UREMIC TOXINS
A focused update 2015

Actualités Néphrologiques
Necker 28 avril 2015

Noémie Jourde-Chiche, Bertrand Gondouin, Marion Sallée, Laetitia Dou, Claire Cerini, Philippe Brunet, Stéphane Burtey
• Uremic toxins:
  – Solutes accumulating in patients with CKD
  – With demonstrated toxicity

• >100 solutes identified as uremic toxins
Classification of Uremic Toxins

Small hydrophilic compounds:
- Urea
- Uric acid
- Guanidine
- Oxalate
- ADMA

Middle molecules:
- Beta2-m
- PTH
- FGF23
- IL-1β
- IL-6
- TNF-α
- K light chain
- L light chain
- Leptin
- Resistin
- Endothelin
- VEGF

Protein-bound uremic toxins:
- P-cresyl sulfate
- Indoxyl-sulfate
- Indole-3 acetic acid
- Homocysteine
- ADMA
- AGEs
- Kynurenic acid
- Pentosidin
- CML
- 5-propyl FPA

Vanholder, Kidney Int 2003
Duranton, JASN 2012
Classification of Uremic Toxins

Small hydrophilic compounds
- Urea
- Uric acid
- Guanidine
- Oxalate
- ADMA

Middle molecules
- Beta2-m
- PTH
- FGF23
- IL-1β
- IL-6
- TNF-α
- K light chain
- L light chain
- Leptin
- Resistin
- Endothelin
- VEGF

Protein-bound uremic toxins
- P-cresyl sulfate (PCS)
- Indoxyl-sulfate (IS)
- Indole-3 acetic acid (IAA)
- Homocystein
- ADMA
- AGEs
- Kynurenin (KYN)
- Pentosidin
- CML
- 5-propyl FPA

Poids moléculaire (Dalton)
- 60 000

Vanholder, Kidney Int 2003
Duranton, JASN 2012
Classification of Uremic Toxins

Indolic uremic toxins: generated by gut bacteria metabolism of Tryptophan
Focused update on uremic toxicity

- **Common Mechanisms**
  - OXIDATIVE STRESS
  - ACTIVATION OF AhR

- **UREMIC TOXINS : A THERAPEUTIC TARGET IN CKD**

- **ENDOTHELIAL DYSFUNCTION**
  - VASCULAR LESION
  - THROMBOSIS
  - INFLAMMATION

- **RENAL FIBROSIS**
  - CKD PROGRESSION
CKD: an independent CV risk factor

CKD patients: traditional risk factors + uremia-related factors:
- Oxidative stress
- Endothelial dysfunction
- Inflammation

UREMERIC TOXINS

Plasma levels associated with CV morbidity and/or mortality

- $\beta_2$m in CKD/HD patients
- IS in CKD/HD patients
- IAA in CKD/HD patients
- PCS in CKD/HD patients
- Hcy in CKD patients
- KYN in patients with stroke
Cardiovascular toxicity of Uremic Toxins

• Endothelial dysfunction
  - observed even in early stages of CKD
  - associated with oxidative stress
  - participates in the accelerated atherosclerosis observed in CKD

• All aspects of endothelial homeostasis are affected in CKD
Endothelial dysfunction in CKD is reflected by:

<table>
<thead>
<tr>
<th>FUNCTIONAL TESTS</th>
<th>SOLUBLE BIOMARKERS</th>
<th>CELLULAR BIOMARKERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated dilation (FMD) related to NO synthesis</td>
<td>Pro-coagulant (vWF, TF, thrombomodulin, PAI-1)</td>
<td>Circulating endothelial cells (CEC)</td>
</tr>
<tr>
<td>Pulse Wave Velocity (PWV) related to arterial stiffness/calcifications</td>
<td>Pro-inflammatory (VCAM-1, ICAM-1, E-selectin...)</td>
<td>Endothelial microparticles (EMP)</td>
</tr>
<tr>
<td>In vivo (animal) thrombus formation, leukocyte adhesion/extravasation, angiogenic capacity</td>
<td>Angiogenesis (VEGF, HGF...)</td>
<td>Endothelial progenitor cells (EPC)</td>
</tr>
<tr>
<td></td>
<td>Glycocalyx dysruption (heparan sulfate, proteoglycans)</td>
<td></td>
</tr>
</tbody>
</table>
Endothelium is normally the cornerstone of vascular homeostasis.

