UCLA Health System
Radiology

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Research Study Protocol v1

Study Title: NOrth american Study for the Treatment of Recurrent epIstaxis with doxycycLine: The NOSTRIL Trial

Study Design: Prospective, Randomized (1:1), Double blind placebo controlled crossover study

Proposed In-life Start: 07-01-2017

Funding Source: Department of Radiology (Exploratory Grant)

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1. Introduction

a. Background and significance

Hereditary Hemorrhagic Telangiectasia (HHT), also called Osler-Weber-Rendu syndrome, is an autosomal dominant disorder characterized by multi-systemic vascular abnormalities. The condition is associated with fragile telangiectasias and arteriovenous malformations (AVMs), which can affect the lungs, liver, brain, and mucosal surfaces. These abnormalities can result in severe and potentially fatal complications, including stroke, brain abscess, heart failure, and pulmonary hypertension. Although it is a rare condition, there is a large affected population with an estimated incidence of 1 in 5000-8000 people.

The most common manifestation of HHT is recurrent epistaxis, which often develops before age 10 and is eventually seen in nearly all HHT patients. Epistaxis results from telangiectasias of the nasal mucosa, which are fragile and can spontaneously rupture. Complications related to epistaxis in HHT include chronic iron deficiency anemia, severe blood loss requiring emergent blood transfusion, and in some cases death. Data also shows that epistaxis is a significant contributor to decreased quality of life in HHT, with HHT patients identifying epistaxis as a priority issue for HHT research. Current therapies for HHT epistaxis include both medical and surgical interventions. Medications that may have benefit include estrogens, progestogens, and antifibrinolytics such as tranexamic acid. These medications, however, are associated with concerning and poorly characterized side effects and their benefit is generally inconsistent. Relevant procedural interventions include laser cauterization, septal dermoplasty, embolization, and nasal closure. Disadvantages of these approaches include their invasive nature and associated risks, as well as a generally temporary benefit.

Anti-angiogenic agents are being increasingly used to control bleeding in HHT patients, since the fundamental problem in HHT is abnormal angiogenesis. Reduced activation of TGF-β and increased levels of vascular endothelial growth factor (VEGF) results in unregulated angiogenesis and leads to formation of disordered and fragile blood vessels on the mucosal surfaces, particularly in the nose and gastrointestinal tract, which are prone to spontaneous and recurrent bleeding (1). Anti-angiogenic agents such as bevacizumab and thalidomide have been used with great success to reduce the formation of these abnormal vessels and reduce bleeding in HHT patients (2-7). Therapeutic effect has been seen even at low doses of these agents, suggesting that the telangiectasias of HHT may be particularly sensitive to anti-angiogenic agents (8,9). However, the existing anti-angiogenic therapies are expensive, difficult to administer, and hard to tolerate due to frequent side effects. There is great need for a drug which can replicate these anti-angiogenic effects but which is well-tolerated, orally-administered, inexpensive and readily available.

Data suggests that doxycycline, a common medication used for decades in the treatment of bacterial infections, may have potential in the treatment of HHT epistaxis. Recent studies reveal that doxycycline has potent anti-angiogenic properties, inhibiting matrix metalloproteinases (MMPs) which function to allow endothelial cells to migrate during angiogenesis. These anti-angiogenic properties have been demonstrated in multiple in-vivo and in-vitro studies (10-14). Doxycycline seems to effect anti-angiogenesis not only by matrix metalloproteinase inhibition, but also by reducing the

effect of VEGF (15) and inhibiting pro-angiogenic macrophages (16). Multiple in-vitro studies have demonstrated suppression of tumor growth and vascularization in cell lines exposed to doxycycline, including lung, cervical, ovarian, pancreatic, and colorectal cancers (17-21).

The potential of doxycycline to suppress angiogenesis and reduce nosebleeds has been previously recognized and studied in a pilot study (22). A retrospective analysis of seven HHT patients treated with oral doxycycline demonstrated that epistaxis severity score (ESS) significantly decreased with treatment, from a pre-treatment ESS of 4.9 to a post-treatment ESS of 2.6. This almost 50% reduction in nosebleed severity underscores the anti-angiogenic properties of doxycycline and supports the potential of this therapy in HHT.

The NOSTRIL study will be the first prospective, randomized trial to assess the control of epistaxis in HHT with prophylactic doxycycline. This therapy has the potential to dramatically improve the health and quality of life of patients with HHT.

