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Preclinical trial of 4-aminopyridine in patients with chronic spinal cord injury

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4-Aminopyridine (4-AP) is a K^+ channel blocking agent that enhances nerve conduction through areas of demyelination by prolonging the duration of the action potential and increasing the safety factor for conduction. We have investigated the effects of 4-AP (24 mg total dose-intravenous) in 6 patients with spinal cord injury (3 complete, 3 incomplete) with the intent of overcoming central conduction block, or slowing, due to demyelination. Vital signs remained stable and only mild side effects were noted. The 3 patients with incomplete injuries all demonstrated enhanced volitional EMG interference patterns and one patient exhibited restored toe movements. The changes were reversed on drug washout. There were no changes in segmental reflex activities. These results are consistent with those obtained from 4-AP trials with animal models of spinal cord injury, showing modest therapeutic benefit attributable to enhanced central conduction.

Keywords: spinal cord injury; 4-aminopyridine; demyelination; conduction block; paralysis.

Introduction

Conduction deficits due to demyelination of axons within the spinal cord are now recognized to constitute a significant component of the pathology of spinal cord injury (SCI).[1–7] As a corollary, restoration of conduction in demyelinated fibres has been identified as an important strategy for promoting functional recovery in SCI patients with compressive or contusion injuries.[8]

4-Aminopyridine (4-AP) is a potassium (K^+) channel blocking agent that has the capability to pass through the blood brain barrier,[9] overcome conduction block in demyelinated neurons,[10–13] improve the neurological function of various animal models of SCI,[14,15] and improve clinical signs in temperature-sensitive patients with multiple sclerosis.[16,17,18] 4-AP has not previously been administered to patients with SCI, although it has undergone widespread testing in other clinical applications[19–23] and there is adequate knowledge of its pharmacokinetics.[24–27] The pharmacological action of 4-AP in demyelinated or dysmyelinated neurons is to prolong the duration of the action potential, by blocking the voltage-sensitive 'fast' K^+ channels, thereby increasing the safety factor for conduction.[11,28–30]

The present open trial of intravenously administered 4-AP in chronic SCI patients was undertaken (a) to identify any side effects of the drug and (b) to provide preliminary observations of the efficacy of the drug in enhancing sensory and motor function. In addition to recording clinical, functional and electrophysiological indices of sensori-motor status, careful monitoring

was undertaken of vital signs and electroencephalograms (EEG) as well as video recording of any restored movement capability.

Methods

Subjects

Six volunteer male SCI patients provided informed consent to participate in the trial. A seventh patient started the trial but his participation was discontinued when unsuccessful venipuncture resulted in the first dose of 4-AP inducing a local vasoconstriction with blanching and cooling of the forearm. The mean age of the group was 29.5 years, and all had experienced traumatic lesions as a consequence of motor vehicle accidents. All were able to discontinue their medications for sufficient time to allow drug washout prior to the 4-AP trial. Three of the patients presented with clinically complete injuries and three were incomplete. Their clinical characteristics are reported in Table I. The inclusion criteria were: traumatic paraparesis, paraplegia, quadriparesis or quadriplegia, neurological level of injury C5–T12, medically stable, aged 18–50 years, and with acknowledgement by their physician of participation. Excluded were patients with brain damage or neuropsychological deficits, including posttraumatic amnesia with a Galveston orientation and amnesia test score less than 76; family or personal history of epilepsy or other seizure disorders; known cardiovascular, metabolic, kidney or respiratory abnormalities; allergy to pyridine-containing medications; excessive pain or spasticity; or nicotine dependence.

Protocol

Patients underwent clinical, electrophysiological and haematologic studies as well as urinalysis (the latter to verify previous medication washout and absence of illicit drugs) prior to hospitalization. On the day of investigation the patients fasted. They underwent hourly assessment of clinical status by standard physical examination, urinalysis and electrophysiological studies of lower limbs (paraplegics) and upper limbs (quadriplegics), while vital signs and EEG were monitored continuously. Each patient received a total dose of 24 mg 4-AP, delivered intravenously (via continuous drip of 3.3% dextrose and 0.3% sodium chloride solution) through a peripheral vein in the dorsal aspect of the hand or forearm at an average rate of 2 mg/20 minutes. The first 3 patients received manual delivery of 2 mg 4-AP every 20 minutes (at a rate of 2 mg/30 s) while the others received continuous infusion (flow volume regulated infusion pump IVAC 560M) at 2 mg/20 minutes. Following the final stages of drug administration, patients were monitored for a period of 2 hours. They then returned to their rooms for a meal, overnight observation of vital signs, and periodic urinalysis to document 4-AP elimination.

