Inhibition of Urinary Angiotensinogen and the Reduction of Blood Pressure by SGLT2 Inhibition in patients with Type 2 Diabetes

NCT Number: NCT02796170

Protocol date: 06/20/2019

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Project Goals: Type 2 diabetes is a complex disease where hyperglycemia occurs as a result of the development of insulin resistance. It is often associated with obesity and is characterized by hyperglycemia, hyperinsulinemia, as well as hyperleptinemia. In addition to the problems associated with poor glucose control, type 2 diabetes is accompanied by a chronic inflammatory state. This chronic inflammatory state leads to a number of associated complications, including cardiovascular disease. Diabetes increases one's risk for cardiovascular disease by 2-4 fold[1], highlighting the importance of developing therapeutics that both control blood glucose levels and reduce one's risk of cardiovascular disease. The monetary impact of diabetes on our healthcare system continues to grow as well. Currently 25.8 million children and adults (8.3% of the population) in the United States suffer from diabetes[2]. In addition to established traditional treatment options, one new approach has focused on blocking glucose reabsorption by the renal tubules to allow substantial excretion of glucose in the urine. This project will elucidate molecular and cellular mechanisms behind the potential cardio protective effects of a new class of antihyperglycemic, sodium glucose co-transporter 2 (SGLT2) inhbitors. We will also investigate clinically a potential mechanism underlying the effect of these drugs on lowering blood pressure (BP) and raising potassium (K).

Rationale: Clinical trials of two SGLT2 inhibitors, canagliflozin and dapagliflozin, have reported drops in systolic blood pressure of ~5 mmHg[3, 4]. Inappropriate activation of intrarenal renin-angiotensin system (RAS) is a major contributor to the increased arterial pressure and tissue injury including diabetic nephropathy. A key factor in the intrarenal RAS activation is stimulation of intrarenal angiotensinogen (AGT) which is the precursor of angiotensin peptides[5]. In animal models of type1 and type 2 diabetes mellitus, hyperglycemia is accompanied by elevated intrarenal AGT and urinary AGT levels[6-8]. Furthermore, direct treatment of rat renal proximal tubular cells (PTC) with high glucose results in stimulation of AGT production[9]. These findings suggest that hyperglycemia augments intrarenal AGT levels in diabetes mellitus leading to the development of high blood pressure and diabetic nephropathy.

By inhibiting glucose reabsorption in the proximal tubule, SGLT2 inhibition has the potential to block the AGT elevating effect of hyperglycemia. We therefore submit the following:

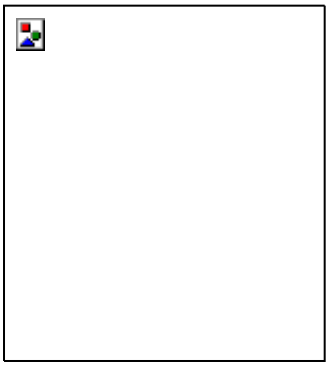
Hypothesis 1: SGLT2 inhibition attenuates renal AGT elevation by hyperglycemia leading to decreased blood pressure, renoprotection and cardio protection in diabetes mellitus. This effect can be elucidated by simultaneously measuring urine AGT and 24 hour ambulatory blood pressure (ABPM)

We will test this hypothesis in a pilot study by:

Aim 1. Exploring the effect of SGLT2 inhibition on intra-renal AGT production in subjects with diabetes mellitus, who are known to have augmentation of intra – renal AGT.

This will be a cross-over pilot study to elucidate whether this mechanism underlies the decrease in BP, as well as the rise in K, in humans with diabetes.

Background



Specific Aim: Explore the effect of SGLT2 inhibition on intra-renal AGT production in subjects with diabetes, who are known to have augmentation of intra – renal AGT.

Rationale: We have recently demonstrated that urinary AGT is increased in patients with diabetes, including patients with diabetes without microalbuminuria and hypertension (not yet published). The increase is greatest in those with established nephropathy and hypertension, suggesting that this activation occurs early and may contribute to both hypertension and proteinuria. Since SGLT2 inhibitors are approved only for type 2 diabetes we propose studying only type 2 patients, stratifying them as to whether they are on RAAS blocker drugs or not. We also propose to study the impact of the BP reduction on vascular compliance and augmentation index – indirect measures of endothelial function that are non-invasive and have been shown to be predictors of CV outcomes.

The RAS has been portrayed as an endocrine, paracrine, autocrine and intracrine system and thus it has been difficult to delineate the quantitative contributions of systemically delivered versus locally formed Ang peptides to the levels existing in any given tissue. Emerging evidence indicates that local formation is of major significance in the regulation of the Ang levels in many organs and tissues. Various studies have demonstrated the importance of tissue RAS in the brain, heart, adrenal glands, vasculature, as well as in the kidney. While every organ system in the body has elements of the RAS, the kidney is unique in having every component of the RAS with compartmentalization in the tubular and interstitial networks as well as intracellular accumulation. In this regard, the kidneys, as well as the adrenal glands, are unique in terms of the tissue concentrations of Ang II which are much greater than can be explained by the concentrations delivered by the arterial blood flow. Indeed, the major fraction of Ang II present in renal tissues is generated locally from AGT delivered to the kidney as well as from AGT locally produced by proximal tubule cells. Ang I delivered to the kidney can also be converted to Ang II.

Additional studies demonstrated that urinary excretion rate of AGT is closely correlated with systolic blood pressure and kidney Ang II content, but not with plasma Ang II concentration. This increase was not just due to increased proteinuria, or increased filtration of AGT into the tubular fluid. The results indicate that urinary AGT originates primarily from the kidneys and not from the plasma.

The principal action of ACE inhibition is a disruption of the conversion of Ang I to Ang II and consequently, inhibition of the Ang II effects such as vasoconstriction. Angiotensin converting enzyme inhibition may trigger additional events as a result of protection of other peptides. Inhibition of ACE also prevents degradation of kinins into inactive peptides. Kinins act as endogenous vasodilators via stimulation of nitric oxide and release vasodilatory prostaglandins; thus kinins contribute to renal vasodilatory actions of ACE inhibitors, particularly in the medullary circulation. Indeed, blockade of ACE or deletion of kidney ACE reduces the severity of the hypertension that develops with chronic Ang II or Ang I infusions showing that ACE mediated de novo formation of Ang II is an essential step in the full development of hypertension. Ang II receptor blockers (ARB) are a specific class of antagonists that block AT_1 receptors at the tissue level. Angiotensin II, generated by non-ACE mechanisms, could be blocked at the receptor level by an ARB. However, increases in Ang II concentration after ARB therapy may stimulate AT_2 receptors if receptors are not fully blocked.

