Original Article

Effect of 4-aminopyridine on gait in ambulatory spinal cord injuries: a double-blind, placebo-controlled, crossover trial

D DeForge[1,3], J Nymark[2], E Lemaire*[,1,2], S Gardner[3], M Hunt[3], L Martel[2], D Curran[2] and H Barbeau[4,5]

[1]Division of Physical Medicine and Rehabilitation, University of Ottawa, Ottawa, Ontario, Canada; [2]Institute for Rehabilitation Research and Development, The Rehabilitation Centre (The Ottawa Hospital), Ottawa, Ontario, Canada; [3]The Rehabilitation Centre, Ottawa, Ontario, Canada; [4]School of Physical and Occupational Therapy, Faculty of Medicine, McGill University, Montreal, Canada; [5]Jewish Rehabilitation Research Centre, Laval, Quebec, Canada

Animal and human research have shown that the drug 4-aminopyridine (4-AP) may improve gait in spinal cord lesions by enhancing nerve transmission to affected muscles.

Study design: Prospective, randomized, double-blind, placebo-controlled, crossover trial.

Objectives: To determine the efficacy of 4-AP in improving lower limb muscle strength and biomechanical gait patterns of chronic spinal cord injuries (SCI).

Setting: The Rehabilitation Centre (Ottawa, Canada).

Methods: In all, 15 chronic, ambulatory SCI persons were randomized to an initial 2 weeks of 40 mg/day, oral medication of either placebo or immediate-release, 4-AP and subsequently crossed over to the alternate medication for the following 2 weeks. Evaluations were conducted at baseline (before starting 4-AP or placebo medication), 2 weeks, and 4 weeks. Measures included dynamometer lower limb isometric muscle force and biomechanical gait measures including temporal–spatial parameters, electromyographic activation patterns, joint kinematics and kinetics. Subjective impressions of the drug by the participants were obtained from an exit survey.

Results: Despite some positive comments from subjects, statistical and clinical analyses showed no within-subject differences between placebo and 4-AP measures of lower limb muscle force and objective gait analyses (ANOVA statistic $P > 0.05$).

Conclusion: Results demonstrated the importance of placebo-controlled trials and quantitative outcome measures for the evaluation of 4-AP aimed to enhance gait for chronic, ambulatory SCI persons. Energy expenditure measures and mood may relate more to subjective comments and is suggested for future investigations.

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Introduction and background

Persons with incomplete spinal cord injuries often have limited mobility. Effective and energy efficient ambulation is frequently a primary goal for these individuals. However, factors such as demyelination of damaged axons may adversely affect the recovery process and contribute to long-term sensory and motor impairments.[1] Demyelination causes an increase in potassium efflux from the affected axons, thereby blocking neuron action potentials. Animal research has shown that residual fibers, following a severed spinal cord, appeared to maintain continuity across the site of injury. However, these neurons may exhibit a loss of function due to demyelination.[2] In human studies, facilitating conduction in surviving central or peripheral nerves may improve gait by increasing muscle strength, coordination, and sensation.[3]

Pharmacological agents such as 4-aminopyridine (4-AP) can increase synaptic transmission and enhance conduction in both peripheral and central demyelinated nerve fibers by blocking the potassium channel at the axon membrane.[4] In experimentally demyelinated peripheral nerves, 4-AP can reverse conduction block and restore action potential transmission.[4,5]

Blight[2] demonstrated that conduction block in partially spinalized animals can be overcome by 4-AP.

*Correspondence: E Lemaire, Institute for Rehabilitation Research and Development, The Rehabilitation Centre, 505 Smyth Road, Ottawa, Ontario, Canada K1H 8M2.

Human case studies have reported that 4-AP may produce neurological improvements in patients with inflammatory demyelinating diseases such as multiple sclerosis (MS) and in neuromuscular junction diseases. Intravenous and oral 4-AP have produced improvements in vision, neurological impairments and reduction of disability for MS patients.[6,7] Within the last 10 years, investigators have turned their focus to the potential benefits of 4-AP in the spinal cord injury (SCI) population.[3] Preliminary, preclinical case studies have provided information on safety, dosage and outcome changes.[8,9] Hayes et al[10] reported enhanced volitional electromyographic (EMG) signals in three incomplete SCI patients following a one time, ongoing intravenous 4-AP dose of 24 mg over 4 h. There was no similar change in the three SCI subjects with complete lesions.

The single group studies led to several controlled trials. Hansebout et al[11] implemented a randomized, double-blind, crossover design to study the effect of intravenous 4-AP over a 2-h period administered to eight, chronic SCI subjects. Five out of six participants, with incomplete injuries, experienced improved sensation, less pain and a trend toward improved motor function. Furthermore, the beneficial effects persisted for up to 48 h following the medication. No improvements were seen in the two complete paraplegic participants. Potter et al[12] studied 26 subjects using a controlled, crossover design. The 4-AP (Fampridine-SR) group revealed improved sensation and responded more positively to the drug efficacy and improved quality of life due to the medication. In a similarly designed crossover study, Donovan et al[13] investigated nonambulatory, chronic SCI subjects and found no evidence of superior gains in the group receiving 30 mg intravenous 4-AP on motor, sensory, spasticity and pain scores.

The above investigations on 4-AP have focussed on sensation, muscle strength and spasticity. However, quantitative gait research related to 4-AP has been very limited. Theoretically, by improving central conduction of action potentials in surviving spinal cord axons, 4-AP may enhance the potential for improved locomotion.[3] MS patients using 4-AP have subjectively reported an increase in walking ability and a decrease in fatigue.[14]

In a noncontrolled study, Segal and Brunnemann[15] measured temporal–spatial gait parameters including velocity, cadence and stride length of nine ambulatory, SCI individuals. The subjects received a single 10 mg dose of 4-AP and were measured regularly over a 24-h period. Perceived increase in smoothness of gait and modest improvements in velocity and stride length were reported for the majority of subjects. However, without a comparative placebo group, the authors recognized that the effect of 4-AP could not be isolated. A more recent placebo-controlled, crossover study investigated gait velocity in 20 chronic, SCI subjects and found no significant increases in the 4-AP group.[16]

Dosage protocols for 4-AP have ranged from single doses to multiple daily intake with quantities varying from 10 to 50 mg. Duration of drug administration has also varied from 2 h up to 4 months. Good tolerance to the various levels of medication have been reported.[3] One study proposed that administration of 25 mg 4-AP closer to the lesion site, intrathecally, may have a greater opportunity for effectiveness.[9] Preliminary results, however, showed that at least at lower doses, there was no appreciable effect.

