Aryl Hydrocarbon Receptor Activation in Chronic Kidney Disease: Role of Uremic Toxins

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Keywords

Aryl hydrocarbon receptor · Uremic toxins · Inflammation · Chronic kidney disease

Abstract

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor involved in the expression of xenobiotic-metabolizing enzymes, inflammatory cytokines and adhesion molecules. Uremic toxins such as indoxyl sulfate and indole acetic acid are derived from tryptophan fermentation by gut microbiota; they accumulate in patients with chronic kidney disease (CKD) on haemodialysis and have recently emerged as potent ligands of AhR. Therefore, AhR can serve as a mediator in inflammation and cardiovascular diseases in these patients. This review discusses current data that support a link between AhR activation and uremic toxins from gut microbiota in CKD.

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Introduction

The aryl hydrocarbon receptor (AhR) was discovered as a mediator of the toxic responses of halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which has carcinogenic and teratogenic effects [1]. Nevertheless, evidence accrued in the past years has shown that AhR is not only a receptor for xenobiotics but it can be activated by several endogenous ligands [2]. In this context, tryptophan metabolism-derived uremic toxins, such as indoxyl sulfate (IS), indole-3-acetic acid (IAA) and indoxyl- β -D glucuronide (IDG), are recognized as endogenous AhR ligands [3, 4] and thus can trigger AhR activation.

Uremic toxins cannot be excreted in urine in chronic kidney disease (CKD) patients or efficiently eliminated by common dialysis modalities; thus, they accumulate

[5]. As a result, tryptophan metabolism-derived uremic toxins are able to activate AhR and, therefore, chronically stimulate oxidative stress and inflammation [4]. Moreover, they also contribute to kidney renal damage and cardiovascular disease (CVD), which is the major cause of death in these patients, particularly for those on dialysis [6, 7]. Thus, uremic toxins have been suggested as a potential “missing link” between CKD and CVD once higher cardiovascular risk in these patients cannot be sufficiently explained by classic factors. In this context, AhR activation by uremic toxins may be linked to these complications in CKD [8, 9].

Taking into account the new evidence about AhR and the importance of understanding the impact of AhR activation in CKD patients, this review aims to discuss how uremic toxins can play a role in the modulation of inflammation and oxidative stress via AhR activation.

Aryl Hydrocarbon Receptor

AhR is a ligand-activated transcription factor involved in the biological detoxification of ligands. AhR has been described as an environment-sensor PAS (Per [period]-Arnt [aryl hydrocarbon receptor nuclear translocator]-Sim [single minded]) protein that belongs to class 1 of the basic helix-loop-helix transcription factors [10].

Under basal conditions, AhR is located in the cytoplasm in an inactive state as part of a complex formed with stabilizer proteins, including 2 molecules of heat-shock protein 90, one co-chaperon p23 (P23) and one X-associated protein 2 [11]. After ligand binding, AhR is activated by a conformational change that exposes its nuclear localization sequence. After its phosphorylation by protein kinase C, the ligand-bound AhR complex is translocated into the nucleus [12]. In the nucleus, this complex releases the protein so that it is able to bind to the AhR nuclear translocator (ARNT) through its PAS domain, resulting in the ligand-bound AhR-ARNT dimer. This heterodimer binds to specific DNA sequences (referred to as DRE or XRE for dioxin- or xenobiotic-responsive element) located within the promoters of target genes, such as cytochrome P450, family 1, member 1A (*Cyp1A1*); cytochrome P450, family 1, member 2A; cytochrome P450, family 1, sub family B (*Cyp1B1*); AhR repressor; and cyclooxygenase-2 (*COX-2*) [6, 13, 14].