The use of ARBs and ACE inhibitors has become common practice in treating patients with diabetes. Because RAS activation plays such a central role in the development and progression of DN, there has been extensive interest in the potential hope for reduction in morbidity and mortality by using agents that block 1 or other ore steps in the RAS. Accordingly, the assessment of urinary AGT as an early biomarker of the status of the intrarenal RAS may be of substantial importance. It may be particularly helpful in serving as a means to determine efficacy of the treatment to reduce intrarenal angiotensin II levels.

In our experience urinary AGT remains elevated in patients treated with ACEI or ARBs and present a novel treatment target (with an easily measurable biomarker)

Hypothesis 2: SGLT2 inhibition will reduce urinary AGT in subjects with 2 diabetes, and this reduction will correlate with reduction in BP.

Rationale:
Progression of hypertension and nephropathy in diabetes mellitus (DM). Elevated levels of angiotensinogen (AGT) are found in the urine of patients with type 1 DM and RAS blockade is commonly used to slow or prevent the development of diabetic nephropathy. AGT is upregulated in proximal tubular cells (PTC) exposed to high glucose conditions in response to the accumulation of reactive oxygen species (ROS). AGT forms angiotensin II (Ang II) which further upregulates AGT via angiotensin II type 1 receptor (AT1R) activation.AT1R signaling is another major source of ROS generation in PTC11 and AT1R blockade attenuates high glucose-induced AGT expression.12 Intrarenal RAS activation is strongly associated with hypertension and kidney injury.1, 10 However, the mechanisms underlying intrarenal RAS activation in DM have not been delineated. Plasma glucose is filtered at the glomerulus and almost completely reabsorbed in the proximal tubules. Sodium-glucose co-transporter 2 (SGLT2) is located in the early segment of the proximal tubule and is responsible for the majority of glucose reabsorption. Increased expression of SGLT2 and glucose reabsorption are observed in DM, but the mechanisms remain unclear. Ang II increases SGLT2 expression in hypertensive rats, but it is not known if AT1R activation in PTC is responsible. Recently, pharmacological blockade of SGLT2 has been developed as a treatment strategy for DM which normalizes blood glucose levels by reducing glucose reabsorption. SGLT2 inhibition also leads to decreased blood pressure and renoprotective effects.22, 23, 24. These findings suggest a potential role for SGLT2 in the activation of the intrarenal RAS. However, it is not known if glucose entry via SGLT2 mediates high glucose-induced AGT expression in PTC.

Preliminary Studies
Immortalized mouse proximal tubule cells consists of cells originating from the early segment of the mouse proximal tubule.To demonstrate the effects of high glucose on AGT expression in this cell line, mPTCs were seeded on 6-well plates at a density of 5.0 x 105 cells per well. Before treatment in high glucose, the cells were rinsed with PBS and treated in 1.5 mL serum-free DMEM (5 mM glucose) for 8 hours. For high glucose treatment, serum-free Dulbecco Modified Eagle's Medium (DMEM) was supplemented with glucose to a final concentration of 5 mM (control), 15 mM, or 25 mM (n = 4). The cells were incubated at 37 ºC for 24 hours. After treatment, cell lysates were prepared. AGT protein was detected via western blot analysis.
The results of this experiment showed that our mPTC cell line exhibits a significant increase in AGT expression under high glucose conditions (15 mM and 25 mM glucose) compared to normal glucose (5 mM glucose).
To test the effects of high glucose on AGT mRNA, cells were cultured as previously described in serum-free DMEM with 5 mM or 25 mM glucose (n = 3) and incubated at 37 ºC.

After 24 hours, AGT mRNA was quantified via qRT-PCR. AGT mRNA relative to GAPDH mRNA was found to be significantly higher after treatment in 25 mM glucose.

The results of these preliminary experiments demonstrate that our mPTC line exhibits very low expression of AGT under normal glucose conditions (5 mM).

The impact of treatment with an SGLT2 inhibitor on intra –renal AGT production is unknown. Additionally, it is unclear what the impact of having significant glycosuria will have on AGT production. Finally, it is important to determine if there are differential effects on AGT production between SGLT2 inhibition and other methods of improving glycemic control, such as Sulfonylurea therapy

Proposed Studies

We propose to conduct a single-center randomized, double blind, cross over study of the effect of Dapagliflozin over 6 weeks, followed by placebo over 6 weeks on the other treatment allocation (those getting placebo first will cross over to Dapagliflozin and vice versa). Treatment will be stratified according to the underlying presence or absence of hypertension.

1. Type 2 diabetes with hypertension and on RAAS blocking drugs with stable blood pressure on therapy for at least 30 days; n= 20
2. Type 2 diabetes without hypertension and not on RAAS blocking drugs n=10

If we are unable to recruit 10 patients without hypertension we will increase the number with hypertension for a total of 30. Stratification by hypertension status will remain and is important in understanding the effect of SGLT2 inhibition in patients not on BP lowering drugs

In addition a Sulfonlyurea (SU) arm will also be included – 10 patients who are on metformin and other background therapy (with the exclusion of SGLT-2 inhibitor and sulfonylurea) will be recruited. This will be an open-labeled arm. Patients will assessed at baseline. They will then receive usual care for 6 weeks. At the end of 6 weeks, patients will then undergo another assessment before being provided SU for 6 weeks. At the end of 6 weeks, patients will undergo assessment again. The aim is to determine whether any effects seen with Dapagliflozin are specific to that drug or related simply to improved glycemic control.

Inclusion criteria;

As per the current approved indications for Dapagliflozin. In addition;

1. Type 2 diabetes with hypertension (history of hypertension in clinical records and on medication for management of hypertension) and on RAAS blocking drugs; or
2. Type 2 diabetes without hypertension and not on RAAS blocking drugs
3. Hemoglobin A1c between 7% - 10% inclusive
4. Background therapy for diabetes to include maximally tolerated dose of metformin.
5. For the dapagliflozin/placebo arm, in addition to metformin, sulfonylureas and/or insulin is allowed
6. Estimated glomerular filtration rate (eGFR) > = 60 ml/min
7. Between the ages of 18 -75
8. Capacity to understand and sign the informed consent.

