4-Aminopyridine for symptomatic treatment of multiple sclerosis: a systematic review

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***Abstract*:** This systematic review summarizes the existing evidence on the effect of 4-aminopyridine (4-AP) as a symptomatic treatment of decreased walking capacity in patients with multiple sclerosis (MS) when administered as an immediate release compound and a slow release compound. It summarizes existing evidence on the basic mechanisms of 4-AP from experimental studies and evidence on the clinical use of the compound. A systematic literature search was conducted of the following databases: PubMed and EMBASE. Thirty-five studies were included in the review divided into 16 experimental studies, two clinical studies with paraclinical endpoints and 17 clinical studies with clinical endpoints. Animal studies show that 4-AP can improve impulse conduction through demyelinated lesions. In patients with MS this translates into improved walking speed and muscle strength of the lower extremities in a subset of patients at a level that is often of clinical relevance. Phase III trials demonstrate approximately 25% increase in walking speed in roughly 40% and improved muscle strength in the lower extremities. Furthermore, 4-AP might have an effect on other domains such as cognition, upper extremity function and bowel and bladder, but this warrants further investigation. Side effects are mainly mild to moderate, consisting primarily of paraesthesia, dizziness, nausea/vomiting, falls/balance disorders, insomnia, urinary tract infections and asthenia. Side effects are worse when administered intravenously and when administered as an immediate release compound. Serious adverse events are rarely seen in the marketed clinical dosages.
In conclusion, 4-AP is easy and safe to use. Slow release 4-AP shows more robust clinical effects and a more beneficial side-effect profile than immediate release 4-AP.

Keywords: 4-aminopyridine, experimental studies, fampridine slow release, multiple sclerosis, translational medical research, treatment outcome

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Introduction

Multiple sclerosis (MS) is the most frequent nontraumatic cause of neurological deficit in young adults [Solari *et al.* 2003]. It is a chronic inflammatory disease of the central nervous system characterized by demyelination which can cause axonal conduction block [Judge and Bever, 2006]. The heterogeneous symptomatology includes paraesthesia, palsy, optic neuritis, diplopia, vertigo and bladder disturbances [Compston and Coles, 2008; McDonald, 1974; Smith and McDonald, 1999].

3,4-Diaminopyridine (DAP) and 4-aminopyridine (4-AP) are potent inhibitors of voltage gated potassium channels (K_v). *In vitro* studies have shown that DAP and 4-AP can improve conduction of action potentials in demyelinated nerve fibres and thereby increase the release of neurotransmitters in synapses and at the neuromuscular junction [Bostock *et al.* 1981; Hayes, 2004]. 4-AP is fat soluble and able to pass the blood–brain barrier [Bever and Judge, 2009; Blight and Henney, 2009], while DAP is water soluble and, therefore, unable to pass the intact blood–brain barrier [Judge and Bever, 2006]. Consequently, 4-AP has been applied in a number of treatment studies related to MS and is the focus of this review. Recently a sustained-release drug (SR-AP) was introduced into the market. An important question to address, therefore, is whether the benefits from a slow-release drug outweigh the

increased cost compared with an immediate-release drug in patients with MS.

