Role of Aryl Hydrocarbon Receptor in Toxicity of PAHs

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Role of Aryl Hydrocarbon Receptor in Toxicity of PAHs

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Abstract - Polycyclic aromatic hydrocarbons (PAHs) are the toxic and ubiquitous environmental pollutants. The most important toxicities are carcinogenicity and endocrine disrupting activity and these toxicities are mediated in part by aryl hydrocarbon receptor (AhR) which is a ligand-activated nuclear transcription factor. In this paper, roles of AhR in endocrine disrupting effect of PAHs and are described.

I. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are toxic and ubiquitous environmental pollutants. They are generally formed and emitted into the environment as a result of incomplete combustion of fossil fuels, wood and other organic materials and from industrial processes. Humans and animals are exposed to PAHs from environmental (air, water), dietary and occupational sources, and also from cigarette smoke. While many PAHs have been shown to be carcinogenic in human and laboratory animals [1, 2] and the principal concern regarding exposure to PAHs has been cancer risk, a new problem on their health effects is endocrine disrupting activity there is increasing concern that man- and nature-made chemicals may cause dysfunctioning of human and wildlife endocrine systems leading to adverse health effects such as increased rates of specific cancers, reproductive system abnormalities and immune system deficiencies. These chemicals have been classified as endocrine disruptors. While a wide variety of chemicals including environmental pollutants, industrial chemicals, and natural products are being studied for their effects on endocrine function, PAHs have also been also investigated for their endocrine disrupting activities, primarily their estrogenic and antiestrogenic characteristics. It has been known that endocrine disrupting activity of PAHs are mediated in part by aryl hydrocarbon receptor (AhR).

II. Mechanisms of AhR-mediated responses

AhR is a ligand-activated transcription factor and a member of basic helix-loop-helix per-arnt-sim family of transcription factors [3]. The action of AhR is very similar to those of steroid hormone receptors. Upon activation by ligand binding, AhR enters the nucleus to form a heterodimer with an AhR nuclear translocator (ARNT) and binds to specific gene regulatory sequences called xenobiotic response elements (XREs) leading to up-regulation of a battery of genes including cytochrome P450 (CYP) 1A1, 1A2 and 1B1.

The mechanism of AhR-mediated responses was initially investigated using CYP1A1 as a model and early studies showed that induction of CYP1A1 by TCDD was preceded by rapid formation of a 180 - 220 kD nuclear AhR complex [4]. Subsequent studies showed that this complex was a heterodimer containing the AhR and AhR nuclear translocator (Arnt) protein, and both AhR and Arnt genes have been cloned and extensively characterized [5, 6]. The nuclear AhR complex interacts with consensus dioxin or xenobiotic response elements (DREs:XREs) in the CYP1A1 gene promoter and in promoters of other Ah-responsive genes, and subsequent recruitment of coactivators and general transcription factors results in transactivation. The consensus DRE contains an N T:G TGCGTG A:C C:G A:T A:G G:C N sequence, and the pentanucleotide core (GCGTG) is required for AhR:Arnt binding and flanking sequences are important for transcriptional activation. Both the AhR and Arnt are members of the basic helix-loop-helix family of transcription factors; the AhR is the only ligand-activated member of this family and Arnt is also known as hypoxia inducible factor-1b (HIF-1b) [7]. Arnt plays a critical

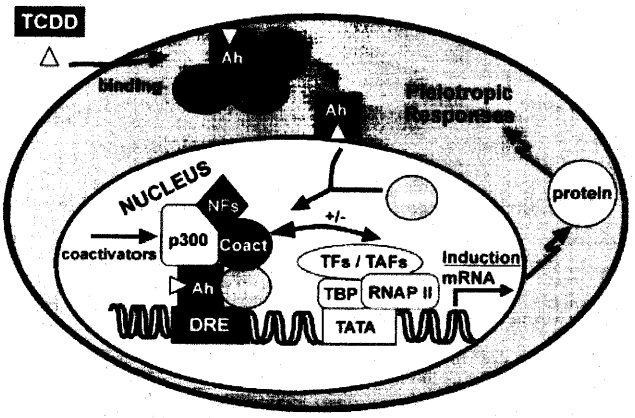

Fig. 1. Proposed mechanism of AhR-mediated gene expression using results from the CYP1A1 gene as a model

role in the cellular response to hypoxia, and the subsequent interaction of Arnt with HIF-1a and their binding to hypoxia

role in the cellular response to hypoxia, and the subsequent interaction of Arnt with HIF-1a and their binding to hypoxia response elements results in the induction of hypoxia-responsive genes. Several Ah-responsive genes that are induced through AhR:Arnt-DRE interactions have been identified, and the overall mechanism (Fig. 1) is similar to that described for ligand-activated nuclear hormone receptors since both nuclear receptor complexes interact with some common proteins including coactivators and corepressors.