2. Objectives and Specific Aims

The objective of this study is to determine whether prophylactic oral doxycycline is effective in reducing epistaxis in Hereditary Hemorrhagic Telangiectasia (HHT).

We will perform a comparative, blinded, randomized (1:1) controlled trial to treat HHT patients with nosebleeds with doxycycline versus placebo. This project has the following specific aims:

1. Determine the effect of doxycycline treatment versus placebo on the frequency, duration, and severity of nasal bleeding in HHT. Bleeding will be quantified in a systematic manner using the Epistaxis Severity Score (ESS), a validated and objective tool for the assessment of epistaxis severity. We will further quantify bleeding using a patient nosebleed diary including frequency, duration and severity of nosebleeds.

2. Determine the effect of doxycycline treatment versus placebo on quality of life in HHT. Data has shown that recurrent epistaxis significantly reduces quality of life in HHT, and HHT patients have identified epistaxis as a key focus for future research in the treatment of the disease. We will evaluate quality of life using the objective, validated Short Form-12 (SF-12) Health Status Questionnaire.

3. Determine the effect of doxycycline treatment versus placebo on objective laboratory measures of bleeding, including hematocrit, hemoglobin, and ferritin.

4. Determine the effect of doxycycline treatment versus placebo on the need for transfusion in HHT. Transfusion is used in cases of severe, life-threatening bleeding, and in many cases is required in HHT-related epistaxis. We will compare the quantitative units of packed red blood cells (PRBCs) required with and without doxycycline therapy.

Hypothesis: Treatment with doxycycline results in significant improvement in HHT epistaxis compared to placebo, which is manifested by reduced clinical bleeding, better quality of life, improvement in objective laboratory values such as hemoglobin levels, and reduced need for blood transfusion.

3. Study Design and Dose Rationale

a. Study Design Rationale

A randomized (1:1), prospective, cross-over design will be used. This guarantees that all participants will receive treatment with the treatment drug, which is important since all patients enrolled will have significant epistaxis and desire treatment. An identical design was used in a previous study of epistaxis reduction in HHT patients (23).

A one-month wash-out period between placebo and treatment drug was selected based on doxycycline pharmacokinetics and mucosa turnover rate. Doxycycline is almost completely absorbed orally with bioavailability of 95% and a time to peak concentration of about 3 hours. The half-life of the drug is about 14 hours, with elimination in both the urine and stool. Thus, the drug will be almost completely absent one week after the last dose (12 half-lives). The nasal mucosa, which could be affected by the drug, regenerates approximately every 5 days (24). Thus the biologic effect of the drug should be virtually absent by 12 days after the last dose. To ensure complete clearance of the drug and its effects between therapy and placebo, an additional two weeks were added, making a total one-month wash-out period.

Subjects who cannot present in person to the clinic due to the COVID-19 virus will conduct the follow-up visit over the phone.

Based on preliminary data (14) the average epistaxis severity score (ESS) for the patients we will recruit is 4.9 ± 2.2. The preliminary study found that doxycycline treatment reduced the ESS by an average of 2.4 ± 1.2 points. Thus, a sample size of 20 patients from the cross over design will achieve 90% power to test the mean differences in ESS between doxycycline and placebo at alpha of 5% using a two-sided t-test. To consider the possible dropout or missing (approximately 10% rate), a total sample size of 22 patients will be randomized. This was the same sample size used in the previous cross-over trial of a different medication in HHT patients with epistaxis (23).

b. Dose Rationale

The dose of doxycycline used in this study will be 100 mg PO BID. This is the standard dose of the drug for anti-bacterial use, and thus the pharmacokinetics and drug safety profile are best known at this dose. The preliminary trial of doxycycline in HHT patients used the same dose (14), as did several clinical trials of doxycycline for treatment of cancer and lymphangioleiomyomatosis (24,25).

