CNS Drug Reviews
Vol. 10, No. 4, pp. 295–316


# The Use of 4-Aminopyridine (Fampridine) in Demyelinating Disorders

Keith C. Hayes

*Department of Physical Medicine & Rehabilitation, The University of Western Ontario, London, Ontario, Canada*

**Keywords:** 4-Aminopyridine — Demyelination — Fampridine — Multiple sclerosis — Spinal cord injury.

## ABSTRACT

4-Aminopyridine (4-AP or fampridine) is a potassium channel-blocking agent that has been shown to restore conduction in focally demyelinated axons. A sustained-release matrix tablet form of 4-AP (fampridine-SR) is currently undergoing multicenter clinical trials in patients with multiple sclerosis or chronic spinal cord injury. This review describes the pharmacology and mechanisms of action of 4-AP, its pharmacokinetics in human subjects, and the outcomes of clinical trials employing either immediate-release or sustained-release formulations of the drug. The randomized clinical trials that have been completed to date indicate that $K^+$ channel blockade may prove to be a useful strategy for ameliorating central conduction deficits due to demyelination. Diverse neurological gains have been reported for both motor and sensory domains. At the present time, however, the clinical trials have not provided sufficiently robust or definitive evidence of efficacy to gain regulatory approval for the symptomatic management of patients with either multiple sclerosis or spinal cord injury.

## INTRODUCTION

4-aminopyridine (4-AP or fampridine) is a potassium ($K^+$) channel-blocking agent that has a long and varied history of applications (59). It has been used as a bird repellant (84,85), a research tool for investigating ion channel function (73,76,103,106,124), an antagonist of neuromuscular blockade (66) and ketamine-diazepam anesthesia (2), as a treatment of verapamil toxicity in veterinary medicine (1,33), and as an investigational new

Address correspondence and reprint requests to: Keith C. Hayes, PhD, Parkwood Hospital/SJHC, 801 Commissioners Rd., E., London, Ontario N6C 5J1, Canada;
Tel.: +1 (519) 685-4292 Ext. 42937; Fax: +1 (519) 685-4060; E-mail: kchayes@uwo.ca
Disclosure: Dr. K. C. Hayes serves as a consultant for Acorda Therapeutics Inc., NY.

drug in various neurologic diseases (3,5,52,62,63,119). 4-AP acts by selectively blocking fast, voltage-gated $K^+$ channels in excitable tissues (and nonexcitable cells such as B cell and T lymphocytes) (46,71,76,122–124). In axons, $K^+$ channel blockade increases the safety factor[1] for conduction across demyelinated internodes and 4-AP can, therefore, restore conduction in focally demyelinated axons (11,15,91,103). 4-AP also increases calcium ($Ca^{2+}$) influx at presynaptic terminals thereby enabling an enhancement of neuroneuronal or neuromuscular transmission in normally myelinated neurons (52,53,61,94, 105,107). These pharmacological properties have prompted extensive investigation of its therapeutic potential for symptom management in disorders of neuromuscular transmission and in demyelinating diseases.

Many of the early clinical applications of 4-AP utilized intravenous delivery or oral gelatin capsules containing the powder form of 4-AP triturated with lactose or microcrystalline cellulose. These all constituted immediate release (IR) formulations with short time-to-peak serum concentration and biological half-life. More recently, a sustained-release (SR) matrix tablet form of 4-AP has been developed (fampridine-SR),[2] and is currently undergoing clinical trials. The SR formulation, with its longer half-life and lower peak serum levels (41–43), was designed to prolong the duration of therapeutic effect, reduce the dosing burden and thereby improve patient compliance, and lower the incidence and severity of unwanted side effects.

The present review summarizes the relevant pharmacology and pharmacokinetic properties of 4-AP and the status of clinical trials in patients with spinal cord injury (SCI) or multiple sclerosis (MS). For the purposes of this report, the term 4-AP or 4-aminopyridine is used to denote the compound in its various IR formulations, in order to distinguish it from fampridine-SR. There have been several previous reviews of 4-AP detailing its historical development, pharmacological properties and early clinical applications (70,98), including SCI (40) and MS (7,97). Since these were published there have been an appreciable number of new clinical trials. The present review is the first to summarize the clinical trial outcomes of fampridine-SR in patients with SCI or MS.

## PHARMACOLOGY OF 4-AP

4-Aminopyridine is a member of a family of mono-amino and di-amino derivatives of pyridine. Its molecular structure is $C_5H_6N_2$. The pyridine ring has an amino substitution in the 4 position (Fig. 1). The pyridine nitrogen of 4-AP has a $pK_a$ value of 9.1 so that at body pH about 98% of the molecules are protonated to form the monocation (70). The charge on the ionic form is delocalized over both nitrogens and this effectively prevents the addition of a second proton. The molecular weight of fampridine is 94.12. This structure constitutes a basic compound that is readily soluble in water and highly ionized at physiologic pH.

The principal mechanism of action of 4-AP is a dose-dependent block of fast, voltage-gated $K^+$ channels (A current) in excitable membranes (70) and in nonexcitable cells such as B cells (122) or T lymphocytes (46). There are several different types of voltage-gated $K^+$ currents and channels in mammalian and human axons (80) and they exhibit varying

[1] "Safety factor" is the ratio of action current generated by an impulse to the minimum amount of action current needed to maintain conduction (104).

[2] Acorda Therapeutics Inc., Hawthorne, New York.

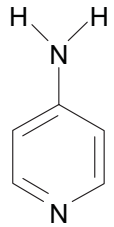


**Fig. 1.** Molecular structure of 4-aminopyridine.

degrees of sensitivity to 4-AP blockade. Kv 3.1, for example, has been reported to have a sensitivity ~150 times greater than that of Kv 2.1 (55). More generally, the sensitivity of different channels varies from micromolar to millimolar concentrations of 4-AP and within each channel the sensitivity depends on its activation state. 4-AP more readily enters open than closed channels and does so from the cytoplasmic side (56). It may remain trapped for an extended period of time in the closed channels accounting for prolonged effects of $K^+$ channel blockade. It can also be rapidly released from the channels when they are activated. Kirsch and Drew (55) suggested that the sensitivity of 4-AP blocking rate to activation may indicate that the drug's site of action is associated with a cytoplasmic region of the pore that couples the voltage sensor to the ion conduction pathway. More recently, Armstrong and Loboda (4) have put forward a model of 4-AP action on $K^+$ channels that proposes that 4-AP enters and leaves the channel only when the activation gate is open. Once in the open channel, its action is to bias the activation gate toward the closed conformation by approximating the energy of a hydrogen bond. The determinants of 4-AP sensitivity in human CNS Kv channels are just being identified (47,48).

In myelinated axons, the blockade of fast, voltage-gated $K^+$ channels has little effect on the action potential and nodal conduction (14,91). Membranes of myelinated axons have a high density of voltage sensitive sodium ($Na^+$) channels clustered at the node of Ranvier and reduced density in the internodal region. Influx of $Na^+$ is responsible for regeneration of the action potential at each node. Voltage-gated $K^+$ channels, on the other hand, are sparsely distributed at the node, but more densely distributed beneath the myelin. The fast voltage-gated $K^+$ channels allow $K^+$ efflux and mediate rapid repolarization of the membrane following the $Na^+$ action current (115,117,118). Their effect is normally attenuated by being covered by myelin. $K^+$ currents do appear to generate an internodal resting potential that contributes electronically to the nodal potential (19,20).

Demyelination alters the structural and functional relationships of voltage-gated ion channels along the axonal membrane. The capacitance of the internodal segment is increased. This results in an impedance mismatch at the transition between the myelinated and demyelinated areas because of the abrupt increase in surface area of axon exposed (115,116). This, in turn, leads to a decrease in action current density. Thus the axon requires greater inward current in order for action potential electrogenesis to occur (114,118). Exposure of $K^+$ channels leads to excessive conductance of $K^+$ with the result being that the cell membrane is held close to $E_k$, the equilibrium potential of $K^+$. There is a shunting of local circuit currents through the $K^+$ channels with impairment of action potential generation and conduction. Additionally, demyelination exposes slow $K^+$ channels and inwardly rectifying channels (mixed $K^+$ and $Na^+$ conductance) leading to disruption of the normal regulation of hyperpolarization and modulation of repetitive axonal discharge (35). As a consequence of demyelination and the altered input impedance, fast $K^+$ channel exposure, and the interaction with unconstrained periods of afterhyperpolarization, the action potential conducts decrementally with either: a) conduction failure; b) marked slowing of conduction velocity; or c) inability to sustain repetitive impulse discharges

(64,82,95,117,118). These conduction deficits contribute to the neurological deficits experienced by SCI and MS patients (117,118).

$K^+$ channel blockade by 4-AP prolongs the duration of the $Na^+$ action current, thereby increasing the safety factor for conduction across the demyelinated internode. Evidence of its effectiveness in restoring conduction in demyelinated axons comes from various sources. Bostock et al. (14,15) examined the effects of 4-AP on nerve roots and single axons from rats in which demyelination was caused by intrathecal injection of diphtheria toxin. 4-AP prolonged the duration of the root compound action potentials and abnormally shaped single fiber action potentials. A late phase of outward current ($K^+$) was abolished by 4-AP and the conduction blocking temperature was raised indicating enhanced conduction (14,15,91). Similar findings were reported by Targ and Koscis (103) who demonstrated restoration of axonal conduction in demyelinated rat sciatic nerves. Demyelination in these studies was induced by lysophosphatidyl choline. Single axon recordings revealed two patterns of change in spike waveform following application of 4-AP: a) broadening and b) bursting of single axon spikes. Blight, using intra-axonal and intra-myelin microelectrode recordings from isolated spinal cord axons from cats that had sustained chronic SCI as a result of a standardized contusion, showed that at 0.1 – 1 mM 4-AP restored conduction at physiological temperatures in critically demeylinated axons (11).

