Arterial Klotho Expression and FGF23 Effects on Vascular Calcification and Function

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Conclusion

Collectively, our results demonstrate that FGF23-Klotho signaling is absent in mouse arteries and that the vascular response was unaffected by FGF23 treatment. Thus, our data do not support Klotho-mediated FGF23 effects in the vasculature although confirmative studies in humans are warranted.

Objective

Recent studies support a role for FGF23 and its co-receptor Klotho in cardiovascular pathology, yet the underlying mechanisms remain largely elusive. This led us to explore and better define the role of FGF23-Klotho in cardiovascular pathology. We addressed this issue by investigating the expression of Klotho in arteries from wild type mice and a novel mouse model with targeted deletion of Klotho in VSMC, and performed functional evaluations of FGF23 treatment on vascular calcification and endothelial function *in vitro* and *ex vivo*.

Methods

We generated a novel mouse model harboring a vascular smooth muscle cell specific deletion of Klotho (*Sm22-KL*$^{-/-}$) using Cre-Lox recombination (Figure 1). Arterial klotho expression was analysed with quantitative real time PCR, immunohistochemistry and Western blot. Egr-1 expression was measured with quantitative real time PCR in aortas and kidneys from wild type mice intravenously injected with FGF23 protein. Calcification were measured with Alizarin red in bovine vascular smooth muscle cells (VSMCs) treated with β-glycerophosphate and FGF23. Vascular responses were tested *ex vivo* with vasoactive drugs.

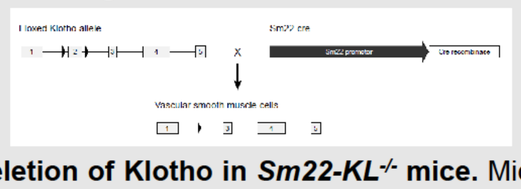

Figure 1. Targeted deletion of Klotho in *Sm22-KL*$^{-/-}$ mice. Mice with loxP sites inserted into intron 1 and 2 of Klotho, were crossed with transgenic mice expressing Cre recombinase under the control of the smooth muscle protein 22-alpha (Sm22) promoter, resulting in targeted deletion of Klotho gene specifically in vascular smooth muscle cells by Cre recombination.

Results

Gross phenotype

Mice were analyzed at 8 weeks of age, and were viable, fertile with normal gross pheotype and no apparent changes in mineral metabolism (Table 1).

Serum Biochemistries			
Parameters	WT (n=10)	*Sm22-KL*$^{-/-}$ (n=7)	*P* value
Calcium (mg/dL)	9.72 (9.09-10.53)	9.30 (8.07-10.43)	0.14
Creatinine (mg/dL)	0.45 (0.30-0.60)	0.50 (0.30-0.60)	0.58
Phosphate (mg/dL)	3.34 (2.96-3.77)	3.19 (2.91-3.39)	0.14
FGF23 (pg/mL)	127.6 (68.1-230.0)	115.7 (56.2-170.5)	0.91
PTH (pg/mL)	39.2 (27.5-86.8)	37.2 (24.8-98.1)	0.35

Table 1. Serum biochemistries in *Sm22-KL*$^{-/-}$ and wild-type mice. At 8 weeks of age *Sm22-KL-/-* mice had normal serum biochemistries reflecting mineral metabolism as compared to wild-type controls. Results are displayed as median (range).

Klotho expression in mouse arteries

Low levels of Klotho transcripts, as measured by quantitative real time PCR, were detected in dissected aortas, femoral arteris, mesenteric arteriess and lungs (Figure 2). Furthermore, immunostaining of the arterial wall and Western blotting of pooled aortas revealed no detectable expression of Klotho (data not shown).

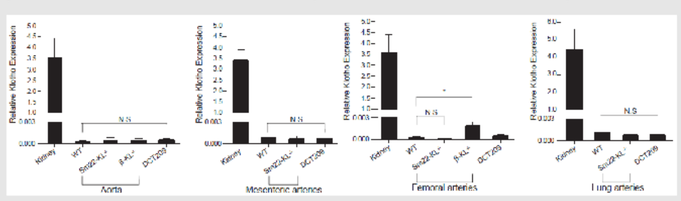

Figure 2. Klotho gene expression in mouse arteries. Gene expresion of Klotho in kidney extracts, arteries and lung of wild type mice, SM22-KL$^{-/-}$ mice, B-KL$^{-/-}$ mice, and DCT209 cells.

Egr-1 expression in response to FGF23 injection

In contrast to kidneys, which demonstrate a distinct rise in Egr-1 mRNA, no increase in in Egr-1 was found in the aortas from mice injected with FGF23 compared to saline (Figure 3).