The purpose of this clinical, controlled trial was to expand on past findings and evaluate the effect of oral 4-AP on lower limb isometric muscle force, biomechanics and EMG activation during gait in chronic, ambulatory, incomplete SCI subjects. By using a randomized, placebo-controlled, crossover design, determination of locomotor improvements, reported in the literature, can be isolated to 4-AP.

Methodology

Subjects
In all, 15 ambulatory individuals (12 male, three female) with chronic, incomplete SCI were recruited from The Rehabilitation Centre, Ottawa, Ontario. Subjects ranged in age from 22 to 70 years ($\bar{x} = 40.1$ years, $SD = 13.6$) and were at least 18 months postinjury. All participants were ASIA D[17] level of recovery demonstrating at least a grade three level of muscle strength in the majority of the muscles below the neurological level.

Ambulation status of all subjects was graded into four categories (manual assistance, walking aids, endurance, gait velocity) using the ambulation subscale of the Clinical Outcome Variables Score (COVS) mobility scale.[18] A maximum score of 28 represented the peak functional locomotor capabilities. Ambulation scores of the subjects ranged from 11 to 27 (Table 1). The participants were either quadriparetic or paraparetic and were able to ambulate with or without a walking aid or ankle–foot orthosis. Persons requiring bilateral knee–ankle–foot orthoses were not included in the trial. Two of the 15 subjects preferred to use their wheelchair for outdoor mobility. Six subjects were previously stabilized on medication for spasticity. Exclusion criteria included cerebral lesions, history of seizures, or liver disease. Subject characteristics are summarized in Table 1.

Study design
The study used a randomized, double-blind, placebo-controlled, crossover design. The subjects acted as their own controls and were randomized to commence the trial with either placebo or 4-AP oral dose. The crossover design allowed for more targeted within-subject changes minimizing the confounding variable of differences in between-subject characteristics. The criteria for the use of the crossover design is satisfied by knowing the short action of 4-AP which minimizes the carryover effect and potential order effect of the drug assignment (half-life of 3 h with 90% washout by 30 h). In addition, the relative stability of the physical status of

Table 1 Subject characteristics (KAFO is the abbreviation for 'knee–ankle–foot orthosis' while AFO stands for 'ankle–foot orthosis')

Subject	Age	Gender	SC level	Etiology	Years since onset	Anti spasticity medications	Walking aids	COVS ambulation score[18]
1	51	M	T12	Familial spastic Paraplegia	13	No	(L) KAFO (R) AFO	25
2	57	M	C3–5	Trauma	3	Clonidine	None	23
3	29	F	C5–6	Trauma	4	Clonidine	None	27
4	70	M	T12	Excision of arterio venous malformation	4	Cyproheptadine	Walker	15
5	50	M	C4–5	Trauma	20	No	None	27
6	42	F	C3–4	Trauma	2	Baclofen	Single cane	26
7	46	M	C5	Syringomyelia	8	No	Single cane	26
8	29	M	C6–7	Trauma	5	No	None	27
9	40	M	C5	Trauma	9	No	Wheeled walker	11
10	40	M	C4–5	Trauma	1	No	None	27
11	45	M	T4	Transverse myelitis	9	No	None	27
12	22	M	L1	Trauma	1	No	2 AFO's	25
13	33	F	C7	Trauma	6	Baclofen	Single cane	25
14	24	M	C4	Trauma	4	No	None	27
15	24	M	T4	Trauma	2	Clonidine	None	25

the majority of the subjects with chronic spinal cord pathologies minimizes the chances of physical changes over a set time period. The critical level of change for the main outcome variable, walking speed, was 0.15 m/s, providing a power of 0.80 and within-subject one-way analysis of variance at a significance level of two-tailed $P < 0.05$. This change and stability of subjects allowed the selection of a relatively small sample size of 15 subjects.[19] Sample size for this study was based on criteria for crossover designs highlighted in Louis et al.[20] The anticipated power for the study was good based on the sample size and the large effect size required for the clinically important change criteria described in the literature.

The subjects and evaluators were blinded to the initial pharmaceutical order. Only the pharmacist was aware of the medication order. Three main assessments were conducted at baseline (prior to medication), following 2 weeks of placebo and following 2 weeks of 4-AP medication. At the end of the first 2 weeks and following the last morning capsule, subjects were evaluated and then crossed over to the alternate medication condition for the following 2 weeks. A final evaluation was completed 2 weeks after the crossover at the same time in the morning as previous tests and following the last capsule. A consumer consultant designed and conducted an exit survey with all subjects following the final evaluation. Owing to the short action of 4-AP, improvements from the use of either medication protocol were unlikely to influence the alternate condition over the full 2 weeks.

Medication protocol
Human trials have reported good tolerance to 4-AP at low doses (< 50 mg/day) and short-term exposure.[3] Minimal side effects included nausea, dizziness and tingling sensations in the extremities. However, a higher risk of liver toxicity or seizures have been reported at higher doses of 4-AP, longer use, or with a history of cerebral lesions.[3] In this study, the medication (placebo or 4-AP) started at 5 mg twice daily for 3 days, increased to 10 mg twice daily for 3 days and finally 10 mg four times daily. The subjects were on the full dosage of 40 mg/day within 1 week. Since 4-AP was not commercially available in an oral dosage form, the Pharmacy Department at The Rehabilitation Centre manufactured capsules using 4-AP powder. The placebo gel capsule consisted of lactose. Blood count (CBC) and liver function tests (LFT = S) were collected at each of the three test intervals. The principal investigator, although unaware of the group assignment, monitored for side effects.

Evaluation protocol
All physical evaluations were performed by blinded evaluators in the Gait and Motion Analysis Laboratory at The Rehabilitation Centre.