Anti-inflammatory and anti-coagulant phenotype

Repair by endothelial progenitor cells (EPC)

Shear-induced NO synthesis

Glycocalyx

Repair by mature EC

Cardiovascular toxicity of Uremic Toxins
Endothelial toxicity of Uremic Toxins

Uremic toxins induce endothelial dysfunction and oxidative stress

- Pro-inflammatory and pro-coagulant phenotype
- Release of EMP
- ROS generation
- Detachment of CEC
- Reduced mobilization and survival of EPC
- Reduced proliferation
- Glycocalyx breakdown
- Reduced NO availability
### Cardiovascular toxicity of Uremic Toxins

**Protein-bound uremic toxins, in particular, display endothelial toxicity**

<table>
<thead>
<tr>
<th>Effects on endothelium in vitro</th>
<th>Protein-bound uremic toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased endothelial NO synthesis</td>
<td>ADMA, AGEs, Hcy</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>IS, IAA, ADMA, AGEs, Hcy</td>
</tr>
<tr>
<td>Increased EMP release</td>
<td>IS</td>
</tr>
<tr>
<td>Pro-inflammatory</td>
<td>IS, IAA, ADMA, AGEs, Hcy</td>
</tr>
<tr>
<td>Leucocyte adhesion/extravasation</td>
<td>IS, PCS, AGEs</td>
</tr>
<tr>
<td>Decreased proliferation</td>
<td>IS, ADMA</td>
</tr>
<tr>
<td>Reduced EPC survival</td>
<td>IAA, AGEs</td>
</tr>
<tr>
<td>Altered EPC differentiation/function</td>
<td>ADMA, AGEs, Hcy</td>
</tr>
</tbody>
</table>
## Cardiovascular toxicity of Uremic Toxins

Protein-bound uremic toxins also display toxic effects on Smooth muscle cells, vascular wall and cardiac remodeling/fibrosis

<table>
<thead>
<tr>
<th>Effects on SMCs/vascular wall/heart</th>
<th>Protein-bound uremic toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>IS, PCS, AGEs</td>
</tr>
<tr>
<td>Vascular calcifications</td>
<td>IS, AGEs</td>
</tr>
<tr>
<td>(SMCs → ostéoblastic phenotype)</td>
<td></td>
</tr>
<tr>
<td>Remodeling of vascular wall</td>
<td>PCS</td>
</tr>
<tr>
<td>Proliferation of vascular wall SMCs</td>
<td>IS</td>
</tr>
<tr>
<td>Increased senescence of SMCs</td>
<td>IS</td>
</tr>
<tr>
<td>Cardiac wall fibrosis</td>
<td>IS</td>
</tr>
</tbody>
</table>

Cardiovascular toxicity of Uremic Toxins

IS and PCS stimulate the crosstalk between leukocytes and the vessel wall (intravital microscopy in rats)

Rolling Leukocytes

Adhering Leukocytes

Extravasated Leukocytes

Pletinck, JASN 2013
Cardiovascular toxicity of Uremic Toxins

IS favors glycocalyx disruption

Soluble heparan sulfate

Aorta

Kidney

Visceral Peritoneum

Pletinck, JASN 2013; Rabelink, Nat Rev Nephrol 2010
IS and IAA induce Tissue Factor expression and TF activity by ECs and PBMCs and reduce proteasomal TF degradation in SMCs
IS and IAA induce TF expression in HUVEC and PBMC and increase TF activity in HUVEC and EMP.

---

**Cardiovascular toxicity of Uremic Toxins**

**Figure a:**
- Control
- IS
- IS + IgG
- IS + anti-TF

**Figure b:**
- Control
- IS
- IS + IgG
- IS + anti-TF

**Figure c:**
- Control
- IS

Gondouin, Kidney Int 2013
Cardiovascular toxicity of Uremic Toxins

IS and IAA promote the pro-coagulant phenotype of endothelial microparticles

Cardiovascular toxicity of Uremic Toxins

This increase in TF expression and activity after IS and IAA exposure is mediated by AhR

Redistribution of AhR from the cytoplasm to the nucleus

Gondouin, Kidney Int 2013
Cardiovascular toxicity of Uremic Toxins

IAA : a new “vasculo-toxin” independantly associated with mortality and cardiovascular events in CKD/HD patients

