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Improvement of Motor, Sensory, and Functional Status of Post-Guillain-Barre Syndrome with the Use of 4-Aminopyridine

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Abstract

Objective: To determine the safety and efficacy of orally delivered 4-aminopyridine for motor weakness due to Guillain-Barre Syndrome (GBS) under a FDA approved protocol (IND No: 58,029).

Setting: Tertiary care outpatient and inpatient rehabilitation center directly attached to a university hospital.

Subjects: Seven subjects who were unable to ambulate more than 200 feet without assistive devices and had residual nonprogressive motor weakness due to GBS more than one year out from the initial episode.

Design: Subjects were randomized to a double-blind, placebo-controlled, cross-over design, which had two four-week treatment arms with a one-week washout. The average dosage at 4 weeks was 30 milligrams (mg) per day.

Data set: Data for motor strength utilizing a traditional 5 point motor scale and handgrip strength were evaluated. Differences over times were assessed via descriptive statistics, Friedman's analysis, Wilcoxon signed-rank, ANOVA and paired Student's t-test. Subjects were evaluated for dose and side effects as required by our FDA approved protocol (IND No: 58,029).

Results: During four weeks of treatment, the averaged lower extremity (LE) motor strength increased from 3.2 SD ± 1.2 to 3.7 SD ± 1.0 (p<0.0001), the average upper extremity (UE) motor strength increased from 3.2 SD ± 1.2 to a maximum of 4.3 SD ± 0.9 (p=0.0073) and the grip strength bilaterally increased from 8.2 lbs. SD+ 9.1 lbs. to 12.2 lbs. SD ± 9.1 lbs. (p=0.0243). There were no statistical changes in the placebo arm regarding LE and UE motor strength or the grip strength at week 4 (p>0.05). Only three laboratory tests had a statistically significant change as the uric acid changed from 6.4 to 6.5, the SGOT went up from 25.1 to 27.9 and the hematocrit dropped from 42.7 to 41.6. None of these results were deemed to be clinically relevant changes. There were no seizures and there was no significant change in the Q – T interval in any of the subjects. Three subjects did report increased paresthesias on 4-aminopyridine.

Conclusion: This Phase IIa trial indicates 4-aminopyridine was generally safe and may be effective in improving the motor function of GBS subjects. Further research requires delineating its biologic half-life, which appears to be longer than two weeks.

Introduction

Guillain-Barre syndrome (GBS) is an immunopathy associated with an acute, often fulminate, evolution of a demyelinating inflammatory polyradiculoneuropathy [1-8]. In developed countries, GBS is the most common cause of acute neuromuscular paralysis, afflicting about 5,000 persons annually in the United States. Over 20% of GBS patients have permanent residual motor deficits that affect their activities of daily living [6,7]. The combined incidence of persistent mono- to tetra-plegia is estimated to be 54% [1,4-6]. The major clinical manifestation is weakness, mainly symmetrical, that evolves over a period of several days. The average period from onset to nadir of illness is 8 days [4].

The duration of the illness is usually less than 12 weeks and most patients are expected to have a favorable outcome [5]. Approximately 10% of GBS patients die, usually from respiratory failure, cardiac arrhythmia, or pulmonary embolism, and 20% are left with deficits in ambulation or respiration one year later [6,7]. Therefore, GBS is a significant cause of new long-term disability for at least 1,000 persons per year in the United States and many more elsewhere. Moreover, given the young age at which GBS sometimes occurs and the relatively long life expectancies following GBS, it is likely that at least 25,000, and perhaps 50,000, persons in the United States are currently experiencing at least some residual effects of GBS [9-15].

Pathologically, GBS is an inflammatory polyradiculoneuropathy that resembles experimental allergic neuritis (EAN) in animals [16]. Both EAN and GBS share common histopathological features

characterized by the presence of perivascular mononuclear cell inflammation, demyelination and edema. Experiments in various animal models have clearly demonstrated that the sensitization of the CD4 subclass T-lymphocytes to proteins in the myelin sheath is necessary for disease induction [16]. The principle electrophysiologic findings, which account for the early weakness noted in GBS is the conduction block produced when a portion of the axon fails to transmit impulses in a segment where myelin has been destroyed or rendered nonfunctional [17].

Currently, there is no approved treatment of any kind for the debilitating fatigue and motor weakness in GBS patients who have not fully recovered, leading to significantly reduced functional status and quality of life for many [18-22].

4-Aminopyridine (4-AP)

4-Aminopyridine (4-AP), a specific blocker of voltage-dependent fast-activating neuronal potassium channels, has been reported to reduce spasticity and improve motor and sensory function in animal models. It is also effective in patients with demyelination of the central nervous system (CNS), such as multiple sclerosis (MS) or spinal cord injury (SCI) [23-27,28]. Double blind trials with 4-AP have demonstrated that patients with MS benefit from long-term administration of 4-aminopyridine, particularly with regard to motor function and endurance [28-30]. Preliminary clinical studies have demonstrated that 4-AP is associated with decreased pain and/or improved motor function [26,28,31]. However, it is entirely possible that in this patient population, an improvement in sensory and pain fiber nerve conduction may result in hypersensitivity and hyperalgesia, similar to that noted in GBS patients during their acute recovery [1]. Animal studies indicate that 4-AP may act through restoration of action potential conduction in damaged, poorly myelinated nerve fibers, directly enhancing synaptic transmission [23-27].