The activation of AhR is primarily known to mediate the expression of phase I and II drug metabolism enzymes, but it is also known to be involved in diverse physiological functions such as the regulation of T-cell differentiation,

conception and embryonic/foetal development, mediation of stress and inflammation responses [15]. In fact, classic AhR signalling is not able to explain all the cellular roles attributed to AhR. Thus, non-canonical AhR signalling functions have been described that include crosstalk with nuclear receptor-like transcription factors such as nuclear factor kappa B (NF- κ B; which promotes the inflammatory response) and activator protein 1 (which regulates different target genes and is involved in cellular proliferation, transformation and cell death functions). AhR also regulates cell cycle, modulates the immune system, interacts with molecular circadian clock and activates other cytosolic proteins, including proteins of the mitogen-activated protein kinase (MAPK) family, extracellular signal-regulated kinase, p38, and Jun-NH2-terminal kinase [8, 16].

There is increasing evidence that chronic exposure to environmental chemicals through diet and air, especially persistent organic pollutants such as coplanar polychlorinated biphenyls, polycyclic aromatic hydrocarbons and halogenated aromatic hydrocarbons, such as TCDD or dioxin, exert negative effects by ligand-activated induction of the AhR signalling pathway [17, 18]. Moreover, there are also endogenous (i.e., physiological) AhR ligand candidates, including eicosanoids such as lipoxin A4, bilirubin, resveratrol and lipopolysaccharides. These endogenous compounds have been identified as relatively weak AhR ligands, and their physiological relevance remains uncertain. In addition to sensing environmental toxins and endogenous ligands and thus inducing detoxifying enzymes and modulating immune cell differentiation and responses, AhR also seems to sense microbial insults. Indeed, it was demonstrated that bacterial virulence factors constitute a new AhR ligand [19].

Uremic toxins derived from tryptophan metabolism by gut microbiota such as IS, IAA and IDG are considered potent activators of AhR transcriptional activity. This observation has considerable physiological relevance due to the presence of high concentrations of these toxins in CKD patients and the association of AhR with vascular disease [2, 3].

Figure 1 illustrates the mechanism of AhR activation by uremic toxins.

Uremic Toxins Derived from Tryptophan Metabolism

Under normal conditions, the human gut microbiota performs several activities essential to the body [20–22]. In contrast, its imbalance, observed in numerous diseases such as CKD [23–26] can cause a cascade of metabolic

Fig. 1. Putative mechanism of aryl hydrocarbon receptor (AhR) activation by uremic toxins derived from tryptophan metabolism. AhR is found in the cytoplasm in inactive state as part of a complex formed by stabilizers proteins, including heat-shock protein 90 (HSP-90), co-chaperon p23 (P23) and X-associated protein 2 (XAP2). Like others AhR agonists, the uremic toxins from tryptophan metabolism enters in the cell through an organic anion transporter (OAT) and binds to AhR complex, which is activated and then translocated into the nucleus. In the nucleus, uremic toxin-AhR complex dissociates from the stabilizer proteins complex to be able to form a heterodimeric complex with aryl hydrocarbon nuclear translocator (ARNT) and therefore, regulate several genes involved in the xenobiotic metabolism and inflammation response. Cyp1A1, cytochrome P450, family 1, member 1A; Cyp1A2, cytochrome P450, family 1, member 2A; Cyp1B1, cytochrome P450, family 1, sub family B; COX-2, cyclooxygenase-2; IS, indoxyl sulfate; IAA, indole-3-acetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; LPS, lipopolysaccharide.

abnormalities, including uremic toxin production [27]. These changes in the gut microbiota profile may be related to the increased influx of urea from the bloodstream to the intestinal lumen, which favours the increase in certain bacterial species that can produce several compounds through protein metabolism, such as IS, IAA, IDG and p-cresyl sulfate [21, 25, 28–31]. In CKD patients, these compounds tend to accumulate in the body fluids once they are protein-bound. Therefore, they can neither be excreted through urine nor be efficiently eliminated by dialysis modalities [5]. These retained compounds are often referred to as uremic toxins because they are cytotoxic, negatively impact biological functions and exert pathological effects on immune systems, kidney, blood vessels and heart [21]. In fact, exposure to uremic toxins has emerged to explain cardiovascular events, overall mortality and renal failure progression [6, 32].