Differential effects of low and high concentrations of 4-AP on axonal conduction in normal and injured guinea pig spinal cord tissue have been reported (92). Whereas at low micromolar range (0.5 – 100 μM) 4-AP increased the amplitude of compound action potentials in injured axons, at higher concentrations (1 – 10 mM) it suppressed conduction. The compound action potentials of uninjured axons were unaffected by 4-AP at concentrations <1 mM. These results were interpreted as evidence that clinically observed effects of 4-AP are likely attributable to enhanced conduction across demyelinated internodes rather than enhanced neuronal transmission. The latter mechanism had been proposed earlier by Felts and Smith (31) on the basis of their observation that enhanced conduction along isolated rat axons demyelinated by ethidium bromide was only evident at high concentrations of 4-AP. The concentration of 4-AP in human cerebrospinal fluid or the extracellular space of cord tissue, at which 4-AP exerts its effects, has not been established in a definitive manner; and there is considerable variance in the levels theorized (31,92). Davis et al. (23) noted that the sensitivity of $K^+$ channels to 4-AP is much greater when applied directly to the cytoplasmic surface than when applied to the external surface. They argued that it is probable that low concentrations of 4-AP outside accumulate inside a neuron over time and block at an internal site having high affinity. The effective concentrations in which 4-AP is applied over a short time *ex vivo* may, therefore, not reflect the lower concentrations required *in vivo*. Also relevant to the issue of the concentration dependency of 4-AP effects on axonal conduction are the observations that there is a redistribution of $K^+$ (and $Na^+$) channels along traumatically demyelinated axons and a modified expression of genes encoding ion channel proteins following axonal trauma (30). These changes in ion channel properties appear to increase the sensitivity of injured axons to $K^+$ channel blockade by 4-AP (30,50,69).

Differences in response to 4-AP between demyelinated sensory and motor fibers have been documented (16,57). The compound action potentials of demyelinated ventral roots showed a prominent post-spike negativity associated with a broadening of single action potentials following application of 4-AP. Whole root responses from myelinated dorsal root axons also developed a late negativity, but individual fibers fired repetitively in re-

sponse to a single stimulus. These observations may explain some of the sensory side effects, e.g., paresthesias, seen in clinical trials (57).

$K^+$ channel blockade by 4-AP has other important physiological consequences with clinical relevance. Prolonged action current results in a larger than normal $Ca^{2+}$ influx at presynaptic terminals (70). This results in increased transmitter release from end terminals and enhanced neuro-neuronal or neuromuscular transmission (52,61,67), Kim et al. (52, 53), for example, showed that 4-AP produced dose-dependent increases in end plate potential amplitude, in rise time to peak, and in the average number of acetylcholine quanta released by nerve terminals. This effect has prompted 4-AP use in the reversal of drug-, toxin-, or pathology-induced neuromuscular blockade (1,3,5,61). The effect on neurotransmission may also underlie some of the observed clinical side effects of 4-AP including epileptiform seizure activity. Moreover, it may account for many physiological effects, such as the induction of rhythmic neural activity within the isolated cord (28) and contribution to observed improvements in neuromuscular performance in clinical trials. The extent to which enhanced neurotransmission contributes to neurologic gains in patients with demyelinating disease, as distinct from overcoming conduction failure, remains controversial (31,92). It awaits resolution with further understanding of the dose dependency of 4-AP induced responses and 4-AP concentrations in CNS tissue.

4-AP induced $K^+$ channel blockade causes spontaneous trains of discharges in sensory axons (16,57). 4-AP can also increase the excitation of cutaneous sensory nerve endings, and it has been suggested that this property derives from the action of 4-AP at or near the action potential generator region of the nerve terminal (54). Either one of these properties may underlie the paresthesias or other sensory changes evident in clinical trials in patients with MS or SCI.

Finally, $K^+$ channel blockade by 4-AP has potent immunomodulatory properties that may have relevance to autoimmune disease-mediated axonal demyelination (46). 4-AP blocks $K^+$ channels in T lymphocytes and modifies their proliferative and effector cell functions (46). $K^+$ channel blockade has been shown, in the rat experimental autoimmune encephalomyelitis model of MS to delay the hypersensitivity response to myelin basic protein and improve the symptoms of the disease (25). $K^+$ channel blockade is thus currently being explored as a novel targeted immunomodulatory approach to the management of neuroinflammatory demyelinating diseases (121).

## Demyelination in Multiple Sclerosis and Spinal Cord Injury

Multiple sclerosis is the archetypical central demyelinating disease, although its etiology, pathogenesis, and pathophysiology are not fully understood. Autoimmune-mediated demyelination within the brain and spinal cord is evident on pathological examination or through imaging. Exposure of paranodal $K^+$ channels contributes to axonal dysfunction (115,117,118). MS yields electrophysiological indicators of central conduction deficits, e.g., long latency visual evoked potentials (24,110) or motor evoked potentials (32), and tends to be responsive to environmental factors or interventions, such as cooling, known to modify ion channel kinetics that increase the safety factor for conduction (21,22). Traumatic and/or compressive SCI is also associated with a prominent inflammatory and autoimmune response (77) and evidence has accumulated of focal myelin thinning, disruption or complete demyelination contributing to axonal conduction failure and neurologic deficits. This evidence derives both from animal models (68,92) and from human post-mortem examination (17,18).

### 4-AP Effects on Animal Models of Demyelinating Disease and SCI

Investigations of the effects of 4-AP on neurologic deficits in animal models of demyelinating disease or SCI have yielded inconsistent results. These studies (12,13,37,49,72, 125) are summarized in Table 1. Some trials have shown indications of potential neurological benefit, such as enhanced motor evoked potentials (125) or reflex activity (12,13), whereas others have yielded no evident gains in function (37). These varied outcomes may be due to differences in models, e.g., acute versus chronic injury, differences in injury mechanism, species-specific differences, or other methodological issues such as drug delivery, or the sensitivity of the outcome measure. With such variation in design it is difficult to extract comparable information or generalize about 4-AP effects from these studies. Seizure induction at higher doses was identified as a significant limitation to therapeutic potential in at least one study (49). The early reports of possible gains in motor function in these animal models were, however, sufficiently persuasive to provide the impetus for investigation of the effects of 4-AP in human patients with demyelinating disease or SCI.

## PHARMACOKINETICS

### Control Subjects

Early reports of the pharmacokinetics of IR 4-AP in control subjects reported plasma concentration (C) parameters following intravenous delivery (0.3 mg/kg over 10 sec or 20 mg over 2 min) or enteric-coated (20 mg) tablets (29,108). Kinetic analysis of the plasma concentration clearance after intravenous dosie of 20 mg (29) resulted in the best

*TABLE 1. Effects of 4-AP in animal models of demyelination and spinal cord injury*

| Investigators | Dose/Delivery | Model | Outcomes |
|---|---|---|---|
| Kaji and Sumner (49) | 1 mg every 20 min injected i.p, | CNS demyelination induced in 5 rats by anti-galactocerebroside serum | • 4-AP partially reversed delayed conduction of compound action potentials through lesion.<br>• High doses induced seizures. |
| Blight and Gruner (12) | 1 mg/kg i.v. | Cats with chronic SCI induced by contusion method | • Augmentation of free fall response |
| Blight et al. (13) | 0.5–1 mg/kg i.v. and oral (gelatin capsule) | Complete and incomplete (chronic) canine paraplegia of various etiology | • Restored hind limb placing, pain appreciation, cutaneous trunci muscle reflex |
| Perez-Espejo et al. (72) | 1 mg/kg i.p. | Compressive SCI in rats 4-AP on days 25–27 post-injury | • nil |
| Haghighi et al. (37) | 2–6 mg/kg for 4 weeks | Compressive chronic (3 w) SCI in rats | • nil |
| Yu et al. (125) | 1 mg/kg i.v. | Varying severity weight drop injury to spinal cord of rats | • Increased amplitude of motor evoked potentials |

fitting of bi- or tri-exponential models and the mean terminal phase elimination half-life $t_{1/2}$ of 3.6 ± 0.9 h. Comparable results were obtained in a more recent report of the pharmacokinetics and excretion of $^{14}$C-labeled 4-AP (15 mg) in four healthy volunteers (51). Following ingestion of enteric coated tablets, 4-AP appeared in the plasma of control subjects ($n$ = 6) at 2.1 ± 0.6 h, reaching $t_{max}$ at 3.2 ± 0.9 h and $C_{max}$ of 62 ± 15 ng/mL. The plasma elimination half-life was 3.0 ± 1.1 h (108). In one of these studies, three subjects who received uncoated tablets became ill with gastric cramps and plasma concentrations could not be determined (108).

In a more recent study six subjects were randomly assigned to receive four single escalating oral doses of 10, 15, 20, and 25 mg 4-AP (gelatin capsule: 4-AP triturated with lactose) with a 1-week washout interval between each dose (42). Their results are summarized in Table 2 and group mean plasma concentration profiles are shown in Fig. 2. 4-AP was rapidly absorbed and $t_{max}$ remained essentially constant across doses, with group mean values between 1.0 – 1.2 h. $C_{max}$ increased linearly with dosage from 46.4 ± 9.7 to 102.3 ± 24.8 ng/mL. Mean $t_{1/2}$, elimination rate ($K_{el}$), and mean residence time at steady state (MRT), were independent of dose.

