**Study Protocol**

**Project Title:**

Effect of Minocycline Treatment on Drug-Resistant Hypertensive Patients

Subtitle: Brain-Gut Microbiome-Immune Axis in Hypertension

**Investigators:**

Principal Investigator Carl J. Pepine, MD
Co-Investigator- Mohan, Raizada, PhD
Co-Investigator-Eileen Handberg, PhD, ARNP-BC
Co-Investigator – Dana Leach, DNP, ARNP-BC
Co-Investigator-Steven Smith, PharmD, MPHCo-Investigator – Margaret Lo, MD
Co-Investigator – Osama Dasa, MD

**Sites:**

University of Florida (Site 1)
College of Medicine
Department of Surgery
Gainesville, Florida

UF Health Shands Hospital (Site 2)
Gainesville, Florida

UF Health Springhill Cardiology Clinic (Site 3)
Gainesville, Florida

Autonomic Reflex Laboratory (Site 4)
Jewish General Hospital
Montreal, Canada

Brain Imaging Centre (Site 5)
Montreal Neurological Institute
Montreal, Canada

**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: (i) perform an open-label dose-range study to determine the lowest effective minocycline dose to lower BP, (ii) perform a double-blind, placebo-controlled cross-over study in a larger cohort of subjects; (iii) test whether the antihypertensive effect of minocycline is associated with a decrease in activated microglia in CNS autonomic region as compared to "control" patients without a diagnosis of neurogenic (treatment-resistant) HTN.

**Research Plan:**

The research will involve three separate studies with the same inclusion and exclusion criteria except for Study 3 which will recruit Two (2) "control" patients without a diagnosis of neurogenic (treatment-resistant) HTN for imaging comparison.

***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.
- (For Study 3 Participants only) Willing to travel to Montreal, Canada for specialized imaging of the participant's brain using magnetic resonance imaging (MRI), positron emission tomography (PET) scanning, Autonomic Nervous System Testing and blood drawing- if participant qualifies.

***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.

**Study 1 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 Study 1 will be performed at Sites 1-3:

Study 1 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.

Study 1 Description: Thirty- five (35) 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

Study 1 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 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 dose that provides with the highest number of responders will be selected for Study 2, the double-blind placebo controlled phase (see below).

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 and (3) ABPM differences in heart rate variability as a measure of autonomic nervous system activity. Pseudo-resistance will be prospectively defined as mean ABPM SBP <130 mm Hg, despite elevated office SBP readings. Participants will be graded according to baseline-dipping pattern into 4 groups: extreme dippers (nighttime BP fall >20%, dippers (nighttime BP fall >10% and <20%), nondippers (nighttime BP fall <10% and >0%), and reverse-dippers (nighttime BP > daytime BP). The BP will be considered at target when daytime and nighttime values were <135/85 and <120/70 mm Hg, respectively.

The proportion of participants who are extreme dippers (decline >20% in night to day BP), dippers (10-20% decline in night to day BP), non-dippers (<10% decline in BP at night), and reverse dippers (night BP >day BP) will be calculated at baseline and at study end-point for responders. Participants in each group will be further categorized according to tertiles of baseline ambulatory SBP. Baseline and 6 month ABPM measurements will be determined for each group.

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. Consequently, the dose that achieves a cumulative total of 27 responders will be used for the crossover study (Study 2). Similarly, if the 75% criteria is achieved using the lower dose only, then the trial will be considered as complete and no higher doses will be tested." The sample size carries an 8% standard error in estimating the proportion for a given dose.

**Study 2 will determine whether minocycline reduces BP and peripheral inflammation in patients with neurogenic hypertension using a double blind, placebo-controlled, crossover study design.**

All study procedures for Study 2 will be performed at Sites 1-3 Study 2 Rationale: A double-blind crossover study using the dose of minocycline found to be optimal in Study 1 will be conducted. The "optimal" dose will be the dose resulting in the larger number of responders from Study 1 based on a daytime SBP reduction of ≥5 mmHg measured through ABPM. The inclusion and exclusion criteria will be applied to a new group of patients, along with Study 1 subjects, who will be screened for participation.

Study 2 Description: Fifty-six (56) 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. Consented participants will be placed in Scheme 1 or Scheme 2 study plans. For scheme 1, participants will be placed on minocycline for 16 weeks followed by 16 weeks placebo with an additional three week "wash out" between treatments. For Scheme 2, participants will be placed on placebo for 16 weeks followed by 16 weeks of minocycline with a three week "wash out" period between treatments.

