A Placebo-Controlled Crossover Trial Evaluating NO Production and Effect of L-NMMA on Orthostatic Intolerance Following Bedrest

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A. Study Purpose and Rationale

The primary objectives of this trial are to determine (1) whether upregulation of nitric oxide (NO) production occurs during bedrest; and (2) whether the nitric oxide synthase (NOS) inhibitor L-NMMA can prevent the development of orthostatic tolerance following bedrest.

B. Background and Hypotheses

Adaptation to spaceflight induces deleterious physiologic changes, most notably affecting the cardiovascular system. During the exposure to microgravity entailed by spaceflight, central and cephalad redistribution of blood volume occurs, heart rate diastolic blood pressure decrease and total peripheral resistance increases. Orthostatic intolerance is one of the most common and problematic sequelae of exposure to microgravity. Two-thirds of astronauts returning to 1G failed post-flight stand testing in one study, some after spaceflight of only 2 days. Numerous mechanisms, including increased venous compliance of the lower extremities, reduced plasma volume and baroreceptor changes, have been proposed to explain the development of orthostatic intolerance; however no clear consensus has emerged.

Head-down bed rest is a useful simulation of microgravity. Bed rest studies varying in length from days to months have demonstrated similar physiologic changes, including central and cephalad redistribution of blood volume and the development of orthostatic intolerance. A technique called rodent hindlimb unweighing has been used as an animal model to mimic both human bed rest and the zero gravity conditions of space flight. Rat studies using this model have shown both an upregulation of nitric oxide synthase, the enzyme that produces nitric oxide (NO) and decreased vascular responsiveness to the vasoconstrictor norepinephrine. Given these findings and our emerging understanding of the importance of NO as a physiologic mediator of vascular tone, we hypothesize that upregulation of nitric oxide synthases (NOS) in response to microgravity also occurs in humans, forming a clinically meaningful part of the development of orthostatic intolerance that can be blocked by administration of the NOS inhibitor L-NMMA.

C. Study Design and Statistical Analysis

This is a prospective, interventional, placebo-controlled, crossover trial enrolling 10 healthy volunteers.

All subjects will undergo 2 ½ days of testing. On Day 1, volunteers will be screened for exclusion criteria and randomized to treatment day. An intravenous catheter will be placed in a forearm vein of all eligible subjects, who will then begin a continuous infusion of L-NMMA and 10 minutes of horizontal bed rest. Following this induction of NOS inhibition, subjects will undergo 60-degree head-up tilt (HUT) with graded application of lower body negative pressure (LBNP). Continuous blood pressure and heart rate monitoring will be performed throughout the protocol. Time measurement will commence with the start of the head-up tilt and will stop at the development of symptomatic hypotension.

The subjects will return for Days 2 and 3. On both days, an intravenous catheter will again be placed in a forearm vein and placebo will be infused. All subjects will undergo LBNP-HUT on each experiment day prior to bedrest, and time to symptomatic hypotension will be recorded. Following

LBNP-HUT, subjects will undergo 24 hour 5-degree head-down bed rest, during which time they will not get up. Ten minutes prior to the completion of bedrest and the commencement of LBNP-HUT, they will begin continuous infusion of either L-NMMA or placebo. Half of the subjects will receive L-NMMA on Day 2 and half will receive L-NMMA on Day 3. Following bed rest, subjects will again undergo 60-degree head-up tilt with graded application of lower body negative pressure and time to symptomatic hypotension will be recorded.

Endpoint.

The primary endpoint of this trial is the occurrence of symptomatic hypotension.

Analyses.

Blood pressure differences between groups (with and without L-NMMA, with and without bedrest) will be analyzed using repeated measures analysis of variance (ANOVA). Time to symptomatic hypotension will be analyzed using both student's t-test and Kaplan-Meier survival curve.

D. Study Procedures

The following procedures are performed for research purposes.

a. Screening

All subjects will be screened on Day 1, at least 30 minutes prior to dosing. Screening will consist of a baseline medical history and physical exam.

b. Dosing

A peripheral intravenous line will be established. The study drug will be mixed and the infusion pump prepared. The antidote, intravenous nitroglycerin, will also be prepared so that it will be immediately available for possible emergent administration. The study drug or placebo will be administered as a continuous infusion at a prespecified rate over 70 minutes.

c. Lower Body Negative Pressure-Head Up Tilt

This is a new test of orthostatic tolerance combining two previously used techniques, which generates adequate orthostatic stress to induce syncope in at least 83% of healthy subjects. Subjects will undergo a standard protocol consisting of 20 minutes of 60-degree head-up tilt (phase 1); then, while remaining in the head-up tilt position, lower body negative pressure is applied at -20mmHg for 10 minutes (phase 2) and –40mmHg for 10 minutes (phase 3). Termination of LBNP-HUT occurs at the completion of phase 3 or the development of symptomatic hypotension; at this time the subject is returned to the horizontal position and LBNP is stopped. This test has a high degree of repeatability, with one study demonstrating average intrasubject differences in time to syncope of 1.1 min +/- 0.6 min.

d. Bedrest

Five-degree head-down bedrest has been demonstrated to approximate the physiologic adaptations seen in astronauts exposed to microgravity, including cephalic redistribution of blood volume and salt and water diuresis leading to contraction of plasma volume. Subjects will be asked to arrive at the laboratory at 11:00AM, having last eaten no sooner than 9AM. Following the pre-experiment LBNP-HUT, they will be asked to void and then recline on a padded tilt table, which will be tilted five degrees head-down. They will not get up during the next 24 hours, urinating into bed pans or urinals as necessary. They will be permitted to engage in activities like reading, writing, television viewing and eating, which can all be pursued in the head-down position.

