Title: Calcium Channel Blockade in Primary Aldosteronism

Detailed Protocol

NCT04179019

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I. BACKGROUND AND SIGNIFICANCE

Primary aldosteronism is the most common cause of secondary hypertension, with studies reporting prevalence rates of ~10% in the general hypertensive population and ~20% among patients with resistant hypertension. The cause of primary aldosteronism can be a unilateral aldosterone-producing adenoma (APA) or bilateral idiopathic hyperaldosteronism (IHA) whereby there is diffuse production of ectopic and non-physiologic aldosterone in the adrenal cortex. IHA likely contributes to >70% of all primary aldosteronism. Whereas surgical cure is the preferred therapy for APA, lifelong mineralocorticoid receptor antagonists (MRAs), such as spironolactone or eplerenone, are used to treat IHA. Treatment is important as patients with primary aldosteronism have an elevated risk of adverse cardiovascular and renal outcomes compared to patients with essential hypertension. It was long thought that curative surgery and lifelong medical therapy were equivalent treatment options, but more recent studies suggest that MRAs may not ameliorate the adverse cardiovascular and renovascular effects of primary aldosteronism to the same extent as surgery. For one, MRAs do not lower aldosterone levels, in fact, aldosterone levels are even higher with MRA therapy. Therefore, for IHA patients with primary aldosteronism in whom surgery is not an option, efforts to improve and optimize medical therapy are important.

In recent years, several mutations in cellular membrane ion channels and pumps have been found to cause primary aldosteronism. Nearly all APAs that cause primary aldosteronism harbor an underlying driver mutation in one or more of the following genes: *KCNJ5* (inward-rectifying potassium channel), *ATP1A1* (Na^+/K^+ ATPase), *ATP2B3* (Ca^{2+} ATPase), *CACNA1D* (L-type voltage-dependent Ca^{2+} channel), *CACNA1H* (T-type voltage-dependent Ca^{2+} channel), or *CLCN2* (chloride channel). The unifying mechanism of all these gene alterations is that the underlying pathology induces increased calcium ion influx into adrenal zona glomerulosa cells which induces excess aldosterone production.

Moreover, recent evidence in surgically removed adrenal glands from patients with primary aldosteronism and IHA has shown that even though IHA adrenal glands do not harbor adrenal tumors, they do harbor foci of ectopic aldosterone production and these foci are enriched for mutations in *CACNA1D*, thereby suggesting that calcium channel mutations are predominant in the pathogenesis of IHA. This represents an intriguing target for medical therapy as blockade of this channel could lower intracellular calcium and hence decrease aldosterone production. Calcium channel blockade would also represent a more upstream therapy than mineralocorticoid receptor antagonists, which block aldosterone's action at its receptor rather than lower its production.

Calcium channel blockers such as amlodipine are a class of medications that have been used in the treatment of essential hypertension for years and are safe with minimal risks. We hypothesize that amlodipine can lower autonomous aldosterone production in primary aldosteronism patients with IHA, a population that is more likely to harbor a mutation in the L-type voltage-gated calcium channel and a population in whom medical therapy is usually the only treatment option.

Preliminary Data to Support the Proposed Study

II. SPECIFIC AIMS

The hypothesis for this **pilot study** is that the calcium-channel blocker amlodipine can specifically decrease aldosterone production in patients with idiopathic bilateral hyperaldosteronism.

- Primary Outcome: Change in 24-hour urinary aldosterone excretion rate and plasma aldosterone in response to maximal amlodipine therapy.

- Secondary Outcomes: Acute change in plasma aldosterone in response to amlodipine therapy.

III. SUBJECT SELECTION

Sample Size:

Up to 15 participants with confirmed or presumed IHA will be recruited. Some patients with IHA will have this diagnosis confirmed via adrenal venous sampling. Many patients have not undergone adrenal venous sampling but have a preumptive diagnosis of IHA because they have no adrenal abnormalities on imaging and/or are already being treated with mineralocorticoid receptor antagonists.