Isometric muscle force Isometric, bilateral muscle strength of the lower limbs was measured using a hand-held dynamometer (Nicholas MMT model 01160, Lafayette Instruments, USA). Standardized subject

positioning was used to test the hip flexors, extensors, abductors and adductors; knee flexors and extensors; ankle dorsiflexors and plantar flexors.[21] The mean score of two repetitions was recorded. Owing to the nature of the testing method and measurement error, the critical threshold level of change was estimated to be 5 kg.[22–24]

Gait analysis data – temporal – spatial measures Subjects were instrumented with bilateral pressure-sensitive footswitches fixed to the soles of their shoes to measure cadence in steps/min. The subject walked along a 10 m walkway between two infra-red light beams set 3.2 m apart. A speed acquisition program calculated average walking velocity between the two light beams. Average stride length was calculated from the velocity and cadence scores. Three trials each at natural and maximum self-selected walking speeds were collected for a total of six trials. Estimated critical thresholds, based on the literature, include: Velocity $\geqslant$ 0.15 m/s; Cadence $\geqslant$ 5 steps/min;
Stride $\geqslant$ 0.15 m/s.[25,26]

Two-dimensional motion analysis All bilateral segment angles, EMG and kinetic data were normalized to 100% of the gait cycle and were collected at natural and maximum walking speeds (three trials per condition). Trunk and lower limb positions were obtained using a video-based marker system (Ariel Performance Analysis System, San Diego, USA) and a Panasonic VHS camcorder. Passive reflective markers were placed bilaterally on the lateral surface of the tip of the shoe, 5th metatarsal head, the heel, the lateral malleolus, knee joint axis in line with the tibial plateau, the greater trochanter and the tip of the acromion. Marker positions from three steps for each side of the body were captured and digitized. The mean of three steps for the motion of the trunk and lower limbs were analyzed. The critical threshold for change was estimated to be 2 degrees for the trunk; 5 degrees for the hip and knee; 3 degrees for the ankle.[25,26]

Sagittal plane joint motion data were combined with ground reaction forces (forceplate by Advanced Mechanical Technology Inc., USA) to calculate 2-D net joint moments (defined as the net effect of a muscle action at a joint) and powers (defined as the rate of muscle force shortening or lengthening).[27] The BIO-MECH software (University of Waterloo, Ontario) was used to obtain bilateral hip, knee and ankle joint moments of force and net joint powers at critical points in the gait cycle.[28]

Surface electromyography (EMG) Simultaneous EMG recordings were obtained using preamplified differential electrode units with two, 1 cm long silver recording surfaces (Delsys Inc., Boston, USA). Bilateral records from a minimum of three steps were collected from the gluteus medius, rectus femoris, vastus lateralis, medial gastrocnemius, medial hamstrings and tibialis anterior (200 Hz sampling rate). Standard skin preparation was used to reduce skin impedance and muscle site location was standardized.[29] EMG signals were full-wave rectified and smoothed using a third-order Butterworth filter with a cut-off frequency of 3 Hz. EMG data from each subject were normalized to 100% of stride and ensemble averaged to provide one representative curve for each muscle.[27] Qualitative analyses included visual inspection of both the raw and processed EMG signals for comparison with normative patterns. Quantification of the EMG patterns used an activation index developed by Fung and Barbeau.[30] The exit survey required yes/no and open-ended responses (Appendix 1). Questions included perceived changes between the first and second medication phases, side effects and recommendations for future research. The survey was designed and administered by a consumer consultant to obtain subjective feedback rather than quantitative results.

Data analysis
Statistical analysis consisted of within-subject one-way analyses of variance (ANOVA) with Bonferroni *post hoc* tests to detect significant differences between baseline, placebo and 4-AP intervals *a priori* two-tailed $P < 0.05$ on:

- mean bilateral, isometric, lower limb muscle force;
- gait parameters at natural and maximum walking speeds including: velocity, stride and cadence; bilateral trunk, hips, knees, ankles kinematics and kinetics; bilateral EMG of six lower limb muscles

Descriptive information was summarized from the qualitative feedback from all the subjects. There was no attempt to quantify the responses for statistical analysis.

Results

Out of 15 subjects, 14 completed all trials. One subject (Table 1, subject #4) dropped out due to side effects during the 4-AP phase. Another subject (#2) did not tolerate the maximum dosage of 40 mg 4-AP and was kept at 10 mg for the trial. All data were statistically analyzed with and without subject #2 with no difference in the final results.

Muscle force measures are illustrated in Table 2. The group mean scores showed a slight increase from baseline to both 4-AP and placebo conditions. No statistically or clinically significant differences were found between baseline and placebo and between baseline and 4-AP ($P > 0.05$).

The temporal–spatial gait measures, at natural and maximum walking speeds, showed slight increases from baseline to placebo and from baseline to 4-AP. However, there was no significant difference between placebo and 4-AP conditions ($P > 0.05$). The statistical significant differences noted in Table 3 between baseline

Table 2 Group mean static, isometric lower limb muscle forces in kg (± 1 SD in brackets)

Muscles	Baseline	Placebo	4-AP	Maximum change score	Normative values[22–24]
Hip flexors	17.3 (5.6)	19.3 (6.4)	19.7 (5.9)	2.4	30
Hip extensors	19.6 (3.3)	22.3 (3.0)	21.7 (2.9)	2.7	29
Hip abductors	16.8 (4.2)	18.5 (3.9)	18.0 (3.8)	1.7	31
Hip adductors	19.3 (6.0)	21.3 (5.6)	20.3 (5.3)	2.0	26
Knee flexors	16.1 (5.2)	18.6 (4.6)	17.1 (4.6)	2.5	37
Knee extensors	22.4 (5.1)	25.1 (7.3)	23.9 (7.0)	2.7	38
Ankle dorsiflexors	11.4 (4.0)	11.9 (4.4)	12.8 (5.1)	1.4	30
Ankle plantarflexors	17.1 (5.9)	19.2 (7.1)	21.5 (7.7)	4.4	43

Right and left limb scores were pooled. 'Maximum Change Score' is the maximum difference between baseline and either of the two conditions

