as amprenavir, carbamazepine, dexamethasone, lovastatin, mifepristone, nifedipine, rifabutin, ritonavir, and simvastatin) are substrates of CYP3A4-mediated metabolism, and these chemicals are also strong inducers of this CYP enzyme [64-66]. Another classical example is phenobarbitals, which act as both substrates and inducers of CYP2B1 [13].

The above prediction that a substrate of an inducible CYP enzyme should also be an inducer of this enzyme seems to be not supported by the experience we have with the uses of various therapeutic drugs in humans. We know that while the metabolism of most drugs involves CYP enzymes, only a relatively small number of the drugs actually cause significant CYP induction in clinical settings. To better understand this apparent discrepancy, it is important to understand the physiological conditions under which chemical induction of CYP enzymes may take place. Hepatocytes in the body normally express a mixture of various metabolizing enzymes at rather high levels, with a combined high capacity for metabolizing many xenobiotics. Therefore, under normal conditions, most chemicals can be effectively metabolized by the existing enzymes, and these drugs will not accumulate in the liver and other parts of the body for extended length of time. As a result, there is no need to further increase the levels of drug-metabolizing enzymes in the liver and/or other organs to accelerate their metabolic disposition because the corresponding receptors will not be activated at sufficient intensity for a sustained period of time. This is the main reason that most pharmacological agents at the recommended safe doses would not substantially induce CYP enzymes in humans. However, if the doses of these drugs continue to increase, eventually they will exceed the metabolic capacity of the existing CYP enzymes. Subsequently, the concentrations of the chemicals will be persistently elevated, and will result in activation of the mediating receptors and increased expression of the CYP enzymes. In comparison, for those drugs that happen to have a very high binding affinity for the mediating receptors, they may elicit a strong induction of the CYP enzyme expression even when they are used at relatively low doses. In fact, some of the clinically-used drugs (such as rifampin, rifapentine, troglitazone, carbamazepine, and paclitaxel) that are well-known strong inducers of the CYP enzymes all share this property. Given the fact that drugs with strong CYP-inducing activity often are more likely to cause hepatotoxicity, these drugs would have a lower chance of surviving the preclinical and clinical stage testings. This is another underlying reason that, in reality, only a relatively small fraction of the clinically-used drugs are strong inducers of the CYP enzymes in humans at the recommended doses.

The proposed hypothesis also logically leads to another suggestion: a chemical's binding affinity for an inducible CYP isoform ought to reflect its binding affinity for the mediating receptor. In fact, a wealth of experimental data (mostly qualitative or semi-quantitative) in the literature concurs with this suggestion. For instance, benzo[*a*]pyrene, 3-methylcholanthrene, and several other polycyclic aromatic hydrocarbons all have high binding affinities (low K_D values)

for the AhR [51], and they all also have high binding affinities for CYP1A1 (as reflected by the low K_M values) [52]. In comparison, some chemicals have relatively low binding affinities for the AhR, and they also have low affinities for CYP1A1-mediated metabolism. It is known that CYP1A1 in rats has a low K_M (high affinity) for benzo[*a*]pyrene hydroxylation, whereas CYP1A2 has a high K_M (low affinity) for benzo[*a*]pyrene hydroxylation [67-69]. As expected, benzo[*a*]pyrene administration induced CYP1A1 to a much greater extent than CYP1A2 in the same tissues of these animals [70,71]. This differential induction of CYP1A1 over CYP1A2 in certain tissues by benzo[*a*]pyrene agrees with the predicted differential binding affinity of this inducer for their corresponding receptors (assuming that each CYP isoform has a corresponding receptor).