## Multiple Sclerosis Patients

Stefoski et al. (101) examined serum concentrations of 4-AP in 13 temperature sensitive multiple sclerosis (MS) patients following oral administration of 7.5 – 52.5 mg (total daily dose). Dosing was based on an optimal-dose-finding approach (24). The terminal half-life ($t_{1/2}$) was 2.92 ± 1.15 h. Peak serum concentrations for clinically determined "responders" ($C_{max}$ = 85 μg/L) did not differ from "non-responders" ($C_{max}$ = = 105 μg/L) and there was no correlation between 4-AP serum level or half-life and the magnitude of improvement among responders. Bever et al. (7,10) reported the pharmacokinetics of orally administered capsules containing 7.5 mg 4-AP and found peak serum concentrations of $C_{max}$ = 55.4 ± 9.8 ng/mL, which occurred at 1.3 ± 0.4 h, with an elimination half-life of $t_{1/2}$ = 3.8 ± 0.5 h. The pharmacokinetics of fampridine-SR were also in-

*TABLE 2. Pharmacokinetics of 4-aminopyridine in control subjects*

| | Dose of 4-aminopyridine (mg) | | | |
|---|---|---|---|---|
| Parameters | 10 | 15 | 20 | 25 |
| $AUC_{0–36}$ (ng · h/mL) | 173.1 (26.6) | 257.2 (10.0) | 329.7 (45.5) | 415.7 (107.8) |
| $AUC_{0–\infty}$ (ng · h/mL) | 184.6 (24.0) | 259.6 (7.2) | 333.2 (40.2) | 420.3 (124.8) |
| $C_{max}$ (ng/mL) | 46.4 (9.7) | 71.5 (17.7) | 86.6 (23.0) | 102.3 (24.8) |
| $K_{el}$ (1/h) | 0.19 (0.03) | 0.21 (0.04) | 0.23 (0.06) | 0.21 (0.07) |
| MRT (h) | 5.49 (0.4) | 5.62 (1.1) | 5.23 (1.0) | 5.88 (1.8) |
| $t_{max}$ (h) | 1.2 (0.4) | 1.0 (0.3) | 1.1 (0.4) | 1.1 (0.3) |
| $t_{1/2}$ (h) | 3.7 (0.7) | 3.4 (0.7) | 3.2 (0.6) | 3.8 (2.1) |

Values are means (±S.D.). Six subjects were treated at each dose level. $AUC_{0–36}$, area under the curve from time 0 to 36 h postdose; $AUC_{0–\infty}$, area under curve from time 0 to infinity; $C_{max}$, maximum observed plasma concentration; $K_{el}$, elimination rate constant; MRT, mean residence time; $t_{max}$, time to maximum observed plasma concentration; $t_{1/2}$, terminal-phase elimination half-life. Reproduced form ref. 41 with permission from *J Clin Pharmacol*.

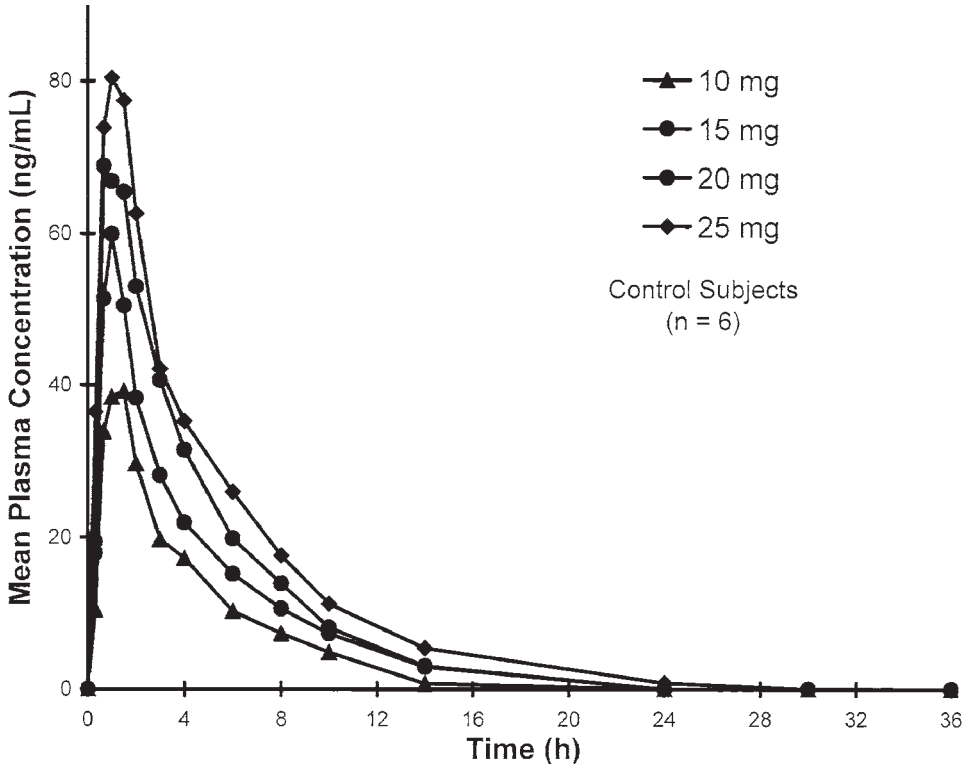


**Fig. 2.** Plasma concentration profiles for varying single doses of 4-aminopyridine in control subjects. With permission from the *J Clin Pharmacol* (41).

vestigated by Bever et al. (10) in 12 MS patients with EDSS scores between 4–7.5. The mean time-to-peak and serum half-life were 5.0 and 5.2 h and maximum tolerable doses varied from 10 mg b.i.d. to 25 mg b.i.d.

## SCI Patients

A study of the pharmacokinetics of a single 10 mg oral dose (gelatin capsules) of 4-AP in SCI patients ($n = 11$), with chronic, incomplete injuries, revealed plasma concentrations similar to those reported for control subjects (Fig. 3) (41). The pharmacokinetic parameters are summarized in Table 3. Of more direct relevance to the understanding and monitoring of bioactivity of the drug are the cerebrospinal fluid (CSF) pharmacokinetic parameters. Following intravenous delivery of 30 mg 4-AP at 15 mg/h in 12 paraplegic SCI patients, CSF levels of 4-AP peaked 30–60 min following peak serum concentration (27). In another report, intrathecal delivery of 4-AP at a rate of 5 μg/h in two SCI patients yielded peak CSF concentrations of 163 and 122 ng/mL. There was no therapeutic benefit or adverse outcome in these patients at these concentrations (38).

The absorption profile of a single 12.5 mg oral dose of fampridine-SR was reported for SCI patients ($n = 25$) with chronic incomplete injuries (89). The overall group mean $C_{max}$ of 27.7 ± 6.2 ng/mL occurred at a $t_{max} = 3.4 \pm 1.4$ h. Area under the serum concentration

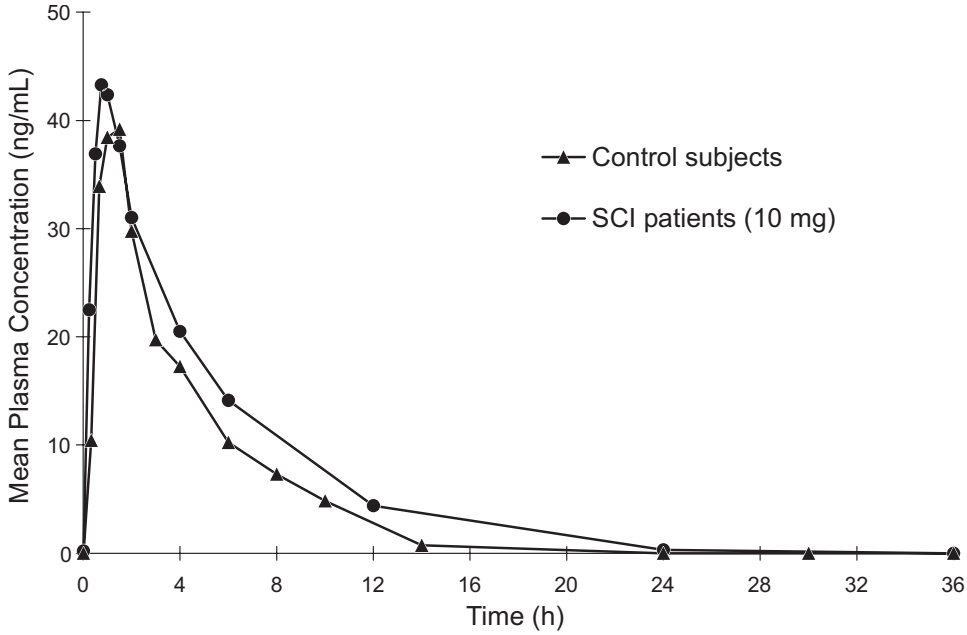


**Fig. 3.** Plasma concentration profiles for a single dose (10 mg) of oral 4-aminopyridine for control subjects and SCI patients. With permission from the *J Clin Pharmacol* (41).

*TABLE 3. Pharmacokinetics of 10 mg 4-AP in spinal cord injury patients*

| Parameters | SCI Subjects means (±S.D.) |
|---|---|
| $AUC_{0-36}$ (ng · h/mL) | 240.3 (37.7) |
| $AUC_{0-\infty}$ (ng · h/mL) | 241.6 (40.0) |
| $C_{max}$ (ng/mL) | 52.6 (20.6) |
| $t_{max}$ (h) | 1.0 (0.6) |
| $t_{1/2}$ (h) | 3.6 (0.6) |

**Note.** $AUC_{0-36}$, area under the curve from time 0 to 36 h postdose; $AUC_{0-\infty}$, area under the curve from time 0 to infinity postdose; $C_{max}$, maximum observed plasma concentration; $t_{max}$, time to maximum observed plasma concentration; $n = 11$; $t_{1/2}$, terminal-phase elimination half-life. Reproduced from ref. 41 with permission from *J Clin Pharmacol*.

curve (AUC) over the first 12 h ($AUC_{0-12}$) was 210.5 ± 49.5 ng/(mL · h). For paraplegics, $AUC_{t_{max}}$ was 76.02 ± 33.28 and for tetraplegics was significantly less at 51.25 ± 20.36 ($p = 0.037$). A statistically significant difference ($p = 0.02$) in the initial rate and extent of absorption, but not in total 4-AP bioavailability over the 12-hour study period, was evident between tetraplegic patients, 0.60 ± 0.23, and paraplegic patients, 0.39 ± 0.14 ($p < 0.05$). There was also a significant linear correlation between the neurological level of injury and $C_{max}/AUC_{t_{max}}$. These results confirmed and extended previous observations of subtly dif-

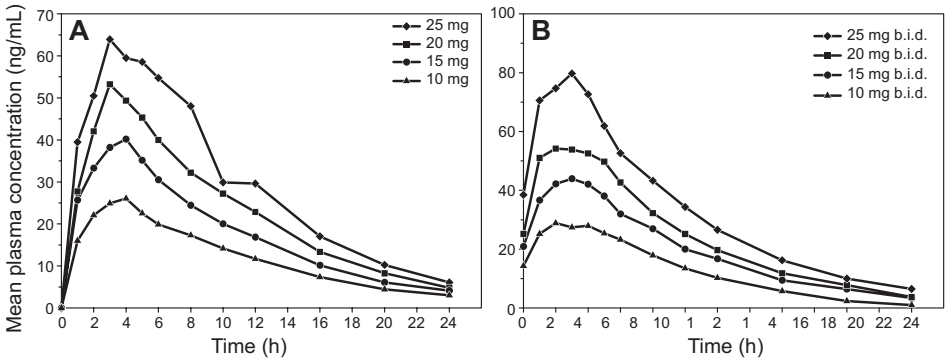


**Fig. 4.** Plasma concentration profile for single doses (**A**) and multiple doses (**B**) of Fampridine-SR in patients with SCI. $N = 13$ to 15 for each dose of each group. Reproduced from ref. 42 with permission from *Clin Neuropharmacol*.

ferent rates of drug absorption among SCI patients with lesions above and below the sympathetic outflow (T6) (89).