A number of reviews have addressed the effects of 4-AP and SR-AP [Bever and Judge, 2009; Blight, 2011; Chwieduk and Keating, 2010; Espejo and Montalban, 2012; Goodman and Stone, 2012; Hayes, 2004, 2007, 2011; Jeffery and Pharr, 2010; Kachuck, 2009; Krishnan and Kiernan, 2012; McDonald and Clements, 2011; Solari *et al.* 2003] in patients with MS, but so far few have paid attention to both experimental and clinical studies. In 2001 a Cochrane review on the topic was published [Solari *et al.* 2001] which was updated in 2003 [Solari *et al.* 2003]. Mainly studies on 4-AP were included and only one study on SR-AP was identified. The conclusion of the Cochrane review was that no reliable statement concerning the safety and efficacy of 4-AP for treating symptoms in patients with MS could be made [Solari *et al.* 2003]. In 2004 Hayes reviewed pharmacokinetic, experimental and clinical studies on 4-AP and one randomized, controlled trial on SR-AP, and concluded that SR-AP most likely yields fewer side effects and more robust clinical gains than 4-AP [Hayes, 2004]. In 2007 the second trial on SR-AP was published [Goodman *et al.* 2007] and the overall conclusion of subsequent reviews [Bever and Judge, 2009; Blight, 2011; Chwieduk and Keating, 2010; Espejo and Montalban, 2012; Goodman and Stone, 2012; Hayes, 2004, 2007, 2011; Jeffery and Pharr, 2010; Kachuck, 2009; Krishnan and Kiernan, 2012; McDonald and Clements, 2011] is that SR-AP has a clinically meaningful beneficial effect on walking speed and muscle strength of the lower extremities. These latest reviews further conclude that SR-AP is generally well tolerated, most adverse events being mild to moderate. Moreover, only limited attention has been drawn to the potential effects on other bodily functions and symptoms than walking, as well as to explaining the clinical effects of the drug by means of the underlying mechanisms of action.

Our aim is to provide a systematic and comprehensive review of the existing evidence of the effects of SR-AP for symptomatic treatment of patients with MS and to compare the safety profile of SR-AP to that of 4-AP.

In addition, our intention is to obtain a better understanding of the therapeutic properties of 4-AP by connecting the results from experimental and clinical studies from which rationales for future trials may arise.

Methods

A systematic literature search was performed of PubMed and EMBASE, applying the subject headings (MeSH terms) multiple sclerosis AND (fampridine OR dalfampridine) on 21 January 2013. Furthermore, the reference lists of all identified reviews and included studies were checked for further relevant studies not captured by the search.

Longitudinal experimental studies testing the effects and safety of 4-AP or SR-AP in either animal studies or clinical trials involving patients with MS were included in the review if a clinical or paraclinical endpoint was applied. Notes, editorials, meeting highlights, case reports, short surveys, letters, comments and non-English papers were excluded.

The search of PubMed yielded 149 hits. Of these, 43 were reviews and 79 did not fulfil the inclusion criteria, being either expert opinions, testing other drugs than AP, applying other patient groups than MS, non-English, case reports, conference highlights or letters. In total, the search yielded 27 relevant hits.

The search of EMBASE yielded 332 hits. Of these, 131 were reviews, most of them not strictly on 4-AP, 62 were conference abstracts or conference letters and 23 were notes, editorials, meeting highlights, case reports, short surveys, letters or non-English. In total, the search yielded 27 relevant hits, all indexed in PubMed. An additional eight studies where identified by searching the reference lists of relevant hits and reviews. In total, this yielded 35 relevant studies (Figure 1).

Studies were grouped into experimental studies including animal studies (n = 16) [Blight, 1989; Bostock *et al.* 1981; Bowe *et al.* 1987; Hirsh and Quandt, 1993; Jensen and Shi, 2003; Kocsis *et al.* 1986; Renganathan *et al.* 2009; Schauf, 1987; Sherratt *et al.* 1980; Shi and Blight, 1997; Shi *et al.* 1997; Smith *et al.* 2000; Targ and Kocsis, 1986; Thomas *et al.* 2010; Thompson, 1982; Tibbs *et al.* 1989]; clinical trials with paraclinical endpoints (n = 2) [Fujihara and Miyoshi, 1998; van Diemen *et al.* 1993b]; and clinical trials with clinical endpoints (n = 17) [Bever *et al.* 1994; Davis *et al.* 1990; Goodman *et al.* 2007,

Table 1. Overview of clinical trial on 4-AP and SR-AP.

