AROMATIC HYDROCARBON RECEPTOR STATUS IN THE METABOLISM OF XENOBIOTICS UNDER NORMAL AND PATHOPHYSIOLOGICAL CONDITIONS

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The aryl hydrocarbon receptor (AhR), a cytosolic protein belonging to the family of nuclear receptors, controls transcription of a wide range of structurally unrelated genes. To date, the most potent AhR ligand is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Exposure to TCDD leads to a number of toxic effects, in particular to tumor promotion and immunosuppression. The function of AhR in cells and living organisms therefore seems to be of paramount importance. Its absence in AhR null mice, results in severe phenotypic abnormalities, such as liver half size with fibrosis, accumulation of retinoic acid and immune system insufficiency. An important role of AhR inheres in its transcriptional control of several biotransformation enzymes (CYP1A1/2,1B1). Hence AhR is the crucial factor in the regulation of xenobiotic metabolism. Under pathophysiological conditions, such as inflammation, the level and activity of the AhR target gene CYP1A is decreased. Thus it is likely, that mediators and/or products of inflammation affect AhR function. This review deals with the role of AhR in xenobiotic metabolism under normal and pathophysiological conditions, with respect to inflammation in particular.

Abbreviations: AhR, aryl hydrocarbon receptor; AhRR, AhR repressor; ARNT, AhR nuclear translocator; bHLH, basic helix-loop-helix; BP, benzo(a)pyrene; CAR, constitutive androstane receptor; CYP, cytochrome P450; DRE (XRE), dioxin (xenobiotic) responsive element; E2, 17 β -estradiol; FXR, farnesyl X receptor; GR, glucocorticoid receptor; GST, glutathione -S- transferase; hsp90, heat shock protein 90 kDa; LXR, lanosterol X receptor; NF- κ B, nuclear factor kappa B; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; PAHs, polycyclic aromatic hydrocarbons; PAS, Per-ARNT-Sim homology; PXR, pregnane X receptor; ROS, reactive oxygen species; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UGTs, UDP-glucuronosyl-transferases; XMEs, xenobiotics metabolizing enzymes

INTRODUCTION

Human populations are exposed to innumerable chemical compounds of natural or synthetic origin, which are foreign to the human body and are called xenobiotics. These include food additives, environmental pollutants, drugs etc. Over the course of evolution, a detoxification system for eliminating xenobiotics from the human organism has developed. This comprises a large group of enzymes, which participate in the excretion process. These, usually divided into two phases, modify the structure of the xenobiotic from a hydrophobic to hydrophilic form for easier excretion.

Biotransformation of xenobiotics is formally divided into three phases. In phase I of biotransformation, a polar

moiety is mostly introduced into the molecule. A major representative of phase I enzymes is the enzyme superfamily of cytochrome P450 (CYP). Many CYP isoforms e.g. CYP3A4, CYP2B6, CYP1A1/2 are induced by xenobiotics and are under transcriptional control of the nuclear receptors PXR (pregnane X receptor), CAR (constitutive androstane receptor) and AhR (aromatic hydrocarbon receptor), respectively. Substantial variations between individuals with respect to CYP enzyme activity arises from a variety of modulating factors, including genetic polymorphisms, age, gender, disease status, pharmacotherapy, and dietary factors such as smoking and alcohol¹.

In phase II of the biotransformation, the oxygenated products of phase I are conjugated to small hydrophilic endogenous molecules such as glucuronic acid, glutathione, sulfate, cysteine or acetate, yielding even more hydrophilic products that can be excreted easily. Examples of phase II enzymes are glutathione transferases, UDP-glucuronosyl-transferases and N-acetyltransferases.

A phase III has recently been described as transmembrane export. This includes transmembrane proteins acting as pumps, which transport non-metabolized xenobiotics or conjugates formed in phase II out of the cell thus decreasing the intracellular concentration of xenobiotics. One of the best-known pumps is P-glycoprotein.

Inflammation, as a pathological state of the organism, affects metabolism of xenobiotics. Inflammation mediators lead almost always, according to all available observations, to decreased levels of cytochromes P450. In this review we have focused on the CYP1A family and AhR,

in terms of their regulation and interplay under normal and under pathophysiological conditions. In addition, we present a brief overview of drug metabolism, comprising main types of reactions, examples of substrates and enzymes participating in xenobiotics metabolism.

