An Investigation Into The Anti-hypertensive And Potential Anti-inflammatory Actions Of Dapagliflozin

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Complete Research Protocol (HRP-503)

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Template Instructions

Sections that do not apply:

- In several sections, the addition of checkboxes for **Not Applicable** have been added to the template as responses.
    - If an N/A checkbox is present, select the appropriate justification from the list.
    - If an N/A checkbox is not present, or if none of the existing checkboxes apply to your study, you must write in your own justification.
- In addition:
    - For research where the only study procedures are records/chart review: Sections 19, 20, 22, 23, 24, 25, 31, and 32 do not apply.
    - For exempt research: Sections 31 and 32 do not apply.

Studies with multiple participant groups:

- If this study involves multiple participant groups (e.g. parents and children), provide information in applicable sections for each participant group. Clearly label responses when they differ. For example:

  Response:

  Intervention Group:

  Control Group:

Formatting:

- Do not remove template instructions or section headings when they do not apply to your study.

If you are pasting information from other documents using the "Merge Formatting" Paste option will maintain the formatting of the response boxes.

Amendments:

- When making modifications or revisions to this and other documents, use the **Track Changes** function in Microsoft Word.
- Update the version date or number **on Page 3.**

PROTOCOL TITLE:

Include the full protocol title.

Response: **An Investigation Into The Anti-hypertensive And Potential Anti-inflammatory Actions Of Dapagliflozin**

PRINCIPAL INVESTIGATOR:

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Telephone Number
Email Address

Response: Paresh Dandona, M.B.B.S., Ph.D., F.R.C.P, F.A.C.P, F.A.C.C
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Co-Investigators: Husam Ghanim, PhD
Manav Batra, MD

VERSION:

Include the version date or number.

Response: 10/2/2018

GRANT APPLICABILITY:

Indicate whether this protocol is funded by a grant (e.g. NIH, foundation grant). For a grant with multiple aims, indicate which aims are covered by this research proposal.

NOTE: This question does not apply to studies funded by a sponsor contract.

Include a copy of the grant proposal with your submission.

Response:

Funded by Astra Zeneca Inc.

RESEARCH REPOSITORY:

Indicate where the research files will be kept, including when the study has been closed. The repository should include, at minimum, copies of IRB correspondence (approval, determination letters) as well as signed consent documents. This documentation should be maintained for 3 years after the study has been closed.

Response: Diabetes Endocrinology Center of WNY

Location: 1000 Youngs Road, Suite 105, Williamsville NY 14221

Department: Diabetes Endocrinology & Metabolism

1.0 Objectives

1.1 Describe the purpose, specific aims, or objectives of this research.

Response: To study the effects of liraglutide (a drug used to treat type 2 diabetes) on glucose (sugar) control over the 26 week study period when used in addition to insulin. The use of Liraglutide in Type 1 Diabetes is investigational

1.2 Describe the purpose, specific aims, or objectives.

Response:

This is a single center, prospective, randomized, placebo –controlled, parallel design and double blind study to investigate The Anti-hypertensive And Potential Anti-inflammatory Actions Of Dapagliflozin in type 2 diabetics.

Study Aims

Aim 1.1: To compare reactive oxygen species (ROS) generation by MNC, protein expression of $p47^{phox}$ subunit of NADPH oxidase, in MNC's prior to and following 12 weeks of dapagliflozin or placebo.

Aim 1.2: To compare intranuclear NFκB binding and the expression of JNK-1, TLR-4, SOCS-3, IL-1β, and TNFα in MNC before and following 12 weeks of treatment with dapagliflozin or placebo.

Aim 1.3: To compare plasma hs-CRP, endotoxin and IL-6 concentrations and oxidized lipids in plasma (TBARS) and in urine (F_2-isoprostane) of obese type 2 diabetic patients prior to and following 12 weeks of dapagliflozin or placebo.

Aim 1.4: To compare postprandial (after a High fat and high carbohydrates meal) changes in ROS generation, NFκB binding in MNC and plasma LPS prior to and following 12 weeks of dapagliflozin and placebo.

Aim 2: to study the effect of single dose of dapagliflozin or placebo on oxidative stress and inflammatory mediators listed in Aim 1.1, 1.2 and 1.3 in MNC from obese type 2 diabetic patients.

