# Protocol NCT01502787

## Title: Attenuation of Angiotensin II-Mediated Vasoconstriction in Hypertension with Nebivolol

## Investigators


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**Participating Institutions:** Univ. of Texas Southwestern Medical Center

**Funding:** Forest Research Institute

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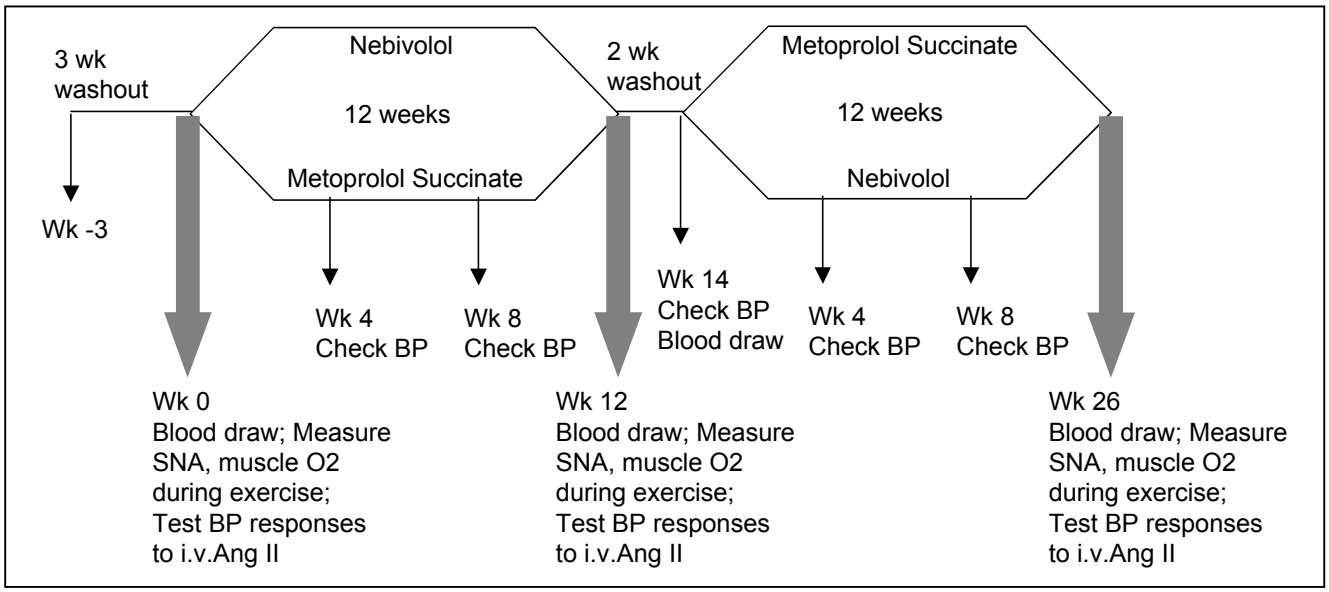
**Objectives:**

The overall objective of this study is to determine if Nebivolol a) attenuates angiotensin II-induced increase in oxidative stress, thereby attenuating angiotensin II-induced vasoconstriction and blood pressure elevation and b) attenuates sympathetic mediated vasoconstriction during exercise, thereby reducing functional skeletal muscle ischemia in hypertensive patients.

**Schema**

In 25 untreated hypertensive subjects, we will measure sympathetic nerve activity (SNA) with direct intraneural recording of sympathetic action potential (microneurography), skeletal muscle oxygenation (Near Infrared spectroscopy), plasma F2-isoprostanes, BP, forearm blood flow (high-resolution ultrasonography), forearm vascular resistance (FVR, the ratio of mean arterial pressure to forearm blood flow), cardiac output (noninvasive impedance plethysmography), total peripheral resistance (TPR, the ratio of mean arterial pressure to cardiac output) in untreated stage I hypertensive subjects at baseline, during handgrip exercise, and during infusion of intravenous Ang II. Then, we will randomize our subjects to receive either 3 months of Nebivolol or Metoprolol, using a crossover design. There will be a 2-week washout period between the two treatments. We will assess SNA and skeletal muscle oxygenation during handgrip exercise and compare the dose response curve of Ang II-induced increase in plasma F2-isoprostanes, FVR, TPR, and BP at baseline, after 3 months of Nebivolol, and after 3 months of Metoprolol treatment in the same subjects.