Exclusion criteria:

1. Current use of Dapagliflozin or any SGLT2 inhibitor
2. History of intolerability to Dapagliflozin
3. Patients with current and past history of bladder cancer
4. Patients with unexplainable baseline hematuria
5. All current contraindications of Dapagliflozin as per the label
6. Severe hepatic insufficiency and/or significant abnormal liver function defined as aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN
7. Total bilirubin >2.0 mg/dL (34.2 µmol/L)
8. Estimated glomerular filtration rate (eGFR): <60 mL/min (calculated by Cockcroft-Gault formula)
9. Recent Cardiovascular Events in a patient:
10. Acute Coronary Syndrome (ACS) within 2 months prior to enrolment
11. Hospitalization for unstable angina or acute myocardial infarction within 2 months prior to enrolment
12. Acute Stroke or TIA within two months prior to enrolment
13. Less than two months post coronary artery revascularization
14. Congestive heart failure defined as New York Heart Association (NYHA) class IV, unstable or acute congestive heart failure. Note: eligible patients with congestive heart failure, especially those who are on diuretic therapy, should have careful monitoring of their volume status throughout the study.
15. Pregnant or breastfeeding patients
16. Patients who, in the judgment of the investigator, may be at risk for dehydration
17. Blood pressure: At enrolment: Systolic BP ≥165 mmHg and/or diastolic BP ≥110 mmHg
    a. At randomization: Systolic BP ≥160 mmHg and/or diastolic BP ≥100 mmHg
18. For patients in the dapagliflozin/placebo arm, use of SGLT-2 inhibitor class of drugs is an exclusion. Patients may continue the background therapy.
    a. For patients that will be included in the sulfonylurea/placebo arm – use of SLGT-2 inhibitor and sulfonylurea class of drugs in an exclusion. Patients can continue the rest of the background therapy.

Methods:

Once the subjects have been provided with the informed consent procedure and have signed the approved consent form and HIPAA form, the screening procedure will commence. Screening will include a completed medical history and list of current medications to determine eligibility for participation in this study. Details of the procedures are listed in the visit schedule section (see below).

Blinding and unblinding:

The research study participants will be blinded throughout the study in the dapagliflozin-placebo arm. Unblinding will be done only in case of emergency for patient safety. In this case, unblinding will be done by the sponsor or the research site as the randomization code will be with the sponsor.

The SU arm will be open label arm.

Storage of investigational product:

All study drugs should be kept in a secure place and under appropriate storage conditions. The Investigational Product label on the bottle specifies the appropriate storage

Accountability:

Storage of IP will be handled by the Investigational Pharmacist, who will maintain both accountability for the IP as well as maintenance of the IP, according to the manufacturer's requirements and recommendations for product safety.

Labeling of drug:
The IP and placebo will be labeled identically. The labels will be prepared according to Good Manufacturing Practice (GMP) and local regulatory guidelines. AZ will provide with prefilled bottles of Dapagliflozin 5 mg and the matching placebo. Labelling will be done by the sponsor.

Randomization:
The randomization code will be generated and provided by the sponsor to the site which the research site personnel will follow.

Dose and treatment regimens: After the subject has started the 5 mg dose of Dapagliflozin or the medication in test period 1, the subject will be called 1 week after initiation of study drug. If the subject is tolerating the medication, the dose will be increased to 2 tabs of placebo or 10mg of Dapagliflozin. If for some reason, the subject is not tolerating the medication the subject will continue on 5 mg daily, particularly if dizziness is present. For the sulfonylurea arm, titration of the sulfonylurea and the placebo will be at the principal investigator's discretion as per usual care. For all the patients, titration of the background medications that have the potential of causing hypoglycemia, titration of the background medications will be at the principal investigator's discretion. The purpose of titration will be to prevent hypoglycemia while the patient is in the study.

Discontinuation of IP:
The investigational drug will be discontinued according to the following criteria:
Discontinuation procedures
Patients with a central laboratory ALT and/or AST >3x ULN will be scheduled for a follow-up visit within 3 days following the receipt of the result. Patients will be discontinued from study if the initial and repeat laboratory tests meet any of the following criteria:
1. ALT and/or AST are >3 x ULN and TB >2 x ULN
2. ALT and/or AST are >5 x ULN for ≥ 14 consecutive days, at any time after initial confirmatory results
3. ALT and /or AST are > 8 x ULN
4. Study drug will be discontinued when eGFR is persistently <60 ml/min/1.73m^2. Clinical judgement by the Principal Investigator will be used to determine if the observed decrease in eGFR < 60 ml/min/1.73m^2 warrants discontinuation after the eGFR is repeated after the initial decrease in eGFR is noted.
5. Pregnancy confirmed by a positive pregnancy test or otherwise verified

Sample size calculation and statistical analysis:
This is a pilot study. Data from this study can be used to derive the sample size for an adequately powered study. Exploratory data analysis: All data will be cleaned and logically checked.
Exploratory analysis will be conducted to examine the distributions of each variable by means of plots (bar charts, box plots, and normal probability plots) and goodness-of-fit test statistics. Means and percentages of baseline variables will be compared between the groups. Possible outliers will be detected. Data transformation will be used to normalize the distribution of continuous variables and to improve linearity in the linear regression analyses if needed.

Comparisons of continuous variables between groups will be performed using t-tests. Chi-square tests will be employed for comparisons between categorical data. Linear regression models will be fit to examine the association between a continuous dependent variables and possible independent co-variables. Data analyses will be performed using SAS version 9 (SAS Institute Inc., SAS/STAT User's Guide, Version 9. Cary, NC: SAS Institute Inc. 2007).

Patient Safety:

A copy of the MedWatch/AdEERs report will be faxed to AstraZeneca at the time the event is reported to the FDA. It will be the responsibility of the investigator to compile all necessary information and ensure that the FDA receives a report according to the FDA reporting requirement timelines and to ensure that these reports are also submitted to AstraZeneca at the same time. When reporting to AstraZeneca, a cover page will accompany the MedWatch/AdEERs form indicating the following:

- Investigator Sponsored Study (ISS)
- The investigator IND number assigned by the FDA
- The investigator's name and address
- The trial name/title and AstraZeneca ISS reference number

The collection of AEs should start after the signing of the informed consent. Investigative site will also indicate, either in the SAE report or the cover page, the causality of events in relation to all study medications and if the SAE is related to disease progression, as determined by the principal investigator. The site research personnel will send SAE report and accompanying cover page by way to AstraZeneca: AEMailboxClinicalTrialTCS@astrazeneca.com or fax number is 302-886-4114.

