Molecular mechanisms of regulation of CYP enzymes of phase I metabolism of xenobiotics – synthetic drugs and herbal preparations

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Summary

The understanding of the pharmacokinetic properties of synthetic drugs as well as herbal preparations used in treatment of many diseases offers a real opportunity to eliminate or minimize the interaction between the active substances. This is of particular clinical importance because it can decrease or increase the action of drugs what is the major cause of failure of pharmacotherapy. Understanding the molecular basis of interaction of substances with enzymes of drug metabolism is extremely helpful in treatment planning and its practical application. The experimental and clinical studies aimed to complete the characterization of the mechanisms of many factors that modulate the activity of cytochrome P450 enzymes, can provide a lot of evidence guaranteeing the safety of herbal products.

Key words: *cytochrome P450, AhR, PXR, CAR, mechanism of regulation*

INTRODUCTION

The interactions of synthetic drug with herbal preparations have become the subject of worldwide scientific discussions concerning molecular pharmacology and toxicology. Polypharmacy that can frequently lead to adverse interactions between the active substances used in medicines is one of the important problems of modern pharmacotherapy. Potential object of the research in this area are cytochrome P450 enzymes involved in detoxification of xenobiotics, their transporters and receptors as well as the mechanism of transcriptional activation of the CYP gene superfamily.

The enzymes of the cytochrome P450 family show the monooxygenase activity with low substrate specificity and are involved in the metabolism of various endogenous substrates (prostaglandins, steroid hormones, bile acids, leukotrienes). A large part of these enzymes is also involved in the metabolism of structurally diverse xenobiotics, including dietary constituents, environmental pollutants, insecticides and drugs [1-3]. The effect of action of CYP enzymes is to give a hydrophilic character of xenobiotics by adding polar functional groups. This is of particular clinical importance in the process of their detoxification. Some substances are harmful to humans but many others are transformed by cytochrome P450 to a highly toxic reactive intermediates which enhances their mutagenic or carcinogenic effect. This can lead to cell dysfunction and ultimate carcinogenesis. These changes occur in the endoplasmic reticulum and mitochondria of organs associated with metabolism of xenobiotics, mainly in liver microsomes and also in lung, intestines and kidneys [3].

There are many CYP isoforms that play a major role in the metabolism of variuos xenobiotics. In mammals, the presence of more than 50 genes of this superfamily was described. CYP3A4/5 isoforms that occur mainly in the liver, are responsible for the biotransformation of more than 60% of clinically used drugs while CYP2D6 isoform metabolizes 25–30% of all drugs used in medicine (tab. 1) [3, 4]. There are reports indicating a correlation between the low activity of CYP2D6 and an increase of toxicity of selected drugs and risk of the development of Parkinson's disease [5, 6].

Furthermore, the CYP1A, CYP2A and CYP2E subfamilies metabolize many protoxins and procarcinogens to their ultimate reactive metabolites [3, 5, 6].

Table 1.