## PROTOCOL FLOWCHART

Visit 1: Inform consent described and signed by subject, check BP, perform physical examination, obtain ECG, and draw 10 cc of blood for Na, K, glucose and creatinine (Cr).

↓

Visit 2 (1-4 wks later): Draw 30 cc of blood for plasma isoprostanes renin, and angiotensin II. Measure SNA, skeletal muscle oxygenation at rest, during LBNP, and during exercise plus LBNP. Measure FVR, TPR, BP at baseline and after intravenous Ang II infusion. Then, randomize to Nebivolol 5 mg once a day, or Metoprolol succinate 100 mg once daily.

↓

Visit 3 (4 wks later): obtain BP (sitting / standing), If SBP < 90 mmHg or DBP < 60 mmHg, reduce the dose of medications by 50%. If SBP is still ≥ 130 or DBP is ≥ 80 mmHg, increase nebivolol to 10 mg/day or Metoprolol succinate to 200 mg once daily.

↓

Visit 4 (4 weeks later): obtain BP (sitting / standing), If SBP < 90 mmHg or DBP < 60 mmHg, reduce the dose of medications by 50%. If SBP is still ≥ 130 or DBP is ≥ 80 mmHg, increase nebivolol to 20 mg/day or Metoprolol succinate to 300 mg once daily.

↓

Visit 5 (4 wks later): Draw 30 cc of blood for plasma isoprostanes renin, and angiotensin II. Measure SNA, skeletal muscle oxygenation at rest, during LBNP, and during exercise plus LBNP. Measure FVR, TPR, BP at baseline and after intravenous Ang II infusion. Then, stop the first medications.

↓

Visit 6 (2 weeks later): obtain BP (sitting / standing). Draw 30 cc of blood for plasma isoprostanes renin, and angiotensin II. Obtain FVR, TPR, BP at baseline. Start drug treatment in the remaining arm.

↓

Visit 7 (4wks later): Repeat procedure in visit 3

Visit 8 (4wks later): Repeat procedure in visit 4

Visit 9 (4wks later): Repeat procedure in visit 5. End of study.

## Background

In normal physiologic states, muscle contraction during exercise blunts alpha-adrenergic mediated vasoconstriction in order to maintain blood flow to the exercising muscles, a concept known as "functional sympatholysis" [1,2]. Studies in animals [3,4] and normal volunteers [5] by Drs. Thomas and Victor from our institution indicated a major role of nitric oxide (NO) derived from neuronal nitric oxide synthase (nNOS) in the skeletal muscle in the prevention of sympathetic vasoconstriction. Recent study in hypertensive rats indicates presence of impaired functional sympatholysis related to Angiotensin II-induced increase in oxidative stress, which inactivates NO [6]. This adverse effect of Ang II can be reversed by superoxide scavenger, tempol [6]. Studies in hypertensive patients in our laboratory also recently confirm presence of impaired functional sympatholysis during rhythmic handgrip exercise, resulting in augmented skeletal muscle hypoxia, as evidenced by Near Infrared (NIR) Spectroscopy. Whether Nebivolol restores increase in forearm blood flow during exercise and improves skeletal muscle oxygenation by inhibiting Ang II-induced superoxide formation remains unknown.