III. Endocrine disrupting effect

PAHs have been also investigated for their endocrine disrupting activities, primarily their estrogenic and antiestrogenic characteristics. Several studies have reported that certain PAHs act as antiestrogens by activating aryl hydrocarbon receptor (AhR) which mediates a broad spectrum of antiestrogenic responses [8] (reviews) or by antagonistically binding to estrogen receptor (ER) in yeast expressing human ER [9]. In contrast, some hydroxylated metabolites of BaP have been shown to be estrogenic in MCF-7 human breast carcinoma cells [10].

Several mechanisms have been proposed to explain AhR-mediated antiestrogenic effects of TCDD and PAHs [8]. These include a reduction in the ERα level by down-regulation of ERα gene expression and/or enhanced proteasome-mediated degradation of ERα, a reduction in the cellular E_2 concentration resulting from enhanced metabolism of E_2 by AhR-induced CYP1 family enzymes, AhR-mediated inhibition of ER-induced gene expression.

On the other hand, we [11] and Vinggaard et al. [12] reported that some PAHs exhibited antiandrogenic effects in LNCaP human prostate cancer cells and CHO-K1 Chinese hamster ovary cancer cells, respectively. Further, we demonstrated that PAHs act as AhR agonists exert their antiandrogenic effect through AhR activation [13].

We investigated whether similar mechanisms may be applicable to the present case. So, effects of the PAHs on AR expression level and cellular DHT concentration were examined in this study. The AR level was not affected by the antiandrogenic PAHs, being consistent with recent findings [14] that the AR mRNA level was not affected by TCDD in LNCaP cells. Cellular DHT concentration was not significantly lowered by Chr, BkF or BaP. While CYP1B1 has been known to metabolize steroids, it may not metabolize DHT so rapidly at its expression level in LNCaP cells.

PAHs did appear to influence AR binding to DNA. The intensity of the AR-ARE(+) complex was significantly reduced in the cells treated with each of the antiandrogenic PAHs in the gel mobility shift assay. Since AR level and cellular DHT concentration were not affected by the PAHs, it is appropriate to consider that the reduced formation of the AR-ARE(+) complex is caused by AhR-dependent pathways. While it has been reported that activated AhR blocks the binding of liganded ER to ERE by binding to XREs adjacent to or overlapping ERE [8], this mechanism is not applicable because the oligonucleotide probe used in the present study does not contain XRE in its sequences. Previous works documented that TCDD induces expression of c-fos and c-jun genes and consequently increase in a transcription factor activator protein-1 (AP-1) [15]. On the other hand, Sato et al. have reported that androgenic induction of PSA gene is repressed by elevating AP-1 level with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) or c-jun expression vector in LNCaP cells and that this effect of AP-1 is based on the inhibition of AR-ARE complex formation by protein-protein interaction between the AR and AP-1 [16]. Elevated mRNA levels of c-jun and c-fos in LNCaP cells treated with Chr, BkF or BaP were observed in the present study, too. The findings in the present study together with the results documented previously present a possible mechanism for the antiandrogenic effects of Chr, BkF and BaP in LNCaP cells that binding of AR to ARE is inhibited through interaction between AR and AP-1 induced by AhR activated with Chr, BkF or BaP.

Another possible mechanism for the cross-talk between AR and AP-1 has been proposed. It is that CREB (cAMP response element binding protein) binding protein (CBP) functions as a coactivator for AR and that the transcriptional interference between AR and AP-1 is the result of competition for limiting amounts of CBP in LNCaP cells [17]. This mechanism could contribute to the antiandrogenic effect of Chr, BkF and BaP observed in the present study.

Adding to the mechanisms described above, interaction between AR and AP-1 may involve a number of mechanisms such as overlapping of DNA binding sites of AR with AP-1 and a composite DNA binding site to which both AR and AP-1 bind. These mechanisms may function in gene-specific or cell-specific manners. To date very little is known about the interaction between AR and AP-1. Further investigation is necessary to understand the potential role of AR-AP-1 interaction in the antiandrogenic effects of PAHs and TCDD.

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