4-AP has also been clinically tested in the Spinal Cord Injury population. All subjects in this population tolerated the drug well and results suggested that conduction within the central nervous system is enhanced by 4-AP. The first double-blind study showed that three of the four subjects with incomplete injuries reported relief of chronic central pain and reduction of spasticity [26]. Clinically, 4-AP has been well tolerated in SCI patients on 30 mg/ per day of the short acting version [32,33] that is on the compounding pharmacy list of the FDA. Long term open label trials in SCI of the compounded short acting version have not demonstrated any toxicity [33].

Patients with chronically disabling GBS provide an ideal model to study the effectiveness of 4-AP in peripheral demyelinating neuropathies. Because these patients do not have a progressive disease, because their neurologic findings are not progressing, these subjects can be used as their own controls. Hence, they are ideally suited to a double-blind crossover study or to a double-blind study under FDA supervision. The effect of 4-AP on the potassium channels appears to be fully reversible [26-31].

The study's goal was to determine if orally delivered 4-AP will improve motor function and is safe in subjects who have suffered Guillain-Barre Syndrome (GBS) [34].

Study Design

This was a Phase IIa double-blind, placebo-controlled, crossover, dose-escalating study of 4-AP in subjects with GBS. The reported study population consisted of 7 subjects (out of 8 recruited patients) between 18 and 75 years of age with GBS injury whose neurological status had been stable for at least 12 months from the last point of neurological deterioration and who had motor weakness which interfered with their activities of daily living. All patients were recruited consecutively and suffered an ascending acute flaccid paralysis with areflexia and had been treated with either intravenous immune globulin or plasma exchange. The 8 patients were randomized to one of the two treatment sequences (A or B) as shown below (Table 1).

Day 1,2	Day 3,4	Day 5,6,7	Day 8,9,10	Day 11,12,13	Day 14-28	Washout Day 28-35
A 5 mg/day	5 mg bid	5 mg tid	5-5-10 mg	5-10-10 mg	10 mg tid	Placebo
B Placebo	Placebo	Placebo	Placebo	Placebo	Placebo	Placebo

Table 1: Dosing sequence for 4-AP during the trial.

All patients were evaluated and treated at a tertiary care outpatient and inpatient rehabilitation center directly attached to a university hospital. All subjects had residual motor weakness from GBS that had not progressed or regressed over the past year.

Inclusion criteria

- Male or Female, 18 to 75 of years of age, irrespective of race.
- Able to and had voluntarily given informed consent prior to the performance of any study specific procedures.
- Have neurological impairment secondary to GBS, which had been stable for at least 12 months.
- Unable to ambulate more than 200 feet without assistive devices.
- Able and willing to comply with the protocol.
- May have profound pain.

- Have abnormal motor or sensory nerve conduction velocities in at least 2 tested nerves [35-40].

Exclusion criteria

A pregnant female (as determined by a urine pregnancy test), a lactating female, or a female of child-bearing potential not using one of the following methods of birth control (oral contraceptive, implantable contraception device or injectable contraceptive agent, barrier method of contraception) or not surgically sterilized.

- A history of seizures.
- A known allergy to pyridine-containing substances.
- Evidence of upper motor neuron involvement.
- Any medical condition, including psychiatric disease, which would interfere with the interpretation of the study monitor.

- Concomitant medications were at a stable dose/regimen for less than 3 weeks, and/or the stable dose/regimen of concomitant medications was expected to be changed during the course of the study.
- A history of drug or alcohol abuse within the past year.
- Received an investigational drug within 30 days prior to the screening visit.
- Taken 4-aminopyridine in the past, whether through participation in a previous study or self-medication [41-43].

Variables to be collected

Motor score: The motor score measures the strength of key muscles bilaterally on a 0-5 scale and is closely based on the ASIA motor scoring technique [44]. Use of these standardized measures has been endorsed throughout the rehabilitation community. This scale grades motor strength of selected muscle groups (for hip abduction, hip adduction, hip flexion, knee flexion, ankle dorsiflexion and plantar flexion) as follows:

- 0 - Absent (total paralysis)
- 1 - Trace (palpable or visible contraction producing little or no movement around joint)
- 2 - Poor (active movement through much or all of the normal range of motion, with gravity eliminated - where relevant)
- 3 - Fair (active movement through full range of motion against gravity)
- 4 - Good (active movement against some resistance)
- 5 - Normal (active movement against full resistance) [45].

