AN UPDATE ON UREMIC TOXICITY: Part 2

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Disclosures

• Dr. Vanholder’s dialysis unit receives unrestricted research grants from Fresenius Medical Care, Baxter Health Care, Gambro, Bellco and Nipro
Summary

• **Removal of protein-bound and middle molecules**
  • Analytical data on adequacy
  • Clinical outcomes
Protein-bound molecules
Protein-bound compounds: functional impact

**AGEs:** inflammation, vascular disease

**CMPF:** PB drugs, detoxification, neuropathy, anemia

**Cytokines:** inflammation, malnutrition, anemia

**Dimethylguanidined**: inhibition Ca$^{2+}$ ATP-ase

**Hippuric acid:** PB drugs, glucose intolerance

**Homocysteine:** vessel disease, detoxification

**Indole-3-acetic acid:** PB drugs, neuropathy, cytotoxicity

**Indoxyl sulfate:** renal and thyroid function decline, PB drugs, detoxification, endothelial function and repair, oxidative stress, osteoblast resistance to PTH, smooth muscle cell proliferation; aortic calcification

**Kinurenine:** neuropathy

**Leptin:** malnutrition

**Phenols:** immune function, neuropathy

**Phenylacetic acid:** nitric oxide synthesis

**Quinolinic acid:** neuropathy
KINETICS OF PROTEIN BINDING

- **bound protein bound compound**
- **free protein bound compound**
### Experimental studies: applying correct concentrations

<table>
<thead>
<tr>
<th>1st Author, year</th>
<th>Cell/organ system</th>
<th>Toxin</th>
<th>Concentration</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1) In vitro, albumin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dou, 2004</td>
<td>Endothelium</td>
<td>IS</td>
<td>25-250 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Odamaki, 2004</td>
<td>Hepatocytes</td>
<td>IS</td>
<td>50-100 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Faure, 2006</td>
<td>Endothelium</td>
<td>IS</td>
<td>256 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Yamamoto, 2006</td>
<td>Smooth muscle cells</td>
<td>IS</td>
<td>250-500 µM</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Dou, 2007</td>
<td>Endothelium</td>
<td>IS</td>
<td>125-250 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Schepers, 2007</td>
<td>Leukocytes</td>
<td>PCS</td>
<td>121.0 mg/L</td>
<td>Whole blood</td>
</tr>
<tr>
<td>Itoh, 2012</td>
<td>Endothelium</td>
<td>IS</td>
<td>29.9-57.2 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td>Chitalia, 2013</td>
<td>Smooth Muscle Cells</td>
<td>IS</td>
<td>25 mg/L</td>
<td>4 g/L</td>
</tr>
<tr>
<td><strong>2) In vitro, low</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tsujimoto, 2010</td>
<td>Liver Microsomes</td>
<td>IS</td>
<td>30 µM</td>
<td>-</td>
</tr>
<tr>
<td>Lekawanvijit, 2010</td>
<td>Cardiac Fibroblasts / Myocytes</td>
<td>IS</td>
<td>&gt; 1µM</td>
<td>-</td>
</tr>
<tr>
<td>Yu, 2011</td>
<td>Endothelium</td>
<td>IS</td>
<td>1.25-125 mg/L</td>
<td>(no m)</td>
</tr>
<tr>
<td>Sun NDT, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS, PCS</td>
<td>1 &amp; 5 mg/L</td>
<td>-</td>
</tr>
<tr>
<td>Sun Plos1, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS, PCS</td>
<td>&gt; 1 mg/L</td>
<td>-</td>
</tr>
<tr>
<td>Sun, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS, PCS</td>
<td>IPCS &amp; IS 1&amp;5 mg/L</td>
<td>(no m)</td>
</tr>
<tr>
<td>Tsujimoto, 2012</td>
<td>Intestinal Cells (hepatic no effect)</td>
<td>IS</td>
<td>20 µmol</td>
<td>-</td>
</tr>
</tbody>
</table>

Vanholder et al, J ASN, in press
### Experimental studies: applying correct concentrations