The pharmacokinetics and safety profile of mid-range doses of fampridine-SR (10–25 mg) administered as a single dose ($n = 14$) or twice daily for 1 week ($n = 16$) in patients with chronic, incomplete SCI have been reported (42). Mean plasma concentrations and area under the plasma concentration-time curve were linearly ($p < 0.05$)proportional to the dose administered, whereas other pharmacokinetic parameters were independent of dose. Fampridine-SR was absorbed slowly (peak plasma concentration shortly after single dosing, 3.2–3.7 h) and eliminated (plasma half-life, $t_{1/2} = 5.8$–$5.9$ h). Multiple dosing reached steady state after 4 days of twice-daily administration. Details of these profiles are shown in Fig. 4 and Table 4 reports the single dose parameters.

*TABLE 4. Pharmacokinetics of fampridine-SR following single dose administration in SCI Patients*

| | Fampridine-SR dose (mg) | | | |
|---|---|---|---|---|
| Parameters | 10 ($n = 14$) | 15 ($n = 14$) | 20 ($n = 14$) | 25 ($n = 13$*) |
| $C_{max}$ (ng/mL) | 27.7 ± 9.1 | 43.5 ± 11.2 | 54.9 ± 11.0 | 67.4 ± 13.3 |
| $t_{max}$ (h) | 3.2 ± 1.0 | 3.5 ± 1.2 | 3.5 ± 1.0 | 3.7 ± 1.2 |
| $AUC_{0-24\,h}$ [(ng · h)/mL] | 285.4 ± 96.8 | 423.0 ± 98.6 | 561.1 ± 117.6 | 715.6 ± 150.0 |
| $AUC_{0-\infty}$ [(ng · h)/mL] | 311.8 ± 93.2 | 460.2 ± 100.2 | 604.3 ± 124.6 | 769.2 ± 154.4 |
| $K_{el}$ (1/h) | 0.13 ± 0.03 | 0.12 ± 0.03 | 0.12 ± 0.02 | 0.13 ± 0.03 |
| $t_{1/2}$ (h) | 5.9 ± 1.5 | 5.9 ± 1.5 | 5.9 ± 1.4 | 5.8 ± 1.6 |
| Cl/F (L/h) | 34.8 ± 10.2 | 34.3 ± 8.9 | 34.7 ± 8.5 | 34.0 ± 8.3 |
| $V_d$/F (L) | 299.8 ± 127.4 | 289.0 ± 93.7 | 289.9 ± 84.1 | 286.2 ± 123.2 |

*One patient was excluded from analysis, as not all blood samples were collected.

$C_{max}$, maximum observed plasma concentration; $t_{max}$, time to reach $C_{max}$; AUC, area under the plasma-concentration time curve; $K_{el}$, elimination rate constant; $t_{1/2}$, plasma half-life; Cl/F, apparent total clearance; $V_d$/F, apparent volume of distribution. Reproduced from ref. 40 with permission from *Clin Neuropharmacol*.

Higher doses of fampridine-SR (25–60 mg b.i.d.) were subsequently studied in chronic incomplete SCI ($n = 16$) patients. Mean steady-state $C_{max}$, $C_{min}$, $C_{av}$, and $AUC_{0–12}$ increased over the entire fampridine-SR dose range and were dose-dependent up to 50 mg twice daily. Fampridine-SR had a mean $t_{max}$ of 2.2 to 3.0 h and a mean $t_{1/2}$ of 5.7 to 6.9 h. Mean apparent volume of distribution ($V_d/F$) (415.4–528.0 L) and apparent total clearance (Cl/F) (51.4–57.7 L/h) were independent of dosage, as were mean $t_{max}$ and $t_{1/2}$ across dosages (43).

## TOXICITY

Extensive data are available on the toxicity of 4-AP in animals (84,85). In high-dose experiments on birds and mammals, toxic responses began as soon as 15 min after dosing and consisted of seizure, tremors, ataxia, dyspnea, dilated pupils, salivation, and prostration (83). Lethal dose (LD) varies widely from species to species. The LD50 ranges from 3.7 mg/kg in the dog to 32 mg/kg in the rat (83), and seizure was the primary cause of death in animals. No long-term animal toxicity data are available.

Adverse reactions to 4-AP have occurred in several human cases of unregulated use by otherwise healthy individuals. Self-administered ingestion of 0.6 mg/kg of 4-AP in powder form led to poisoning in three men who mistook 4-AP for an aphrodisiac (99). One man developed weakness, nausea, profuse sweating, thirst, dyspnea and vomiting, became unconscious and exhibited tonic-clonic seizures, hypertension, a metabolic acidosis and a raised serum glutamic oxaloacetic transaminase concentration. Similar, but appreciably milder effects, were seen in the other two men. In another report, a healthy 22-year-old weight lifter was hospitalized with severe poisoning after taking an excessively high dose of 4-AP which he thought was an anabolic steroid (93). He exhibited epileptic seizure activity, confusion, cardiac arrhythmias, conduction disorders, and severe hypertension with a serum concentration of 335 ng/L. Finally, an 8-month-old boy ingested a capsule with 4-AP and presented on arrival to an emergency department as jittery, with tachycardia, and tachypnea (112). The patient developed dramatic opisthonic posturing and vermiform tongue fasciculations, but no EEG evidence of seizure. His symptoms responded well to therapy with benzodiazepines (two doses of 0.05 mg/kg lorazepam).

Stork and Hoffman (102) reported three cases of MS patients who experienced seizures while taking various and unknown doses of 4-AP. One of the patients was a 28-year-old woman who had been maintained on 4-AP 2 mg q.d. for two years before stopping her medication for three weeks. On resumption (6 mg) she experienced multiple seizures and had a serum concentration of 136.3 ng/mL. All of these patients responded to benzodiazepines, some with the addition of phenytoin and/or phenobarbital. Similarly, a 32-year-old female patient with MS who noted improvements in her symptoms at a self-administered dosage of 4-AP 8–16 mg t.i.d. became toxic after ingesting 28–36 mg in two doses roughly four hours apart. She presented with delirium and disorientation, and continuous dystonic choreoathetoid-type movements (74). In this patient serum concentration of 4-AP was 233.6 ng/mL. She also responded favorably to benzodiazepines at anticonvulsant dosages.

These cases of toxicity associated with 4-AP that was prescribed for MS patients highlight the considerable variability that exists in the peak serum concentrations of 4-AP obtained following even low doses. This was evident in control subjects (Table 2) and in the pharmacokinetic studies conducted with SCI patients by Hayes et al. (41) and in MS patients by Stefoski et al. (101) and Bever et al. (9). It was particularly notable in the case of the woman who took a low dose of 6 mg and had a serum concentration of 136.3 ng/mL. Polman et al. (75) have also reported on a patient who experienced generalized tonic-clonic seizure after having taken just two 5-mg capsules of 4-AP. Clearly there are concerns about generalizing expected serum concentration from oral dosing; concerns that have led to the suggested need for serum concentration control in clinical trials and prescribing practices (9). The cases also highlight the potential risks of self-medication and the need for clear education about the benefit-risk trade-off of 4-AP and for careful quality control over potency and shelf-life when 4-AP is prepared in compounding pharmacies.

## CLINICAL TRIALS

### Early Stage and Phase I Clinical Trials of 4-AP in MS and SCI Patients

A series of early stage (small number of subjects, open-label, case cohort) trials of 4-AP administered to patients with MS indicated that there was potential for functionally relevant gains in a number of sensory or motor domains (24,45,100). Visual function, as measured by luminescence threshold or variability, improved following oral 4-AP (20–60 mg) in patients with labile visual symptoms (45). Vision, oculomotor function and motor function (power, coordination, gait) improved following intravenous injection of 7–35 mg in temperature-sensitive patients (100). Other measures noted to respond to 4-AP included muscle strength (24).

In SCI patients, early stage 4-AP trials also provided some electrophysiological evidence of neurological gains. The gains were observed in the motor domain, e.g., increase in amplitude and reductions in latency of motor evoked potentials (44,79), and in assessments of sensory function, e.g., somatosensory evoked potentials of patients with chronic incomplete injuries (6,44). Clinical indicators of spasticity, bowel and bladder function, erectile function (44,78), motor function (e.g., locomotor status) (89), and pulmonary function (88) were all reported to respond to 4-AP although not all outcomes were the same in each patient, nor did all patients respond positively or without side effects. There was evidence that the efficacy of 4-AP was sustained over many months (78,90). These early observations helped shape the design of more formal randomized clinical trials (RCTs).

### Randomized Clinical Trials (RCTs) of 4-AP and Fampridine-SR in Patients with MS

The results of RCTs of 4-AP in MS patients are summarized in Table 5 (9,81,96,111). Although some of the trials confirmed the types of neurologic gains reported in earlier trials, the outcomes were generally constrained or confounded by appreciable placebo effects, the inherent variability of neurological status associated with the chronic pro-

gressive or relapsing-remitting form of MS, and the need to use clinically useful functional outcomes, such as the Expanded Disability Status Scale (EDSS), when they are often less sensitive to change than electrophysiological measures or other measures of impairment per se. Appreciable side effects were also encountered in the RCTs with the IR formulation.

