Effects of inorganic phosphate and FGF23 on C2C12 myoblast cells

A. Raimann[1], A.Dangl[1], S. Greber-Platzer[1], G.Haeusler[1]

1 Department of Pediatrics and Adolescent Medicine, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Wien, Austria

MEDICAL UNIVERSITY OF VIENNA

Die menschliche Größe

Department of Paediatrics and Adolescent Medicine

Introduction

Inorganic phosphate is a crucial component of mineralized tissues and critical for a broad spectrum of biological processes. Due to the importance of phosphate in metabolism, phosphate homeostasis is coordinated by a complex orchestration of both systemic and local factors regulating renal excretion, bone efflux and influx as well as intestinal reabsorption. Disturbed phosphate levels, as seen in various pathologic conditions, lead to typical symptoms: While hypophosphatemia leads to hypophosphatemic rickets, hyperphosphatemia has been shown to be associated to increased mortality and morbidity in patients with chronic kidney disease (CKD).
Muscle fatigue, a constant feature of hyperphosphatemic conditions, is attributed to many factors such as associated calcium levels and potentially toxic factors as being present in uremia.

Fibroblast growth factor 23 (FGF23) is a key regulator of serum phosphorus concentration. It suppresses proximal tubular phosphate reabsorption via the fibroblast growth receptor 1 (FGFR1) isoform IIIc, a canonical FGF receptor that is converted by α- Klotho. Thereby, the expression of type IIa sodium-phosphate cotransporters (NaPi2a) is reduced.
Elevated serum FGF23 concentrations are associated with mineralization defects due o phosphate loss, whereas deficiency of systemic FGF23, caused by inactivation FGF23 mutations, leads to elevated serum phosphorus and pathologic tissue calcification.

FGF23 is highly elevated in most hyperphosphatemic conditions, such as CKD. Inorganic phosphate and FGF23 have been shown to act via similar signaling pathways e.g. ERK-MAPK. (Fig 1) in several cell types.
So far, the role of FGF23 in skeletal muscle cell viability, differentiation and potential interactions with phosphate signaling, remains unknown. We therefore investigated the effect of inorganic phosphate and FGF23 on skeletal muscle cells in a murine *in vitro* model system.

Study aims

1. **To characterize effects of high phosphate loads on muscle cell differentiation**
2. **To evaluate the role of increased FGF23 levels on muscle cell gene expression and proliferation**
3. **To identify interaction between inorganic phosphate and FGF23 on muscle cell differentiation**

Significance

Knowledge of phosphate-and FGF23 induced effects on skeletal muscle contributes to a better understanding of disease featuring hyperphosphatemia. Revealing cellular responses to excess of FGF23 and phosphate may help to modify existing treatment approaches such as FGF23 inactivating antibodies or phosphate binders, esp. in patients with severe muscle fatigue.

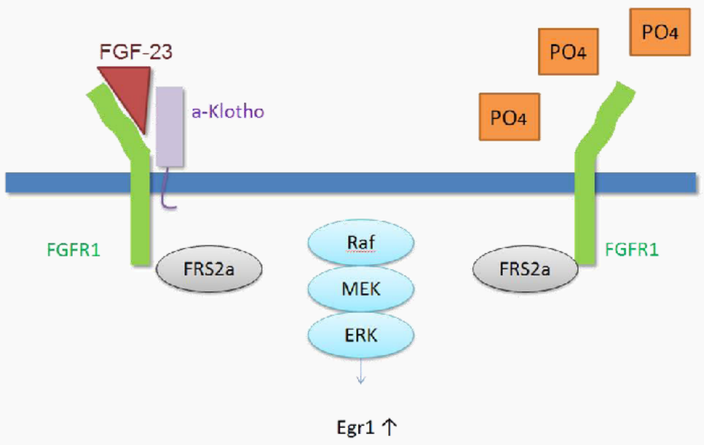

Fig 1: FGFR activation by phosphate and FGF23/Klotho
Scheme of FGFR-activation by both inorganic phosphate and FGF23. Activation has been shown in multiple cell lines including HEK392 and MC3T3 cells (1,2).

