Aryl hydrocarbon receptor is activated in patients and mice with chronic kidney disease

Laetitia Dou[1], Stéphane Poitevin[1], Marion Sallée[1,2], Tawfik Addi[1], Bertrand Gondouin[2], Nathalie McKay[1], Michael S. Denison[3], Noémie Jourde-Chiche[1,2], Ariane Duval-Sabatier[2,4], Claire Cerini[1], Philippe Brunet[1,2], Françoise Dignat-George[1] and Stéphane Burtey[1,2]

[1]Aix-Marseille University, INSERM, UMR-S 1076, VRCM, Marseille, France; [2]Centre de Néphrologie et Transplantation Rénale, AP-HM, Marseille, France; [3]Department of Environmental Toxicology, University of California, Davis, California, USA; and [4]Association des dialysés Provence-Corse, Marseille, France

Patients with chronic kidney disease (CKD) are exposed to uremic toxins and have an increased risk of cardiovascular disease. Some uremic toxins, like indoxyl sulfate, are agonists of the transcription factor aryl hydrocarbon receptor (AHR). These toxins induce a vascular procoagulant phenotype. Here we investigated AHR activation in patients with CKD and in a murine model of CKD. We performed a prospective study in 116 patients with CKD stage 3 to 5D and measured the AHR-Activating Potential of serum by bioassay. Compared to sera from healthy controls, sera from CKD patients displayed a strong AHR-Activating Potential; strongly correlated with eGFR and with the indoxyl sulfate concentration. The expression of the AHR target genes *Cyp1A1* and *AHRR* was up-regulated in whole blood from patients with CKD. Survival analyses revealed that cardiovascular events were more frequent in CKD patients with an AHR-Activating Potential above the median. In mice with 5/6 nephrectomy, there was an increased serum AHR-Activating Potential, and an induction of *Cyp1a1* mRNA in the aorta and heart, absent in $AhR^{-/-}$ CKD mice. After serial indoxyl sulfate injections, we observed an increase in serum AHR-AP and in expression of *Cyp1a1* mRNA in aorta and heart in WT mice, but not in $AhR^{-/-}$ mice. Thus, the AHR pathway is activated both in patients and mice with CKD. Hence, AHR activation could be a key mechanism involved in the deleterious cardiovascular effects observed in CKD.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2017.11.010

KEYWORDS: aryl hydrocarbon receptor; cardiovascular disease; chronic kidney disease; indoxyl sulfate; mouse model

Correspondence: *Pr Stéphane Burtey, Team «Dysfonction endothéliale et Insuffisance rénale chronique» VRCM, UMR_S1076 Aix-Marseille Université, Faculté de pharmacie, 27 bd Jean Moulin 13005 Marseille, France. E-mail: stephane.burtey@univ-amu.fr*

Received 13 November 2016; revised 18 October 2017; accepted 9 November 2017

Chronic kidney disease (CKD) is an emerging epidemic. CKD increased by 73% from 1990 to 2013 as a cause of deaths worldwide.[1] CKD is associated with an increased risk of death, especially from cardiovascular disease (CVD).[2,3] Classical CVD risk factors do not explain the increased rate of cardiovascular events in CKD.[4] Uremic toxins that accumulate during CKD could be the missing link between reduced ability of the kidney to eliminate waste and CVD.[5] They are divided into 3 groups: small soluble compounds, middle molecules, and protein-bound molecules.[6] Among this last group, increased levels of the indolic toxins indoxyl sulfate (IS) and indole-3 acetic acid (IAA) are associated with increased risk of death and cardiovascular events.[7,8] In addition, numerous studies have demonstrated the deleterious effect of indolic toxins on renal and vascular cells.[9] The cellular receptor of indolic solutes was identified as aryl hydrocarbon receptor (AHR).[10,11] AHR is an intracellular receptor for xenobiotics such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the dioxin-like 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), and benzo[a] pyrene, a chemical found in tobacco smoke.[12] AHR also binds endogenous ligands, including metabolites of arachidonic acid and tryptophan, such as kynurenine.[13,14] AHR resides in the cytoplasm of mammalian cells in a multiprotein complex that includes HSP90 and AHR-interacting protein.[12,15,16] AHR-ligand complex translocates to the nucleus, where it forms a heterodimeric complex with the aryl hydrocarbon nuclear translocator.[12,15] The complex binds to a DNA consensus sequence[12,15] present in the promoters of a wide variety of genes, including those coding for enzymes involved in xenobiotic detoxification (*CYP1A1*, *CYP1A2*, and *CYP1B1*) and the AHR repressor, *AHRR*.[12,15] A genomic, aryl hydrocarbon nuclear translocator–independent pathway for AHR-mediated gene expression has recently been reported,[17] as well as a role of AHR as a component of numerous signaling pathways, independent of its ability to bind to DNA.[17]