Aim 3.1: To evaluate effects of treatment with dapagliflozin for 12 weeks on blood pressure and the requirement of anti-hypertensive medication.

Aim 3.2: To compare the in plasma concentrations of cGMP, cAMP, ANP, BNP, and a reduction in plasma concentrations of angiotensinogen, renin and angiotensin II before and following 12 weeks of dapagliflozin or placebo.

1.3 State the hypotheses to be tested, if applicable.

NOTE: A hypothesis is a specific, testable prediction about what you expect to happen in your study that corresponds with your above listed objectives.

Response:

Hypothesis 1: Dapagliflozin treatment suppresses basal and meal induced oxidative and inflammatory stress in mononuclear cells (MNC) of patients with type 2 diabetes.

Hypothesis 2: Dapagliflozin **acutely** suppresses oxidative and inflammatory stress in MNC of patients with type 2 diabetes

Hypothesis 3: Treatment of patients with type 2 diabetes with dapagliflozin will reduce blood pressure and increase vasodilators and suppress vasoconstrictors concentrations.

2.0 Scientific Endpoints

2.1 Describe the scientific endpoint(s), the main result or occurrence under study.

NOTE: Scientific endpoints are outcomes defined before the study begins to determine whether the objectives of the study have been met and to draw conclusions from the data. Include primary and secondary endpoints. Some example endpoints are: reduction of symptoms, improvement in quality of life, or survival. Your response should **not** be a date.

Response: The primary endpoint of the study is to detect a significant difference in the percent change in fasting NFκB activation (DNA binding activity) in MNC before and after dapagliflozin use (0 week vs. 12 weeks) as compared to placebo. Secondary endpoints include comparing changes in expression of $p47^{phox}$, SOCS-3, IL-1β, JNK-1, TLR4; and plasma concentrations of LPS between dapagliflozin and placebo groups. In addition, plasma concentrations of TNF-α, IL-6, IL-1β, CRP, ANP, b-NP, cGMP, cAMP, angiotensinogen, renin and angiotensin II will also be measured at 0, 6 and 12 weeks following dapagliflozin and changes will be compared to that in the placebo group.

3.0 Background

3.1 Provide the scientific or scholarly background, rationale, and significance of the research based on the existing literature and how it will contribute to existing knowledge. Describe any gaps in current knowledge. Include relevant preliminary findings or prior research by the investigator.

Response: Dapagliflozin is an inhibitor of SGLT-2 mediated glucose transport. This action inhibits the reabsorption of glucose from proximal convoluted tubules and hence induces glucosuria. This action has recently been utilized in the treatment of diabetes since with the reduction of renal threshold for glucosuria following the inhibition of SGLT-2, glucose concentration falls. This action is independent of insulin and β-cell function and thus, may potentially be of use in both type 2 and type 1 diabetes.

Since glucose and hyperglycemia induce oxidative and inflammatory stress (1,2), it is likely that dapagliflozin will reduce oxidative and inflammatory stress. Since oxidative and inflammatory stresses are the basic mechanisms underlying atherosclerosis, a potential anti-oxidative and inflammatory action would be anti-atherogenic. Furthermore, dapagliflozin is also known to

reduce blood pressure (3,4), the mechanism underlying which is not known. An anti-hypertensive action is also anti-atherogenic. Since dapagliflozin is currently licensed for use in type 2 diabetes and since this condition has a markedly increased risk of atherosclerosis and cardiovascular disease, it is important that the potential anti-oxidative and anti-inflammatory effects of dapagliflozin be investigated.

It is also important that the mechanisms underlying the blood pressure lowering effect be investigated. Our recent work has shown that blood pressure lowering effect of GLP-1 receptor agonists, which was first described by us (5), is associated with an increase in plasma concentrations of known vasodilators, ANP, cGMP and cAMP; in addition, there is a reduction in plasma concentrations of angiotensinogen, renin and angiotensin II. Although the blood pressure lowering effect of SGLT-2 inhibitors is attributed to osmotic diuresis secondary to glucosuria and hypovolemia, it is possible that other mechanisms, as enumerated above, are involved.