Serious adverse events that do not require expedited reporting to the FDA will be reported to AstraZeneca preferably using the MedDRA coding language for serious adverse events. In the case of blinded trials, AstraZeneca will request that the Sponsor either provide a copy of the randomization code/ code break information or unblind those SAEs which require expedited reporting. All SAEs have to be reported to AstraZeneca, whether or not considered causally related to the investigational product. All SAEs will be documented. The investigator is responsible for informing the IRB and/or the Regulatory Authority of the SAE as per local requirements.

Visit Schedule:

Following is the schedule of the study visits:

1. **Visit 1(screening visit)**: Screening and informed consent will be conducted during this visit. If subjects meet the inclusion criteria they will come back within 4 weeks for randomization. If the required laboratory tests to establish whether the subject meets the inclusion criteria are available and have been drawn within the past ninety days, no laboratory tests will be required at this visit. However, if not, laboratory tests will be drawn at this visit for the purpose of establishing that the subject meets the criteria for inclusion in the study. The patient will be asked to keep a log of the blood sugars while participating in the study.

2. **Visit 2 (baseline visit)**: During this fasting baseline visit, subject will undergo history and physical, blood draw, urine sample, EndoPAT test and given a bottle for 24 hour urine collection. Subjects will

be given the monitor for 24 hr. BP monitoring. The patient will return the monitor along with a 24 hour urine sample

If however during the screening visit, the laboratory results are available for review and the patient qualifies for the study, the screening and the baseline visits will be combined.

3. **Visit 3 (randomization visit)** - subject will return the ABPM monitor and urine bottle and will be randomized to either dapagliflozin or placebo in a double-blind fashion. The first arm of the study will be labeled test period 1 and the second arm of the study will be labeled test period 2. All subjects will be educated on the recognition and management of hypoglycemia at this visit.

4. **Test period 1:** Test period 1 will start once the subject has taken the first dose of the drug to which the subject was randomized. Test period 1 will continue for 6 weeks.

5. **Visit 4 (Telephone visit)**. After the subject has started the 5 mg dose of the medication in test period 1, the subject will be called 1 week after initiation of study drug. If the subject is tolerating the medication, the dose will be increased to 2 tabs (10mg). If for some reason, the subject is not tolerating the medication the subject will continue on 5 mg daily, particularly if dizziness is present. The patients will also be asked if they have had any low blood sugars and what time the low blood sugars were. The blood sugars will be relayed to the PI in case there is any hypoglycemia. The PI will then make the necessary changes to the patient's background therapy to prevent further hypoglycemia.

6. **Visit 5 (telephone visit):** Visit 5 will be a telephone visit that will occur 2 weeks after the visit 4 telephone visit. The purpose of the telephone visit is to ensure that the subject is tolerating the medication. This is particularly important if the dose had been increased to the maximum dose of 2 tablets during visit 4. The patients will also be asked if they have had any low blood sugars and what time the low blood sugars were. The blood sugars will be relayed to the PI in case there is any hypoglycemia. The PI will then make the necessary changes to the patient's background therapy to prevent further hypoglycemia.

7. **Visit 6 (cross over visit)** –Visit 6 will occur 6 weeks +/- 7 days after randomization. The subject will come in fasting for this visit. During this visit, subject will undergo a brief physical examination. Blood and urine sample will be collected and the EndoPAT procedure will be conducted. Subject will take the last dose of the first lot of medication on the day of visit 6. Subject will start the24 hr. AMBP and 24 hour urine collection on the day of visit 6 after taking the last dose of the medication for test period 1.

8. **Visit 7**: Next day. Return AMBP monitor and then start the washout period which will last at least 14-28 days.

9. **Test period 2:** Test period 2 will start with visit 8

10. **Visit 8**: After the washout period of 14-28 days, the subject will come to the research suite in a fasting state and undergo blood and urine sample collection. Patient will receive the second medication and enter test period 2. The subject will start the new lot of medication- dapagliflozin at 5 mg (1 tablet) or placebo. During this visit, the subject will undergo EndoPAT procedure and will be started on ABPM.

11. **Visit 9 (the day after visit 8):** The subject will return the ABPM.

12. **Visit 10 (telephone visit)**: After the subject has started the medication in test period 2, the subject will be called 1 week later. If the subject is tolerating the medication, the dose will be increased to 2 tablets daily (10 mg). If for some reason, the subject is not tolerating the medication the subject will continue on 5 mg daily, particularly if dizziness is present. The patients will also be asked if they have had any low blood sugars and what time the low blood sugars were. The blood sugars will be relayed to the PI in case there is any hypoglycemia. The PI will then make the necessary changes to the patient's background therapy to prevent further hypoglycemia.

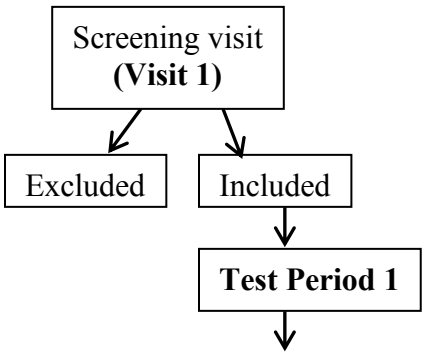
13. **Visit 11 (telephone visit)**: will be a telephone visit that will occur 2 weeks after visit 10 which is also a telephone visit. The purpose of the telephone visit is to ensure that the subject is tolerating the medication. This is particularly important if the dose had been increased to the maximum dose of 2 tablets (10 mg) during visit 7. The patients will also be asked if they have had any low blood sugars and what time the low blood sugars were. The blood sugars will be relayed to the PI in case there is any hypoglycemia. The PI will then make the necessary changes to the patient's background therapy to prevent further hypoglycemia.