Hand-held dynamometer: The hand-held dynamometer is a reproducible method for evaluating maximal grip strength [46-48]. The inter-rater correlations are .996 for the right hand and .999 for the left hand. Test-retest reliability is .88 for the right hand and .93 for the left hand. Validity was studied by suspending known weights from the dynamometer and determining what it weighed on the tool. A 3% variation was found, which indicates a high validity for the Jamar dynamometer. Patients were evaluated in each hand with a calibrated Jamar dynamometer. The highest grip strength of three trials was recorded.

GBS Disability Scale: The first measure of functional outcome selected for this study was based on the 6 point ordinal scale utilized for evaluation of clinical intervention in the early stages of GBS [5,46-51]:

- healthy;
- minor symptoms or signs;
- able to walk five meters without assistance;
- able to walk five meters with assistance;
- chair- or bed-bound;
- requiring assisted ventilation for at least part of the day or night;
- dead.

The Get Up and Go Test: The Get Up and Go Test is a measurement of functional mobility, which measures how long it takes to get up from a standard chair and walk 10 meters with turning around. The patient may use his/her ambulatory assistive devices for this test. This test for quantifying functional mobility is also useful in following clinical change over time [52].

Nerve Conduction studies: All patients had two upper and lower extremities motor and sensory nerve conductions (total 4 motor, 4 sensory) performed at the enrollment period and after 4 weeks of drug delivery in both the A and B phases. Nerve conduction velocities and amplitudes were performed for the right median and peroneal motor and sensory nerves according to previously established methodologies [53-58].

Other treatment variables collected

Changes in pain complaints were rated weekly on the visual analog scale (VAS) on a 1-10 scale weekly [59]. A five-point scale documenting deep tendon reflexes at the biceps, patella and achilles [33] was as follows:

- Reflexes absent
- Hyporeflexia
- Normal
- Mild hyperreflexia
- 3 or 4 beats clonus only
- Clonus
- Safety Measurements
- Safety endpoints were as follows:
- Vital signs
- Laboratory tests
- Adverse events

The vital signs height and body weight were noted at screening.

Clinical laboratory tests

- The Health Sciences Center laboratory carried out clinical laboratory tests. The following parameters were determined:
- **Hematology:** hemoglobin, hematocrit, red blood cell count, white blood cell count with differential and platelet count; serum pregnancy test (at screening only if applicable).
- **Blood Chemistry**: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, total protein, albumin, SGOT/AST, SGPT/ALT, alkaline phosphatase, total bilirubin and LDH.
- Electrocardiogram evaluated for any changes with particular attention paid to Q – T segment duration changes.
- Nerve conduction studies assessed for both efficacy and changes in nerve conduction velocities and amplitudes.

Study medication

Once a patient met the entry criteria, he/she was provided with the arm 1 study medication (4-AP or placebo) and was instructed to begin taking the medication that evening. The patients were randomized to one of two treatment arms in a double blind randomized fashion via coin toss. The patient was reminded to call the study coordinator if he/she had questions regarding the titration schedule or experienced any adverse events. 4-AP was supplied as 5.0 mg white tablets. Placebo tablets, identical to the 5.0 mg 4-AP tablets, were supplied. Following the baseline examination and meeting the entry criteria, patients were given written and oral instructions to begin the study medication (4-AP) or placebo as a double blind treatment. The initial dose was one tablet once daily at bedtime. The dosage was increased slowly with a target dose of 30 mg per day after 2 weeks of treatment. At the completion of arm I there was a one-week washout period where the patients were instructed to continue taking an identified placebo tablet

three times per day. After the one-week washout, patients were crossed over to the second arm of the study.

Dosing sequence

4-aminopyridine was produced locally by a pharmaceutical compounding company. This compound is stable for several months (Table 1). The dose escalation only applied when the subject did not exhibit a dose-limiting toxicity defined as a drug-related adverse event severe enough to interfere with the subject's daily activity. In the event a subject experienced such toxicity, he/she would have been instructed to reduce the dose to the next lowest level. If dose-limiting toxicity occurred, the subject was to be discontinued from the study. Dosing did not exceed 30 mg/day.

Evaluations by visit

Patients were evaluated weekly throughout the study. On clinic visit days, patients were instructed to take their morning dose prior to coming to the clinic. Instruction was provided on the dose titration schedule. Patients were reminded to call the study coordinator if they had questions regarding the titration schedule or experienced any adverse events.

Adverse events

An adverse event (AE) was any undesirable event that occurred to a participant during the course of the study (or a reasonable time after study termination), whether or not that event was considered study drug-related.

Examples include

Any treatment related emergent signs and symptoms (events marked by a change from the patient's baseline/entry status [e.g., an increase in severity or frequency of pre-existing abnormality or disorder]).

All reactions from study drug, an overdose, abuse of drug, withdrawal phenomena, sensitivity or toxicity to study drug.

Apparently unrelated illnesses

Injury or accidents (note: if a medical condition was known to have caused the injury or accident, the medical condition and the accident were to be reported as two separate medical events [e.g., for a fall secondary to dizziness, both "dizziness" and "fall" were to be recorded separately]).