These data will provide us with novel information on the potential anti-inflammatory effects of dapagliflozin; in addition, they will also provide information on post prandial glycemia, insulinogenesis and incretin secretion. Finally, it may provide us with information on the mechanisms underlying the anti-hypertensive effect of this drug

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3.2 Include complete citations or references.

Response:

1. Mohanty, P., Hamouda, W., Garg, R., Aljada, A., Ghanim, H., and Dandona, P. 2000. Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. J Clin Endocrinol Metab 85:2970-2973.

2. Aljada, A., Friedman, J., Ghanim, H., Mohanty, P., Hofmeyer, D., Chaudhuri, A., and Dandona, P. 2006. Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumor necrosis factor alpha messenger RNA by mononuclear cells in healthy human subjects. Metabolism 55:1177-1185.

3. Nauck, M.A., Del Prato, S., Duran-Garcia, S., Rohwedder, K., Langkilde, A.M., Sugg, J., and Parikh, S.J. 2014. Durability of glycaemic efficacy over 2 years with dapagliflozin versus glipizide as add-on therapies in patients whose type 2 diabetes mellitus is inadequately controlled with metformin. Diabetes Obes Metab.

4. Lambers Heerspink, H.J., de Zeeuw, D., Wie, L., Leslie, B., and List, J. 2013. Dapagliflozin a glucose-regulating drug with diuretic properties in subjects with type 2 diabetes. Diabetes Obes Metab 15:853-862.

5. Viswanathan, P., Chaudhuri, A., Bhatia, R., Al-Atrash, F., Mohanty, P., and Dandona, P. 2007. Exenatide therapy in obese patients with type 2 diabetes mellitus treated with insulin. Endocr Pract 13:444-450.

6. Chaudhuri, A., Ghanim, H., Vora, M., Sia, C.L., Korzeniewski, K., Dhindsa, S., Makdissi, A., and Dandona, P. 2012. Exenatide exerts a potent antiinflammatory effect. J Clin Endocrinol Metab 97:198-207.

7. Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Assian, E., and Ahmad, S. 2001. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J Clin Endocrinol Metab 86:3257-3265.

8. Ghanim, H., Garg, R., Aljada, A., Mohanty, P., Kumbkarni, Y., Assian, E., Hamouda, W., and Dandona, P. 2001. Suppression of nuclear factor-kappaB and stimulation of inhibitor kappaB by troglitazone: evidence for an anti-inflammatory effect and a potential antiatherosclerotic effect in the obese. J Clin Endocrinol Metab 86:1306-1312.

9. Makdissi, A., Ghanim, H., Vora, M., Green, K., Abuaysheh, S., Chaudhuri, A., Dhindsa, S., and Dandona, P. 2012. Sitagliptin exerts an antinflammatory action. J Clin Endocrinol Metab 97:3333-3341.

4.0 Study Design

4.1 Describe and explain the study design (e.g. case-control, cross-sectional, ethnographic, experimental, interventional, longitudinal, observational).

Response: This is a single center, prospective, randomized, placebo –controlled, parallel design and double blind study. The study will be conducted at the Diabetes – Endocrinology Center of Western New York under the direction of Dr. Paresh Dandona, M.D.

Two groups of 26 patients each (total 52 patients) with type 2 diabetes on oral agents will be included in the study. One group will be randomized to dapagliflozin (a dose of 5 mg daily will be titrated to 10 mg daily during the first week) while the other will be placebo. The patients will be treated for 12 weeks. Randomization will take in consideration even distribution of patients in regards to background medications (statins, ACE inhibitors, TZDs and ARBs). Only half the patients (equal numbers in both groups) will be tested for the secondary endpoints related to postprandial and single dose induced changes.

5.0 Local Number of Subjects

5.1 Indicate the total number of subjects that will be enrolled or records that will be reviewed locally.

Response: 52

5.2 If applicable, indicate how many subjects you expect to screen to reach your target sample (i.e. your screen failure rate).

Response: 65-75. All screened and qualified patients will be enrolled and randomized up to 52 enrolled patients.

5.3 Justify the feasibility of recruiting the proposed number of eligible subjects within the anticipated recruitment period. For example, how many potential subjects do you have access to? What percentage of those potential subjects do you need to recruit?