BIOTRANSFORMATION AND XMEs

Phase I – oxido/reduction

In phase I of biotransformation, xenobiotics are converted to more polar compounds by oxidation (aromatic and aliphatic hydroxylation, epoxidation, dealkylation, nitrogen oxidation, oxidative dehalogenation), reduction (reductive dehalogenation, nitroreduction, azoreduction) and hydrolytic reactions.

Cytochrome P450 (CYP) enzymes catalyze oxidation reactions. The total number of P450 substrates exceeds 200 000, including the majority of drugs, other xenobiotics, together with eubiotics such as steroids, fatty acids, eicosanoids, retinoids and prostaglandins². The nomenclature of P450s is based on homology in amino acid sequences³. In man, the superfamily of P450s comprises four families of enzymes (CYP1, CYP2, CYP3 and CYP4) that are involved in xenobiotic metabolism⁴. Several CYPs are inducible, particularly by xenobiotics that bind to specific intracellular receptors and consequently modulate expression. These enzymes are expressed in many tissues, but the highest levels are found in the liver. Within the cell, CYPs are localized on the cytosolic side of the endoplasmic reticulum.

Flavin monooxygenases (FMO)⁵ which catalyse the oxygenation of nitrogen-, sulphur- and other nucleophilic heteroatom-containing chemicals and xenobiotics found in foodstuffs. The nomenclature of this enzyme is based on the primary amino acid sequence identity.

Amine oxidases⁵ may be distinguished by their cofactor requirements, substrate specificities and inhibitor sensitivities. Despite involvement of amino oxidases in the metabolism of some xenobiotics, the products of the reaction, ammonia, hydrogen peroxide and an aldehyde are themselves toxic.

Hydroxylases⁵ are represented by aldehyde oxidase and xanthine oxidase, complex molybdoflavo-proteins with similar catalytic properties but with differences in their substrate/inhibitor specificities. They catalyze both oxidation and reduction reactions. Unlike P450, oxidative hydroxylation catalysed by the molybdenum hydroxylases generates reducing equivalents, and the source of the oxygen atom inserted into substrates is water and not O₂. In most cases, these enzymes have complementary substrate specificities with microsomal monooxygenases.

Phase II – conjugation

The oxidized product of phase I is further “polarized” in phase II by conjugation with certain molecules. Phase II enzymes include arylamine N-acetyltransferase, UDP-glucuronosyltransferases (UGTs), glutathione S-transferases (GSTs), sulpho-transferases (STs), and methyl-transferases (catechol O-methyltransferases, thio-

purine S-methyltransferase, and N-methyltransferases). UGTs, STs, and GSTs are gene families with multiple individual isoforms, each having its own preferred substrates, mode of regulation and tissue-specific patterns of expression⁶.

UDP-Glucuronosyltransferases (UGTs)⁵ catalyse the transfer of glucuronic acid from UDP-glucuronic acid to phenols, hydroxylamines, carboxylic acid etc. UGTs are a gene family of integral proteins of the endoplasmic reticulum membranes and the nuclear envelope which are present in many tissues of vertebrates. Two UGT enzyme families termed UGT 1 (bilirubin, phenol) and UGT2 (steroids) have been described. Both families are inducible enzymes, where enzymes of UGT1 family are induced by methylcholanthrene, while UGT2 enzymes are induced by phenobarbital⁷.

Glutathione-S-transferases (GSTs)⁵ catalyse the conjugation of reduced glutathione (GSH) with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH and the substrate. Two superfamilies of mammalian enzymes have evolved and on the basis of their sequence identity, they have been assigned to eight families.

Sulphotransferases (STs)⁵ transfer the sulpho moiety (SO₃⁻), usually from 3'-phosphoadenosine-5'-phosphosulphate (PAPS) as the donor, to a nucleophilic group of the acceptor molecule. The introduced negative charge affects important properties of the acceptor molecule, such as interaction with receptors and transport proteins, water solubility and penetration of the cell membranes.

Phase III – transport

For a long time, biotransformation was formally divided into two phases only. Recently, the process of drug export from the cell is considered as phase III of biotransformation. Thus, membrane proteins transporting xenobiotics across cell membranes are called “phase III enzymes”. The most notorious example of membrane protein with toxicological significance is P-glycoprotein (multidrug resistant protein, MDR1), which mediates the trans-epithelial efflux of xenobiotics. It pumps hydrophobic neutral or positively charged molecules from the cells and plays a significant role in drug absorption and fate⁸. Inhibition and induction of P-glycoprotein leads to drug-drug interactions and has always much greater impact on tissue distribution than on plasma concentrations. Owing to the overlapping substrate specificity of CYP3A4 and P-glycoprotein, and because of similarities in P-glycoprotein and CYP3A4 inhibitors and inducers, many drug interactions affect both P-glycoprotein and CYP3A4⁹.

