Vitamin K: From Coagulation to Calcification

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Annual Dialysis Conference
presented by the University of Missouri Division of Nephrology

Seattle, Washington
February 28th, 2016
Disclosures statement:

- Consultant: Ardelyx, Becker Professional Education
- Grant support: Sanofi-Aventis
- Speaker honoraria: Sanofi-Aventis
Objectives

1. To discuss application of vitamin K biology to various clinical disorders in patients with kidney disease
2. To discuss therapeutic application of vitamin K for vascular calcification
Cardiovascular mortality in the CKD population has not improved
Ancient Egyptian Mummy
Lancet. 2013 Volume 381, Issue 9873, Pages 1211–1222
Cardiovascular disease pathobiology in CKD is different than in non-CKD

- Carbamylated LDL-C
- Oxidative stress
- Proinflammatory state
- Sympathetic drive
- Cardiotonic steroids
- 1,25 Vitamin D anemia
- ADMA and NO

Atherosclerosis

Uremic cardiomyopathy

Left ventricular hypertrophy

Nat Med. 2010 Jan;16(1):38-40
Vascular calcification in dialysis patients

70% patients with significant coronary artery and aortic calcification

50% patients with calcified valves
Vascular Calcification Causes Cardiac Disease

VC

↑ Vessel Stiffness

↑ Pulse Wave Velocity  ↓ Coronary Perfusion

↑ Pulse Pressure  ↑ Afterload

Left Ventricular Hypertrophy

Cardiac Disease Events
Arterial calcification is associated with higher mortality risk in end-stage renal disease.
Calcification of the superficial femoral artery is associated with reduced survival in CKD patients

CJASN 2007;2:1241-1248
Vascular calcification in dialysis patients

70% patients with significant coronary artery and aortic calcification

50% patients with calcified valves

50% cardiovascular deaths that may be associated with abnormal tissue calcification in patients treated with dialysis
Chronic Kidney Disease- Mineral Bone Disease (CKD-MBD)

- **Laboratory Abnormalities**
  - Abnormal Ca, P, PTH, vitamin D metabolism

- **Abnormal Calcification**
  - Vascular or other soft tissue calcification

- **Bone Disease**
  - Abnormal bone turnover, mineralization, volume, linear growth, or strength

**Clinical Outcomes**

- Bone pain
- Fractures
- Secondary
- Hyperparathyroidism
- Progression to ESRD
- Cardiovascular events
- Hospitalization
- Mortality

Recent advances and clinical trials in the field of CKD-MBD have been largely disappointing.
FGF-23 levels are associated with mortality

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Temporal aspects of disordered phosphorus metabolism in progressive CKD and after kidney transplantation

Fibroblast Growth Factor 23
1,25 Dihydroxyvitamin D
Parathyroid Hormone
Phosphate

GLOMERULAR FILTRATION RATE
(ml/min/1.73m²)

TIME POST-TRANSPLANT (MONTHS)

>90
75
60
45
30
15
0
3
6
>12

>10,000
1,000
100
90
80
70
60
50
40
30
20
10
0

ANALYTE CONCENTRATION

Wolf M JASN 2010;21:1427-1435
FGF23-Ab improved hyperparathyroidism but increased mortality in CKD-MBD rat model

J Clin Invest. 2012 Jul 2;122(7):2543-53
FGF23-Ab treatment promoted aortic calcification in CKD-MBD rat model
Phosphate binders reduced serum and urinary phosphate but promoted the progression of vascular calcification in CKD.
Serum PTH levels achieved the recommended KDIGO target in EVOLVE Trial

Cinacalcet did not reduce the risk of death or major cardiovascular events in patients undergoing dialysis.
Time to go back to the drawing board
"Connecting this rare disease and normal aging is bearing fruit in an important way...valuable biological insights are gained by studying rare disorders such as Progeria. Our sense from the start was that Progeria had a lot to teach us about the normal aging process."