Summary of selected substrates for human cytochrome P450

Isoforms	Substrates for selected CYP enzymes
CYP3A4	▪ antiarrhythmic drugs (quinidine, propafenone) ▪ calcium channel blockers (nifedipine, diltiazem, verapamil) ▪ statins (lovastatin, simvastatin, not fluvastatin) ▪ steroids (hydrocortisone) ▪ benzodiazepines (midazolam, alprazolam) ▪ dihydropyridines (nicardipine, felodipine) ▪ protease inhibitors (indinavir, ritonavir) ▪ antineoplastic (cyclophosphamide, ifosfamide, tamoxifen) ▪ analgesics (methadone) ▪ antidepressants (fluvoxamine, fluoxetine) ▪ others (cyclosporin A, erythromycin)
CYP2D6	▪ neuroleptics (clozapine, haloperidol) ▪ antidepressants (amitriptyline, fluoxetine, paroxetine) ▪ analgesics (codeine, tramadol) ▪ antiarrhythmics (encainide, mexiletine) ▪ beta-blockers (metoprolol, propranolol) ▪ antihypertensive agents (guanoksan) ▪ others (dextromethorphan, tamoxifen)
CYP2C9	▪ NSAIDs (diclofenac, ibuprofen, piroxicam) ▪ antidiabetics (tolbutamide, glipizide) ▪ angiotensin II receptor blockers ▪ tamoxifen ▪ phenytoin ▪ others (fluvastatin, warfarin)
CYP1A2	▪ antipsychotics (clozapine) ▪ antidepressants (imipramine, fluvoxamine) ▪ antiarytmics (mexiletine) ▪ NSAIDs (naproxen) ▪ methylxanthines (theophylline) ▪ caffeine ▪ warfarin ▪ polycyclic aromatic hydrocarbons (PAHs) ▪ heterocyclic aromatic amines
CYP2E1	▪ paracetamol ▪ chlorzoxazone ▪ acetaminophen ▪ anesthetics (enflurane, halothane) ▪ alcohols (ethanol, pentanol) ▪ aldehydes (acetaldehyde) ▪ organic solvents (chloroform, carbon tetrachloride) ▪ aromatic hydrocarbons (benzene, toluene) ▪ nitrosamines (N, N -dimethylnitrosamine)

NSAIDs – nonsteroidal anti-inflammatory drugs

The mechanism of regulation of CYP enzymes

The activity of many cytochrome P450 isoforms can show the variability resulting from the polymorphisms of genes encoding these enzymes or regulation by xenobiotics acting as inhibitors or inducers (tab. 2) [7, 8]. The aim of numerous scientific studies is to clarify the mechanism of the biotransformation of foreign substances. Differences in the activity of CYP isoforms in the metabolism of selected drugs and chemicals can make a significant impact on the interactions between herbal preparations and synthetic drugs, activation of drugs and carcinogens as well as detoxification. The potential contribution of CYP enzymes in the generation of toxic metabolites has become a subject of many studies in the aspect of safety of chemical pollution and drugs used in therapy.

Table 2.

Inductors and inhibitors of the activity of selected human CYP isoforms

Isoforms	Inhibitors	Inductors
CYP3A4	▪ macrolide antibiotics (erythromycin, clarithromycin not azithromycin) ▪ azole antifungal drugs (ketoconazole, miconazole, fluconazole) ▪ dihydropyridine calcium channel blockers ▪ selective serotonin reuptake inhibitors (fluoxetine, fluvoxamine, paroxetine ▪ quinolones (ciprofloxacin, norfloxacin) ▪ protease inhibitors ▪ others (cimetidine, ranitidine)	▪ barbiturates (phenobarbital) ▪ glucocorticoids (dexamethasone, prednisone) ▪ isoniazid ▪ rifampicin
CYP2D6	▪ antiarrhythmics (quinidine, propafenone, amiodarone) ▪ selective serotonin reuptake inhibitors (paroxetine, fluoxetine, sertraline) ▪ neuroleptics (chlorpromazine, triflupromazine, chlorprothixen) ▪ others (cimetidine, diphenhydramine, hydroxychloroquine, celecoxib)	▪ not defined
CYP2C9	▪ amiodarone ▪ cimetidine ▪ diclofenac ▪ fluvastatin ▪ isoniazid ▪ ketoconazole ▪ metronidazole ▪ ketoprofen ▪ lovastatin ▪ sertaline ▪ sulfamethoxazole ▪ sulfonamides	▪ rifampicin ▪ barbiturates

Isoforms	Inhibitors	Inductors
CYP1A2	▪ enoxacin ▪ ciprofloxacin ▪ furafylline ▪ fluvoxamine ▪ cimetidine ▪ clarithromycin ▪ erythromycin ▪ enoxacin ▪ ofloxacin	▪ tobacco smoke ▪ omeprazole ▪ rifampin ▪ karbamazepine ▪ TCDD ▪ hyperforin ▪ insulin, ▪ lamotrigine ▪ lansoprazole
CYP2E1	▪ diethyldithiocarbamate ▪ cimetidine ▪ disulfiram ▪ propofol ▪ bergamotine ▪ bergapten ▪ kaempferol ▪ 6,7-dihydroxybergamottin	▪ ethanol ▪ acetone ▪ isoniazid ▪ clofibrate

Table 3.