Table 3 Group mean (± 1 SD in brackets) for self-selected natural and maximum gait velocity, cadence and stride length of 14 subjects and difference score from baseline at three intervals

	Normative stride parameters[25,26]	Baseline mean (s)	Placebo mean (s)	Difference	4-AP mean (s)	Difference
Velocity (m/s)	Natural 1.4±0.2	0.81 (0.31)	0.87 (0.33)**	0.06	0.89 (0.32)**	0.08
	Maximum 1.9±0.1	1.01 (0.39)	1.10 (0.44)**	0.09	1.15 (0.45)**	0.14
Cadence (steps/min)	Natural 117±2	89.30 (15)	90.90 (19)	1.60	93.80 (16)**	4.50
	Maximum 134±3	102.20 (18)	106.20 (20)	4.00	108.30 (18)**	6.10
Stride (m)	Natural 1.4±0.03	1.12 (0.24)	1.18 (0.26)*	0.06	1.18 (0.22)**	0.06
	Maximum 1.7±0.04	1.23 (0.25)	1.29 (0.26)*	0.06	1.30 (0.26)**	0.07

Analysis of variance results indicated by the asteriks
*$P < 0.05$ compared to baseline
**$P < 0.01$ compared to baseline

and either 4-AP or placebo generally did not reach the critical threshold levels cited in the Methods section.

The studied population varied greatly in their gait performance since baseline, natural gait speeds ranged from 0.29 to 1.25 m/s. This heterogeneity prompted a more detailed study of individual stride parameters to segregate the sample into higher and lower functioning groups. No trends were noted in the results of subjects who walked at baseline with either slower or faster velocity. Furthermore, the individuals with the slowest baseline walking speeds were limited in their ability to increase to maximum walking speed compared to all other subjects. Table 4 illustrates the subjects segregated by speed in three categories: slow = ≤0.40 m/s: moderate = >0.40 <0.90 m/s and faster = ≥0.90 m/s at the baseline, natural speed. This table highlights that the subjects with moderate and faster walking speeds altered both their natural and maximum walking speeds more than the subjects with the slowest walking speed.

The order of 4-AP administration had no effect on the outcomes. Raw scores of each subject for walking velocity in Table 4 illustrate that by separating the subjects in two groups, dependent on their order of medication, there was no obvious influence of whether they first received 4-AP or placebo. The order of drug administration was not a significant factor in the final results as illustrated in Table 5.

Two-dimensional motion of the trunk, hip, knee and ankle did not reveal significant within-subject changes

across the three conditions at both natural and maximum walking speeds ($P > 0.05$). Table 6 shows minimal changes in mean total range of motion at natural walking speed for all subjects. Similar results were observed at maximum walking speed.

Bilateral, group kinetic analyses showed no significant differences in peak moment or power values at the key events in the gait cycle (refer to Figure 1), across the three conditions, and at natural and maximum walking speeds ($P > 0.05$). Group mean results of the left limb kinetics ($n = 11$) are illustrated in Table 7. Kinetic analyses could not be obtained from three subjects due to technical or feasibility difficulties. Similar results were found for the right limb. Data from subject #8 is displayed in Figure 1 (kinetic analysis) and Figure 2 (EMG patterns). This subject was selected since the stride parameters were close to the baseline, group mean velocity (0.81 m/s). EMG analyses for all subjects were visually inspected for abnormal phasic activity patterns. No within-subject differences were found. The activation indices on a subgroup of subjects were extremely sensitive and too variable for further analysis.

Exit interview
Out of 14 participants, 12 responded to the exit interview. In total, 75% of the respondents correctly identified the 4-AP condition mainly because of the various negative effects such as nausea or dizziness.

Table 4 Individual subjects velocity (m/s) data separated by order of group assignment: A Group = Placebo → 4-AP and B Group = 4-AP → Placebo

A Group (Placebo → 4-AP)

	Natural walking speed (m/s)			Maximum walking speed (m/s)		
Subjects	Baseline	Placebo	4-AP	Baseline	Placebo	4-AP
S 15	1.00	1.08	1.07	1.14	1.21	1.26
S 3	1.12	1.19	1.20	1.32	1.46	1.43
S 5	1.08	1.11	1.13	1.36	1.53	1.70
S 10	1.25	1.31	1.35	1.69	1.89	1.97
S 8	0.88	0.86	0.85	1.02	1.13	1.14
S 7	0.89	1.13	1.11	1.25	1.35	1.43
S 13	0.52	0.59	0.63	0.68	0.78	1.00
S 1	0.48	0.58	0.76	0.87	0.91	1.02

B Group (4-AP → Placebo)

	Natural walking speed (m/s)			Maximum walking speed (m/s)		
Subjects	Baseline	4-AP	Placebo	Baseline	4-AP	Placebo
S 11	0.98	1.10	1.12	1.19	1.36	1.35
S 14	1.10	1.11	1.14	1.33	1.33	1.36
S 12	0.73	0.80	0.77	0.85	0.91	0.87
S 6	0.65	0.71	0.71	0.81	0.89	0.88
S 2	0.36	0.36	0.29	0.41	0.44	0.40
S 9	0.29	0.29	0.32	0.29*	0.29*	0.32*

*Unable to walk faster than natural speed

Table 5 Results of testing the order effect of the drug administration where 'order' was the independent variable

	Mean square	F	P-value
Velocity	0.001	0.26	0.62
Cadence	3.6	0.13	0.73
Stride	0.003	0.26	0.62
Motion ROM			
Trunk	0.73	0.71	0.42
Hip	0.14	0.03	0.86
Knee	20.5	1.33	0.27
Ankle	0.32	0.08	0.78

Overall, 50% of respondents reported positive effects on gait, endurance, and leg strength in spite of the negative effects. Three subjects reported increased sensation and coordination, while one subject experienced increased sexual function, pain relief and limb flexibility. Interestingly, all of the subjects that received 4-AP first, experienced negative effects of nausea or dizziness while only one subject in the group receiving placebo first, reported dizziness. Thirty percent indicated no changes in physical function or gait performance. The most consistent recommendation from the participants was that 4-AP should be taken over a longer duration.