E. Study Drug

The investigational drug N^G-methyl-L-arginine (L-NMMA) is an arginine analogue that inhibits nitric oxide synthase in vascular endothelial tissue, likely by competitive inhibition of endogenous L-arginine. The drug will be obtained as the acetate salt from Sigma Chemical Co. and reconstituted in sterile saline solution for systemic administration in a continuous infusion through a peripheral intravenous line.

L-NMMA has been used in a variety of animal models, including dogs, rats and rabbits, in systemic and regional infusions with doses ranging from 0.3mg/kg to 300mg/kg. Administration was associated with transient increases in peripheral vascular resistance but no adverse effects were reported.

L-NMMA has been extensively in humans as a regional infusion in the brachial artery:

L-NMMA has also been used systemically in humans:

1. Haynes et al (1993)—L-NMMA was administered intravenously at a dose of 3mg/kg over 5 minutes to 8 healthy subjects. Mean arterial pressure was increased by 10%, heart rate was decreased by 19%, cardiac index was decreased by 25% and total peripheral resistance was increased by 46%. These physiologic effects were maximal at 10-15 minutes after starting L-NMMA infusion and there were no adverse events reported.
2. Habib et al (1994)—L-NMMA was administered intravenously in 12 patients being investigated for heart failure as an escalating dose from 4□mol/min for 5 min to 32□mol/min for 5min. Eight patients achieved the maximal cumulative dose of 300□mol; three patients stopped dose escalation because of a rise in systolic pressure and one patient stopped after experiencing a decrease in cardiac output. All four patients had heart failure. There were no adverse symptoms noted and no subjects developed ischemia.

F. Medical Device

No medical devices will be used in this study.

G. Study Questionnaire

No questionnaires will be used in this study.

H. Study Subjects

We expect to enroll ten healthy volunteers to participate in this study.

a. Inclusion criteria

- include age 18 years old or greater and ability to comprehend and sign the informed consent document.

b. Exclusion criteria

- include a history of cardiac dysrhythmias, coronary artery disease, congestive heart failure, diabetes mellitus, marked gastroesophageal reflux disease or other systemic illnesses. Patients taking medications other than oral contraceptive agents will be excluded or asked to discontinue those medications for at least one week prior to the study.

I. Recruitment of Subjects

Subjects will be recruited through public advertisement of the study.

J. Confidentiality of Study Data

Subject records will be kept confidential and stored in a locked facility accessible only to members of the research team. Study information may be reviewed by the research team and potentially by the FDA. Data resulting from this study may be used in scientific publications or presentations but patients' identity will remain confidential.

K. Potential Conflict of Interest

There is no conflict of interest.

L. Location of the Study

Study information will be collected in the inpatient unit of the Irving Center for Clinical Research, utilizing the tilt lab.

M. Potential Risks

Potential risks include the following:

a. L-NMMA administration

In patients with septic shock associated with either a MAP consistently less than 70mmHg for at least 30 minutes (despite fluid resuscitation to a pulmonary artery occlusion pressure of greater than 12mmHg) or a requirement for vasopressor support (either greater than 0.1 g/kg/min of norepinephrine or an equivalent dose of another vasopressor agent) to maintain a MAP of 90mmHg or less, administration of L-NMMA in a phase III prospective, randomized, double-blind, placebo-controlled trial was associated with a significantly increased incidence of mortality, necessitating early termination of the study. Significant adverse events have not been reported in studies with healthy volunteers. L-NMMA has been reported to elevate mean arterial blood pressure in healthy subjects; in studies using a dose of 3mg/kg, MAP increased 10 +/-4%. A theoretic possibility exists that L-NMMA administration could induce hypertensive urgency or pulmonary hypertension.

b. Antidote

Systemic administration of IV nitroglycerin, which is not affected by L-NMMA, should reverse any adverse effects of the study drug.

c. LBNP-HUT

Syncope and transient hypotension are expected outcomes of this trial. At the onset of symptomatic hypotension, LBNP is immediately terminated and subjects are placed in a horizontal position with subsequent spontaneous restoration of normotension. Rarely, seizure-like activity can be seen following syncope.

d. Intravenous line placement

Placement of an intravenous line always carries the risk of infection, bleeding or discomfort. These risks are minimized by careful IV catheter placement by a skilled health care professional under sterile conditions.

N. Potential Benefits

There is no direct benefit to the subjects.

O. Alternatives

The alternative to participating in this study is to not participate.

P. Compensation to Subjects

Subjects will be monetarily compensated for participation in this study in a pro-rated fashion. They will receive $50 for completion of Day 1, $150 for completion of Days 1 and 2, and $300 for completion of the entire protocol.

Q. Costs to Subjects

There will be no costs to subjects for participating in this study.

R. Minors as Research Subjects

No minors will be enrolled in this study.

S. Radiation or Radioactive Substances

No radiation or radioactive substances will be used in this study.

T. References

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