Dou, Sallée et al, JASN 2014
Cardiovascular toxicity of Uremic Toxins

IAA induces COX-2 production by endothelial cells through AhR activation

**A**

![Graph showing COX-2 mRNA expression (fold change) for different cell lines and treatments.](image)

**B**

![Graph showing normalized COX-2/ß-actin ratio for different treatments.](image)

**C**

![Graph showing COX-2 mRNA expression (fold change) for different concentrations of IAA and HSA.](image)

- **CH**: AhR inhibitor
- **SB**: p38/MAPK inhibitor
- **BAY**: NF-KB inhibitor

Dou, Sallée et al, JASN 2014
AhR mediated toxicity of uremic toxins: a «dioxin-like» poisoning

Exogenous ligands: pollutants
- DIOXIN (TCDD)
- PCBs, PCDDs, PCDFs
- Industrial activities >> volcano/forest fire

Endogenous Ligands: products of Tryptophan metabolism
- Indoles (IS, IAA)
- Kynurenins

**GENOMIC PATHWAY**
- Nuclear translocation
- XRE → Induction of «detoxification» genes
  - CYP1A1, CYP1B1

**NON GENOMIC PATHWAY**
- Inflammation (NFkB, AP-1...)
- Signalisation (MAPK, ERK, p38, JNK)
- Cell cycle (p27, Cyclin D...)

**AhR**

 Ligand

2,3,7,8-TCDD [1746-01-6]
AhR mediated toxicity of uremic toxins: a « dioxin-like » poisoning

AhR

Xenobiotic response
- Induction of xenobiotic metabolizing enzymes (CYP)
- Regulation of AhR pathway activity

Cell cycle
- p27Kip1 transcription
- E2F targets
- RB1 phosphorylation

Estrogen response
- Estrogen-like activity of AhR ligands (ER-α interaction)
- ER-α protein levels (CUL4B)

Antioxidant response
- NRF2 activation
- NRF2 synergy

Pro-inflammatory
- IEL/ILC homeostasis
- Th17 development (RORyt synergy, STAT1 activation or inhibition, IL-22 production)

Anti-inflammatory
- IDO/IL-10 production in DCs
- LPS response (STAT1, NF-κB)
- RelB stabilization
AhR mediated toxicity of uremic toxins: a «dioxin-like» poisoning

DIOXIN POISONING
- ACUTE: chloracne, hepatitis
- CHRONIC:
  - Immunological perturbances
  - Neurological/developmental abnormalities
  - Hormonal perturbation (thyroid/steroids/reproductive)
  - Carcinogenesis (Agent orange, Vietnam)
  - Cardiovascular disease

WHO: levels authorized in food/beverage

TOXINS FROM TRYPTOPHAN METABOLISM: similar toxicity
AhR mediated toxicity of uremic toxins: a « dioxin-like » poisoning

<table>
<thead>
<tr>
<th>AhR-activating-pollutants</th>
<th>TRP-derived uremic toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Association with cardiovascular events</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular mortality</td>
<td>Cardiovascular mortality</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Hospitalization for ischemic stroke</td>
<td>Hospitalization for ischemic stroke</td>
</tr>
<tr>
<td>Number of atherosclerotic carotid plaques</td>
<td>Size of atherosclerotic carotid plaques</td>
</tr>
<tr>
<td>Decreased endothelial-dependent vasodilation</td>
<td>Decreased endothelial-dependent vasodilation</td>
</tr>
<tr>
<td>Induction of cardiomyopathy in rodents</td>
<td>Induction of cardiac fibrosis in rodents</td>
</tr>
<tr>
<td>Induction of atherosclerotic lesions in mice</td>
<td>Induction of atherosclerotic lesions in mice</td>
</tr>
</tbody>
</table>

Sallée, Toxins 2014
AhR mediated toxicity of uremic toxins: a « dioxin-like » poisoning