4-AP side effects
One subject dropped out of the study due to the severity of 4-AP side effects (dizziness, weakness, regression in walking ability). This individual had a non-traumatic etiology and was functioning using a walker with limited community mobility skills. Side effects were reported in 50% of the subjects including symptoms of nausea, dizziness and sleeping difficulties. One subject did not tolerate the daily 40 mg total dosage due to nausea and dizziness and was kept to the minimum dose of 10 mg. All other subjects tolerated the 4-AP side effects.

Discussion

This placebo-controlled, clinical trial resulted in no statistically or clinically significant differences in lower limb muscle strength or biomechanical gait measures in 14 chronic, ambulatory SCI persons when on a 2-week trial of 4-AP. This 4-AP trial expands and replicates past work and is unique in providing quantitative muscle strength and biomechanical gait analysis in evaluating the efficacy of 4-AP.

Past preclinical trials on 4-AP with chronic SCI subjects reported increased muscle strength based on clinically feasible manual muscle grading using the 0–5 ordinal scale.[8–10] Appendix 2 outlines the extent of the published preclinical and clinical trials of 4-AP in SCI. There has not been an attempt, in the 4-AP literature, to quantify isometric muscle force using a more sensitive and accurate dynamometer. The present study showed slight changes in muscle force, but did not exceed clinically relevant levels of change. Segal and Brunnemann[15] studied changes in stride parameters of nine SCI

Table 6 Group mean range of motion in degrees (± 1 SD in brackets) of trunk, hip, knee and ankle at natural speed

	Baseline	Placebo	Difference	4-AP	Difference
Trunk					
Right	10.6 (3.3)	12.4 (2.5)	1.8	12.3 (3.2)	1.7
Left	12.5 (3.5)	14.5 (2.2)	2.0	14.1 (2.0)	1.6
Hip					
Right	36.5 (6.5)	38.1 (8.3)	1.6	38.3 (6.8)	1.8
Left	36.9 (7.8)	37.2 (9.3)	0.9	37.6 (7.5)	0.7
Knee					
Right	50.7 (13.8)	53.4 (17.8)	2.7	53.6 (13.9)	2.9
Left	54.7 (16.6)	55.6 (16.5)	0.9	56.4 (15.4)	1.7
Ankle					
Right	28.7 (6.8)	28.4 (5.9)	0.3	28.5 (6.5)	0.2
Left	27.3 (5.9)	27.7 (5.3)	0.4	26.6 (5.6)	0.7

Table 7 Group mean peak moment and power values (± 1 SD in brackets) for 11 subjects (left side) at natural speed including P-value from ANOVA statistic

	Baseline	Placebo	4-AP	P-value
Peak moment				
Ankle 1	4.54 (5.28)	5.88 (4.28)	5.57 (3.14)	0.85
2	−80.47 (21.06)	−90.22 (25.20)	−82.54 (22.93)	0.61
Knee 1	20.41 (25.82)	41.66 (33.00)	20.99 (20.79)	0.32
2	−9.72 (10.28)	−18.52 (13.50)	−20.19 (15.88)	0.19
3	23.11 (20.67)	26.48 (18.75)	23.36 (23.06)	0.98
4	−15.37 (7.05)	−17.33 (6.68)	−15.55 (7.43)	0.97
Hip 1	−63.84 (28.30)	−74.61 (34.67)	−70.38 (41.70)	0.82
2	52.70 (31.92)	50.20 (26.20)	61.20 (44.18)	0.77
Peak Power				
Ankle 1	−6.17 (9.05)	−3.47 (1.69)	−4.22 (3.44)	0.80
2	4.27 (2.70)	6.46 (3.36)	5.58 (5.37)	0.79
3	−52.81 (32.45)	−68.74 (32.67)	−50.76 (32.34)	0.49
4	78.87 (56.32)	98.74 (96.92)	77.64 (44.91)	0.73
Knee 1	−18.16 (26.40)	−49.62 (39.92)	−14.44 (11.96)	0.18
2	28.25 (26.20)	52.14 (34.46)	29.29 (24.56)	0.33
3	7.16 (2.79)	6.98 (5.23)	10.03 (6.72)	0.40
4	−94.77 (79.87)	−102.67 (99.69)	−90.61 (66.10)	0.95
5	−42.67 (29.17)	−46.66 (36.07)	−39.17 (22.91)	0.93
6	12.07 (13.17)	10.95 (9.52)	9.15 (8.79)	0.85
Hip 1	64.70 (41.55)	89.23 (48.94)	78.01 (48.08)	0.51
2	−36.10 (44.74)	−24.10 (26.84)	−51.46 (54.58)	0.43
3	50.22 (33.62)	51.74 (36.72)	55.96 (38.79)	0.95

subjects in a noncomparative group design. The authors reported modest positive changes in stride variables following a single, oral dose of 10 mg of immediate-release 4-AP. However, similar to the present study, velocity and stride length measures did not exceed the critical threshold of clinical importance proposed in this paper. The present study confirmed a more recent past 4-AP gait investigation that found a lack of walking velocity changes in 20 chronic SCI ambulators[16] reinforcing the necessity for placebo-controlled studies in evaluating the efficacy of 4-AP. Appendix 2 illustrates that less than half of the cited trials were true placebo-controlled.

Potter et al[12] recognized that 4-AP trials have demonstrated a large placebo effect, perhaps due to the nature of the clients and expectations of improvement. From the present data (refer to Table 3), the 'placebo effect' for natural walking speed may account for at least 0.14 m/s in velocity, 0.12 m in stride and 6 steps/min in cadence. These values approximate the critical thresholds of change, estimated from the literature and proposed in the present study. The stride parameter thresholds resulting from the present study represent important values for future gait trials in SCI. Drug intervention trials related to gait enhancements in SCI would need to exceed these

placebo-effect-corrected values in order to report an important clinical change. Similarly, neurophysiological tests need to consider functionally meaningful outcomes.[3,31]