Response: The Diabetes and Endocrinology Center of WNY is the largest Diabetes center in the WNY area, seeing between 60 and 100 type 2 diabetic patients every month. Therefore, majority of recruited patients are our clinic patients. We do recruit a few patients through advertisement and researchmatch.org. These sources will suffice to recruit the needed number to subjects.

6.0 Inclusion and Exclusion Criteria

6.1 Describe the criteria that define who will be **included** in your final study sample.

NOTE: This may be done in bullet point fashion.

Response:

- Age 20-80 years inclusive.
- Type 2 diabetes
- BMI ≥30 kg/m^2
- Subjects on statins, ACE inhibitors, ARBs, diuretics, DPP-IV inhibitors, thiazolidenediones and antioxidants will be allowed as long as they are on stable doses (same dose for last 3 months) of these compounds and the dosage in not changed during the course of study. Patients will be evenly distributed between the 2 groups based on statins, ARBs, TZDs and ACE inhibitors use.
- HbA1c ≤ 8.0%

6.2 Describe the criteria that define who will be **excluded** from your final study sample.

NOTE: This may be done in bullet point fashion.

Response:

- Use of GLP-1 agonists or SGLT-2 inhibitors therapy in the last 3 months.
- Risk for pancreatitis, i.e., history of gallstones, alcohol abuse, and hypertriglyceridemia.
- Coronary event or procedure (myocardial infarction, unstable angina, coronary artery bypass, surgery or coronary angioplasty) in the previous 3 months.
- Hepatic disease: Severe hepatic insufficiency and/or significant abnormal liver function defined as:
  1. aspartate aminotransferase (AST) >3x upper limit of normal (ULN) and/or alanine aminotransferase (ALT) >3x ULN
  2. Total bilirubin >2.0 mg/dL (34.2 µmol/L)
  3. Positive serologic evidence of current infectious liver disease including Hepatitis B viral antibody IGM, Hepatitis B surface antigen and Hepatitis C virus antibody
- (liver function tests more than 3 times the upper limit of normal)
- Renal impairment (serum eGFR <60 ml/min)
- Any other life-threatening, non-cardiac disease
- Uncontrolled hypertension (BP > 160/100 mm of Hg)
- Congestive Heart Failure class III or IV.
- Use of an investigational agent or therapeutic regimen within 30 days of study
- Participation in any other concurrent clinical trial
- pregnant or breastfeeding patients

- Volume depleted patients. Patients at risk for volume depletion due to co-existing conditions.
- Those with a history of bladder cancer, Diabetic Ketoacidosis (DKA)

6.3 Indicate specifically whether you will include any of the following special populations in your study using the checkboxes below.

NOTE: Members of special populations may not be targeted for enrollment in your study unless you indicate this in your inclusion criteria.

Response: None of the below populations will be enrolled

☐ Adults unable to consent

☐ Individuals who are not yet adults (infants, children, teenagers)

☐ Pregnant women

☐ Prisoners

6.4 Indicate whether you will include non-English speaking individuals in your study. **Provide justification if you will exclude non-English speaking individuals.**

In order to meet one of the primary ethical principles of equitable selection of subjects, non-English speaking individuals may **not** be routinely excluded from research as a matter of convenience.

In cases where the research is of therapeutic intent or is designed to investigate areas that would necessarily require certain populations who may not speak English, the researcher is required to make efforts to recruit and include non-English speaking individuals. However, there are studies in which it would be reasonable to limit subjects to those who speak English. Some examples include pilot studies, small unfunded studies with validated instruments not available in other languages, studies with numerous questionnaires, and some non-therapeutic studies which offer no direct benefit.

Response: We have no non-English speaking patients in this population. We have patients that English is a second language, but they are able to read, write and understand it. This population is less than 10% of the total population.

7.0 Vulnerable Populations

If the research involves special populations that are considered vulnerable, **describe the safeguards included to protect their rights and welfare.**

NOTE: You should refer to the appropriate checklists, referenced below, to ensure you have provided adequate detail regarding safeguards and protections. You do not, however, need to provide these checklists to the IRB.

7.1 For research that involves **pregnant women,** safeguards include:
NOTE CHECKLIST: Pregnant Women (HRP-412)

Response: We will not be using subjects from vulnerable populations

☒ **N/A**: This research does not involve pregnant women.