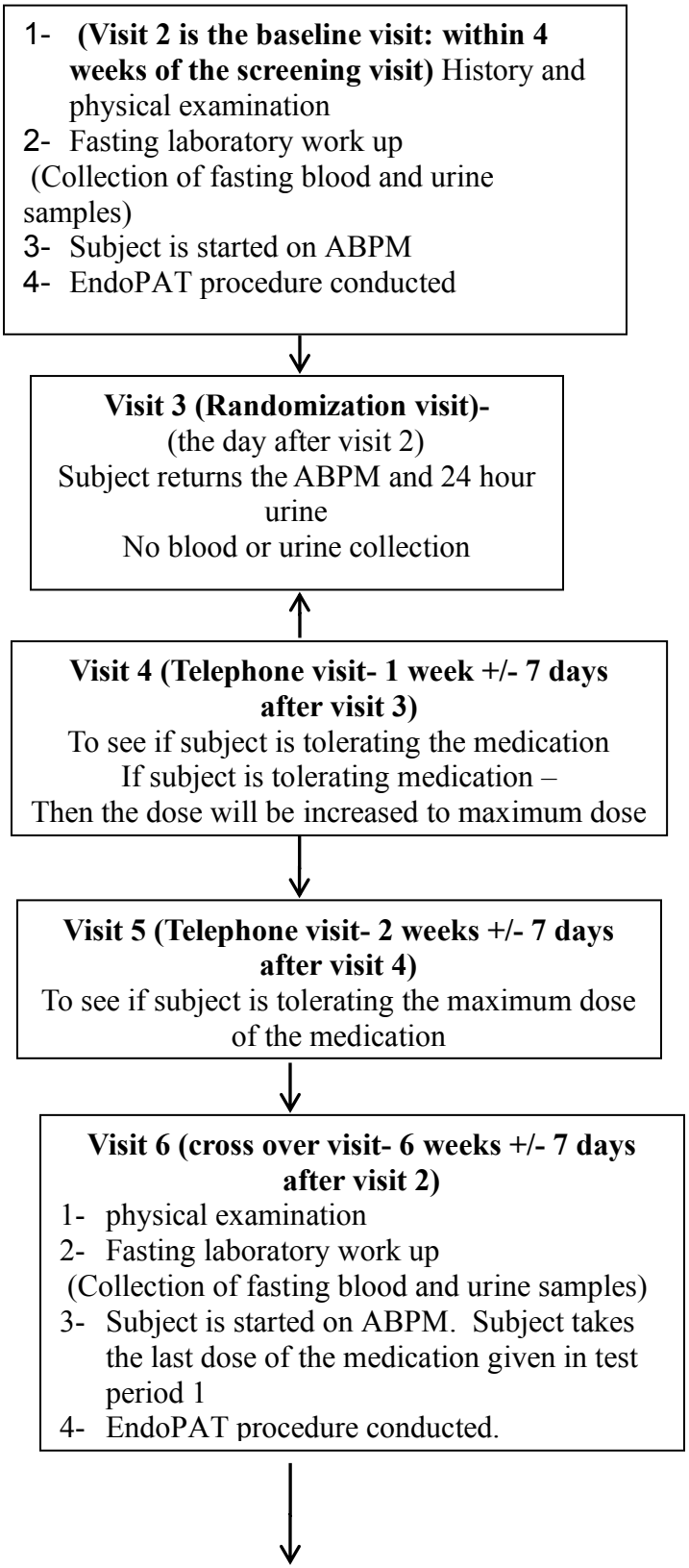
14. **Visit 12; (end of treatment visit)**: This visit will conclude test period 2 six weeks after the washout period. During this fasting visit, a brief physical examination will be done, blood and urine samples will be collected, and the EndoPAT procedure will be conducted. Subjects will take the last dose of the second lot of medication on the day of visit 11. Subject will start the 24 hr. AMBP and urine collection after taking the last dose of the medication for test period 2.

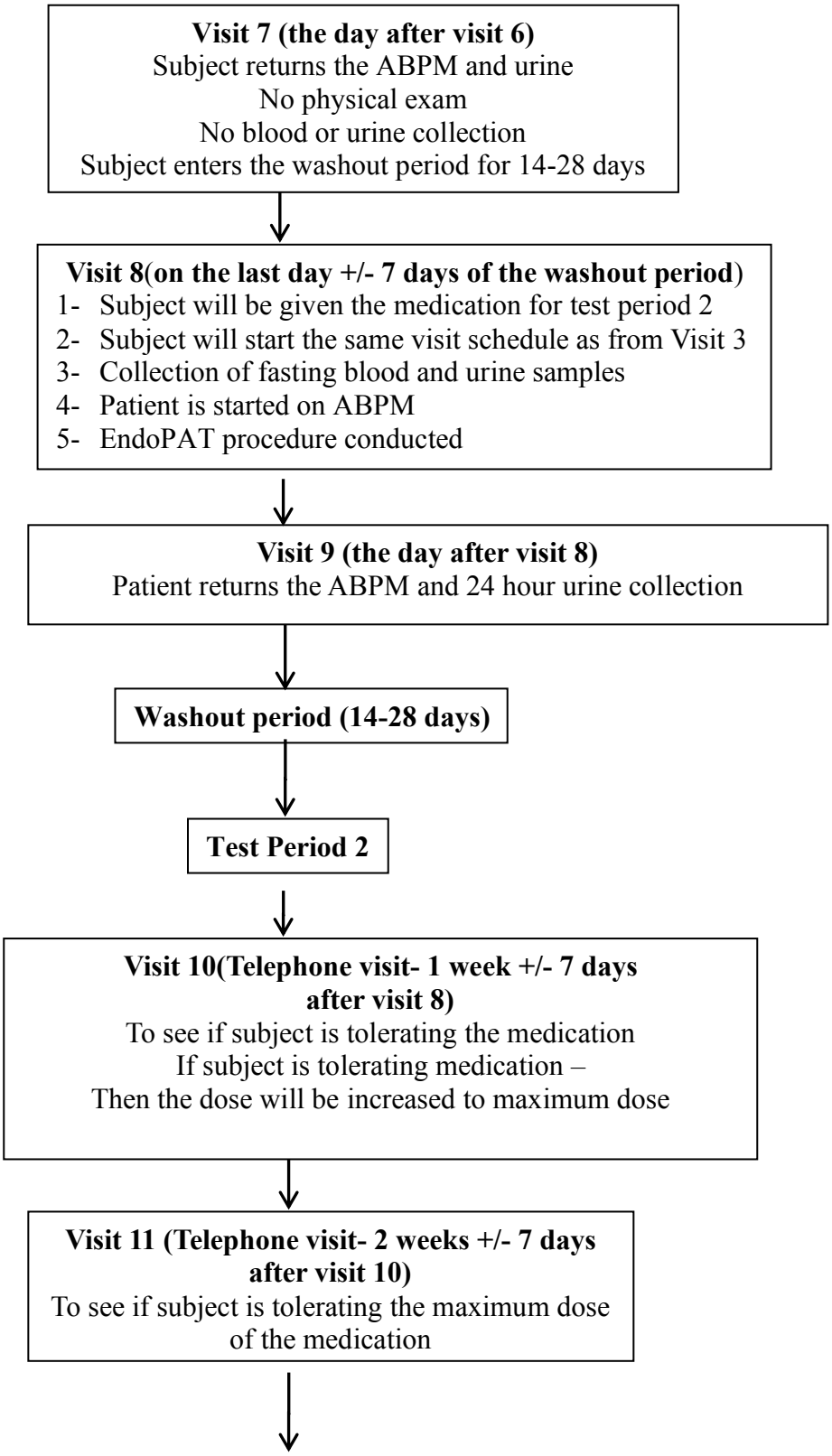
15. **Visit 13**: The day after visit 12, the subject will return the AMBP monitor. This concludes the study.

