Evaluation of Nicotinamide Riboside in Prevention of Small Fiber Axon Degeneration and Promotion of Nerve Regeneration

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Study Protocol including statistical analysis

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1. Abstract

Small fiber neuropathy (SFN) is a type of peripheral neuropathy that affects the small umyelinated fibers, including both somatic innervation of the skin and autonomic nerves. Although diabetes and prediabetes are the two most common causes, up to 50% of all SFN remain idiopathic. Currently there is no effective treatment that prevents it or reverses it through regeneration of nerve fibers.

Recent advances in understanding the molecular machinery that mediates Wallerian degeneration (i.e. degeneration of nerve fibers after physical transection) showed that key players in this pathway (e.g. NMNAT2, Sarm1) and NAD+ metabolites play a similar role in degeneration of axons in a distal-to-proximal manner seen in many peripheral neuropathies including SFN. Pre-clinical studies have shown that rapid depletion of NAD initiates a cascade of molecular events that leads to axon degeneration and that supplementation of a NAD precursor, nicotinamide riboside (NR) can prevent this degeneration.

In this study we plan to evaluate the ability of NR to prevent degeneration of small somatic sensory axons innervating the epidermis as well as its ability to promote regeneration of these same fibers in a human experimental model of nerve degeneration and regeneration. This experimental human model has been used previously to evaluate the rate of nerve degeneration and regeneration in several peripheral neuropathies and in healthy subjects.

Since NR is available as a nutritional supplement, if successful, this research can lead to development of a therapy for a variety of peripheral neuropathies very rapidly.

2. Objectives (include all primary and secondary objectives)

We propose to use an established model of capsaicin-induced axonal degeneration and regeneration to assess the potential of nicotinamide riboside (NR) to delay small unmyelinated nerve fiber degeneration as well as to promote regeneration. The study will test two hypotheses:

(1) NR-treatment will reduce capsaicin-induced axonal degeneration in the epidermis in healthy controls.

(2) NR-treatment will enhance axonal regeneration after capsaicin-induced epidermal denervation in healthy controls.

3. Background

Small fiber neuropathy (SFN) manifests in a variety of different diseases and often results in symptoms of burning pain, shooting pain, allodynia, and hyperesthesia (Tavee and Zhou, 2009). Although strength remains preserved in patients with SFN, the pain and paresthesia's are often disabling. Diabetes mellitus (DM) and impaired glucose tolerance (IGT) in combination with metabolic disease are the most common identified causes of SFN (Hovaguimian and Gibbons, 2011). In these cases, besides strict glucose control, no other treatment option has demonstrated the ability to slow or prevent axon loss in people with diabetic patients with peripheral neuropathy.

Pathologically, the peripheral nerve damage in patients with SFN affects predominantly or exclusively the small myelinated (Aδ) fibers or unmyelinated C fibers. Many small fiber neuropathy patients test normally on conventional electrophysiological tests because intraepidermal nerve fiber function is not assessed by routine nerve conduction studies. Thus, in many patients, the only objective evidence of neuropathy is by skin biopsy determining the intraepidermal nerve fiber density (IENFD) in the collected skin tissue samples (Holland et al., 1998),

The capsaicin cream is an FDA approved medication available over the counter for treatment of various conditions including arthritis related joint pain and post herpetic neuralgia (Fernandes et al., 2016). It is the active ingredient in hot chili peppers and has been shown to bind vanilloid receptors on C- and A-delta nerve fibers leading to calcium entry and subsequent axonal degeneration (Marsh et al., 1987; Pini et al., 1990). The application of over-the-counter strength capsaicin cream (0.1%) for 48 hours leads to loss of intraepidermal nerve fibers mimicking the changes seen in small fiber neuropathies (Simone et al., 1998; Nolano et al., 1999). Associated with the loss of intraepidermal nerve fibers is a decrease in sensation in the area of the patch. Over weeks to months, the fibers slowly regenerate, repopulating the treated area (Polydefkis et al., 2004; Hahn et al., 2007). This regenerative response is associated with improved sensory function as well.