Angiotensin II has also been shown to promote peripheral vasoconstriction and elevated blood pressure (BP) in both hypertensive and normotensive subjects by increasing circulating levels of oxidative stress [7,8]. This effect of angiotensin II (Ang II) was reversed by concomitant infusion of antioxidant Vitamin C in healthy volunteers [8]. Previous study also demonstrated that Ang II-induced BP elevation was attenuated by oral administration of carvedilol, but not by metoprolol in patients with congestive heart failure [9]. Mechanisms underlying this observation is unknown but could be mediated by antioxidant effects of carvedilol. Nebivolol has been shown to inhibit Ang II-induced superoxide formation by inhibiting NADPH-oxidase in rats [10] and decrease markers of oxidative stress in hypertensive patients [11]. However, efficacy of Nebivolol in attenuating increase in oxidative stress, vasoconstriction, and hypertension during Ang II infusion has not been previously assessed in hypertensive patients.

## Agent/Device

1. Nebivolol
Trade Name: Bystolic
Source: Forest Pharmaceuticals, Inc
Pharmacology: Nebivolol is a beta1 selective adrenergic receptor blocker approved by the FDA for treatment of hypertension (2.5-40 mg/day). Nebovolol lacks intrinsic sympathomimetic activity at clinical relevant doses with no alpha-1 adrenergic blocking activity. Mechanisms of antihypertensive effects of beta blockers in general include 1) peripheral inhibition of beta 1 receptors leading to decreased cardiac output, 2) a central effect leading to reduced sympathetic outflow to the periphery, and 3) suppression of renin activity. Nebivolol may also reduce BP by increasing endothelium-dependent release of nitric oxide in hypertensive patients, which was not observed with other beta blockers [12].

Toxicity: In placebo-controlled clinical trials comparing nebivolol with placebo, discontinuation of therapy due to adverse events was reported in 2.8% of patients treated with nebivolol and 2.2% of patients given placebo. The most common adverse events that led to discontinuation of nebivolol were headache (0.4%), nausea (0.2%) and bradycardia (0.2%). Other adverse events associated with higher dose than recommended by the FDA include bronchospasm and heart block.

2. Metoprolol Succinate
Trade name: Toprol XL
Source: Astra Zeneca

Pharmacology: Toprol XL is a beta1 selective adrenergic receptor blocker approved by the FDA for treatment of hypertension (25-400 mg/day). Mechanisms of antihypertensive effects of beta blockers in general include 1) peripheral inhibition of beta 1 receptors leading to decreased cardiac output, 2) a central effect leading to reduced sympathetic outflow to the periphery, and 3) suppression of renin activity.

Toxicity: Tiredness and dizziness have occurred in about 10 of 100 patients. Depression has been reported in about 5 of 100 patients. Mental confusion and short-term memory loss have been reported. Headache, somnolence, nightmares, and insomnia have also been reported. Shortness of breath and bradycardia have occurred in approximately 3 of 100 patients. Cold extremities; arterial insufficiency, usually of the Raynaud type; palpitations; congestive heart failure; peripheral edema; syncope; chest pain; and hypotension have been reported in about 1 of 100 patients. Wheezing (bronchospasm) and dyspnea have been reported in about 1 of 100 patients. Musculoskeletal pain, blurred vision, decreased libido, and tinnitus have also been reported.

3. Angiotensin II
Tradename: N/A
Source: Merck Biosciences, Switzerland
Pharmacology: Angiotensin II causes direct vasoconstriction via angiotensin receptor subtype I, resulting in elevated BP. Angiotensin II also stimulates release of aldosterone from adrenal gland, resulting in increased sodium absorption and potassium secretion from the renal tubules. Angiotensin II will be administered intravenously at the dose of 1, 2, and 3 ng/kg/min for 15 minutes at each dose to test vascular sensitivity in hypertensive patients.

IND # 53,558
Toxicity: Potential side effects are hypertension and hypokalemia. However, the dose of 3 ng/kg/min of Angiotensin II has been administered to more than 400 untreated essential hypertensive patients and was shown to increase mean arterial pressure by 3-20 mmHg [13-15]. None of these subjects experienced any complication from the drug. A higher dose of Angiotensin II of 5 ng/kg/min has been administered to patients with hypertension from aldosterone producing adenoma on 3 separate days without complications [16]. The increase in mean arterial pressure in that study was between 10-20 mmHg [16].

