REVIEW

Vascular Senescence in Chronic kidney Disease; Association of Aryl Hydrocarbon Receptor Activated by Indoxyl Sulfate

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The impact of chronic kidney disease (CKD) on the occurrence of cardiovascular disease (CVD) is a major concern and this reciprocal relation is currently so called "cardio-renal syndrome". More detailed understanding in its mechanism may have a possibility to reduce the global burden of CVD. Of note, uremic toxins have been known to accumulate in the progression of CKD and play an important role for worsening renal function, on the other hand, recent studies suggest that they also negatively affect cardiovascular system. In this review, we delve into the role of aryl hydrocarbon receptor (AhR) in uremic toxicities, as highlighted in our latest work and give a new insight for the mechanism of cardio-renal syndrome.

Keywords: Cardio-renal syndrome; Uremic toxin; Indoxyl sulfate

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Indoxyl sulfate serves as a cardiovascular toxin

Correlation between cardiovascular disease (CVD) and chronic kidney disease (CKD) is well-known as cardio-renal syndrome [1, 2]. Currently, numerous uremic toxins have been identified, many of which are found associated with worsening renal function [3,4] However, recent studies suggested those toxins also interact with cardiovascular cells [5, 6, 7]. Indoxyl sulfate (IS) is one of uremic toxins which is an end-metabolite of tryptophan. Indole, a precursor of IS is metabolized from dietary tryptophan by intestinal bacterias and it is synthesized to IS with sulfate conjunction in liver [8]. In the healthy subjects, IS is excreted into urine whereas with the progression of glomerular filtration ratio (GFR), which in turn is gradually elevated in CKD patients and known as "un-dialyzed uremic toxin" [9, 10]. Mainly, a cellular toxicity of IS attributes to enhanced oxidative stress as well as other ureic toxins [11, 12]. Notably, IS has been recently identified to be involved in the onset of cardiovascular disease [13-16]. For example, IS induces the expression of chemokines, cytokines and cell adhesion molecules which have vasoactive properties, leading to atherogenesis [17, 18]. In addition, in human endothelial cells, IS induces nitric

oxide (NO) depletion, resulting in the endothelial dysfunction [19]. Others also showed, in the clinical setting, IS is found to correlates with carotid intima-medina thickness in patients with coronary artery disease [20] and elevated serum IS level is observed in patients with dilated cardiomyopathy [21]. Thus, IS associates cardiovascular disease and investigations in search for the signal pathway connecting increased IS and vascular toxicities are very important. According to studies, serum IS is introduced into cells through organic anion transporters (OATs) [22, 23]. Ito *et al*. reported IS significantly enhanced the adhesion of human monocytic cells in tumor necrosis factor-α (TNF-α)-activated HUVEC[17]. On the other hand, Liu *et al*. showed blockade of OATs prevents formation of fibrosis induced by IS in neonatal cardiac myocytes [24]. It is very clear that OATs have an pivotal role in many of vascular toxicities by IS, however the pathway cannot explain everything which occurs in vasculature secondary to increased IS.

Indoxyl sulfate is a potent agonist of AhR in human endothelial cells

Recent studies have responded to this interesting concern, focusing on an association between aryl hydrocarbon receptor (AhR) and IS [25, 26]. AhR is a ligand activated, basic helix-loop-/per-ARNT-sim transcriptional factor. It is well-known that the activation of AhR contributes to the various physiological processes such as tumor promotion, inflammatory response, drug metabolism and the development of organs [27,28]. Through the stimulation by biological receptor agonists, cytosol AhR translocates into nucleus with subsequent dimerization with AhR nuclear translocater (ARNT) and the AhR/ARNT complex binds to dioxin response element (DRE) in promoter region which in turn trans-activates the target gene expressions [29]. Halogenate aromatic hydrocarbons such as 2, 3, 7, 8-tetrachlorod-ibenzo-para-dioxin (TCDD) and polycyclic aromatic hydrocarbons, such as benzo (a) pyrene are classic environmental compounds for the signal transduction of AhR. Notably, AhR activation by those agonists has been shown to develop atherosclerotic changes, mainly through inflammatory responses in vascular cells [30, 31, 32]. Wu *et al*. reported the association between AhR activation and atherogenesis which shows that TCDD promotes cholesterol accumulation in U937 macrophages and enhances atherosclerotic formation in the aorta of the ApoE knockout mouse [33]. The effect of Benzo (a) pyrene on vascular cells is also significant as is shown in the report by Knaapen *et al*. that this compound induces monocyte chemoattractant protein-1 (MCP-1) expression in human umbilical venous endothelial cell (HUVECs) as well as the increase in atherosclerotic plaque formation in the animal model [32]. Others also showed inducible endothelial dysfunction through AhR to demonstrate that cytochrome P450 (CYP) 1A1, a representative gene induced by TCDD promotes ROS generation with subsequently reduced NO production in human aortic endothelial cells [34]. Thus, these studies indicate that ligand-activated AhR interacts with cardiovascular cells, particularly in the process of atherosclerosis. Interestingly, as well as other tryptophan metabolites, IS is currently reported to activate AhR as a receptor agonist in human hepatocyte with very high binding affinity [25]. Considering AhR is found in many types of human cells, IS possibly activates AhR in human endothelial cells. According to our previous work [35], in HUVECs, 500μmol/L of IS which is a similar concentration in patients on hemodialysis and frequently used in studies to exert oxidative stress in human cardiovascular cells [36-39] significantly enhanced the mRNA expression CYP 1A1 and 1B1 which served as AhR responsive genes. On the other hand, the induction of these genes was canceled by AhR inhibitors. Western blot analysis using the nuclear and cytosol fraction of IS-treated HUVECs showed AhR expression in the nucleus was increased whereas that in the cytoplasm was reduced in a time dependent manner. Taken together, these results clearly indicate that IS behaves as a receptor agonist of AhR and conducts a signal transduction with subsequent expression of AhR-specific genes. In addition, there we also investigate if AhR activation contributes to IS-induced oxidative stress. Intriguingly, AhR inhibitors countered against the induction of NADPH oxidase4 (NOX4) expression and reactive oxygen species (ROS) production respectively in HUVECs incubated with IS. Thus, these results suggest that AhR is essential in endothelial cells to conduct a signal by IS. But what and how is IS-AhR responsible to developing atherosclerotic related changes in vasculature?