Study	Evidence level	Jadad score	Design	Intervention	Sample size	Disability (EDSS)	Type of MS	Outcome measures	Main findings
Immediate release AP (*n* = 12)									
Jones *et al.* [1983]	IIc	1	Open-label placebo-controlled trial	4-AP up to 60 mg a day or lactose placebo	10 (5 with labile visual symptoms and 5 with spastic paraperesis)	N/A	N/A	Perimetry Luminance threshold Visual temporal resolution VEP Visual acuity	Overall improvement in visual measurements apart from VEP and visual acuity
Stefoski *et al.* [1987]	IIc	0	Single-blind placebo-controlled trial	Intravenously 4-AP up to 35 mg or saline	22 (4-AP administered to 12 patients and 5 healthy subjects. 5 additional MS patients serve as single-blinded placebo controls)	N/A	N/A	Motor function* CFF Visual acuity Visual fields	Improvement in motor function, vision and oculomotor function
Davis *et al.* [1990]	IIb	2	RCT	4-AP up to 25 mg a day or lactose placebo	20	N/A	N/A	Motor function* CFF Visual acuity Visual fields VEP	Improvement in motor and visual functions and in VEP latencies
Stefoski *et al.* [1991]	IIb	2	RCT/crossover	4-AP up to 52.5 mg a day or lactose placebo	17	N/A	N/A	Motor function* CFF VEP	Improvement in motor function, CFF and VEP
Van Diemen *et al.* [1992]	Ib	4	RCT/crossover	4-AP in concentrations up to 0.5 mg/kg or placebo	70	2.0–7.5	RRMS, PMS	Motor function (evaluated by EDSS) Visual acuity CS VEP EMR Subjective effect	Improvement in EDSS, VEP latencies and EMR
Van Diemen *et al.* [1993a]	Ib	4	RCT/crossover	Phase I: 4-AP intravenously up to 40 mg followed by placebo Phase II: 4-AP orally up to 15 mg three times daily	70	2.0–7.5	RRMS, PMS	Smooth pursuit eye movements	Improvement in smooth pursuit eye movements
Polman *et al.* [1994b]	IIc	1	Open-label trial	4-AP in concentrations up to 0.5 mg/kg, total dose up to 40 mg/day	31	4–7.5	RRMS, PRMS	Patient reported effects in long-term treatment	Improvement in motor function and fatigue

(Continued)

Table 1. (Continued)

Study	Evidence level	Jadad score	Design	Intervention	Sample size	Disability (EDSS)	Type of MS	Outcome measures	Main findings
Polman *et al.* [1994a]	IIb	2	RCT/crossover	Up to 70 mg DAP and up to 35 mg 4-AP	24	4.0–7.5	RRMS, PMS	CS and patient-related outcomes	4-AP is superior to DAP
Bever *et al.* [1994]	IIb	4	RCT/cross over	4-AP in low concentration (30–59 ng/ml), high concentration (60–100 ng/ml) or placebo containing lactose	8	3–8	PRMS, PMS	CS FFF LEMS evaluated manually and quantitatively	Improvement in CS Improvement in LEMS in the high-concentration arm
Smits *et al.* [1994]	IIb	3	RCT/ crossover	4-AP up to 10 mg four times a day or placebo	20	2.5–8	RRMS, PMS	SRT PASAT SDMT WLG VMT	No significant changes
Rossini *et al.* [2001]	IIb	2	RCT/crossover	4-AP up to 8 mg four times a day or placebo	62	4–7.5	PPMS, SPMS	EDSS FSS VEP SSEP Neuropsychological evaluation HDS	Slight positive effect on fatigue in individuals with high serum concentrations
Romani *et al.* [2004]	IIb	5	Parallel group design	4-AP 8 mg three times a day or fluoxetine 20 mg + 0 + 0	60 (40 fatigued patients with MS who are randomized to either 4-AP or FLX and 20 nonfatigued controls not receiving treatment)	Median 3.1–3.3 (SD 2.3–2.5)	RRMS	FSS FIS/ HDRS BDI Neuropsychological test battery Fatigability MVC by thumbadduction	Improvement in fatigue in both treatment arms Improvement in depression in both treatment arms, more pronounced in the FLX arm Improvement in MVC in the AP arm
Sustained–release AP (n = 5)									
Schwid *et al.* [1997]	IIb	4	RCT/crossover	SR-AP 17.5 mg twice a day or placebo	10	6–7.5	N/A	Muscle strength evaluated manually and quantitatively Grip strength Walking speed Stair climbing	Improvement in walking speed