Among uremic toxins, those derived from tryptophan metabolism are particularly of importance because they are associated with cardiovascular toxicity and, moreover, have been shown to be potent AhR ligands [33]. Tryptophan is an essential amino acid found in the diet and can be metabolized through different pathways: 2 from endogenous metabolism and 1 from gut microbiota fermentation, the indolic pathway. In the gut, tryptophan

is metabolized by tryptophanase to indole, which is then absorbed and further converted into IS and IDG in the liver [34]. IAA is produced from tryptophan directly in the gut or in tissues through the tryptamine pathway [35].

A study conducted by Schroeder et al. [3] showed for the first time that IS is a human AhR ligand and a potent activator of its transcription activity, opening new paths to stimulate clinical studies in CKD patients and raising the question of whether AhR antagonists can block the negative effects of IS-AhR binding, thereby making it a therapeutic target. Since then, several other studies have found correlation between the negative effects of IS and AhR [3].

Uremic toxins have been associated with inflammation and the incidence of CVD [36–38], the progression of CKD [39, 40], the endothelial dysfunction [41, 42] and bone diseases in CKD patients [43]. Some studies have further shown that these toxins are independently associated with mortality [44, 45].

It has been suggested that high levels of protein-bound uremic toxins are a strong and independent risk factor for cardiovascular events in CKD patients [38]. In particular, the accumulation of indolic uremic toxins in these patients may be a persistent stimulus for the activation of AhR [8], and their exposure has emerged to explain CVD in CKD patients through pro-oxidant, pro-inflammatory

ry, pro-coagulant and pro-apoptotic effects of AhR activation on the cardiovascular system [8, 13]. However, the exact mechanisms by which higher levels of these solutes contribute to CVD and mortality have still not been elucidated. Nevertheless, a growing number of publications have confirmed the negative effects of these compounds.

Barreto et al. [7] showed that there is a gradual rise in serum IS with the severity of CKD from the very earliest stages of the disease. In addition, higher serum IS was a powerful predictor of overall and cardiovascular mortality risk within the cohort study, and this effect was found to be independent of age, gender, diabetes mellitus, phosphate, albumin and haemoglobin levels, vascular stiffness, and aortic calcification. Additionally, in a recent study, it was observed that CKD patients with high IS plasma levels presented a higher incidence of first heart failure event [46].

A recent meta-analysis suggested that IS might suppress the activity of activated leucocytes, inhibit the release of platelet-activating factor by macrophages and contribute to endothelial dysfunction and oxidative stress [28]. Moreover, it was also shown that IS increased oxidative stress and induced the production of reactive oxygen species (ROS) via the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, as it was prevented by the NADPH oxidase inhibitor apocynin. Furthermore, exposure to IS can induce monocyte-mediated inflammation as well as secretion of tumour necrosis factor- α (TNF- α) and interleukin (IL)-6 by adipose cells in patients with renal dysfunction. Therefore, IS can induce adipocyte perturbation and promote inflammatory state mainly through the induction of oxidative stress [47]. In fact, we recently showed that IS and IAA were associated with higher IL-6 and monocyte chemoattractant protein-1 expression (MCP-1) in CKD patients, potentially playing a role in the development of CVD [48].

It was also observed in CKD patients that IAA predicted clinical outcomes (mortality and cardiovascular events) and exerted prooxidant and proinflammatory effects. Additionally, it was demonstrated in cultured human endothelial cells that IAA induced endothelial oxidative stress and inflammation by activating an AhR/p38MAPK/NF- κ B pathway, leading to COX-2 upregulation [6].

A study performed by Bolati et al. [49] demonstrated that IS downregulated the renal expression of factor 2-related erythroid nuclear factor 2 (which control antioxidant and anti-inflammatory cellular responses) through the activation of NF- κ B, followed by the downregulation of haeme oxygenase-1 and NAD(P)H:quinone oxidoreductase 1, thereby increasing the production of ROS.

