REVIEW

Oxazaphosphorine bioactivation and detoxification: the role of xenobiotic receptors

Duan Wang, Hongbing Wang*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, USA

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Abstract Oxazaphosphorines, with the most representative members including cyclophosphamide, ifosfamide, and trofosfamide, constitute a class of alkylating agents that have a broad spectrum of anticancer activity against many malignant ailments including both solid tumors such as breast cancer and hematological malignancies such as leukemia and lymphoma. Most oxazaphosphorines are prodrugs that require hepatic cytochrome P450 enzymes to generate active alkylating moieties before manifesting their chemotherapeutic effects. Meanwhile, oxazaphosphorines can also be transformed into non-therapeutic byproducts by various drug-metabolizing enzymes. Clinically, oxazaphosphorines are often administered in combination with other chemotherapeutics in adjuvant treatments. As such, the therapeutic efficacy, off-target toxicity, and unintentional drug–drug interactions of oxazaphosphorines have been long-lasting clinical concerns and heightened focuses of scientific literatures. Recent evidence suggests that xenobiotic receptors may play important roles in regulating the metabolism and clearance of oxazaphosphorines. Drugs as modulators of xenobiotic receptors can affect the therapeutic efficacy, cytotoxicity, and pharmacokinetics of coadministered oxazaphosphorines, providing a new molecular mechanism of drug–drug interactions. Here, we review current advances regarding the influence of xenobiotic receptors on the metabolism and clearance of oxazaphosphorines.

KEY WORDS Oxazaphosphorine; Cyclophosphamide; Ifosfamide; CAR; PXR; CYP2B6

Abbreviations: ALDH, aldehyde dehydrogenase; AKR, aldo-keto reductase; AhR, aryl hydrocarbon receptor; BCRP, breast cancer resistance protein; CAA, chloroacetdehyde; CAR, constitutive androstane receptor; CPA, cyclophosphamide; CYP, cytochrome P450; CMF, cyclophosphamide-methotrexate-fluorouracil; CITCO, 6-(4-chlorophenyl)imidazo-[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl)oxime; GST, glutathione S-transferase; IFO, ifosfamide; 4-OH-CPA, 4-hydroxycyclophosphamide; 4-OH-IFO, 4-hydroxyifosfamide; MRP, multidrug resistance-associated protein; MDR1, multidrug resistance 1; PXR, pregnane X receptor; RXR, retinoic X receptor; R-CHOP, rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone; SNP, single nucleotide polymorphism; UGT, UDP-glucuronosyltransferases

*Corresponding author. Tel.: +1 410 706 1280; fax: +1 410 706 5017.
E-mail address: hwang@rx.umaryland.edu (Hongbing Wang).

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1. Introduction

Oxazaphosphorines are a class of bi-functional alkylating agents that have been extensively investigated in the past 50 years for their anticancer and immune-regulating activities, with the most successful representatives including cyclophosphamide (CPA), ifosfamide (IFO), and to a lesser extent trofosfamide. Most oxazaphosphorines are designed prodrugs, which require cytochrome P450 (CYP) enzyme-mediated bioactivation to generate highly reactive alkylating nitrogen mustards that exert their chemotherapeutic effects by attacking specific nucleophilic groups of DNA molecules in target cancer cells. CPA is the first oxazaphosphorine agent that achieved great success in its clinical application in many cancer patients. Although CPA has been clinically available for over a half century, it continues to be amongst the front-line choices of chemotherapy for solid tumors, such as breast cancer, for which it is used as an important component of the CPA-methotrexate-fluorouracil (CMF) regimen, and hematopoietic malignancies, such as non-Hodgkin lymphoma, for which it is applied as a critical constituent of the rituximab-CPA-doxorubicin-vincristine-prednisone (R-CHOP) multidrug regimen. Additionally, CPA has also been used at higher doses in the treatment of aplastic anemia and leukemia prior to bone marrow transplantation and as a therapeutic immunosupressor for several autoimmune disorders.

