Role of Klotho in Kidney Diseases: An Overview

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KLOTHO: BASIC INTRODUCTION

- Klotho was discovered in 1997 when silencing this gene in mice resulted in multiple organ dysfunction and premature aging with shortened life span (Kuro-o M, et al. Nature 1997;390:45-51)

- Mainly expressed in:
  - **Kidney**: Distal tubular cells
  - **Brain**: Epithelium of the Choroid plexus

- Also expressed in:
  - Bronchial and alveolar epithelial cells
  - Vascular smooth muscles
  - Pituitary gland
  - **Heart (not much), Parathyroid Gland**, inner ear, urinary bladder, pancreas, testis, ovary, colon
Klotho Deficient Mice and CKD: Similarities

- Pulmonary Emphysema
- Neurodegeneration
- Hypogonadism
- Premature Thymic involution
- Ectopic calcification
- Impaired angiogenesis
- Endothelial dysfunction
- Cardiac hypertrophy and fibrosis
- Growth Retardation
- Impaired bone mineralization
- Skin atrophy
- Abnormal Blood chemistry (Ca, P, FGF23)
- Hearing loss

- Neurodegeneration
- Ectopic calcification
- Impaired angiogenesis
- Endothelial dysfunction
- Growth Retardation
- Impaired bone mineralization
- Abnormal Blood chemistry (Ca, P, FGF23)
- Cardiac hypertrophy and fibrosis
The KL gene locus is located on chromosome 13 in humans. It exists in 3 main forms:

- Membrane Klotho
- Soluble Klotho
- Secreted Klotho

The kidney is a major contributor to maintain Soluble Klotho which is the main functional form in circulation.
Klotho acts as an obligatory co-receptor for FGF23 in the Kidney
Klotho: A Highly Pleiotropic Protein

• Regulation of energy metabolism
• Anti-inflammatory and anti-oxidative effects
• Modulation of ion transport (Ca^{2+}, P, K^+)
• Regulation of mineral metabolism (FGF23-dependent and independent)
• Prevents vascular calcification (promoting phosphaturia and direct effects on vascular smooth muscle)
• Modulates cardiomyocyte hypertrophy and cardiac hypertrophy (mostly FGF23-independent)
• Helps maintain normal renal function (prevents CKD progression)
KLOTHO AND CKD

• CKD is a state of accelerated aging (by its antioxidant effects)
• Kidneys are the main source of Klotho
• The abundance of Klotho is markedly decreased in the kidneys from patients with CKD and CKD mouse models indicating that CKD is a Klotho-deficient state
• CKD and ESRD patients have low levels of circulating Klotho
• Renal Klotho deficiency in the early stages of CKD is mainly due to suppression of renal expression rather than loss of viable renal tubules.
Reduction of renal α-Klotho (α-KL) expression with progression of CKD


http://www.plosone.org/article/info:doi/10.1371/journal.pone.0086301
Soluble Klotho levels are significantly decreased already in stage 2 CKD

N=292 adults with CKD
Klotho was positively correlated with eGFR and negatively with creatinine

The decline in renal α-KL levels was followed by reductions in 1,25VitD₃
Circulating Klotho is already Reduced during the Early Stages of CKD
Klotho in Children with CKD

Median (IQR) s-klotho levels in children with CKD (n=105)

<table>
<thead>
<tr>
<th>CKD1–3</th>
<th>CKD 4-5</th>
<th>Dialysis</th>
<th>Transplantation</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1736.5 (1272–2737)</td>
<td>1019 (502–1592)*</td>
<td>1148 (779–1515)*</td>
<td>1698 (1110–2123)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* P< 0.05 vs. CKD Stages 1-3

Nephrology Dialysis Transplantation 2013; 28: 153–161
Klotho and Other Hormones in Children on Peritoneal Dialysis (n=31)

<table>
<thead>
<tr>
<th></th>
<th>Month 1 (n:31)</th>
<th>Month 6 (n:25)</th>
<th>Month 12 (n:15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klotho (normal controls 320 ± 119.4 pg/mL)</td>
<td>132.1 ± 58*</td>
<td>133.3 ± 29.2</td>
<td>130.3 ± 34.4</td>
</tr>
<tr>
<td>FGF23 (normal controls 9.4 ± 5.7 pg/mL)</td>
<td>215.1 ± 303.6*</td>
<td>229.8 ± 252.6</td>
<td>194.8 ± 300.9</td>
</tr>
<tr>
<td>PTH (recommended for CKD-5D children 200–300 pg/mL)</td>
<td>330.8 ± 273.4</td>
<td>349.9 ± 283.3</td>
<td>320.8 ± 205.1</td>
</tr>
<tr>
<td>1,25 (OH)D (normal controls 43 ± 2 pg/mL)</td>
<td>26.7 ± 22.2</td>
<td>27.5 ± 21.4</td>
<td>N/A</td>
</tr>
</tbody>
</table>

