Protocol Title: A single-center, randomized, double-blind clinical study to evaluate the effect of sulfasalazine on painful neuropathy.

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Detailed Protocol

BACKGROUND AND SIGNIFICANCE

The rationale for our study comes from the fusion of a number of separate observations that we have combined together to generate an exciting and readily testable hypothesis for the treatment of neuropathic pain, such as pain due to nerve injury or diabetic peripheral neuropathy. We first describe the identification of tetrahydrobiopterin (BH4) and its synthesis pathway as an important modulator in pain following nerve injury in animals and humans. We then explain how the FDA-approved drug sulfasalazine was found to inhibit a key enzyme in BH4 production, sepiapterin reductase (SPR). In the last section, we describe an animal study showing a marked beneficial effect of sulfasalazine in a common rodent model of neuropathic pain, streptozotocin-induced painful diabetic neuropathy. Together, these three observations suggest that sulfasalazine may reduce neuropathic pain by inhibiting BH4 production. Our goal for this study is to test whether sulfasalazine reduces pain in subjects with painful diabetic neuropathy.

a. Historical background

Neuropathic pain is defined as pain due to nerve in injury. Common neuropathic pain conditions occur following direct nerve injury such as occurs in trauma and radiculopathy, and painful neuropathy such as painful diabetic neuropathy. Using an animal model of neuropathic pain, Tegeder, Woolf, and their colleagues performed transcription array expression profiling to identify genes that were regulated following peripheral nerve injury (Tegeder et al., 2006). Because the peripheral nerve injury model causes a pronounced and long-standing increase in pain, genes upregulated after nerve injury could be associated with increased pain while genes down-regulated after nerve injury could be associated with decreased pain. The investigators found that several genes in the tetrahydrobiopterin (BH4) synthesis family were differentially regulated such that genes that promoted BH4 production, including the rate-limiting GTP cyclohydroxylase (GCH1) and SPR, were strongly upregulated while genes that reduced BH4 production were downregulated following nerve injury. BH4 itself was markedly increased after nerve injury, and drugs that blocked BH4 increase after nerve injury reduced pain levels. Finally, they found that human haplotypes of GCH1 that produced increased BH4 were pain-promoting while haplotypes of GCH1 that reduced BH4 were pain-protective.

In a recent screen to identify drug/protein interactions, Chidley and his colleagues identified that sulfasalazine binds to and inhibits the catalytic activity of SPR (Chidley et al., 2011). Interestingly, sulfasalazine, but not other related sulfa drugs such as mesalamine have this activity. Sulfasalazine is an FDA-approved drug with indication for treatment of ulcerative colitis. A long-acting formation is also approved for the treatment of juvenile and adult rheumatoid arthritis. Indeed, sulfasalazine has been found both to reduce inflammatory indices like the

erythrocyte sedimentation rate and to decrease pain levels in rheumatoid arthritis (Suarez-Almazor et al., 2000).

b. Previous pre-/clinical studies supporting the proposed research

In the streptozotocin rodent mouse model of painful diabetic neuropathy, rats develop findings consistent with neuropathic pain such as tactile allodynia, pain in response to nonpainful, low-intensity stimuli. Tactile allodynia is a common feature of patients with neuropathic pain, including painful diabetic neuropathy. Berti-Mattera and her colleagues tested whether or not sulfasalazine could block tactile allodynia in streptozotocin-treated rats (Berti-Mattera et al., 2008). While rats treated with both streptozotocin and sulfasalazine did not withdraw from a more than 12 g stimulus (similar to untreated rats), rats treated with streptozotocin alone withdrew from only a 4 g stimulus. Common anti-inflammatories such as acetylsalicylic acid (aspirin) increased the threshold of streptozotocin treated rats only slightly.

Furthermore, sulfasalazine has been shown to be effective in treating pain due to rheumatoid arthritis (Suarez-Almazor ME et al., 2000). The fact that sulfasalazine has widely been used over long courses emphasizes the safety of the medication.

c. Rationale behind the proposed research, and potential benefits to patients and/or society.

Neuropathic pain is a common, severe, and treatment-refractory chronic disease. Rodent and human data has implicated BH4 production as an important pathway in the development of neuropathic pain. The recent identification of sulfasalazine, a well-tolerated FDA-approved drug, as a potent inhibitor of BH4 production suggests a novel and safe potential way to reduce neuropathic pain, and animal data already supports the efficacy of sulfasalazine in reducing pain due to diabetic neuropathy. We propose to evaluate the potential of sulfasalazine for treating neuropathic pain in humans.