(Continued)

Table 1. (Continued)

Study	Evidence level	Jadad score	Design	Intervention	Sample size	Disability (EDSS)	Type of MS	Outcome measures	Main findings
Goodman *et al.* [2007]	IIb	5	RCT	SR-AP in 5 mg increments per week up to 40 mg twice a day or placebo	36	2.5–6.5	RRMS, SPMS	BFI MFIS MSFC LEMS Safety	Improvement in walking speed Improvement in LEMS Evaluation of AEs (Table 2)
Goodman *et al.* [2008]	IIb	4	RCT	SR-AP 10, 15 or 20 mg twice a day or placebo	206	2.5–6.5	RRMS, SPMS	MSFC LEMS Spasticity T25FW	Improvement in walking speed in a *post hoc* analysis Improvement in LEMS
Goodman *et al.* [2009]	IIb	5	RCT	SR-AP 10 mg twice a day or placebo	301	2.5–7.0	RRMS, SPMS, PPMS	Response to treatment Spasticity LEMS MSWS-12 T25FW	Treatment response in 35% Improvement in walking speed Improvement in MSWS-12 Improvement in LEMS
Goodman *et al.* [2010]	IIb	4	RCT	SR-AP 10 mg twice a day or placebo	239	1.5–7.0	RRMS, SPMS, PPMS, PRMS	Response to treatment LEMS T25FW MSWS-12	Treatment response in 42.9% Improvement in walking speed

AE, adverse event; 4-AP, 4-aminopyridine; SR-AP, sustained relaese aminopyridine; BDI, Beck's Depression Inventory; BFI, Brief Fatigue Inventory; CFF, critical flicker fusion; CS, contrast sensitivity; DAP, diaminopyridine; EDSS, Expanded Disability Status Scale; EMR, eye movement registration; FFF, flicker fusion frequency; FIS, Fatigue Impact Scale; FLX, fluoxetine; FSS, Fatigue Severity Scale; HDS/HDRS, Hamilton Depression Scale/Hamilton Depression Rating Scale; LEMS, lower extremity muscle strength; MFIS, Modified Fatigue Impact Scale; MS, multiple sclerosis; MSFC, Multiple Sclerosis Functional Composite; MSWS-12, 12-Item MS Walking Scale; MVC, maximal voluntary contraction; N/A, not applicable; PASAT, Paced Auditory Serial Addition Test; PMS, progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; PRMS, progressive relapsing multiple sclerosis; RCT, randomized controlled trial; RRMS, relapsing remitting multiple sclerosis; SD, standard deviation; SDMT, Symbol Digit Modalities Test; SPMS, secondary progressive multiple sclerosis; SRT, Spatial Recall Test; SSEP, somatosensoric evoked potentials; T25FW, Timed 25 Foot Walk Test; VEP, visual evoked potential; VMT, Verbal Memory Test; WLG, Word List Generation.

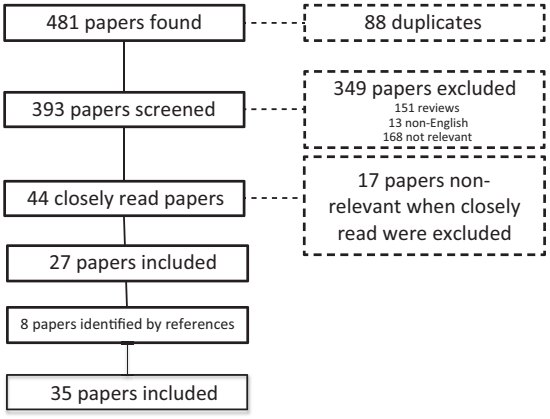

Figure 1. Consort diagram of papers identified and included.