The most important transcription factors responsible for regulation the expression of selected genes involved in metabolism and transport of drugs

Transcription factor	Protein involved in heterodimer formation	Exemplary ligands	Regulated genes
CAR (constitutive androstane receptor)	RXR	phenobarbital, androstenol, polychlorinated biphenyls	CYP2B, CYP3A, CYP2C9, GST, SULT, UGT
PXR (pregnane X receptor)	RXR	phenobarbital, dexamethasone, pregnenolon, corticosterone, bile acids	CYP3A, CYP2B, SULT, UGT, MDR1
AhR (aryl hydrocarbon receptor)	ARNT	dioxins, PAHs, bilirubin	CYP1A,CYP1B, GST, UGT
HNF4 (hepatocyte nuclear factor 4)	_	fatty acid derivatives	CYP2A, CYP2C, CYP2D, CYP3A
HNF1 (hepatocyte nuclear factor 1)	_	not defined	CYP1A2, CYP2E1

The molecular mechanism responsible for the expression of certain genes is regulated by environmental and physiological factors, among which there are factors inducing their own biotransformation or metabolism of other substances. Moreover, it has been shown that many foreign substances affect the activity of

specific receptors as ligands that activate transcription factors [9]. In this regard, special attention is paid to the possible modulation of transcription factors, such as aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) pregnane X receptor (PXR) that are involved in regulation of the expression of cytochrome P450 isoforms in order to understand the molecular mechanism of interaction between the herbal preparations and synthetic drugs (tab. 3) [10, 11].

Aryl hydrocarbon receptor (AhR)

Aryl hydrocarbon receptor (AhR) belongs to a large family of transcription factors and possesses DNA-binding domain with the structure of basic helix-loop-helix (bHLH) and PAS domain (called Per-Arnt-Sim) responsible for the dimerization of the protein. The AhR induces expression of human CYP1A1, CYP1A2, CYP1B1 and some genes of phase II enzymes of drug metabolism [9]. AhR ligands are mainly polycyclic aromatic hydrocarbons (PAHs) and organic compounds of dioxins (e.g. 2,3,7,8-tetrachlorodibenzodioxines - TCDD) with strong toxic properties [12]. In most cells, AhR exists as an inactive complex with HSP90 chaperones, XAP2 fusion protein and p23. These proteins are involved in the process of folding and stabilization of AhR [13]. After joining the ligand (dioxin or PAH), the receptor is released from the complex of chaperone proteins and is translocated to the nucleus, in which forms a heterodimer with ARNT (AhR nuclear translocator) [14]. The binding of the heterodimer to the xenobiotic response elements (XRE) leads to the activation of the CYP gene transcription. The PAS protein as a AhR repressor inhibits signal transduction by competing with AhR but repressor is induced by AhR indicating a negative feedback loop for the regulation of AhR [13]. Protein kinase C and tyrosine kinase are also involved in the AhR signal transduction which inhibit the induction of target genes. Studies have shown that the body's response to TCDD, including teratogenesis, immunosuppression, defects in reproduction and development of cancer, requires the action of AhR [15].

Pregnane X receptor (PXR)

The nuclear orphan pregnane X receptor (PXR) is a transcription factor involved in regulation of the expression of CYP isoforms primarily responsible for the metabolism of drugs. It participates in the induction of CYP3A, CYP2B and selected drug transporters (MRP1) as well as phase II enzymes of drug metabolism (SULTs, UGTs) [16-18]. PXR is mainly expressed in liver and intestine where detoxification reactions occur [18]. Ligands of this receptor are structurally diverse exogenous and endogenous compounds, such as steroid hormones and metabolites of steroids (e.g. progesterone, estrogen, corticosterone, 5β-pregnan and androstanol) and dietary components, such as kumestrol and carotenoids [17, 19, 20].

Pharmaceutical PXR activators include antibiotics (rifampicin), calcium channel blockers (nifedipine), HIV protease inhibitors (ritonavir), anti-cancer drugs (paclitaxel) [21].