4-aminopyridine

4-AP was obtained directly from Regis Chemical Company, Morton Grove, Illinois, USA and prepared for injection using methods described by Uges and Huizinga.[31] The vials were sterilized by steam autoclaving for 20 minutes at 121 °C and the infusate subsequently examined for pyrogenicity (<175 endotoxin units per total

Table I Clinical characteristics of patients

Patient	Age (years)	Level of lesion	Frankel class	Motor index	Years post injury
1	26	T6	C	59	1
2	29	T7–8	D	85	5
3	29	T3	A	50	1.7
4	22	T7	A	50	3
5	32	C5–6	C	54	6
6	39	C5–6	C	46	1.5

dose), stability, potency (using HPLC) and pH. The experimental protocol and injectable drug formulation procedures were approved by the Health Protection Branch of the Government of Canada under IND #5HP894935.

Physical, medical and functional evaluation
Medical history, demographic information and current physical and functional status were determined by a specialist in physical medicine and rehabilitation. Patients were classified according to the functional assessment criteria of the American Spinal Injury Association (ASIA) and to the motor index score.[32]

Electrodiagnostic assessment
Peripheral nerve examinations (sensory and motor) of posterior tibial and peroneal nerves were conducted bilaterally using conventional NCV, H-reflex, M-wave, flexion reflex[33] and clinical EMG assessment techniques.[34] The primary purpose of the examination was to identify any peripheral neuropathy or disorders of neuromuscular transmission. Maximal voluntary contraction EMG interference patterns were recorded. Jendrassik's maneuver[35] was employed to reinforce voluntary efforts and reflex activity. Central sensory conduction was assessed using tibial nerve SEPs (3.1 Hz stimulation with Cz-Fz recording), or in 2 cases using median nerve stimulation with C_3-Fz recording, and voluntary contraction EMG interference patterns were recorded from tibialis anterior (TA), lateral gastrocnemius (LG) and rectus femoris (RF) muscles. In addition, central motor conduction was evaluated by motor evoked potentials (MEPs), elicited using transcranial magnetic stimulation of motor cortex, and reinforced with target and remote muscle contraction.[36,37] Cortical stimulation was delivered from a Cadwell MES-10 magnetic stimulator through a 9 cm focal point coil electrode with posterior rim over vertex.[33,37] The central motor conduction tests were only recorded prior to drug adminstration because of the potential combined risk of both 4-AP and cortical stimulation lowering seizure threshold.

Vital signs
Oral temperature, systolic and diastolic blood pressure, respiratory rate and electrocardiogram (ECG) were monitored periodically throughout the testing by a nurse and emergency medicine physician. EEG recordings were made continuously using a Grass model 8–10D electroencephalograph with 8 channel montage.

Urinalysis for 4-AP
Detection of urinary concentration of 4-AP was done using a Hewlett Packard 1090 HPLC with UV @ 254 nm. Urine samples were diluted 1:11 (100 µl in 1.0 ml MeOH) and injected directly onto the column (15 cm × 4.6 mm 1 D Beckman Ultrasphere 1 P.C18, 5 u). The retention time was 3.5 min. Since no extraction was used and injections were automated no internal standard was used. The coefficient of variation for quality control samples (1000 $\mu g L^{-1}$) was 3.7%.

Results

All 6 patients tolerated the drug administration well and without significant side effects (see below). The investigations lasted approximately 6 hours from the beginning of pre-drug testing, through 4 hours of drug administration and 2 hours post-drug tests. Most patients spontaneously reported fatigue at the end of the testing.

