[6]Laboratory of Epigenetic and Metabolic Research, Department of Medicine, Howard University College of Medicine

OBJECTIVES/GOALS: This study aimed to characterize urinary exosomal miRNA content in African American adults with diabetic kidney disease. METHODS/STUDY POPULATION: Male and female participants between the ages of 18 and 65 were recruited from the Diabetes Treatment Center and the Nephrology Clinic at the Howard University Hospital. Exosomes were isolated from cleared urine of healthy controls (n=3), type 2 diabetics (n=3), and participants with chronic kidney disease (n=3). The purity and size of isolated microparticles was evaluated using NanoSight technology (30nm to 120nm size range) and western blot analysis for exosome-specific markers (TSG101 and CD81) RESULTS/ANTICIPATED RESULTS: Expression of 5 selected microRNAs, miR-4534, miR-320c, miR-451, miR-362-3p and miR-877-3p were evaluated by qRT-PCR. miR-4534 and miR-451 was increased between healthy controls and the type diabetic group. MiR-320c was increased in the CKD group, in comparison to healthy controls. Conversely, there was no difference in miR-877-5p between the three groups. DISCUSSION/SIGNIFICANCE: These findings will provide insight into the use of circulating miRNAs as early markers of DKD, ultimately creating more effective treatments and preventive measures.

498

Bruton's tyrosine kinase (BTK) inhibitors impede platelet aggregation but not adhesion to collagen.

Thomas Kartika, Lorena Buitrago, Jihong Li and Barry S. Coller
Rockefeller University

OBJECTIVES/GOALS: The research objectives of this project are to elucidate the effects of Bruton's tyrosine kinase inhibitors (BTKi) of varying target specificity on platelet function with regard to platelet aggregation, adhesion, spreading, and intracellular signaling as measured by kinase phosphorylation. METHODS/STUDY POPULATION: Blood from healthy volunteers was obtained and processed to obtain both washed platelets and platelet-rich plasma. The samples were then treated with one of the BTKi drugs or with vehicle (DMSO) at concentrations matching patient blood concentrations derived from clinical trials and pharmacokinetic studies. The incubated samples were then analyzed in an aggregometer using one of several agonists. Aggregation was stopped after five minutes with a perchloric acid-based lysis buffer. The samples were then analyzed by SDS-PAGE and immunoblotting to quantify BTK protein and BTK phosphorylation. Adhesion was assessed by incubating washed platelets treated with BTKi on microtiter wells coated with fibrinogen or collagen and quantifying adherent platelets by their endogenous acid phosphatase activity. RESULTS/ANTICIPATED RESULTS: We found that ibrutinib, zanubrutinib, and pirtobrutinib all completely inhibited collagen-induced platelet aggregation, whereas they did not inhibit aggregation induced by thrombin, ristocetin, arachidonic acid, or the PAR1 activator peptide SFLLRN (T6). Acalabrutinib inhibited collagen-induced platelet aggregation only at high concentrations (1-2 micromolar). At the lower concentration of 200 nanomolar, comparable to the concentration required for the other BTK inhibitors to completely inhibit platelet aggregation, acalabrutinib failed to inhibit aggregation but did inhibit auto-phosphorylation, indicating an impact on signaling. None of the BTKi drugs inhibited adhesion of platelets to collagen-coated surfaces. DISCUSSION/SIGNIFICANCE: Our data show the inhibitory effect of BTKi on collagen-induced platelet aggregation and signaling. However, it remains unclear whether the inhibition is due to an effect on BTK itself or other related kinases. Better insight into the mechanisms of platelet inhibition by BTKi may help guide the development of BTKi with a lower risk of hemorrhage.

499

Physiological and Metabolomic Effects of a Community-Based Cardiorenal Protective Diet Intervention in African Americans with Chronic Kidney Disease and Hypertension

Meera J. Patel, Xuan Wang, Baylor Scott and White Health Teodoro Bottiglieri, Baylor Scott & White Health Heather Kitzman
University of Texas Southwestern Medical Center

OBJECTIVES/GOALS: Chronic kidney disease (CKD) impacts 15% of US adults and African American (AA) persons are disproportionately affected with more than 3 times higher risk of kidney failure when compared to Caucasian persons. This study evaluated the physiological and metabolomic effects of increased fruits and vegetables (F&V) on cardio-renal risk factors. METHODS/STUDY POPULATION: This pilot trial used a prospective, 2-group, randomized study design to evaluate a F&V intervention (N=46), where participants received a prescribed amount of fresh, base-producing F&V compared to a wait-list control (WL) condition (N=45). All participants were African American adults (≥18 years), had self-reported hypertension, and had CKD (Stage 1-3) on screening spot-urine microalbumin test. Participants were measured at baseline and 6 weeks post-intervention. Clinical data (i.e., systolic and diastolic blood pressure, lipid panel, hemoglobin A1C, BMI [body mass index], and albumin to creatinine ratio) were collected. Targeted metabolomic quantitative analysis was performed followed by LC-MS/MS and FIA-MS/MS. Linear mixed models evaluated analyte expression and clinical data. RESULTS/ANTICIPATED RESULTS: AA participants (N=91) were aged 58 ± 10.2 years, 66% female, and 54% had incomes ≤$50,000. T-tests compared change scores (baseline to 6-weeks) between groups. The F&V group demonstrated a significant reduction in BMI of -4.7 ± 10.5 kg/m² compared to a 1.9 ± 8.3 kg/m² increase in the WL group, $p<.01$. Further, the F&V group demonstrated a reduction in total cholesterol of -15.4 ± 58.8 mg/dL compared to a 17.7 ± 68.8 mg/dL increase in the WL group, $p<.05$. Non-significant reductions in hemoglobin A1c were found in the F&V versus the WL group. Metabolomic analysis indicated significant variation with an increase of suggestive key biomarkers for worse CKD in the WL versus F&V groups at 6-weeks. DISCUSSION/SIGNIFICANCE: Consumption of only 2 cups of F&V via a community-based intervention reduced CVD risk factors in AA adults with CKD and HTN and resulted in molecular/biochemical changes which may improve long-term kidney health. Further investigation may lead to development of cost-effective dietary intervention models to improve CKD outcomes in AA persons.

https://doi.org/10.1017/cts.2024.422