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<th>Concentration</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adijang, 2008</td>
<td>Aorta, kidney</td>
<td>IS</td>
<td>23.1 mg/L</td>
<td>rat</td>
</tr>
<tr>
<td>Ito, 2010</td>
<td>Endolium/leukocyte interaction</td>
<td>IS</td>
<td>15.7 mg/L</td>
<td>mouse</td>
</tr>
<tr>
<td>Adijang, 2010</td>
<td>Aorta</td>
<td>IS</td>
<td>15-20 mg/L</td>
<td>rat</td>
</tr>
<tr>
<td>Bolati, 2011</td>
<td>Proximal Tubular Cells</td>
<td>IS</td>
<td>9.4-18.9 mg/L</td>
<td>rat</td>
</tr>
<tr>
<td>Sun Plos1, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS, PCS</td>
<td>No concentration</td>
<td>mouse</td>
</tr>
<tr>
<td>Watanabe, 2012</td>
<td>Renal Tubular Cells</td>
<td>PCS</td>
<td>32.6 mg/L</td>
<td>rat</td>
</tr>
<tr>
<td>Sun, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS, PCS</td>
<td>IS 8.5, PCS 5.6 mg/L</td>
<td>mouse</td>
</tr>
<tr>
<td>Shimizu, 2012</td>
<td>Proximal Tubular Cells</td>
<td>IS</td>
<td>9.4-18.9 mg/L</td>
<td>rat</td>
</tr>
</tbody>
</table>
Leukocyte recruitment is enhanced in response to LPS, IS, pCS and pCSpCG

Pletinck et al, JASN, doi: 10.1681/ASN.2012030281
Red blood cell velocity is hampered in indoxylsulfate treated rats

Pletinck et al, JASN, doi: 10.1681/ASN.2012030281
INTRAVITAL MICROCOPY: VIDEO PICTURES

Pletinck Anneleen et al, JASN 2013
INTRAVITAL MICROCOPY

CONTROL

INDOXYLSULFATE

Pletinck Anneleen et al, JASN 2013
P-CRESYLSULFATE INDUCES INSULIN RESISTANCE

Koppe et al, JASN, 24: 88-99; 2013
P-CRESYL SULFATE INDUCES INSULIN RESISTANCE

Koppe et al, JASN, 24: 88-99; 2013
Free P-cresylsulfate is a predictor of mortality in patients at different stages of chronic kidney disease

Liabeuf et al, NDT, 25: 1183-1191; 2010

**Number of patients at risk**

<table>
<thead>
<tr>
<th></th>
<th>Days of follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Free p-cresylsulphate ≤ 0.051 mg/100mL</td>
<td>70</td>
</tr>
<tr>
<td>Free p-cresylsulphate &gt; 0.051 mg/100mL</td>
<td>69</td>
</tr>
</tbody>
</table>
THE SORBENT AST-120 DECREASES SERUM INDOXYL SULFATE

Schulman G. et al, AJKD, 47: 565-577; 2006
HDF (POST AND PRE-DILUTION) IS SUPERIOR TO PREDILUTION HEMOFILTRATION AND HIGH FLUX DIALYSIS FOR REMOVAL OF PROTEIN BOUND TOXINS

* p < 0.05 vs hemofiltration

Meert et al, NDT, 24: 562-570; 2008
Total clearances in HD (n=20) and PD (n=50) patients. & P < 0.01, # P < 0.0005, and $ P < 0.0001, respectively, PD vs HD.

Evenepoel et al, KI, 70: 794-799; 2006
**P-CRESOL: CONCENTRATIONS**

Mid-day (PD) and time-averaged (HD) serum concentrations in HD (n=20) and PD (n=50) patients. *P < 0.05 and $P < 0.0001, respectively, PD vs HD.