Gait analyses involving EMG, segmental motion and kinetic measures did not result in substantial changes across the three conditions either at natural or maximum walking speeds. The lack of quantitative gait improvement contradicts studies that reported gait improvement of subjects.[8,12] This discrepancy between perceived changes in function and a lack of quantitative changes was also seen in the present trial and may be due to several issues. Positive comments from subjects may be attributed to a Hawthorne effect, high expectations[12] or differences in the definition of improvement. Clinicians equate improvement with quantitative measures while subjects may perceive general positive changes due to an increase in energy level and mood. Segal et al[32] reported enhanced pulmonary function in SCI subjects after 4-AP. Walking efficiency, which was not measured in the present trial, may be worth future

study. A possible psychotropic effect has been previously reported and the authors postulated that 4-AP may have an analgesic effect similar to morphine.[12] One subject in the present study experienced an increase in a positive mood. Despite the lack of objective changes in the present trial, 50% of the subjects reported improved gait when on the 4-AP condition. Other positive comments included perceived increased strength, co-ordination, sensation, sexual function, and endurance. Past work has also reported similar positive comments from subjects when taking 4-AP.[3] The present study showed more positive comments from respondents during the 4-AP condition in contrast to the placebo. However, two subjects reported positive changes, and one subject reported negative effects, when on the placebo condition.

Drug administration
Dosage parameters in the past have widely varied, making comparisons difficult at this early stage of
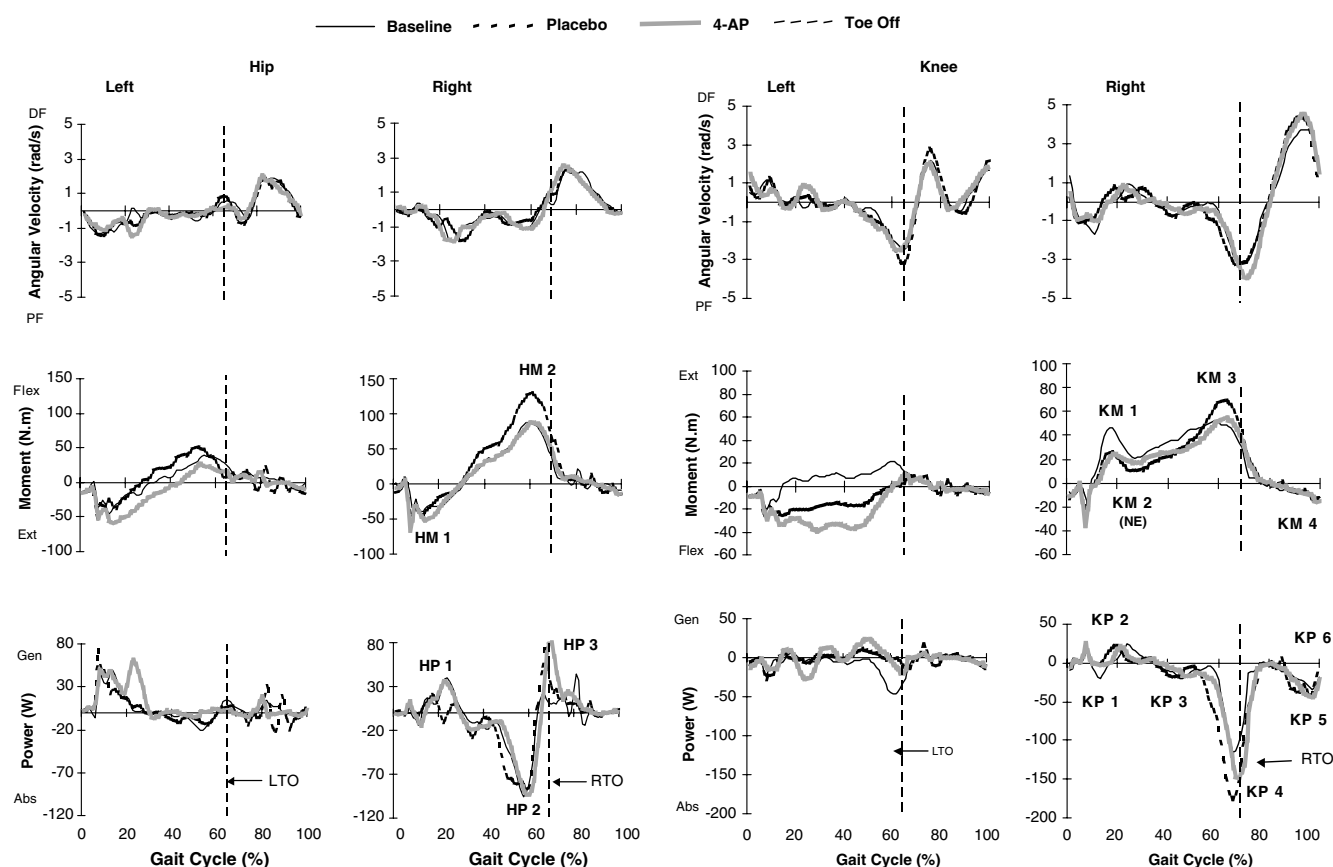