A systematic review and meta-analysis of the outcomes from the RCTs (97) concluded that the available information did not allow unbiased statement about safety or efficacy of aminopyridines for treating MS symptoms. The analysis identified the fact that few common endpoints were available across trials, and because of lack of information on individual periods within studies, none of the data could be pooled for quantitative analysis. It should be noted that this analysis included trials with 3–4 diaminopyridine, a $K^+$ channel blocker that does not pass so readily through the blood brain barrier (60) and which had been shown to be inferior to 4-AP in symptom management in MS (75). It also included one trial with fampridine-SR. The meta-analysis of 4-AP is thus itself constrained by the small number of trials ($n = 4$), and diverse outcome measures, that limit generalization. The efficacy of 4-AP in the symptomatic management of MS remains, therefore, neither proven nor disproven.

The side effects, or adverse event profiles, of 4-AP in MS patients are significant. Most frequently reported side effects include dizziness/lightheadedness, paresthesias, gait instability, nausea/vomiting, abdominal pain, and restlessness/anxiety (75,100,101,111). Many of these are transient, occurring at the onset of treatment or on increases in dosage, and resolve within a few days. Serious adverse events include hepatitis, acute encephalopathy, episodes of confusion and epileptic (tonic-clonic) seizures (75). Problematic side

*TABLE 5. Randomized clinical trials of 4-aminopyridine in patients with multiple sclerosis*

| Investigators | Dose (mg) | Design* | *N* | Positive Outcomes |
|---|---|---|---|---|
| Bever et al. (10) | Serum concentration 30–59, 60–90 ng/mL*** | Double-blind, placebo-controlled concentration controlled, crossover, trial | 8 | • Ambulation<br>• Leg strength<br>• Ocular contrast sensitivity (No difference in outcome at low and high serum concentrations of 4-AP) |
| Rossini et al. (81) | 32/day | Double-blind, placebo-controlled concentration controlled, crossover, trial | 54 | • Fatigue**<br>• Motor evoked potentials |
| Smits et al. (96) | 20–40/day | Double-blind, placebo-controlled concentration controlled, crossover, trial (2 weeks) | 20 | • nil (neuropsychological tests) |
| Van Diemen et al. (111) | Max 0.5 mg/kg | Double-blind, placebo-controlled concentration controlled, crossover, trial (12 weeks) | 70 | • Subjective ADL<br>• Visual evoked potentials<br>• Motor evoked potentials |

* Each study involved only one center;
** In patients with high (>30 ng/mL) serum concentrations;
*** Daily dose and AUC constant across arms.
ADL, Activities of Daily Living.

effects or adverse events have tended to be associated with high serum concentrations, i.e., >100 ng/mL (9) and are generally, but not necessarily, associated with higher doses.

There have been three RCTs conducted on the efficacy of fampridine-SR in MS. The first of these was unpublished, but mentioned by Schwid et al. (87). It involved 161 MS patients in a 6-week, double-blind, placebo-controlled, parallel group trial, with the EDSS, a composite measure of function, as the primary outcome variable. Twenty-two percent of patients on drug improved, but an equal percentage improved on placebo. Schwid et al. (87) subsequently demonstrated positive fampridine-SR effects over placebo on a timed gait task in a small ($n$ = 10) double-blind, placebo-controlled, crossover, pilot trial. Most recently, Goodman et al. (34) reported on a trial of fampridine-SR that was conducted in patients with clinically definite MS with disability (EDSS < 6.5 and Fatigue Severity Scale > 4). Significant improvements were reported in the Lower Extremity Manual Muscle Test and there was a strong positive trend, relative to placebo, on the primary endpoint of walking speed, as measured by a timed 25-foot walk (34). These trials are summarized in Table 6. An earlier open-label trial (over 18 months) established that fampridine-SR is safe and well tolerated (8). Adverse events (e.g., dizziness, insomnia, paresthesias, and seizure) tended to be more severe at doses > 50 mg/d. Clinical trials are ongoing in this area.

## Randomized Clinical Trials (RCTs) of 4-AP and Fampridine-SR in Patients with Spinal Cord Injury

RCTs of 4-AP in SCI patients have been conducted by several different groups of investigators (36,39,90,109,113,120). Each of these studies had rather small numbers of subjects enrolled ($n$ = 8–60) and employed a variety of outcome measures to capture evidence of neurologic benefit. Whereas the majority of trials employed double-blind, placebo-controlled, crossover designs, two used an active-treatment, dose-blinded, format.

The outcomes of several of these studies indicated that 4-AP can produce neurologic gains in motor function (motor scores, motor evoked potentials, and central motor conduction), pulmonary function, spasticity (reductions in the modified Ashworth Scale), sensory function (sensory scores), and sexual dysfunction (36,39,90). One trial yielded no evidence of therapeutic benefit (109) and another found no change in cardiac function (113). The magnitude of treatment effect seen in trials with positive outcomes was gen-

*TABLE 6. Randomized clinical trials of fampridine–SR in patients with multiple sclerosis*

| Investigators | Dose (mg) b.i.d. (duration of treatment) | Design | Number of patients | Positive outcomes |
|---|---|---|---|---|
| Unpublished (Schwid) | 12.5–17.5 (6 weeks) | Double-blind, placebo-control, parallel groups | 161 | • n/a |
| Schwid et al. (87) | 17.5 (one week) | Double-blind, placebo-control, crossover | 10 | • Timed gait |
| Goodman et al. (34) | 10–40 (7 weeks) | Double-blind, placebo-controlled, parallel group, dose ranging, multi-center | 36 | • LEMMT |

b.i.d., twice a day; LEMMT, Lower Extremity Manual Muscle Test; n/a, not available.

erally small, but given the severity of impairment in many of the patients, was sometimes sufficient to provide some functional benefit. Placebo effects were prominent, although generally less than 4-AP effects. The types of side effects events reported were similar to those seen in MS patients or controls and were, for the most part, mild to moderate, and transient. There were no serious adverse events warranting hospitalization. The studies are summarized in Table 7.

A clear limitation of 4-AP as a drug is its high peak serum concentrations and its short half-life. There is only a short time window in which therapeutic effects might be expected with single doses, and the pharmacokinetic profile of multiple dosing is not well documented. With b.i.d. or t.i.d. dosing there is likely to be considerable variability in the serum and CSF concentrations of 4-AP. The time of outcome assessment, relative to CSF levels, is, therefore, critical in determining biologic effects, and in some of the trials the timing of assessment was not specified.

Three multicenter RCTs of fampridine-SR in SCI patients have been conducted and are summarized in Table 8. The numbers of subjects enrolled were relatively high (26–91) for this type of trial in SCI patients and the designs included both crossover and parallel group formats (26,58,78). These Phase II trials revealed consistent (in each of three trials) benefit of fampridine-SR over placebo in the reduction of spasticity (lower modified Ash-

*TABLE 7. Randomized clinical trials of 4-aminopyridine in patients with incomplete spinal cord injury*

| Investigators | Formulation | Dose (mg) | Design | Number of patients | Positive outcomes |
|---|---|---|---|---|---|
| Hansebout et al. (39) | IV | 18–33.5 | Double-blind, placebo-controlled, crossover | 8 | • Sensory score<br>• Breathing<br>• Pain<br>• Spasticity |
| Segal et al. (90) | IR | 6–30/day** | Open-label, active-treatment control, dose-blinded | 21 | • Motor scores<br>• Sensory scores<br>• Ashworth Scale<br>• Breathing |
| Wolfe et al. (120) | IR | 10 | Double-blind, placebo-controlled, crossover | 25 | • Motor evoked potentials<br>• Central conduction |
| Van der Bruggen et al. (109) | IR | 15–45/day* | Double-blind, placebo-controlled, crossover | 21 | • Nil (multiple domains) |
| Grijalva et al. (36) | IR | 5–30/day | Double-blind, placebo-controlled | 27 | • Motor function<br>• Sexual function<br>• Functional independence |
| Wakana and Segal (113) | IR | <30/day for 4 weeks | Randomized, active-treatment-control, dose-blinded | 60 | • nil (no change in parameters of cardiac function) |

IV, Intravenous; IR, Immediate-release capsule.

* Total dose per day: Range based on 0.5 mg/kg body weight;

** Total dose per day: 6 mg active treatment control.

worth Scale). In addition, sensory scores, motor scores, erectile dysfunction, and bowel function were all shown to benefit from fampridine-SR in at least one of the trials. Full reports of two of the studies have yet to be published but brief reports appear in the proceedings from various conferences.

Two large scale Phase III RCTs of fampridine-SR in chronic, (>18 months) incomplete SCI patients have been completed. These involved over 70 clinical centers across the United States and Canada with approximately 400 subjects enrolled. The admission criteria included having moderate to severe spasticity and the primary outcome measures were the modified Ashworth Scale and the subject's global impression. Preliminary reports of the outcomes have been posted (www.acorda.com). They show an unexpectedly large placebo response with consequent failure to show a significant drug treatment effect.

In one trial, there was a strong positive trend ($p < 0.069$) in the primary endpoint of reducing muscle spasticity when analyzed across all observations during the double-blind study drug period, however neither trial achieved statistical significance. When analyzed based on the subjects' last observation carried forward, a commonly used method of analysis, the modified Ashworth Scale in that study was significantly reduced ($p = 0.006$). The fampridine-SR groups in both trials showed a progressive mean improvement in the Ashworth score during the drug period. There was a more pronounced reduction in spasticity, than was expected, in the placebo group in one of the studies. The results of the secondary endpoints of improvement in bowel, bladder, and sexual functioning have not been reported.