IFO, the second anticancer drug in the oxazaphosphorine class, was introduced to clinics in the early 1970s. Developed as an analog of CPA, IFO only differs chemically from CPA by one chloroethyl group transpositioned from the mustard nitrogen to the ring nitrogen. Like CPA, IFO also requires CYP-mediated metabolism to produce active alkylating moieties before manifesting its antitumor effects. Clinically, IFO has been used in young adult and pediatric tumors along with other chemotherapeutics in adjuvant treatment. In a number of malignant diseases, IFO exhibits a higher therapeutic response rate, with less myelosuppression, in comparison with its parent analog CPA. Trofosfamide is another derivative of CPA and an orally administered oxazaphosphorine prodrug with high bioavailability. As a congener of CPA and IFO, the antitumor cytotoxicity of trofosfamide also relies on its metabolic activation by “ring” oxidation, using the hepatic mixed-function oxidase system. Trofosfamide is often used clinically in adult soft tissue sarcomas and non-Hodgkin lymphomas with relatively low toxicity profiles.

In addition to these traditional oxazaphosphorines, several new analogs of CPA and IFO such as mafosfamide and glufosfamide have been designed, aiming to achieve increased therapeutic selectivity and reduced off-target toxicity, in comparison with their ascendants. Unlike traditional oxazaphosphorines, mafosfamide and glufosfamide do not require hepatic oxidative enzyme-mediated bioactivation. For instance, mafosfamide is a 4-thioethane sulfonic acid salt of 4-hydroxycyclophosphamide (4-OH–CPA), a key bioactive intermediate metabolite of CPA; while glufosfamide is a glucose conjugate of ifosfamide, in which isophosphoramide mustard, the bioactive alkylating metabolite of ifosfamide, is covalently linked to β-D-glucose. At present, several Phase I studies have shown favorable outcomes from intrathecal administration of mafosfamide in the treatment of meningeal malignancies, although further comprehensive clinical evaluation is needed. In the case of glufosfamide, the development of this oxazaphosphorine agent was based on the rationale that cancer cells are active in importing and utilizing glucose. Thus, the differential expression of transmembrane glucose transporters between cancer and normal cells accounts for the target selectivity of glufosfamide. Recent clinical trials revealed beneficial effects of utilizing glufosfamide in the treatment of pancreatic adenocarcinoma, non-small cell lung cancer, as well as head and neck squamous cell carcinoma. Together, these promising anticancer activities of mafosfamide and glufosfamide indicate that new generation of oxazaphosphorine agents, with better target selectivity and less unwanted cytotoxicity, could be clinically available in the near future.

The journey of our understanding of the mechanisms underlying oxazaphosphorine action started with the development of CPA. After many years of intensive investigation, although a number of new oxazaphosphorine derivatives displayed promising therapeutic features, currently, CPA and IFO remain to be the most successful and widely used oxazaphosphorines in the treatment of an array of various malignancies. As aforementioned, CPA and IFO are prodrugs requiring metabolic activation by hepatic drug-metabolizing enzymes. The expression and functional perturbation of these enzymes can dramatically influence the metabolism, clearance, and pharmacokinetics of these oxazaphosphorines. Recently, accumulating evidence suggests that nuclear receptors, in particular, a group of so-called “xenobiotic receptors”, are the primary regulators governing the transcription of most hepatic drug-metabolizing enzymes and drug transporters. Drugs as modulators of xenobiotic receptors can affect the metabolism rate and pharmacokinetics of coadministered oxazaphosphorines, and dictate their therapeutic efficacy and toxicity, accordingly. The current review tends to highlight the recent advances in elucidating the roles of xenobiotic receptors in mediating the bioactivation and deactivation of oxazaphosphorines with the focus on the roles of the constitutive androstane receptor (CAR, NR1I3), the pregnane X receptor (PXR, NR1I2), and the Aryl hydrocarbon receptor (AhR) on metabolism and clearance of CPA and IFO. This review however is by no means a comprehensive coverage of all findings of oxazaphosphorine research.
2. Metabolism and transport of oxazaphosphorines