Indoxyl sulfate, AhR and vascular senescence

A key to answering these questions is an accelerated cellular senescence induced by IS. It is reported that oxidative stress mediates atherosclerosis via the process of vascular senescence in the animal model of renal dysfunction, suggesting that the onset of premature vascular senescence in the setting of CKD is deeply related to the pathogenesis of cardio-renal syndrome [37]. Sirtuin (Sirt1) is a key player in cellular senescence, which is the closet homologue of silent information regulator2 (Sirt2), identified to be a NAD^+- dependent deacetylase. Sirt1 plays an important role in the process of senescence, apoptosis and cell cycle modulation by regulating the acetylation of lysine groups of many transcriptional factors and proteins such as histones, p53 and FOXO transcriptional factors [41-43]. In addition, studies suggest

that increased oxidative stress impairs Sirt1 activity through suppressing intracellular nicotineamide phosphoribosyltransferase (iNampt), the rate-limiting enzyme for NAD^+ biosynthesis derived from nicotinamide (NAM), with subsequent decease in cellular NAD^+ contents [44-47]. Modulation of Sirt1 activity has been reported to associate cardiovascular disease. For example, reduced Sirt1 activity is negatively associated with NO dependent-vasodilation in hypertensive patients [48]. Sin TK et.al demonstrated restored Sirt1 activity counters against fibrosis formation in the heart of aged mice [49]. Of note, enhanced oxidative stress has been shown to affect sirt1 activity. In HUVECs, H_2O_2 induction suppresses Sirt1 activity with subsequent senescent changes [50]. Csiszar. A reported that enhanced oxidative stress by cigarette smoking up-regulates inflammatory markers, such as intracellular adhesion molecular-1, interleukin-6 and TNF-α in human coronary endothelial cells [51]. But so far, very few studies have referred to the relation between the uremic toxicity with inducible oxidative stress and Sirt1 regulation involved in vascular senescence. In our latest work [52], we saw how IS-AhR is involved in the endothelial senescence particularly, focusing on iNampt-NAD^+-Sirt1 system. Although there were no significant changes in Sirt1 protein expression, as determined on an immunoblotting analysis, Sirt1 activity detected by using an HDAC calorimetric assay was significantly suppressed by IS in HUVECs. The effect of IS on NAD^+ contents and iNampt was also significant. Calorimetric assay showed that both NAD^+/NADH ratio and iNampt activity were reduced after 24h incubation with IS in HUVECs, on the other hand, the recruitment of NAM showed very effective restoration of NAD^+ contents and Sirt1 activity. Thus, these results indicate IS suppressed sirt1 activity through deceasing the cellular NAD^+ and iNampt activity.

We also investigated the association between IS-induced oxidative stress and cellular senescence. According to the studies, oxidative stress has been demonstrated to cause cellular senescence due to impairment of the iNampt-NAD^+-Sirt1 system. Of note, the effect of apocynin, a specific NADPH oxidase inhibitor was significant. In HUVECs, IS increased senescence-associated β-galactosidase positive cells in a similar pattern to H_2O_2, a positive control of oxidative stimulation, whereas this change was obviously reversed by the addition of apocynin. Furthermore, immunoblotting analysis showed apocynin clearly reversed IS-inducible acetylated p53 expression as a result of suppressed sirt1 activity, and that restored decrease in cellular NAD^+ contents and iNampt activity, respectively. Taken together, these results indicate enhanced oxidative stress following IS generated ROS production impairs iNampt-NAD^+-Sirt1 system and results in cellular senescence in HUVECs.

Finally, we used AhR blockade against IS-induced cellular senescence to further confirm the involvement of AhR in the mechanism. The addition of AhR inhibitors to HUVECs incubated with IS was significantly effective on the senescent change determined by SA-Bgal staining. In addition, they also abolished the reduction in the iNampt and NAD^+/NADPH ratio, which in turn restored both the sirt1 activity determined on HDAC calorimetric assay and the protein expression of acetylated p53. Thus, we conclude that IS- induced endothelial senescence is AhR-dependent.

AhR, as a therapeutic target for cardiovascular disease

In summary, the onset of CVD, comorbid with the progression of CKD is developed by various factors including up-regulation of cytokines and growth factors, the renin-angiotensin system (RAS), sympathetic nervous system and oxidative stress. Among them, current studies have focused on uremic toxins, many of which are accumulated with CKD and found deeply involved in the progression of atherosclerotic changes in vasculature. Thus, revealing the detailed mechanism of uremic toxicity may contribute to CVD outcome strategy. As a part of this concept, here we show the relevance of IS-AhR in vascular senescence, suggesting further investigation is required to confirm if blocking AhR is effective on CVD in the clinical setting. This finding will give a profound understanding for the uremic toxin and may provide the novel therapeutical tool for preventing cardio- renal syndrome.

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