Vital signs
During administration of 4-AP the group mean oral temperature, systolic and diastolic blood pressure, respiratory rate and heart rate did not differ significantly from the baseline values (Table II). There were no obvious changes in the ECG. EEG recordings typically showed normal baseline EEG consisting of alpha and some beta activity and in one subject there was evidence of a decrease in amplitude of alpha activity associated with subjective reports of lightheadedness after ~14 mg 4-AP. It is not entirely clear whether the lightheadedness was due to the drug, fasting, or other factors, although Stefoski *et al*[16] have also

Table II Vital signs during 4-AP administration

		Pre drug	4-aminopyridine				Post drug
			6 mg	10 mg	18 mg	24 mg	
Heart rate (beats per minute)	$\bar{x}$	62.8	65.8	62.3	61.3	61.7	61.7
	SD	7.3	9.5	7.1	7.6	9.0	4.2
Temperature (centigrade)	$\bar{x}$	36.9	36.7	36.7	36.4	36.6	36.9
	SD	0.4	0.7	0.3	0.5	0.6	0.4
Respiration (breaths per minute)	$\bar{x}$	19.0	19.2	19.7	20.3	19.7	19.0
	SD	2.8	2.7	2.7	2.9	2.3	2.8
Systolic blood pressure (mmHg)	$\bar{x}$	110.3	115.7	113.7	115.0	114.7	112.5
	SD	7.8	14.3	10.4	12.6	14.9	11.9
Diastolic blood pressure (mmHg)	$\bar{x}$	67.5	70.7	69.3	71.3	71.7	71.5
	SD	8.8	13.1	8.7	12.9	14.6	13.2

Pre drug refers to immediately prior to first 2 mg dose of 4-AP.
Post drug refers to 20 min after last 2 mg dose of 4-AP.
All measurements based on $n = 6$ and SD refers to the between-patient standard deviation.

reported lightheadedness during adminstration of 4-AP. The episode passed quickly and the subject continued to receive the total dose (24 mg).

Somatosensory evoked potentials

SEPs were elicited from only one patient (5) in the pre-drug testing. This was a median nerve SEP with low amplitude (~2.5 μV) and normal latency (N_1 = 19 ms). The amplitude of the SEP was maintained constant with the infusion of 4-AP. Tibial nerve SEPs could not be recorded from any patients. On clinical examination one subject (5) with Brown-Séquard syndrome reported subjective changes in the distribution of sensory loss, viz improved quality of nociception to pinprick on the more affected side, ie less asymmetry. No significant changes in peripheral sensory conduction velocity (tibial or median nerve) were noted in any patients.

Motor evoked potentials and electromyography

In pre-drug testing MEPs were elicited from lower limb muscles (TA, LG and RF) in 2 of the patients with incomplete lesions (1 and 5) but not in any of the 3 patients with complete lesions. In each case MEPs were only elicitable with reinforcement from target muscle contraction.[37] Figure 1 shows the long latency and low amplitude MEPs recorded from patient 5 together with his pre-drug maximum voluntary contraction EMG interference pattern. The latency of the MEP in this patient's right TA ($\bar{x}$ = 38 ms) was appreciably longer than normal (range = 27–29 ms).[36] This patient was able to induce voluntary recruitment of one or two motor units in his right TA when reinforcing the contraction with Jendrassik's maneuver.[36]

During administration of 4-AP each one of the 3 patients with incomplete motor loss (1, 2 and 5) showed enhanced volitional motor unit recruitment and EMG interference patterns. The time course of changes varied appreciably across patients. Patient 5 revealed a progressive enhancement of motor unit recruitment in TA. This was even more striking when the contractions were reinforced by Jendrassik's maneuver (Fig 2). The enhancement of volitional motor unit recruitment was in a muscle that in pre-drug testing was electrically quiescent, but which revealed MEPs following cortical stimulation reinforced with attempted target muscle contraction (Fig 1). The patient also reported restored capability to move the fourth and fifth toes of his right foot which he could not do prior to receiving 4-AP. The restored flexion/exten-