II. SPECIFIC AIMS
a. Specify objective and hypotheses to be tested in the project

To determine whether sulfasalazine reduces pain in peripheral neuropathy:

Based on strong and relevant existing data from both animals and humans, we hypothesize that sulfasalazine treatment will reduce pain due to painful peripheral neuropathy, a type of neuropathic pain.

III. SUBJECT SELECTION
a. Inclusion/exclusion criteria.
Inclusion Criteria:

- Patients with Painful Peripheral Neuropathy
- Age 18 years or older
- Able to provide informed consent
- Current or prior hemoglobin A1c > 6.5% and some degree of glucose control (HgA1c<11) for patients with painful neuropathy from diabetes.
- Michigan Neuropathy Screening Instrument score of at least 3.
- Have a cell phone and be willing and able to receive and reply to text messages (up to 5 text messages incoming and 5 text messages outgoing per day for 21 days of surveying)
- Subjects who have average pain score equal to or greater than 4/10 in daily pain record for the week between the screening and baseline visits will be randomized.

Exclusion criteria:
- Other severe pain
- Depression, other psychiatric disorder, substance dependence or abuse within one year, as determined by the Mini International Neuropsychiatric Interview 6.0 (MINI).
- Any current or historical diagnosis of mania, dipolar disorder or psychosis, as determined by the MINI.
- Anticipated difficulty weaning off of opioid medications. In particular, we will exclude patients taking long-acting opioids such as morphine sulfate sustained release (MS Contin), oxycodone sustained release (Oxycontin), or methadone. Such medications typically require more than one week to wean off.
- Contraindications to sulfasalazine such as:
    - hypersensitivity to sulfasalazine, sulfa drugs, salicylates, or any component of the formulation; porphyria
    - G6PD deficiency (risk of hemolytic hemolytic anemia)
    - hepatic impairment to be defined as LFTs 1.5 times the upper limit of normal.
    - blood dyscrasias – clinically significant anemia (hematocrit < than 32%), thrombocytopenia (platelets < 130,000/mm^3) or leukopenia (WBC < 4,000/mm^3)
    -significant renal impairment (risk of crystalluria) (estimated GFR<40 mL/min)
    - clinically significant asthma or severe allergies
    - Concomitant use of isoniazid or clinically significant immunosuppression due to treatment or disease (including etanercept and HIV).

- Inability to monitor home blood glucose if taking a sulfonylurea such as glimepiride, glyburide, or glipizide, as sulfasalazine may increase the risk for low blood sugar when taken in combination with sulfonylurea.
- Because of the heavy use of questionnaires, some of which have not been validated in languages other than English, we will exclude subjects who are not proficient in reading and speaking English.

- Sulfasalazine is a Pregnancy Class B (FDA) and Class A (AUS) drug. Generally speaking, drugs that fall into either class A or B are considered safe and are often used in pregnancy. Nonetheless, all women of child-bearing potential will have a serum pregnancy test. We will exclude pregnant or breast-feeding women.

b. Source of subjects and recruitment methods.
See section IV.

IV. SUBJECT ENROLLMENT
a. Methods of enrollment, including procedures for patient registration and/or randomization.
We will recruit patients with painful neuropathy using the Research Patient Data Registry (RPDR) and the RSVP for Health registry of MGH and Brigham and Women's Hospital (BWH). We will also distribute flyers at centers attending to patients with painful neuropathy, including relevant clinics affiliated with Partners, Beth Israel Deaconess Medical Center (BIDMC), Boston Medical Center, and Dana Farber Cancer Institute**.** The flyer is informational in nature and will direct interested individuals to contact study staff at MGH where all recruitment, consent and study activities will occur. We will also advertise the study in the local press.

We will review patient records at the MGH Center of Pain Medicine to find potential candidates with a painful neuropathy and a letter with an opt-out card enclosed from the attending physician or clinic chief will be sent to the patient describing the study. A two-week follow up telephone call to potential subjects may occur for those potential subjects who have not returned the opt-out post card.

Potential subjects will be invited to contact the study staff who will explain the protocol in greater detail. If a potential subject is interested, an informed consent form will be sent via USPS or e-mail and the screening visit will be scheduled.

b. Procedures for obtaining informed consent (including timing of consent process).

Subjects interested in participating in the study will be asked to give written informed consent. We will mail/e-mail the informed consent form to potential subjects so that they have at least 24 hours to study the form before enrollment.

When potential participants arrive for their study visit, a physician investigator will explain the study in detail and answer any questions related to study procedures, research purpose, potential risks and benefits for the participant or the consent form.

The subject and the physician investigator will sign the consent form and a copy of the signed form will be given to the subject.

