**Study Protocol**

**Project Title:**

Effect of Minocycline Treatment on Drug-Resistant Hypertensive Patients

Subtitle: Brain-Gut Microbiome-Immune Axis in Hypertension

NCT02133872

**Investigators:**

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**Abstract:**

Hypertension (HTN) is the single most modifiable risk factor for cardiovascular disease and stroke but also implicated in diseases such as diabetes, metabolic syndrome, dementia, and many other conditions. Recent statistics indicate that about one-third of all adults, and up to two-thirds

of older adults in the USA have HTN[1]. Despite advances in life style modification and multi-drug therapies, ~20% of hypertensive patients may remain resistant to treatment[2,3]. These individuals exhibit autonomic dysregulation due to elevated sympathetic outflow, norepinephrine spillover, and low parasympathetic activity.[1-6] It is generally accepted that this "treatment resistant" HTN is primarily "neurogenic" in origin, involving sympathetic nervous system over activity that initiates and sustains HTN.[1-6] An invasive approach, such as the recently developed "Simplicity Catheter"[7] assisted renal denervation remains one of the few options available to these patients. But several recent randomized controlled trials (Symplicity-HTN3 and Prague15) showed disappointing results. Thus, a mechanism-based breakthrough is imperative to develop novel strategies to prevent and perhaps eventually cure (treatment resistant) neurogenic hypertension.

This study is designed to determine what dose of minocycline produces antihypertensive effects in treatment-resistant neurogenic hypertensive individuals. Minocycline was selected because it is a safe, time-tested, small molecule that penetrates the blood barrier to inhibit microglial activation.[8] No other available compound appears to be safer and/or display specificity inhibiting microglial activation better than minocycline. Thus, the potential therapeutic benefits of this inexpensive, well-tolerated, long time FDA-approved (in 1971) drug that has minimal side effects would be enormous.

**Background and Significance:**

As many as 20% of all hypertensive patients may remain refractory to multiple hypertensive drugs plus life-style modification[2,3]. For these "treatment resistant" patients invasive intervention remains the only available option, but with very limited success. The proposed study will test the hypothesis that minocycline, a small molecule, central nervous system (CNS)-penetrable anti-inflammatory antibiotic, inhibits activity of brain microglia attenuating sympathetic nervous system activity to lower blood pressure in patients with neurogenic (treatment-resistant) HTN.

This project is extremely significant in that it seeks to investigate the hypothesis that neuroinflammatory processes involving activated microglial cells in the autonomic brain regions play a critical role in the initiation and establishment of neurogenic (treatment-resistant) HTN. In addition, it is further hypothesized that this is associated with a dysfunctional neural-bone marrow (BM) communication which perpetuates HTN pathophysiology. This study will conduct both fundamental physiological genomic studies in experimental models to elucidate the mechanism of neural-BM dysfunction and translational studies in patients with resistant HTN, to provide clinical validation for possible novel therapeutics for neurogenic HTN. This innovative hypothesis is supported by published/preliminary studies[9-13]: (i) High blood pressure (BP) in angiotensin II (Ang II)-dependent animal models of neurogenic HTN is associated with rapid and sustained increases in microglial activation and proinflammatory cytokines (PICs) in the paraventricular nucleus (PVN); (ii) Ang II, via angiotensin II type 1 receptor ($AT_1R$), increases production and selection of C-C chemokine ligand 2 CCL2), that acts via its C-C chemokine receptor 2 (CCR2) to stimulate microglial migration towards neurons; CCL2 levels in the PVN are also elevated in HTN; (iii) inhibition of brain mitochondrial reactive oxygen species (ROS) or microglial activation by minocycline decreases PICs in the PVN and attenuates Ang II-induced HTN.

**Specific Aims:**

The overall aim of this study is to determine if targeting brain microglial activation by minocycline would produce beneficial outcomes in neurogenic (treatment-resistant) HTN. This will be achieved by addressing the following objectives in patients with neurogenic treatment-resistant HTN: perform an open-label dose-range study to determine the lowest effective minocycline dose to lower BP

**Research Plan:**

The following are the inclusion and exclusion criteria

***Inclusion:***

- Greater than 18 years of age
- Participant is receiving a stable antihypertensive medication regimen defined as-
    - Fully-tolerated doses of 3 or more antihypertensive medications of different pharmacologic classes, one of which must be a diuretic.
    - With no changes for a minimum of two months prior to screening
    - That is expected to be maintained without changes for at least 3 months.
- The participant agrees to have all study procedures performed.
- Patients participating in UF IRB approved protocol # IRB201400233 will be eligible to participate.

