

Effects of a Potassium Channel Blocker, 4-aminopyridine, on Lipid Profiles and Cardiovascular Risk Factors in Patients with Spinal Cord Injury

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Abstract

Study Objective: Spinal cord injured patients are at a 3.7 fold increase risk of death due to cardiovascular disease. To characterize baseline cardiovascular risk factors, including lipid profiles and to detail the effects of the long-term administration of a potassium channel blocker 4-aminopyridine (4-AP) on the risk factors with long-standing spinal cord injury (SCI).

Design: Randomized, active-treatment control, dose-level blinded study.

Setting: University-affiliated, tertiary-level care medical center.

Interventions: Oral administration of an immediate-release formulation of 4-AP.

Patients: Thirty-nine healthy men and women (27 quadriplegic and 12 paraplegic) SCI-outpatients suffering from traumatic SCI (≥ 1 year) who had completed six months of treatment with 4-AP.

Measurements and Main Results: 4-AP had no effect on body weight or blood pressure at the end of six-months. The high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), triglyceride (TG), LDL/HDL and TC/HDL at baseline and after six months of treatment with 4-AP were compared. A paired t-test was carried out to test for the significance of differences between means and variances. There were no statistically significant differences between HDL-C, TC, LDL-C, and TC/HDL at baseline and after six months of treatment with 4-AP. TG values (mean \pm standard deviation) went from 81.95 ± 63.78 mg/dL to 101.18 ± 66.17 mg/dL ($p = 0.003$) after six months administration of 4-AP.

Conclusion: During the six month time period that 4-aminopyridine was administered, changes in HDL-C, LDL-C, TC, and TC/HDL levels were not statistically significant, with the exception of an increase in TG levels. 4-AP does not appear to influence HDL-C,

LDL-C, TC and TC/HDL levels. However, the potential association of treatment with 4-AP to the statistically significant increase in TG levels after six months of drug intake needs further investigation and has yet to be clearly elucidated.

Keywords: spinal cord injury; 4-aminopyridine; serum lipids, coronary heart disease; lipoproteins.

Running Head: Spinal Cord Injury: Effects of 4-aminopyridine on Serum Lipids

Introduction

Spinal cord injury (SCI) patients have a higher risk of coronary heart disease (CHD) because of an unfavorable lipid profile and a characteristically high prevalence of low level high-density lipoprotein (HDL) cholesterol.^{1,2} It has been suggested that the low HDL-C levels in SCI patients are due to extreme physical inactivity,^{3,5} resulting from a sedentary life-style.² Several studies also suggest that low HDL-C levels increase the risk of CHD,⁴ lessening the protective effect of HDL-C in transporting oxidized small dense cholesterol particles to the liver.^{5,6} The ratio TC/HDL-C has been found to be associated with CHD risk if it is above the desired ratio of 4 or lower.^{6,7} Many clinicians also use the ratio of LDL/HDL as a marker of risk with a ratio equal to or above 3 at risk. Typically, the threshold risk for CHD is HDL-C less than 35 mg/dL.⁷ In SCI, varying degrees of sensorimotor impairments as well as physiological and metabolic changes are evident.^{1,8} SCI causes the disruption of pathways from the brain to the sympathetic nervous system. Cardiovascular changes in SCI patients can be attributed to changes in sympathetic activity.¹

4-aminopyridine (4-AP) is a potassium channel blocker that has the capacity to improve the propagation of action potentials in demyelinated nerve fibers.^{8,9} Some of the benefits of 4-AP in humans with SCI are improved muscle strength, decreased spasticity,

enhanced energy and endurance. The capability of 4-AP to facilitate conduction of impulses within the damaged spinal cord in humans has been associated with modest improvements in electrophysiologic parameters and clinically evident improvement in neurologic and sensorimotor function.^{8,9} Evidence has been found that 4-AP exhibits unique, potentially beneficial pharmacologic properties.^{9,10} This study was implemented to test the hypothesis that the long-term administration of 4-AP might cause alteration of the lipid profile of spinal cord injured humans.

Methods

Subject Selection and Characteristics

The study was conducted with the authorization of the U.S. Food and Drug Administration (FDA) under IND #47,305. Thirty-nine healthy men and women volunteers (27 quadriplegic, 12 paraplegic; Table 1) with traumatic SCI for more than one-year duration completed the six-month study. Efficacy data on these 39 patients was reported earlier.¹⁴ All patients gave informed consent. Spinal cord injuries were complete in 14 patients and incomplete in 25 patients. Before the screening evaluation, the medical records of each potential participant and their completed prescreening questionnaire were reviewed. Each participant underwent a comprehensive history and physical examination and detailed neurologic examination before enrolling. Laboratory tests were resting electrocardiogram (ECG), electroencephalogram (EEG), a comprehensive biochemical profile, urinalysis, a lipid panel, and a 5-hour glucose tolerance test. Patients who were selected for enrollment repeatedly took these tests and examinations at each visit. Exclusion criteria were seizures, epilepsy, or an abnormal EEG; recreational or illicit drug use, including ethanol abuse; maintenance treatment with bronchodilators; use of anticholinergic (atropinic) or antihistaminic drugs; psychiatric disorders; and pregnancy or inadequate or unverifiable gender-specific contraceptive measures. Subjects' usual lifestyle, such as activity levels, sleep-wake cycles, and dietary patterns, for the duration of the study, were not altered except when they returned for scheduled testing, which was defined as time of day (circadian rhythm), activity level and diet content-dependent. The study was conducted with IRB approval at Long Beach Veterans and Harbor-UCLA Medical Centers.

