An Approved Protocol to Evaluate 4-Aminopyridine (4-AP) for use as a Single-Dose Diagnostic Agent to Diagnose Peripheral Nerve Continuity in Humans

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BACKGROUND

- Traumatic peripheral nerve injury (TPNI) presents particular diagnostic dilemmas to the treating surgeon and thus represents a major health problem with significant functional impairment and permanent disability.
- Injured nerves may be stretched, crushed, or transected.
- There is no adequate measure to distinguish nerve injuries where the nerve is intact but not functioning from injuries which completely sever the nerve.
- Current state of the art in diagnosis of this critical matter is left to electrodiagnostic (EDX) studies which are not sensitive for weeks after the injury.
- We have found that 4-aminopyridine (4-AP) dosing can allow early distinction in multiple animal species with TPNI.
- We propose a randomized crossover trial to test single dose 4-AP (vs. placebo) during a time when patients have nerve dysfunction after TPNI in two distinct problem subpopulations: Patients who present to the hospital with nerve injuries sustained in trauma and patients who sustain TPNI as a result of surgical intervention.
- **The key question we aim to answer:** Is early distinction between severed nerves and non-severed non functional nerves possible with pharmacodiagnostic challenge using 4-AP?

STUDY DESIGN

- This is an investigator-initiated, prospective, single-center randomized, double-blind, placebo-controlled cross-over study in patients with possible peripheral nerve discontinuity (Figures A-B).
- This study is to assess the effect of single-dose 4-AP on both subjective and objective measures of function before and after administration of 4-AP or placebo.
- Because this is a preliminary study designed primarily to assess the functional improvements of the injured nerve, a 10-mg dose of 4-AP will be tested based on previous clinical trials in multiple sclerosis (MS) patients.
- A crossover design is chosen because it requires fewer participants than a parallel arm study and participants serve as their own controls to reduce the effects of interpatient variability.

STUDY DESIGN

- This trial consists of two groups of patients: Post traumatic (**Aim 1**) and post-surgical (**Aim 2**) nerve dysfunction with possible nerve discontinuity.
- In both groups: After screening, participants will be randomized in a 1:1 ratio to 1 of 2 blinded crossover treatment sequences: Single-dose 4-AP followed by placebo or placebo followed by single-dose 4-AP (Figure A).
- After baseline assessment of the nerve functions and blood sampling, participants will receive a single-dose 4-AP or placebo (**period 1**) and will be re-evaluated for the same measures hourly for 3 hours, after which the low level of drug is known to have no expected direct effect.
- After a 2-hour break and washout, participants will receive their crossover treatment (**period 2**) and will be evaluated for the exploratory functions and blood sampling as described above.
- Patients will be discharged after conclusion of period 2, and follow-up will include the current standard of care and bi-weekly assessment to monitor the recovery and progress after the injury.
- Patients will receive EDX testing at 6 weeks intervals after discharge (Figure B).
- This study is approved by institutional board review of the Penn State University College of Medicine and all participants will provide written informed consent prior to enrollment.
- The study will be conducted in accordance with the principles of Good Clinical Practice.

Aim 1: Trauma patients presenting with possible nerve discontinuity.

Aim 2: Non-trauma patients presenting with post-intervention nerve dysfunction indicating possible discontinuity.

Figure A. Both aims will follow the similar study design on Testing Day:

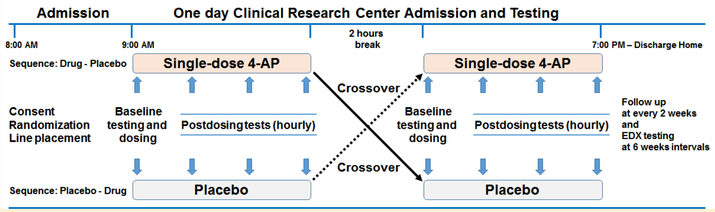
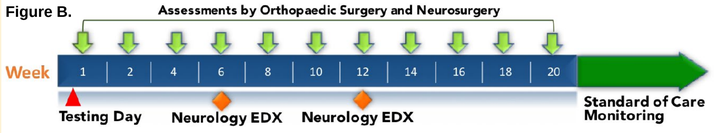


Figure B.