Extensions or exacerbations of symptomatology, subjective patient-reported events, new clinically significant abnormalities in clinical laboratory, physiological testing or physical examination.

At each visit the Investigator asked the patient a non-specific question (e.g., "Have you noticed anything different since your last visit?") to assess whether any AEs had been experienced since the last report or visit. AEs were identified and documented on the AE comprehensive report form (CRF) in appropriate medical terminology. The severity of the AE and the relationship to the study drug were determined and reported on the CRF (see below).

Adverse events - severity rating

The severity of each AE was characterized and then classified into one of three clearly defined categories as follows:

- Mild - the AE did not interfere in a significant manner with the patient's normal functioning level. It may have been an annoyance.
- Moderate - the AE produced some impairment of functioning, but is not hazardous to health. It is uncomfortable or an embarrassment.
- Severe - the AE produced significant impairment of functioning or incapacitation and is a definite hazard to the patient's health.

These three categories are based on the Investigator's clinical judgment, which, in turn, depends on consideration of various factors such as the patient's report, the physician's observations and the physician's prior experience.

Serious adverse events (SAEs) and unexpected adverse events

Each AE was classified by the Investigator as "serious" or "not serious." A serious adverse event (SAE) was one that:

- is fatal or immediately life-threatening;
- is permanently [or substantially] disabling;
- requires [or prolongs] hospitalization;
- is a congenital anomaly [in an offspring];
- results in cancer;
- is a medication overdose [intentional or accidental]

An SAE may also include any other event that the Investigator or Medical Monitor judges to be serious or that suggests a significant hazard, contraindication, side effect or precaution. An unexpected AE is one that is not identified in nature, severity or frequency in the current Investigator's Brochure.

Protocol review

The study was reviewed and approved by The University of Alabama at Birmingham Health Sciences Human Investigation Committee (new drug and device investigations). Prior to testing and after being familiarized with the experimental method and the potential risks as well as the potential benefits of the procedure, each patient signed an informed consent form. This study was performed under an FDA approved investigational drug treatment protocol, IND# 58,029.

Statistical methods

The statistical study design was a randomized double-blind, placebo-controlled, cross-over with each patient utilized as his or her own control. All data were reported as the mean + one standard deviation (SD). The Wilcoxon signed-rank test was used to test the significance of observed differences between baseline and after 6 weeks of continuous treatment for ordinally measured data (deep tendon reflexes, the disability scale, motor strength, VAS). Changes over time were assessed utilizing Friedman's analysis, which is the nonparametric equivalent of the ANOVA. A two-tailed test was utilized with $p<0.05$ considered for significance. Although nonparametric tests were used, data were presented as means and standard deviations to facilitate the interpretation of the magnitude and clinical significance of the results. Rather than consider each muscle separately, average scores for muscle tone, spasms, motor strength and reflexes were averaged for the upper extremities or the lower extremities for each patient.

All laboratories, the nerve conduction studies, and the grip strength were parametric measures and were assessed utilizing a two-tailed

Student's t-test and/or a one way ANOVA with repeated measures. Motor strength changes and changes in the deep tendon reflexes scores were averaged over all joints bilaterally for the UE or the LE.

Results

Eight patients (3 males, 5 females) were recruited for the double blind trial and were within 3 years of their initial diagnosis. One female patient left the study due to the development of CIDP, which may have been partially masked by the 4-AP (Reported in Adverse Events below). The remaining seven patients (mean age = 57; range 27-73) completed the double-blind, randomized protocol.

Motor function

Lower extremity strength for hip abduction, hip adduction, hip flexion, knee flexion, ankle dorsiflexion and plantar flexion increased from an average motor score of 3.2 standard deviation (SD) ± 1.2 to a motor score of 3.7 SD ± 1.0 ($p < 0.0001$, Friedman's) during the active treatment with 4-AP (Figure 1). There was also a statistically significant increase in lower extremity motor strength after 4 weeks treatment with the active drug ($p < 0.0001$, Wilcoxon Signed Rank), following which time there was no statistically significant difference between the active drug and placebo after 4 weeks of treatment ($p > 0.05$, Wilcoxon Signed Rank).

Figure 1: The average motor score in the lower extremities (LE) bilaterally for hip abduction, hip abduction, hip adduction, hip flexion, knee flexion, ankle dorsiflexion and plantar flexion. Data are reported for patients, during the 4-AP (blue line) and placebo (red line) arms, at the start of treatment and after 1 week, 2 weeks, 3 weeks and 4 weeks of treatment with one standard error bars.

