EffIcacy, mechaNisms and saFety of SGLT2 INhibitors in kIdney Transplant recIpients: The INFINITI study

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Objectives

1. Primary objective:

The primary objective is to determine if acute and chronic dapagliflozin use causes a change in systolic blood pressure compared to placebo. **Acute** means changes from baseline to Visit 4 (1 week), and **chronic** means changes from baseline to Visit 9 (12 weeks).

2. Secondary objectives:

2.1. We will characterize the safety of dapagliflozin use compared to placebo.

We will determine acute and chronic effects of dapagliflozin vs. placebo from baseline on:

2.2. Calcineurin inhibitors (CNI) levels, a class of immunosuppressants measured by tacrolimus or cyclosporine
2.3. Systemic hemodynamics
2.4. Total and proximal renal tubular natriuresis measured by sodium/lithium clearance
2.5. Sympathetic nervous system (SNS) activation
2.6. Metabolic parameters
2.7. Kidney function indicated by iohexol-measured and estimated glomerular filtration rate (GFR)
2.8. Neurohormones
2.9. Glucose-lowering effects

3. Exploratory objectives:

3.1. We will determine acute and chronic effects of dapagliflozin vs. placebo from baseline on estimated plasma volume (ePV).
3.2. We will assess whether the proximal renal tubular natriuretic effect of dapagliflozin leads to volume contraction compared to placebo. To this end, we will determine the association between acute and chronic changes to fractional sodium handling and acute and chronic changes in volume markers.
3.3. We will assess whether the proximal renal tubular natriuretic effect of dapagliflozin is associated with changes in kidney function compared to placebo. To this end, we will determine the association between acute and chronic changes to fractional sodium handling and acute and chronic changes in GFR.
3.4. We will assess whether volume contraction with dapagliflozin leads is associated with SNS activation compared to placebo. To this end, we will determine the association between acute and chronic changes to volume markers and acute and chronic changes in markers of SNS activation.
3.5. We will determine whether changes in neurohormones and volume markers are associated with changes in kidney function. To this end, we will determine the

association between acute and chronic changes in absolute measures of kidney function and the acute and chronic changes in neurohormones and volume markers with dapagliflozin compared to placebo.

3.6. We will determine whether changes in neuro-hemodynamic & volume markers are associated with changes in arterial stiffness. To this end, we will determine the association between acute and chronic changes in arterial stiffness and the corresponding acute and chronic changes in neurohormones and volume markers with dapagliflozin compared to placebo.

3.7. We will determine whether changes in neurohormones and volume markers are associated with changes in blood pressure. To this end, we will determine the association between acute and chronic changes in blood pressure and the acute chronic changes in neurohormones and volume markers with dapagliflozin compared to placebo.

3.8. We will determine acute and chronic effects of dapagliflozin vs. placebo from baseline on urine and plasma chemistry marker levels.

4. Exploratory subgroup analyses:

4.1. Compare diabetes vs non-diabetes status groups separately.

5. Pre-specified post-hoc analyses:

We will determine the acute and chronic effects of dapagliflozin vs. placebo from baseline on:

5.1. Urine adenosine

5.2. Whether changes in proximal renal tubular natriuretic effect are associated with changes in afferent arteriolar tone.

Outcomes

1. Outcomes for the primary objective:

1.1. The primary outcome is measured by the acute and chronic change in seated systolic blood pressure (measured using automated sphygmomanometer) from baseline with dapagliflozin vs placebo.
1.2. We will also measure the primary objective on these secondary outcomes:
   a. Seated diastolic blood pressure
   b. Seated mean arterial pressure