Variability of the nucleotide sequence in the ligand binding domain leads to significant interspecies differences in the pharmacological activation of PXR. Rifampicin and troglitazone are effective activators of human and rabbit PXR receptor, but cause a small induction of the rat and mouse PXR. The human PXR receptor shows 96% of a sequence homology in the DNA-binding domain (DBD) of the rat and 76% of a sequence homology in the ligand binding domain (LBD). After binding ligand, the activated PXR forms a heterodimer with the retinoid X receptor α (RXRα) which binds to the DNA sequence of the promoter of target genes containing xenobiotic response element (XRE) and acts as a regulator of transcription [10]. The binding of PXR/RXR with DNA region by everted repeat (ER6) and direct repeat (DR3) follows by joining the coactivator proteins such as SRC-1 (steroid receptor coactivator-1). It leads to the activation of transcription of the target genes [10, 22]. In addition, a mutation in the sequence of ER6 or DR3 causes lowering the activity of the CYP3A4 gene by 20–50% higher, whereas the mutation present both in the DR3 and ER6 reduces the activity of this gene more than 80%.

Moreover, PXR and RXRα are induced by glucocorticoid receptor (GR) that participates in the indirect regulation of CYP3A. Activation of GR by glucocorticoids (e.g. dexamethasone) leads to the induction of PXR/RXR and CAR while the opposite effect takes place under the influence of inflammatory cytokines IL-6 [23, 24]. This partly explains the occurrence of repression of some CYP enzymes by cytokines. Studies on experimental animals have shown that mice lacking PXR showed no induction of CYP3A after administration of the classic inducers but the lack of PXR did not change the basic level of expression of this subfamily [25]. Furthermore, transgenic mice with human PXR exhibited induction of CYP3A after administration of specific inducers such as rifampicin.

In addition, PXR regulates the gene expression of OATP2 and MRP2 transporters involved in bile acid homeostasis. It was shown that secondary bile acid (lithocholic acid – LCA) activates PXR suggesting the involvement of this receptor in protecting liver against pathophysiologic levels of bile acids [26].

Constitutive androstane receptor (CAR)

The nuclear orphan receptor CAR (constitutive androstane receptor) is also involved in the process of CYP transcriptional regulation. It is mainly expressed in liver and mediates the induction of CYP2B, CYP3A, CYP2C and phase II enzymes of drug metabolism (GSTs, SULTs and UGTs) [27, 28]. This receptor is regulated by IL-6 which decreases its activity [29, 30]. Hence, it is claimed that inflammatory factors may cause the repression of CYP enzymes. A possible activator of human

CAR is 5β-pregnan. Deactivators and agonists of this receptor are androstanol and clotrimazole [19, 31]. CAR is activated differently from the majority of receptors because it is constitutively active without ligand. It is suggested that a complex with cofactor is required for this activation.

Upon binding agonist, CAR is deactivated by the release of coactivator SRC-1 from the ligand binding domain [19]. Similarly to PXR, CAR requires heterodimerization with RXR in order to bind to the DNA sequence of the promoter of target genes. Heterodimer CAR/RXR binds to the conservative sequence called PBREM (phenobarbital-responsive enhancer module) in the 5' region of the gene CYP2B and to a place ER6 and DR3 in the CYP3A4 gene sequence. Moreover, PXR can bind to the DR4 sequence in PBREM and regulate the expression of CYP2B [27, 32, 33].

Phenobarbital is a classic example of a compound inducing xenobiotic metabolizing enzymes and influencing many cellular processes by the nuclear receptor CAR, although, the exact mechanism of induction of this factor by this ligand is not fully understood yet [19, 21, 32]. There are data indicating that phenobarbital not only facilitates the translocation of CAR into the nucleus but also activates the receptor in the nucleus [33, 34]. This phase is dependent on phosphorylation since the translocation and activation are inhibited by inhibitors of protein phosphatase (PP) and CaM kinase (CK) [35]. This model was confirmed in the studies which demonstrated that in mouse hepatocytes, CAR is localized in the cytoplasm and translocated to the nucleus only after the application of inducer [32].