Participants will be included if they are aged 18-80 years, have a confirmed diagnosis of primary aldosteronism, a suppressed plasma renin activity (<1.0 ng/mL/h), and confirmed or presumed IHA. Participants taking a calcium-channel blocker will be included if they are willing to undergo a washout protocol (described below). Participants taking mineralocorticoid receptor antagonists will be included if their plasma renin activity is <1.0 ng/mL/h; if it is not, they may still be included if they are able to lower the dose of this medication such that their renin activity falls below 1.0 ng/mL/h.

Participants will be excluded if they have discrete or large bilateral adrenal adenomas on cross-sectional imaging (done as a part of standard clinical care) to avoid including participants who may have APAs, which predominantly have *KCNJ5* mutations rather than *CACNA1D* calcium channel mutations. Participants with anemia, leukopenia, or thrombocytopenia (based on our acceptable ranges) or a blood pressure greater than 180/100 mmHg, will be excluded. Pregnant or breastfeeding women will also be excluded.

IV. SUBJECT ENROLLMENT

The study will take place at Brigham and Women's Hospital (BWH). Our research team has extensive experience and an established infrastructure for recruiting individuals with IHA. Participants will be recruited from 2 sources.

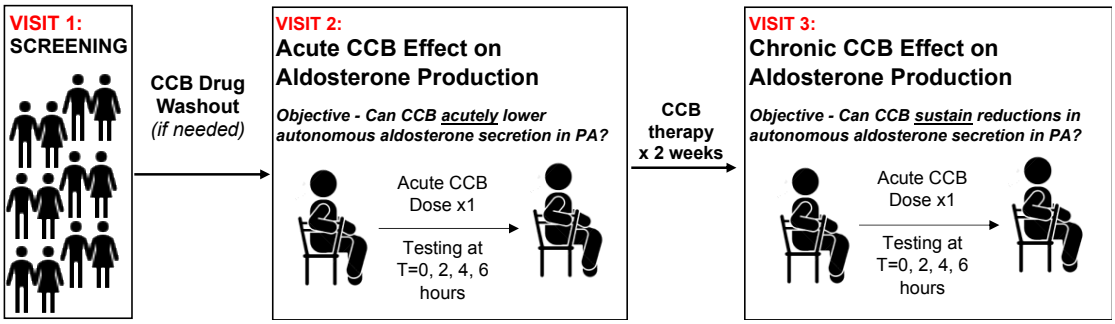
1. Center for Adrenal Disorders at BWH: The PI sees primary aldosteronism patients in the clinic. In addition, participants undergoing adrenal venous sampling for primary aldosteronism will be contacted if they have IHA. Patients with IHA will be contacted regarding interest in participation by the PI's research team with a letter.

2. Partners Research Patient Data Registry (RPDR): The Brigham & Women's Hospital and Partners affiliates i.e. Massachusetts General Hospital, Faulkner Hospital, Newton Wellesley, etc. have a shared database to identify potentially eligible patients that can be used for clinical research studies. We will search for patients with primary aldosteronism

in the RPDR and with IRB committee approval, conduct targeted chart reviews to assess which patients likely meet inclusion criteria for this study. Once we have identified potentially eligible patients, they will be invited to participate via the Mass General Brigham Patient Gateway secure online portal or we will identify their primary care physician, or endocrinologist, or other internist with close ties to the patient in our system, and request permission to contact their potentially eligible patient with our IRB approved letter (co-signed by the PI and physician) including information regarding the study. The letter is used to ask permission to send their patients a co-signed letter inviting them to learn more about and potentially participate in our study. In the letter, participants will be notified that we will be calling within about 2 weeks after receipt of the letter. Our phone number will be provided so individuals may contact us with questions or to opt out of receiving further information about the study. Interested patients will be invited for a screening visit to formally assess eligibility.