Preliminary results

Based on publications on FGFR- related pathway activation in C2C12 myoblasts (3, 4), myogenic differentiation was induced with supplementation of inorganic phosphate, FGF23/α- Klotho or combined treatments.

Gene expression

Phosphate treatments inhibited the expression of differentiation markers in C2C12 cells in a dose-dependent manner. The most marked effect was seen at one of the hallmark genes of myoblast differentiaion, *myogenin* (Fig 2). FGF23/Klotho treatment showed inhibition if *myogenin* exoression in a comparable effect of 0,5mM phosphate, while higher phosphate loads led to a more pronounced decrease of mRNA transcription.
Myf5 and *MyoD* expression was significantly decreased during high phosphate exposure, while 0,5mM phosphate treatment as well as FGF23/Klotho treatment just showed a tendency to decreased mRNA amount compared to controls (Fig 2)

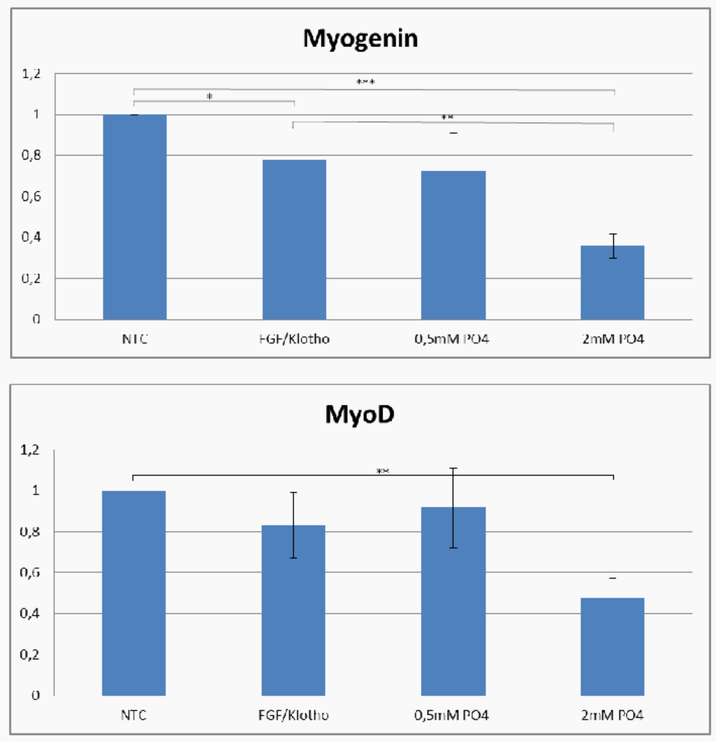

Fig 2: Gene expression in C2C12 myoblasts
RT-PCR analysis of mRNA expression of differentiation markers with different supplementations to standard differentiation media (n= 5, * $p < 0,05$; ** $p < 0,01$, *** $p < 0,001$)