## Treatment of Other Demyelinating Diseases

There have been no published Phase II RCTs of the efficacy of 4-AP or fampridine-SR in patients with other demyelinating conditions. There are clearly other patient groups with demyelinating pathology, such as those with cervical myelopathy, the leukodystrophies, compressive myelopathy due to tumors, or myelopathy secondary to arteriovenous malformations, where 4-AP or fampridine-SR may be indicated. In a preliminary commu-

*TABLE 8. Randomized clinical trials of fampridine–SR in patients with incomplete spinal cord injury*

| Investigators (reference) | Dose (mg) b.i.d. | Design | Number of patients | Positive outcomes |
|---|---|---|---|---|
| Potter et al. (78) | 12.5–17.5 | Multicenter (2) double-blind, placebo-controlled, crossover | 26 | • Motor score<br>• Sensory score<br>• Ashworth Scale<br>• Quality of Life<br>• Patient satisfaction |
| Ditunno et al. (26) | 10–25 | Multicenter (6) double-blind, placebo-controlled, crossover, dose escalation | 60 | • Erectile dysfunction<br>• Ashworth Scale |
| Lammertse et al. (58) | 25–40 | Multicenter (11) double-blind, placebo-controlled, parallel group | 91 | • Subject global impression<br>• Bowel function<br>• Ashworth Scale |

b.i.d., twice a day.

nication, Meythaler et al. (65) described the outcomes of a randomized, double-blind, placebo-controlled crossover Phase 1 trial of the safety and efficacy of oral 4-AP for motor weakness due to Guillain-Barré Syndrome (GBS). Patients with severe residual (>12 months) nonprogressive motor weakness due to GBS received 4 weeks of treatment with 4-AP (average dose, 30 mg/d) followed by a 1-week washout and 4 weeks of placebo (or vice versa). Significant increases in lower and upper extremity muscle strength were noted following the 4-AP arm of the trial, but not following the placebo arm. There were no clinically relevant changes in laboratory values, including liver function, following 4-AP. These results were interpreted as suggesting a possible therapeutic role for 4-AP in GBS.

## Current Status of 4-AP and Fampridine-SR as Investigational New Drugs

4-Aminoprydine (4-AP), by virtue of its orphan drug status, continues to be prescribed widely throughout North America for patients with MS or SCI, and is prepared by compounding pharmacies. This is despite equivocal research outcomes from RCTs, the high dosing burden, and side effects that are most likely made worse by high peak serum levels. While there are no formal comparisons between 4-AP and fampridine-SR with respect to efficacy or safety, the indications are that the SR formulation yields fewer side effects, when matched for dosage, and more robust clinical gains.

The diversity of reported benefit from 4-AP and fampridine-SR, in MS and SCI patients, has presented challenges for the choice of endpoints in clinical trials, and in the filing of "indication" with the Food & Drug Administration (U.S.) and other regulatory bodies. While many patients report, or are shown to derive some form of clinical benefit from one or other formulation, the different types of gains are widely distributed across heterogeneous patient subtypes. This presents an additional challenge for the design of trials with appropriate power to detect treatment effects and the designation of the most appropriate indication. Spasticity has been the choice to date for Phase III trials in SCI patients, but spasticity itself is notoriously variable. A broad-spectrum indication such as "neurological gain" is insufficient to satisfy regulatory requirements.

Finally, the variable nature of the symptoms of MS, in its various forms, together with the heterogeneity of lesions of SCI, i.e., varying amounts of axonopathy or myelinopathy, and the inherent variability of the symptoms (spasticity, for example, is influenced by the presence or absence of urinary tract infection) challenges the design of clinical trials. This is further confounded by the presence of relatively large Hawthorne or placebo-type responses to involvement in clinical trials in these patient populations. In addition, when a clinical endpoint such as "spasticity" is identified in SCI patients there is the implicit assumption that demyelination underlies or somehow contributes to this deficit. This causal link has not been established. Many individuals with spasticity may have little or no demyelination, whereas others do. Imaging of demyelination in the cord is not yet sufficiently precise to be of assistance. The benefits derived by some SCI patients for spasticity may simply indicate that some patients do have (reversible) conduction deficits due to demyelination that contribute to their spasticity, whereas others do not. Variable rates of absorption and peak serum concentrations introduce additional complications for effective prescription or design of clinical trials.

## SUMMARY

In summary, the clinical efficacy of 4-AP or fampridine-SR has yet to be established in a rigorous series of RCTs. Safety issues are considerable but appear manageable with proper regulation and monitoring. Phase III clinical trials of fampridine-SR in MS patients are continuing; and if they yield positive outcomes, and regulatory approval is obtained, fampridine-SR will most likely become a useful pharmacotherapy for symptomatic management of this form of demyelinating disease and potentially others. In the case of MS, fampridine-SR may prove to be a useful adjunctive and/or complementary therapy to immunomodulatory approaches designed to slow disease progression.

## REFERENCES

1. Agoston S, Maestrone E, van Hezik EJ, Ket JM, Houwertjes MC, Uges DR. Effective treatment of verapamil intoxication with 4-aminopyridine in the cat. *J Clin Invest* 1984;73:1291–1296.
2. Agoston S, Salt PJ, Erdmann W, Hilkemeijer T, Bencini A, Langrehr D. Antagonism of ketamine-diazepam anaesthesia by 4-aminopyridine in human volunteers. *Br J Anaesth* 1980;52:367–370.
3. Agoston S, van Weerden T, Westra P, Broekert A. Effects of 4-aminopyridine in Eaton Lambert Syndrome. *Br J Anaesth* 1978;50:383–385.
4. Armstrong CM, Loboda A. A model for 4-aminopyridine action on K channels: Similarities to tetraethylammonium ion action. *Biophys J* 2001;81:895–904.
5. Ball AP, Hopkinson RB, Farrell ID, et al. Human botulism caused by Clostridium botulinum type E: the Birmingham outbreak. *Quart J Med* 1979;48:473–491.
6. Barros TE, Oliveira R, Barros EMK, Cristante AF, Marcon RM, Camargo A. Somatosensory evoked potential in the evaluation of the effects of 4-aminopyridine. *J Spinal Cord Med* 2003;26:S33.
7. Bever CT, Jr. 4-Aminopyridine: Use in Multiple Sclerosis. *CNS Drug Rev* 1995;1:261–279.
8. Bever CT, Jr., Katz E, Tierney D, Johnson K. Experience with slow release 4-aminopyridine in multiple sclerosis patients: Long term tolerability and safety. *J Neuroimmunol* 1995;1(Suppl):58.
9. Bever CT, Jr., Young D, Anderson PA, et al. The effects of 4-aminopyridine in multiple sclerosis patients: Results of a randomized, placebo-controlled, double-blind, concentration-controlled, crossover trial. *Neurology* 1994;44:1054–1059.
10. Bever CT, Jr., Young D, Tierney D, et al. The pharmacokinetics and tolerability of a slow-release formulation of 4-aminopyridine in multiple sclerosis patients. *Neurology* 1995;45(Suppl 4):A351.
11. Blight AR. Effect of 4-aminopyridine on axonal conduction-block in chronic spinal cord injury. *Brain Res Bull* 1989;22:47–52.
12. Blight AR, Gruner JA. Augmentation by 4-aminopyridine of vestibulospinal free fall responses in chronic spinal-injured cats. *J Neurol Sci* 1987;82:145–159.
13. Blight AR, Toombs JP, Bauer MS, Widmer WR. The effects of 4-aminopyridine on neurological deficits in chronic cases of traumatic spinal cord injury in dogs: A phase I clinical trial. *J Neurotrauma* 1991;8: 103–119.
14. Bostock H, Sears TA, Sherratt RM. The effects of 4-aminopyridine and tetraethylammonium ions on normal and demyelinated mammalian nerve fibres. *J Physiol* 1981;313:301–315.
15. Bostock H, Sherratt RM, Sears TA. Overcoming conduction failure in demyelinated nerve fibres by prolonging action potentials. *Nature* 1978;274:385–387.
16. Bowe CM, Kocsis JD, Targ EF, Waxman SG. Physiological effects of 4-aminopyridine on demyelinated mammalian motor and sensory fibers. *Ann Neurol* 1987;22:264–268.
17. Bunge RP, Puckett WR, Becerra JL, et al. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. In: Seil FJ, eds. *Advances in Neurology.* Vol. 59. New York: Raven Press, 1993;75–89.
18. Byrne TN, Waxman SG. *Spinal cord compression: Diagnosis and principles of management.* Philadelphia: F. A. Davis Co., 1990.
19. Chiu SY, Ritchie JM. Evidence for the presence of potassium channels in the paranodal region of acutely demyelinated mammalian single nerve fibres. *J Physiol* 1981;313:415–437.