2.1. Cytochrome P450

Prototypical oxazaphosphorine cytostatics such as CPA and IFO are chemically and pharmacologically inactive transport forms of alkylating nitrogen mustards that are biotransformed to their active forms predominantly in the liver\(^47,48\). As one of the key mechanisms of action, hepatic metabolism of CPA, has been extensively studied during the past several decades, utilizing human and animal liver microsomes, primary hepatocytes, recombinant CYP enzymes, CYP-selective chemical and antibody inhibitors, as well as whole animal models\(^48–50\).

Upon administration, CPA undergoes hepatic oxidation to form the pharmacologically active intermediate metabolite 4-OH-CPA, which enters blood circulation and is transported to target tissues by binding to erythrocytes\(^51–54\). 4-OH-CPA is further tautomerized to aldophosphamide, followed by spontaneous \(\beta\)-elimination to release the phosphoramide mustard as the final DNA-cross-linking metabolite\(^10,55\). Notably, hydroxylation of CPA at the 4-carbon position is the rate limiting step of its bioactivation, and blood concentration of 4-OH-CPA has often been used as a biomarker monitoring the efficacy of CPA therapeutics\(^56–58\). Multiple CYP isozymes are involved in the hydroxylation of CPA, including CYP2A6, CYP2B6, CYP3A4, CYP3A5, CYP2C9, CYP2C18, and CYP2C19\(^49,50,59,60\), with CYP2B6 being the primary player, which contributes approximately 45% of CPA bioactivation\(^48–50\). To a lesser extent, CYP3A4 and CYP2C9 also contribute around 25% and 12% of CPA 4-hydroxylation, respectively\(^49,50\). Alternatively, CPA is subject to significant side-chain oxidation, primarily \(N\)-dechloroethylation, by a number of CYPs to generate the inactive dechloroethyl-CPA and the toxic byproduct chloroacetaldehyde (CAA)\(^27,49,58,60\) (Fig. 1). The predominant CYP enzyme responsible for the \(N\)-dechloroethylation of CPA is CYP3A4, which was reported to be responsible for up to 95% of this reaction, followed by CYP3A7 and CYP3A5\(^52,49,53,60\). On the other hand, CYP2B6 only provides negligible contribution to this non-therapeutic biotransformation of CPA\(^50\).

Metabolism of IFO shares a generally CYP-based pathway in common with that of CPA, but exhibits differential CYP affinity and metabolism rates\(^23,34\). Akin to CPA, IFO is bioactivated by CYP3A4 and CYP2B6 to form the 4-hydroxy-ifosfamide (4-OH-IFO), which subsequently goes through a series of biochemical reactions to yield the ultimate therapeutic alkylating agent, ifosfamide mustard\(^48,61\). More detailed characterization, however, revealed that CYP3A4 plays a major role in the 4-hydroxylation of IFO with CYP2B6 as a supplementary isozyme\(^56,62\). Moreover, these two CYP enzymes also control the \(N\)-dechloroethylation of IFO forming the neurotoxic CAA\(^39,60\), with CYP3A4 contributing approximately 70% of liver microsomal CAA formation and CYP2B6 accounting for roughly 25%\(^34,49\). Additionally, CYP3A5 was also reported to be involved in the dechloroethylation of IFO. Polymorphic mutations of CYP3A5 can affect the rate of CAA formation as well\(^63\).

