Diabetic Complications Consortium

Application Title: Treating complications of hyperphosphatemia in diabetic kidney disease

Principal Investigator: Timo Rieg

1. Project Accomplishments:

For the first time we have successfully generated db/db-Npt2a knockout mice which allow to be used a proof-of-concept model to test a new therapeutic approach, pharmacological Npt2a inhibition, as a treatment for hyperphosphatemia.

2. Specific Aims:

Specific Aim 1&2. Specific Aim I and II will determine the therapeutic effect of Npt2a inhibition on the devolvement of hyperphosphatemia as well as associated vascular calcification, hypertension, and cardiac function in the mouse models described above.

Results: Body weight of db/db mice was ~2-fold greater compared to control (db/+) mice. No significant differences in body weight between db/db and db/db-Npt2a$^{-/-}$ mice were observed. Of note, plasma P_i was ~1 mM greater in db/db compared to control mice and db/db-Npt2a$^{-/-}$ mice show a restored plasma P_i level as seen in control mice. Despite hyperphosphatemia in db/db mice, urinary P_i/creatinine ratio was not significantly different from control mice. In contrast, db/db-Npt2a$^{-/-}$ mice show ~2.2-fold greater urinary P_i/creatinine ratios compared to control and db/db mice. Plasma Ca^{2+} was higher in db/db compared to control mice and db/db-Npt2a$^{-/-}$ mice showed greater plasma Ca^{2+} levels compared to db/db mice. Because P_i and Ca^{2+} homeostasis are hormonal regulated processes, we studied parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels. Chronic kidney disease is normally associated with elevated PTH and FGF23 levels. PTH levels were not significantly different between db/db and control mice; however, in db/db-Npt2a$^{-/-}$ mice PTH levels were substantially suppressed reaching levels close to zero. The phosphaturic hormone FGF23 was slightly but significantly elevated in db/db compared to control mice. Of note, db/db-Npt2a$^{-/-}$ mice showed a ~70% reduction compared to control and db/db mice. Bone is the biggest P_i storage compartment in the body, which links impaired P_i homeostasis to disturbed bone mineralization. Bone formation (procollagen type I N-propeptide and osteocalcin) and resorption (type I collagen cross-linked C-telopeptide) markers were not significantly different between groups. However, the bone formation marker tartrate-resistant acid phosphatase 5b was significantly higher in db/db and db/db-Npt2a$^{-/-}$ mice compared to control mice. To test for the first time if hyperphosphatemia in db/db mice can be treated by pharmacological Npt2a inhibition, we performed a baseline blood collection followed by oral administration (30 mg/kg) of Npt2a-inhibitor. After 2 hours another blood collection was performed, and plasma P_i determined. In db/db mice no significant changes in plasma P_i were observed in response to vehicle application. In contrast, the Npt2a-inhibitor decreased plasma P_i in db/db mice by ~1.2 mmol/L. Plasma P_i in db/db-Npt2a$^{-/-}$ mice was comparable to levels seen in db/db mice after Npt2a-inhibitor administration and the Npt2a-inhibitor was without a significant effect on plasma P_i. In summary, we found that db/db mice are hyperphosphatemic and we have generated a novel proof-of-concept model to study renal Npt2a inhibitor effects. In the next steps we will determine possible preventive effects of Npt2a inhibition on cardiovascular outcomes.

3. Publications:

Published

1) Physiopathology of Phosphate Disorders. Portales-Castillo I, Rieg T, Khalid SB, Nigwekar SU, Neyra JA. Adv Kidney Dis Health. 2023;30(2):177-188.
2) Sodium phosphate cotransporter 2a inhibitors: potential therapeutic uses. Xue J, Thomas L, Dominguez Rieg JA, Rieg T. Curr Opin Nephrol Hypertens. 2022;31(5):486-492.
3) Intestine-Specific NHE3 Deletion in Adulthood Causes Microbial Dysbiosis. Xue J, Dominguez Rieg JA, Thomas L, White JR, Rieg T. Front Cell Infect Microbiol. 2022;12:896309.

4) NHE3 in the thick ascending limb is required for sustained but not acute furosemide-induced urinary acidification Xue J, Thomas L, Dominguez Rieg JA, Fenton RA, Rieg T. Am J Physiol Renal Physiol. 2022;323(2):F141-F155.
5) SGLT2 inhibition effect on salt-induced hypertension, RAAS, and Na^+ transport in Dahl SS rats. Kravtsova O, Bohovyk R, Levchenko V, Palygin O, Klemens CA, Rieg T, Staruschenko A. Am J Physiol Renal Physiol. 2022;322(6):F692-F707.
6) Npt2a as a target for treating hyperphosphatemia. Thomas L, Dominguez Rieg JA, Rieg T. Biochem Soc Trans. 2022;50(1):439-446.
7) Enhanced phosphate absorption in intestinal epithelial cell-specific NHE3 knockout mice. Xue J, Thomas L, Murali SK, Levi M, Fenton RA, Dominguez Rieg JA, Rieg T. Acta Physiol (Oxf). 2022;234(2):e13756.

Submitted/under revision
8) Genetic deletion of the renal Na^+/H^+ exchanger-3 (NHE3) does not alter calcium and phosphate balance due to compensatory responses. Poulsen SB, Murali SK, Thomas L, Nielsen R, Dimke H, Rieg T*, Fenton RA*. Kidney Int. 2023. *Shared senior authorship. Revision under review
9) Intravenous ferric carboxymaltose and ferric derisomaltose alter the intestinal microbiome in female iron deficient anemic mice. Rieg T, Xue J, Stevens M, Thomas L, White JR, Dominguez Rieg JA. Biosci Rep. 2023. Under review.
10) Epac Induces Ryanodine Receptor-Dependent Intracellular and Interorganellar Calcium Mobilization in mpkCCD cells. Yip KP, Ribeiro-Silva L, Cha B, Rieg T, Sham JSK. Front. Physiol. 2023. Revision under review.
11) Kravtsova O, Levchenko V, Klemens C, Rieg T, Liu R, Staruschenko A. Effect of SGLT2 inhibition on salt-induced hypertension in female Dahl SS rats. Am J Physiol Regul Integr Comp Physiol. 2023. Under review.