V. STUDY PROCEDURES

The study will take place in the outpatient clinical research centers of the BWH. There will be one screening visit, and 3 study visits. The duration of the entire study is 2-10 weeks (see protocol schema).



Screening visit (Visit 1): Participants will meet with a study staff member (licensed physician or nurse practitioner or physician assistant) to review the study protocol, risks, and benefits. They will have received the consent form ahead of this visit and it will be reviewed in detail and signed prior to proceeding. The screening visit will entail a brief review of the patient's medical history, a physical examination, blood pressure measurements, an EKG will be perfomed, and phlebotomy to assess eligibility. Women between the ages of 18-50 will have a pregnancy test that must be negative.

Blood pressure medication washout and optimization period: Many participants may already be treated with a calcium-channel blocker per standard of care. To assess the effect of amlodipine on aldosterone production, these participants will washout their prescribed calcium-channel blocker for 4-8 weeks and will have their blood pressure managed with other anti-hypertensive agents in the interim. After stopping their calcium-channel blocker, participants will be provided with a home blood pressure machine and taught how to measure their blood pressure at home and how to report daily readings to study staff. The target blood pressure for this washout period, and throughout the entire study protocol, will be 120-150/60-90 mmHg. The upper limit of this blood pressure range exceeds the normal range because many patients with primary aldosteronism have chronically elevated and poorly controlled blood pressure; although study staff will make every effort to maintain their blood pressure in a normal range (<130/80 mmHg),

this goal may not be achievable in this short study. If home blood pressure exceeds 130/80 mmHg, participants will be prescribed doxazosin (in 2 mg daily increments up to 8 mg daily) and/or hydralazine (in 25 mg daily increments up to 25 mg three times daily) in efforts to keep blood pressure in the target range. These medications will be used to substitute calcium-channel blockers since they do not interfere with the renin-angiotensin-aldosterone system and are the recommended medications by the international Endocrine Society guidelines.

In addition to blood pressure monitoring during this washout period, serum potassium will be monitored to ensure it remains in a target range of 3.5-4.5 mEq/L. Most patients with primary aldosteronism are hypokalemic and already taking daily potassium supplements. Study staff may increase the dose of potassium supplementation (using potassium chloride in 20 mEq supplements) to keep serum potassium in the target range. Serum potassium will be checked at 2-4 weeks, and if not in range, again at 4-8 weeks.

Participants already taking a mineralocorticoid receptor antagonist (such as spironolactone or eplerenone) will be included and studied as long as their renin activity remains suppressed (<1.0 ng/mL/h). Participants who have a renin activity greater than or equal to 1.0 ng/mL/h while on mineralocorticoid receptor antagonists may still participate if their dose can be safely decreased such that renin activity decreases to <1.0 ng/mL/h. If needed, renin will be measured at 2-4 weeks, and if not in range, again at 4-8 weeks.

This washout and optimization period will range from 4-8 weeks to provide sufficient time for optimization of washout, blood pressure, potassium, and renin levels. Participants who are unable to tolerate a washout or maintain blood pressure or potassium in the target range, will be withdrawn from the study.

Study Visit 2-Dexamethasone Suppression Test/Cosyntropin stimulation test: After completing the washout period, participants will be scheduled to undergo a dexamethasone suppression test and cosyntropin stimulation test. This will be conducted prior to the high sodium diet given before visit 3.

The dexamethasone suppression test is often used in the clinical setting to measure whether there is excess cortisol secretion. Dexamethasone (1mg) is a synthetic adrenal steroid and is taken the night before the cosyntropin stimulation test between 11pm and 12am. When the participant arrives to the CCI the following morning between 8-9am, initial biochemical testing will include ACTH and cortisol levels. These hormone values will demonstrate how well cortisol can be suppressed.

