**Effects of pNaKtide in 5/6 Partial Nephrectomy mouse model having benefits on Red Blood Cells survival and Cardiac Function**

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**Objectives of the study**: We have shown previously that Na+/K+-ATPase has a signaling function in addition to its pumping function. We have also demonstrated that this signaling function amplifies oxidants and increases cellular oxidant stress; conversely the blockage of this signal cascade with a designed peptide, pNaKtide, attenuates oxidant stress. In this study we show that pNaktide ameliorates the phenotypical changes in experimental chronic kidney failure (5/6 partial nephrectomy (PNx) mouse model. Interstitial fibrosis represented by collagen fibers both in heart and kidney are hallmark of pathological changes. Our PNx model showed non-iron deficient anemia without elevation in the blood pressure compared to Sham surgery animals. Administration of pNaktide in sham and PNx mice induced an increase in the Red Blood Cells (RBCs) half life and reduced fibrosis in heart and kidney.

**Methods:** C57BL/6 mice were randomly divided into 4 experimental groups; Sham surgery, PNx surgery, Sham+pNaKtide, and PNx+pNaktide. Surgery was performed in two steps; Surgery One for pole ligations of left kidney and Surgery Two a week later to remove the right kidney. Day3 post-surgery two biotin was injected via cardiothoracic puncture. Subsequently, pNaKtide was injected subcutaneously (25mg/kg mixed with normal saline one week after second surgery and repeated weekly for a total of three weeks). Blood samples were collected through submandibular vein puncture at post biotin injection days 3, 7, 14, 21, 28, and 35 and RBC half-life was calculated based on the values of biotin-labeled RBCs measured by flow cytometry with Streptavidin-PE. All mice were sacrificed 5 weeks of surgery two. Heart and kidney were collected both for western blots and histological studies

**Results:** Comparing with Sham, PNx surgery significantly stimulates cardiac hypertrophy and anemia determined by the heart/body weight ratio and hematocrit (HCT) percentage, respectively. However, there is no significant difference concerning plasma iron level, TIBC, UIBC and iron saturation rate. RBC half-life was significantly reduced in PNx animals. pNaKtide treatment animals increased RBC half-life of Sham and PNx. Plasma EPO analysis by ELISA showed that PNx increased EPO level compared to Sham group (Sham 14.63±1.175 pg/mL, vs. PNx 20.49±1.452 pg/mL; p=0.0077), as well as that pNaKtide does not significantly change comparing to Sham group (Sham 14.63±1.175 pg/mL, vs. Sham+pNaKtide 11.42±3.525 pg/mL; p=0.3060), but significantly increased compared to PNx group (PNx 20.49±1.452 pg/mL, n=12, vs. PNx+pNaKtide 27.63±3.189 pg/mL, n=7; p=0.0322).   
**Conclusions:**  PNx induced anemia is independent of iron. Heart and renal fibrosis and anemia in these mice might not be dependent on iron homeostasis rather due to the generation of ROS which is the case in heart and kidney fibrosis in our previously published studies. We show here in this study that Biotin labeled RBC survival by Streptavidin-PE/biotin complex can be measured using flowcytometry and use of pNaKtide had significant effect on its half-life. This can be explained by preventing the generation of ROS by blocking the Na+/K+-ATPase–Src signaling pathway with pNaKtide. Work is In Progress to elucidate this signaling pathway and beneficial effects of pNaKtide.