Conjugates with glucuronic acid, sulphate or glutathione are excreted from the cell by export pumps known as **OATPs** (Organic anion transporting polypeptides). OATPs are a gene superfamily and they transport amphipathic organic solutes¹⁰. Some members of this superfamily are selectively expressed in the liver where they are involved in hepatic clearance of albumin-bound compounds from the portal blood plasma, but most OATPs are expressed in multiple tissues including the blood-brain barrier, choroid plexus, lung, heart, intestine, kidney, placenta

and testis¹⁰. Individual members of the OATP family represent polyspecific organic anion carriers with partially overlapping and partially distinct substrate preferences for a wide range of amphipathic organic solutes including bile salts, organic dyes, steroid conjugates, thyroid hormones, anionic oligopeptides, numerous drugs and other xenobiotic compounds. So far, 9 human OATPs have been identified¹⁰. It was predicted that all OATPs would consist of 12 transmembrane domains. However, this has not been proven experimentally for any OATP¹⁰. OATP1 expression is controlled at transcriptional and post-transcriptional levels and it is, at least in part, tissue-specific. In the kidney, OATP1 expression is stimulated by testosterone and inhibited by estrogens, while hepatic OATP1 expression is not influenced by sex hormones¹⁰. Rat Oatp2 expression is induced by phenobarbital. Recently, it has been found that OATP2 expression is controlled by PXR¹¹. At the protein level, functional down-regulation of rat Oatp1 occurs via serine phosphorylation¹⁰. Analogically to OATPs, there exists a transporter system called organic cation transporters (OCTs). Small organic cations like tetramethylammonium, tetraethylammonium, tetrabutylammonium, *N*-methyl-nicotinamide, thiamine, choline, dopamine, cimetidine, serotonin, histamine, adrenalin and noradrenalin are transported by specialized organic cation transporters (OCT1 in humans and Oct1 in rats). In man, OCT1 is expressed mainly in the liver. The activity in rat Oct1 is modulated by phosphorylation¹².

XENOBIOTICS METABOLIZING ENZYMES REGULATION

A common mode for XMEs regulation is transcriptional control by receptors. In the generalised model of transcriptional regulation of P450s, a receptor interacts with a low molecular weight ligand in the cytosol, consequently changes its conformation and binds to a cognate sequence in DNA where it triggers target gene transcription. Several receptors including PXR^{13, 14}, CAR^{13, 14, 15}, AhR, retinoid X receptor (RXR)¹⁴, vitamin D receptor (VDR)^{13, 14}, retinoic acid receptor (RAR)¹⁴ and GR^{13, 14} are implicated in XMEs regulation. In this review, we have focused on the role of the AhR receptor in XMEs regulation, both under normal and under pathophysiological conditions.

Aryl hydrocarbon receptor (AhR)

The aryl hydrocarbon receptor is a transcription factor belonging to the PAS (Per-ARNT-Sim) family of transcription factors which controls the expression of human CYP1A1, CYP1A2 and CYP1B1, as well as several phase II metabolizing enzymes (NAD(P)H: quinone-oxidoreductase, GST, UGT)^{16, 17}. The human AhR gene is located on chromosome 7¹⁸. There are 12 exons, which encode 848 a.a. protein with a theoretical mass of 96,147 Da^{18, 19}. Typical AhR ligands are PAHs and TCDD. These trigger a number of toxic effects including cancer, immunosuppression and endocrine disruption in rodents²⁰.

AhR functioning

The unliganded AhR exists in the cytosol in a multiprotein complex consisting of one AhR, two hsp90 chaperones, a small protein (p23) and an immunophilin-like protein termed XAP, AIP or ARA9, the latter being involved in the correct folding and stabilization of AhR. Hsp90 acts as chaperone protein system that prevents transcriptional activation of the AhR but keeps the receptor in a conformation that facilitates ligand binding (Figure 1). Upon ligand binding, the chaperon proteins dissociate and AhR translocates to the nucleus, where it rapidly forms a 180–220 kDa²¹ heterodimer with aryl hydrocarbon receptor nuclear translocator (ARNT), known as the hypoxia inducible factor-1 β (HIF-1 β)²². This heterodimer binds to the specific DNA region termed dioxin or xenobiotic response element (DRE or XRE), which has a core sequence of 5'-TNGCGTG-3'¹⁶, and thereby activates gene expression.