Study 2 Research Visit and Procedures:

**Visit 1 Baseline 1 (Day 0)**

- Medical history and medication review will be performed.
- Brief Physical exam will be performed.
- Office SBP, DBP and PP will be performed.
- Female participants will have a pregnancy test performed to verify a negative result before continuing.
- Blood drawn (4 tablespoons approximately). 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 flow cytometry analysis and additional biomedical tests.
- 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.
- 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 14 ± 3 days)**

- Review of medications and hospitalizations will be performed.
- 24-hour ambulatory blood pressure monitor (ABPM).
- Randomization to either Group A or Group B.
    - Group A
        - Minocycline (dose to be determined by Study 1) for 16 weeks
        - 3 weeks of no study drug (wash out period)
        - Placebo (an inactive substance) for 16 weeks
    - Group B
        - Placebo for 16 weeks
        - 3 weeks of no study drug
        - Minocycline (dose to be determined by Study 1)

- Dispense study medication (depending on which group the participants are placed into)
- 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 (Week 16, ±7 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,
- Review HBPL.
- 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.
    - Blood draw (4 tablespoons approximately).
    - 24 hour ambulatory blood pressure monitor (ABPM).

No study drug will be dispensed at this visit.

**Visit 4 (Week 19, ± 7 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,
- Review HBPL.
- Dispense study medication (depending on which group the participants are placed into)
- 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.
- Blood draw (4 tablespoons approximately).
- 24 hour ambulatory blood pressure monitor (ABPM).

**Visit 5 (Week 35, ±7 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,
- Review HBPL.
- 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.  Physical examination and vital signs.
- Blood draw (4 tablespoons approximately).
- 24 hour ambulatory blood pressure monitor (ABPM).

No study drug will be dispensed at this visit.

**Visit 6 (Week 38, ±7 days, Final Visit)**

- 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.
- Review of HBPL.
- 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.
- Blood draw (4 tablespoons approximately).

Sample size requirements for the cross-over study are based on a two-sided two-sample t-test assuming a standard deviation of 12, and with level of significance 0.05. We have greater than 0.80 power to detect a daytime SPB difference of ≥5mmHg (from ABPM measurements) with a sample size of twenty-four (24) in each dose group. To allow for a 15% dropout rate, the study plans to randomize a total of fifty-six (56) subjects.

**Study 3 - will determine whether Minocycline decreases microglia activation in the PVN and other autonomic brain regions of patients with resistant NH as compared to "control" participants without a diagnosis of** neurogenic (treatment-resistant) HTN**.**

Study 3 Description: Nine (9) adult patients who are resistant to hypertensive medication and meet all inclusion criteria and no exclusion criteria will be offered an opportunity to participate in this study. Participants may have completed participation in study 1 or they are currently being recruited to participate in study 1. Study procedures will be reviewed with the participant and

once written consent is obtained the subject will be enrolled into the study. Study 3 for patients taking study medication will include up to two (2) trips to Montreal, Canada to undergo specialized imaging of the brain. All imaging studies will be performed at the Brain Imaging Centre of the Montreal Neurological Institute (MNI), in Montreal Canada, using established protocols. The MNI is one of only 14 centers worldwide that hosts a high-resolution research tomograph (HRRT) which will allow PET imaging of small brainstem nuclei and has established the synthesis of microglia ligands. These studies will be done to determine how the brain is affected by this medication and how it impacts blood pressure. . Two (2) patients will be recruited as "controls" (without a diagnosis of neurogenic (treatment-resistant)HTN and have not been treated with Study drug. Control participants will be asked to make one(1) trip to Montreal, Canada to undergo specialized imaging of the brain. All imaging studies will be performed at the Brain Imaging Centre of the Montreal Neurological Institute (MNI), in Montreal Canada, using established protocols. The MNI is one of only 14 centers worldwide that hosts a high-resolution research tomograph (HRRT) which will allow PET imaging of small brainstem nuclei and has established the synthesis of microglia ligands. These studies will be done as a comparison with patients who have taken study medication and have a diagnosis of neurogenic (treatment-resistant)HTN in order to compare how the brain is affected by study medication and how it impacts blood pressure between the two groups. This is described in more detail below.

Sympathetic activity in HTN patients will be done by autonomic function tests (such as ratio of standing BP to supine BP, immediate HR response to standing (30:15 ratio), HR variation with respiration, Valsalva maneuver will be performed. The primary study endpoint will be a reduction in microglia activation.

Study procedures for participants currently being recruited to participate in Study 1 and Study 3:

AND/OR

Study Procedures for participants who have completed participation in Study 1 and have not taken minocycline for 2 months will be approached to enroll in Study 3.

