Combined Familial Hypercholesterolemia and Mineral Bone Disorder in Chronic Kidney Disease: a Mouse Model
Alexandra H. Tsivitis OMS-II, Eric J. Schram OMS-II, Mohnish Singh MS, Gregory D. Oliva OMS-II, Ryan J. Senese DO, Olga V. Savinova PhD

Introduction

Patients with chronic kidney disease (CKD) are at increased risk for cardiovascular events compared to the general population. CKD is often associated with dyslipidemia and increased atherosclerosis. CKD also affects the musculoskeletal system, which presents as mineral bone disorder (MBD) as a consequence of hyperphosphatemia. To study this complex relationship, we developed a mouse model of CKD in combination with familial hypercholesterolemia (FH) making subjects more susceptible to kidney injury, dyslipidemia, atherosclerosis, and vascular calcification.

Abstract

Patients with chronic kidney disease (CKD) are at increased risk for cardiovascular events compared to the general population. CKD is often associated with dyslipidemia and increased atherosclerosis. CKD also affects the musculoskeletal system, which presents as mineral bone disorder (MBD) as a consequence of hyperphosphatemia. To study this complex relationship, we developed a mouse model of CKD in combination with familial hypercholesterolemia (FH) making subjects more susceptible to kidney injury, dyslipidemia, atherosclerosis, and vascular calcification.

Hypothesis

Hyperlipidemic mice with CKD will have increased dysregulated calcium-phosphate metabolism, MBD, and cardiac hypertrophy compared to hyperlipidemic mice without CKD.

Methods

- LDL-R Knockout Mice → FH Mice
- 8 weeks 18 weeks
  Western Diet
  6 Males (M) 6 Females (F)
- 8 weeks 18 weeks
  Western Diet + 0.2% Adenine
  6 Males (M) 6 Females (F)

Echocardiographic Studies

- 8 weeks 18 weeks
  Harvesting Plasma (fasted)

Harvesting

- Kidney, Heart, Liver, Femur

Tissue Investigations

- Femur → MicroCT w/ Reconstruction
- Kidney → H&E Stain
- Liver & Aortic Root → Oil Red O Stain

Statistical Analysis

- GraphPad Prism 8

Raw Data Analysis

- Femur → Dragonfly
- Kidney → Bright-Field Microscopy → ImageJ
- Liver & Aortic Root → Bright-Field Microscopy → ImageJ

Acknowledgements

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Blood Chemistry Collection
• Increased BUN, plasma calcium, and plasma phosphate in FH + CKD mice when compared to FH mice.

Renal Histopathology

Histological Assessments of Mice Renal Cortices with H&E Staining.
Magnification bar corresponds to 100 µm
• FH: Renal tubules, glomeruli, and microvasculature have a uniform architecture and normal appearance.
• FH + CKD: Findings are noted below, suggesting evidence for renal failure:
  A. Karyolysis of renal tubule epithelial cells
  B. Leukocytic infiltration into renal tubules and glomeruli, forming ring-like clusters in late-stages, suggesting structural destruction
  C. Karyorrhexis and epithelial cell detachment from basement membrane with dropout into the interstitium of renal tubules
  D. Hypertrophic microvasculature
  E. Thyroidization, suggesting renal tubule destruction (black arrows in Figure E)
  F. Luminal expansion of renal tubules with non-uniform architecture (black arrows in Figure F)
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Introduction

Results (1)

Results (2)

Conclusion

Echocardiography

Long Axis

Short axis

Lipids & Atherosclerosis

TG, mg/dl (F+M)  
Baseline FH  
FH + CKD  
0  
500  
1000  
1500

CHOL, mg/dl (F+M)  
Baseline FH  
FH + CKD  
0  
1000  
2000  
3000

Aortic root plaque area, µm²

Surprisingly, the CKD group had significantly reduced atherosclerosis of the aortic root and a significant reduction in plasma triglycerides compared to controls, whereas plasma cholesterol was not different between the groups.

Liver Chemistry & Histopathology

Histological assessment of mice livers revealed that CKD female mice had reduced lipid deposits, whereas there was no significant change in lipid deposits in CKD males when compared to controls.

Statistical Significance:

Comparison to Baseline: *p < 0.05, **p < 0.01, ***p < 0.001

Comparison to FH: ¹p < 0.05, ²p < 0.01, ³p < 0.001

Abbreviations: Body weight (BW), Heart rate (HR), Stroke volume (SV), Left ventricular internal diameter (LVID), Ejection fraction (EF), Left ventricular anterior and posterior wall end-diastolic thickness (LVAD and LVPWd), cardiac output (CO)

Echocardiography Parameter Findings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Western Diet (FH)</th>
<th>Adenine-Western diet (FH + CKD)</th>
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</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>20.1 ± 2.0</td>
<td>24.5 ± 2.665 ***</td>
<td>14.23 ± 1.8411 **</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>440 ± 40</td>
<td>359 ± 46 ***</td>
<td>286.1 ± 59 ***</td>
</tr>
<tr>
<td>SV/BW, µl/g</td>
<td>1.97 ± 0.36</td>
<td>1.55 ± 0.21 **</td>
<td>2.06 ± 0.27</td>
</tr>
<tr>
<td>LVID, mm/g</td>
<td>0.197 ± 0.016</td>
<td>0.161 ± 0.009 ***</td>
<td>0.233 ± 0.025 *</td>
</tr>
<tr>
<td>EF, %</td>
<td>58.7 ± 7.481</td>
<td>57.45 ± 9.457</td>
<td>67.89 ± 12.76 §</td>
</tr>
<tr>
<td>LVAD, mm/g</td>
<td>0.039 ± 0.009</td>
<td>0.034 ± 0.009</td>
<td>0.057 ± 0.009 **</td>
</tr>
<tr>
<td>LVPWd, mm/g</td>
<td>0.034 ± 0.005</td>
<td>0.030 ± 0.005</td>
<td>0.046 ± 0.013 §</td>
</tr>
<tr>
<td>CO/BW, µl/min</td>
<td>0.872 ± 0.209</td>
<td>0.553 ± 0.115 ***</td>
<td>0.608 ± 0.206 **</td>
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</table>
Discussion

Increased levels of plasma calcium and phosphate in mice treated with adenine indicate the presence of MBD. Additionally, decreased cortical and trabecular bone volume, further confirm MBD.

Western diet feeding resulted in hypercholesterolemia. Plasma triglycerides were increased in the FH group, but not in the FH + CKD group, suggesting a possible side effect of adenine diet on triglyceride synthesis in the liver.

Moving Forward...

1) Mice with adenine induced CKD developed less atherosclerosis in the aortic root compared to mice without CKD. This indicates that adenine induced CKD may not be an appropriate model to study atherosclerosis in animals.

2) Understanding the role of purine synthesis in CKD and why it reduces lipid deposits in tissues may have a relevance to the pathophysiology of atherosclerosis.

3) Further quantification and research of the presence of osteoporosis in MBD-CKD mice may have clinical applications to patient comfort and care.