Other potential mechanisms of regulation of CYP enzymes

Another possible mechanism of the regulation of cytochrome P450 isoforms is based on tissue-specific expression of these proteins with transcription factors. It should be noted that the P450 protein show its activity in fully differentiated hepatocytes where transcription factors such as C/EBP, HNF4, HNF1, HNF3 and HNF6 can participate in their constitutive expression. It was shown that the effect of transcriptional activation of CYP2C9 is a result of action of HNF4, although HNF1 stimulates the expression of CYP1A2 [36, 37]. It also showed that the factor HNF1α activates the CYP2E1 gene expression in rat hepatocytes [38]. It is suggested that the CYP2E1 isoform is regulated in a comprehensive manner on the stage of transcription, translation and post-translationally [39]. The stabilization of the mRNA and protein is probably the most important and regulation on the stage of transcription appears to be less important in contrast to other isoforms. The starvation and long-term ethanol intake increase the level of CYP2E1 gene transcript and affect the stability of the protein. It should be emphasized that many cytokines including IL-1β, IL-6, TNF and interferon-γ reduce the level of expression of CYP1A2, CYP2C, CYP2E1 and CYP3A in contrast to IL-4 which induces human CYP2E1 in hepatocytes [23].

Hepatocyte nuclear factor (HNF4) also belongs to orphan receptors that bind to DNA as a homodimer in the region HPF1 and participates in the regulation of enzyme expression of CYP2A, CYP3A, CYP2D subfamily. Many studies suggest that HNF4α controls the expression level of CYP2D6 gene [40]. The results showed that HNF4 is a major regulator of CYP2C gene expression in the liver of rabbit. It is claimed that HNF4 and the HPF1 motif may significantly affect the activation of human CYP2C9 and rat CYP3A [41]. Moreover, it was shown that HNF4α can activate gene transcription in the absence of exogenous ligand [42]. However, it should be emphasized that the role of this factor in regulating the expression of these genes is not fully characterized yet.

Effect of herbal substances on the CYP gene expression

The exogenous and endogenous substances can modulate the activity of CYP through potential effect on transcription factors involved in their regulation. Thanks to intensive studies several synthetic inducers and inhibitors of specific enzymes of the CYP superfamily became understood and described. However, their expression can be modulated by the active compounds of commonly used herbal preparations such as *Hypericum perforatum*, *Valeriana officinalis*, *Echinacea purpurea*, *Allium sativum*, *Camellia sinensis*, *Panax ginseng*, *Gingko biloba* and *Glycine max*.

St. John's wort (*Hypericum perforatum*) or its active substances are important in the interaction with synthetic drugs especially metabolized by human CYP3A4 enzyme such as indinavir (HIV protease inhibitor), cyclosporin (immunosuppressant) and oral contraceptives [43]. This well-known antidepressant contains many active substances of which hyperforin and hypericin are the most important ones. The *in vitro* studies have shown that St. John's wort can modulate the activity of human CYP1A2, 2C9, 2C19, 2D6 and 3A4 [18]. This fact explains that hyperforin is a strong ligand for PXR receptor regulating the expression of CYP3A4 [19].

In the treatment of many diseases, especially of respiratory and immune system, preparations from garlic (*Allium sativum*) are often used. Their pharmacological activities are associated with the presence of sulfur-containing compounds (allicin, allin) as well as flavonoids (quercetin, routine). The analysis of the interactions showed that they are mainly related to drug metabolism by the CYP2B1 enzyme. Administration of the garlic oil causes an increase of rat CYP2B1 activity and inhibition of CYP2E1 activity in mice [25]. The results of clinical studies show no association between the intake of garlic extract and the activity of CYP2D6 and CYP3A4, although there have been reports concerning the possibility of the induction of CYP3A4 under the influence of this raw material [33].