Figure 1 Bilateral motion analysis results of one subject (#8) at baseline, placebo and 4-AP conditions at natural walking speed. Mean of three strides for each condition of hips, knees and ankles are normalized to 100% of the gait cycle with RTO = right toe off; LTO = left toe off. Left of the vertical line delineates the stance phase. Angular velocity is represented in rad/s; moment curves are in N m; power curves are in W. The peak values of moment and power curves are represented on the right hip, knee and ankle only as an example for all subjects. The peaks are indicated as follows: **AM** 1,2 = 2 peak ankle moments; **KM** 1–4 = 4 peak knee moments; **HM** 1,2 = 2 peak hip moments; **AP** 1–4 = 4 peak ankle powers; **KP** 1–6 = 6 peak knee powers; **HP** 1–3 = 3 peak hip powers. The code '**NE**' refers to nonevent. Corresponding mean velocities are: baseline (0.88 m/s); placebo (0.86 m/s) and 4-AP (0.85 m/s)
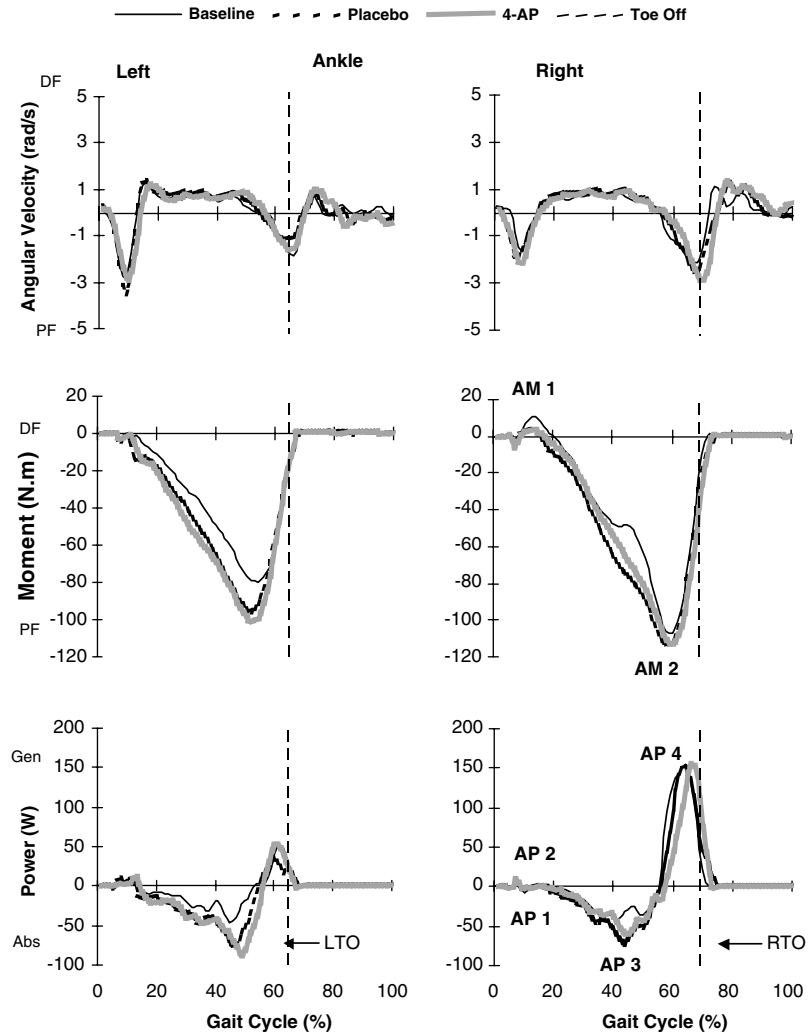
Figure 1 Continued

clinical trials. Past trials have varied in the drug administration from intrathecal, intravenous or oral tablet. In the present study, ramping up to a maximum 40 mg daily oral dose was well tolerated by the majority of subjects. However, the medication duration could have been longer, as suggested by positive participant feedback. Past researchers have reported that carryover may last up to 48 h.[3,11,13] Currently, the optimum dosage and administration for 4-AP remains unclear.[33] A complete dose response curve has not yet been investigated.

Conclusion

The present study found that 4-AP did not produce a significant improvement in lower limb isometric muscle strength or quantitative gait patterns for 14 persons with chronic, incomplete, SCI. There were no clear positive trends beyond the minimal critical levels of change in outcome measures for the 4-AP group. These results

demonstrated the importance of placebo-controlled trials to test 4-AP efficacy for gait enhancement in chronic, ambulatory SCI persons. The placebo effect may account for close to 10% of changes in walking velocity, cadence and stride variables and is relevant for future trials. The physical and biomechanical gait results generally agreed with the most recent controlled trial and did not support the past preclinical 4-AP studies that reported positive ambulation effects. Future clinical crossover gait trials may consider several factors: a more selective and homogeneous group of subjects based on moderate to mild functional disabilities; drug dosage clarification of duration, method of intake such as oral, intravenous or intrathecal and known dose–response curve; and finally, consideration of ambulation energy expenditure measures and training options. There may be the possibility that 4-AP could facilitate the locomotor training process by creating a permissive situation such as with clonidine.[34]
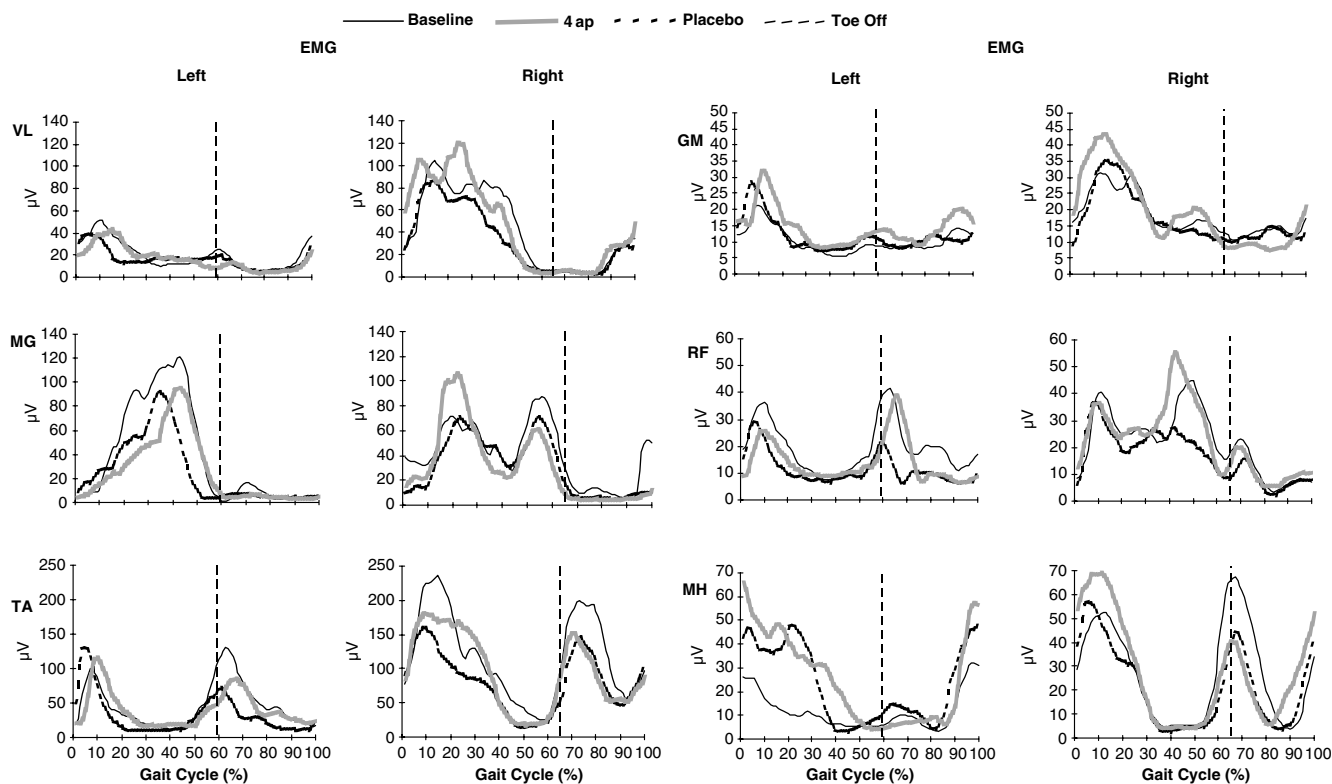
Figure 2 Mean EMG linear envelope of six muscles of subject #8 at baseline, placebo and 4-AP at natural walking speed. All data represent the mean of at least three strides and normalized to 100% of the gait cycle. Left of the vertical line delineates the stance phase. Muscles include gluteus medius (GM), rectus femoris (RF), medial hamstrings (MH), vastus lateralis (VL), medial gastrocnemius (MG) and tibialis anterior (TA). Units are in microvolts (μV). Corresponding mean velocities are: baseline (0.88 m/s); placebo (0.86 m/s) and 4-AP (0.85 m/s)