In comparing the biotransformation of CPA and IFO, only 10% of CPA is subject to \(N\)-dechloroethylation, whereas approximately 25–60% of IFO undergo this metabolic pathway, generating more toxic byproducts\(^58,64,65\). This rather distinct profile of metabolism also contributes to the clinically observed side-effect, in which CAA-mediated neurotoxicity occurs in ~20% of IFO-treated patients, while happens quite rare in CPA-treated patients\(^66,67\). Importantly, CYP enzymes are involved differentially in the biotransformation of these oxazaphosphorines; for instance, CYP2B6 selectively activates CPA over IFO and only exhibits a negligible effect on CPA-\(N\)-dechloroethylation. Therefore, it might be possible to design novel CPA-based therapeutic regimens by modulating these metabolic pathways to achieve greater bioactivation without concurrent augmentation of unwanted cytotoxicity.

**Figure 1** Schematic summary of cyclophosphamide (CPA) and ifosfamide (IFO) metabolism. The prodrugs CPA and IFO are biotransformed through a group of CYP and non-CYP drug-metabolizing enzymes to form their therapeutically active DNA-crosslinking mustards, as well as non-therapeutic metabolic byproducts (modified from Wang et al.\(^120\)).
2.2. Other drug-metabolizing enzymes

Following CYP-mediated 4-hydroxylation, both CPA and IFO can be further activated to their corresponding therapeutic mustards or inactivated to different byproducts through sequential metabolic processes mediated by other non-CYP drug-metabolizing enzymes. First, 4-OH-CPA quickly reaches equilibrium with its acyclic form, aldophosphamide, which can be spontaneously decomposed through β-elimination to form the ultimate active alkylating product phosphoramid mustard and a urotoxic byproduct acrolein, which is commonly associated with clinically important hemorrhagic cystitis. Intracellular phosphoramid mustard then attacks host DNA to exert expected cytotoxicity. Alternatively, phosphoramid mustard can undergo detoxification by glutathione S-transferase (GST)-mediated conjugation, hydrolysis of the chloroethyl side chain to form alcohols, or cleavage of the phosphorus-nitrogen bond to release 3-(2-chloroethyl)-1,3-oxazolidin-2-one (CNM), which are all metabolic byproducts without antitumor activity. The acrolein, meanwhile, is converted to acrylic acid by aldehyde dehydrogenases (ALDH), ALDH1A1 and ALDH3A1. An important detoxification pathway for 4-OH-CPA is the conversion of its tautomer, aldophosphamide, to the less toxic carboxyphosphamide (CEPM), which represents a major stable non-therapeutic metabolite of CPA found in clinical samples. This oxidative reaction is primarily catalyzed by ALDH1A1 and to a lesser extent by ALDH3A1 and ALDH5A1. Alternatively, aldophosphamide can be oxidized to form alcohosphamide by alcohol dehydrogenase (ADH) and ald-keto reductase (AKR1). An additional detoxification pathway occurs through reversible dehydration to form iminocyclophosphamide, which is further conjugated with glutathione mediated by GSTA1, GSTA2, GSTM1, and GSTP1, and eventually generates the nontoxic 4-glutathionylcyclophosphamide. This major difference regarding the metabolism of CPA and IFO happens in the CYP-mediated 4-hydroxylation and N-dechloroethylation. Metabolic destinations of these oxazaphosphorines thereafter are highly comparable. As with 4-OH-CPA, 4-OH-IFO exists in equilibrium with its tautomer aldoifosfamide, which decomposes through β-elimination to yield ifosfamide mustard and acrolein. Ifosfamide mustard is also subject to further degradation, forming the inactive metabolite CNM and chloroethylamine. Similarly, 4-OH-IFO can also be biotransformed to carboxyifosfamide by ALDH1A1, to 4-keto-IFO by AKR1, or to alcofosfamide by ADH and AKR1. Glutathione conjugation represents another important detoxification mechanism of ifosfamide mustard.

Collectively, it is evident now that both CYP and non-CYP drug-metabolizing enzymes can contribute to the bioactivation and detoxification of CPA and IFO. Although liver contains the most abundant drug-metabolizing enzymes and plays predominant roles in the biotransformation of oxazaphosphorines, extrahepatic tissue-specific expression of these enzymes also contribute to the targeted “selective cytotoxicity” which is one of the leading motive in developing safe and effective chemotherapeutics.