Similar characteristics have also been observed with other CYP isoforms such as CYP2B1 and CYP2B2: (i) CYP2B1 has a high K_M (low affinity) for the metabolism of phenobarbital, and as expected, the concentrations of phenobarbital required for CYP2B1 induction in cultured rat hepatocytes are also very high ($EC_{50} = 15 \mu\text{M}$ [60]). (ii) *p*-Hydroxyphenobarbital, a major metabolite of phenobarbital formed by CYP2B1, understandably has a much lower affinity than phenobarbital (the substrate) for CYP2B1, and has little or no activity for the induction of CYP2B1 [72]. (iii) Purified CYP2B1 has a much higher affinity and a higher catalytic activity than purified CYP2B2 for metabolizing many of the prototypic substrates [73,74], and as expected, all these substrate chemicals induce CYP2B1 more strongly than they induce CYP2B2 [57,60-62]. The high degree of correlation between the inducing activity of the different substrates for CYP2B1 and CYP2B2 and their rates of metabolism by these two CYP isoforms is in accord with the proposed receptor-mediated induction mechanism. It is possible that the past failure to identify the phenobarbital receptor(s) by using radiolabeled phenobarbital as a probe might be due to the fact that its binding affinity was too low to allow successful detection of the mediating receptor(s).

It is known that many inducible CYP isoforms (*e.g.*, isoforms of the CYP3A and CYP2B families) have low substrate specificities, *i.e.*, one CYP isoform often metabolizes multiple substrates and the same reaction can often be catalyzed by multiple CYP isoforms. The proposed theory predicts that the mediating receptors would also have low specificity for their inducers, *i.e.*, one inducer chemical may bind multiple types of receptors, or one receptor may interact with various inducers. This suggestion, in general, is consistent with numerous experimental findings showing that one inducer chemical can often induce multiple CYP enzymes and, likewise, a given inducible CYP enzyme can almost always be induced by a variety of chemicals.

Mechanistically, when a chemical binds and activates more than one type of mediating receptors, it is almost certain that the binding affinities of this chemical for the multiple types of receptors will differ vastly. Consequently, the induction profile of each of the multiple CYP isoforms by different chemicals would also be very different. This prediction is in agreement with numerous experimental observations that consistently showed that: (i) when *multiple* CYP isoforms were co-induced, usually one CYP isoform was predominantly induced while other isoform(s) was only induced to a much smaller extent, and (ii) the induction pattern (ratio) for multiple CYP isoforms was almost always different with different inducers. Based on the mechanistic explanation tendered above, it is readily understood that some CYP1A1 inducers, which are AhR agonists, may also activate the mediating receptor for CYP1A2, thereby resulting in the co-induction of both CYP enzymes. However, because different chemicals would have different binding affinities for these two mediating receptors (as these chemicals would have different K_m values for CYP1A1 and 1A2), the ratios of CYP1A1 induction to CYP1A2 induction in the same cell or tissue are expected to vary with different inducers. The same explanation can be used to explain the co-induction of CYP2B1 and 2B2 by various chemical inducers.

It is conceivable that, in some cases, an inducer chemical may interact with multiple receptors in very different ways: activating one or several type(s) of the mediating receptors but inhibiting other type(s) of the mediating receptors. Many previous studies with various chemical inducers have shown that while the chemicals would stimulate the biosynthesis of one or several CYP isoform(s), they often also simultaneously inhibit the biosynthesis of certain other CYP isoform(s). For the ease of understanding of some of the complex interactions discussed above, a schematic depiction is presented in Fig. 2.

Lastly, it is worth mentioning that the current CYP isoform classification is based on the degree of homology of the amino acid sequences. Different CYP isoforms of the same subfamily share a greater degree of homology in their amino acid sequences than those in different subfamilies, and often isoforms within a subfamily also can catalyze the same reactions. However, the degree of similarity of their amino acid sequences may not always reflect the degree of their *functional* similarity, which is largely determined by their steric structures. Therefore, the steric structures of the inducer-binding sites of the receptors for the CYP isoforms of the same subfamily may not always share a higher degree of similarity compared to those receptors for the CYP isoforms of different subfamilies.

