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 influx and influx as well as intestinal reabsorption. Disturbed phosphate levels, as seen in various pathological conditions, lead to typical symptoms: While hypophosphatemia leads to hypophosphatemic rickets, hypophosphatemia has been shown to be associated to increased mortality and morbidity in patients with chronic kidney disease (CKD).

Muscle fatigue, a common feature of hyperphosphatemic conditions, is attributed to many factors such as accelerated 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) also known as a canonical FGF receptor that is converted by α-Klotho. Thereby, the expression of type II sodium-phosphate cotransporters (Npctc2a) is reduced.

Elevated serum FGF23 concentrations are associated with mineralization defects due to 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. ERK1/2/3, (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.

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

Materials and Methods

Cell cultures: 2C12 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 α-Klotho.

Treatments: Cells were treated with recombinant human FGF23 (100ng/ml) and α-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 OD260/280 ratio. Real time (RT-PCR) amplification will be performed and monitored using a 7500 fast real time PCR system. Expression of differentiation markers was analyzed by RT-PCR against β3 and β 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.

References

Preliminary results

Based on publications on FGF23 related pathway activation in C2C12 myoblasts (1, 2), inorganic 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 differentiation, myogenin (Fig 2). FGF23/α-Klotho treatment showed inhibition if myogenin expression 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 in high phosphate exposure, while 0.5mM phosphate treatment as well as FGF23/α-Klotho treatment just showed a tendency to decreased mRNA amount compared to controls (Fig 2).

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/α-Klotho treatments showed a tendency to increase BrdU incorporation. Regarding metabolic activity, EZAU assay revealed a tendency of inorganic phosphate supplementations to stimulate glucose metabolism. This tendency was observed both with and without addition of FGF23/α-Klotho (Fig 3).

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.

The authors declare no actual or potential conflict of interest related to this poster.

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