The proteolytic degradation of transcription factors is an established mechanism for regulating signal transduction pathways. The AhR protein is degraded following ligand exposure and nuclear export both *in vivo* and *in vitro*. The magnitude of degradation appears to differ among various tissues and cells and it is proteasome-ubiquitin dependent. The concentration of AhR and ARNT protein detected in nuclear lysates of culture cells is highest following 1–2 h of TCDD exposure²³ but then rapidly drops as AhR is degraded. Indeed, cells co-treated with 26S proteasome inhibitors (MG-132 or lactacystin) and TCDD exhibit high levels of AhR and ARNT protein in the nucleus up to 8 h after TCDD treatment²³. When complexed with hsp90 and immunophilin-like proteins, AhR is inactive and these proteins participate in masking the nuclear localization signal (NLS)^{16, 24}, resulting in the cytoplasmic retention of AhR. Furthermore, AhR contains a nuclear export signal (NES)¹⁶, which is important for AhR removal from the nucleus.

The relationship of these proteins to the degradation of AhR seems to be relevant, since their association with AhR may mask key domains for nuclear import, nuclear export, ubiquitination, or phosphorylation. Recently a mouse protein has been identified which is closely related to the AhR, designated aryl hydrocarbon receptor repressor (AhRR). AhRR is localized in the nuclei and does not bind TCDD. AhRR is able to interact with ARNT but AhRR-ARNT dimers are not transcriptionally active. The AhRR is induced by AhR, thus serving as negative feedback loop¹⁶ (Fig. 1).

AhR family

PAS family is a gene family of a structurally related transcription factors with characteristic structural motifs designed as basic helix-loop-helix (bHLH). AhR, ARNT and AhRR are members of the PAS family. The bHLH motif is characteristic for a large number of proteins such as modulators of cell proliferation and differentiation, neurogenesis, myogenesis, B-cell differentiation, and sex determination²⁵. The bHLH motif, localized in the N-terminal region, consists of two amphipathic α -helices separated by a non-helical loop. The HLH region medi-

ates dimer formation, while the basic region is required for binding to DNA. The PAS domain, which has been identified as a conserved sequence among *Drosophila* transcription factors PER (period), SIM (single-minded) and human protein ARNT, is involved in the formation of AhR-ARNT heterodimers, binds a ligand and interact with heat shock protein 90 kDa (hsp90). The ligand binding domain (LBD) of AhR is located in the sequence of 230–431 a.a. from the N-terminus, partly overlapping with the binding site for hsp90 which keeps AhR structurally competent to bind ligands¹⁶.

AhR – a member of the orphan receptor family

To date, no physiological endogenous ligand of AhR has been identified. However, its existence is indirectly supported by numerous observations demonstrating AhR-dependent responses in the absence of exogenous ligand²⁴. Nevertheless, perhaps the best evidence for the existence of an endogenous AhR ligand comes from studies on AhR null mice. Growth rates of the AhR-null mice was retarded compared to wild type mice for the first 3 weeks of life,

and the mutant mice were defective in liver and immune system development¹⁶. Several phenotypic defects have been reported for adult AhR-null mice, such as retinoid accumulation in liver and abnormal hepatic and kidney vascular structures, decreased liver size and hepatic fibrosis¹⁶. Concerning xenobiotic metabolism, the absence of AhR abolished the inducible expression of CYP1A1 and 1A2 genes in response to TCDD or PAHs and resulted in a loss of teratogenesis caused by TCDD and susceptibility to chemical carcinogenesis by BP¹⁶. The loss of CYP1A1, 1A2 and 1B1 inducibility is considered a cause of resistance to chemical carcinogenesis in AhR-null mice.