Acknowledgements

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Appendix 1. Excerpts from the consumer-designed exit survey

1. Why did you become involved in this experiment?
2. Did you have specific personal set expectations/goals from this research?
3. When you took the medication for the first time, how did you feel?
4. Did you experience any side effects from the medication?
   Yes ☐   No ☐
   If yes, what did you feel?
   Was the side effect with the first medication ☐ or the second one ☐?
5. Did you observe and feel any changes in your body?
   motor strength ☐   sensation ☐   co-ordination ☐   spasticity ☐
   blowel ☐   bladder ☐   sexuality ☐   endurance ☐   pain ☐
   Did these changes occurred with the first medication ☐   or second ☐?
   Comments
6. While you were on the medication, were there any changes in your emotions?
7. Do you think that your walking ability has improved?
   Yes ☐   No ☐
   If yes, when did you feel the change? (describe)
8. Do you have any recommendations/comments to add about this or future studies?

Appendix 2. Relevant clinical research in 4-AP and spinal cord injury (reference number in brackets) (* sensory-evoked potentials (SEP) and motor-evoked potential (MEP))

Authors	Subjects	Design	Max. Dose	Washout	Measures	Results	Conclusions
Hansebout et al (1993)[11]	n = 8	Placebo-controlled double-blind crossover	Single 33.5 mg i.v. 2 h infusion	2 weeks	SEP* MEP* -Ordinal scale (sensori-motor) -Subjective	Modest ↑neurological deficits 4-AP	Suggest oral dosing with titration for an increased duration -No change in most severely impaired -4/8 wanted to stay on
Hayes et al (1993)[10]	n = 6	Noncomparative single group	Single 24 mg i.v. 4 h infusion	N/A	Electro-physiological -Motor scale -Subjective	Modest ↑ in less impaired	4-AP may be beneficial in temperature-sensitive central conduction deficits
Hayes et al (1994)[3]	n = 6	Noncomparative single group	Single 25 mg i.v. 2 h infusion	N/A	SEP* MEP*	↑ in some subjects but functional implications unknown	Need more functional outcomes
Qiao et al (1997)[29]	n = 19	Noncomparative single group	Single 10 mg oral	N/A	MEP* -Relexes	Positive trends in central conduction in less severely impaired	Need to study therapeutic efficacy to match positive electrophysiological tests
Potter et al (1998)[12]	n = 26	Placebo-controlled double-blind crossover	35 mg daily oral for 2 weeks	1 week	Composite score of senori-motor impairment -Patient satisfaction -Impact on quality of life	Large placebo effect -Some ↑ individual impairments -Subjective versus objective relationship unclear	Increase dosing 40–50 mg daily -Need larger scale clinical trials
Segal et al (1998)[15]	n = 9	Noncomparative single group	Single 10 mg oral	N/A	Gait temporal–spatial parameters	Modest ↑ in stride parameters	Need to study functional importance of modest changes
Potter et al (1998)[8]	n = 3	Case studies	30 mg daily oral for 4 months	N/A	Ashworth -McGill pain -ASIA sensori-motor score -Subjective	Positive trends in most scores with increased mood	Need more placebo-controlled trials
Segal et al (1999)[30]	n = 21	Controlled (nonplacebo) comparative groups	Low dose: 6 mg daily High dose 30 mg daily Oral: 3 months	N/A	Ashworth -ASIA sensori-motor -Pulmonary -Subjective	Mixed ↑ in sensori-motor Pulmonary more positive	40 mg daily well tolerated. Need population specific pharmacokinetics
Donovan et al (2000)[13]	n = 12	Placebo-controlled double-blind crossover	Single 30 mg i.v.	2 weeks	Ashworth -EMG -Reflex scale -ASIA sensori-motor -McGill pain	No significant changes	Need to assure stable baseline. IV may not be best dosing method
Halter et al (2000)[9]	n = 6	Noncomparative single group	Intrathecal 25 mg 4–5 h	N/A	Ashworth -McGill pain -Reflex scale -ASIA sensori-motor	Mixed individual positive changes in less severely impaired	Less severely impaired do better. Low-dose intrathecal may cause only local physiological changes. Need to study higher doses in selective subjects to study functional changes
van der Bruggen et al (2001)[16]	n = 20	Placebo-controlled double-blind crossover	50 mg/daily oral 2 weeks	4 weeks	Standardized functional status scale -Natural and maximum walking speed -Vibration perception	No statistical or clinically important change in outcomes	Unclear benefit in this population. May have a role in possible pain relief.