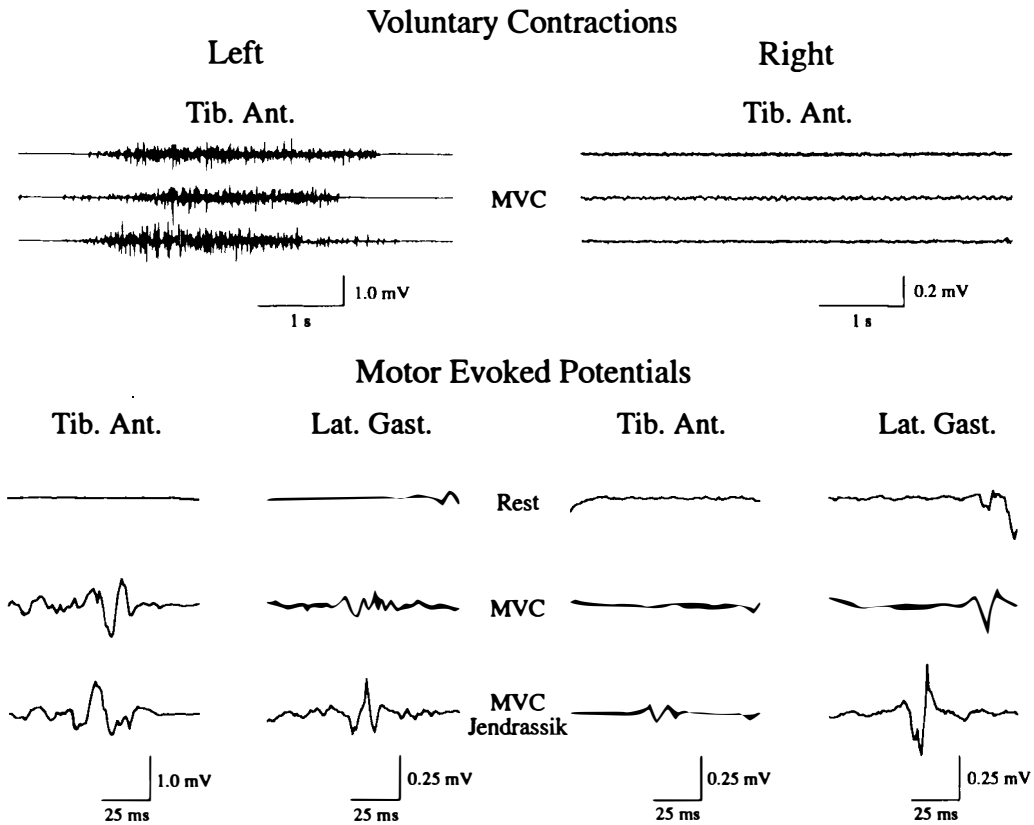

Figure 1 Maximal voluntary contraction (dorsiflexion), EMG interference patterns, and motor evoked potentials in left and right tibialis anterior and gastrocnemius, recorded pre-drug administration in patient 5 with C5–6 quadriparesis. Patient was unable to generate EMG interference pattern in TA of right leg, but did reveal low amplitude MEPs in TA when cortical stimulation was delivered with patient attempting target muscle contraction reinforced with Jendrassik's maneuver.[36]

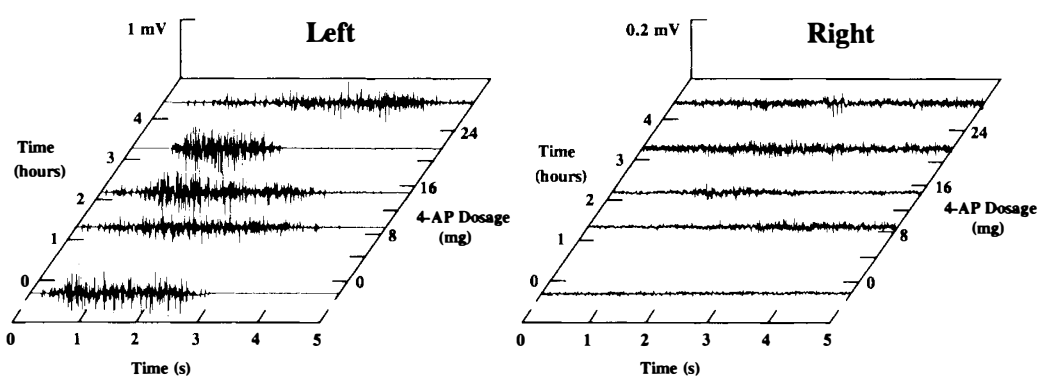

Figure 2 Maximal voluntary contraction (dorsiflexion) EMG interference patterns, with Jendrassik type reinforcement in left and right tibialis anterior of patient 5, recorded before, during, and after administration of 4-aminopyridine. Right leg showed a progressive increase in voluntarily initiated motor unit activity.

sion movements were lost on drug washout. Video analysis verified the changes in movement capability.