C .Treatment assignment, and randomization (if applicable).
There are two factors to consider in the randomization schema. First, we want the study to be performed in a double-blind manner; that is, both the patient and treating physician will not know whether a particular treatment is drug or placebo. Second, we want to make sure that our results do not suffer from bias due to the order of treatment. For example, patients could potentially respond better to the first treatment. There are two possible permutations, placebo followed by sulfasalazine (group A) and sulfasalazine followed by placebo (group B). We therefore want to maintain equal numbers within the two groups.
To meet these two considerations, we will use block randomization with blocks of 4.

There will be 4 study visits, 6 telephone contacts, and up to 5 text messages per day to complete three week-long pain diaries throughout the course of the study (see schematic).

Participants will be instructed to record their level of pain and medication taking behavior by responding to the text messages they receive each day on their personal cellular phones. Participants who do not respond to the daily pain assessment text message within 15 minutes will receive a second text message reminding them to complete the pain assessment. If participants do not respond to the pain and medication taking behavior assessments for 2 consecutive days (48 hours), a member of the research staff will call to inquire if they are having problems with the system. Staff will encourage participants to complete their assessments as prescribed during study enrollment (see attached text message algorithm and flowchart). If subjects are still unable to respond via text messaging remaining pain assessments will be completed in a manner preferable to the subject, either active phone calls or equivalent written diaries (see attached phone script). Similarly, if a technical problem occurs with the text messaging system, a written diary will be given to subjects to record their answers to these questions.

Visit #1- Screening visit, inclusion/exclusion criteria, history and physical, laboratory testing, including baseline LFTs, Cr, BH4 and CBC with differential to make sure that we are not starting the drug if there is a clinically significant abnormality, determination of pregnancy status followed 2 – 3 days later with a telephone call to discuss findings of laboratory tests, remind subjects of instructions regarding pain diary text messages and medication weaning. HgbA1c will be checked if there is not a documented value in LMR within the prior year.

Wean off current pain medications if necessary (1 week). We will allow patients to continue non-opioid "neuropathic pain" medication typically used for treatment of painful neuropathy including: amitriptyline, carbamazepine, desipramine, duloxetine, gabapentin, imipramine, milnacipran, nortriptyline, pregabalin, oxcarbazepime, sodium valproate, topiramate, venlafaxine. Topical treatments such as capsaicin, diclofenac and lidocaine may not be continued for the trial and must be

stopped. Opioids including tramadol as well as non-steroidal anti-inflammatory drugs may not be continued for the trial.

Acetaminophen will be breakthrough pain treatment and will be documented by subjects responding to pain diary text messages. Use of acetaminophen will be limited to no more than 3 gm per 24-hour period. We will ask that study doctor be notified if any new medications are being considered.

Visit #2 - Baseline visit - collection of initial data, subjects may continue to participate if average pain score >=4 as indicated by subject's pain diary text messages. Provision of study drug. Two weeks course of study drug with collection of data during last week. Completion of pain diary as noted above. Sulfasalazine will be provided to subjects as a two week supply of pills each containing 500 mg of study drug and to be taken as two pills twice a day with meals. This is a typical initial dose for FDA-approved indications. Placebo will be provided to subjects as a two week supply of pills and will be similar in appearance to pill containing sulfasalazine. Placebo will also be taken as two pills twice a day with meals. There will also be research labs drawn at this visit (approximately 15 ml or 1 Tbsp of blood).

Following 14 days of study drug, there will be a one week washout period followed by two weeks of second study drug and collection of data during last week as noted above.

The last study visit will include laboratory testing for safety. We will repeat labs (LFTs and CBC) at the end of the study to confirm that there are no sustained lab abnormalities. Values of interest would be an increase in LFTs to greater than 2 times the upper normal limit or a decrease in WBC or HCT (more than 10% below the lower limit of normal). Results will be conveyed in final telephone contact.

All time intervals, including initial medication wean, baseline assessment, drug treatment periods and washout may be extended up to three days in order to better accommodate subject schedules.

We will also use phone calls to remind patients to take medication.

We will also utilize blood from the samples collected for research related to pain. Depending on the results of the study, we may analyze these samples for protein, RNA, and genetic analysis. Such studies will include quantification of serum BH4 levels, RNA and protein measurements of genes in the BH4 pathway (and control genes), and single nucleotide polymorphism (SNP) analysis to determine correlation with pain assessments, as has been performed in other pain cohorts in the studies documenting the role of BH4 in pain. However, we may also measure additional protein and RNA levels as well as other SNP and sequence analysis.