Due to non-specific immune stimulant, purple coneflower (*Echinacea purpurea*) can be a source of drug interactions with immunostimulatory or immunosuppressive agents. *In vitro* studies have shown that active ingredients of this raw materials may alter the metabolism and effectiveness of drugs that are substrates

for CYP3A4 enzyme. Gorski et al. showed the induction of CYP3A4 activity and a decrease in the activity of CYP1A2 and CYP2C9 after the application of *Echinacea purpurea* supplementation [32].

Valerian (*Valeriana officinalis*) is a popular dietary supplement which in addition to *Echinacea purpurea* and *Ginkgo biloba*, has been recognized as a potential inhibitor of CYP2D6 [38]. There are also suggestions indicating no effect of valerian extract on the activity of CYP2D6 and a slight impact on CYP3A4 [40].

The analysis of the influence of standardized *Panax ginseng* (4% ginsenosides) and soybean (*Glycine max*) containing 50 mg of isoflavones on cytochrome CYP3A activity showed no effect on CYP3A4 enzyme induction after 14 days of the experiment. However, results of studies on human liver microsomes suggest that non-hydrolysed soy extract causes a slight inhibition of CYP1A2, CYP2A6, CYP2D6 and CYP3A4 while the hydrolyzed extract causes inhibition of these isoforms particularly CYP2C9 and CYP3A4. The authors underline the lack of correlation between induction of CYP3A4 observed *in vitro* and a lack of activation of this isoform after application of *Panax ginseng* and *Glycine max* extracts *in vivo* [41].

Understanding of the pharmacokinetic properties of synthetic drugs as well as herbal supplements used in the treatment of many diseases poses a real chance to eliminate or minimize the interactions between active substances. This is of particular clinical importance because it may lead to a weakening or intensification of the drug action. The aim of many experimental and clinical studies is to understanding the mechanisms of action of many factors that can modulate the activity of cytochrome P450.

CONCLUSION

The interactions between herbal preparations and synthetic drugs lead to change in the activity of cytochrome P450 isoforms, although the mechanism of this regulation is not fully understood. Up to day, there are few reports concerning the analysis of the impact of these plant extracts on the level of expression of transcription factors involved in the activation of expression of cytochrome P450 isoforms. In the case of St. John's wort, it has been shown that hyperforin is a ligand of PXR and induces CYP3A4 gene transcription. The consequence of this process is an increase in expression of CYP3A4 protein and rapid metabolism of drugs which are substrates of this enzyme [21]. It was shown that active substances of St. John's wort may cause the interactions with HIV protease inhibitor – indinavir, immunosuppressant cyclosporin as well as some oral contraceptives. It may lead to decreased biological half-life of drugs metabolised by CYP3A4 enzyme or can also decrease their therapeutic effect.

Therefore, full knowledge of mechanisms of transcriptional regulation of cytochrome P450 isoforms allows to increase the safety of herbal preparations and dietary supplements in combination with classical pharmacotherapy.

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MOLEKULARNE MECHANIZMY REGULACJI ENZYMÓW CYP I FAZY METABOLIZMU KSENOBIOTYKÓW – LEKÓW SYNTETYCZNYCH I PREPARATÓW ROŚLINNNYCH

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Streszczenie

Zrozumienie właściwości farmakokinetycznych leków syntetycznych, jak również preparatów roślinnych stosowanych w leczeniu wielu schorzeń stwarza realną szansę na eliminację lub zminimalizowanie interakcji pomiędzy substancjami aktywnymi. Ma to szczególne znaczenie kliniczne, ponieważ może prowadzić do osłabienia bądź nasilenia działania leków znajdujących się u podłoża niepowodzeń farmakoterapii. Poznanie podłoża molekularnego interakcji substancji z enzymami metabolizmu leków jest niezwykle pomocne podczas planowania terapii i jej praktycznego zastosowania. Wiele dowodów gwarantujących bezpieczeństwo stosowania produktów zielarskich mogą dostarczyć badania eksperymentalne oraz kliniczne zmierzające do pełnej charakterystyki mechanizmów działania wielu czynników modulujących aktywność enzymów cytochromu P450.

Słowa kluczowe: *cytochrom P450, AhR, PXR, CAR, mechanizm regulacji*