**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.

**Prior to starting study medication:** Participants will be asked to travel to Montreal Canada to have specialized imaging of the brain performed. Upon completion of the first trip to Montreal, Canada, participants will return to the UF site and continue study visits as outlined in study 1.

**After Study 1 Visits are complete:** After completion of study 1, or after achieving blood pressure lowering on Minocycline as determined by the Investigator the participant will return to Montreal, Canada for repeat specialized imaging of the brain. Upon completion of the second trip, participant will return to the UF site for their final visit.

**Final Visit**

- 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.

**Study 3 Procedures:**

Travel: All travel arrangements will be made by the University of Florida, Cardiology Division and the research staff will assist the subject in getting prepared for the trip. There will be no expense to them for airfare, hotel, meals, and travel to and from the airport. Any incidentals will be at their own discretion.

Informed Consent at Canadian Site: Participants will also be asked to provide research informed consent for the procedures that will occur at the Canadian Sites. This portion of the research will be overseen by the McGill University Faculty of Medicine Institutional Review Board, Montreal, Canada.

MRI: The MRI will be performed on a 3 Tesla Siemens Trio scanner at the baseline visit and the final study visit. T1 weighted volumetric data-sets with 1mm resolution for co-registration with the PET data to localize the brain structures of interest will be acquired.

PET: Prior to scanning, two fine needle-catheters will be inserted into the veins of the participant arm. One will be used to administer small amounts of a radioactive substance, the other to take four blood samples of 10 mL, equal to two teaspoons each. A portion of the blood drawn will be

used to determine blood levels related to the imaging and a portion of the sample will be used to determine if a specific genetic component is present that may affect the imaging so that all participants can be categorized according to the specific genotype group that they belong to. This will allow proper interpretation of the PET-image results. The radioactive substance the participant will receive prior to commencing the study medication and then at the end of the study is $^{11}$C-PBR28. This substance is labelled with the short-lived radioactive atom Carbon 11 (physical half-life = 20 minutes). The dose of $^{11}$C-PBR28 you will receive is 600 MBq. The participant will receive this dose initially at the baseline visit and then again at the end of the study. MBq is short for megabecquerels, a unit used to measure how much radioactivity there is in a sample; the quantity the participant will receive is towards the low end of what is used for diagnostic clinical tests performed on a regular basis in Nuclear Medicine. The substance being injected to perform the PET study is not currently approved for general human use in Canada; however, its use for research purposes is permitted by Health Canada.

PET scanning will be performed at the baseline visit and the final study visit. The PET scans will be performed on a CTI/Siemens HRRT scanner in 3D mode (63 parallel planes, resolution at 1cm from the center: 4.4mm radial, 5.1mm axial). All Participants will receive an intravenous injection of 600MBq of $^{11}$C-PBR28 at each scanning session for a total of 1200MBq's over an iv line. Prior to injection of the tracer, the participant will be brought inside the scanner and a transmission scan will be performed. This transmission scan will only be used to record the position of the participant's head in the scanner and is needed for attenuation correction emission scans. After this transmission scan of approximately 10 minutes, the participants will be injected with 600MBq of $^{11}$C-PBR28 and a dynamic scan will be performed over 90 minutes. Venous blood samples will be taken in the steady state phase following radiotracer injection. During the $^{11}$C-PBR28 PET scan, four venous samples at 30, 50 ,70 and 90 minutes (about 3 tablespoons) will be drawn. The subject is the allowed to leave the scanner for 35 minutes. After 80 minutes, the subject will once again be placed in the scanner and three frames from 90-120 minutes will be performed followed by a transmission scan.

Since a genetic polymorphism can influence the binding affinity of the TSPO-receptor for $^{11}$C-PBR28, all participants will be genotyped for this polymorphism and DV/plasma ratios will be corrected according to the respective genotype groups. A one-time blood sample (about half a table spoon) is required to perform this genotyoping and will be taken when the IV line is inserted at the time of the baseline scan. This is only necessary at the baseline scan. This blood sample will be sent to the McGill University and Genome Quebec Innovation Centre for analysis. The blood sample will be stored there for the duration of the study. After this time, the blood sample will be destroyed. The sample will be used only for the purposes of this research protocol.

The scanning session will take up to four hours to complete, during which time the participant will be asked to lie still on the mattress in the scanner. All procedures during the PET study will be carried out by a qualified nuclear medicine technician, and supervised by a qualified nuclear medicine physician.