The greatest exposure to AhR ligands comes from food. There are numerous reports of naturally-occurring dietary chemicals (e.g. flavonoids, carotenoids, phenolics) that activate the AhR signaling pathway, although the majority of these chemicals appear to be relatively weak AhR ligands. Recently, the presence of AhR agonists/antagonists in extracts from a variety of different fruits, vegetables, herbs and teas has been reported²⁴. Certain flavonoids, e.g. flavone and diosmin,

Fig. 1. Basic scheme of AhR signalling. 1) After entering the cell, the ligand binds to AhR, chaperone proteins dissociate and AhR-ligand complex translocates into the nucleus. 2) In the nucleus complex heterodimerizes with ARNT and binds to dioxine responsive element (DRE). 3) Higher transcription rate leads to production of many proteins, including CYP1A subfamily and AhRR. 4) AhRR returns to the nucleus and acts as a competitive inhibitor for ARNT dimerization with AhR, resulting in AhRR-ARNT transcriptionally inactive dimer. 5) CYP1A metabolizes ligand. Activated metabolites form adducts with DNA. Simultaneous formation of H₂O₂ leads to oxidative stress, which in turn activates NF-κB factor, similarly as the cytokines do. Active NF-κB inhibits AhR and decreased AhR activity leads to inhibition of CYP1A production.

act as AhR agonists and induce CYP1A1, 1A2 and 1B1 activities²⁶.

Classical exogenous AhR ligands such as TCDD, 3-MC (3-methylcholanthrene), BP or α -naphthoflavone²⁷ share several common structural features: they are hydrophobic, planar or co-planar molecules of polycyclic structure. Nevertheless several non-polycyclic and non-planar compounds like omeprazole, thiabendazole or carotenoid canthaxanthin activate the CYP1A1 gene as well. However, they are very weak AhR ligands²⁷.

The most studied AhR-mediated expression is that of CYP1A1 (benzpyrene hydroxylase, aryl hydrocarbon hydroxylase), however, other XMEs like paraoxygenase are induced as well²⁸. It seems that there exists cross-talk between AhR and retinoid acid receptors (RARs) signaling pathways, the existence of which is supported by facts: i) AhR expression in keratinocytes is affected by retinoic acid and this depends on the state of differentiation, where an increased level of AhR transcript and activation of CYP1A1 have been observed²⁷, ii) retinoid accumulation in liver has been observed in AhR null mice¹⁴, iii) CYP1A1 is induced through a retinoid acid responsive element (RARE). Indeed, functional interplay and transcriptional cross-talk between AhR and RAR, the vitamin D receptor (VDR) and peroxisome proliferator-activated receptors (PPARs) have recently been reported²⁹, (PPARs are important factors in human health³⁰).

AhR and beyond

Post-transcriptional modification of transcription factors is a common regulatory mechanism of gene expression in eukaryotic cells. Many AhR ligands stimulate signaling cascades similar to those initiated at the cell surface by growth factors, hormones, neurotransmitters and extracellular signals, and share with classical mitogenic factors the propagation of the signal including increased ion fluxes across the membrane, elevation of intracellular Ca^{2+} and cAMP levels, and the activation of protein kinases (PKs) and protein phosphatases (PPs). AhR/ARNT appears to be regulated via phosphorylation, because the binding activity of the AhR/ARNT heterodimer to the XRE is abolished by the phosphatase treatment³¹. These observations are consistent with the idea that the AhR and PK coexist in the cytosol as a part of a multimeric protein complex. TCDD causes an immediate increase in protein kinase C (PKC) and tyrosine kinase activities in hepatocyte membranes. Evidence for PKC involvement in AhR activation comes from the finding, that specific inhibition of PKC by staurosporine²⁷ prevents the ligand-induced DNA-binding of AhR/ARNT heterodimers and leads to suppression of CYP1 expression. Phosphorylation of AhR *in vitro* has been localized to the C-terminal part of the peptide³¹. AhR and ARNT are both phosphorylated on threonine residues³¹, suggesting that a serine/threonine kinase may be directly or indirectly involved in the regulation of the functions of these proteins.

Cell cycle and AhR

The high degree of conservation in the primary structure of the AhR suggests that this protein is crucial for

such life functions, as cell signaling, proliferation and apoptosis¹⁹. The AhR interacts with the retinoblastoma protein (RB1), and the AhR-RB1 complex hinders progression from the G1 to the S phase of the cell cycle by blocking E2F transcription factor-mediated expression of S phase genes. Thus, activated AhR obviously participates in cell cycle arrest at the G1/S boundary. The level of CYP1As in these cell types is critical, because CYP1As degrade AhR ligands and thus prevent the block in progression in G1/S. Constitutive CYP1A1 mRNA is detectable in the mature oocyte, in liver and many other mouse tissues¹⁷. It is likely that abundance of constitutive CYP1A1 mRNA in the fertilized ovum may be important for maintaining sufficient amounts of the CYP1A1 enzyme during the transition from maternal to zygotic control¹⁷.