Patients 1 and 2 both revealed increased amplitude and density of EMG interference patterns during maximal voluntary contractions in TA or RF after 12–16 mg 4-AP, but this enhancement appeared to lessen during the final stages of drug administration (20–24 mg). Associated contractions in the contralateral leg reduced in intensity (ie reduced amplitude and density of EMG interference patterns) as the strength of the ipsilateral leg contractions increased. There were no appreciable changes in the motor index scores or Frankel class ratings of any patients.

Segmental reflexes

In all patients from whom posterior tibial nerve H-reflexes and M-waves could be recorded ($n = 5$), the amplitude and latency of posterior tibial nerve H-reflexes and the amplitude of M-waves generally remained constant during or after adminstration of 4-AP (Table III). Analysis of variance, with Bonferroni post hoc tests, revealed a consistent reduction in M-wave latency ($p < .05$) at all levels of drug adminstered, compared to the pre-drug value. Since the change did not appear to be dose dependent, and was not accompanied by commensurate changes in H-reflex latency, or M-wave amplitude, we interpret this effect to be artifactual. There were indications of a reduced amplitude of the H-reflex after 8 mg 4-AP but this was not significant ($p > .05$). Similarly there was a reduction in flexion reflex amplitude and elevated stimulation threshold in some patients after 8 mg 4-AP, but this was not consistent across patients. There were no clear indications of any individual patients showing altered segmental reflex activity. Particular attention was focused on the segmental reflex properties of patient 3 who had a surgically verified anatomically complete severance of the cord at T3. This 'spinal' man provided an opportunity for detailed examination of any peripheral effects of 4-AP, eg on neuro–neuronal transmission in the isolated cord or neuromuscular transmission. No consistent changes were observed in the latency or amplitude of his H-reflexes, M-waves, or polysynaptic flexion reflexes evoked by cutaneous stimulation in the lower limbs.

Side effects

All patients complained of localized mild to moderate discomfort around the insertion site of the intravenous line. Two patients exhibited behaviours associated with mild perioral paraesthesia and one reported a transient period of lightheadedness at 12 mg 4-AP associated with periodic low amplitude EEG activity. There were no other

Table III H-reflex and M-wave values during administration of 4-aminopyridine

		Pre drug	4-aminopyridine			
			8 mg	14 mg	20 mg	24 mg
H-reflex latency (ms)	Mean	31.20	30.30	29.80	29.80	29.90
	SD	1.35	1.04	1.44	0.82	1.39
H-reflex amplitude (mV)	Mean	4.16	2.57	3.97	3.82	4.53
	SD	4.48	2.22	3.99	4.04	3.95
M-wave latency (ms)	Mean	5.13*	3.63	3.50	4.00	3.75
	SD	1.35	0.74	0.89	0.22	0.42
M-wave amplitude (mV)	Mean	8.68	7.89	10.29	9.11	8.63
	SD	4.37	3.82	5.30	4.60	2.87

All measures based on $n = 5$. Posterior tibial nerve H-reflex and M-waves were not recorded from one patient who had an L4 vertebral fracture in addition to a thoracic level neurological lesion.
*The M-wave latency at each level of drug administration was significantly ($p < .05$) reduced from the pre-drug value.

side effects apart from a generalized fatigue and some mild irritability, associated with the prolonged duration of intense investigation.

Urinary excretion

The urinary excretion profiles exhibited considerable variability in 4-AP concentration levels, among patients. They showed a large peak urinary concentration of 4-AP occurring 2–3 hours after the start of drug administration (16–20 mg). There was also some evidence of a second late peak ($2969\ \mu gL^{-1}$) occurring 10 hours after the initial dose in one quadriplegic patient (6). The sparse intermittent sampling precluded careful delineation of the time course of either the first of second peak. By the following morning the urinary concentration of 4-AP was reduced to close to zero μgL^{-1} in all patients.

Discussion

This open, preclinical trial of intravenous 4-aminopyridine was undertaken to identify any adverse side effects when delivered to SCI patients, and to document any enhancement in sensory or motor status.

