Protein bound uremic toxins promote vascular calcification by glucose mediated activation of inflammation and coagulation pathways

Background and Aim

Vascular calcification is frequently seen in patients with chronic kidney disease (CKD). Protein-bound uremic toxins indoxyl sulfate (IS) and p-cresyl sulfate (PCS) have been associated with cardiovascular morbidity and mortality in this patient population (1-2). Both uremic toxins originate from protein fermentation in the intestine and accumulate in CKD patients due to impaired renal clearance and increased intestinal production (3). We aimed to provide evidence for an etiological role for IS and PCS as major contributors to the onset and development of calcification in the vessel wall of CKD patients by use of a CKD rat model.

Study Design and Methods

• Induction of CKD by daily oral gavage of adenine sulfate (600 mg/kg/day)
• Exposure to vehicle versus IS or PCS (150 mg/kg/day)
• Biochemical analysis
  - Serum creatinine, phosphorus and calcium levels
  - Serum IS and PCS levels by LC-MS/MS
• Serum glucose levels
• Evaluation of vascular calcification in the aorta, femoral and carotid arteries
• Unraveling the complex molecular signaling events associated with toxin-mediated arterial calcification by a high-dimensionality unbiased proteomic approach

Results

1. Renal function and mineral metabolism

CKD was present in all groups as reflected by a significant increase in serum creatinine, however with less pronounced in IS exposed CKD rats. Serum phosphorus levels were increased over time in CKD rats, while serum calcium levels were decreased. IS exposed CKD rats showed lower phosphorus levels and higher calcium levels compared to vehicle exposed CKD rats.

<table>
<thead>
<tr>
<th>Time (week)</th>
<th>Vehicle</th>
<th>IS</th>
<th>PCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.63±0.12</td>
<td>1.83±0.12</td>
<td>1.63±0.12</td>
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<tr>
<td>3</td>
<td>1.85±0.12</td>
<td>2.05±0.12</td>
<td>1.85±0.12</td>
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<tr>
<td>7</td>
<td>2.15±0.12</td>
<td>2.35±0.12</td>
<td>2.15±0.12</td>
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Serum creatinine, phosphorus and calcium levels. Data are presented as mean ±SEM. *P <0.05 versus vehicle, same time point. °P<0.05 versus week 0.

2. Time averaged serum concentrations of IS and PCS

Based on the IS and PCS serum levels at week 0, 3, 5 and 7 time averaged concentration (TAC) of IS and PCS were calculated. CKD rats exposed to IS showed a significant increase in TAC of IS as compared to vehicle exposure. Similarly, TAC of PCS was also significantly elevated in PCS-exposed CKD rats as compared to vehicle exposure.

<table>
<thead>
<tr>
<th>Time (week)</th>
<th>Vehicle</th>
<th>IS</th>
<th>PCS</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4.83±0.79</td>
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<td>9.20±0.67</td>
<td>10.80±0.67</td>
<td>9.20±0.67</td>
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</tbody>
</table>

Time averaged concentration of IS and PCS. Data are presented as mean ±SEM. *P<0.05 versus vehicle, same time point.

3. Glucose metabolism

IS and PCS exposed CKD rats had increased serum glucose levels and lower expression of GLUT1, a major glucose uptake transporter, in the aorta. IS and PCS exposure caused alterations in glucose metabolism.

4. Effect of IS and PCS exposure on calcification in the aorta and peripheral arteries

Development of moderate to severe vascular calcification, as indicated by a distinct Von Kossa positivity and significant increase in calcium content, was observed in the aorta and peripheral vessels of CKD rats exposed to IS and PCS.

5. Molecular signaling pathways associated with toxin-induced aortic calcification

Proteome analyses of arterial samples revealed that calcification events were likely associated with the common IS/PCS proteome. This was performed by gene ontology and ingenuity pathway analysis with the amount of upregulated (black bars) or downregulated (grey bars) proteins in each pathway.

References


Conclusion

IS and PCS directly promote vascular calcification via activation of inflammation and coagulation pathways in the arterial wall which was strongly associated with impaired glucose homeostasis.