Upper Extremity strength for shoulder abduction, elbow extension, elbow flexion, wrist dorsiflexion and flexion increased from 3.2 (SD) ± 1.2 to a maximum of 4.3 SD ± 0.9 ($p = 0.0065$, Friedman's) at week three before returning to baseline at week four on 4-AP (Figure 2). There was also a statistically significant increase in upper extremity motor strength after 3 weeks of treatment with the active drug ($p < 0.0073$, Wilcoxon Signed Rank), following which time there was an inexplicable falling off of motor strength in the fourth week ($p > 0.05$, Wilcoxon Signed Rank). There was no statistically significant difference between the active drug and placebo after 4 weeks of treatment ($p > 0.05$, Wilcoxon Signed Rank).

Bilateral grip strength also increased significantly as measured by a hand held dynamometer. After four weeks of treatment with 4-AP, grip strength increased from 8.2 lbs. SD ± 9.1 lbs. to 12.2 lbs. SD ± 9.1 lbs. ($p = 0.0243$, paired Student's t-test). Over the four week course, the repeated measures ANOVA approached statistical significance ($p = 0.0715$, MANOVA) (Figure 3). There was no statistically significant difference between the active drug and placebo after 4 weeks of treatment ($P > 0.05$, paired Student's t-test).

Figure 2: The average motor score in the upper extremities (UE) shoulder abduction, elbow extension, elbow flexion, wrist dorsiflexion and flexion. Data are reported for patients, during the 4-AP (blue line) and placebo (red line) arms, at the start of treatment and after 1 week, 2 weeks, 3 weeks and 4 weeks of treatment with one standard error bars.

Figure 3: The average grip strength bilaterally utilizing a Jamar hand held dynamometer. The strongest of three trials was recorded for each hand.

There was no statistically significant change in the timed Get Up and Go test due to the lack of numbers of patients (N=4) able to ambulate 10 meters at the start of the study. There was also no statistically significant change in the GBS disability scale over the course of the four week study, although there was a trend towards improvement with the mean score dropping from 8.0 SD ± 1.0 to 4.6 SD ± 1.3 ($p = 0.0948$, Friedman's).

Placebo

There were no statistically significant findings while patients were on the placebo agent. The consistently elevated placebo starting point for the second arm of the study is believed to be due to the one week wash out, which was probably inadequate (Figures 1-3).

For those patients on the active agent during their first arm of the study, the motor strength and grip strength continued to remain improved for up to two additional weeks after the one week washout period (Figures 1-3). It was not until week 2 on the placebo arm that motor strength began to return to the initial levels noted at the very start of the study. This finding indicates that the neurologic effects of 4-AP can last for more than 2 weeks.

Pain

There was a trend towards an improvement in the mean perceived pain scores from 8.0 SD ± 2.6 to 4.7 SD ± 2.0, which approached statistical significance ($p = .1202$, Friedman's). However, pain also decreased on the placebo side of the study from 8.0 SD ± 2.6 at the initiation of the study to 4.6 SD ± 1.3 ($p = 0.0948$, Friedman's), and there was no statistical difference between the placebo and the active drug treatment after 4 weeks of treatment ($p > 0.05$, Wilcoxon Signed Rank). Data were analyzed from the initiation of the study as it was thought that just taking a pill, even if the pill was identified as a placebo medication, would have an effect. Furthermore, the data indicate that the one week washout was not sufficient to washout the effect of the medication for those who were on the active medication in the first part of the study (See Figures 1-3). This appears to be supported by the data as the average pain intensity of those patients after the one week washout was 5.3 SD ± 2.1.

Nerve conduction studies

There was a trend towards an improvement in motor conduction amplitudes for the median motor nerves. The amplitude of the median motor evoked potential increased from 4.5 millivolts (mv) SD ± 2.8 to 6.3 SD ± 1.5 (p=0.1645, Paired Student's t-test) and the peroneal motor evoked potential improved from 0.8 mv SD ± 0.9 to 2.9 mv SD ± 6.2 (p=0.47, Paired Student's t-test) while the patients were on the active drug (Table 2).

Test/Variable	Value
Median Motor Lateral	
Baseline Mean ± SD/SE (range)	4.429 ± 1.154/0.436 (3.100 – 6.400)
Week 4 Mean ± SD/SE (range)	5.100 ± 1.975/0.806 (3.900 – 9.100)
Mean Difference	0.517
DF	5
t-Value	0.95
P-Value	0.3856
Median Motor Amplitude:	
Baseline Mean ± SE (range)	4.454 ± 2.766/1.046 (0.83 – 8.439)
Week 4 Mean ± SE (range)	6.281 ± 1.504/0.614 (4.481 – 8.399)
Mean Difference	-1.098
DF	5
t-Value	-1.628
P-Value	0.1645
Median C V:	
Baseline Mean ± SE (range)	52.667 ± 8.066/3.293 (44.000 – 66.000)
Week 4 Mean ± SE (range)	52.500 ± 5.753/2.349 (42.000 – 59.000)
Mean Difference	-0.167
DF	5
t-Value	-0.063
P-Value	0.9521

Table 2: Median Motor Nerve Conduction Studies.