## Eligibility Criteria

Experiments will be performed in stage I (140-159/90-99 mmHg) untreated hypertensive subjects, age 18-65, without target organ disease.

Criteria for inclusion of subjects

(1) Patients with stage 1 primary hypertension (BP between 140-159/90-99 mmHg) without treatment.

Criteria for exclusion of subjects

(1) Congestive heart failure or coronary artery disease.
(2) Blood pressure averaging >159/99 mmHg or resting heart rate < 55 bpm
(3) Serum creatinine > 1.4 mg/dL
(4) Asthma or chronic obstructive pulmonary diseases
(5) Left ventricular hypertrophy by echocardiography or ECG
(6) Pregnancy
(7) Hypersensitivity to beta blockers or angiotensin
(8) Any history of substance abuse (other than tobacco)
(9) Concomitant drug treatment which raise endogenous nitric oxide levels such as nitrates or phosphodiesterase V inhibitors (Viagra, Levitra)
(10) History of symptomatic bradycardia or heart block

## Material and Data to be accessioned:

**Visit 1 (week # –3 to -1):** Complete history will be taken and a physical examination will be done. Laboratory safety screen, height, weight, sitting blood pressure, vital signs, urine pregnancy tests, and urinalysis will be obtained by the investigators in all subjects prior to entering the study. Laboratory safety screen will include electrocardiogram, random blood glucose, BUN, creatinine, total bilirubin, electrolyte, alkaline phophatase, SGOT, and urinalysis. The presence of inclusion criteria and absence of exclusion criteria will be verified and documented on the case report form. Subjects who receive pharmacologic treatment of hypertension will be required to all stop antihypertensive medications for at least 3 weeks prior to the study. After medications are stopped for 2 weeks, these subjects will be asked to return to laboratory for BP check. Subjects with BP of ≥ 160/100 mmHg will be excluded from the study and they will be instructed to resume their medications. Subjects who are not on hypertension treatment during visit 1 can proceed to visit 2 without delay.

**Visit#2 (week #0):** Subjects who meet inclusion/exclusion criteria of the study will undergo baseline evaluation during this visit. During visit 2, each subject will undergo the following procedures:

1) Microneurographic Recording of Muscle Sympathetic Nerve Activity. Multiunit recordings of postganglionic SNA will be obtained with unipolar tungsten microelectrodes inserted selectively into muscle nerve fascicles of the peroneal nerves using the microneurographic technique of Valbo et al [17]. The nerve signals are amplified, filtered (bandwidth 700-2000 Hz), rectified and integrated to obtain a mean voltage display of SNA. The criteria for an acceptable recording of muscle SNA are: 1) electrical stimulation through the microelectrode evokes twitch contractions of the peroneal muscles without paresthesias; 2) stretching the muscles or tendons elicits mechanoreceptor discharge; 3) the inter-burst interval is equal to or a multiple of the cardiac cycle length, indicating baroreceptor modulation; and 4) the activity increases during the decrease in arterial pressure produced by phases II and III of the

Valsalva maneuver and decreases during the overshoot in pressure produced by release of the maneuver (phase IV). Sympathetic bursts will be detected by inspection of the filtered and mean voltage neurograms. A deflection on the mean voltage display is counted as a "burst" if it has a minimal signal to noise ratio of 2:1. The interobserver and intraobserver variations in identifying bursts are <10% and < 5% [18]. When a given subject is studied on repeated occasions, the intra-subject variability is less than 15% [19].