- Dr. Francis Collins, Director of the National Institutes of Health

www.progeriaresearch.org
Calciphylaxis is a disorder of calcification and thrombosis of dermal arterioles leading to painful skin lesions.
Histopathological features of calciphylaxis

- Calcification and subintimal fibroplasia of arteriole
- Thrombotic occlusion of arteriole
- Cutaneous ischemic necrosis
Calciphylaxis patients have diffuse vascular calcifications

Courtesy Drs. Novak and Harisinghani
Why Vitamin K?
The Nobel Prize in Physiology or Medicine 1943

Henrik Carl Peter Dam
Prize share: 1/2

Edward Adelbert Doisy
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 1943 was divided equally between Henrik Carl Peter Dam "for his discovery of vitamin K" and Edward Adelbert Doisy "for his discovery of the chemical nature of vitamin K".

Henrik Dam and Edward A. Doisy received their Nobel Prize one year later. In 1944, during the selection process in 1943, the Nobel Committee for Physiology or Medicine decided that none of the year's nominations met the criteria as outlined in the will of Alfred Nobel. According to the Nobel Foundation's statutes, the Nobel Prize can in such a case be reserved until the following year, and this statute was then applied. Henrik Dam and Edward A. Doisy therefore received their Nobel Prize for 1943 one year later, in 1944.
Forms of vitamin K

Phylloquinone (vitamin K1)

Menaquinone-4 (a form of vitamin K2)

Menaquinone-7 (a form of vitamin K2)

Menadione (vitamin K3)
Why Vitamin K?

• Association between warfarin (vitamin K antagonist) therapy and increased odds of calciphylaxis

<table>
<thead>
<tr>
<th>Study</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hayashi et al</td>
<td>Odds Ratio: 10.1; 95% Confidence Intervals: 1.63–62.7</td>
</tr>
<tr>
<td>Nigwekar et al</td>
<td>Odds Ratio: 4.30; 95% Confidence Intervals: 1.57-11.74</td>
</tr>
</tbody>
</table>

• Reports of calciphylaxis in patients with normal renal function (non-uremic calciphylaxis) but with the following risk factors:
  • Liver disease
  • Inflammatory bowel disease
  • Gastric bypass surgery

Potential biological link between warfarin and calciphylaxis

Matrix Gla Protein
- 84 amino acid protein
- Requires vitamin K dependent carboxylation for its activation
- Vascular calcification inhibitor
- Proposed to inhibit BMP signaling; inhibits release of membrane bound vesicles and also has been shown to prevent progression of calcification by direct binding to calcium phosphate
- Produced in vascular smooth muscle cells and chondrocytes

Vitamin K deficiency

Dermal arteriolar calcification
Effects of vitamin K deficiency on MGP gamma-carboxylation in calciphylaxis

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Definition</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin K deficiency</td>
<td>PIVKA-II ≥ 2 ng/mL</td>
<td>ELISA assay (Stago, USA)</td>
</tr>
<tr>
<td>MGP gamma-carboxylation status</td>
<td>% carboxylated MGP = [carboxylated MGP ÷ (carboxylated MGP + uncarboxylated MGP)] X 100</td>
<td>ELISA assays for carboxylated and uncarboxylated MGP (VitaK BV, Netherlands)</td>
</tr>
</tbody>
</table>
### Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n=20)</th>
<th>Controls (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>58 ± 16</td>
<td>62 ± 14</td>
<td>0.899</td>
</tr>
<tr>
<td>Female sex, %</td>
<td>35.0</td>
<td>35.0</td>
<td>1.00</td>
</tr>
<tr>
<td>White race, %</td>
<td>90.0</td>
<td>90.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>50.0</td>
<td>75.0</td>
<td>0.103</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.9 ± 8.7</td>
<td>31.7 ± 6.9</td>
<td>0.474</td>
</tr>
<tr>
<td>Macrovascular disease, %</td>
<td>40.0</td>
<td>15.0</td>
<td>0.077</td>
</tr>
<tr>
<td>Warfarin, %</td>
<td>30.0</td>
<td>30.0</td>
<td>1.00</td>
</tr>
<tr>
<td>Serum calcium (albumin corrected), mg/dL</td>
<td>9.3 ± 0.9</td>
<td>9.3 ± 0.9</td>
<td>0.827</td>
</tr>
<tr>
<td>Serum phosphorous, mg/dl</td>
<td>4.4 ± 1.0</td>
<td>4.1 ± 1.0</td>
<td>0.256</td>
</tr>
<tr>
<td>Serum parathyroid hormone, pg/mL</td>
<td>110 (57, 481)</td>
<td>169 (127, 265)</td>
<td>0.172</td>
</tr>
<tr>
<td>Serum alkaline phosphatase, U/L</td>
<td>132.6 ± 69.4</td>
<td>115.6 ± 55.2</td>
<td>0.330</td>
</tr>
<tr>
<td>Serum albumin, g/dL</td>
<td>3.2 ± 0.7</td>
<td>3.8 ± 0.7</td>
<td>0.013</td>
</tr>
<tr>
<td>Catheter access, %</td>
<td>20.0</td>
<td>25.0</td>
<td>0.719</td>
</tr>
</tbody>
</table>

