A PAI-1 Antagonist Ameliorates Hypophosphatemia in the Hyp Mouse, a Vitamin D-Resistant Rickets Model Mouse

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Abstract: Congenital FGF23-related hypophosphatemic rickets/osteomalacia is a rare disorder characterized by hypophosphatemia due to excessive FGF23 secretion. Using Hyp mice, a model for X-linked hypophosphatemia (XLH), this study explores the therapeutic potential of TM5614, a PAI-1 antagonist, in ameliorating hypophosphatemia. After a single oral administration of TM5614 (10 mg/kg) to female Hyp mice, serum phosphate concentration increased with a peak at 6 hours. TM5614 administration decreased intact FGF23 concentration, normalized the expression of 25-hydroxyvitamin D-1α-hydroxylase protein, and Cyp27b1 mRNA in the kidneys. The results suggest that TM5614 may be an effective treatment for congenital FGF23-related hypophosphatemic rickets and osteomalacia.

Introduction: FGF23, a phosphaturic hormone secreted by osteoblasts and osteocytes, plays a pivotal role in regulating phosphate metabolism. X-linked hypophosphatemia (XLH), the most common inherited rickets, results from mutations in the phosphate-regulating gene PHEX. In Hyp mice, an XLH model, elevated FGF23 levels lead to increased renal phosphate excretion, causing hypophosphatemia. TM5614's potential as a therapeutic agent lies in its ability to counteract PAI-1, a key player in FGF23 homeostasis.

Methods: The study utilized a single oral administration of TM5614 (10 mg/kg) to 17-week-old female Hyp mice and WT mice, with blood samples collected at various time points. Additionally, continuous daily administration of TM5614 (10 mg/kg) was performed over a 10-day period, with blood samples obtained at specified intervals. The effects on serum phosphate levels, intact FGF23 concentration, and gene expression related to vitamin D metabolism were assessed. Serum phosphate concentration was measured using an automated clinical chemistry analyzer. Intact FGF23 levels were quantified using a mouse iFGF23 ELISA kit. Gene expression analyses of Fgf23 and Cyp27b1 in femur and kidney tissues were conducted through RNA isolation, reverse transcription, and quantitative PCR. Immunohistochemistry was employed to assess 25-hydroxyvitamin D-1α-hydroxylase protein expression in kidney tissues. Statistical analyses were performed using GraphPad Prism 9 software.

Results: Upon a single oral administration of TM5614 (10 mg/kg) to Hyp mice, a significant increase in serum phosphate levels occurred, peaking at 6 hours post-administration. This was accompanied by a notable reduction in intact FGF23 concentration, as validated by ELISA. Continuous daily administration over 10 days sustained elevated serum phosphate levels in Hyp mice, contrasting with the vehicle-treated group. Importantly, TM5614 demonstrated its ability to decrease intact FGF23 levels persistently. TM5614 treatment showcased a pivotal role in the regulation of vitamin D metabolism. The expression of Cyp27b1, encoding 25-hydroxyvitamin D-1α-hydroxylase, was enhanced in the kidneys of Hyp mice treated with TM5614. Immunohistochemistry further confirmed the normalization of 25-hydroxyvitamin D-1α-hydroxylase protein levels in TM5614-treated Hyp mice, emphasizing the potential of TM5614 in addressing FGF23-related dysregulation.

Discussion: TM5614 as a Therapeutic Candidate: The observed effects of TM5614 in ameliorating hypophosphatemia in Hyp mice present a promising outlook for its therapeutic potential. As a small-molecule PAI-1 antagonist with proven safety in human trials, TM5614's oral availability and persistent efficacy make it a viable alternative for addressing FGF23-related hypophosphatemic disorders. The reduction in intact FGF23 levels, sustained phosphate elevation, and modulation of vitamin D metabolism highlight TM5614's multifaceted impact on the underlying pathology. The limitation of this experiment is that the effect of TM5614 on bone metabolism could not be verified due to the short duration of TM5614 administration.

Conclusion: This study demonstrates that the PAI-1 antagonist TM5614 may be an effective treatment for vitamin D-resistant rickets and osteomalacia caused by excessive production of FGF23. TM5614 is in advanced human clinical trials and has completed safety studies, positioning it as a promising oral therapeutic agent.

Significance/Clinical Relevance: This study demonstrates the potential of TM5614, a PAI-1 antagonist, as a promising treatment for congenital FGF23-related hypophosphatemic rickets/osteomalacia. The observed improvement in hypophosphatemia, coupled with TM5614's oral availability and completed safety trials, suggests a clinically significant advancement. This novel therapeutic approach could enhance patient adherence and presents a hopeful alternative for addressing rare disorders associated with excessive FGF23 secretion, contributing to the expanding landscape of treatment options for vitamin D-resistant rickets and osteomalacia.