Interventions and Treatments

Prior to the study, three SCI subgroups were defined:

- Group 1: 7 subjects, dosage-cognizant, who received 30 mg/day and were at steady state at three and six months;
- Group 2: 16 subjects, dosage-blinded, 4-AP naive were titrated to 30 mg/day and were at steady state at three and six months;
- Group 3: 16 subjects, dosage-blinded, 4-AP naive, who received 6 mg of active drug/day in divided doses, and served as a low-dose, active treatment control group.

With the approval of the FDA, this trial used an active treatment control group instead of a placebo control. The active treatment control was chosen because 4-AP causes subject discernible paresthesias at all reasonable doses. Dosages were titrated to tolerance in increments of 2, 5, or 10 mg over two weeks using an immediate-release formulation of crystalline 4-AP (Regis Technologies, Morton Grove, IL) in admixture (w/w) with pharmaceutical-grade microcrystalline cellulose (Avicel; PH-101, NF, FMC Corp., Philadelphia, PA).

Anthropometric Factors

Patients' weight was measured using a calibrated bed scale. Height was determined by placing the patient in a supine posture, and the position of the top of the patient's head and feet (heel) were marked on the bed sheet. The subject's height is the distance between the two marks and was measured in centimeters. The Body Mass Index (BMI) was defined as body mass in kilograms divided by the height in meters squared.

Randomization

Individuals were randomized in accordance with a computer-generated allocation schedule. Allocation was concealed in sequentially numbered, sealed envelopes that were not opened until randomization, one day before enrollment.

Masking

All dosages were prepared using no. 2 gelatin capsules and microcrystalline cellulose. All capsules had the same appearance and taste, and did not vary in weight. The investigational drug was stored and dispensed by the Research Pharmacy Service, and was issued in identical plastic containers. The allocation schedule was accessible only to the research pharmacist throughout the study.

Blood Lipids

The TC and TG concentrations were measured using enzymatic tests and HDL-C concentration was measured using a unique detergent combined with enzymatic tests on the SYNCHRON LX20 (Beckman Coulter,

Inc., Fullerton, CA, USA). The coefficient of variation for HDL-C, TC and TG measurements is 4.5%. The LDL-C concentration was calculated using the Friedewald Formula: $TC - HDL-C - TG/5$.

Statistical Analyses

Sample size and power calculations were used to determine the sample size needed to support statistical inferences. Means and standard deviations (SD) were calculated using Microsoft Excel. A paired t-test was done to compare group differences at baseline (pre-treatment) to those at six months. A p value (two-tailed) of 0.05 or less was required to assign statistical significance to the differences between means and variances. Means are expressed as \pm SD.

Results

The homogeneity of the study population is shown in Table 1. Baseline blood pressure measurements were normal and there was no effect on six-month blood pressure measurements. Furthermore, there was no effect on body weight over the six-month period when compared to baseline measurements (Table 1). No statistical significance was observed between LDL-C, TC, TG and TC/HDL (Table 2, 3, 5, 6) at baseline and after six months treatment with 4-AP. Likewise there was no significant increase in LDL-C/HDL-C ratio (data not shown). However, statistical significance was seen in triglyceride concentrations for all SCI subjects and increased from 81.95 ± 63.78 mg/dL to 101.18 ± 66.17 mg/dL ($p = 0.003$, Table 4) following six months administration of the drug.

Discussion

Spinal cord injury, also referred to as traumatic spinal myelopathy, generates cataclysmic changes in physiological and metabolic homeostasis in humans.⁸

Coronary heart disease (CHD) is prevalent in individuals with SCI and contributes to the high morbidity and mortality rates in this population.^{2,4,6,11,15} It has recently been demonstrated that SCI patients have a 3.7-fold increase mortality from cardiovascular diseases,¹⁵ and it is likely that this increased risk is due in part to the extremely low HDL-C levels and physical inactivity of spinal cord injured persons.^{6,12,13}

4-aminopyridine has been designated as an FDA Orphan Drug. The safety and efficacy of 4-AP in restoring and enhancing sensorimotor function in persons with SCI are currently under investigation.¹⁴ The present study is the first study to examine the influence of the long-term administration 4-AP on the blood lipid profiles of SCI patients and was implemented as a component of an ongoing process of drug discovery and population-specific assessment of drug safety. Our primary goal was to quantitatively assess whether 4-AP negatively or adversely affects serum lipid concentrations following SCI, especially HDL-C levels which are strongly related to CHD risk,^{4,6} since an adverse effect on serum lipids, especially those associated with an increased risk of CHD, could increase the risk-benefit ratio of administration or make 4-AP unfit for use as a pharmacological intervention in SCI. For the same reasons, the influence of 4-AP on critical ratios such as TC/HDL-C and LDL/HDL, during six months of drug intake was also investigated as both ratios are considered to be stronger predictors of CHD than isolated elevations in HDL-C concentration.³ It has been shown that a one unit increase in the TC/HCL-C ratio increases the risk of myocardial infarction by 53%.⁷