Other effects reported

Three of the patients reported an increased paresthesia on the active drug, described as a "tingling sensation." One patient reported that he felt as if he were recovering again from GBS. Four of the patients reported considerably improved motor endurance on the active agent. One reported that they could "peel several potatoes" instead of just one. Another patient reported being able to walk the length of the local shopping mall without resting. For the previous four years, he had only been able to walk 1/3 of the distance.

Adverse events monitoring

Laboratories: Only two laboratory values had a statistically significant change. The SGOT went up from 25.1 U/L SD ± 9.7 to 27.9 U/L SD+ 11.6 (p=0.0341, Paired Student's t-test) and the hematocrit dropped from 42.7% SD ± 2.8 to 41.6% SD ± 2.6 (p=0.03, Paired Student's t-test). Neither of these changes was deemed to be clinically relevant and may reflect statistical chance as so many laboratory values were tested. There were no other significant changes while on the active agent. No laboratory values during the study were considered to fall in the abnormal range per our institutional laboratory values.

There were no seizures, nor were there any significant changes in the Q – T interval on any of the electrocardiograms (EKG) recorded. Subjects did report increased paresthesias on 4-AP. There were no statistically significant findings noted in either amplitude or velocity nerve conduction studies, but this failure could be due to the small patient numbers. There was a trend towards increased amplitudes in the median motor conduction studies.

Adverse events

One patient had to drop out of the study. She was two weeks into the first arm of the study and on the active medication when some tingling in her extremities was noted. No significant change in motor strength was noted; there was no progression at week 3, although she reported more tingling. At week 4 there was a drop in hand grip strength as recorded on the hand held dynamometer. The motor nerve conduction studies demonstrated a decrease in both the amplitude and the nerve conduction velocity. The blinding was broken, and she was found to be on the active drug. The patient was reviewed with the Neurologist on this study, as well as with her personal Neurologist. It was believed that there was a possibility that the patient was having a relapse of her inflammatory demyelinating polyneuropathy more than one year from the initial event. Because the following week was the "washout week," they decided to observe her for another week. Her strength dropped significantly during the next week, and she was placed on prednisone and removed from the study. Over the next three months she underwent two treatments with intravenous immunoglobulin (IVIg) for what was believed to be chronic demyelinating polyneuropathy. A review of the literature indicates that the development of a relapse this far out from acute GBS is very rare. It was the conclusion of the research team that while the 4-AP probably did not cause the relapse, it masked the relapse by improving her motor function. A serious adverse event report was sent to the FDA.

Discussion

This small pilot study demonstrated that there were statistically significant improvements in motor strength using oral 4-AP for persistent motor weakness due to GBS. This is truly remarkable considering the small number of patients enrolled in the study and outcome measures utilized. Indeed a change in motor strength from a level where one only has strength to move against gravity (motor score 3) to one that can give resistance against the examiner (motor strength 4) has consistently been proven to result in a major functional change in the disabled from a motor impairment (100). In our study we improved motor strength in the upper limbs from 3.2 to 4.3 (p=0.0065,

Friedman's) on the active agent and in the lower extremities from 3.2 to 3.7 (p<0.0001, Friedman's) on the active agent. In the upper limbs there was a statistically significant improvement on the active agent versus the placebo with regard to both motor strength (p<0.0073, Wilcoxon Signed Rank) and the grip strength improved on the active agent while there was no change in the placebo arm. In some patients this resulted in functional status changes with regard to ambulating without a cane or improved independent living skills. It has to be acknowledged that there is variability in motor performance testing day to day, which is why a larger study is warranted. Studies in the spinal cord injury (SCI) population have concluded that changes in motor strength that have been less than this may be clinically relevant, and have been recommended continued study or use to improve motor recovery or function [98,99]. Changes in motor strength of less than those reported here led to the recommendations for the use of methylprednisolone in SCI [99] but unless there is fair to good strength, most functional activities will not be changed [100]. Furthermore, the trends in functional improvement are encouraging as it has been demonstrated that even small increases in motor strength or endurance can reduce functional limitations and improve the quality of life of the disabled.

It must be stressed that in a Phase IIa FDA trial (10-20 patients) the primary focus is on safety not efficacy with a secondary goal of assessing drug dosing levels. This pilot study, despite its small size, indicates that 4-AP may be effective in improving the motor function of GBS subjects. The results in this GBS patient population are clearly more dramatic than that seen in MS or SCI, the original study populations for the agent [27-33,37,38]. Most patients reported a subjective improvement in motor endurance, including increased ambulation and repetitive hand function. These activities were not adequately tested in this study and will need to be studied in more detail in a future study. Nerve conduction studies revealed a trend towards increased amplitude in the motor nerve conduction studies, which also may warrant further study. It has been suggested in some studies that 4-AP may improve nerve conduction [37,39,40], or the synchronous firing of motor units [41], thereby improving motor function.