20. Chiu SY, Ritchie JM. On the physiological role of internodal potassium channels and the security of conduction in myelinated nerve fibres. *Proc R Soc Lond B Biol Sci* 1984;220:415–422.
21. Davis FA. Axonal conduction studies based on some considerations of temperature effects in multiple sclerosis. *Electroencephalogr Clin Neurophysiol* 1970;28:281–286.
22. Davis FA, Jacobson S. Altered thermal sensitivity in injured and demyelinated nerve. A possible model of temperature effects in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 1971;34:551–561.
23. Davis FA, Stefoski D, Quandt FN. Mechanism of action of 4-aminopyridine in the symptomatic treatment of multiple sclerosis. *Ann Neurol* 1995;37:684.
24. Davis FA, Stefoski D, Rush J. Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. *Ann Neurol* 1990;27:186–192.
25. Devaux J, Beeton C, Beraud E, Crest M. Ion channels and demyelination: Basis of a treatment of experimental autoimmune encephalomyelitis (EAE) by potassium channel blockers. *Rev Neurol (Paris)* 2004;160: S16–S27.
26. Ditunno JF, Jr., Graziani V, Katz MA, Blight AR, Group S-S. Double-blind, placebo-controlled, dose-escalating study evaluating the safety and efficacy of oral doses of fampridine-SR (sustained-release 4-aminopyridine) in patients with chronic spinal cord injury. *J Spinal Cord Med* 2002;25: S1-S38.
27. Donovan WH, Halter JA, Graves DE, et al. Intravenous infusion of 4-AP in chronic spinal cord injured subjects. *Spinal Cord* 2000;38:7–15.
28. Dubuc R, Rossignol S, Lamarre Y. The effects of 4-aminopyridine on the spinal cord: Rhythmic discharges recorded from the peripheral nerves. *Brain Res* 1986;369:243–259.
29. Evenhuis J, Agoston S, Salt PJ, de LA, Wouthuyzen W, Erdmann W. Pharmacokinetics of 4-aminopyridine in human volunteers: A preliminary study using a new GLC method for its estimation. *Br J Anaesth* 1981; 53:567–570.
30. Fehlings MG, Nashmi R. Changes in pharmacological sensitivity of the spinal cord to potassium channel blockers following acute spinal cord injury. *Brain Res* 1996;736:135–145.
31. Felts PA, Smith KJ. The use of potassium channel blocking agents in the therapy of demyelinating diseases. *Ann Neurol* 1994;36:454.
32. Fujihara K, Miyoshi T. The effects of 4-aminopyridine on motor evoked potentials in multiple sclerosis. *J Neurol Sci* 1998;159:102–106.
33. Gay R, Algeo S, Lee R, Olajos M, Morkin E, Goldman S. Treatment of verapamil toxicity in intact dogs. *J Clin Invest* 1986;77:1805–1811.
34. Goodman AD, Blight A, Cohen JA, et al. Placebo-controlled double-blinded dose ranging study of fampridine-SR in multiple sclerosis. *Neurology* 2003;60:A167.
35. Gordon TR, Kocsis JD, Waxman SG. Electrogenic pump ($Na^+/K^+$-ATPase) activity in rat optic nerve. *Neuroscience* 1990;37:829–837.
36. Grijalva I, Guizar-Sahagun G, Castaneda-Hernandez G, et al. Efficacy and safety of 4-aminopyridine in patients with long-term spinal cord injury: A randomized, double-blind, placebo-controlled trial. *Pharmacotherapy* 2003;23:823–834.
37. Haghighi SS, Clapper A, Johnson GC, Stevens A, Prapaisilp A. Effect of 4-aminopyridine and single-dose methylprednisolone on functional recovery after a chronic spinal cord injury. *Spinal Cord* 1998;36:6–12.
38. Halter JA, Blight AR, Donovan WH, Calvillo O. Intrathecal administration of 4-aminopyridine in chronic spinal injured patients. *Spinal Cord* 2000;38:728–732.
39. Hansebout RR, Blight AR, Fawcett S, Reddy K. 4-Aminopyridine in chronic spinal cord injury: A controlled, double-blind, crossover study in eight patients. *J Neurotrauma* 1993;10:1–18.
40. Hayes KC. 4-Aminopyridine and spinal cord injury: A review. *Rest Neurol Neurosci* 1994;6:259–270.
41. Hayes KC, Katz MA, Devane JG, et al. Pharmacokinetics of an immediate-release oral formulation of fampridine (4-aminopyridine) in normal subjects and patients with spinal cord injury. *J Clin Pharmacol* 2003; 43:379–385.
42. Hayes KC, Potter PJ, Hansebout RR, et al. Pharmacokinetic studies of single and multiple oral doses of fampridine-SR (sustained-release 4-aminopyridine) in patients with chronic spinal cord injury. *Clin Neuropharmacol* 2003;26:185–192.
43. Hayes KC, Potter PJ, Hsieh JT, Katz MA, Blight AR, Cohen R. Pharmacokinetics and safety of multiple oral doses of sustained-release 4-aminopyridine (Fampridine-SR) in subjects with chronic, incomplete spinal cord injury. *Arch Phys Med Rehabil* 2004;85:29–34.
44. Hayes KC, Potter PJ, Wolfe DL, Hsieh JTC, Delaney GA, Blight AR. 4-aminopyridine-sensitive neurologic deficits in patients with spinal cord injury. *J Neurotrauma* 1994;11:433–446.
45. Jones EG. The nature of the afferent pathways conveying short-latency inputs to primate motor cortex. *Adv Neurol* 1983;39:263–285.

46. Judge SI, Paterson PY, Mannie MD, Yeh JZ. Modulation of outward $K^+$ conductance is a post-activational event in rat T lymphocytes responsible for the adoptive transfer of experimental allergic encephalomyelitis. *J Biomed Sci* 1997;4:98–110.
47. Judge SIV, Yeh JZ, Goolsby JE, Monteiro MJ, Bever CT, Jr. Determinants of 4-aminopyridine sensitivity in a human brain kv1.4 $K^+$ channel: Phenylalanine substitutions in leucine heptad repeat region stabilize channel closed state. *Mol Pharmacol* 2002;61:913–920.
48. Judge SI, Monteiro MJ, Yeh JZ, Bever CT. Inactivation gating 4-AP sensitivity in human brain Kv1.4 potassium channel. *Brain Res* 1999;831:43–54.
49. Kaji R, Sumner AJ. Effects of 4-aminopyridine in experimental CNS demyelination. *Neurology* 1988;38: 1884–1887.
50. Karimi-Abdolrezaee S, Eftekharpour E, Fehlings MG. Temporal and spatial patterns of Kv1.1 and Kv1.2 protein and gene expression in spinal cord white matter after acute and chronic spinal cord injury in rats: Implications for axonal pathophysiology after neurotrauma. *Eur J Neurosci* 2004;19:577–589.
51. Katz MA, Butler J, Blight AR, Devane J. Pharmacokinetics and excretion of a single oral dose of $^{14}C$-labeled fampridine (4-aminopyridine) in health volunteers. *J Spinal Cord Med* 2002;25(Suppl 1):S29.
52. Kim YI, Goldner MM, Sanders DB. Facilitatory effects of 4-aminopyridine on neuromuscular transmission in disease states. *Muscle Nerve* 1980;3:112–119.
53. Kim YI, Goldner MM, Sanders DB. Facilitatory effects of 4-aminopyridine on normal neuromuscular transmission. *Muscle Nerve* 1980;3:105–111.
54. Kirchoff C, Leah JD, Jung S, Reeh PW. Excitation of cutaneous sensory nerve endings in the rat by 4-Aminopyridine and tetraethylammonium. *J Neurophysiol* 1992;67:125–131.
55. Kirsch GE, Drewe JA. Gating-dependent mechanism of 4-aminopyridine block in two related potassium channels. *J Gen Physiol* 1993;102:797–816.
56. Kirsch GE, Shieh CC, Drewe JA, Vener DF, Brown AM. Segmental exchanges define 4-aminopyridine binding and the inner mouth of $K^+$ pores. *Neuron* 1993;11:503–512.
57. Kocsis JD, Bowe CM, Waxman SG. Different effects of 4-aminopyridine on sensory and motor fibers: Pathogenesis of paresthesias. *Neurology* 1986;36:117–120.
58. Lammertse D, Graziani V, Katz MA. Safety and efficacy of oral fampridine-SR (sustained-release 4-aminopyridine) in patients with chronic motor-incomplete spinal cord injury. *J Spinal Cord Med* 2002;25:S5.
59. Lechat P. Historical review. In: Lechat P, Thesleff S, Bowman WC, eds. *Aminopyridines and similarly acting drugs*: *Effects on nerves*, *muscles and synapses*, *Advances in Biosciences*. Vol. 35. Oxford: Pergamon Press, 1982;3–7.
60. Lemeignan M, Millart H, Lamiable D, Molgo J, Lechat P. Evaluation of 4-aminopyridine and 3,4-diaminopyridine penetrability into cerebrospinal fluid in anesthetized rats. *Brain Res* 1984;304:166–169.
61. Lundh H. Effects of 4-aminopyridine on neuromuscular transmission. *Brain Res* 1978;153:307–318.
62. Lundh H, Nilsson O, Rosen I. 4-aminopyridine — a new drug tested in the treatment of Eaton-Lambert syndrome. *J Neurol Neurosurg Psychiatry* 1977;40:1109–1112.
63. Lundh H, Nilsson O, Rosen I. Effects of 4-aminopyridine in myasthenia gravis. *J Neurol Neurosurg Psychiatry* 1979;42:171–175.
64. McDonald WI, Sears TA. The effects of experimental demyelination on conduction in the central nervous system. *Brain* 1970;93:583–598.
65. Meythaler JM, Guin-Renfroe S, Johnson A, Brunner RM. The safety and efficacy of 4-aminopyridine for motor weakness due to Guillain-Barré Syndrome: A double-blind cross-over Phase I drug trial. *Arch Phys Med Rehabil* 2000;81:1293.
66. Miller RD, Booij LH, Agoston S, Crul JF. 4-Aminopyridine potentiates neostigmine and pyridostigmine in man. *Anesthesiology* 1979;50:416–420.
67. Molgo J, Lundh H, Thesleff S. Potency of 3, 4-diaminopyridine and 4-aminopyridine on mammalian neuromuscular transmission and the effect of pH changes. *Eur J Pharmacol* 1980;61:25–34.
68. Nashmi R, Fehlings MG. Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord. *Neuroscience* 2001;104:235–251.
69. Nashmi R, Fehlings MG. Mechanisms of axonal dysfunction after spinal cord injury: With an emphasis on the role of voltage-gated potassium channels. *Brain Res Brain Res* Rev 2001;38:165–191.
70. Paskov DS, Stojanov NA, Micov VZ. 4-aminopyridine hydrochloride (Pymadin). In: Charkevich DA, eds. *New neuromuscular blocking agents — Basic and Applied Aspects*, *Handbook of Experimental Pharmacology*. Vol. 79. New York: Springer-Verlag, 1986;679–717.
71. Pelhate M, Pichon Y. Proceedings: Selective inhibition of potassium current in the giant axon of the cockroach. *J Physiol* 1974;242:90P-91P.

