Repurposing Nervonic acid: a Potential Therapy to Attenuate VLCFA Accumulation in Adrenoleukodystrophy - Translational and Clinical Pharmacology Considerations

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Introduction

➢ Adrenoleukodystrophy (ALD) is an X-linked disorder due to defective peroxisomal transporter, ALDP
➢ Causes accumulation of very long-chain fatty acids (VLCFA, C26:0)
➢ Variable presentation with majority of men and carrier women eventually getting affected
➢ 35% affected by the devastating childhood cerebral form of ALD
➢ Early detection is crucial to prevent morbidities

Figure 1: Newborn Screening in the U.S. Green indicates the states currently screening for ALD, orange have initiated a pilot, while red will start screening later this year. Blue indicate the states that are mobilizing efforts to start ALD screening. (https://adrenoleukodystrophy.info/clinical-diagnosis/ald-newborn-screening)

➢ Newborn screening for ALD is expanding in the US
➢ However, there are no approved treatments available for pre-symptomatic patient that can prevent or delay the onset of the cerebral form.
Dietary Therapy in ALD

➢ Lorenzo’s Oil (LO), a mixture of oleic (C18:1) and erucic (C22:1) acids dosed at (2-3mg/kg/day), can lower plasma C26:0 levels in asymptomatic ALD patients (Ahmed et al., 2016)

Figure 3: Effect of erucic acid (the active ingredient in LO) on normalizing C26:0 levels. The colored bars indicate the median of data (N=97). The dashed line indicates the mean normal C26:0 levels and the solid lines are the deviations from the mean.

➢ Relatively safe profile although erucic acid has the potential to cause cardiotoxicity (Kramer et al., 1992). Thus, its consumption is banned in the US

➢ However, the efficacy of LO in preventing the onset of cerebral disease is unclear

➢ This is largely due to the lack of knowledge of LO pharmacology

Long-term Goal: Develop therapy for presymptomatic ALD that can normalize VLCFA and arrest or delay disease progression by characterizing drug pharmacology early in development.

Objective: Determine whether monounsaturated fatty acid, nervonic acid (C24:1) can decrease C26:0 and the total lipid saturated VLCFA levels in ALD cell models
**Methods**

**Human Skin Fibroblasts Cell Lines and Assays**
- Neonatal Human Dermal Fibroblast (NHDF) from foreskin - Control
- GM17819 from cALD
- GM04904 from adrenomyeloneuropathy (AMN)

- **Nervonic Acid Treatment:** 5, 20 & 50μM (in <0.1% ethanol) for 5 days using 2% FBS Minimum Essential Media (MEM)
- **Bioanalytical Methods:** The total fatty acids in cell lysate extracts measured using GC/MS. The complex lipids were measured by LC/MS. (Lagerstedt et al., 2001; Hubbard et al., 2009)
- **Cellular Assays:** Cell viability was determined using CyQUANT NF assay (Invitrogen, CA, USA) and ATP production measured using ATPlite luminescence assay (PerkinElmer, MA, USA)

**Results**

Nervonic Acid Decreases C26:0, Total Saturated VLCFA and Complex Lipids

**Figure 4.** Decreases in C26:0 (A), total lipid saturated VLCFA (B), C26:0-ceramides(C), C26:0-LPC (D); C26:0-sphingomyelin (E) and increases in beneficial C24:1-sphingomyelin (F) following 5-day incubation with increasing nervonic acid concentrations. Data (mean ± SEM) is normalized to total cellular proteins. Data analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. * p<0.05; ** p<0.01; *** p<0.001
Nervonic Acid Offers Cellular Protection of ALD Fibroblasts from Oxidative Insults via Increases in ATP Production

**Figure 5:** Effect of Nervonic acid (NA) on cytoprotection against oxidative stress (A, B) and ATP production (C, D) in ALD cells. Erucic acid (EA) was used as a comparator. Cells were pretreated with fatty acid for 5 days and then stressed with hydrogen peroxide (H$_2$O$_2$) for 24h. Cell viability is expressed as % viability of untreated cells and relative ATP production is shown as percentage of vehicle treated cells (considered 100%) after normalization with cell numbers. Data (Mean ± SEM) is from 3 independent assays and analyzed using 2-tailed unpaired student’s t-test. * p<0.05; ** p<0.01

**Summary & Discussion**

➢ Our results show that nervonic acid can attenuate the accumulation of total free and complexed VLCFA

➢ It can also protect cells from oxidative stress probably by improving cellular bioenergetics

➢ Nervonic acid is an important component of the sphingolipids of white matter in human brains.

➢ It is found to be decreased in ALD post-mortem brains (Sanger et al., 1994)

➢ Recently it has been shown to correct motor disorder in a mouse model of Parkinson disease (Hu et al., 2021)

➢ Nervonic acid occur naturally and thus is considered safe
  ➢ Primarily derived from the diet
  ➢ Endogenously produced in human body (found in maternal milk)
  ➢ Found in multiple plant and animal sources

➢ Nervonic acid containing dietary supplements are available

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Next Steps

➢ Examine the effect of nervonic acid in ALD mouse
  ➢ Effect on tissue C26:0 levels
  ➢ Potential benefit in alleviating AMN symptoms
  ➢ Understand nervonic acid exposure (concentration)- response (C26:0) relationships
  ➢ Assess safety in animals

➢ We are initiating clinical trial readiness studies in ALD mouse models
➢ Goal is to fully understand nervonic acid pharmacology that can guide formulation, dose selection and regimen for studies in patients
➢ We will conduct acute and long-term exposure studies in mouse to assess safety and efficacy in ALD

Pharmacological Considerations for Optimal Drug Repurposing

Formulation ➔ Mechanism of action ➔ Dose finding studies ➔ Exposure-response analysis ➔ Long-term safety & efficacy studies

Route of administration ➔ Tissue uptake studies

Acknowledgements

Kennedy Krieger Institute
• Ann Moser, MS
• Paul Watkins, MD, PhD

University of Minnesota
• Troy Lund, MD, PhD
• Julianne Tieu, PharmD
• Chenwei Yan (undergraduate student)