The data indicate that the placebo group might have been affected by the inadequate washout period. There have been some claims that the biologic effects of 4-AP on the CNS may last as long as two weeks [33]. The loss in motor strength in the upper limbs at 4 weeks of treatment was not consistent with the increased grip strength. This variability may reflect the lack of patient numbers, which could result in test-retest variability playing a significant factor.

Adverse effects associated with oral administration of 4-AP have included mild dizziness, light-headedness, paresthesia/dysesthesia, nausea and mild agitation [30,31,42,43]. Doses above 30 mg. per day have induced confusional states (disorientation, agitation, anxiety), respiratory distress (dyspnea, hyperventilation), locomotor and balance problems and epileptiform seizures (34-36,39), which is why we elected to not go above 30 mg per day. Seizures appear most commonly in those who have multiple sclerosis [31,42,43]. Cardiac abnormalities have been reported only with extremely high doses, beyond what is now recommended for treatment [39].

Most reports of GBS describe pain as a prominent clinical feature of the diagnosis and it has been reported to be the sole initial symptom in some [19]. The types of pain described include paresthesia, dysesthesia, axial and radicular pain, meningism, myalgia, joint pain and visceral discomfort [20]. In one small prospective study early pain was reported in 55% of the patients and in 72% of the patients throughout the whole course of illness [21]. In this study there was improvement of pain symptoms in both arms of the study. Of significance, some patients reported increased paresthesias while on the active medication. Three of the patients described a dysaesthetic type of pain as if they were again in the first stages of recovery from GBS.

There were no untoward side effects noted in our study. Although there have been reports of changes in the electrocardiograms of MS patients [37], there were no changes noted in our study. We had no significant laboratory abnormalities in our study. 4-AP has been linked to increased seizure activity in MS patients [37]. Because our patients are not prone to seizures, as are MS patients, it is not expected that seizures will be a significant impediment in the use of 4-AP for those patients who have suffered from an inflammatory polyneuropathy. The incidence of one patient having a relapse and developing CIDP was not thought to be related to the agent. However, the 4-AP could have masked the relapse by maintaining the motor strength.

Pharmacokinetics of different 4-AP formulations have been evaluated in healthy adults, and in individuals with MS, administration of 4-AP in the immediate release form in doses significantly above 30 mg. per day has resulted in plasma levels in excess of 100ng/ml [37,38]. These plasma levels were associated with seizure activity in some MS subjects [37,38]. Clinically, 4-AP has been well tolerated by patients whose plasma concentration was below 100ng/ml. Dosages of up to 30 mg per day of the Immediate Release form have generally been well tolerated in the MS or SCI populations [28,29,33,37,38,60-74].

In animal studies, aminopyridines such as 4-AP are able to block the fast K+ channels and are able to improve conduction in regions of nerve demyelination [75,76]. 4-AP apparently has little effect on the action potential waveform or the firing properties of mature myelinated axons but 4-AP does increase the duration and amplitude of the compound action potential in myelinated fibers [73,76]. In the normal mammalian myelinated axon, voltage-sensitive sodium channels are densely clustered in the area of the node of Ranvier [69]. On the other hand fast K+ channels are present under the endoneurial sheath and the myelin [70-72]. The K+ channels are believed to assist in the generation of the internodal resting potential [72] to prevent re-excitation and stabilized firing properties after the action potential [73]. When the recovery process of the action potential is slowed, such as by a block of the K+ channels, the amplitude of the compound action potential is increased. In regions of the nerve paranodal and internodal areas if these channels are disrupted then there is a conduction block [74,75,77]. By increasing the duration and amplitude of the action potential, 4-AP may be capable of blocking sufficient K+ channels so that the action potential can be propagated beyond the point of demyelination and trigger another "all or none" action potential at the next intact node of Ranvier [78-85]. It has been well demonstrated in post-polio patients that one may only need 10 to 20% of the active motor fibers to have some "functional strength" [86-94]. Restoring only a few percent of the initial motor units may significantly improve motor strength in this population. In animal studies, 4-aminopyridine is able to block the fast K+ channels and is able to improve conduction in regions of nerve demyelination [74,75]. The effect of 4-AP on nerve conduction is felt to be due to its ability to increase the duration and amplitude of the compound action potential [73,76]. Blocking K+ channels by 4-AP improves the safety factor in nerve conduction and may result in improved conduction in those fibers with a reduced conduction, or restore conduction in those

nerves with a conduction block [77-80]. In patients with multiple sclerosis 4-AP increased the mean amplitudes and decreased the variability of onset latencies in the peripheral nerves [40]. It appeared to improve impulse conductivity in evoked potentials in either peripheral and/or central nervous system [40].