NOTE: In order to give patients flexibility all visit will have a window of +/ - 7 days for visits
Study Flow chart:

A: For the study comparing Dapagliflozin and placebo:




1- (Visit 2 is the baseline visit: within 4 weeks of the screening visit) History and physical examination
2- Fasting laboratory work up
(Collection of fasting blood and urine samples)
3- Subject is started on ABPM
4- EndoPAT procedure conducted
Visit 3 (Randomization visit)-
(the day after visit 2)
Subject returns the ABPM and 24 hour urine
No blood or urine collection
Visit 4 (Telephone visit- 1 week +/- 7 days after visit 3)
To see if subject is tolerating the medication
If subject is tolerating medication –
Then the dose will be increased to maximum dose
Visit 5 (Telephone visit- 2 weeks +/- 7 days after visit 4)
To see if subject is tolerating the maximum dose of the medication
Visit 6 (cross over visit- 6 weeks +/- 7 days after visit 2)
1- physical examination
2- Fasting laboratory work up
(Collection of fasting blood and urine samples)
3- Subject is started on ABPM. Subject takes the last dose of the medication given in test period 1
4- EndoPAT procedure conducted.


Visit 7 (the day after visit 6)
Subject returns the ABPM and urine
No physical exam
No blood or urine collection
Subject enters the washout period for 14-28 days
Visit 8(on the last day +/- 7 days of the washout period)
1- Subject will be given the medication for test period 2
2- Subject will start the same visit schedule as from Visit 3
3- Collection of fasting blood and urine samples
4- Patient is started on ABPM
5- EndoPAT procedure conducted
Visit 9 (the day after visit 8)
Patient returns the ABPM and 24 hour urine collection
Washout period (14-28 days)
Test Period 2
Visit 10(Telephone visit- 1 week +/- 7 days after visit 8)
To see if subject is tolerating the medication
If subject is tolerating medication –
Then the dose will be increased to maximum dose
Visit 11 (Telephone visit- 2 weeks +/- 7 days after visit 10)
To see if subject is tolerating the maximum dose of the medication

Visit 12 (cross over visit- 6 weeks +/- 7 days after visit 8)

5- physical examination
6- Fasting laboratory work up
(Collection of fasting blood and urine samples)
7- Subject is started on ABPM. Subject takes the last dose of the medication given in test period 2
8- EndoPAT procedure conducted.