2.3. Drug transporters

It is believed that all oxazaphosphorine prodrugs are highly hydrophilic and thus are not easily diffused across cell membranes. Mounting clinical and experimental evidence, however, agreed that both CPA and IFO can be readily administered orally or intravenously with high bioavailability and decent intracellular concentrations. These phenomena suggest that active uptake transporters may contribute to the absorption of these oxazaphosphorines though direct scientific support is limited thus far. Conversely, the circular proactive metabolites of these oxazaphosphorines, 4-OH-CPA, aldoifosphamide and 4-OH-IFO, can easily cross the lipid bilayer membranes of many cells through passive diffusion. In contrast to uptake, more research efforts have been centered on the efflux transportation of these alkylating agents from cancer cells, which is pivotal in multidrug resistance of cancer chemotherapy. In this regard, a number of ATP-binding cassette transporters have been identified as transmembrane modulators associated with exporting CPA, IFO, and their metabolites.

In vitro studies, utilizing HepG2 cells stably transfected with multidrug resistance-associated protein 4 (MRP4, ABCC4) expression vector, have clearly established that CPA and IFO are substrates of this efflux transporter. Overexpression of MRP4 in HepG2 cells led to increased resistance to CPA- and IFO-induced cytotoxicity, while inhibition of this transporter by dicyfenac or celecoxib, two known inhibitors of MRP4, significantly sensitized the MRP4-HepG2 cells to CPA and IFO. Notably, glutathione, the most abundant cellular redox molecule, plays an important role in the function of MRP4 and depletion of intracellular glutathione can significantly affect the export of cAMP by MRP4. Since glutathione is pivotal in detoxification of phosphamide and ifosfamide mustards, it was speculated that MRP4-mediated resistance to CPA and IFO might be glutathione-dependent. Indeed, addition of buthionine sulfoximine, a glutathione synthesis inhibitor, considerably reversed MRP4-mediated resistance to CPA and IFO in MRP4-HepG2 cells.

The multidrug resistance-associated protein 2 (MRP2, ABCC2) has been reported to export a detoxified CPA metabolite, 4-glutathionylcyclophosphamide, from hepatocytes into the bile in rats; this biliary excretion appears to compete with the bioactivation pathway that generates the active alkylating agent. In addition, clinical studies have shown that multidrug resistance-associated protein 1 (MRP1, ABCB1) and the breast cancer resistance protein (BCRP, ABCG2) are involved in the resistance to chemotherapy in breast cancer patients receiving CMF regimen. However, whether CPA and/or its metabolites are substrates of BCRP and/or MRP1 requires further investigation, given that methotrexate and 5-fluorouracil in the CMF regimen are known substrates of BCRP.

MRP1, MRP2, and BCRP are all expressed in the apical (canalicular) membrane of hepatocytes and are in charge of hepatic biliary excretion of many drugs and endobiotics into the bile. Conversely, MRP4 is localized in the basolateral (sinusoidal) membrane of hepatocytes and are in charge of hepatic biliary excretion of many drugs and endobiotics into the bile. Collectively, it is evident that the oxazaphosphorine type of alkylating prodrugs require hepatic biotransformation mediated by many
drug-metabolizing enzymes to produce the ultimate DNA-alkylating mustards, with CYP2B6 and CYP3A4 predominantly governing the initial and rate-limiting step of their bioactivation. Although induction of CYP expression generally increases the elimination of drugs and leads to therapeutic failure, in the case of CPA and IFO, increasing CYP-mediated biotransformation can generate more cytotoxic intermediate metabolites with and without therapeutic potentials, which may lead to comprehensive diagnostic outcomes. Many drugs and environmental chemicals can influence the expression of these CYP enzymes, which are transcriptionally regulated by a group of transcription factors termed xenobioc receptors. Unlike traditional endocrine hormone receptors, xenobioc receptors, functioning as sensors of toxic byproducts derived from both endogenous and exogenous chemical breakdowns, are typically activated by abundant but low-affinity lipophilic molecules at rather high (micromolar) concentrations, without real endogenous ligands identified thus far. Major xenobioc receptors, including PXR, CAR and AhR, predominantly localized in the liver and intestines, have been documented as important xenobioc sensors mediating the transcription of drug-metabolizing enzymes and transporters associated with the metabolism and clearance of oxazaphosphorines.