This was the first study on the use of 4-AP in patients whom have suffered GBS. There are reports on the use of a similar compound, 3,4-diaminopyridine (DAP) in demyelinating polyneuropathy. In two studies with DAP there was a failure to improve motor or sensory nerve conduction and it was not successful in improving motor strength [74,85]. Both DAP and 4-AP may increase chemical transmission at peripheral synapses by increasing transmitter release and thereby improve strength similar to the effect that 4-AP in patients who have failure of neuromuscular transmission, as seen in Lambert-Eaton syndrome [83-86]. Because 4-AP crosses membranes so much better than DAP due to its lipophilic nature it may be superior to DAP with regard to its effects at the neuromuscular junction. Both mechanisms of action are likely to benefit the functional status in the post-GBS patient population. Unlike 4-AP, DAP does not cross the blood nerve barrier (BNB) sufficiently to block the fast K^+ channels underneath the myelin to improve nerve conduction. This is because DAP is very polar and does not cross the lipophilic blood-brain barrier (BBB) (74,86) and likely does not cross the BNB. It was initially felt that this was an advantage as DAP would contribute to fewer central nervous system side effects, such as seizures, than 4-AP [74,86]. Indeed DAP was developed initially because it had a much lower level of penetration than 4-AP and thus DAP was felt to be "safer" than 4-AP [92]. However, this may have resulted in its failure to demonstrate efficacy in trials with patients who were suffering from the chronic effects of motor weakness from only partial remyelination following an inflammatory neuropathy [74,85]. Although the BNB long has been considered more "leaky" than the BBB, recent in vivo studies has established that the BNB may be almost as effective in occlusive function as the BBB [87-89]. Experimental studies on Wallerian degeneration have demonstrated that the BNB loses structural integrity during the early phases of axonal degeneration and gradually recovers over several months and then is reestablished [89-91].

During the acute phase the BNB is very permeable [89]. Indeed there is recent evidence that the initial disruption of the BNB affects the ion channels underneath the epineurium and contributes to the early conduction block noted in GBS [93]. When the antibodies that contribute to this inflammatory reaction are removed the conduction block may be reversed [40,93]. However, over several months the structural integrity of the BNB is restored [89-91]. This time course of events may explain why experimental studies utilizing DAP during the acute inflammatory stages of demyelination in animals demonstrated improved nerve conduction but progressively decreases over time as the nerve "heals" [74,75]. In those patients who do not have an ongoing inflammatory demyelination it is unlikely DAP will penetrate to the axonal K^+ channels underneath the Schwann cells. However, because 4-AP is lipophilic and crosses the BNB easily it is likely that it will affect the K^+ channels under the myelin whereas DAP will not. This explains the failures of the previous studies that utilized 3.4-diaminopyridines to improve nerve conduction in patients who had suffered a demyelinating polyneuropathy [95-100].

It may be argued that the predominate effects of 4-AP may be more at the neuromuscular junction rather than by improving transmission at the level of the axon [101]. This is based on one animal study

utilizing radiation myelopathy [101]. First, this study [101] acknowledges that it is conflict with the study by Shi and Blight [102,103]. In their animal model of demyelinated spinal cord injury there was an improvement in conduction at the level of the axon with 4-AP [103]. It was admitted that the demyelination caused by a crush injury might be very different than that caused by radiation [103]. We agree and it is likely that both models of demyelination above have little in common with demyelination of a peripheral nerve caused by an autoimmune disorder. Indeed, it can also be argued that both models have little in common with autoimmune CNS lesions of demyelination such as MS. In a rodent model, radiation to the spinal cord induces a late myelopathy that not only has effects on the oligodendrocytes, but also has effects on vascular tissue and progenitor cells that may impede central nervous system recovery from injury [102]. In GBS there often is a small area of focal demyelination. If the segment demyelinated does not involve too many nodes of Ranvier there is a possibility of restoring conduction across the area of demyelination with even partial remyelination. This is not the case in a CNS model where the radiation may disrupt myelin, neurons and vascular flow in a large area of the central nervous system [102-109].

Finally, similar to DAP, 4-AP may increase chemical transmission at peripheral synapses by improving neuromuscular transmission, as seen in Lambert-Eaton syndrome [83,84,86]. Because 4-AP crosses membranes so much better than DAP due to its lipophilic nature it may be superior to DAP with regard to its effects at the neuromuscular junction. Both mechanisms of action are likely to benefit the functional status in the post-GBS patient population.

Clearly, eight patients are not enough to establish efficacy for approval of a medication. The fact that the trends in the functional outcome scales were so significant with the active drug is encouraging. A change in the Motor score of even a quarter point may result in significant functional changes that may reduce the disability of patients. Finally, we acknowledge that the placebo group might have been affected by the inadequate washout period. The loss in motor strength in the upper limbs at 4 weeks was not consistent with the increased grip strength. This variability may reflect the lack of patient numbers, which could result in test-retest variability playing a significant factor.

Currently, there is no approved treatment of any kind for the debilitating fatigue and motor weakness in GBS patients who have not fully recovered, leading to significantly reduced functional status and quality of life for many. This medication may be compounded in the United States. However, this study was an early phase study focused mostly on safety and some preliminary clinical efficacy data. A more extensive randomized trial over a longer period of time is necessary obtain data on the potential efficacy of 4-AP in the GBS patient population.

Acknowledgement

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