2) Measurement of Skeletal Muscle Oxygenation during exercise by Near Infrared (NIR) Spectroscopy: The NIR method is based on the principle that laser light with wavelengths in the 700–900 nm range penetrates tissues with relative ease, and is absorbed by the iron-porphyrin moieties in hemoglobin and myoglobin. Changes in NIR light absorption are proportional to changes in the relative concentrations of oxygenated hemoglobin and myoglobin (HbO2+MbO2). To monitor the tissue absorption of NIR light, two fiber optical bundles will be placed on the skin over the left flexor digitorum profundus muscle, which is the main muscle recruited during handgrip exercise. NIR signals at four different wavelengths will be sequentially sampled at a rate of 1 Hz, converted to optical densities by using known algorithms, and stored digitally for analysis (NIRO 500, Hamamatsu Photonics, Hamamatsu City, Japan).

Skeletal muscle oxygenation will be measured at rest and during lower body negative pressure application to evoke reflex sympathetic activation. The subject's lower body will be enclosed in a negative pressure chamber to the level of the iliac crest. Pressure inside the chamber will be measured by a Statham transducer (Gould, Oxnard, CA). Lower body negative pressure (LBNP) at -20 mmHg simulates mild orthostatic stress (e.g., the transition from the supine to the seated position). Then, subjects will be asked to perform 5 minutes of rhythmic handgrip exercise at 30% of maximal voluntary contraction. During the last 2 minutes of exercise, we will apply lower body negative pressure (LBNP) to activate sympathetic nerve activity.

Blood pressure, SNA, heart rate, respiration, handgrip force, and NIR signals will be recorded in response to 2 min of LBNP applied at rest and during the 3rd and 4th min of each 5-min exercise period. Each exercise period will be followed by at least 2 min of forearm circulatory arrest during inflation of a pneumatic cuff on the upper arm to 250 mmHg to establish the maximal decrease in muscle tissue oxygenation. The total labile signal will be defined as the difference in tissue oxygenation in the forearm at rest and during sustained circulatory arrest. Changes in the NIR signals in response to LBNP alone and LBNP plus handgrip exercise will be expressed as a percentage of the total labile signal.

3) Assessment of vascular sensitivity to angiotensin II: To avoid confounding influence of sodium intake on BP, each subject will be given diet containing 200 mmol sodium, 100 mmol potassium, 800 mg calcium, and 200–300 g carbohydrate for 7 days prior to the study. On the day of the study, two intravenous catheters will be inserted in antecubital veins (one in each arm) for infusion of angiotensin II and measurement of levels of isoprostanes, renin, and angiotensin II. Forearm blood flow (FBF) will be measured with high resolution Doppler Ultrasonography (Phillips HDI 5000, Bothell, WA, USA) with a 12 MHz linear array transducer. All 2D imaging and Doppler data will be digitized into our computer and analyzed off-line, using an automatic wall-tracking system (Vascular Analysis Tools, 5.0, Medical Imaging Analysis, Coralville IA). Beat-to-beat MBV will be calculated by integrating the total area under the Doppler waveform divided by RR interval. Forearm vascular resistance (FVR) will be calculated as mean arterial pressure (MAP) divided by forearm blood flow. BP will be measured noninvasively with oscillometric method (Welch Allyn, Vital Signs moitor). Cardiac output (CO) will be measured noninvasively by thoracic electrical bioimpedance (Bioz, CardioDynamics International Corporation). Stroke volume will be derived from change in impedance/time measured during electrical systole. Cardiac output will be determined as the product of stroke volume and heart rate. Total peripheral

resistance (TPR) will be calculated by multiplying mean arterial pressure (MAP) by 80 and dividing the product by CO.

Changes in MAP, FVR, TPR, renin, plasma $F_2$-isoprostanes, and angiotensin II will be assessed at baseline and in response to intravenous infusion angiotensin II of at the dose of 1, 2, and 3 ng/kg/min for 15 minutes at each dose.

**Treatment**
After visit#2, each subject will be randomized to receive Nebivolol at the dose of 5 mg once daily or Metoprolol succinate (Toprol XL) 100 mg once daily.

**Visit#3 (4 weeks later):** Subjects will return for BP check. If BP is < 90/60 mmHg or resting heart rate < 50 bpm, the dose of drug will be reduced by 50%. If BP > 130/80, the dose of Nebivolol will be increased to 10 mg and Toprol XL will be increased to 200 mg once daily.