### 3.1. Pregnane X receptor (PXR)

As one of the important components of the body’s adaptive defense mechanism against xenobiotics, PXR represents the most promiscuous xenosensor among all xenobioc receptors and can be activated by a broad spectrum of ligands including prescription drugs, herbal medicines, environmental pollutants, and endobiotic derivatives. The structural diversity of PXR ligands stems mainly from the unusually large, spherical, and flexible ligand binding pocket of the receptor. Drug-mediated activation of PXR is associated with the inductive expression of many target genes including drug-metabolizing enzymes, such as CYP3A4, CYP2B6, CYP2Cs, and UDP-glucuronosyltransferases (UGT); and drug transporters, such as the multidrug resistance 1 (MDR1, ABCB1), and MRPs by recognizing and binding to specific xenobioc response elements located in the promoters of these genes.

Among others, CYP3A4 and CYP2B6 are highly inducible PXR target genes, which exhibit marked inter- and intra-individual variations in their expression. As many clinically used drugs as PXR activators can influence the pharmacokinetics of CPA and IFO when coadministered in multidrug regimens. Additionally, accumulating evidence suggests that hepatic bioactivation of CPA and IFO is auto-inducible upon repeated application of these oxazaphosphorines, which contribute significantly to the observed autoinduction of CYP2B6 and CYP3A4 by which their own metabolism and clearance are increased. In this process, CPA and IFO bind to the ligand binding domain of PXR that leads to the release of PXR-bound corepressors, such as the silencing mediator of retinoic acid and thyroid hormone receptor (SMRT) and the nuclear receptor corepressor 1 (NCOR1), and the recruitment of its heterodimer partner, the retinoic X receptor (RXR), and other coactivators, such as steroid receptor 1. The PXR/RXR heterodimer directly interacts with specific promoter sequences of CYP2B6 and CYP3A4 genes and stimulates their transcription. Additionally, Harmsen et al. recently reported that CPA and IFO can also induce the expression of MDR1 through PXR transactivation. Although MDR1 represents one of the major mechanisms associated with the multidrug resistant phenotype in response to many chemotherapeutics, CPA, IFO, and their protractive metabolites 4-OH-CPA and 4-OH-IFO, are not typical substrates of MDR1; thus, this induction may not directly affect the intracellular levels of these oxazaphosphorines and their active metabolites.

In addition to drug-induced activation, genetic polymorphisms of PXR may also affect the metabolism and pharmacokinetics of CPA and IFO in different patients. The presence of PXR variants was investigated by Huestert et al., in two Caucasian and African ethnic groups; three PXR protein variants (V140M, D163G, and A370T) were identified to be functionally associated with altered basal and/or induced transactivation of CYP3A promoter reporter genes. In a separate study, Lim et al. reported that a Q158K variant of PXR, found in Chinese population, impairs drug-mediated induction of CYP3A4 by altering ligand-dependent PXR interaction with the steroid receptor coactivator-1. Although autoinduction of CYP3A4 by CPA and IFO was not directly investigated in these two studies, CPA and IFO are known activators of PXR, and inducers of CYP3A4 that enhance their own metabolism and clearance. Thus, these naturally occurring PXR genetic variants may play a role in the observed interindividual variability of CYP3A4 expression and therefore, influence the varied bioactivation of chemotherapeutic prodrugs including CPA and IFO.