REDCap will be used in data collection. REDCap (Research Electronic Data Capture) is a free, secure, HIPAA compliant web-based application hosted by the Partners HealthCare Research Computing, Enterprise Research Infrastructure & Services (ERIS) group. Vanderbilt University, with collaboration from a consortium of academic and non-profit institutional partners, has developed this software toolset and workflow methodology for electronic collection and management of research and clinical study data. Data collection projects rely on a study-specific data dictionary defined by members of the research team with planning assistance from Harvard Catalyst | The Harvard Clinical and Translational Science Center EDC Support Staff. The iterative development and testing process results in a well-planned data collection strategy for individual studies. Using REDCap, the research team can also design web-based surveys and engage potential respondents using a variety of notification methods. REDCap provides flexible features that can be used for a variety of research projects and provides an intuitive interface to enter data with real time validation (automated data type and range checks). The system offers easy data manipulation with audit trails, reports for monitoring and querying participant records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

VI. BIOSTATISTICAL ANALYSIS

a. Specific data variables being collected for the study (e.g., data collection sheets).

Daily pain diary, appendix 1. and questionnaires, Brief pain inventory interference scale, Euroquality of Life, Beck Depression Inventory and Patient Global Impression of Change scale comprise the data to be collected.

b. Study endpoints.

- primary endpoint: difference in mean average pain scores, on 11 point scale, averaged over the last week of placebo and sulfasalazine treatment periods (parallel study)

Secondary

- % of patients with >=50% pain reduction
- physical functioning – Brief pain inventory interference scale, Euroquality of Life
- emotional functioning - Beck Depression Inventory (pearsonassessments.com)
- overall improvement – Patient Global Impression of Change scale
- categorical rating of pain intensity (none, mild, moderate, severe)
- documented use of rescue medication (acetaminophen)

Additional secondary endpoints will include mean pain score and the above secondary endpoints including the crossover component of the study.

c. Statistical methods.

We will apply 2-sided t-test to compare mean pain score between the two arms. We will conduct normality test of outcomes. If non-normal distribution is detected, we

will apply nonparametric tests such as the Wilcoxon rank sum test for comparison. In secondary analyses of the cross-over data, we will apply ANOVA with repeated measurements to analyze the effect of sulfasalazine, and to test whether there is any pattern (whether patient is given placebo fist or sulfasalazine first) effect.

d. Power analysis (e.g., sample size, evaluable subjects, etc.)

Our planned sample size of 42 patients (21 per arm) would lead to 80% power to detect a difference in mean pain score using two-sided t-test with alpha of 0.05, assuming standard deviation of 2.2 and mean difference of 2. A standard deviation of 2.2 has been used in other studies of painful neuropathy (Goldstein et al., 2005). Assuming a dropout/ineligibility rate of about 50%, we anticipate that enrollment of 84 patients would yield 42 who complete the study. Reasons for this expected dropout/ineligibility rate are as follows:

- Renal impairment is a contraindication of sulfasalazine. Over 35% of 20+ year olds with diabetes have chronic kidney disease (Centers for Disease Control and Prevention, 2010)
- Those individuals we recruit from RSVP for Health and flyers may not necessarily be part of the Partners Health Care system, meaning we will not be able to pre-screen them via medical records. We won't be able to determine whether or not these individuals have renal impairment until after consenting and blood work.
- High frequency of nausea associated with sulfasalazine (about one-third of treated patients).
- Patients may drop out of the study on account of the relatively long study duration for the cross-over study

VII. RISKS AND DISCOMFORTS (STRATIFY BY COMMON AND UNCOMMON)

a. Complications of surgical and non-surgical procedures, etc.

Not relevant.

b. Drug side effects and toxicities

Sulfasalazine is a safe drug that has been prescribed for many years. We will use 1 g bid, which is the typical initial dose for FDA approved indications. Nonetheless, there are occasional side effects and rare but serious side toxicities.

Side effects (from micromedex):

Common:

Dermatologic: Pruritus (3% to 4%), Rash (3% to 13%)
Gastrointestinal: Abdominal pain (8%), Indigestion (13% to 33%), Loss of appetite (33%), Nausea (19% to 33%), Stomatitis (4%), Vomiting (8% to 33%)
Neurologic: Dizziness (4%), Headache (9% to 33%)
Renal: Discolored urine
Reproductive: Oligozoospermia, Reversible (33%)
Other: Fever (3% to 5%)

Serious:
Dermatologic: Stevens-Johnson syndrome (rare)
Hematologic: Agranulocytosis, Aplastic anemia, Pure red cell aplasia
Hepatic: Fulminant hepatic failure, Hepatotoxicity
Immunologic: Hypersensitivity reaction, Systemic lupus erythematosus
Neurologic: Disorder of the central nervous system, Myoneural disorder
Renal: Kidney disease
Reproductive: Male infertility
Respiratory: Diffuse interstitial pulmonary fibrosis

Risk of Acetaminophen:

Risks of taking acetaminophen (Tylenol) include rare but serious allergic reactions in which there may be swelling of the face, mouth, and throat; difficulty breathing; itching; and rash. Severe liver damage may occur when taking more than 4,000 mg in a 24 hour period or at lower doses when combined with alcohol.