2. Outcomes for the secondary objectives:

2.1. Safety will be assessed by the number of hypoglycemic episodes, acute rejection episodes, volume depletion, UTI or genital infection, acute kidney injury, ketoacidosis and serious adverse events.
2.2. CNI levels will be measured by tacrolimus or cyclosporine levels
2.3. Systemic hemodynamic assessments include the following group of outcomes:
   a. NICOM parameters (chronic change only)
   b. arterial stiffness
      i. radial, aortic and carotid augmentation index (AIx)
      ii. carotid-radial pulse-wave velocity (PWV)
      iii. carotid-femoral PWV
2.4. Natriuretic effect will be measured using the following group of outcomes:
   a. Lithium clearance
      i. $FE(Li^+) = 100\ X\ ([(urine\ Li^+)\ X\ (creatinine\ plasma)]/[(plasma\ Li^+)\ X\ (creatinine\ urine)]$
   b. Total overall (proximal and distal) tubular sodium handling
      i. $FE(Na^+) = 100\ X\ ([(urine\ Na^+)\ X\ (creatinine\ plasma)]/[(plasma\ Na^+)\ X\ (creatinine\ urine)]$
   c. Distal sodium handling:
      i. $FE(Na^+) - FE(Li^+)$
2.5. SNS activation will be measured by norepinephrine, epinephrine, dopamine, heart rate and heart rate variability (RMSSD and SDNN).
2.6. Metabolic parameters will be measured by weight and waist circumference
Lipid profile will be measured by cholesterol, LDL, HDL and triglyceride (use screening instead of baseline value).
2.7. Renal hemodynamic function will be measured using the following groups of outcomes:
   a. Iohexol measured GFR
      i. *mGFR*
      ii. *Hematocrit (HCT)*
   b. 24 hour urine measured GFR
      i. *24-hour Creatinine (mmol/day)*
      ii. *Creatinine clearance (ml/min)*
   c. Estimated GFR (eGFR) calculated by CKD-EPI 2021 equation
2.8. Neurohormones will be measured by plasma aldosterone and plasma renin concentration

2.9. Glucose-lowering effect will be measured by HbA1c and fasting blood glucose

3. Outcomes for the exploratory objectives:

3.1. Plasma volume will be estimated [ePV] by Strauss estimation:
   a. $([\{Hb_{baseline}/Hb_{end}\}\times\{(100-Ht_{end})/(100-Ht_{baseline})\}]-1)\times 100$
3.2. Relationship between proximal renal tubular natriuretic effects and volume reduction: proximal renal tubular natriuretic effects will be evaluated by lithium clearance FE(Li+); volume change will be measured using the following outcome:
   a. Estimated plasma volume (ePV)
      i. *Estimated percent change by Strauss estimation*

$([\{Hb_{baseline}/Hb_{end}\}\times\{(100-Ht_{end})/(100-Ht_{baseline})\}]-1)\times 100$

3.3. Relationship between proximal renal tubular natriuretic effects and kidney function: proximal renal tubular natriuretic effects will be evaluated by lithium clearance FE(Li+); Kidney function will be measured using measured GFR.
3.4. Relationship between volume and SNS activation: Volume will be measured by ePV. Hormones will be measured by:
   a. Neurohormones: Plasma aldosterone and renin
   b. SNS markers: norepinephrine, epinephrine, dopamine, heart rate, heart rate variability (SDNN and RMSSD)
3.5. Relationship between neurohormones and volume markers with kidney function. Hormones will be measured by serum aldosterone and renin. Volume will be measured by ePV. Absolute measure of renal function outcomes is measured by measured GFR.
3.6. Relationship between arterial stiffness and neuro-hemodynamic & volume markers. Arterial stiffness outcomes will be measured by radial, aortic and carotid AIx, carotid-radial PWV and carotid-femoral PWV. Neuro-hemodynamic and volume markers will be measured by:
   a. Seated: SBP, DBP, and MAP
   b. NICOM: SBP, DBP, and MAP (chronic change only)
   c. SNS: epinephrine, norepinephrine, dopamine, heart rate, heart rate variability (SDNN and RMSSD)
   d. Neurohormones: Plasma aldosterone and renin
   e. Volume will be measured by ePV
3.7. Relationship between neurohormones & volume markers and blood pressure. Hormones will be measured by plasma aldosterone and renin. Volume will be measured by ePV. Seated and NICOM (chronic change only) measures of SBP, DBP, and MAP will be the blood pressure outcomes.
3.8. The blood and urine biomarkers are:
   a. Plasma chemistry:
      i. *Sodium, potassium, urea, phosphate, chloride, calcium*
      ii. *AST, ALT, ALP, amylase*
      iii. *Hemoglobin, hematocrit, creatinine, total bilirubin, albumin, bicarbonate,*
      iv. *Glucose*
   b. Urine chemistry
      i. *Spot: UACR, albumin, creatinine*

ii. *Spot: urea, protein, potassium, sodium – normalized to urine creatinine*
iii. *24-hour urine: total volume, albumin, protein, creatinine, UACR, urea, urate, potassium, sodium, glucose*

4. Exploratory subgroup analyses

4.1. Compare diabetes vs non-diabetes status groups separately, and include test of interaction
   a. Primary objectives
   b. Secondary objectives
   c. Exploratory objectives

5. Outcomes for pre-specified post-hoc analyses:

5.1. Urine adenosine will be normalized to urine creatinine
5.2. Relationship between proximal renal tubular natriuretic effects and markers of afferent arteriolar tone: proximal renal tubular natriuretic effects will be evaluated by lithium clearance FE(Li+); afferent arteriolar tone will be measured by urine adenosine normalized to urine creatinine.