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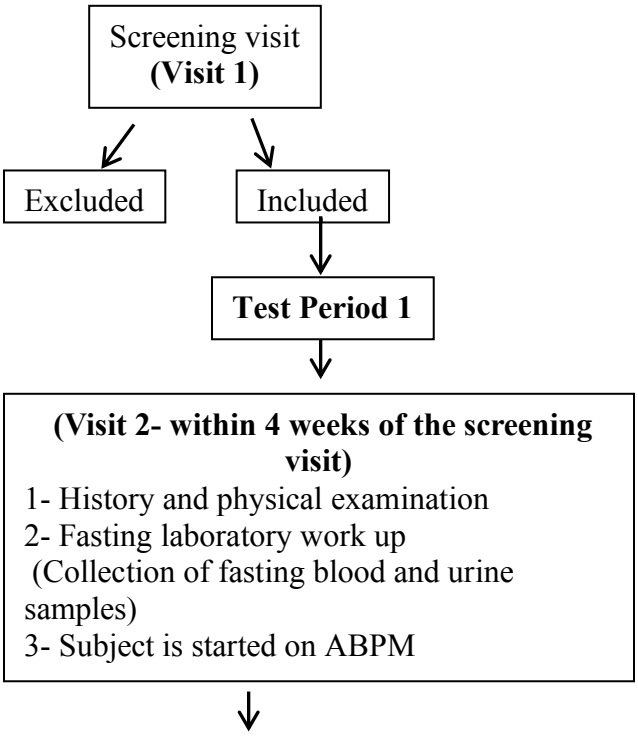
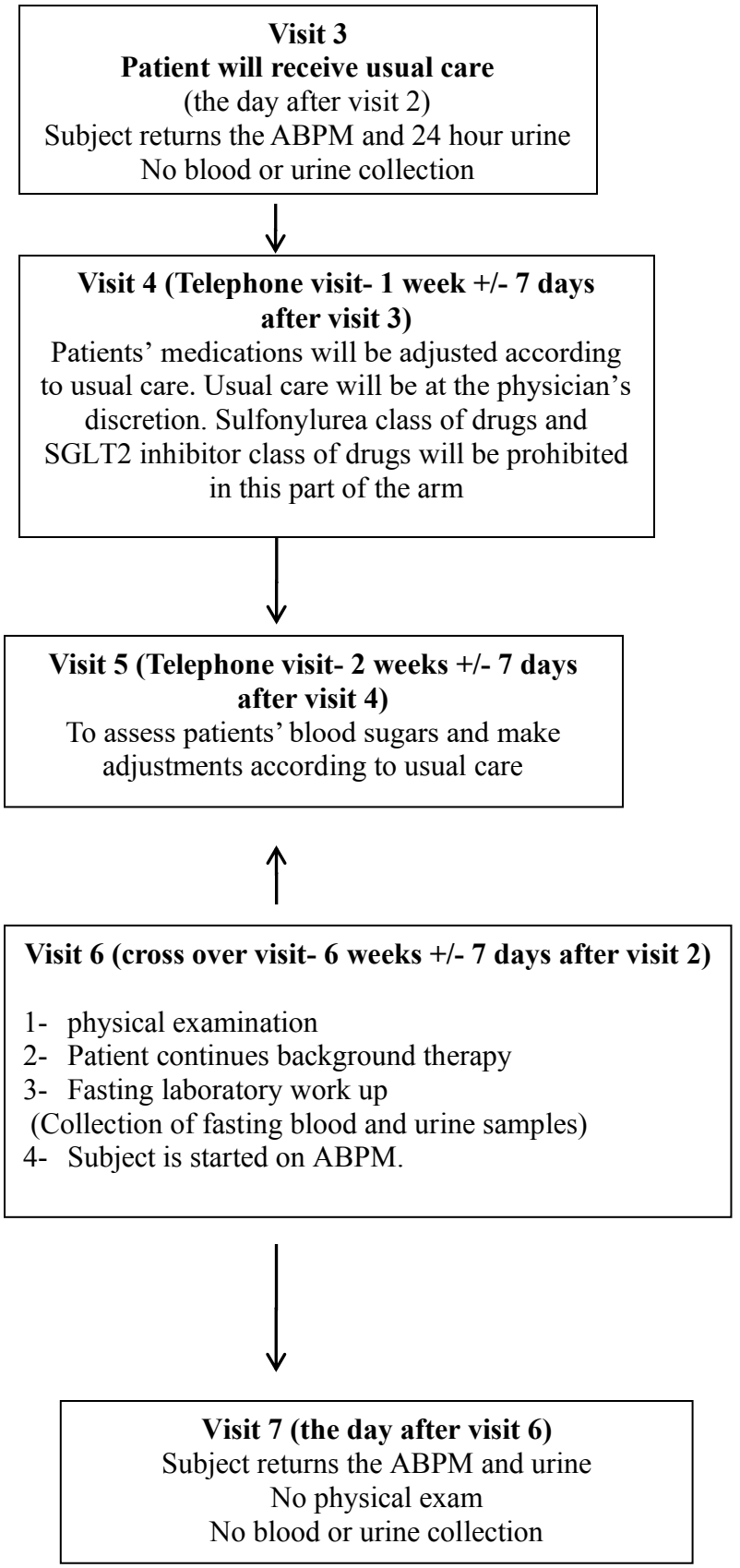
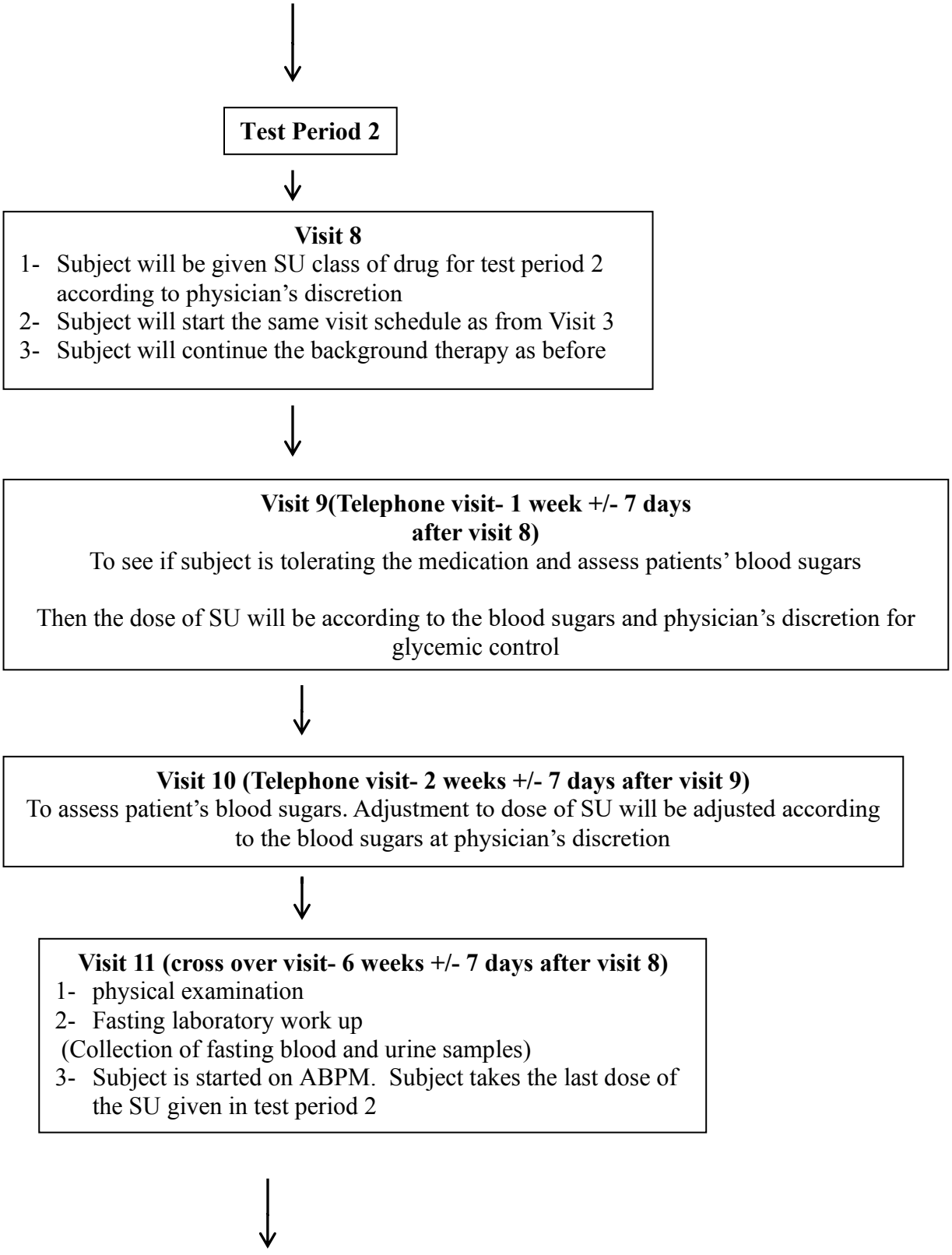
Visit 13 (the day after visit 12)