AhR and estrogen receptor

It has been reported that TCDD induced hepatocellular carcinogenesis in female but not male Sprague-Dawley rats and the tumorigenic response in females was estrogen-dependent²². Decreased formation of estrogen dependent uterine and mammary tumors in rats fed TCDD in the diet suggested that TCDD blocked estrogen receptor (ER) action. Indeed, existence of crosstalk between AhR and ER has been proposed. The AhR is expressed in the rodent uterus and several studies have shown that TCDD and other AhR agonists inhibited 17 β -estradiol (E2)-induced uterine weight increase, cell proliferation and progesterone receptor binding (PR)²². E2 does not affect AhR protein, whereas TCDD induces degradation of ER α , and in cells co-treated with TCDD and E2, cellular ER α protein levels were considerably reduced²². Inhibitory AhR-ER crosstalk affects multiple estrogen-dependent pathways in breast cancer cell lines. For instance, CYP1A induction by AhR agonists results in rapid metabolism and depletion of E2 in the cell culture but not *in vivo*²².

AhR polymorphism

It is well known that there are marked strain and species differences in sensitivity to TCDD in mice, when different responsiveness to TCDD depends on the AhR alleles. Convincing evidence comes from inbred mouse studies where allelic differences in the AhR gene resulted in striking inter-individual differences in susceptibility to cancer, mutagenesis, birth defects and cell type-specific toxicity. Heavy exposures of human populations to dioxin, halogenated hydrocarbons or cigarette smoke have been associated with increased rates of malignancies, birth defects and mental problems³². AhR shows polymorphism, but no dramatic effects of AhR polymorphisms on human health have yet been reported. The extraordinary toxicity of the potent AhR ligand, TCDD, in laboratory animals creates great concern that humans exposed to dioxin-like substances also may suffer from a variety of adverse effects¹⁹. Exposure to dioxin-like compounds has been clearly shown to result in the severe skin disorder chloracne. However, most people who have been exposed to TCDD, even at substantial levels, did not develop

chloracne¹⁹. The basis of the large variation in human susceptibility to chloracne is not known but may be related to genotypic/phenotypic differences in the AhR. Only a few epidemiological studies have focused on association of AhR polymorphisms to cancer risk in men and to date, no study has revealed an increased cancer risk. The polymorphisms might reduce susceptibility to PAHs that are bioactivated by CYP1A1. There are little genetic variations in the AhR in human populations. However, more human AhR polymorphisms certainly remain to be discovered.

Polymorphism

Low-level exposure to carcinogenic chemicals is a frequent, if not ubiquitous, occurrence in Western countries. Environmental tobacco smoke and vehicle exhaust are widespread sources of PAHs and other carcinogens³³. Induction of P450 activities enhances the metabolic activation of substrates, which indirectly determines their toxic, mutagenic and carcinogenic effects. The induction level depends on genetic, dietary, physiological and environmental factors. There is considerable variability in human CYP activity levels due to the polymorphism of some human CYPs. A significant percentage of the population is deficient in a specific enzyme (e.g. CYP2D6) or have a functional enzyme with altered amino acid sequence (CYP2C9)³⁴. Allelic variants of P450s exist due to point mutations in the coding region, but deletion of an entire gene can also occur. Polymorphism plays an important role in the homeostasis/protein level control of some P450s (e.g. CYP2D6), while others are regulated predominantly transcriptionally (e.g. CYP3A4). However, the most P450s are both polymorphic and inducible³⁵. Expression levels of specific CYP enzymes vary substantially among individuals. In men, polymorphisms can result in a population which is either highly sensitive or resistant to the biological effects of chemicals. This is well established in drug therapeutics where individuals lacking a particular P450 exhibit hypersensitivity to drugs having narrow therapeutic indexes. The role of polymorphisms in xenobiotic-metabolizing enzymes in cancer susceptibility is being actively investigated³³.