-At baseline: Klotho levels: 41% lower vs. controls; FGF23 markedly higher vs. controls
-At month 12, FGF23 and Klotho levels remained similar to baseline values

*p<0.001 vs. controls

Klotho Deficiency in CKD

• Serum, urine and renal levels associated with eGFR decline (positively correlated)
• Associated with CKD progression (inversely correlated)
• Correlated negatively with FGF23 and serum phosphate
• Significantly reduced in ESRD
• Associated with cerebrovascular disease
• Associated with cardiovascular disease and cardiac hypertrophy
Klotho and Cerebrovascular Disease in Hemodialysis Patients

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 88)</th>
<th>With cerebrovascular disease (n = 28)</th>
<th>Without cerebrovascular disease (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Klotho (pg/ml)</td>
<td>119.10 ± 47.29</td>
<td>91.65 ± 28.19**</td>
<td>131.90 ± 49.09</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>72 (81.82%)</td>
<td>24 (85.71%)</td>
<td>48 (80%)</td>
</tr>
<tr>
<td>HD vintage (months)</td>
<td>34.55 ± 18.57</td>
<td>40.86 ± 17.09</td>
<td>31.60 ± 18.63</td>
</tr>
<tr>
<td>Age, years</td>
<td>57 ± 14</td>
<td>66 ± 10**</td>
<td>54 ± 14</td>
</tr>
</tbody>
</table>

**Serum klotho level was significantly lower and age was older in the participants with cerebrovascular disease.

Cerebrovascular diseases were diagnosed by CT or MRI scans; 23 cases had cerebral infarction, 5 cases had cerebral hemorrhage. Average age of the participating patients was 57 ± 14 years.

Wei et al. BMC Nephrology (2019) 20:63
OXIDATIVE STRESS IN CKD AND KLOTHO

• An imbalance between the processes of formation of free radicals and their removal with a predominance of the production of reactive oxygen species (ROS) is referred to as oxidative stress.

• ROS include superoxide radical, hydrogen peroxide, and hydroxyl radical. The role of antioxidants in the body is fulfilled by enzymes: superoxide dismutase, catalase, oxidase, and glutathione peroxidase, and other substances such as glutathione, vitamins E and C, magnesium ions, zinc, albumin, ferritin, transferrin and others.

• The uncontrolled increase in the concentration of free radicals is postulated to be one of the pathophysiological mechanisms of many diseases, and is associated with cardiovascular complications in CKD.
A trend toward higher median oxLDL values in children with LVH was present (88.60 (76.24; 107.07) versus 81.06 (61.13; 98.35) U/L; p = 0.084)
Klotho Suppresses ROS Formation

- Klotho gene
  - Transmembrane Klotho protein
  - Secreted Klotho protein

- Klotho
  - Cell surface
  - + Phospho-FOXO3a

- FOXO3a
  - MnSOD promoter

- ROS↓

Secreted Klotho protein → ROS↓ → insulin↓ → IGF-1↓ → Senescence↓
Kidney is the Principal Source of Klotho

Neyra & Hu 2016, 2017
Yzydorczyk et al. 2008
Haruna et al. 2007
Klotho and Prematurity: Background

• Preterm neonates are often treated with supplemental oxygen and exposed to high–oxygen levels relative to the hypoxic intrauterine environment.

• Hyperoxia + inherently low antioxidant concentrations → oxidative stress and cellular damage → bronchopulmonary dysplasia, retinopathy, and necrotizing enterocolitis.

• Preterm infants are born during active nephrogenesis and exposure to high-oxygen levels has been proposed to cause kidney injury and impaired kidney development.