<table>
<thead>
<tr>
<th>AhR-activating-pollutants</th>
<th>TRP-derived uremic toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelial dysfunction</strong></td>
<td></td>
</tr>
<tr>
<td>Inhibition of endothelial cell proliferation</td>
<td>Inhibition of endothelial cell proliferation</td>
</tr>
<tr>
<td>Inhibition of endothelial NO production</td>
<td>Inhibition of endothelial NO production</td>
</tr>
<tr>
<td>Inhibition of endothelial cell migration</td>
<td>Inhibition of endothelial cell migration</td>
</tr>
<tr>
<td>Decrease in endothelial progenitor cells</td>
<td>Decrease in progenitor cells</td>
</tr>
<tr>
<td>Induction of stress fiber formation</td>
<td>Induction of stress fiber reorganization</td>
</tr>
<tr>
<td><strong>Oxidative stress</strong></td>
<td></td>
</tr>
<tr>
<td>Association with oxidative stress markers</td>
<td>Association with oxidative stress markers</td>
</tr>
<tr>
<td>Induction of endothelial ROS</td>
<td>Induction of endothelial ROS</td>
</tr>
<tr>
<td>Induction of VSMC migration by ROS</td>
<td>Induction of VSMC migration by ROS</td>
</tr>
<tr>
<td>Induction of T cell differentiation</td>
<td>Induction of T cell differentiation</td>
</tr>
<tr>
<td>AhR-activating-pollutants</td>
<td>TRP-derived uremic toxins</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td></td>
</tr>
<tr>
<td>Induction of TF production and activity</td>
<td>Induction of TF production and activity</td>
</tr>
<tr>
<td><strong>Inflammation</strong></td>
<td></td>
</tr>
<tr>
<td>Association with inflammatory markers</td>
<td>Association with inflammatory markers</td>
</tr>
<tr>
<td>Increase in monocyte expression of inflammatory cytokines</td>
<td>Increase in monocyte expression of inflammatory cytokines</td>
</tr>
<tr>
<td>Increased monocyte adhesion</td>
<td>Increased monocyte adhesion</td>
</tr>
<tr>
<td>Induction of VCAM-1, MCP-1, E-selectin, ICAM-1 and COX-2</td>
<td>Induction of VCAM-1, MCP-1, E-selectin, ICAM-1 and COX-2</td>
</tr>
<tr>
<td>Induction of urinary COX-2 metabolites in mice</td>
<td></td>
</tr>
</tbody>
</table>
ENDOTHELIAL DYSFUNCTION
VASCULAR LESION
THROMBOSIS
INFLAMMATION

RENAL FIBROSIS
CKD PROGRESSION

UREMIC TOXINS : A THERAPEUTIC TARGET IN CKD

Common Mechanisms
OXIDATIVE STRESS
ACTIVATION OF AhR
# Uremic Toxins and Renal Fibrosis / CKD Progression

<table>
<thead>
<tr>
<th>Effects on kidney/CKD progression</th>
<th>Protein-bound uremic toxins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidative stress</td>
<td>IS, IAA</td>
</tr>
<tr>
<td>Expression of PAI-1, NF-kB</td>
<td>IS, IAA</td>
</tr>
<tr>
<td>Tubular senescence, renal fibrosis</td>
<td>IS, PCS</td>
</tr>
<tr>
<td>Glomerulosclerosis after nephrectomy</td>
<td>IAA</td>
</tr>
<tr>
<td>EGF-R activation and renal remodeling</td>
<td>IS, PCS</td>
</tr>
<tr>
<td>Reduced expression of Klotho</td>
<td>IS, PCS</td>
</tr>
<tr>
<td>Epigenetic silencing of Klotho</td>
<td>IS, PCS</td>
</tr>
<tr>
<td>Effect on renin-angiotensin system</td>
<td>IS</td>
</tr>
<tr>
<td>Podocyte injury</td>
<td>IS</td>
</tr>
<tr>
<td>Progression of CKD in patients</td>
<td>IS, PCS</td>
</tr>
</tbody>
</table>

Uremic Toxins and Renal Fibrosis / CKD Progression

IS induces tubulo-interstitial fibrosis in mouse kidney (8 weeks)
IS induces lesion of podocytes in mice, through AhR activation.
Toxicity of IS and PCS on proximal tubular epithelial cells could be mediated by reduced Klotho expression (epigenetic silencing).