Other regulatory mechanisms

Albeit transcriptional control is the predominant mean of gene regulation, post-transcriptional regulation plays a part as well. It is more difficult to obtain evidence for mRNA stabilization than for enhanced mRNA synthesis. However, there is evidence that certain proteins selectively bind the mRNA of some P450s and stabilise it^{5, 36}. Moreover, some chemicals inducing one P450 down-regulate other P450s at the same time (e.g. CYP2C11 and PAHs) by a mechanism involving mRNA level lowering⁵. Another means for P450s regulation is post-translational regulation, whereby the sensitivity of P450s to proteolytic degradation can be attenuated by ligand binding or enhanced by protein alkylation. Incorporation of heme, a post-translation event, plays a role in P450s stability. Evidence for destabilisation of P450s by serine

phosphorylation has been demonstrated for rat CYP2B1 and 2E1⁵. The degradation of at least some P450s does involve ubiquitination⁵. Finally, gender-specific expression of CYP2C11 in male rats has been described, while in human males, the gender effects of drug metabolism are rather small.

EXPRESSION AND XMEs ACTIVITY UNDER PATHOPHYSIOLOGICAL CONDITIONS

There are a large number of factors and conditions which modify CYPs levels and/or activities. It has been widely reported that the factors involved in host defense and inflammatory responses interact with and alter the levels and activities of drug metabolizing enzymes. Since inflammation is an accompanying feature of many pathological states, the alteration of drug biotransformation during any inflammatory process should be considered in clinical therapeutics. Although the majority of reports describe a down-regulation of the enzymes, there are examples whereby a specific CYP isoform is induced during such responses. Cytokines which typically modulate P450s expression are interferon (IFN)- α , β , η and δ , interleukins 1 and 6 (IL-1, IL-6), and the tumor necrosis factor alpha (TNF α)³⁷.

Inflammatory processes are complex, and occur in response to a number of pathological disorders, including infection, tissue damage, burns, trauma, tumors, and autoimmune diseases. The process is characterized by the release of cytokines, mediators, acute phase proteins, and hormones. Some of these agents down-regulate (e.g. *Staphylococcal* enterotoxin B, TNF- α , *Escherichia coli* lipopolysaccharide) or up-regulate (e.g. IL-1 β vs. CYP3A1, hepatitis B or C infection vs. CYP2A6) XMEs³⁸.

Cytokines

Definitive proof that IFN suppresses hepatic drug metabolising systems has been provided using human IFNs recombinant preparations (IFN- α , IFN- β , IFN- γ)³⁸. IFNs down-regulate the expression of constitutive enzymes, such as CYP3A2, CYP2C11, CYP2C12 and CYP2E1 in the rat, and CYP1A2 and CYP2C6 in the mouse. Inducible levels of CYP4A1 were depressed by IFN as well. However, the majority of inducible enzymes, e.g. rat CYP3A1 or mouse CYP1A1, CYP1A2, CYP26C, and CYP2B1, were resistant to IFN in the rat³⁸. Recently, endogenous IFN- α / β has been shown to down-regulate all of the major inducible hepatic CYPs in rodents, including CYP1A1, CYP1A2, and CYP2E1, CYP2B and CYP3A1. Recombinant IFN- α administration to humans depresses a number of CYP-dependent drug biotransformation pathways. In addition, down-regulation of induced CYPs by recombinant IFNs also occurs in human and rat hepatocyte cultures³⁸.

In primary rat and human hepatocytes cultures, the media obtained from activated Kupffer cells as well as IL-1 depressed CYP. Subsequently, recombinant cytokines, including IL-1 α , IL-1 β , IL-6, TNF, and TGF

(tumor growth factor), were shown to suppress the expression of CYP1A1, CYP1A2, CYP2B1/2, CYP2C12, and CYP3A3 in rodents and human cell lines (HepG2)³⁸. IL-1 also induced CYP3A1 activity in female rats, while in male rats it depressed CYP3A2, CYP2C11, and CYP1A1. Selective depression of CYP1A1, CYP1A2, and CYP3A6 was attained, when IL-1 and IL-2 were added to cultured rabbit or human hepatocytes³⁸. As compared to other cytokines, the effects of IL-6 on CYP expression are much less pronounced. IL-6 suppresses CYP2C11 and CYP2E1. It is only after dexamethasone pretreatment that IL-6 depresses CYP2C12 in female rats. In primary human hepatocytes, IL-6 specifically inhibited rifampicin and phenobarbital mediated CYP2B6, 2C8/9, and 3A4 expression by a mechanism involving impairment of CAR and PXR transcriptional regulation³⁸. Since both CAR and PXR are under GR transcriptional control, and their transactivation in cell lines was not affected by IL-6, P450s suppression it is a logical consequence of combat between GR and antiglucocorticoid factor IL-6. By contrast, the same study showed that TCDD dependent CYP1A2 expression was only weakly disturbed by IL-6, suggesting role of GR in AhR functioning to be minor.