**Visit#4 (4 weeks later):** Subjects will return for BP check. If BP is < 90/60 mmHg or resting heart rate < 50 bpm, the dose of drug will be reduced by 50%. If BP > 130/80, the dose of Nebivolol will be increased to 20 mg and Toprol XL will be increased to 300 mg once daily.

**Visit#5 (4 weeks later):** Subjects will return for measurement of SNA and skeletal muscle oxygenation at rest, during LBNP, and during exercise plus LBNP. Measurement of MAP, FVR, TPR, renin, plasma isoprostanes, and angiotensin II at baseline and in response to intravenous infusion angiotensin II of at the dose of 1, 2, and 3 ng/kg/min each for 15 minutes at each dose will be repeated as outlined in visit #2. Then the medications will be stopped for 2 weeks. This duration of washout period was chosen because previous crossover studies have demonstrated it to be adequate to allow detection of the difference in endothelial function between Nebivolol and other beta blockers without carryover effect.[12,20]

**Visit#6 (2 weeks later):** Subjects will return for measurement of MAP, FVR, TPR, renin, plasma isoprostanes, and angiotensin II at baseline only. Then subjects will be given BP medications in the remaining arm (either Toprol XL 100 mg once daily or Nebivolol at the dose of 5 mg once daily).

**Visit#7 (4 weeks later):** Subjects will return for BP check. If BP is < 90/60 mmHg or resting heart rate < 50 bpm, the dose of drug will be reduced by 50%. If BP > 130/80, the dose of Nebivolol will be increased to 10 mg and Toprol XL will be increased to 200 mg once daily.

**Visit#8 (4 weeks later):** Subjects will return for BP check. If BP is < 90/60 mmHg or resting heart rate < 50 bpm, the dose of drug will be reduced by 50%. If BP > 130/80, the dose of Nebivolol will be increased to 20 mg and Toprol XL will be increased to 300 mg once daily.

**Visit#9 (4 weeks later):** Subjects will return for measurement of SNA and skeletal muscle oxygenation at rest, during LBNP, and during exercise plus LBNP. Measurement of MAP, FVR, TPR, renin, plasma isoprostanes, and angiotensin II at baseline and in response to intravenous infusion angiotensin II of at the dose of 0.5, 1, and 3 ng/kg/min for 30 minutes at each dose will be repeated as outlined in visit #2 and 5. End of study.

***Potential Risks:***
The potential risks are related to: a) microneurography, b) venous cannulation, c) intravenous infusion of angiotensin II, d) administration of nebivolol and metoprolol, e) lower body negative pressure, f) handgrip exercise and forearm circulatory arrest during inflation of a pneumatic cuff.

a) The potential risks of microneurography: 10% of subjects may experience transient leg tiredness, or increase sensitivity to touch (hyperesthesia), motor weakness in the leg lasting less than 1 week. Since 1979, microneurography has been performed on more than several thousand subjects without permanent complications related to procedure.

b) The risks of intravenous catheters are infection, bleeding, and vasovagal reaction.

c) The potential risks of infusing i.v. angiotensin II (IND #53,558) are hypertension and hypokalemia with chronic infusion.

d) The potential risks of nebivolol and metoprolol are hypotension, bradycardia, fatigue, dizziness, and depression.

e) Potential risks of lower body negative pressure application is vasovagal syncope if negative pressure is applied at -40 mmHg or lower for a prolonged period of time. However, in our study we will applied pressure at the lowest level of –20 mmHg for less than 5 minutes. Therefore, vasovagal syncope is unlikely to occur.

f) Potential risks of handgrip exercise and forearm circulatory arrest during inflation of a pneumatic cuff are muscle ache or cramp.