Adijiang, Am J Nephrol 2010; Sun, Kidney Int 2012; Young, Kidney Int 2012
UREMIC TOXINS: A THERAPEUTIC TARGET IN CKD
Uremic Toxins: a therapeutic target in CKD

1. Decrease plasmatic levels of uremic toxins

- Reduce diet intake of TRP, TYR
- AST-120
- Pre/probiotic
- Reduce gut generation and absorption of toxins

Improve:
- Renal elimination
- Dialysis removal
Uremic Toxins: a therapeutic target in CKD

1. Decrease plasmatic levels of uremic toxins

- **Reduce diet intake of TRP, TYR**
  - Low-protein diet/low TRP intake? (but essential AA)

- **Improve protein-bound uremic toxins removal:**
  - Free fraction only in conventional hemodialysis
  - Frequent dialysis
  - Online hemofiltration
  - Preservation of residual renal function

Fagugli, Am J Kidney Dis 2002; Meert, NDT 2011; Meert, NDT 2009
Decrease plasmatic levels of uremic toxins:
Modulate expression of renal transporters of uremic toxins

« Organic Anion Transporters » (OAT) : uptake of uremic toxins at the baso-lateral membrane of renal proximal tubular cells

IS and PCS : mainly OAT1 (SLC22A6) and OAT3

Modulation of SLCO4C1 expression in rat/mice : increased elimination of ADMA, guanidine... and reduced cardiovascular effects

Toyohara, JASN 2009 ; Suzuki, J Pharm Sci 2011 ; Akiyama, PloSOne 2013
Decrease plasmatic levels of uremic toxins: role of AST-120

AST-120 (Kremezin®): oral adsorbant of uremic toxins
- Reduces plasma concentrations of IS and PCS
- Reduces cardiovascular effects and renal fibrosis of CKD
- 2 randomized control trials in Asia: slowing of CKD progression
- approved in Japan and Korea for the prevention of CKD progression
- 2 large randomized international trials (EPICC 1 and 2): no benefit
Uremic Toxins: a therapeutic target in CKD

Trials EPICC 1 and 2: no benefit of AST-120 on CKD progression

Schulman, JASN 2014
Uremic Toxins: a therapeutic target in CKD

EPICC 1 and 2: unexpected slow progression in the placebo group

Design assumption: 31 months (124 weeks) 50% event free in placebo

Design assumption: 43 months (172 weeks) 50% event free in AST-120

Disparity in event occurrence between projected and actual in placebo group

HR, 0.97 (95% CI, 0.83-1.12)
P=0.64

Patients at risk, n

<table>
<thead>
<tr>
<th></th>
<th>AST-120</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000</td>
<td>999</td>
</tr>
<tr>
<td></td>
<td>990</td>
<td>983</td>
</tr>
<tr>
<td></td>
<td>953</td>
<td>929</td>
</tr>
<tr>
<td></td>
<td>912</td>
<td>876</td>
</tr>
<tr>
<td></td>
<td>849</td>
<td>820</td>
</tr>
<tr>
<td></td>
<td>788</td>
<td>758</td>
</tr>
<tr>
<td></td>
<td>737</td>
<td>708</td>
</tr>
<tr>
<td></td>
<td>691</td>
<td>645</td>
</tr>
<tr>
<td></td>
<td>611</td>
<td>572</td>
</tr>
<tr>
<td></td>
<td>491</td>
<td>459</td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>364</td>
</tr>
<tr>
<td></td>
<td>276</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>77</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Schulman, JASN 2014
Uremic Toxins: a therapeutic target in CKD

1. Decrease plasmatic levels of uremic toxins: Modulation of gut microbiome

CKD: proliferation of urease-producing bacteria, which produce uremic toxins

Prebiotics or probiotics: modification of gut microbiome
Uremic Toxins: a therapeutic target in CKD

1. Decrease plasmatic levels of uremic toxins

2. Control downstream toxic cardiovascular and renal damage

- Decrease plasmatic levels of uremic toxins
- Control downstream toxic cardiovascular and renal damage

- NAC, Vit E, Vit C, Sevelamer, RAS inhibitors, Statins
- Selective AhR inhibition?
- EPO, RAS inhibitors, Statins
- Oxydative stress
- Inflammation and thrombosis
- Altered repair (EPC)

Uremic Toxins (UT)
Conclusion

- Protein-bound uremic toxins participate in endothelial dysfunction, vascular disease, thrombosis and inflammation in CKD

- They induce renal fibrosis and favor CKD progression

- Toxins derived from tryptophan metabolism activate AhR and exert a « dioxin-like » chronic poisoning of CKD patients

- Targetting uremic toxins is a promising field to reduce cardio-vascular morbidity and slow disease progression of CKD patients
Remerciements

Stéphane Burtey
Laetitia Dou
Bertrand Gondouin
Marion Sallée
Claire Cerini
Stéphane Poitevin
Bertrand Dussol
Philippe Brunet
Françoise Dignat-George