The cosyntropin stimulation test will measure how well cortisol can be stimulated. A cosyntropin stimulation test is a common procedure that is performed routinely in the ambulatory setting at BWH in the primary care, endocrine, and oncology clinics. It involves the measurement of adrenal hormones before, and one hour after, the administration of cosyntropin (an analogue of the peptide ACTH: “ACTH 1-24”). Participants will arrive to the CCI at 8-9am in the morning and will have an IV placed in the arm. A baseline blood measurement will be analyzed for cortisol, aldosterone, plasma renin activity, direct renin concentration, ACTH, and basic metabolic chemistries. The standard dose of cosyntropin (250 mcg) will then be administered by IV bolus and blood work for cortisol and aldosterone measured again 60mins later. This will conclude the visit which is expected to last ~2hours.

Study Visits 3 and 4: Following screening and washout/optimization, there will be two study visits separated by 2 weeks (see Schema Figure). Critical determinants of aldosterone production

include body posture, dietary sodium intake, serum potassium, and blood pressure. Thus, this protocol ensures that all 4 of these confounders are controlled to ensure that outcome measures are reliable and reproducible.

Study Visit 3*:* The objective for study visit 3 is to establish the magnitude of autonomous aldosterone production at baseline and evaluate the *acute* impact of amlodipine on aldosterone production.

To ensure rigor and reproducibility, all aldosterone (and other measurements) will be conducted under conditions where diet and posture are controlled. Five days before study visit 3, participants will be provided with sodium supplements (in the form of 3 broth packets approximating 3,000 mg of sodium daily) to consume daily. The average daily sodium consumption in the United States is 3.8-4.5 grams daily, and thus this sodium intake will ensure that all participants are consuming a typical U.S. sodium diet and for research purposes, ensure that the 24 hour urinary sodium excretion at study visit 3 is >200 mmol Na^+/24h. This sodium loading is a standard of care clinical practice referred to as an "oral sodium suppression test" and the internationally recommended test to diagnose primary aldosteronism by the Endocrine Society.

After consuming this sodium diet, participants will arrive at the BWH Clinical Research Center at 8AM, after having collected their urine for 24 hours. An EKG will be performed if the participant had a remote screening visit. Participants will remain in an upright seated position for a total of seven hours. After one hour of seated rest, baseline blood measures will be obtained, and a single dose of amlodipine 10 mg will be administered. In order to reflect amlodipine pharmacodynamics, blood measurements will be repeated every 2 hours to assess the serial change in outcome measures (t=2,4,and 6 hours after the amlodipine dose). This serial blood testing may be performed via repeated venipuncture, or by placing an intravenous line to draw, whichever is preferable to the participant. Blood tests will include serum aldosterone, plasma renin activity, basic metabolic panel, adrenocorticotropic hormone, cortisol, and other hybrid adrenal steroids. Extra plasma and serum will be saved for future use and DNA will be extracted from peripheral leukocytes for genetic analyses of aldosterone-related genes. The 24h urine will be analyzed for aldosterone excretion rate, cortisol, sodium, potassium, and creatinine.

During the study visit, participants will be able to drink water and after a 4-hour period be able to eat a light snack. After the study is completed, participants will be able to eat and encouraged to bring food with them for this.

Treatment phase: Following completion of Visit 3, participants will be prescribed amlodipine 10mg daily for 2 weeks. Home blood pressure monitoring will continue to ensure blood pressure remains in the target range of 120-150/80-90 mmHg. If blood pressure falls below this range with amlodipine, doxazosin and/or hydralazine doses may be reduced or stopped. If blood pressure remains low even after stopping doxazosin and hydralazine, the dose of amlodipine may be lowered to 5mg daily. Potassium chloride supplements may be titrated based on the values obtained at Study visit 3.

Study Visit 4: The objective for study visit 4 is to establish the magnitude of autonomous aldosterone production after 2 weeks of amlodipine treatment.