3.3. Hypothesis 3: Each inducible CYP gene may have a different genomic response element that interacts selectively with the corresponding receptor

An alternative premise to this hypothesis is that the same genomic response element for a mediating receptor is present in multiple inducible CYP genes. If this is the case, then the transcription of multiple CYP genes would always be simultaneously activated by a series of inducers (*i.e.*, agonists for the same mediating receptor), and the rank order of potency for the induction of these CYP isoforms with various inducers should be identical or very similar. As discussed earlier, most experimental observations in the literature strongly disagreed with this alternative premise. In addition, earlier studies have shown that the same aryl hydrocarbon response element (AhRE) identified for the CYP1A1 gene did not appear to be present in the CYP1A2 and CYP1B1 gene promoters. This finding disagrees with this alternative premise.

The proposed hypothesis, however, does not rule out the possibility that an activated mediating receptor may interact with a homologous regulatory region in other functionally-similar CYP genes. Such cross interactions may provide certain degrees of coordination for the induction of other functionally-similar CYP isoforms.

In addition, it is known that the AhRE for CYP1A1 gene is also present in genes encoding several Phase II enzymes, such as glucuronosyltransferase and glutathione S-transferase [14, 15]. Similarly, a recent study [75] showed that the nuclear PXR not only mediated the induction of certain CYP3A isoform, but it also mediated the induction of certain Phase II conjugating enzymes, such as dehydroepiandrosterone sulfotransferase [75]. These interesting findings demonstrated a general mechanism for the coordinated induction of certain Phase I oxidative enzymes, Phase II conjugative enzymes, and possibly Phase III drug transporters that are functionally coupled. Understandably, if elevated oxidative metabolism of a drug (primarily catalyzed by cellular CYP isoforms) is uncoupled to a *selective* increase of its conjugative metabolism, it may be potentially hazardous to cells, whereas coordinated upregulation of CYP isoforms (such as CYP1A1), certain conjugation enzymes that can effectively metabolize the oxidation products, and transporters would favorably detoxify lipophilic chemicals such as polycyclic aromatic hydrocarbons and their oxidative metabolites.

In brief, the proposed hypothesis suggests that a mediating receptor protein would have both ligand specificity and certain degrees of target gene selectivity, and these two features would enable the receptor to mediate the selective induction of a CYP isoform by the inducer chemicals. The chemically-activated receptors will act as transcription factors leading to the

initiation of selective transcriptional activation of target *CYP* genes. The relative selectivity at the target gene is enabled by the specific response element usually present in the promoter of an inducible *CYP* gene. Although this mechanism has been demonstrated with the AhR-mediated chemical induction of CYP1A1 as well as the receptor-mediated induction of CYP3A and CYP4A family members, it is believed that the same mechanism is shared in the chemical induction of other *CYP* isoforms.

4. CONCLUDING REMARKS AND EXPERT OPINIONS

Selective induction of *CYP* isoforms by inducer chemicals has attracted intense research interest throughout the past half century. Such inductive responses of various organs or cells in the body can have a major impact on drug metabolism, on pharmacokinetics and drug-drug interactions, on the toxicity and carcinogenicity of foreign chemicals, and on the potency and disposition of circulating hormones [3-9]. In this paper, a general theoretical framework is proposed concerning the mechanisms of selective induction of *CYP* isoforms by chemicals. Specifically, it is suggested that: (i) each chemically-inducible *CYP* isoform may have a receptor that interacts with the inducer chemical and mediates the selective induction of this *CYP* isoform; (ii) each chemically-inducible *CYP* isoform and its corresponding receptor may share a highly similar steric structure in their substrate/inducer binding sites; and (iii) each inducible *CYP* gene may have a distinct genomic response element that interacts selectively with the activated receptor.

While the proposed theories are strongly supported by the existing experimental data on CYP1A1 induction (known to be mediated by the AhR), they are also largely in agreement with a considerable body of experimental observations scattered in scientific literature concerning the chemical induction of several other *CYP* enzymes. For some of the *CYP* enzymes, their mediating receptors are already known, but for many others their mediating receptors are presently unknown. Ultimately, for the verification of each of the proposed theoretical elements, one needs to identify the inducer receptor for each of the inducible *CYP* isoforms, and one also needs to directly compare the steric structures of the inducer-binding site of the mediating receptor with the substrate-binding site of the corresponding *CYP* enzyme. These key pieces of experimental evidence are needed to verify the proposed mechanistic explanation for the complex process of selective *CYP* enzyme induction by many structurally-diverse chemicals.