### 3.2. Constitutive androstane receptor (CAR)

The constitutive androstane receptor, also denoted as the constitutively activated receptor (CAR; NR1I3), is the closest relative of PXR in the nuclear receptor superfamily and they share a panel of overlapping target genes, including a number of Phase I and II drug-metabolizing enzymes, as well as drug transporters that are involved in the metabolism and clearance of the oxazaphosphorines. PXR and CAR also share many xenobioc activators, such as the sedative phenobarbital, the anti-malaria artemisinin, the synthetic opioid methadone, as well as the oxazaphosphorine CPA but not IFO. As such, the extensive cross-talk between PXR and CAR may form a compensatory biological safety net that ensures comprehensive protection against various exogenous and endogenous chemicals. On the other hand, CAR also holds several unique features that separate itself from PXR and many other nuclear receptors. First, in line with its designated name, CAR is constitutively activated and spontaneously localized in the nucleus of nearly all immortalized cell lines independent of chemical stimulation. Secondly, unlike activation of PXR that is prototypically ligand-dependent, CAR could be transactivated by either direct binding to ligands such as the human CAR selective agonist 6-(4-chlorophenyl)imidazo-[2,1-b][1,3]thiazole-5-carbaldehyde-O-(3,4-dichlorobenzyl) oxime (CITCO) or ligand-independent indirect mechanisms such as the prototypical CYP2B1 inducer, phenobarbital. In fact, the majority of CAR activators identified thus far are actually phenobarbital-type of indirect activators.
without binding to the receptor. Last but not least, differing from the situation in immortalized cells, CAR is predominantly localized in the cytoplasm of hepatocytes cultured in vitro or in the liver in vivo without activation and only translocates to the nucleus upon chemical stimulation. Together, these features of CAR make the identification of its activators more challenging, particularly towards a high-throughput format in vitro. As a result, only a limited number of CAR activators thus far have been reported, in comparison to the numerous drugs and environmental toxicants documented as agonistic ligands of PXR.

Recent studies from our laboratory have shown that CPA but not IFO is a phenobarbital-like activator of CAR. Treatment of human primary hepatocytes at clinically relevant concentration of adenovirus-expressing enhanced yellow fluorescent protein-tagged human CAR supported by additional agonistic ligands of PXR. Studies in our lab revealed that human CAR but not IFO primarily transactivates PXR. Assuming this selective autoinduction also occurs in vivo, these findings may potentially be of clinical importance where drug-mediated manipulation of hCAR activity could alter the autoinduction and pharmacokinetics of selective oxazaphosphorines.

Similar to that of PXR, accumulating evidence suggests that both polymorphism and alternative splicing of human CAR play an important role in the modulation of its gene expression. To date, approximately 30 single nucleotide polymorphisms (SNPs) of the human CAR gene have been identified, residing in the 5’-flanking regulatory regions, coding exons, and non-coding introns. Functional analysis of four SNPs, localized in the ligand binding domain of CAR, (His246Arg, Leu308Pro, Asn323Ser, and Val133Gly) revealed that His246Arg is associated with decreased CAR activation by CITCO, while Leu308Pro affect basal but not chemically stimulated CAR activation in cell-based reporter assays. Recently, a number of naturally occurring alternative splicing variants of human CAR have been identified, residing in the 5’ untranslated regions of the gene, without binding to the receptor. Some of these spliced CAR transcripts revealed that some are associated with altered expression, cellular localization, and chemical response of the receptor.

Functioning as another xenosensor dictating the inductive expression of many drug-metabolizing enzymes, AhR is actually classified into the basic helix-loop-helix protein of the PER-ARNT-SIM (PAS) family not to the nuclear receptor superfamily. Nevertheless, AhR shares a number of comparable characteristics with CAR and PXR, which are important in modulating the toxicity and biological functions of many environmental aromatic hydrocarbons and clinically used drugs. Upon activation, ligand-bound AhR dissociates with its cytoplasmic chaperon partners and translocates to the nucleus. There, it forms a heterodimer with the aryl hydrocarbon nuclear translocator and stimulates the expression of its target genes. Along with the ever growing list of AhR activators, transactivation of AhR is associated with altered expression of many genes including but not limited to CYP1A1, CYP1A2, CYP1B1, UGT1As, GST, ADH, ALDH3A1, and BCRP.