***Exclusion***

- Older than 85 years of age
- Average office blood pressure less than 120 mmHg
- eGFR <45mL/min/1.73m$^2$, using the MDRD equation.
- More than one in-patient hospitalization for an antihypertensive crisis/emergency within the past year.
- More than one episode(s) of orthostatic hypotension (>20mmHg systolic BP [SBP] reduction or, >10mmHg diastolic BP [DBP] within 3 minutes of standing).
- Known hypersensitivity or contraindication to minocycline or another tetracycline.
- Evidence of alcoholism or drug abuse.
- Concurrent severe disease (such as neoplasm, HIV positive, or AIDS).
- Women of childbearing potential, who are not using 2 forms of birth control.

**This study will determine if a low dose of minocycline is effective to reduce SBP and peripheral inflammation in patients with "neurogenic" or resistant HTN**.

All study procedures for this study will be performed at Sites 1-3:

Rationale: is the dose-escalation phase with the objective of determining the potential anti-hypertensive effects of minocycline in neurogenic (treatment-resistant) HTN by analyzing the

doses: 50mg/d, 100mg/d and 200mg/d. This is necessary because the side effects to this antibiotic, although infrequent, can be dose and duration dependent. Minocycline, a semisynthetic tetracycline derivative, has been in clinical use for ~45 years and is known for its excellent oral bioavailability and tissue distribution. Its efficient blood brain barrier passage (CNS/ plasma distribution rate in the range of 0.3-0.6) allows CNS levels up to the micromolar range after repeated daily standard oral doses of 100–200 mg [1–3]. Thus, it is important to determine if a lower dose is sufficient to enter the brain, have anti- inflammatory effects, and result in BP lowering.

Description: Thirty- four (34) adult patients who are resistant to hypertensive medication and meet all inclusion criteria and no exclusion criteria will be offered the opportunity to participate in this study. Study procedure will be reviewed with the participant and once written informed consent has been obtained, the participant will be enrolled in the study

Research Visits and List of Procedures:

**Visit 1 Baseline 1**

- **(Screen)**Medical history and medications review will be performed.
- Brief physical exam will be performed.
- Office Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and pulse pressure (PP) will be performed.
- A total of four tablespoons of blood will be collected. Three tablespoons of blood will be collected and analyzed for the following: lipid panel, high sensitivity-C reactive protein, high sensitivity troponin, a complete metabolic profile, cystatin C and albumin. An additional tablespoon of blood will be drawn for additional biomedical tests.
- Female participants will have a pregnancy test performed to verify a negative result before continuing.
- Participants will be fitted with an ambulatory blood pressure monitor (ABPM) and will be educated on how to take their blood pressure at home. They will also be instructed to start study drug after completing a 24 hour ABPM period. Once completed, subjects will mail the monitor back to the research site. Mailing material will be provided by the study.
- Participants will be provided with a home blood pressure log (HBPL) and will be instructed to take their blood pressure at least 3 times/week. Around the same time and document the results in the log. They will be asked to bring their HBPL to every clinic visit.

**Visit 2 Baseline 2**

**(Day 0)**

- Review of medications and hospitalizations will be performed.
- Brief physical exam will be performed.
  Office SBP, DBP and PP readings will be performed.
- Study drug (minocycline 50mg) will be dispensed
- Review HBPL will be performed.

- Participants will be provided with a home blood pressure log (HBPL) and will be instructed to take their blood pressure at least 3 times/week around the same time and document the results in the log. They will be asked to bring their HBPL to every clinic visit. Participants will be asked to bring any unused medications and bottles (including empty bottles) to every clinic visit.

**Visit 3, 7, and 11**

- **(2 Week ± 7 days, 90 ± 14 days, and 150 ± 14 days)** Review of any unused study medication and bottles including empty bottles.
- Review of medications and hospitalizations will be performed.
- Brief physical examination will be performed.
- Office SBP, DBP and PP readings will be performed.
- Assessment of medication compliance and tolerance will be performed.
- Review HBPL will be performed.
- Study drug (Visit 3, 2 week – minocycline 50mg and Visit 7, Day 90 – minocycline 100mg) will be re-dispensed
- Participants will be provided with a home blood pressure log (HBPL) and will be instructed to take their blood pressure at least 3 times/week around the same time and document the results in the log. They will be asked to bring their HBPL to every clinic visit.
- Participants will be asked to bring any unused medications and bottles (including empty bottles) to every clinic visit.

**Visit 4, and 8**
**(Day 60 ±14 days and 120 ±14 days)**

- Review of any unused study medication and bottles including empty bottles Review of medications and hospitalizations will be performed.
- Brief Physical Exam will be performed.
- Office SBP, DBP and PP readings will be performed,
- One tablespoon of blood will be drawn for additional biomedical tests.
- Participants will be fitted with the ABPM and instructed to complete a 24-hr reading cycle as performed during visit 1.
- Review HBPL.
- Study drug (Visit 4 Day 60 – minocycline 50mg/d / Visit 8 Day 120 – minocycline 100mg/d) will be re-dispensed.
- Participants will be provided with a home blood pressure log (HBPL) and will be instructed to take their blood pressure at least 3 times/week. Around the same time and document the results in the log. They will be asked to bring their HBPL to every clinic visit.
- Participants will be asked to bring any unused medications and bottles (including empty bottles) to every clinic visit.

