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In the phase II clinical studies, ASSERT & ASSURE, high risk CVD events (MACE) in patients with cardiovascular disease (CVD) in phase II clinical trials, and progression of CKD. Apabetalone is a first-in-class orally active bromodomain and allele inhibitor, demonstrated an even greater reduction of FGF23 vs. placebo (n=23): -82.5 RFUs and -17.3 RFUs, -3.7% vs. placebo patients (n=47), -8.6 RFUs, -1.7% (ANCOVA p-values for its biological property, such as anti-oxidant, anti-fibrosis and regulating IGF-1 pathway, etc. Previously, we reported that repeated minor kidney injury results in more reduction of klotho and cause of CKD in klotho haploinsufficiency mice. In this study with this model, we investigated the effects of phosphorus on the progression of CKD under the condition of modulating klotho expression mediating induction by gene delivery or suppression by siRNA technique.

INTRODUCTION: It has been established that klotho protein is a key molecule in the axis of Ca/P metabolism in CKD-MBD, on the other hand, klotho is speculated to be implicated in the mechanism of preceding the CKD, in which klotho suppression is likely to be a result and a cause of CKD, since klotho protein has several potentialities for its biological property, such as anti-oxidant, anti-fibrosis and regulating IGF-1 pathway, etc. Previous study we reported that repeated minor kidney injury results in more reduction of klotho and cause of CKD in klotho haploinsufficiency mice. In this study with this model, we investigated the effects of phosphorus on the progression of CKD under the condition of modulating klotho expression mediating induction by gene delivery or suppression by siRNA technique.

METHODS: Short time clamping of renal artery for 20 minutes was performed and repeated once a week for 3 weeks in the klotho gene heterozygous mice (kl/-/+). The kidney damage was assessed by serum creatinine level and tissue injury score. The expression levels of fibrosis related markers were estimated by immunostaining and RT-PCR. Klotho expression in the kidney was monitored by immunohistochemistry, and serum FGF23 level was measured by enzyme immunoassay, in addition, its expression in the kidney was confirmed as to whether it was expressed in mRNA and protein level. Phosphorus loading was altered in the diet (normal diet: 0.35% and high phosphate diet: 2.5%). Then, the Klotho expression in the kidney was modulated by hydrodynamic method. An adenovirus harboring the mouse klotho gene (ad-kl(1.6×10^9tu)) was intravenously injected into mice (n=5), comparing with ad-LacZ group. And an expression vector carrying synthetic klotho-siRNA was injected via intravenously and analyzed the resulting changes in gene expression by real-time PCR.

RESULTS: Repeated ischemia reduced the renal function and worsen tissue score in kl (-/+ +) mice than in wild type mice. Klotho expression assessed by immunostaining level and mRNA level quickly diminished after each ischemic maneuver. High phosphate diet (2.5%) reduced the expression of klotho mRNA and accelerated renal damage of ATN score. On the other hand, higher serum FGF23 levels were detected, and its protein and mRNA expression were confirmed in tubular epithelium in those from the HP groups, despite FGF23 mRNA was not confirmed in the kidneys from the normal P
groups. Klotho delivery maneuver improved kidney tissue damage and reduced serum FGF23 level and slightly but not significantly reduced tissue expression of FGF23. In contrast, siRNA treatment reduced klotho expression and tended to increase serum FGF23 levels.

**CONCLUSIONS:** In mice in which Klotho expression is reduced by half, higher renal injury is induced even in renal injury of minor and frequent longitude, suggesting that Klotho protein has the property of tissue protection. At that time, since Klotho#39;s original property is handling and regulating phosphorus, it is possible that the toxicity of phospholipids may be noticeable when the expression of Klotho is reduced. It is also presumed that induction of FGF23 at the RNA and protein level in kidney tissue with phosphorus load is a compensatory mechanism of phosphorus excretion in coordination with Klotho. There may be a possibility that changing the expression of FGF23 in the kidney is induced by artificially regulating the expression of Klotho.