Nicotinamide riboside (NR) is a pyridine-nucleoside form of vitamin B3 that functions as a precursor to nicotinamide adenine dinucleotide or NAD+. Studies in mice have shown that boosting the levels of NAD+ can produce multiple health benefits, including reducing nerve damage (Trammell et al., 2016a; Hamity et al., 2017). In humans, the levels of NAD+ diminish with age and it has been suggested that loss of this metabolite may play a role in age-related health decline (Johnson and Imai, 2018). NR is FDA approved as a dietary supplement and can be purchased over the counter.

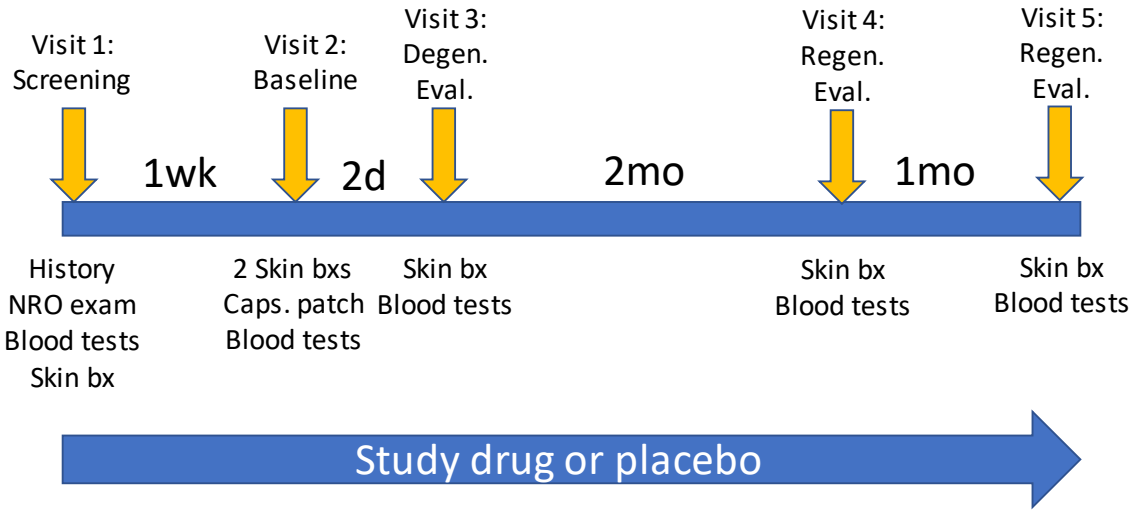
4. Study Procedures

a. Study design:

This is a randomized double-blinded study to evaluate the effects of NR on experimental axon degeneration and regeneration. After the initial screening (visit 1) is completed, the patients will undergo a skin biopsy and be randomized to either the study drug or placebo and will be instructed to take it for a week prior to visit 2. Based on existing pharmacokinetic studies, this should be sufficient to raise the plasma NAD levels at the time of experimental denervation of the skin. At visit 2, patients will undergo two baseline skin biopsies and application of the capsaicin patch for 48 hours and will be instructed to continue the study drug. At visit 3 (48 hours after visit 2), capsaicin patch will be removed, and a repeat skin biopsy will be done to evaluate the degree of epidermal denervation. Patients will be instructed to continue the study drug for another 3 months and will undergo repeat skin biopsies at 2 months (visit 4) and 3

months (visit 5). Blood samples will also be drawn at each visit to measure NAD levels to confirm compliance and to correlate with degree of epidermal innervation. Figure 1 depicts a schema of visits and timelines.

Figure 1: Study Timeline



b. Study duration and number of study visits required of research participants.
As outlined above, there will be a total of 5 visits over 3 and ½ months.

c. Blinding, including justification for blinding or not blinding the trial, if applicable.
The study will be done in a blinded manner where the clinical evaluation and the histopathological evaluation of degeneration and regeneration will be done by different teams. Patients will be randomized and given unique patient identifiers by the research coordinators. The staff at the cutaneous nerve laboratory carrying out the histopathological evaluation will not know the identities of the patients or the samples. At the end of the study, the code will be broken and the PI will have access to the histopathological evaluations and patient identities to carry out the statistical analysis.

d. Justification of why participants will not receive routine care or will have current therapy stopped.
The patients will continue with their current routine care. They will not be asked to stop any medication or care.

e. Justification for inclusion of a placebo or non-treatment group.
Since human nerves degenerate and regenerate at different rates, in order to find out if NR has any therapeutic value, we do need to include a placebo control group.

f. Definition of treatment failure or participant removal criteria.
No participant will be removed from the study. Even if the treatment does not lead to faster nerve regeneration, previous research has shown that after topical capsaicin, epidermis fully reinnervates within 6 months. We will examine whether NR can speed this regeneration.

g. *Description of what happens to participants receiving therapy when study ends or if a participant's participation in the study ends prematurely.*
Once the study is completed, the participants will continue with their regular life and medical care. If a participant leaves the study early, the impact on epidermal reinnervation is minimal as it will regenerate.