For the fairness of the discussion of the proposed theories, it should be noted that there are also experimental and clinical observations concerning the chemical induction of *CYP* enzymes that may not be readily explained by the proposed theories. In some of the cases, it appears that other non-receptor mediated processes may be involved. For instance, omeprazole is a strong inducer of the CYP1A1 and 1A2, but this chemical does not appear to have a binding affinity for AhR [76]. The exact mechanism for the induction of the CYP1A family enzymes by omeprazole is still poorly understood at present, although it has been suggested that it may depend on its effect on tyrosine kinase activity [77]. In many other cases, however, the apparent inconsistencies may not contradict the theories proposed; rather they are largely due to the involvement of many other regulatory pathways and factors that could affect the expression of the *CYP* genes. The presence of these confounding factors sometimes obscures the proposed mechanistic core elements. A brief discussion of some of the confounding factors using concrete examples would be helpful and is provided below.

- Theoretically, a high-affinity substrate for an inducible *CYP* enzyme (usually determined *in vitro* with microsomes or recombinant *CYP* enzymes) would also be a high-affinity activator of its mediating receptor. However, there are also other underlying factors that, at times, can cause discrepancies when these inducers are tested *in vivo*. The poor absorption of an inducer chemical, its slow transport across

the plasma membrane into the cells, or its high-affinity binding to plasma proteins, for example, are among many common causes of the low intracellular concentrations of the inducer chemicals, and consequently, for their low *in vivo* enzyme-inducing activity.

- Based on the proposed theory, the density of the mediating receptor in a cell would be an important determinant of the magnitude of the maximal induction of the respective CYP isoform in that cell. This would be true if we compare two cells that have different levels of the mediating receptor but everything else remains exactly the same. However, in real biological situations, often other factors that are involved in transcriptional regulation of a CYP gene may not be exactly the same. Besides the ligand-activated receptor (which functions as a gene-specific transcriptional factor), the transcriptional activation of an inducible *CYP* gene also involves other *trans*-acting factors (such as co-activators and co-repressors). These accessory factors are usually less cell-specific and less gene-specific but their levels could be altered under different conditions. Tissue- or cell-specific differences in these regulators are expected to affect the rate of constitutive as well as inducer-stimulated transcription of a given *CYP* gene [31]. This notion is exemplified in many recent studies of the selective estrogen receptor modulators (SERMs), which highlights the complexity of the transcriptional regulation of intracellular receptor-controlled genes [78,79]. As a result of these complex regulations, the magnitude of the maximal induction of a given CYP isoform by a chemical in two different types of cells or tissues may not be exactly proportional to the density of the mediating receptor present. However, it is expected that the rank order of potency for a given series of chemical inducers will still be highly similar in different responsive cells, which would reflect the critical role of a specific receptor in determining the inducibility of a given *CYP* gene following exposure to chemical inducers.
- Chemical induction of CYP isoforms is also subject to modulation by a variety of factors that alter the overall growth status of a cell, which include, but are not limited to, growth hormones, thyroid hormone, certain interleukins, intracellular levels of cAMP, and many others. It is believed, however, that modulation of chemical induction of CYP enzymes by these growth-related factors is generally nonspecific (or far less specific) with respect to the inducer chemicals used or the CYP isoforms involved. For instance, studies have shown that rapidly proliferating hepatocytes would have much lower ability to express CYP enzymes compared to well-differentiated, slow-proliferating hepatocytes. Therefore, in the presence of these growth-regulating factors, the magnitude of induction of various CYP enzymes following exposure to various chemical inducers would be greatly reduced, which has been repeatedly observed in many studies. However, in these cells, the rank order of potency for the induction of a given CYP enzyme almost always remain nearly the same, which agrees with the speculated role of the corresponding receptor in selectively mediating the induction of a target CYP enzyme.
- There were experimental observations showing that the binding affinity of a substrate to the catalytic site of a CYP enzyme may not always parallel its rate of metabolism. TCDD is a typical example in this regard. TCDD is a very potent inducer of CYP1A1 (because it is a very potent, high-affinity agonist for the AhR), and it also has a high affinity for binding to CYP1A1 [51,53]. However, TCDD is essentially not metabolized by CYP1A1. This unusual discrepancy between its high binding affinity for CYP1A1 and its low rate of metabolism by this isoform may lie in the possibility that the unusually strong electron-withdrawing effect of the multiple halogen atoms in this rare, man-made molecule makes its oxidative reaction via the electrophilic