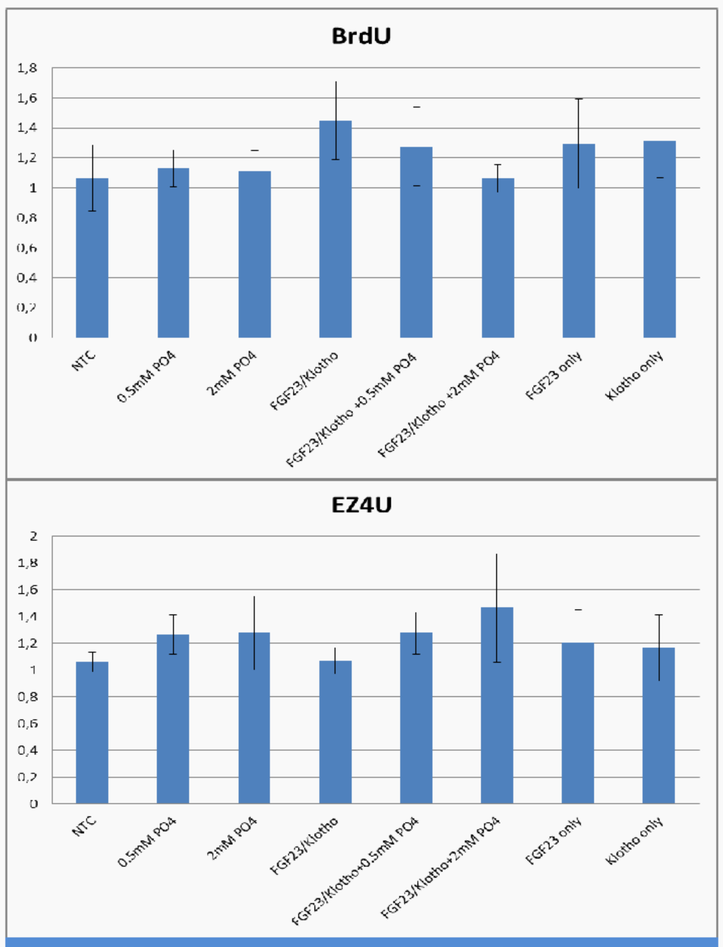

Fig 3: Proliferation rate and metabolic activity
Phosphate and FGF23/Klotho supplementation did not alter metabolic activity or proliferation rates significantly. (n=3)

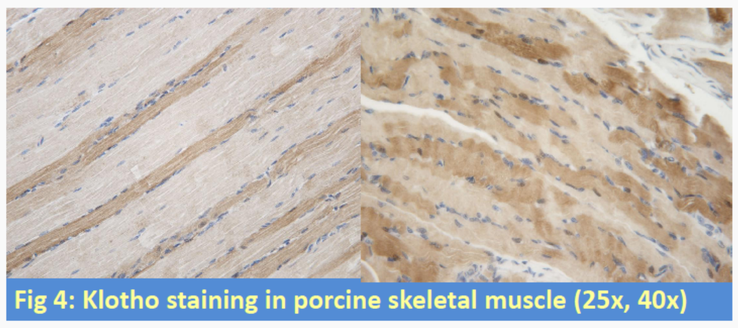

Fig 4: Klotho staining in porcine skeletal muscle (25x, 40x)
Immunohistochemistry revealed presence of α-Klotho in porcine skeletal muscle tissue, confirming previous data on α- Klotho mRNA expression (5, Fig 4).

Proliferation / Metabolic activity

Supplementation of media did not alter cell proliferation rates significantly. Importantly, the observed effects of phosphate treatments on gene expression were not associated with any impaired metabolic or proliferative capacity of myoblasts.
FGF23/ Klotho treatments showed a tendancy to increase BrdU incorporation. Regarding metabolic activity, EZ4U assays revealed a tendancy of inorganic phosphate supplementations to stimulate glucose metabolism. This tendancy was observed both with and without addition of FGF23 / α- Klotho (Fig 3).

Materials and Methods

Cell culture: C2C12 myoblast cells were differentiated in DMEM +2% horse serum muscle progenitor cells were differentiated under single and combined treatments with inorganic phosphate and/or FGF23 and Klotho.
Treatments: Cells were treated with recombinant human FGF23 (100ng/ml) and Klotho (50ng/ml) as supplementation to standard media during 6 days of differentiation.
Real time PCR: RNA isolation was performed according to the TRI® reagent kit instructions. The purity and amount of RNA was determined by measurement of the $OD_{260/280}$ ratio. Real time (RT)-PCR amplification will be performed and monitored using a 7500 fast real time PCR system. Expression of differentiation markers were analyzed by RT-PCR against 18S and b Actin expression
Statistics: All data were analyzed by one-way ANOVA and Student's t-test with Bonferroni correction. $P < 0,05$ was assumed as significant.

Conclusions

High **phosphate** loads **inhibited expression of differentiation markers** in a **murine myoblast** model system. FGF23/α- Klotho supplementation did in part mimic this effect.

Proliferation and metabolic activity was **not affected** significantly by inorganic phosphate or FGF23/α- Klotho treatments. Further experiments will be performed to analyze the mechanisms of the observed results.

Knowledge of the distinct effects of phosphate could help us to optimize treatment of hyperphosphatemia and ultimately to prevent musculoskeletal diseases.

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Adalbert Raimann

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