Lee et al. 2005
Sutherland et al. 2014
Silver et al. 2015
Abitbol et al. 2004
Collaborative Research: Nephro-Neonatology at UM

- *Mohammed Farhan Ali*
- Sunil Kumar Bathally Venkatarayappa
- Merline Benny
- Karen Young
- Shathiyah Kulanadavelu
- Claudia Rojas
- Naimeh Da Silva
- Michael Freundlich
- *Carolyn Abitbol*
- *Marissa DeFreitas*
Hypothesis

Administration of exogenous Klotho will attenuate hyperoxia induced glomerular and tubular injury in rats via an increase in antioxidant capacity.
Experimental design

Newborn Rat Pups

Hyperoxia 85%

IP administration of PBS or Klotho 30 mcg/kg every other day

Normoxia 21%

3 Weeks Hyperoxia

3 Weeks Normoxia

3 Weeks Normoxia

Rat Pups sacrificed

Glomerular/Tubular Injury

Placebo = PBS: Phosphate-buffered saline
Kidney mRNA Klotho expression decreased with hyperoxia exposure

* p < 0.05
Klotho prevents hyperoxia-induced glomerulomegaly

**Bar Graph:**
- **Placebo:**
  - Normoxia: 5000 μm²
  - Hyperoxia: 10000 μm²
- **Klotho:**
  - Normoxia: 5000 μm²
  - Hyperoxia: 7000 μm²

**Images:**
- Placebo: Normoxia (left), Hyperoxia (right)
- Klotho: Normoxia (left), Hyperoxia (right)

**Legend:**
- White bar: Normoxia
- Black bar: Hyperoxia

**Note:** N = 10 per group

**Statistical Significance:**
- * p < 0.05
Klotho attenuates hyperoxia-induced tubular injury

N= 10 per group

* p <0.05
Klotho increases kidney antioxidant capacity

Placebo Klotho

Kidney MnSOD Expression Normalized to β-Actin

MnSOD

β- Actin

* p <0.05

ASN 2018
Conclusions

• The findings demonstrate that hyperoxia exposure in an animal model of nephrogenesis results in increased glomerular size and tubular injury.
• Hyperoxia significantly reduced kidney Klotho expression.
• Exogenous Klotho attenuates hyperoxia induced tubular injury and prevents glomerulomegaly.
• Exogenous Klotho administration increases antioxidant capacity.
• The demonstrated novel nephroprotective properties of exogenous Klotho administration during nephrogenesis may open opportunities for future clinical studies in critically ill premature infants requiring oxygen supplementation.
Klotho Administration Improves Cardiac Hypertrophy and Function in AKI by Ischemia Reperfusion Injury

Figure 1. αKlotho administration after acute kidney injury (AKI) maintained higher plasma and renal αKlotho levels and improved cardiac function in ischemia-reperfusion injury (IRI)–induced AKI mice.

Ming Chang Hu, Mingjun Shi, Nancy Gillings, Brianna Flores, Masaya Takahashi, Makoto Kuro-o, Orson W. Moe

Recombinant α-Klotho may be prophylactic and therapeutic for acute to chronic kidney disease progression and uremic cardiomyopathy

Effects of Exogenous Klotho Administration on Cardiac Hypertrophy in Hyperoxia Model

Klotho attenuates pulmonary hypertension and RV hypertrophy

Klotho increases RV anti-oxidant expression
The role of mineral and bone disorder in uremic cardiomyopathy
Mechanisms of Klotho downregulation in CKD and beneficial effects of soluble Klotho

Chronic kidney disease
- Progressive renal mass loss
- Oxidative stress
  - ↑ ROS
- ↑ Inflammatory cytokines
  - TNF
  - IFN
  - IL-1
- Dyslipidemia & hyperglycemia
- Accumulation of uremic toxins
  - Indoxyl sulfate
  - p-cresyl sulfate
- Abnormal mineral metabolism
  - High serum Pi
  - Low serum vitamin D3
- Activated RAA system

Progression to end-stage renal disease
- ↓ Renal Klotho
  - ↓ Soluble Klotho
  - Maladaptive repair
  - ↓ Normal repair

End-stage renal disease

Potential protective effects of soluble Klotho
- Effects on cells
  - Anti-oxidation
  - ↓ Cell senescence
  - ↓ Cell apoptosis
  - ↑ Cell autophagy
- Effects on mineral homeostasis
  - ↓ Serum phosphate
  - ↓ Serum FGF23
- Effects on vessel
  - ↑ Vascular injury
  - ↑ Angiogenesis
  - ↓ vascular permeability
- Inhibition of tubulointerstitial fibrosis