Indoxyl sulfate contributes to vascular calcification in rats with chronic renal failure

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Background and Aim

Except for phosphate and inflammation, other uremia-related factors may contribute to vascular disease. Among uremic retention solutes, indoxyl sulfate (IS) and p-cresyl sulfate (PCS) have recently been associated with cardiovascular disease and mortality [1-2]. Both uremic toxins originate from protein fermentation in the intestine and accumulate in chronic kidney disease (CKD) patients due to impaired renal clearance and increased intestinal production. Indoxyl sulfate induces upregulation of bone-specific proteins and cell senescence in vascular smooth muscle cells [3,4], two mechanisms involved in the calcification process. In hypertensive rats, indoxyl sulfate promotes medial calcification with expression of bone-specific proteins in the aorta [5].

These findings suggest that protein-bound uremic retention solutes, which can not be cleared by dialysis, may play an important role in vascular calcification and thus may have a significant impact on the high cardiovascular disease burden in patients with end-stage renal failure.

To clarify the potential causal role of IS and PCS in the development of vascular calcification, the impact of both uremic toxins on arterial calcification was studied in CKD rats.

Results

1. Mortality

Table 1: Mortality.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of dead rats</th>
<th>Mortality percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=12)</td>
<td>2</td>
<td>16.6%</td>
</tr>
<tr>
<td>Low-IS (n=12)</td>
<td>4</td>
<td>33.3%</td>
</tr>
<tr>
<td>Low-PCS (n=12)</td>
<td>3</td>
<td>8.3%</td>
</tr>
<tr>
<td>High-IS (n=12)</td>
<td>3</td>
<td>33.3%</td>
</tr>
<tr>
<td>High-PCS (n=12)</td>
<td>6</td>
<td>50%</td>
</tr>
</tbody>
</table>

2. Renal function and mineral metabolism

Figure 1: Study setup

- CKD induced by 600 mg/kg adenine via daily gavage for 10 days
- High phosphorus diet (1.2% P)
- IS and PCS via drinking water in a dose of 150-200 mg/kg/day

Figure 2: Creatinine clearance (A), serum phosphorus (B) and serum calcium (C). Data are presented as mean ± SD. *P<0.05 vs vehicle. °P<0.05 vs week 0.

3. Vascular calcification

Figure 3: Calcium aorta (A), area% calcified tissue on Von Kossa stained sections (B), calcium carotid artery (C) and calcium femoral artery (D). *P<0.05 vs vehicle.

Figure 4: Von Kossa stained section of a CKD rat exposed to the high IS dose. Chondrocyte-like cells have been microscopically found in the vessel wall of CKD rats exposed to a high dose of IS.

4. Cell biological changes

Conclusions

- Exposure of CKD rats to IS from the start of CKD induction onwards increased calcification in the aorta and carotid artery
- Serum IS and PCS levels still need to be analyzed
- Chondrocyte-like cells in the aorta of rats exposed to high IS suggests a transformation of vascular cells towards a mineralization competent phenotype which may refer to a direct cell biological effect of the toxin on the arterial wall.
- mRNA analysis of markers of osteoblast and chondrocyte-like cells are needed to confirm these microscopic observations
- Despite the reported association between PCS and cardiovascular mortality of CKD patients, no effect of PCS on vascular calcification in CKD rats was found.

References


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