72. Perez-Espejo MA, Haghighi SS, Adelstein EH, Madsen R. The effects of taxol, methylprednisolone, and 4-aminopyridine in compressive spinal cord injury: A qualitative experimental study. *Surg Neurol* 1996;46: 350–357.
73. Pichon Y, Meves H, Pelhate M. Effects of aminopyridines on ionic currents ad ionic channel noise in unmyelinated axons. In: Lechat P, Thesleff S, Bowman WC, eds. *Aminopyridine and similarly acting drugs*: *Effects on nerves*, *muscles and synapses*. Vol. 35. Oxford: Pergamon, 1982;53–68.
74. Pickett TA, Enns R. Atypical presentation of 4-aminopyridine overdose. *Ann Emerg Med* 1996;27:382–385.
75. Polman CH, Bertelsmann FW, van Loenen AC, Koetsier JC. 4-aminopyridine in the treatment of patients with multiple sclerosis. Long-term efficacy and safety. *Arch Neurol* 1994;51:292–296.
76. Pongs O. Molecular biology of voltage-dependent potassium channels. *Physiol Rev* 1992;72:S69–S88.
77. Popovich PG, Stokes BT, Whitacre CC. Concept of autoimmunity following spinal cord injury: Possible roles for T lymphocytes in the traumatized central nervous system. *J Neurosci Res* 1996;45:349–363.
78. Potter PJ, Hayes KC, Segal JL, Hsieh JTC, Brunnemann SR, Delaney GA, Tierney DS, Mason D. Randomized double-blind crossover trial of fampridine-SR (sustained-release 4-aminopyridine) in patients with incomplete spinal cord injury. *J Neurotrauma* 1998;10:837–849.
79. Qiao J, Hayes KC, Hsieh JTC, Potter PJ, Delaney GA. Effects of 4-aminopyridine on motor evoked potentials in patients with spinal cord injury. *J Neurotrauma* 1997;14:135–149.
80. Reid G, Scholz A, Bostock H, Vogel W. Human axons contain at least five types of voltage-dependent potassium channel. *J Physiol* 1999;518(Pt 3):681–696.
81. Rossini PM, Pasqualetti P, Pozzilli C, et al. Fatigue in progressive multiple sclerosis: Results of a randomized, double-blind, placebo-controlled, crossover trial of oral 4-aminopyridine. *Mult Scler* 2001;7: 354–358.
82. Sakatani K, Iizuka H, Young W. Randomized double pulse stimulation for assessing stimulus frequency-dependent conduction in injured spinal and peripheral axons. *Electroencephalogr Clin Neurophysiol* 1991;81: 108–117.
83. Schafer EW, Brunton RB, Cunningham DJ. A summary of the acute toxicity of 4-aminopyridine to birds and mammals. *Toxicol Appl Pharmacol* 1973;26:532–538.
84. Schafer EW, Burnton RB, Lockyer NF. The effects of subacute and chronic exposure to 4-aminopyridine on reproduction in coturnix quail. *Bull Environ Contam Toxicol* 1975;758–764.
85. Schafer EW, Burnton RB, Lockyer NF. Hazards to animals feeding on blackbirds killed with 4-aminopyridine baits. *J Wild Manage* 1974;38:424–426.
86. Schauf CL, Davis FA. Impulse conduction in multiple sclerosis: A theoretical basis for modification by temperature and pharmacological agents. *J Neurol Neurosurg Psychiatr* 1974;37:152–161.
87. Schwid SR, Petrie MD, McDermott MP, Tierney DS, Mason DH, Goodman AD. Quantitative assessment of sustained release 4-Aminopyridine for symptomatic treatment of multiple sclerosis. *Neurology* 1997;48: 817–821.
88. Segal JL, Brunnemann SR. 4-Aminopyridine improves pulmonary function in quadriplegic humans with longstanding spinal cord injury. *Pharmacotherapy* 1997;17:415–423.
89. Segal JL, Hayes KC, Brunnemann SR, et al. Absorption characteristics of sustained-release 4-aminopyridine (fampridine SR) in patients with chronic spinal cord injury. *J Clin Pharmacol* 2000;40:402–409.
90. Segal JL, Pathak MS, Hernandez JP, Himber PL, Brunnemann SR, Charter RS. Safety and efficacy of 4-aminopyridine in humans with spinal cord injury: A long-term, controlled trial. *Pharmacotherapy* 1999;19: 713–723.
91. Sherratt RM, Bostock H, Sears TA. Effects of 4-aminopyiridine on normal and demyelinated mammalian nerve fibres. *Nature* 1980;283:570–572.
92. Shi R, Blight AR. Differential effects of low and high concentrations of 4-aminopyridine on axonal conduction in normal and injured spinal cord. *Neuroscience* 1997;77:553–562.
93. Smeets JW, Kunst MW. Severe poisoning by 4-aminopyridine in a body builder. *Ned Tijdschr Geneeskd* 1995;139:2667–2669.
94. Smith KJ, Felts PA, John GR. Effects of 4-aminopyridine on demyelinated axons, synapses and muscle tension. *Brain* 2000;123(Pt 1):171–184.
95. Smith KJ, Hall SM. Nerve conduction during peripheral demyelination and remyelination. *J Neurol Sci* 1980;48:201–219.
96. Smits RC, Emmen HH, Bertelsmann FW, Kulig BM, van Loenen AC, Polman CH. The effects of 4-aminopyridine on cognitive function in patients with multiple sclerosis: A pilot study. *Neurology* 1994;44: 1701–1705.
97. Solari A, Uitdehaag B, Giuliani G, Pucci E, Taus C. Aminopyridines for symptomatic treatment in multiple sclerosis. *Cochrane Database Syst Rev* 2003;CD001330.
98. Soni N, Kam P. 4-Aminopyridine — a review. *Anaesth Intensive Care* 1982;10:120–126.

99. Spyker DA, Lynch C, Shabanowitz J, Sinn JA. Poisoning with 4-aminopyridine: Report of three cases. *Clin Toxicol* 1980;16:487–497.
100. Stefoski D, Davis FA, Faut M, Schauf CL. 4-Aminopyridine improves clinical signs in multiple sclerosis. *Ann Neurol* 1987;21:71–77.
101. Stefoski D, Davis FA, Fitzsimmons WE, Luskin SS, Rush J, Parkhurst GW. 4-Aminopyridine in multiple sclerosis: Prolonged administration. *Neurology* 1991;41:1344–1348.
102. Stork CM, Hoffman RS. Characterization of 4-aminopyridine in overdose. *J Toxicol Clin Toxicol* 1994;32: 583–587.
103. Targ EF, Kocsis JD. 4-aminopyridine leads to restoration of conduction in demyelinated rat sciatic nerve. *Brain Res* 1985;328:358–361.
104. Tasaki I. *Nervous Transmission*. Springfield, IL: Charles C. Thomas, 1953.
105. Thesleff S. Aminopyridines and synaptic transmission. *Neuroscience* 1980;5:1413–1419.
106. Thompson S. Aminopyridine block of transient potassium current. *J Gen Physiol* 1982;80:1–18.
107. Thomsen RH, Wilson DF. Effects of 4-aminopyridine and 3,4-diaminopyridine on transmitter release at the neuromuscular junction. *J Pharmacol Exp Ther* 1983;227:260–265.
108. Uges DRA, Sohn YJ, Greijdanus B, Scaf AHJ, Agoston S. 4-Aminopyridine kinetics. *Clin Pharmacol Ther* 1982;31(5):587–593.
109. van der Bruggen MA, Huisman HB, Beckerman H, Bertelsmann FW, Polman CH, Lankhorst GJ. Randomized trial of 4-aminopyridine in patients with chronic incomplete spinal cord injury. *J Neurol* 2001; 248:665–671.
110. van Diemen HA, Polman CH, van Dongen MM, et al. 4-Aminopyridine induces functional improvement in multiple sclerosis patients: A neurophysiological study. *J Neurol Sci* 1993;116:220–226.
111. van Diemen HA, Polman CH, van Dongen TM, et al. The effect of 4-aminopyridine on clinical signs in multiple sclerosis: A randomized, placebo-controlled, double-blind, cross-over study. *Ann Neurol* 1992;32: 123–130.
112. Velez L, Shirazi F, Goto C, Shepherd G, Roth BA. Opisthotonic posturing with neuromuscular irritability attributable to 4-aminopyridine ingestion by a healthy pediatric patient. *Pediatrics* 2003;111:e82–e84.
113. Wakana CI, Segal JL. Effects of 4-aminopyridine on cardiac repolarization, PR interval, and heart rate in patients with spinal cord injury. *Pharmacotherapy* 2003;23:133–136.
114. Waxman SG. Conduction in myelinated, unmyelinated, and demyelinated fibers. *Arch Neurol* 1977;34: 585–589.
115. Waxman SG. Membranes, myelin, and the pathophysiology of multiple sclerosis. *N Engl J Med* 1982;306: 1529–1533.
116. Waxman SG. Demyelination in spinal cord injury. *J Neurol Sci* 1989;91:1–14.
117. Waxman SG. Demyelinating diseases — new pathological insights, new therapeutic targets [editorial; comment]. *N Engl J Med* 1998;338:323–325.
118. Waxman SG. Ion channels and neuronal dysfunction in multiple sclerosis. *Arch Neurol* 2002;59: 1377–1380.
119. Wesseling H, Agoston S, Van Dam GB, Pasma J, DeWit DJ, Havinga H. Effects of 4-aminopyridine in elderly patients with Alzheimer's disease. *N Engl J Med* 1984;310:988–989.
120. Wolfe DL, Hayes KC, Hsieh JT, Potter PJ. Effects of 4-aminopyridine on motor evoked potentials in patients with spinal cord injury: A double-blinded, placebo-controlled crossover trial. *J Neurotrauma* 2001; 18:757–771.
121. Wulff H, Beeton C, Chandy KG. Potassium channels as therapeutic targets for autoimmune disorders. *Curr Opin Drug Discov Devel* 2003;6:640–647.
122. Wulff H, Knaus HG, Pennington M, Chandy KG. $K^+$ channel expression during B cell differentiation: Implications for immunomodulation and autoimmunity. *J Immunol* 2004;173:776–786.
123. Yeh JZ, Oxford GS, Wu CH, Narahashi T. Dynamics of aminopyridine block of potassium channels in squid axon membrane. *J Gen Physiol* 1976;68:519–535.
124. Yeh JZ, Oxford GS, Wu CH, Narahashi T. Interactions of aminopyridines with potassium channels of squid axon membranes. *Biophys J* 1976;16:77–81.
125. Yu K, Li J, Rong W, et al. Recording of spared motor evoked potentials and its augmentation by 4-aminopyridine in chronic spinal cord-injured rats. *Chin Med J (Engl)* 2001;114:155–161.