7.2 For research that involves **neonates of uncertain viability or non-viable neonates,** safeguards include:
NOTE CHECKLISTS: Non-Viable Neonates (HRP-413), or Neonates of Uncertain Viability (HRP-414)

Response:

☒ **N/A:** This research does not involve non-viable neonates or neonates of uncertain viability.

7.3 For research that involves **prisoners**, safeguards include:
NOTE CHECKLIST: Prisoners (HRP-415)

Response:

☒ **N/A:** This research does not involve prisoners.

7.4 For research that involves **persons who have not attained the legal age for consent to treatments or procedures involved in the research ("children")**, safeguards include:
NOTE CHECKLIST: Children (HRP-416)

Response:

☒ **N/A:** This research does not involve persons who have not attained the legal age for consent to treatments or procedures ("children").

7.5 For research that involves **cognitively impaired adults**, safeguards include:
NOTE CHECKLIST: Cognitively Impaired Adults (HRP-417)

Response:

☒ **N/A:** This research does not involve cognitively impaired adults.

7.6 Consider if other specifically targeted populations such as students, employees of a specific firm, or educationally or economically disadvantaged persons are vulnerable. **Provide information regarding their safeguards and protections, including safeguards to eliminate coercion or undue influence.**

Response: No specific populations or vulnerable groups will be targeted. All subjects enrolled in this study will be of legal adult consenting age with the ability to speak, read and interrupt the English language. Patients will have the ability to speak with the research team regarding any questions or concern they have before signing the consent. Patients are made aware that this study is voluntary and they are able to stop participating at any time they feel uncomfortable. Patients are not be pressured into participating and their clinic standard of care will remain the same if they participate or choose not to participate.

8.0 Eligibility Screening

8.1 Describe **screening procedures** for determining subjects' eligibility.
Screening refers to determining if prospective participants meet inclusion and exclusion criteria.

Include all relevant screening documents with your submission (e.g. screening protocol, script, questionnaire).

Response: :

Prospective participants will be asked to read and understand the consent and any questions they may have regarding the protocol will be answered. If the subject wants to participate in the study, they will be asked to sign the informed consent form. The subject's medical history and current medications will be obtained as well as their blood pressure and vitals. A physical examination will also be done. Fasting labs before 10:00am include; Pregnancy test, urine and baseline blood draw to measure CBC, CMP and HbA1c

All qualifying patients will be randomized to drug or placebo groups according a randomization chart prepared by Microsoft Excel software on a 1:1 ratio. All patients will start receiving study medication at visit 1B. 24 hr urine collection container will be provided and patients will be instructed to start collecting urine 24 hours prior to visit 1B if qualified.

☐ **N/A:** There is no screening as part of this protocol.

9.0 Recruitment Methods

☐ **N/A:** This is a records review only, and subjects will not be recruited. NOTE: If you select this option, please make sure that all records review procedures and inclusion/exclusion screening are adequately described in other sections.

9.1 Describe when, where, and how potential subjects will be recruited.

NOTE: Recruitment refers to how you are identifying potential participants and introducing them to the study. Include specific methods you will use (e.g. searching charts for specific ICD code numbers, Research Participant Groups, posted advertisements, etc.).

Response: Participants will be identified by prescreening clinical charts, patient doctor interaction at the time of their visits, flyers advertisements and researchmatch.org. Diabetes Endocrinology Center of WNY Locations include:

1. 1020 Youngs Road, Williamsville NY 14221

2. 705 Maple Road, Williamsville NY 14221

3. 462 Grider Street, Buffalo NY 14215

4. 1000 Youngs Road, Suite 105, Williamville NY 14221

The study clinical team will evaluate their clinic patients for possible participation in this study according to the inclusion and exclusion criteria at the Diabetes and Endocrinology Center of WNY. Patients that may qualify for the study are referred to the research team for further eligibility evolution. Patients meeting the inclusion and exclusion criteria based on preliminary phone evaluation will be invited to participate in the study.

9.2 Describe how you will protect the privacy interests of prospective subjects during the recruitment process.

NOTE: Privacy refers to an individual's right to control access to him or herself.