3.3. Aryl hydrocarbon receptor (AhR)

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Although AhR-modulated CYP and UGT1A enzymes, and efflux transporter BCRP only have moderate effects on the bioactivation and clearance of CPA and IFO, other AhR target genes such as ADH and ALDH3A1, are proved enzymes that play critical roles in the detoxification of these two oxazaphosphorines. Along with the ever growing list of AhR activators, transactivation of AhR is associated with altered expression of many genes including but not limited to CYP1A1, CYP1A2, CYP1B1, UGT1As, GST, ADH, ALDH3A1, and BCRP.

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to chemical-stimulated induction, these ALDH enzymes demonstrate tissue specific distribution and developmental changes, and are also over-expressed in certain types of cancer cells.154,155. Thereby, manipulating tissue-specific expression of ALDH may alter the cellular sensitivity to oxazaphosphorines. Notably, recent studies revealed that other than the prevailing mechanisms of AhR activation stimulated by exogenous ligands, elevation of intracellular second messenger cAMP could also lead to the nuclear translocation of AhR.156. Nevertheless, cAMP-mediated translocation of AhR acts as a repressor in lieu of an activator of AhR, which leads to repression of AhR target genes including ALDH156,157. As such, drugs and endogenous signaling molecules differentially modulating the function of AhR may affect the expression of ALDH enzymes one way or the other and eventually influence the clinical responses to CPA- and IFO-containing regimens.

4. Concluding remarks

The oxazaphosphorines CPA and IFO represent the most widely used chemotherapeutic alkylating agents with a history of clinical application for more than 50 years. To date, extensive studies have elucidated the general pharmacology, metabolism, pharmacokinetics and cytotoxicity of these oxazaphosphorines. However, because of the increased polypharmacy in general and in oxazaphosphorine-based chemotherapy in particular, drug-drug interactions associated with CPA and IFO multidrug regimens have become rising concerns in clinical practice. Accumulating evidence thus far established clearly that hepatic CYP2B6 and ALDH enzymes are over-expressed in certain types of cancer cells.154,155. Thereafter, a number of studies have demonstrated that such strategy could be successful over CYP3A4 in the liver.142. This notion might be clinically attractive in directed modulation of CPA-based chemotherapy, given the fact that CPA is predominantly bioactivated by CYP2B6 while deactivated through CYP3A4.

Selective cytotoxicity towards tumor but not normal cells is the ultimate goal for all chemotherapeutic agents to achieve. Realizing the specific role of CYP2B6 in the bioactivation of CPA, Waxman and colleagues have reported that locally delivery of adenovirus- or retrovirus-encoding CYP2B expression cassette into tumor cells resulted in increased intracellular CPA 4-hydroxylation and cytotoxicity.158,159. Thereafter, a number of studies have demonstrated that such strategy could be successful in cell cultures in vitro, tumor xenografts in animal, and to a certain extent in initial clinical trials.158,159. The current reality, however, is that clinically used CPA and IFO rely predominantly on hepatic CYP-mediated biotransformation and the activated metabolites are transported by erythrocytes to tumors and normal tissues via blood circulation. Moreover, unlike localized solid tumors, systemic chemotherapy is necessary for hematopoietic malignancies such as lymphoma and leukemia, in which CPA continues to be used among the first-line R-COUP regimen. Therefore, understanding the role of xenobiotic receptors in the regulation of key drug-metabolizing enzymes in the liver involving the bioactivation and deactivation of oxazaphosphorine agents is of both scientific significance and clinical importance.

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