Subject returns the ABPM and 24 hour urine collection
No physical exam
No blood or urine collection

Protocol for the sulfonylurea arm:




Visit 3
Patient will receive usual care
(the day after visit 2)
Subject returns the ABPM and 24 hour urine
No blood or urine collection
Visit 4 (Telephone visit- 1 week +/- 7 days after visit 3)
Patients' medications will be adjusted according to usual care. Usual care will be at the physician's discretion. Sulfonylurea class of drugs and SGLT2 inhibitor class of drugs will be prohibited in this part of the arm
Visit 5 (Telephone visit- 2 weeks +/- 7 days after visit 4)
To assess patients' blood sugars and make adjustments according to usual care
Visit 6 (cross over visit- 6 weeks +/- 7 days after visit 2)
1- physical examination
2- Patient continues background therapy
3- Fasting laboratory work up
(Collection of fasting blood and urine samples)
4- Subject is started on ABPM.
Visit 7 (the day after visit 6)
Subject returns the ABPM and urine
No physical exam
No blood or urine collection


Test Period 2
Visit 8
1- Subject will be given SU class of drug for test period 2 according to physician's discretion
2- Subject will start the same visit schedule as from Visit 3
3- Subject will continue the background therapy as before
Visit 9(Telephone visit- 1 week +/- 7 days after visit 8)
To see if subject is tolerating the medication and assess patients' blood sugars
Then the dose of SU will be according to the blood sugars and physician's discretion for glycemic control
Visit 10 (Telephone visit- 2 weeks +/- 7 days after visit 9)
To assess patient's blood sugars. Adjustment to dose of SU will be adjusted according to the blood sugars at physician's discretion
Visit 11 (cross over visit- 6 weeks +/- 7 days after visit 8)
1- physical examination
2- Fasting laboratory work up
(Collection of fasting blood and urine samples)
3- Subject is started on ABPM. Subject takes the last dose of the SU given in test period 2

Visit 12 (the day after visit 11)

Subject returns the ABPM and 24 hour urine collection
No physical exam
No blood or urine collection

Measurements:

1- Blood pressure will be measured as per clinical practice and we will also do a 24 hr. ambulatory blood pressure monitoring before and after 6 weeks of treatment in the Dapagliflozin/ placebo arm as well as before and 6 weeks after treatment in the Sulfonylurea/placebo arm
2- We will also measure vascular compliance/ endothelial function using the EndoPAT test at the same visits as the ABPM only in the Dapagliflozin/placebo arm.
3- Spot and 24 hour urine will be collected. Urine samples will be obtained, before and after treatment. After appropriate processing, the samples will be quickly stored at -20°C, and analyzed for concentrations of AGT, albumin, and creatinine within one week of collection as previously described. The urinary AGT concentrations and urinary albumin concentrations will be normalized by urinary creatinine concentrations. Urine creatinine will also be measured and urine AGT/ creatinine calculated to correct the AGT for volume of excretion. Plasma K, renin and AGT will be measured at these visits.
4- Fasting plasma glucose will be measured for the Dapagliflozin/placebo and sulfonylurea arm at the following time points: a) at baseline and end of period 1 and b) at baseline and end of period 2.

Blood and urine will be stored at -70°C, for future testing of other biomarkers of renal and cardiovascular disease.

The table below summarizes the timings of the measurements:

Measurements	Timings
ABPM and 24 hour urine collection	<u>Dapagliflozin/Placebo arm</u> - Before randomization visit (in test period 1) - At the end of test period 1 (before entering washout period) - At the beginning of test period 2 - At the end of test period 2 (at the end of the study)
	<u>Sulfonylurea arm</u> - Before usual care arm visit (ie visit 2 in test period 1)

	- At the end of test period 1 - At the end of test period 2 (at the end of the study)
Fasting glucose, blood and urine samples	<u>Dapagliflozin/placebo</u> - At the beginning of test period 1 - Before the end of test period 1 - At the beginning of test period 2 - At the beginning of test period 2
	<u>Sulfonylurea arm</u> - Before the usual care arm visit (in test period 1) - At the end of test period 1 - At the end of test period 2 (at the end of the study)
EndoPAT Procedure	- <u>Dapagliflozin/placebo arm only</u> - Before randomization visit (in test period 1) - At the end of test period 1 - At the beginning of test period 2 - At the end of test period 2

The **primary end point** will be reduction in urine AGT comparing the AGT before and after treatment using a paired ANOVA Test.

Exploratory endpoints will be:

1. Examine the relationship between change in plasma K and change in urine AGT
2. Examine the relationship between change in BP and change in endothelial function/vascular compliance using the EndoPAT procedure.

Depending on results of this pilot study we will plan further definitive studies of the effect of SGLT2 inhibition on urine AGT, in a study with appropriate statistical power.

Forecasted key milestones dependent on IRB/IEC approval and contracting

1. Research Agreement executed Feb 28, 2016
2. Projected IRB/IEC approval Feb 28, 2016
3. First Subject In Apr 29, 2016
4. Last Subject In (100% enrollment) Apr 30, 2017
5. Last Subject Last Visit (Treatment end) Jun 30, 2017
6. Publication Jan 30, 2018

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Budget

We will need support in the form of

1. Study drug and placebo pills
2. Funding –

Appendix A: Identity of investigational product(s)

Investigational Product	Manufacturer	IMP/ NIMP	Type	Route of administration	Formulation	Comment
Dapagliflozin 5 mg	AstraZeneca	IMP	Active Drug	Oral	Film Coated Tablet	Green, plain, diamond shaped
Matching placebo for Dapagliflozin 5 mg	AstraZeneca	IMP	Placebo	Oral	Film Coated Tablet	Green, plain, diamond shaped

AstraZeneca will provide the Dapagliflozin and matching placebo tablets. Dapagliflozin and matching placebo tablets will be supplied in bottles, each containing 35 tablets. The tablets contain lactose, which may cause discomfort in lactose-intolerant individuals.