The side effects observed in the present trial, of localized forearm discomfort, mild perioral paraesthesia, and, in one subject, transient lightheadedness, were anticipated and have all been noted before.[16–22] None was sufficient for the patients to discontinue the trial. The small number and the mildness of the side effects noted were most likely related to the conservative total dose of 24 mg (which corresponded to ~0.3 $mg \cdot kg^{-1}$) and the rate of delivery (over 3.6 hours). Elsewhere there have been reports of paraesthesia, giddiness, ataxia, agitation and even epileptiform seizure activity when appreciably higher doses of 4-AP have been administered and at faster rates.[16–22,24,38] The present observations indicate that with a conservative total dose and rate of delivery, and with rigorous criteria applied to the selection of patients, 4-AP appears safe to administer to SCI patients.

Urinary excretion profiles were generally consistent with previously reported results, although the appearance of a second peak in 4-AP urinary concentration ($\sim 2969\ \mu gL^{-1}$) as late as 6 hours after the final 2 mg dose in one quadriplegic patient was not expected. Secondary peaks in 4-AP plasma concentration levels at 20–60 min have been noted previously and attributed to entero-systemic recirculation.[27] The prolonged excretion profile may simply reflect normal variability or may indicate some pathophysiological change such as reduced renal perfusion with lowered renal clearance rate. There is little or no biotransformation of 4-AP in man.

There was no electrophysiologic evidence of any sensory changes, although one patient reported qualitative changes in response to pinprick. Enhancement of visual evoked potentials (with enhanced visual acuity) by 4-AP has been reported in temperature sensitive multiple sclerosis (MS) patients.[16–18]

All 3 patients with incomplete injuries exhibited enhanced volitional motor unit activity during the administration of the drug. In one patient, this improvement was reflected in the *de novo* voluntary activation of motor units. This improvement was sustained throughout the course of delivery of the drug and appeared dose dependent. The enhanced volitional effort was also manifest in restored toe flexion and extension; a movement capability that reversed after 24 hours when the drug had been eliminated. In the other 2 patients the increase in amplitude and density of voluntary effort EMG interference patterns reached a maximum at 12–16 mg 4-AP but diminished with further increments in the drug.

Davis *et al*[17] have reported maximal therapeutic benefit of 4-AP in MS patients at doses of 12–14 mg, and noted declining motor function at higher doses using the same rate of delivery as the present study.[18] This has been attributed to the inadequate summation of drug effects, given the acknowledged short half life of 4-AP (~4 hours)[25,27] and the considerable between-patient variability that exists in plasma concentration and urinary excretion rates[25,27] (Stefoski, personal communication). A similar interpretation would apply to our observations in SCI patients if pa-

tients (1 and 2) had relatively short half life for 4-AP availability. The urinary excretion data obtained from patients 1 and 2 are consistent with this interpretation, but not definitive by virtue of sparse sampling.

The observation that there were no changes in segmental reflex excitability, particularly in the patient (3) with surgically verified complete disruption of his cord, suggests that the observed changes in motor function in patients 1, 2 and 5 could be attributable to enhanced central conduction. 4-AP induced alterations in neuro–neuronal and neuromuscular transmission are known to occur,[26] but these effects are typically evident at higher dose levels, or higher rates of delivery than those used in the present study. Conduction failure due to demyelination has been shown to be overcome by 4-AP in various *in vitro* preparations[13] and has been assumed to underlie the improvements in clinical signs in MS[16–18] and the improved function in animal models of SCI.[14,15]

The relatively modest changes in electrophysiological indices of motor function seen with 4-AP in the present study may be attributable to the conservative rate of drug administration (relative to its half life). Alternatively, the patients who volunteered may have had injuries principally involving axonal damage, as distinct from demyelination or dysmyelination. In order to assist with future identification of patients most likely to benefit from 4-AP or other K^+ channel blocking agents, a test has now been developed for revealing temperature-sensitive central conduction deficits.[39] With either induced hypothermia or with 4-AP, procedures which both prolong the duration of the action potential, there is an increase in the safety factor for conduction,[30] and in both instances there has been tangible evidence of enhanced conduction and restored voluntary motor function in SCI patients.[39] The principle of attempting to manage conduction failure due to demyelination in SCI patients thus appears to be promising and to warrant continued study.

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