Risk to privacy:

Data will be coded and treated with strict adherence to professional standards of confidentiality and be kept by the investigator under adequate security and restricted access. Specifically, only research team members who are part of this study will have access to this information. All have participated in HIPAA and institution specific training on the importance of confidentiality and are CITI certified. Data obtained from text message responses will be stored with RipRoad, a patient information management program. This program is HIPAA compliant.

Venipuncture:

Subjects will have blood drawn as part of this research study (up to 90 cc.). This can cause pain, bruising, or infection at the spot where the blood was taken. Some people may feel faint when they have blood taken.

Questionnaires:

Subjects occasionally become tired or anxious when completing questionnaires. All subjects will be advised they are free to refrain from answering any questions they do not wish to answer.

c. Device complications/malfunctions
Not relevant.
d. Psychosocial (non-medical) risks
e. Radiation Risks (statement provided by Radiation Safety Committee)
Not relevant.

VIII. POTENTIAL BENEFITS

a. Potential benefits to participating individuals.
Participation in this study will not guarantee any benefit to the subject, although we hope some individuals will experience decreased pain.

b. Potential benefits to society (e.g., increased understanding of disease process, etc.)
We hope that individuals with painful neuropathy will benefit in the future from what we learn in this study.

IV. MONITORING AND QUALITY ASSURANCE
a. Independent monitoring of source data
Because potential risks to subjects are not great, we do not plan to employ a Data Safety Monitoring Board.

The Principal Investigator will assess the quality and completeness of data regularly over the course of the study, and consistent problems will be identified and corrected as needed.

b. Safety monitoring (e.g. Data Safety Monitoring Board, etc.)
Safety of subjects will be discussed actively and longitudinally as needed during the period that the study is active.

Progress and potential safety or other concerns for subjects will be discussed at protocol meetings to be held as needed during the duration of the protocol activity.

c. Outcomes monitoring

d. Adverse event reporting guidelines.
Adverse events and unanticipated problems involving risks to subjects or others will be reported to the PHRC in accordance with PHRC adverse event and unanticipated problems reporting

X. References

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APPENDIX 1:

<u>Chronological List of Visits/ Phone Calls</u>

Visit 1: Initial Clinical Visit with Study Physician

- describe study
- obtain informed consent
- take history
- review existing relevant lab tests (recent HgbA1c, B12, ?HIV)
- brief physical exam and completion of screening instruments:
    - Michigan Neuropathy Screening Instrument (30 min)
    - MINI (Mini International Neuropsychiatric Interview) (15 min)
- obtain initial labs
- subject will be excluded at any step if inclusion condition not met/exclusion criteria identified. For example, if history reveals anticipated difficulty weaning pain medications, patient will not undergo examination and labs
- subject instructed that he will receive phone call in 2-3 days with lab results, and that if appropriate he will begin medication wean after that phone call

Phone Call 1:

- review initial lab tests
- exclude subject if lab tests indicate that patient should not be included
- if patient is to be included, patient is instructed to begin medication wean

Phone Call 2:

- Reminds patient to complete Baseline Pain Diary

Visit 2:

- review subject Baseline Pain Diary with Study Coordinator
- if inclusion criteria met, patient given initial treatment drug
- patient completes Baseline Pain Data Forms (EuroQol 5D, Beck Depression Inventory

Phone Call 3:

- Reminds subject to complete Primary Pain Diary

Visit 3:

- Subject returns completed Primary Pain Diary
- Subject completes Primary Pain Data Forms (EuroQol 5D, Beck Depression Inventory, Patient Global Impression of Change, adverse event and symptoms)
- Subject receives second drug to be started after 1 week washout

Phone Call 4:

- Reminds subject to begin taking second drug

Phone Call 5:

- Reminds subject to complete Secondary Pain Diary

Visit 4:

- Subject returns completed Secondary Pain Diary
- Subject completes Secondary Pain Data Forms (EuroQol 5D, Beck Depression Inventory, Patient Global Impression of Change, adverse event and symptoms)
- Subject has final lab tests

Phone Call 6:

- Review final lab tests
- Arrange subsequent follow up if deemed medically appropriate