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STUDY PARTICIPANTS and COHORT SIZES

- Eligible subjects will be recruited based on well-defined inclusion and exclusion criteria (below) by a co-investigator on the study team.
- Patients will come from two sources within 4 days (96 hours) of injury:
  - Traumatized patients while in the emergency room (**Aim 1**)
  - During consultation as inpatients (**Aim 2**)
- Patients will all be consented in face-to-face interviews inside the Penn State Milton S. Hershey Medical Center.
- To minimize the opportunity for coercion a study, coordinator listed on the study will conduct the consent process. The subject will be told about the study and given ample time to ask questions and review the consent form before deciding about participation.
- Statistical forecasts of the number of patients needed to demonstrate our desired effects were based on the rates of success of our preliminary murine studies in conjunction with the loose definition of post-injury EDX at 6 weeks as a "gold-standard" examination. Using these data and standard drop-out rates (25%), our qualified biostatistical team concluded that we need 34 patients/aim for this trial.

PARTICIPANTION CRITERIA

Inclusion Criteria:

- Adults aged 18-90 years with the ability to give written consent.
- Known limb trauma which resulted in nerve injury or post-operative/post intervention nerve injury.
- Closed soft tissue envelope obscuring direct observation of the affected nerve.
- Able to complete single day dosing within four days (96 hours) of nerve injury.
- Cognitive ability to report sensory and motor deficit during examination.
- Eligible for standard of care plan of monitoring vs. surgical exploration of the nerve.
- Capable of safely undergoing electrodiagnostic testing (EDX) and availability for all testing days and subsequent follow-up.

Exclusion Criteria:

- Distracting injury which prevents adequate examination.
- Surgical exploration of the nerve as part of another surgical procedure within 48 hours of evaluation.
- Intoxication during examination or evidence of cognitive deficit.
- History of multiple sclerosis, stroke, brain injury, seizure or neurological disorder.
- History of hypersensitivity to AMPYRA® or 4-aminopyridine.
- Patients unable to communicate return or loss of sensation.
- Patients unable to exhibit motor control on the affected limb at baseline.
- Pregnancy, breastfeeding or incarcerated individuals.

PARTICIPANT RETENTION

- Patients will be incentivized to complete their timeline.
- The trial budgets a compensation fee for the testing day and for gold-standard EDX at 6 and 12 weeks (proposed nominal fee of 100 dollars per day, along with meals for accompanying family members).
- The fee schedule is standard in the Penn State Clinical and Translational Science Institute (CTSI) and represents a non-coercive compensation for the patient's time.
- This study is designed as an intent-to-test (treat) study. Therefore drop-outs will not be excluded from appropriate analyses.

TESTING and DATA ANALYSIS

- **Blood tests:** Circulating level of 4-AP will be determined using a validated high performance liquid chromatography method.
- **Sensory function testing:** The most complete set of clinical examination tests must be employed to achieve our aims at understanding the mechanism of 4AP-mediated return of function (or lack thereof).
- **Motor function testing:** As with sensory testing, a complete motor assessment which includes all the voluntary motor function tests used in the routine assessment of these patients must be here applied to our trial patients within the 1 hour testing periods in between serum tests.
- **Sudomotor function testing:** We will attempt to obtain electrical conductance measurements in skin areas known to be innervated by the injured nerve during muscle testing. Scores from this test will be compared (as controls) against the same measurements in well innervated and from clothing and or hair (non-innervated).
- **EDX testing:** Patients enrolled in the trial will undergo serial EDX testing to specifically assess the continuity of the injured limb nerves.
- Data analysis for both aims will be identical, and will involve a generalized linear mixed-effects model with a logit link function for the bivariate binary response in a 2×2 crossover design.

OUTCOME MEASURES

- **The primary outcome measure is the return of sensorimotor function and EDX sensitivity with 4-AP in the setting of nerve trauma and early distinction between severed and non-severed nerves.**
- **Possible outcomes ranked by likelihood:**
  - **4-AP:** Functional return *without* any recordable EDX or other evidence of continuity in patients with non-severed (continuous) nerves AND no functional return in patients with severed nerves. **Placebo:** No function or EDX positivity in both patients with severed and non-severed nerves.
  - **4-AP:** Functional return *with* any recordable EDX or other evidence of continuity in patients with non-severed (continuous) nerves AND no functional return in patients with severed nerves. **Placebo:** No function or EDX positivity in both patients with severed and non-severed nerves.
  - **4-AP:** Functional return *with* any recordable EDX or other evidence of continuity in *only some* patients with non-severed (continuous) nerves AND no functional return in patients with severed nerves. **Placebo:** No function or EDX positivity in both patients with severed and non-severed nerves.
  - **4-AP:** No functional return and no recordable EDX or other evidence of continuity in *any* patient. **Placebo:** Identical to 4-AP treatment.