attack of the substrate molecule catalyzed by the CYP1A1 enzyme exceptionally difficult (nearly impossible).

- Many other vitally-important biological systems usually have a built-in redundant mechanism. The proposed theory shows that there is also a built-in redundancy in the chemical induction of CYP enzymes. Here let us use benzo[a]pyrene as an example, which is a well-known chemical carcinogen that can be metabolized by several CYP1A family enzymes, including 1A1, 1A2, and 1B1. When benzo[a]pyrene is given to an animal (such as i.p. injection into rats), it will induce the expression of all three CYP1A isoforms (1A1, 1A2, and 1B1) to varying degrees. In fact, there are considerable data in the literature showing that the induction ratio of these three CYP1A isoforms matches very closely their preference for metabolizing benzo[a]pyrene as a substrate. The possible explanation (based on the proposed theory) is that the receptors for CYP1A1, 1A2, and 1B1 also have similar binding sites as the corresponding enzymes such that these receptors can all bind the inducer chemicals (albeit with varying binding affinities). It is apparent that there is redundancy here in the induction of the metabolizing enzymes, *i.e.*, three CYP enzymes that possess similar catalytic functions are all induced to metabolize the same inducer chemical. Such redundancy is crucial when one CYP enzyme system cannot be induced due to genetic defects, other functionally-similar enzyme systems can be induced to compensate. Understandably, when three functionally similar enzymes (such as CYP1A1, 1A2, and 1B1) are all induced, each one may be induced at a relatively low level in order to achieve the intended biological purpose, but when one of the enzyme systems cannot be induced due to genetic defects, then the other two systems may need to be induced to a higher level to achieve the same goal.
- Lastly, as a unique case that may stimulate further thinking, it is worth noting that there is a phenomenon in antibody immunology that bears some resemblance to the selective induction of CYP isoforms by chemicals. It is known that all antibody molecules produced by a B cell (or a clone of B cells) have the same antigen-binding site. In fact, the first set of antibodies produced by a newly-formed B cell are not secreted, instead they are modified and inserted into the plasma membrane where they serve as the *receptor* for recognizing antigens. In this way, each clone of the antibodies has a corresponding clone of membrane receptors with exactly the same antigen-binding site. When the antigen binds specifically to the receptor antibodies present in the surface of a virgin B cell or a memory B cell, it initiates a series of intracellular events culminating in cell proliferation to produce antibody-secreting B cells. In this case, because the receptor antibodies present in the surface of the antibody-secreting B cells have exactly the same antigen-binding site as the antibodies to be secreted, this assures that the secreted antibodies can selectively recognize the antigen to which the organism is exposed. The overall resemblance between the antigen induction of specific antibodies and the chemical induction of CYP isoforms is intriguing, and it adds biological credence to the hypothesis that a CYP isoform and its corresponding receptor may share a highly similar steric structure in their substrate/inducer binding sites. However, the specificity of a CYP enzyme for various substrates generally is far lower than an antibody's specificity for the antigens. The high degrees of specificity of antibodies are primarily due to the existence of very large numbers of different clones of B cells that can be selectively stimulated for antibody production. In comparison, the numbers of CYP isoforms are far less. In fact, the ability of such small numbers of CYP isoforms to metabolize almost unlimited numbers of endogenous or exogenous chemicals is fulfilled by the relatively low substrate specificity of many of the inducible CYP isoforms together with their suitable biochemical properties as having high metabolic capacity (*i.e.*, high $V_{\max}$) but generally low binding affinity (*i.e.*, high K_m).

