Follow-up of osteoporosis and vascular calcifications in rats with chronic kidney disease

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Introduction. Impaired bone mineralization and vascular calcification (VC) are frequently seen together in patients with chronic kidney disease (CKD) or postmenopausal women. Moreover, in CKD patients osteoporosis often is seen superimposed upon the presence of renal osteodystrophy. A close relationship exists between osteoporosis and VC, the latter being generally accepted as an active and strictly regulated process with close relation to bone mineralization. Apparently, patients with the highest amount of bone loss have the greatest progression of VC¹,². Additionally, medications used to treat osteoporosis, such as bisphosphonates, seem to prevent VC development³. This is the so called calcification paradox.

The present study focused on osteoporosis complicated with VC in CKD-rats. To induce chronic kidney disease and VC, the adenine-low protein (AD) diet was administered to rats. Since it is known that adenine-induced CKD animals develop a high bone turnover caused by hyperparathyroidism, we included also the ‘remnant kidney rat’ (RK) model. In this model, CKD is induced by a 5/6th nephrectomy and bone turnover will stay rather normal. This was combined with either ovariectomy (OVX,) to induce osteoporosis, or sham operation.

Method. 50 female Wistar rats (ca. 250 g) were divided into 6 groups: OVX (ovariectomy, n=8), CKD combined with OVX (AD+OVX, n=12) or sham operated (AD+sham, n=8). CKD and VC were induced by administration of the 0.75% adenine-2.5% low protein diet during 4 weeks. Two additional groups were added to the study: ‘remnant kidney’ combined with either OVX (RK+OVX, n=12) or sham operated (RK+sham, n=10). Two weeks before starting and throughout the study, rats were fed a 1.03% phosphate diet.

Bone status and aortic calcification (AC) were evaluated in vivo at week -2, 2, 4, 5, 6, 8 and 10 by a SkyScan 1076 micro-CT scanner. A thoracal scan was made to evaluate AC, at 35 µm, with Ti-filter and 90 kV/110 µA source energy⁴. Scan lasted about 19 min. Subsequently, a short scan of the tibia was performed after fixation of the leg, at 35 µm, with Al 0.5 mm filter and source energy of 100 kV/100 µA. Bone loss was calculated using image analysis software CTAnalyzer (SkyScan) of the metaphyseal trabecular and cortical bone of the tibia. Urine and blood samples were collected at regular time points to determine Ca, P and creatinine. Adenine animals were sacrificed at week 6, ‘remnant kidney’ rats at week 10. Abdominal aorta was isolated and digested in nitric acid for bulk Ca analysis. Ca in aorta was also quantified after ex vivo scanning at 35 µm, with Al 0.5 mm filter and 100 kV/100 µA. AC was then calculated as the number of voxels above density threshold of 65 Houndsfield units. Additionally, preserved aortas and tibiae were analyzed histologically after Von Kossa or Goldner staining resp.

Results. At sacrifice, renal function was decreased in CKD rats compared to OVX (serum creatinine OVX 0.47 mg/dl vs. AD+OVX 1.19 mg/dl and AD+sham 1.11 mg/dl, RK+OVX 1.07 mg/dl and RK+sham 1.08 mg/dl, p<0.01) and serum phosphate was not significantly higher in CKD rats compared to OVX. Excretion of phosphate in urine was decreased (OVX 576.35 mg/24h vs. AD+OVX 224.66 mg/24h, RK+OVX 286.42 mg/24h and RK+sham 290.95 mg/24h, p<0.001).
According to micro-CT bone analysis, trabecular bone volume (TBV) of all rats declined during the first 4 weeks, probably induced by disturbances in Ca and P metabolism after onset of renal failure. Subsequently, TBV of adenine animals dramatically increased to initial values between week 4 and 6, coinciding with the switch to high P diet and appearance of AC. This is confirmed by histomorphometry. In contrast, a clear deterioration of the cortical bone could be detected from week 4 in the in vivo scans (fig. 1). There was no clear effect of OVX between both groups with adenine induced CKD. This suggests that the impairment of the renal function exceeded the effect of OVX on the bone. Also for RK+sham, TBV increased from week 4 to 10 but not for RK+OVX. Since renal function was only moderate in RK rats compared to adenine rats, the effect of OVX was visible.

Half of the adenine rats manifested AC from week 5 as detected by in vivo micro-CT and only 2 RK+sham rats. Ca bulk analysis revealed 2 more AD+OVX animals with mild calcifications, probably below the detection limit of in vivo micro-CT whereas ex vivo micro-CT did detect these calcifications. No AC could be distinguished by micro-CT or Ca bulk analysis in OVX and RK+OVX animals.

Direct correlations were found between the Ca content in the aorta and TBV (r = 0.510, p < 0.01) and cortical bone (r = -0.482, p < 0.05).

### Table 3: Vascular calcification. p<0.05 versus a control with normal renal function (NRF), b none – CKD, c mild, d moderate calcifications.

<table>
<thead>
<tr>
<th>Vascular calcification</th>
<th>Number animals</th>
<th>Area % calcification</th>
<th>mg/g wet tissue</th>
<th>Calcified volume (mm³)</th>
<th>Trabecular bone volume (%)</th>
<th>Bone area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe</td>
<td>5</td>
<td>26.70&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>63.73&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>48.38&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>14.68&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.95&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>8.84&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>21.91&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>12.94&lt;sup&gt;a,b,d&lt;/sup&gt;</td>
<td>12.44&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mild</td>
<td>2</td>
<td>0.87&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.39&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>15.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>None – CKD</td>
<td>17</td>
<td>0.04</td>
<td>0.52</td>
<td>0.02</td>
<td>6.03</td>
<td>0.50</td>
</tr>
<tr>
<td>None – NRF</td>
<td>8</td>
<td>0.03</td>
<td>0.47</td>
<td>0.02</td>
<td>4.02</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**Conclusion.** In uremia, VC development seems to be accompanied by inverse disturbances in the cortical and the trabecular bone. Cortical rather than trabecular bone loss is determining for VC accession. Animals establish a high bone turnover, probably due to secondary hyperparathyroidism, and seem to be predestined to develop VC.

**References.**
1. Kiel DP et al., Calcified Tissue Int., 2001
3. Price PA et al., Kidney Int., 2006