Although these results in CKD patients show an association between indolic uremic toxins and pro-inflammatory/pro-oxidant effects and CVD, to the best of our knowledge, there have been no studies showing the direct role of AhR in CKD patients. However, studies using cell culture and animal models provide evidence that indolic uremic toxins, especially IS, induce AhR activation.

AhR Activation Through Tryptophan Metabolism-Derived Uremic Toxins

Uremic toxins derived from tryptophan metabolism, such as IS and IAA, are able to activate the AhR-complex pathway through direct binding, which in turn induces the nuclear translocation of AhR complex-ligand (uremic toxins). Using microarray analyses of endothelial cells, it was found that IS and IAA induced upregulation of 8 client genes of AhR, including *Cyp1A1* and *Cyp1B1* [13]. Moreover, AhR activated by IS induced progressive glomerular damage, podocytes injury and pro-inflammatory phenotype in vivo and in vitro [50].

Enoki et al. [51] demonstrated that IS entered muscle cells via organic anion transporter and accumulated in the cytoplasm. By this action, IS activated NADPH oxidase and the AhR pathway to cause an increase in ROS production. This then triggered the production of inflammatory markers and induced the expression of myostatin and atrogin-1, which were involved in muscle wasting [51].

A study conducted by Adelibieke et al. [52] in human umbilical vein endothelial cells (HUVECs) and human aortic smooth muscle cells (HASMCs) treated with IS showed that AhR activation was responsible for IS-induced IL-6 expression through the activation of p65 (subunit of NF- κ B) signalling transduction in these cell cultures.

Another study in HUVECs found that IS activated AhR and induced MCP-1 through ROS production [53]. In addition, a model of IS-induced vascular inflammation showed that IS induced ROS formation, which led to TNF- α -triggered Jun N-terminal kinase and NF- κ B activation. In addition, IS-AhR binding directly stimulated activator protein-1 transcriptional activity. Both pathways acted synergistically to induce E-selectin expression, causing the aggravation of leukocyte-endothelial interaction, a common state found in CDK patients [33].

Most recently, it was demonstrated that IS is involved in the downregulation of MAS receptor, a component of renin angiotensin system axes that is involved in vasodi-

lation and in the development of benefits effects via AhR/NF- κ B activation in rat aorta and HASMCs [54]. Furthermore, Hwang et al. [55] demonstrated that IS regulated Th2 differentiation in an AhR-dependent manner in an allergic asthma model. These results may be important in CKD once patients develop a dysfunctional immune system, as indicated by an increased Th1/Th2 ratio [55].

Ochi et al. [56] observed that IS suppressed the expression of fetuin-A via AhR in human hepatoma HepG2 cells. Fetuin-A is a liver-derived circulating protein that has a potent calcification-inhibitory activity and is decreased in uraemic patients, increasing vascular calcification and cardiovascular mortality [56].

Shivanna et al. [57] demonstrated that IS is a potent CKD-specific prothrombotic metabolite that induced tissue factor in vascular smooth muscle cells through AhR signalling, thereby suggesting that AhR is an antithrombotic target.

Finally, Koizumi et al. [58] showed that IS decreased intracellular nicotinamide phosphoribosyl transferase, the NAD(+)/NADPH ratio and Sirt1 activity, resulting in

an increase in the percentage of senescence-associated β -galactosidase positive cells, which are associated with vascular senescence, a phenomenon that contributes to the development of the cardio-renal syndrome. The authors also observed that antagonists ameliorated the senescence-related changes [58].

Conclusion

On the basis of these findings in the literature, it can be concluded that the uremic toxins-AhR pathway exerts several negative effects, most of which are related to CKD with consequences in the cardiovascular system. Therefore, the uremic toxins-AhR pathway is an important target of investigation in order to improve the health of CKD patients.

Disclosure Statement

The authors have no conflicts of interest to declare.

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