5. Inclusion/Exclusion Criteria

Study will be conducted in healthy adult patients aged 18-65 without any symptoms, signs or risk factors of peripheral neuropathy.

Inclusion criteria

- Age: 18-65
- BMI<32
- Normal neurological examination defined as NIS≤2 (Neuropathy Impairment Score)

Exclusion criteria

- History of peripheral neuropathy
- Any peripheral neuropathy risk factor including diabetes, Vitamin B12 deficiency, HIV-infection, chronic kidney or hepatic disease, hypothyroidism, chemotherapy or other know neurotoxic exposure.
- Pregnancy
- Abnormal Labs: HIV, B12, HgA1c, Thyroid Stimulating Hormone (TSH), Complete Blood Count (CBC), Comprehensive Metabolic Panel (CMP)

6. Drugs/ Substances/ Devices

a. *The rationale for choosing the drug and dose or for choosing the device to be used.*
As outlined in background section NR is commercially available as a nutritional supplement and has been evaluated in humans in a Phase 1 pharmacokinetic study (Trammell et al., 2016b). It is safe at the doses used in that study (100 mg, 300 mg and 1000 mg) over the course of 36 days with no significant side effects except 2 patients reporting flushing sensation. Since NR is able to elevate the NAD levels in plasma, we expect that this correlates with elevated tissue levels of NAD and able to prevent distal axonal degeneration of small unmyelinated fibers induced by capsaicin.

b. *Justification and safety information if FDA approved drugs will be administered for non-FDA approved indications or if doses or routes of administration or participant populations are changed.*
NR is available over the counter as a nutritional supplement as a form of vitamin B3 and is sold under various brand names including Tru Niagen at a dose of 300 mg per capsule. It is FDA approved as a nutritional supplement and recommended daily dose is 15 mg. However, based on the study by Trammell and colleagues, effect of NR on NAD metabolism was best observed at the highest dose of 1000 mg/day. There were no significant side effects or toxicity at that dose over 36 days of observation. Furthermore, a more recent clinical trial of NR in obese men looked at the effects of NR on insulin sensitivity over 12 weeks and utilized a dose of 1000 mg twice a day without any significant side effects or drop out from the study (Dollerup et al., 2018). In this study there were increases in multiple NAD metabolites in the serum and urine with this dose. Given that both studies did not observe any side effects or toxicity doses above 1000 mg a day, and that the study by Dollerup and colleagues showed increases in NAD metabolites, we plan to use 1000 mg orally (four 250mg capsules) twice a day for convenience

of dosing using the commercially available NR. The placebo capsules will be prepared by ChromaDex who is also supplying the study drug.

c. *Justification and safety information if non-FDA approved drugs without an IND will be administered.*
Not applicable

7. Study Statistics

a. *Primary outcome variable.*
We are testing whether NR will reduce axonal degeneration as well as promote regeneration. The primary outcome will the measurement of epidermal nerve fiber counts.

b. *Secondary outcome variables.*
Plasma NAD levels will be measured and correlated with primary outcome measure

c. *Statistical plan including sample size justification and interim data analysis.*
Aim 1 focuses on the ability of NR to reduce or prevent nerve fiber degeneration in the capsaicin model. From past studies, we know that following topical capsaicin application, the epidermis is nearly completely denervated. A previous study of 66 healthy control subjects, the baseline intraepidermal nerve fiber density was 22.7 $\pm$ 10.1 fibers/mm (Polydefkis et al., 2006). Following 2 days of topical capsaicin application, the post-capsaicin IENFD was 0.25 $\pm$ 0.04 fibers/mm (nearly 99% reduction in nerve fiber density).