In humans exposed to AHR agonists such as TCDD or PCB 126, the risk for cardiovascular events is increased.[18] This association was recognized by the US government after exposure of veterans to Agent Orange.[19] In $ApoE^{-/-}$ mice, AHR activation by TCDD is associated with acceleration of atherosclerosis.[20] In rats, PCB 126 exposure increases CVD risk factors: serum cholesterol, blood pressure, and heart weight.[21]

The activation of AHR by IS and IAA has been demonstrated to contribute to vascular dysfunction.[8,22] In endothelial cells and in vascular smooth muscle cells, AHR activation increases the expression and activity of tissue factor, leading to a procoagulant state.[22,23] It also induces an increased expression and activity of the pro-inflammatory enzyme cyclooxygenase-2 in endothelial cells.[8] The vascular dysfunction induced by AHR activation could lead to atherothrombosis and plays a role in the increased risk of myocardial infarction, peripheral artery disease, and stroke observed in CKD.[24] Despite strong evidence of AHR activation by indolic uremic toxins *in vitro*, studies of AHR activation in CKD are scarce. The present study aimed to demonstrate that AHR is activated in patients with various stages of CKD. In CKD mice, we also studied AHR activation in the vascular wall and the role of IS as a representative uremic AHR agonist.

RESULTS

Uremic serum induced AHR activation

We studied a cohort of 116 patients with CKD (51 with stage 3–5 CKD and 65 with stage 5D CKD) (Table 1) and compared these patients with 52 healthy controls. We analyzed the activation of AHR by serum samples using the AHR-responsive chemically activated luciferase expression cell bioassay, a method commonly used for the screening of samples for the presence of TCDD, dioxin-like compounds, and AHR agonists and/or antagonists.[25,26] The serum AHR-activating potential (AHR-AP) was significantly higher in patients with stage 3 to 5 CKD ($P < 0.05$) and stage 5D CKD ($P < 0.0001$) than in controls (Figure 1a). Mean ± SD values of AHR-AP were 22 ± 9 arbitrary units (AU) (range: 5–44 AU), 37 ± 24 AU (range: 6–121 AU), and 79 ± 56 AU (range: 8–259 AU), respectively, in controls, in patients with stage 3 to 5 CKD, and in patients with stage 5D CKD (Figure 1a). We then examined whether AHR-AP of uremic serum could be counteracted by an AHR antagonist. The addition of the AHR antagonist CH223191 reduced by 46% the AHR-AP of serum from patients with stage 5D CKD ($P < 0.01$) (Figure 1b). CH223191 alone had no effect, and induced a slight, not significant decrease in the AHR-AP of control serum (Figure 1b).