Statistical Analysis Plan (SAP)

0. Summary of baseline (Visit 3) demographic and clinical characteristics separated by dapagliflozin group and placebo group will be generated. Significance testing between-group will be performed. The following variables will be included:
    - *Age, sex, weight, waist circumference, BMI, HbA1c, hemoglobin, fasting blood glucose*
    - *race (white/black/Hispanic/Asian/other)*
    - *SBP, DBP, MAP, HR*
    - *Mean eGFR calculated by CKD-EPI 2021 equation*
    - *UACR (median and IQR)*
    - *Concomitant medication use (ACEi, ARB, Diuretic, statin)*

1. ***Analysis for the primary objective:***

1.1. To assess dapagliflozin-related acute and chronic changes in the primary outcome of seated systolic blood pressure compared to placebo, linear mixed-effects models will be used. Two separate models will be fit. For acute change, the vector of outcomes will include the baseline and 1-week values (i.e. Visit 3 and Visit 4 values). For chronic changes, the vector of outcomes will include the baseline and 12-week values (i.e. Visit 3 and Visit 9 values). The covariates will include the visit and treatment-by-visit interaction; due to randomization, a covariate for the main effect of treatment will not be included. The treatment-by-time coefficient, its 95% confidence interval, and its p-value will be used to assess the effect of treatment.

1.2. We will repeat this analysis with:
   a. Seated diastolic blood pressure as an outcome
   b. Seated mean arterial pressure as an outcome

2. ***Analysis for secondary objective:***

2.1. The numbers of hypoglycemic episodes, acute rejection episodes, volume depletion, UTI or genital infection, acute kidney injury, ketoacidosis and serious adverse events will be reported for both the placebo group and dapagliflozin group. No statistical comparisons will be performed.

2.2. – 2.10. The same models described in the analysis of primary objective 1.1 will be completed for each of the different outcomes.

3. ***Analysis for exploratory objective:***

3.1. ePV should be tested for equality of variances using the Welch–Satterthwaite equation.

3.2. – 3.7. Linear regression will be performed, with treatment as a covariate. Two separate models will be fit for acute and chronic changes. Acute changes will use the baseline and 1-week values for both the dependent and independent variable values; chronic changes will use baseline and 12-week values. Exceptions include NICOM parameters – will only evaluate chronic changes.

3.8. The same models described in the analysis of primary objective 1.1 will be performed for each of the different outcomes. *Note 3.8.a.i, blood urea was not collected at V4. Only perform V3-V9 change.*

4. ***Exploratory Subgroup Analysis.***

Analyses following the forms described in 1.1 will be conducted by adding a main effect of diabetes-status and a third-level interaction term for diabetes-status by time by treatment. For 2.1, the numbers of adverse events will be calculated according to diabetes status. Analyses following the forms described in 3.2 – 3.7 will be conducted by adding a main effect of diabetes-status and an interaction term for diabetes-status by treatment.

5. ***Pre-specified post-hoc analyses:***

5.1. The same models described in the analysis of primary objective 1.1 will be performed for the outcome.
5.2. The same models described in the analysis of exploratory objective 3.2 will be completed for each of the different outcomes.

Analysis population: The intention to treat population will be used for all analyses.

Hypothesis testing: For hypothesis testing, a 5% significance level will be used (two-sided). No interim analyses will be performed. For secondary objectives, nominal p-values will be reported.

General model assumptions and model diagnostic procedures: For the linear mixed-effects models with visit and treatment-by-visit interaction as covariates, a participant-level random intercept will be included. Restricted-maximum-likelihood will be used for estimation and the missing-at-random framework will be assumed. Plots of marginal and conditional residuals will be used for model diagnostics and assessment. Similar diagnostic procedures will be used for the linear regression models.