Blood Draws: Blood will be drawn (3 tablespoons) during the PET scan to determine metabolite corrected plasma activity, and average plasma activity will be used to calculate simplified distribution volume (DV)/plasma ratios. Since a genetic polymorphism can influence the binding

affinity of the TSPO-receptor, all participants will be genotyped for this polymorphism and DV/plasma ratios will be corrected according to the respective genotype groups.
Autonomic Function Testing:

The Autonomic Nervous System Testing will take place at the Autonomic Reflex Laboratory of the Jewish General Hospital. Prior to the test, the participant's height and weight will be obtained. The participant will then be required to lie on a special table that can be tilted to a standing position. Electrodes will be placed on the participant's chest and abdomen to record heart function. The participant will wear a finger cuff that continuously measures blood pressure. A small device will be placed near the participant's nose to record breathing. An elastic headband will be placed around the participant's forehead to measure brain blood flow and oxygen. The participant will then lie quietly for 10 minutes. The tilt table will then be placed upright for 30 minutes. After the 10 minutes of standing, the tilt table will be returned to the lying position. After a few minutes of rest, the participant will be asked to take several slow deep breaths for about 1 minute. The participant will then be asked to blow into a tube on 2 separate occasions for 15 seconds each time. During these breathing tests the medical staff will continue to measure blood pressure and heart function. The tilt table test and the breathing tests are all routinely used in clinical practice to diagnose participants with low blood pressure or other autonomic nervous system disorders.

Participants will be assessed at the autonomic lab at the Jewish General Hospital. A standard test battery that is used on a daily basis to assess clinical cardiovascular and sudomotor autonomic function will be performed using standard non-invasive techniques. The battery comprises cardiovascular response 40min of 80° head-up tilt, followed by response to Valsalva maneuver and rhythmic deep breathing. During these tests beat-to-beat BP will be measured from the finger using photoplathysmography, cerebral blood flow (transcranial Doppler and NIRS) and HR from routine 3-lead EKG. Postganglionic sympathetic sudomotor function will be assessed from 4 standard sites using the QSART. The total duration of testing is about 2h including participant preparation, device placement, calibration and actual testing.For the second part of the test, small capsules will be placed on the participants forearm, leg and foot. These capsules will be filled with a liquid containing a drug called acetylcholine. The capsules will be connected to a small battery and for 5 minutes the participant will feel a mild tingle or buzzing sensation over the area of the capsule. This test causes sweating over the area covered by the capsule. The sweating lasts about 15 minutes and the medical staff will measure it for 10 minutes. The capsules are then removed. This test is called a QSART and is also routinely used to diagnose participants with autonomic nervous system disorders.

The PET and MRI will take place at the Brain Imaging Centre of the Montreal Neurological Institution. These tests will take place over one or possibly two days, whichever is easier to schedule.

The Autonomic Nervous System Testing will take place at the Jewish General Hospital, also in Montreal and will take approximately 1 ½ to 2 hours.

Study 3 Data Analysis Plan: T1 MR images will be used to localize the respective nuclei in the brain and to place volumes of interest for quantitative PET analysis. (cite Heiss WD et al

Metabolic Rates in Small Brain Nuclei Determined by High-Resolution PET, J Nucl Med 2004 v45 p1811).

PET Images will be reconstructed using a filtered back-projection algorithm (Fourier rebinning and 2D backprojection with Hanning filter: kernel FWHM=3 mm), supplied by the manufacturer, with a Hanning filter cut-off frequency of 0.4 cycles/pixel. After correction for scatter, attenuation and random coincidences, image volumes comprising 21 frames with matrix dimensions of 128x128x63 voxels and a voxel size of 2.06 x 2.06 x 2.43 mm will be obtained. For each participant, PET images will all be registered to the T1 weighted anatomical MRI in native space for localization purposes. For integration and coregistration of the different image modalities, a new software package (VINCI 2.55, http://www.mpifnf.de/vinci/) developed at the Max-Planck-Institute for Neurological Research in Cologne will be used. This tool has been developed for integration of multimodality imaging data sets with positioning information from neuronavigation systems (like the one used in this study). Using this tool, all relevant parameters can be measured at defined points within the coregistered imaging volumes to test the specific hypotheses.

The DV/plasma ratio for all brain regions in each subject at baseline will be compared to the DV/plasma ration at follow up using a one-factor repeated measures ANOVA on ranks (Friedmann test) with respective post-hoc tests for pairwise comparisons. A similar non parametric repeated measures design will be used for assessing differences in the physiological parameters acquired during the autonomic testing.