Protection Against Risks:

a) Microneurography will be performed under the supervision of Dr. Vongpatanasin who has extensive experience. A stimulus isolation unit (special electronic circuit) will be used to prevent electrocution from the recording apparatus. Subjects will be given a microneurographic questionnaire to report any adverse reactions occurring up to one week after the study.

b) All subjects will have a routine history and physical examination performed by the physician-investigators. In addition, hospital medical records will be reviewed prior to the initiation of these studies.

c) ACLS-certified personnel, will be present during all experiments. Resuscitative equipment and drugs will be available in the laboratory. Blood pressure and the subject's heart rate will be monitored continuously during the microneurographic experiments. Infusions of angiotensin II will be discontinued if mean arterial pressure decreases or increases by more than 20 mmHg above the baseline level.

d) During treatment with antihypertensive medication with Nebivolol and Metoprolol, patients will be seen at the cardiovascular physiology research laboratory for monitoring of BP.

e) Venous cannulation will be performed by an experienced nurse practitioner, Debbie Arbique, RN.

**Procedures to Maintain Confidentiality**

1. Information will be given only to the physician-investigators.
2. The nature of the information concerns pertinent medical and social history.
3. The purpose of the disclosure is solely for research purposes, in particular to determine whether potential subjects meet criteria for inclusion or exclusion and to identify specific subgroups of subjects.
4. The subject's right of confidentiality will be given strict priority. No mention of the subjects' identities will be made either directly or indirectly in oral or written presentation of this work. The investigators, who are physicians, will be responsible for providing medical care in the event of any adverse effects to the subjects.

5. All medical and biographical information will be held strictly confidential and no disclosures of personal identity will be allowed unless specifically requested by the subject. Copies of executed consent forms, as well as the experimental log book, will be kept in a locked file cabinet in our research laboratory.

## Evaluation Criteria

The study is designed to evaluate SNA, skeletal muscle oxygenation, BP, FVR, and TPR, and neurohormonal responses to exercise and angiotensin II during treatment with two antihypertensive medications which are already approved by the FDA for treatment of hypertension. The study is not designed to evaluate survival, efficacy, or toxicities of a new drug. The maximal dose of each drug used in this study is described under treatment section above.

## Off-study Criteria

**Stop Points:** The following will constitute stop points indicating permanent discontinuation of study drug by participant and scheduling of close out visit.

- Withholding of study medication for > 4 weeks for whatever reason.
- Any reason including serious adverse events that results in participant's inability to continue with study protocol and procedures.
- Severe hypertension defined as persistent elevation of systolic BP > 180 mmHg or diastolic BP > 110 mmHg on 3 consecutive measurements after randomization
- Refractory hypotension defined as persistent systolic BP < 90 mmHg
- Depression requiring drug treatment or psychiatric evaluation
- Asthma or brochospasm
- Symptomatic bradycardia with heart rate persistently below 50 beats/min
- Pregnancy
- Death

**Conditions for temporarily halting study medications:** These include 1) BP < 90/60 mmHg; c) Severe uncontrolled hypertension in the outpatient setting defined as elevation of systolic BP > 180 mmHg or diastolic BP > 110 mmHg on two consecutive occasions measured at least 48 hours apart despite effort to lower BP by diet or pharmacologic intervention.

**Conditions for permanently halting study medication in an individual:** A stop point will be defined as any serious adverse event that in the opinion of the Principal investigator could be reasonably attributed to the study intervention and could be made worse or recur by continued administration of the study medication. At this time the participants study medication will be permanently discontinued and a close out visit will be scheduled.

**Conditions for stopping the protocol entirely:** Conditions for stopping the entire protocol are 1) cumulative incidence of severe uncontrolled hypertension (>180/110 mmHg) or heart rate < 50 bpm of > 50% of the study cohort after a minimum of 10 patients have been randomized to study drug, (2) any evidence of myocardial ischemia or infarction (chest pain, EKG changes), (2) any new onset of sustained cardiac arrhythmia including heart block, atrial fibrillation, supraventricular tachycardia, ventricular tachycardia, or ventricular fibrillation; (3) any death. Under such circumstances, the study will not resume without IRB approval.