**Phone Calls**

**Visit 5 (within 10 days of visit 4) and 9 (within 10 days of visit 8)**

- Participants will be given results of their ABPM blood pressures by the study team and told if their participation in this study will end or continue on to the next visit.

If they are told by the study staff that their participation is continuing, they will be asked to return to clinic within approximately 1 week for dose titration of their study medication.

If they are told by the study staff that their participation has ended, they will be asked to return to clinic within approximately 1 week for a final visit (see Visit 12 Final Visit for description)

**Dose Titration**

**Visit 6 (within 7 days of visit 5), and 10 (within 7 days of visit 9)**

- Review of any unused study medication and bottles including empty bottles. Review of medications and hospitalizations will be performed. Review of medications and hospitalizations will be performed.
- Brief physical examination will be performed.
- Office SBP, DBP and PP readings will be performed.
- Review HBPL
- Patient will be dispensed study medication (Visit 6 - minocycline 100mg/d / Visit 10 – minocycline 200mg/d).
- Participants will be provided with a home blood pressure log (HBPL) and will be instructed to take their blood pressure at least 3 times/week around the same time and document the results in the log. They will be asked to bring their HBPL to every clinic visit.
- Participants will be asked to bring any unused medications and bottles (including empty bottles) to every clinic visit.

**Visit 12 Final Visit**

**(Day 180 ±14 days or at any time participation ends)**

- Review of any unused study medication and bottles including empty bottles. Review of medications and hospitalizations will be performed. Review of medications and hospitalizations will be performed.
- Brief Physical Exam will be performed.
- Office SBP, DBP and PP readings will be performed.
- Review HBPL
- A total of approximately four tablespoons of blood will be collected, as performed in Visit 1.
- Participants will be fitted with the ABPM and instructed to complete a 24-hr reading cycle as performed during Visit 1.5 line

Office blood pressure (BP) readings will be taken in a seated position after 5 minutes of rest with feet flat on the floor according to JNC-7 Guidelines. At the baseline visit, BP will be measured in each arm, and the arm with the higher SBP will be used for all subsequent readings. All BP recordings will be made in triplicate. The initial reading will be discarded and the second and third readings for SBP and DBP and HR will be averaged and used for analysis.

Home blood pressure monitoring via the ABPM will be performed using an oscillometric Spacelabs 90207 monitor (Spacelabs Healthcare, Issaqua, WA) with readings taken every 30 minutes in daytime and every 60 minutes at nighttime. ABPM readings will be averaged for, daytime (7 AM to 10 PM), and nighttime (10 PM to 7 AM).

Participants will be assessed while adhering to their usual diurnal activity and nocturnal sleep routine. Antihypertensive drugs and doses taken will be recorded at each visit on standardized forms along with any reports of adverse experiences known to occur with the drugs used (e.g. lightheadedness, dizziness, syncope, etc.).

Based on our treatment design, enrolled participants will start the study at the lowest dose of 50 mg/d and maintained in this regimen for approximately 60 days. At Visit 4, if the participant is asymptomatic and average daytime SBP is not reduced by ≥5.0 mmHg measured through ABPM, then drug dosage will be increased to 100 mg/d until Visit 8. Similarly, if the average daytime SBP is not reduced (≥5.0 mmHg), then drug dosage will be increased to 200 mg/d until the end of the trial at Visit 12. Subjects that successfully achieve a drop of ≥5 mmHg in average daytime SBP by Visit 4, 8, and 12 will be classified as a responder and their study participation will be terminated. At Visit 12, the same blood tests performed at the baseline visit will be repeated and a last ABPM cycle will be recorded.

**Statistical Analysis**

The primary measure of interest will be the categorical classification of subjects into responders vs. non-responders upon treatment with a specific minocycline dose: 50, 100 or 200 mg/d. Responders are defined as subjects who achieve a drop of >5 mmHg in mean daytime SBP, based on daytime ABPM measurements (7 am to 10 pm). For these participants, the discontinuation or lowering of the dose of a concurrent anti-hypertensive drug due to excessive SBP reduction will also be assessed. Excessive SBP reduction is defined as an office SBP <120 mmHg or >10 mmHg SBP decrease associated with symptom(s). On the other hand, non-responders are defined as the participants that fail to show any change in their average daytime SBP measured through ABPM despite being exposed to the different minocycline doses evaluated.

The secondary measures for the responders group will assess changes in the following based from baseline to study participation end point: (1) Evaluation of changes in office SBP recordings. (2) Changes in daytime and nighttime ABPM differences.