We expect participants will exhibit increased $^{11}$C-PBR28 binding in the autonomic brain regions (paraventricular nucleus (PVN), rostral ventrolateral medulla (RVLM), nucleus of the solitary tract (NTS), etc.) prior to treatment. This should appear as a well-defined area of increased radioactivity. These areas of increased $^{11}$C-PBR28 would show a decreased uptake on the follow-up scan 52 weeks after initiation of minocycline. We do not anticipate any difficulty in subject recruitment or expect any significant side effects of minocycline.

**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. “Control” patients in study 3 will not take this medication..

**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.**

**MRI**: During the MRI-study, participants will be exposed to a strong magnetic field. No long-term negative side effects have been observed from this type of study. Participants with transdermal patches will have to remove the patch before the procedure and replace the patch after the procedure. If necessary, the respective drug will be substituted orally or intravenously for the duration of the scan. If substitution or interruption of transdermal drug application is not possible, these participants will not be included in the study. The MR is very noisy and the subjects will wear headphones to reduce this effect. Claustrophobic subjects will not be included.

**Positron Emission Tomography (PET):**

Participants may experience some discomfort caused by the insertion of the fine needle catheter into their veins used to inject the radioactive substance as well as to draw blood. Although the taking of the blood sample causes no serious problems for most people, it can cause some bleeding, bruising and/or discomfort at the site where the needle is inserted. Also, participants may experience some discomfort as a result of lying on the mattress in the scanner for a long time without moving.

The main risk of taking part in this study is exposure to radiation from the short-lived tracer substance injected for the PET scan. The administered radioactive substance $^{11}$C-PBR28 will expose patients to a maximal dose of 4mSv per scan, total of 8mSv for the two scans according to our best scientific estimates. This small dose of radiation 8mSv, is above that which participants are inevitably exposed to in daily life (natural radiation in the environment, cosmic rays, etc.), or to that which participants might receive for medical reasons (diagnostic x-rays, radiation therapy). Nationally accepted limits on radiation doses which can be administered for research purposes have been defined (50 mSv/year), and in order to ensure that participants do not go above the recommended limit participants must make sure to let the investigators know about any other research protocol that participants might have been part of that would have involved radiation exposure, as well as to mention the current protocol, if participants do take part in it, to any investigator asking participants to take part in another protocol in the future. Most of the radioactivity participants will receive will be gone from their body in a matter of hours. The risk which is alluded to when discussing risk associated with radiation exposures of the level seen in PET scanning (specifically in the current study, the radiation exposure is

estimated at 8mSv) is that of developing a cancer at some point in the future, which would not have happened if participants had not received that radiation dose. Although radiation clearly increases the risk of developing cancer over certain doses, its ability to do so at the levels used in PET imaging has never been observed, is certainly at most very low and could conceivably not even exist.

The substance being injected to perform the PET study is not currently approved for general human use in Canada. However, its use for research purposes is allowed by Health Canada.

**Autonomic Nervous System Testing:** The most important risk is the potential for syncope during tilt testing. As the participant is continuously monitored during the test, the prodrome to syncope is easily detectable and the test can terminated by returning the table to the supine position. Objective criteria for test termination include: decline of abrupt decline of heart rate by more than 20 BPM or a decline in systolic pressure or 30 mmHg. In addition continuous monitoring of cerebral blood flow with transcranial Doppler and NIRS ensures that cerebral perfusion in the up-right position is adequate at all times during the test.
A minor skin irritation can occur from application of the EKG electrodes or during the iontopheresis of acetylcholine during QSART testing.

**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

**Contraindications:**

**PET Study:**

The following are contraindications for this procedure: If you are pregnant or breast feeding, under 18 years of age, previous radiation absorbed doses received within the past (12 months) from other experiments that would lead with inclusion of this study, to an aggregate radiation absorbed dose exceeding 20 mSv.

**MRI Study:**

The following are contraindications for this procedure: The presence of a cardiac pacemaker, an aneurysm clip, heart / vascular clip, prosthetic valve, metal prosthesis, metal in your eye or body, tattoos, body piercing or dental braces, of if you suffer from acute claustrophobia. Pregnant and breast feeding women should not undergo an MRI. Transdermal patches are also a contraindication. If you need to have a patch on most of the time, you should bring one with you for application after the scanning session. It is imperative that you fill out a detailed questionnaire aimed at identifying any contraindications prior to undergoing this procedure.

**Effects of participation in this study on your health:**

Both the PET and MRI scans should not interfere with any treatment or other diagnostic test you may require.

**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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