Participants will again complete 5 days of sodium supplements and arrive to the BWH Clinical Research Center at 8AM, bringing with them a 24-hour urine collection. Participants will be instructed to not take their daily amlodipine dose prior to arriving, they will take it as a part of the study visit. They will repeat the exact same study protocol completed at Visit 3. Participants will

remain in an upright seated position for a total of seven hours. After the first hour of seated rest, baseline blood measures will be obtained and a single dose of amlodipine (the dose will be the same dose they took for the preceding 2 weeks) will be administered. Blood and urinary measurements will again be performed exactly as they were at visit 3.

VI. BIOSTATISTICAL ANALYSIS

The analytic plan for this pilot study is to compare the change in plasma aldosterone (Aldo) and the change in 24h urinary aldosterone excretion rate (AER). The primary analytic plan will be to evaluate ΔAldo and ΔAER between visit 2 and 3. Wilcoxon signed rank, or paired T-test statistics, will be used for univariate statistical analyses to assess significant differences between the paired measurements of ΔAldo and ΔAER. Adjustment for potential confounders (age, sex, race/ethnicity, BMI, BP) will be considered using generalized linear mixed random effects models (SAS 9.4). Secondary analyses to assess the acute change in plasma aldosterone after a single dose of amlodipine will be conducted using the same methodological approach.

Sample size calculations are based on an expected ΔAldo of 5 ng/dL, with up to a ΔS.D. of 4-5 ng/dL. It is thus estimated that 7-10 participants are needed in this paired design to reject the null hypothesis that ΔAldo does not significantly change with amlodipine therapy with 80% power. Similarly, for an expected ΔAER of 7 mcg/24h, with a ΔS.D. of 5-6 mcg/24h, it is estimated that 6-8 participants are needed in this paired design to reject the null hypothesis that ΔAER does not significantly change with amlodipine therapy with 80% power.

We aim to enroll 15 participants to provide sufficient sample size and power to detect differences even if 2-5 participants (up to 33%) are non-responders to amlodipine. *As this is a pilot study, any information regarding the influence of amlodipine on aldosterone production may be useful in designing future studies.*

VII. RISKS AND DISCOMFORTS

Sodium supplementation

The sodium intake is not intrinsically harmful, and in fact contains a similar amount of sodium to the diet consumed by the average American. This sodium intake is also the standard of care, and international guideline recommended approach, to assess aldosterone production in patients with primary aldosteronism. Possible side effects include a rise in blood pressure or drop in serum potassium which study staff will be monitoring closely and will be able to titrate medications to prevent or treat.

Risk of Washout Period/Risk of Stopping Regular Blood Pressure Medications

During the washout/optimization period, there is a risk that the participants' blood pressure may rise despite initiation of an alternative antihypertensive agent. Risks of high blood pressure include headache and in rare and extreme circumstances stroke, heart attack, and death. Home blood pressure will be closely monitored, and study staff will actively titrate alternative antihypertensive agent(s) to ensure blood pressure remains well-controlled. This is a commonly performed procedure in clinical practice and routine.

Calcium channel blocker treatment (amlodipine)

Amlodipine is a commonly prescribed medication that has been used to treat essential hypertension for years. The most common side effect is swelling in the legs and ankles (which usually takes 1-2 weeks or longer to manifest). It can also cause low blood pressure particularly at a high dose, but this is unlikely in the current study. Other rare side effects include fluid in the lungs in heart failure patients, palpitations, fatigue, skin rash, and stomach upset.

Hydralazine
Hydralazine has been used for many years in the treatment of essential hypertension. It is an international guideline recommended medication for treatment in primary aldosteronism when diagnostic testing is conducted. Side effects include low blood pressure, fast heart rate, stomach upset, and headache. Rare side effects include an autoimmune lupus-like syndrome, liver damage, and decreased white blood cell count. It should not be used in patients with coronary artery disease.

Doxazosin
Doxazosin has been approved for the treatment of essential hypertension for many years. It is an international guideline recommended medication for treatment in primary aldosteronism when diagnostic testing is conducted. Side effects include low blood pressure, leg swelling, nausea, dizziness, and headache. Rare side effects include liver inflammation and angioedema (swelling of the lips and tongue).