Article highlights box

- A general theoretical framework regarding the mechanisms of selective induction of CYP enzymes by chemical inducers is presented. Briefly, this theory suggests that each chemically-inducible CYP isoform may have a specific receptor that can interact with the inducer chemical to mediate the selective induction of this CYP isoform. Moreover, each chemically-inducible CYP isoform and its corresponding receptor may share a highly similar steric structure in their substrate/inducer binding sites.
- Each of the proposed theoretical elements is formulated on the basis of strong scientific rationale and is also supported by a large body of direct or indirect experimental observations.
- There are also experimental and clinical observations of the chemical induction of CYP enzymes that may not be readily explained by the proposed theories. While in some of the cases non-receptor mediated processes may be involved, most of the other inconsistencies actually do not contradict the theories proposed; rather they are largely due to the involvement of many other regulatory pathways and factors that could affect the expression of the *CYP* genes. These confounding factors sometimes may obscure the core elements.

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Abbreviations used

CYP	cytochrome P450
AhR	aryl hydrocarbon receptor
AhRE	AhR response element
PXR	Pregnane X receptor
CAR	Constitutive androstane receptor.

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Figure 1.

A schematic illustration of the three theoretical elements (see text) that govern the selective chemical induction of CYP isoforms. **Inset A** depicts a mediating receptor that has an inducer-binding site and a highly selective xenobiotic response element (XRE)-binding site. **Inset B** depicts a CYP isoform with a substrate-binding site that is identical (or highly similar) in steric structure to the inducer-binding site of its corresponding receptor.

Figure 2.

Some deducible pharmacological characteristics that are associated with the hypothesis that each chemically-inducible CYP isoform is mediated by a corresponding receptor. The upper panels (**A, B, C**) depict a possible situation where an inducer chemical (**I**) can bind and then activate a number of structurally-similar receptors (R_1 , R_2 , R_3) with different binding affinities. The induction potency of a chemical for a given CYP isoform will largely depend on its binding affinity for its mediating receptor. As depicted, the binding affinity of inducer **I** for R_1 , R_2 , and R_3 is assumed to follow the rank order of $R_1 > R_2 > R_3$. It is expected that inducer **I** is a more potent inducer for the induction of R_1 -mediated CYP isoform than R_2 - or R_3 -mediated CYP isoforms. In comparison, the maximal induction of a given isoform (achieved at high inducer concentrations) may be, in a significant part, determined by the levels of the inducer receptor present. Note that in this hypothetical case since the receptors R_1 , R_2 , and R_3 bind to the same chemical moiety of the inducer, it is expected that the induced CYP isoforms will also have the ability to catalyze the same metabolic reaction (albeit they may have rather different K_M values).

The middle panels (**D, E, F**) depict a situation where multiple chemical moieties of the same inducer chemical may interact with different inducer receptors. The binding affinity of each moiety for the corresponding receptor will basically determine the relative potency of induction of a corresponding CYP isoform, and the total receptor levels may, in part, determine the magnitude of induction of a given CYP isoform. There is considerable experimental evidence showing that a chemical inducer (such as phenobarbital) can induce multiple CYP isoforms with widely different potencies and efficacies. Notably, since in this case different moieties of the inducer chemical interact with different receptors to induce different CYP isoforms, it is

expected that each of the induced CYP isoforms will also have a preferential ability to metabolize the corresponding moiety of the inducer chemical.

The bottom panels (**G, H, I**) depict a situation where an inducer chemical may activate multiple inducer receptors (such as R_1 and R_2), resulting in the induction of corresponding CYP isoforms, but it may also competitively block some other inducer receptors (such as R_3), resulting in a reduced expression of the corresponding CYP isoform. This phenomenon has been observed with some inducer chemicals, such as phenobarbital.