## Registration and Study Monitoring

The PI will immediately report all unexpected and any serious adverse events and the recommendations derived from data and safety monitoring (such as continuation or conclusion of the trial) to the IRB.

The PI will submit copies of adverse events to the IRB.

The PI will anonymize all reports of local adverse events submitted to the IRB to protect a subject's privacy.

The PI will educate all key personnel about the requirements to report unexpected and serious adverse events to the IRB.

## Statistical Methods

SAS (SAS Institute, Cary, NC) statistical software will be used for statistical analyses, particularly PROC MIXED for linear models. Model assumptions such as normality and covariance structure will be carefully assessed. Data transformations or nonparametric tests may be performed to meet analysis assumptions. Hypothesis tests will be two-sided using the 0.05 significance level. Bonferroni type adjustments for multiple testing will be implemented to control Type I errors as necessary.

### *Sample size, power estimates, statistical method*

Power calculations were based on the within-person variability of the angiotensin II infusion rate required to raise mean arterial pressure by 20 mm Hg (Pd20). These values were previously determined by studies in our laboratory and others [21]. Normotensive men had intra-individual coefficients of variation that averaged 18% for angiotensin II [21]. Assuming a greater BP variability in hypertensive patients' of approximately 25%, it was estimated that a crossover study of 20 patients would have 80% certainty of demonstrating a 15% difference in cardiovascular reactivity between nebovolol and toprol XL at the alpha level of 0.05.

To allow for 25% attrition during the study, total of 25 subjects will be enrolled in the study. Treatment order will be randomized using blocked randomization.

Statistical method: Descriptive statistics will be used to summarize the responses over infusion time for each phase of this study. To compare responses between the study phases, repeated measure analysis of variance models with repeated factors for both study phase and infusion time will be constructed. Polynomial contrasts will be constructed to compare the linear trends or slope of the MAP, TPR, and FVR responses to infusion of Ang II over time between the study phases. A between group factor will be included in these models to assess treatment order effects. A mixed linear model approach will be used for repeated measures analysis that allows flexibility in the specification of the covariance structure over time and can accommodate missing data in most situations.

## Gender/Minority Analysis:

## Inclusion of Women

Approximately 50% of our subjects will be women.

## Inclusion of Minorities

We are over-sampling non-Hispanic Blacks because (1) prevalence of hypertension in the U.S. are higher in blacks than nonblacks, (2) the class of drugs we are studying is thought in general to be more effective in lowering blood pressure in blacks than other ethnic groups. Our aim is to obtain a 50:50 split in ethnicity between non-Hispanic blacks ("blacks") and all other ethnicities ("non-blacks"). The non-black group will be ~60% white, 35% Hispanic, and 5% other.

## Inclusion of Children

Children will not be included because: (1) the safety of microneurography in children has not been established and children are not likely to undergo successful microneurographic procedure which requires staying still in bed while keeping the leg absolutely motionless for 3-4 hours to allow adequate nerve recording, (2) the safety of administration of angiotensin II is unknown, and 3) children are much more likely to have secondary hypertension than adults.

## Targeted/Planned Enrollment Table:

**Study Title: Attenuation of Angiotensin II-Mediated Vasoconstriction in Hypertension with Nebivolol**

**Total Planned Enrollment:** 25

| **Ethnic Category** | **Sex/Gender** | | |
|---|---|---|---|
| | **Females** | **Males** | **Total** |
| Hispanic or Latino | 2 | 2 | 4 |
| Not Hispanic or Latino | 11 | 10 | 21 |
| Ethnic Category Total of All Subjects* | 13 | 12 | 25 |
| **Racial Categories** | | | |
| American Indian/Alaska Native | 0 | 0 | 0 |
| Asian | 0 | 0 | 0 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 |
| Black or African American | 7 | 6 | 13 |
| White | 6 | 6 | 12 |
| Racial Categories: Total of All Subjects * | 13 | 12 | 25 |

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