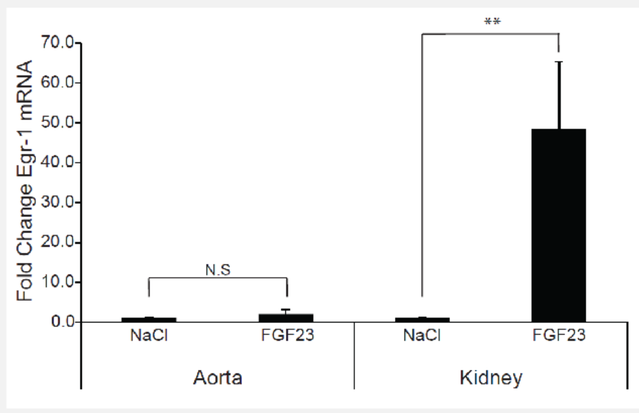

Figure 3. Vascular Egr-1 response to FGF23 injcetion. Transcript levels of Egr-1 were analyzed in aorta and kidneys from wild type mice injected with 0.9% NaCl or 0.15 mg/kg FGF23

Impact of FGF23 on vascular calcification

FGF23 treatment (0.125-2 ng/mL) did not affect vascular calcification induced by addition of calcification medium to bovine vascular smooth muscle cells (Figure 4).

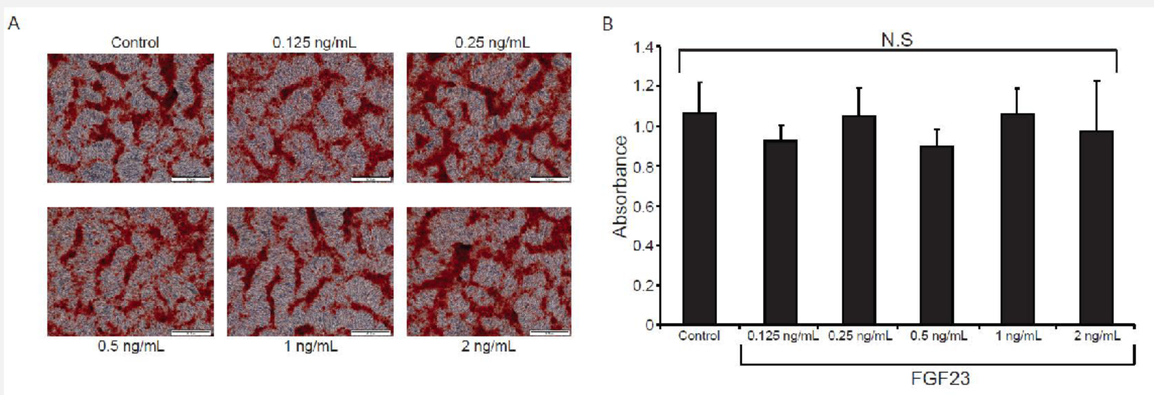

Figure 4. No effect of FGF23 on β-glycerophosphate induced mineral deposition by VSMCs. (A) Alizarin red stained cultured VSMCs incubated with medium containing 5mM βGP supplemented with FGF23 in increasing concentrations. (B) Mineralization was quantified by eluting the dye and measuring the absorbance.

Impact of FGF23 on vascular function

Isolated arteries from wild type mice were exposed to high doses of FGF23 (6 ng/mL) followed by assessment of contractile and dilatory functions *ex vivo* by addition of other vasoactive substances (Figure 5).

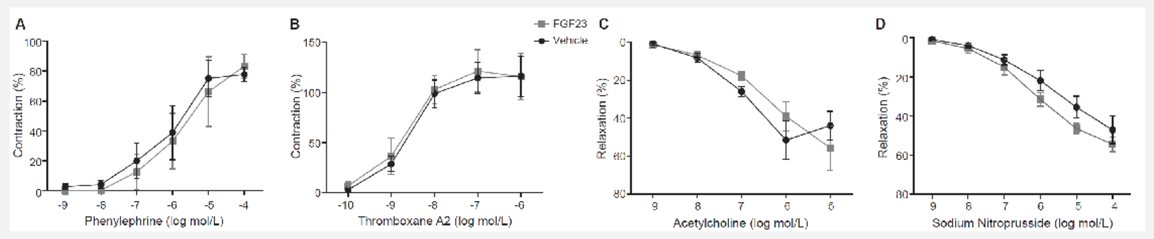

Figure 5. Short-term effects of FGF23 on induced contractions or relaxations. Short term effects of FGF23 (6 ng/mL) or vehicle (DMSO 0.17% v/v) on phenylephrine (A), thromboxane A2 analog U46619 (B), acetylcholine (C) and sodium nitroprusside (D) induced contractions or relaxations.

Summary

- Klotho transcript and protein expression is low or absent in mouse arteries
- The low levels of Klotho in arteries do not mediate FGF23 signaling as evidenced by lack of Egr-1 upregulation
- FGF23 does not influence vascular calcification *in vitro* in bovine vascular smooth muscle cells
- FGF23 has no influence on endothelial dilatory and contractile function *ex vivo*

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