Sample size requirements for the dose determination study are based on consideration of the primary measure of interest, change in SBP in response to treatment with minocycline. In order to achieve our primary measure of interest (responder vs. non-responder) and assuming a 15% drop out rate, we will target to enroll 35 participants to participate in this study. This sample size

estimation is based on our interest to target a dose where 75% (27/35) of all participant's response to treatment and are therefore classified as responders. For this purpose, we will use a cumulative response approach where participants who respond to the lower doses will be assumed to also respond to higher doses.

**Possible Discomforts and Risks:**

**Minocycline** is a well-tolerated antibiotic, which has been in continuous use in the USA since 1971 when approved by the FDA, and adverse effects are anticipated to be infrequent. Side effects in some subjects may be gastrointestinal (abdominal cramping, diarrhea, nausea and dry mouth), central nervous system (CNS) (headache, vestibular reaction) or hypersensitivity e.g. rash, itching; sun sensitivity, new signs of infection e.g. fever, chills, persistent sore throat, oral thrush or new yeast infection; ringing in the ears. The drug can cause fetal harm when taken during pregnancy. The drug treatment will immediately be discontinued with the experience of any of these side effects.

**Blood draws** may cause pain, bruising, lightheadedness, or, on rare occasions, infection. Varying amounts of blood will be collected for each study (3 tablespoons at each draw).

**Special note to women.** Being part of this study while pregnant may expose the unborn child to significant risks, some of which may be currently unforeseeable. Therefore, pregnant women will be excluded from the study. If you are a woman able to become pregnant, a pregnancy test will be done and it must be negative before you can continue in this study. If sexually active, you must agree to use appropriate contraceptive measures while taking part in this study. Medically acceptable contraceptives include: (1) surgical sterilization (such as tubal ligation or hysterectomy), 2) approved hormonal contraceptives (such as birth control pills, patches, implants or injections), (3) barrier methods (such as a condom or diaphragm) used with a spermicide, or (4) intrauterine device (IUD). If you become pregnant while taking part in this study or if you have unprotected sex, you must inform the study doctor immediately. A pregnancy test will be repeated at the 6-month visit. **Due to the possible drug interaction of minocycline with oral contraceptives, women of child bearing age who are using oral contraceptives will be required to utilize a second form of birth control while participating in this study.**

**Risks of twenty-four-hour ambulatory blood pressure cuff**

The risks to the ambulatory blood pressure monitoring generally relate to the annoyance of the repeated inflations and may become uncomfortable and/or could irritate the skin. Careful monitoring of the arm and removal during non-recording periods can alleviate any irritation

**Study Monitoring:**

This study will be conducted to meet strict requirements approved by the Institutional Review Board (IRB-01) of the University of Florida. It will be conducted according to the ethical principles stated in the Declaration of Helsinki (2008). IRB approval will be obtained before initiating the study. The consent forms will take into consideration the well-being, free-will and respect of the participants, including respect of privacy. We agree to respect the requirements of the McGill University Faculty of Medicine Institutional Review Board, the Tri-Council Policy

Statement: Ethical Conduct for Research Involving Humans, 2010. The IRB will also monitor the lab performance during the approved period. In addition, the IRB committee performs annual reviews of all human studies for safety related issues. An independent DSMB will be formed and operate under a pre-specified set of rules for monitoring.

**Data Safety:**

Each subject will be assigned a code when they are recruited into the study. Copies of case report forms, original test results, participants' medical records, correspondence, participant informed consent and any other documentation relevant to the study collected from the subjects will be coded and secured in a safe place (in a locked filing cabinet inside a locked office). Access will only be granted to investigators and study coordinators involved in the study. All electronic files will be encrypted. Data recorded with computers, will be transferred and kept on computer disks with limited access. This information will be kept for a maximum of 10 years after the study is completed, after which they will be destroyed. The research data will appear only in the form of a scientific presentation or publication, without the participant's name, or any potentially identifying information being disclosed. These measures will protect subject confidentiality.

All information obtained about the participant during this research study at the Canada sites will be performed under the oversight of the McGill University Faculty of Medicine Institutional Review Board. The participant will sign an informed consent which will describe the safety plan for all research data. The data collected from all study procedures performed at the Canada sites will be de-identified and transferred on to computer disks with limited access. A copy of each participant's de-identified data will be shipped to the University of Florida site. The study code key, study documents and these disks will be kept for a maximum of 10 years at the Canada site following per policy after which they may be destroyed.

**Possible Benefits:**

Subjects may or may not benefit from participating in this study. They could potentially benefit from taking minocycline and see improvement in their hypertension.

Incidental Findings:

Research scans are not subject to clinical review. However, any incidental finding will be communicated with the participant and if requests the participants primary physician.

**Conflict of Interest:**

None.

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