4. Study procedures

This trial will be performed with UCLA Institutional Review Board approval and in accordance with the Declaration of Helsinki, with all patients providing written informed consent.

a. Study Design:

i. This is a single-center, prospective, randomized (1:1), controlled, cross over, double-blinded study

ii. Total N = 22 Patients

iii. The following information will be collected during the study:

1- Demographic information

2- Medical history
3- Physical Examination
4- Concomitant medication
5- Adverse events
6- Vital signs
7- SF-12 quality of life questionnaire
8- ESS score
9- Labs

iv. Eligible patients will have a diagnosis of definite HHT by the Curacao criteria or a positive DNA test for HHT (relevant mutation in the genes coding for SMAD-4, ALK-1, or endoglin). Other inclusion criteria include age 18 or older, avoidance of other experimental therapies during the trial, and significant epistaxis (minimum of 3 bleeding episodes per week, total duration of nosebleeds at least 15 minutes per week). Female patients with childbearing potential will require a negative pregnancy test on Day 1 of the trial and at the Crossover visit. Female patients with childbearing potential will also agree to use birth control during the study from Day 1 of the trial (First and Second arm) and for 28 days following cessation of the study medications (Placebo and Doxycyclyne). Patients will also be excluded if they have used certain medications within 14 days prior to the study, including barbiturates, tegretol, dilantin, warfarin, isotretinoin, and a variety of other medications that are contra-indicated with doxycycline use.

v. Consented subjects will be randomized and blinded to the study medications. Subjects will be randomized to either the Doxycycline arm (100mg PO BID) or to the placebo arm (2 placebo pills/day). After randomization, subjects will have 15 days to 1 month run in period to establish baseline characteristics (bleeding) prior to treatment initiation. As this is a crossover study, subjects will start with one treatment for 2 months followed by a 1 month washout period and then switch to the second treatment for another 2 months and a 1 month follow-up visit.

vi. Both Doxycycline and placebo drugs will be dispensed by the Ronald Reagan UCLA Medical Center (RRUMC) investigational pharmacy. The exploratory grant is covering for the cost of the drugs.

b. Outcomes

The primary outcome measures will be the frequency and duration of epistaxis as determined by a nosebleed diary and the Epistaxis Severity Scale (ESS), a validated objective measure of epistaxis severity. The ESS metric has been recommended for the evaluation of new epistaxis therapies. There will also be a number of secondary measures. First, we will evaluate patient quality of life using the objective Short Form-12 (SF-12) Health Status Questionnaire. Laboratory markers of bleeding will represent an additional secondary outcome measure and include ferritin, hemoglobin, hematocrit and MMP-9. To evaluate for severe bleeding requiring transfusion, we will quantify the units of packed red blood cells (PRBCs) transfused over each one-month period of observation. Finally, we will evaluate for treatment failure, including need for nasal surgery or other epistaxis treatments.

c. Inclusion/Exclusion Criteria

i. Inclusion Criteria
- Age 18 and older
- Diagnosis of definite HHT by the Curacao criteria or positive genetic test for HHT
- Epistaxis severity during the observation month is at least moderate by Epistaxis Severity Score (ESS) evaluation, a validated objective index of bleeding severity
- Female patients with childbearing potential will require a negative pregnancy test on the first day of the trial and at the crossover visit. They will also agree to use birth control during the study medication period (placebo and Doxycycline) and for 28 days post study medication intake. This is due to the fact that doxycycline is a Pregnancy Category D medication and since this is a double blinded study, patients need to be on birth control on both arms

ii. Exclusion Criteria
- Patients will be disqualified for use of certain medications within 14 days prior to the study, including barbiturates, tegretol, dilantin, warfarin, isotretinoin, and several other medications that are contra-indicated with doxycycline use.

5. Monitoring and Safety Mechanisms

a. The study will follow the OHRPP Data Security in Research guidance and procedures for data security. The study data will be coded and no personal information will be recorded.
b. Patient safety monitoring will be performed by the principal investigator. Any events or unforeseen complications during and after the treatment period will be promptly assessed and any required intervention will be performed to minimize any compromise to patient care and safety.

6. Statistical Analysis and Method of Data Analysis

All data will be summarized with descriptive statists (number of subjects, mean, standard deviation, minimum, median, and maximum) for continuous endpoints and frequency and percentage for categorical endpoints.

The differences between outcome measures in the doxycycline group versus the placebo group will be contrasted using Student's t-test for each of the primary and secondary outcome measures. If the outcome measurements do not meet the normality assumption even after a transformation, non-parametric approach of Wilcoxon sign-rank tests can be used to test the differences between doxycycline and placebo. Although we will have enough washout time before cross over to other treatment arm, we can test the effects of sequence and carryover in a model.

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