ACTH infusion
Cosyntropin is a synthetic derivative of naturally occurring adrenocorticotrophic hormone (ACTH), specifically the first 24 of the 39 amino acids of the hormone. Very rarely, patients with a history of asthma or other forms of allergy may be predisposed to hypersensitivity reaction or anaphylaxis during Cosyntropin administration; bradycardia, tachycardia, hypertension, peripheral edema, and rash have also been reported with cosyntropin. A physician or nurse will be present for the test. This cosyntropin stimulation test is a common ambulatory test that is performed in the clinical setting.

Dexamethasone
Dexamethasone is a synthetic glucocorticoid. Very rarely dexamethasone may cause anaphylactic reactions in those with hypersensitivity to dexamethasone; infection; slow wound healing; diaphoresis; nausea; headache; and mood changes. Most of these reactions are cause from chronic use, not the one-time administration as in our study.

Blood Draws/Phlebotomy
Some may experience a mild sting or painful feeling at the site of needle or intravenous catheter insertion. Although uncommon, it can also lead to a blood clot or infection. Standard sterile precautions will be taken to minimize these risks.

Pregnancy/Fetus
Women cannot take part in this study if they are pregnant or breastfeeding. Women between the age of 18-50 years will be required to have a negative pregnancy test prior to taking part in the study. Women who are postmenopausal and amenorrheic for one year or who have had a surgical sterilization procedure are exempt from submitting to a pregnancy test. Women will be counseled about using abstinence or contraception during participation in this study to avoid exposure to potentially teratogenic medications.

Genetic Information

Genetic information about this study does not have medical or treatment implications at this time. However, information about participation in a genetic study may influence insurance and/or employers. These risks are minimized by not sharing information about participation and by using de-identified study codes. We will not place genetic information in the medical record. All results and samples will be coded and the key to the code kept in a separate locked file.

In order to allow researchers to share test results, the National Institutes of Health (NIH) and other central repositories have developed special data (information) banks that analyze data and collect the results of whole genome studies. These banks may also analyze and store DNA samples, as well. These central banks will store genetic information and samples and give them to other researchers to do more studies. Although it is impossible to predict how genetic information will be used in the future, there are many safeguards in place to ensure that all genetic information is protected and de-identified.

Unknown Risks
There may be other risks or side effects that are not known at this time.

VIII. POTENTIAL BENEFITS

Although patients are not expected to directly benefit from the trial, their participation will contribute to medical research. Because of the close monitoring, supervision, and pro-active blood pressure and potassium therapies, participants will likely see an improvement in their primary aldosteronism therapy goals, even though this is not the primary objective of the study.

IX. REMUNERATION

Patients will receive monetary compensation for participating in the study and parking and/or transportation costs will also be covered. Participants will receive $100 for completing study visit 3 and $100 for completing study visit 4. Parking at BWH lots will be covered. For participants who need transportation, reimbursement for public transportation and/or livery services (i.e. Uber, Lyft, taxi, etc) will be strongly considered on a case-by-case basis based on feasibility and location.

X. MONITORING AND QUALITY ASSURANCE

The Principal Investigator will be responsible for reviewing the safety and/or efficacy data and determining whether the research should be altered or stopped.

Safety data will be reviewed on every case studied in the PI's monthly research group meeting. The PI's research group, the Cardiovascular Endocrinology Research Group (CERG), meets once a month on a Tuesday. The CERG includes more than 5 patient-oriented clinical investigators who are all endocrinologists with detailed knowledge of human research protocols, safety monitoring, and managing hypertension. At each CERG meeting, each PI presents recruitment status, adverse events/safety issues, minor deviations, and other pertinent study-related issues to the group of PI's. Research coordinators also attend this meeting. Discussion of issues and a plan are formulated for each adverse event. The PI will be responsible for executing any plans agreed upon during these meetings.

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