We demonstrated that after six months of 4-AP administration, only the triglyceride(TG) fraction of the total lipid profile of 39 patients with SCI was significantly increased. The differences in HDL-C, LDL-C, TC and TC/HDL at baseline and six months showed some non-significant trends that were neither statistically nor clinically significant. An increase in TG levels is associated with a decrease in HDL-C levels.⁷ And this association was true for Group 1 and Group 3 (Table 2). While, HDL-C and TG levels for all SCI and Group 2 increased by 23% after six months, only the increase in TG is note-

Table 1: Anthropometric data of the SCI subjects.

Groups	Demographics				Injury Level		
	Mean \pm Standard Deviation				C2-T2	T3-T12	L1, S4-5
	Age (yrs)	Height(cm)	Weight(kg)	BMI (kg/m ²)			
All SCI, n=39	42.59 \pm 12.08	176.48 \pm 8.63	74.79 \pm 14.41	24.48 \pm 5.53	27	11	1
Group 1, n=7	49.57 \pm 10.21	174.53 \pm 7.14	75.64 \pm 14.841	24.78 \pm 4.36	4	3	0
Group 2, n=16	37.81 \pm 13.50	177.28 \pm 9.86	73.47 \pm 14.31	24.83 \pm 7.42	11	4	1
Group 3, n=16	44.31 \pm 9.77	176.53 \pm 8.30	75.21 \pm 15.23	24.00 \pm 3.79	12	4	0

worthy and is probably of negligible importance. (Table 2, 4).

It is reasonable to conclude from the results of our

study that the peroral administration of an immediate release formulation of 30 mg per day of 4-AP for up to six months does not cause clinically significant changes in HDL-C, LDL-C, TC and TC/HDL levels, i.e., pre-

Table 2: HDL-C concentrations of SCI subjects.

Groups	HDL-C (mg/dL)		p Value
	Mean ± Standard Deviation		
	Baseline	6 months	
All SCI, n=39	47.90 ± 11.56	48.33 ± 13.42	0.65
Group 1, n=7	45.14 ± 15.51	44.86 ± 16.82	0.84
Group 2, n=16	47.75 ± 8.77	50.06 ± 11.40	0.21
Group 3, n=16	49.25 ± 12.65	48.12 ± 13.42	0.41

Table 3: TC concentrations of SCI subjects.

Groups	TC (mg/dL)		p Value
	Mean ± Standard Deviation		
	Baseline	6 months	
All SCI, n=39	185.72 ± 33.57	190.13 ± 37.46	0.15
Group 1, n=7	187 ± 41.67	183.86 ± 45.35	0.66
Group 2, n=16	183.19 ± 31.01	187.69 ± 33.57	0.08
Group 3, n=16	191.87 ± 44.60	191.12 ± 26.8	0.48

Table 4: Triglyceride concentrations of SCI subjects.

Groups	TG (mg/dL)		p Value
	Mean ± Standard Deviation		
	Baseline	6 months	
All SCI, n=39	81.95 ± 63.78	101.18 ± 66.17	0.003
Group 1, n=7	150.29 ± 117.32	193.86 ± 159.45	0.11
Group 2, n=16	69.06 ± 31.50	80.19 ± 43.36	0.06
Group 3, n=16	64.94 ± 33.78	81.62 ± 85.35	0.09

Table 5: Low density lipoprotein Cholesterol concentrations of SCI subjects.

Groups	LDL (mg/dL)		p Value
	Mean ± Standard Deviation		
	Baseline	6 months	
All SCI, n=39	117.87 ± 35.16	121.64 ± 33.18	0.37
Group 1, n=7	111.71 ± 28.78	100.29 ± 31.80	0.10
Group 2, n=16	121.50 ± 30.09	125.87 ± 38.22	0.28
Group 3, n=16	125.50 ± 35.16	126.75 ± 25.78	0.73

Table 6: Total cholesterol and high density lipoprotein cholesterol ratio of SCI subjects.

Groups	TC/HDL		p Value
	Mean ± Standard Deviation		
	Baseline	6 months	
All SCI, n=39	4.10 ± 1.22	4.21 ± 1.37	0.19
Group 1, n=7	4.65 ± 2.04	4.67 ± 2.15	0.92
Group 2, n=16	3.96 ± 1.00	3.95 ± 1.07	0.93
Group 3, n=16	3.98 ± .096	4.28 ± 1.37	0.11

dictors of CHD, in spinal cord injured humans and does not appear to adversely influence a 23% risk-benefit ratio for prescribing this drug. The significance of the increase in TG after six months of drug intake is unknown and requires further investigation in larger populations of patients.

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