Cytokines versus AhR

Cytokines regulate the expression of a number of genes via different transcription factors, such as nuclear factor kappa beta (NF- κ B), signal transducer and activator of transcription (STAT), and CCAAT-enhancer-binding protein alpha (CEBP α). It has been postulated that the endogenous ligand of AhR is affected by certain factors, several of them apart from AhR ligands, such as hormones, cytokines and chemicals have a modulation effect. Several studies have revealed that the cytokines TNF- α and IL-1 β repress CYP1A1^{38,40}. It has been demonstrated that the suppressive effects of TNF- α and lipopolysaccharide (LPS) on the CYP1A1 promoter are at least in part due to direct NF- κ B action⁴⁰. NF- κ B, a "pro-inflammatory" transcription factor plays a role in a wide array of physiological and pathological processes including immune modulation, inflammatory responses and apoptosis.

In the past few years, evidence has emerged to show that the AhR and NF- κ B interact and transcriptionally modulate each other⁴⁰. A few studies have suggested that CYP mRNA degradation may be accelerated following the inflammatory process. The cytokine-mediated loss of CYP1A1 and CYP1A2 at accelerated rates in hepatocytes as compared with hepatocytes treated with a transcriptional inhibitor may also be explained by a more rapid turnover of mRNA³⁸.

It has been shown, that dioxin causes chronic, sustained oxidative stress in animals, probably due to production of reactive oxygenated metabolites by CYP1 enzymes¹⁴. The production of reactive oxygen species (ROS) occurs during inflammation or infections. ROS produced *in vivo* and *in vitro* have been implicated in the cascade of events, leading to a loss of CYP during inflammation. Hydrogen peroxide and oxidative stress cause the loss of hepatic CYP1A1 and CYP1A2 mRNAs

in isolated hepatocytes³⁸. The role of oxidative stress on CYP2A5 expression has been examined⁴¹. These results indicated that antioxidants (e.g. vitamin E) prevent and pro-oxidants (buthioninesulfoximine, BSO) stimulate CYP2A5 induction.

Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria⁴², provokes an inflammatory response in the infected host which sometimes leads to septic shock and multiple organ failure. Exposure to LPS rapidly activates the synthesis and release of several pro-inflammatory cytokines including IL-1 β , IL-6 and TNF- α by lymphocytes and macrophages and decreases drug clearance when administered to humans. The most frequently used model of inflammation is the response to LPS, and almost universally, a loss of CYPs has been observed in a variety of species and cell culture systems, e.g. phenobarbital-inducible CYP2B³⁸.

Proinflammatory cytokines such as IL-1, TNF- α , IL-6, interferons, stimulate the production of **nitric oxide (NO)** by increasing expression of inducible nitric oxide synthase (iNOS). NO depresses CYPs mRNAs levels and in parallel binds to the heme moiety of CYPs, which results in loss of CYP activity⁴³. When NO biosynthesis was stimulated in cell lines derived from chinese hamster fibroblasts, benzo(a)pyrene turnover (CYP1A activity) was dramatically reduced to almost unmeasurable levels⁴³.

CONCLUSIONS

Research on biotransformation enzymes regulation has been, is and will be an important and compelling issue. This is a problematique studied at a multidisciplinary level comprising chemistry, biochemistry, biophysics, pharmacology, toxicology, molecular biology, and clinical studies. The discovery and description of the complicated interplay and cross-talk between drug metabolizing enzymes and several nuclear receptors, in particular PXR, CAR, GR, and RXR, has led to the rapid development of current pharmacology. Recently, the role of receptors taking part in normal physiological processes such as farnesyl X receptor (FXR), lanosterol X receptor (LXR), and the vitamin D receptor (VDR), has been elucidated and this brings new insights into the classical concept of xenobiotic metabolism. In this review we have attempted to summarize and discuss current knowledge of the aromatic hydrocarbon receptor (AhR) function and its role in the organism under normal and pathophysiological conditions. As described above, AhR's role in XMEs regulation is undisputed. However, its physiological function remains to be unveiled.

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