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Methods: Uninephrectomized Wistar rats were treated with adenine (200 mg/kg/day) for 14 days. After stabilization with normal salt diet (NSD, 0.3% NaCl), adenine-induced CKD rats were fed high salt diet (HSD, 8% NaCl). Mean arterial pressure (MAP) was continuously monitored by a telemetry system. Low frequency (LF) of systolic arterial pressure (SAP) was also analyzed, which reflects the SNA.
Results: In adenine-induced CKD rats, HSD for 5 days significantly increased MAP from 106 ± 2 to 148 ± 3 mmHg, but MAP was decreased to 96 ± 3 mmHg within 24 hours after switching to NSD (n=7). Treatment with an SGLT2 inhibitor, luseogliflozin (10 mg/kg/day, p.o., n=7), significantly attenuated the HSD-induced elevation of MAP, which was associated with reduction in LF of SAP.
Conclusions: These data suggest that treatment with an SGLT2 inhibitor attenuates the salt-sensitivity of BP which is associated with the inhibition of SNA in non-diabetic CKD rats.

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APOA1-MIMETIC PEPTIDES ENHANCE CELLULAR CLEARANCE OF CALCIPROTEIN PARTICLES AND ATTENUATE INFLAMMATORY PRIMING

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Introduction: Calciprotein particles (CPP) - colloidal nanoaggregates of calcium phosphate mineral, proteins and other biomolecules - have emerged as potential mediators of pro-inflammatory mineral stress in CKD. Transformation of CPP to the crystalline state (secondary CPP), as favoured by uraemia, enhances their inflammatory potential due to an enrichment of endogenous and bacteria-derived cargos that are able to ligate the innate immune sensor, Toll-like receptor 4 (TLR4). Unless overwhelmed, rapid removal of particles from the cell surface by scavenger receptor (SR)-A-mediated endocytosis restrains activation of TLR4 and licensing of downstream inflammatory cascades. Strategies aimed at augmenting immunologically-inert clearance of CPP by SR-A may abrogate the reaction to chronic mineral excess and lessen the inflammatory burden in CKD. However, in order to test whether exploiting such pathways may be beneficial, the native ligand for SR-A needed to be identified.
Methods: We used a combination of genetic and biochemical approaches to elucidate the ligand for SR-A-mediated endocytosis of secondary CPP. Ligand-depleted sera were generated by immunoaffinity chromatography. CPP were synthesized in uraemic human serum supplemented with supersaturating concentrations of calcium and phosphate salts, isolated by gel filtration chromatography and dosed according to concentrations determined by nanoparticle tracking analysis (NTA). We used flow cytometry and live-cell confocal imaging to monitor the binding and internalisation of fluorescently-labelled CPP in BM-derived WT and SR-A-deficient murine macrophages as well as in human lines in which either SR-A or TLR4 had been deleted using CRISPR/Cas9 technology. A peptide mimetic of the ligand was synthesised along with scrambled control peptides. NFkB induction was evaluated using a luciferase reporter and inflammatory cytokine mRNA expression by qPCR.
Results: Ripening of CPP crystalline state is associated with an enrichment of Apo-A1. Exposure of human macrophage to labelled CPP synthesised in serum depleted of Apo-A1, resulted in an 80% decrease in cell-associated fluorescence relative to CPP from Apo-A1-replete serum. A similar reduction in uptake of Apo-A1-replete CPP2 was observed in macrophage derived from SR-$A^{-/-}$ mice and SR-A knockout human macrophage relative to their respective wild-type controls. Two patients with genetically confirmed Apo-A1 deficiency were found to have modestly elevated serum CPP levels compared to age- and gender-matched controls (n=20), but a markedly increased CPP-2 to CPP-1 ratio, consistent with an important role for Apo-A1 in CPP2 metabolism. Compared with Apo-A1-replete CPP2, those synthesised from Apo-A1-depleted, uraemic serum induced greater NFkB induction and cytokine mRNA levels (TNF-alpha & IL-6) at equivalent doses, but not in TLR4-knockout cells. Addition of an 18mer Apo-A1 mimetic peptide dose-dependently increased CPP2 uptake in macrophage, but this effect was absent in murine and human cells lacking SR-A. Reconstitution of Apo-A1-deplted CPP2 with mimetic peptide restored uptake and inhibited inflammatory signalling.
Conclusions: Therapeutic enhancement of SR-A-dependent CPP uptake using Apo-A1 mimetics may help ameliorate the inflammatory response to chronic mineral dysregulation\