We expect a very similar study subject profile in the current study. If that is true, 20 subjects/arm will have a power of 0.8 to detect a difference in post-capsaicin epidermal nerve fiber density between NR and placebo of as low as 0.035 fibers/mm. If our subjects show more variability (standard deviation of 0.06 instead of 0.04) we would still have a power of 80% to detect a difference of 0.055 fibers/mm. Therefore, we are confident that a sample of 20 subjects/ group is sufficient to determine whether NR decreases axonal degeneration in our model.

Aim 2 focuses on the potential for NR to promote IENFD regrowth. Following denervation with topical capsaicin, epidermal nerve fibers re-innervate the epidermis in a predictable fashion. Biopsies taken at two and three months following denervation have higher IENFD, the rate of regrowth is determined by calculating the slope of the line of IEND against time. Across several studies, this rate of nerve fiber regrowth has been 0.18 $\pm$ 0.08 fibers/mm/day (Polydefkis et al., 2004). A sample of 20 subjects/group will have a power of 0.8 to detect a 40% or greater change in nerve fiber regeneration. We will have a power of 0.6 to detect a 30% improvement in regeneration.

d. *Early stopping rules.*
The study will be terminated early if the subjects experience any significant side effects. NR is produced as a supplement by a number of different manufacturers. There have been no side effects reported. Additionally, it was well tolerated in several published studies. Therefore we do not anticipate any toxicity. If a subject complains of side effects, the potential for any causation will be reviewed by Drs. Hoke and Polydefkis and reported to the IRB.

8. Risks

a. Medical risks, listing all procedures, their major and minor risks and expected frequency.

The capsaicin denervation model and other study procedures have been successfully performed in numerous Johns Hopkins IRB-approved studies (including as part of six industry-sponsored multicenter clinical trials with over 600 subjects total. Therefore, we do not anticipate any complications.

Neurological exam: There are no risks associated with a neurological examination.

Capsaicin cream: Capsaicin is the active ingredient in hot peppers and may cause a local burning sensation to the area that it is applied. The degree of burning is generally less than mild sunburn. The affected area will become less sensitive to sensory stimuli due to local nerve fiber degeneration. With time the area should return to normal or near normal with subsequent nerve regeneration and reinnervation of the skin. We have obtained IRB approval for similar studies in the past (RPN00-10-25-01, RPN 99-11-02-01, NA_00010088 (multicenter trial), WIRB Pr. No.: 20060008 (multicenter trial)) and have a FDA exemption letter for the use of topical capsaicin. The study participants may use over-the-counter, non-steroidal pain medications to manage any pain associated with the use of capsaicin cream.

Skin biopsy testing requires taking a 3mm-punch biopsy. The affected areas are anesthetized with subcutaneous 1% lidocaine with epinephrine, and the site will be sterilely dressed after the procedure. No sutures are involved. The patients will receive instructions how to care for each biopsy site after the procedure. The risk of infection is estimated at 1:500 and is likely less in healthy subjects. Punch skin biopsy may result in a small scar that generally fades by one year.

b. Steps taken to minimize the risks.
The skin biopsies will be done under sterile conditions. Patients will be given clear instructions on how to care for the biopsy sites.

c. Plan for reporting unanticipated problems or study deviations.
If the patients experience any signs of infection at the site of biopsies, they will be asked to call the study coordinator and/or principal investigator. They will be seen in the clinic and appropriate medical care will be provided. Similarly, if the patients experience any side effects from the study drug, they will be asked to call the study coordinator and/or the principal investigator and report it.

d. Legal risks such as the risks that would be associated with breach of confidentiality.
In order to preserve participant confidentiality, all study participant records will be stored in a locked file cabinet located in the study coordinator's office. All subjects will be given a study-specific unique identifying number and this will be used to label study documents. Data spreadsheets will use deidentified subject study numbers and will be password protected and stored on the study coordinator's computer which is backed up regularly.

e. Financial risks to the participants.
There is no cost to participate in the study.

9. Benefits

There is no direct benefit to subjects. We are testing the potential benefit of NR on human nerve degeneration and regeneration.

10. Payment and Remuneration

There will be no cost to study subjects to participate. Subjects will be paid $100/session for each study visit ($500 total) plus $100 if they complete all 5 visits of the study. Each subject will be paid $300 after the 3rd visit and the remaining $300 at the completion of the 5th visit. If subjects decide not to continue with the study, they will be paid for the sessions they completed but they will not be eligible to receive the study completion bonus of $100.

11. Costs

a. There is no cost to participate in the study.

12. References

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