Mean and standard deviations are reported for all continuous variables except for serum parathyroid hormone and dialysis vintage where median and interquartile ranges are reported.
Vitamin K deficiency is more prevalent in calciphylaxis patients independent of warfarin exposure.

![Bar charts showing prevalence of vitamin K deficiency in calciphylaxis cases and controls.](chart.png)
Vitamin K deficiency is associated with impaired MGP gamma-carboxylation.
Calciphylaxis is characterized by impaired MGP gamma-carboxylation.
Calciphylaxis is characterized by impaired MGP gamma-carboxylation even in the absence of warfarin exposure.
Vitamin K-calciphylaxis Pilot double-blind RCT
NCT02278692

Identification of eligible study patients (d-ESRD patients with newly diagnosed skin-biopsy confirmed CUA)

Review of medical records to confirm study eligibility

Informed consent

Randomization

Vitamin K 10 mg orally three times a week (on alternating days) after dialysis for 3 months (n = 12)

Oral placebo three times a week (on alternating days) after dialysis for 3 months (n = 12)

Study visits at baseline and then at every month for 3 months

Study activities at each visit: history and physical, plasma/serum sample collection, pain assessment, CUA lesion(s) measurements, and adverse events assessment
Roadmap for vitamin K research

Brandenburg et al. Atherosclerosis, 2015-05-01, Volume 240, Issue 1, Pages 10-16
Discussion points

• Vitamin K deficiency is a risk factor for calciphylaxis development
  – Likely involved in the pathogenesis of dermal arteriolar calcification

• Future investigations in calciphylaxis are needed:
  – To determine the etiology of vitamin K deficiency
  – To examine the effects of vitamin K deficiency and supplementation on vitamin K dependent proteins
  – To examine the diagnostic relevance of vitamin K biology

• Examination of vitamin K biology and supplementation in calciphylaxis will provide insights applicable to other types of vascular calcification in patients with kidney disease
Acknowledgements

• MGH Multidisciplinary Calciphylaxis Team

• Vitamin K Laboratory
  – Sarah Booth, PhD

• Bloch Laboratory

• Thadhani Laboratory

This work was conducted with the support of a KL2/Catalyst Medical Research Investigator Training award (an appointed KL2 award) from Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award KL2 TR001100). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health.
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Extra slides
Calciphylaxis is characterized by increased arteriolar BMP signaling

A von Kossa (vK) positive arteriole in a patient with CUA is shown at 10x (a) and 60x magnification (b). Strong nuclear expression of PSMAD was detected in the corresponding vessel (c, d). Low or absent PSMAD expression was present in arterioles of control skin samples (e, f). Note that in some arterioles from CUA patients, von Kossa staining was negative (g), while the corresponding vessels had cells with high levels of PSMAD (h).

*Nigwekar, Bloch, and Malhotra. AHA 2014*