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MUTATION OF P66SHC IN RATS CAUSES INCREASED H2O2 PRODUCTION AND LEADS TO REDUCED NUMBER OF GLOMERULI

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Introduction: Shc proteins, products of *Shc1* gene, exists in three functionally distinct isoforms (p46Shc, p52Shc, and p66Shc) that serve as intracellular adaptors for several key signaling pathways involved in renal pathologies. Adaptor protein p66Shc contributes to the pathogenesis of oxidative stress-related diseases and is known to regulate the sensitivity to oxidative stress. The objective of this study was to test the hypothesis that abnormal p66Shc-mediated ROS production could be critically involved in the development of nephrons during nephrogenesis. p66Shc ability to promote oxidative stress-related diseases requires phosphorylation of serine 36 residue (Ser36), which causes conformation change and consequent translocation of p66Shc to the mitochondria where it increases the production of ROS.
Methods: Using embryo microinjection of Zinc Finger Nucleases, we have generated unique mutant rats (termed $p66Shc^{del29\text{-}37}$), which express endogenous p66Shc with a 9-amino acid deletion containing the regulatory Ser36 on the genetic background of Dahl salt-sensitive (SS) rats.
Results: $p66Shc^{del29\text{-}37}$ rats display increased H_2O_2 renal production, as measured by enzymatic microelectrode biosensors when compared with parental SS rats. Since p66Shc is known to oxidize cytochrome *c* in mitochondria, rendering it unavailable to reduce oxygen to water and promoting deviation of a fraction of mitochondrial electron flow to the production of H_2O_2, likely $p66Shc^{del29\text{-}37}$ is constitutively translocated to mitochondria. The kidney is particularly sensitive to increases in oxidative stress, and $p66Shc^{del29\text{-}37}$ rats are characterized by increased susceptibility to renal pathologies and reduction in renal function. We quantitated nephron numbers in fully developed homozygote and heterozygote $p66Shc^{del29\text{-}37}$ rats as well as in the SS rats using the acid maceration method. We also quantified ureteric bud branching morphogenesis in embryos from corresponding strains using the ureteric bud marker calbindin-D-28K. Metanephric kidney ureteric bud branching is suppressed in embryos, and the number of glomeruli is significantly reduced in adults $p66Shc^{del29\text{-}37}$ rats when compared to SS.
Conclusions: The mechanism of nephron number control is not well characterized. Our data suggest that H_2O_2 renal production caused by abnormal signaling of p66Shc could be critical in regulating nephrogenesis and constitutive p66Shc signaling negatively impacts kidney development and renal function.

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ELECTRO-ACUPUNCTURE INCREASES PROTEIN SYNTHESIS THROUGH DOWNREGULATION OF LET-7 MICRORNA IN CHRONIC KIDNEY DISEASE MICE

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Introduction: Our previous study found that acupuncture with low frequency electrical stimulation (Acu/LFES) can prevent muscle atrophy by attenuation of protein degradation in chronic kidney disease. However, it is not clear whether Acu/LFES can increase protein synthesis in skeletal muscle.
Methods: Normal and 5/6-nephrectomized (CKD) C57/BL6 mice were given Acu/LFES treatment, applied for 30 minutes once. Gastrocnemius and triceps brachii muscles were harvested at 0, 6, 24, 48 and 72 hours after treatment. Protein synthesis was measured by the surface sensing of translation (SUnSET) assay. Exosomes were harvested using serial centrifugations and subjected to microRNA deep sequencing. The mature microRNA library was validated using a High Sensitivity DNA chip. The Student's t-test and ANOVA were used for statistics.
Results: Acu/LFES prevented soleus and extensor digitorum longus muscle weight loss and increased the hind-limb muscle grip function in CKD mice. Protein synthesis in the gastrocnemius muscle was enhanced in both normal control and CKD mice. In normal mice, protein synthesis was also increased in forelimb muscle (triceps brachii). To identify how