Evenepoel et al, KI, 70: 794-799; 2006
RESIDUAL RENAL FUNCTION HAS AN IMPACT ON CONCENTRATION

Marquez et al, CJASN, 6:290-296; 2011
THE COLON CONTRIBUTES TO UREMIC TOXIN GENERATION

<table>
<thead>
<tr>
<th>Colon-derived uremic solutes</th>
<th>Colectomy/with colon</th>
<th>Hemodialysis/normal</th>
<th>Dialytic reduction ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS</td>
<td>0.01</td>
<td>11</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>±-N-phenylacetyl-L-glutmaine</td>
<td>0.07</td>
<td>91</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>IS</td>
<td>0.02</td>
<td>23</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>Indoxyl glucuronide</td>
<td>0.02</td>
<td>--</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>5-hydroxyindole</td>
<td>--</td>
<td>--</td>
<td>0.83 ± 0.08</td>
</tr>
</tbody>
</table>

Aronov et al, JASN, 22:1769-1776; 2011
CONCLUSIONS WITH REGARDS TO PROTEIN BOUND UREMIC TOXINS

• The toxicity of protein bound uremic solutes has been demonstrated in a large array of experimental studies applying correct conditions.

• They have also been associated to hard outcomes in observational trials.

• Several methods are available to decrease their plasma concentration: hemodiafiltration, intestinal adsorption, and peritoneal dialysis.

• Most of these methods are not specifically removing protein bound solutes, and as a consequence the direct impact of protein bound solutes on hard outcomes has not been tested in controlled trials.
Middle molecules
(Molecular weight ≤ 500 Dalton)
Pre-dialysis $^2$ -2 M and outcome: HEMO Study

- The mean cumulative pre-dialysis serum $^2$ 2-microglobulin concentration levels over time were associated with greater mortality:
  - Adjusted* RR (95% CI): 1.11 (1.05-1.11; $p=0.001$) per 10- mg/L increase in concentration level

*adjusted for age, gender, race, diabetes, time on dialysis, ICED score, albumin, residual renal function, baseline high-flux dialysis, ultrafiltration volume, modeled body urea distribution, and high Kt/V arm

Cheung et al, J ASN 17: 546-555; 2006
IL-6 and mortality in CKD

(a) Kaplan–Meyer estimates of overall mortality for all patients (n=125) as a function of median plasma IL-6 levels. (b) Kaplan–Meyer estimates of cardiovascular mortality for all patients (n=125) as a function of median plasma IL-6 levels.

Barreto et al, Kidney Int, 77: 550-556; 2010
Middle molecules with biological potential

Adrenomedullin
AGE
Angiogenin
AOPP
Atrial natriuretic peptide
Cholecystokinin
Clara cell protein
Complement factor D
Cystatin C
Cytokines
Delta sleep inducing protein
Endothelin
Endorphin
Ghrelin
Glomerulopressin
GIP I
GIP II
Leptin
Lipotropin
Macrophage-colony-stimulating factor
Methionine-enkephalin
β2-Microglobulin
Neuropeptide Y
Orexin A
Retinol binding protein
Evolution of $^2$-2 microglobulin over time

Locatelli et al, KI, 50: 1293-1302; 1996
Kaplan-Meier survival analysis

\( \leq 4\text{g/dl AI} \)

\[ P=0.0320 \]

<table>
<thead>
<tr>
<th>No. at risk</th>
<th>Months since month 0</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-flux</td>
<td>250 212 173 134 85 44 26 7</td>
</tr>
<tr>
<td>Low-flux</td>
<td>243 202 152 117 67 41 15 3</td>
</tr>
</tbody>
</table>

Locatelli et al, JASN, 20: 645-654; 2009
HDF: Survival advantage vs. hemodialysis

ESHOL- trial

Death: 22.8%
HR: 0.7 [0.53-0.92]

Maduell et al, JASN, 24:487-497; 2013
Increasing length of dialysis without changing other parameters improves removal

Percentage change vs. 4 hrs

<table>
<thead>
<tr>
<th></th>
<th>4 hrs</th>
<th>6 hrs</th>
<th>8 hrs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>QB and QD</td>
<td>72L</td>
<td>72L</td>
<td>72L</td>
<td>NS</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.4 ±0.3</td>
<td>1.6 ±0.6</td>
<td>1.5 ±0.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Eloot et al, KI, 73:765-770; 2007
Conclusions

- In bench studies, compounds which are difficult to remove by standard dialysis have a patho-physiologic potential for vascular damage.
- Removal can be enhanced by opening pore size and adding convection.
- Recent data show the likelihood of an improvement of outcome by increasing this pore size.
- Observational data suggest the same for adding convection.