At baseline, AHR-AP from all patients with stage 3 to 5D CKD negatively correlated with hemoglobin ($r = -0.31$,

Table 1 | Baseline characteristics of the CKD population

Characteristics	All patients ($n = 116$)	AHR-AP < 44AU	AHR-AP ≥ 44AU	*P* value
Age (yr)	68 (23; 89)	63 (31; 89)	72 (23; 89)	<0.05
Gender ratio (W:M)	40:76	19:36	21:40	1
Body mass index (kg/m^2)	24.6 (15.8; 47)	24.9 (16.8; 37.9)	24.5 (15.8; 47)	0.7
Dialyzed patients (%)	65 (56%)	19 (34%)	46 (75%)	<0.0001
eGFR[a] (ml/min per 1.73 m^2)	25 (8; 59)	30 (11-59)	14 (8; 53)	<0.01
Kidney disease				
Glomerulonephritis	22 (19%)	8 (15%)	14 (23%)	0.3
ADPKD	11 (9%)	6 (11%)	5 (8%)	0.7
Vascular	32 (28%)	13 (24%)	19 (31%)	0.4
Interstitial	23 (20%)	15 (27%)	8 (13%)	0.06
Other hereditary	7 (6%)	5 (9%)	2 (4%)	0.2
Unknown	21 (18%)	8 (14%)	13 (21%)	0.4
Hypertension	101 (87%)	47 (85%)	54 (89%)	0.8
Systolic blood pressure (mm Hg)	141± 24	143 ± 28	139 ± 19	0.5
Diastolic blood pressure (mm Hg)	77 ± 14	79 ± 15	74 ± 12	<0.05
Current smokers	47 (41%)	20 (36%)	27 (44%)	0.4
History of cardiovascular diseases	41 (35%)	17 (31%)	24 (39%)	0.4
Phosphate binders	60 (52%)	19 (34%)	41 (67%)	<0.001
Antihypertensive drugs	86 (74%)	43 (78%)	43 (70%)	0.4
Statins	37 (32%)	13 (24%)	24 (39%)	0.07
Antiplatelet drugs	47 (40%)	17 (31%)	30 (49%)	0.058
Anticoagulant drugs	26 (22%)	11 (20%)	15 (24%)	0.6
Erythropoietin therapy	58 (50%)	17 (31%)	41 (67%)	<0.001
Serum CRP level (mg/l)	4 (0; 78)	4.6 (0.1; 54)	4 (0; 78)	0.5
Hemoglobin (g/dl)	12.0 (8.8; 16.3)	12.4 (9.5; 16.3)	11.3 (8.8; 14.4)	<0.001
Serum bicarbonate level (mmol/l)	22.7 ± 3.0	23.7 ± 3.1	21.8 ± 2.6	<0.01
Serum albumin level (g/l)	36 (26; 44)	36 (26; 44)	36 (28; 43)	0.7
Serum calcium level (mmol/l)	2.34 ± 0.12	2.34 ± 0.10	2.34 ± 0.14	0.9
Serum phosphate level (mmol/l)	1.33 (0.65; 3.17)	1.23 (0.65; 3.17)	1.5 (0.7; 2.9)	<0.05
Serum cholesterol level (mmol/l)	4.6 (1.9; 9.1)	5.5 (2.7; 9.1)	4.3 (1.9; 7.3)	<0.001
Serum LDL cholesterol level (mmol/l)	2.9 ± 1.1	3.2 ± 1.1	2.6 ± 0.9	<0.01
Serum triglyceride level (mmol/l)	1.5 (0.4; 5.9)	1.4 (0.4; 5.9)	1.6 (0.5; 3.6)	0.7
Serum β2 microglobulin level (mg/l)	22.6 (2.8; 66.9)	7.9 (2.8; 66.9)	27.6 (3.8; 56.4)	<0.0001
Serum Indole-3 acetic acid level (μM)	2.9 (0.6; 19.1)	2.2 (0.6; 16.3)	3.5 (0.7; 19.1)	<0.05
Serum indoxyl sulfate level (μM)	43.7 (0.2; 256.2)	13.4 (0.2; 157.8)	78.9 (1.2; 256.2)	<0.0001

AHR, aryl hydrocarbon receptor; AP, activating potential; ADPKD, autosomal dominant polycystic kidney disease; AU, arbitrary units; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; M, men; W, women.
Results are given as mean ± SD if distribution is Gaussian, or in median (min; max) if not.
[a]Calculated by Modification of Diet in Renal Disease (MDRD) study formula for nondialyzed CKD patients ($n = 51$).