2008, 2009, 2010; Jones *et al.* 1983; Polman *et al.* 1994a, 1994b; Romani *et al.* 2004; Rossini *et al.* 2001; Schwid *et al.* 1997; Smits *et al.* 1994; Stefoski *et al.* 1987, 1991; Van Diemen *et al.* 1992, 1993a].

Side effects reported in trials investigating 4-AP (Table 2) and SR-AP (Table 3) were summarized. Three trials did not report the exact number of side effects [Jones *et al.* 1983; Rossini *et al.* 2001; Stefoski *et al.* 1987].

Clinical trials with clinical endpoints were categorized and quantitatively scored according to the Modified Oxford Centre for Evidence-based Medicine Levels of Evidence and the quality of randomization and blinding was rated by the Jadad score if applicable [Jadad *et al.* 1996].

Results

Experimental studies including animal studies

Sherratt and colleagues examined the effect of 4-AP in normal and demyelinated rat nerve fibres isolated from both ventral and dorsal sacral roots. They found that 4-AP could prolong the action potentials in both unmyelinated and demyelinated fibres, and that 4-AP could abolish the late phase of outward potassium current in demyelinated fibres [Sherratt *et al.* 1980].

Bostock and colleagues examined the spinal roots of rats, where they also found that 4-AP can increase peak amplitudes in C fibres and abolish late phase outward potassium current and raise blocking temperatures in demyelinated fibres [Bostock *et al.* 1981].

On pleural axons from sea lemon, Thompson demonstrated that aminopyridine blockade of fast

Table 2. Side effects to immediate release 4-AP.

Side effect	Immediate release 4-AP (n = 300)	Placebo (n = 217)
Paraesthesia	104 (34.7%)	10 (4.6%)
Dizziness	101 (33.7%)	8 (3.7%)
Nausea/vomiting	20 (6.7%)	1 (0.5%)
Restlessness/anxiety	12 (4%)	0
Gait problems	11 (3.7%)	1 (0.5%)
Abdominal pain	7 (2.3%)	0
Vascular pain	6 (2%)	0
Discomfort	6 (2%)	1 (0.5%)
Hyperkalemia	5 (1.7%)	0
ECG changes	4 (1.3%)	0
Generalized tonic clonic seizure	3 (1%)	0
Diarrhoea	3 (1%)	0
Constipation	1 (0.3%)	0
Headache	1 (0.3%)	1 (0.5%)
Hepatitis	1 (0.3%)	0
Tremor	1 (0.3%)	0
Fatigue	1 (0.3%)	0
Encephalopathy	1 (0.3%)	0

Analysis is based on Davis et al. 1990; Stefoski et al. 1991; Van Diemen et al. 1992; Van Diemen et al. 1993a; Polman et al. 1994a; Polman et al. 1994b; Bever et al.1994; Smits et al. 1994 and Romani et al. 2004.

Table 3. Side effects of slow release 4-AP.

Side effect	Slow release 4-AP (*n* = 533)	Placebo (*n* = 249)
Falls/balance disorder	107 (20.0%)	40 (16.0%)
Urinary tract infection	72 (13.5%)	22 (8.8%)
Insomnia	62 (11.6%)	10 (4.0%)
Dizziness	57 (10.7%)	12 (4.8%)
Asthenia	52 (9.8%)	11 (4.4%)
Headache	51 (9.6%)	10 (4.0%)
Nausea	46 (8.6%)	7 (2.8%)
Fatigue	34 (6.4%)	7 (2.8%)
Paraesthesia	30 (5.6%)	6 (2.4%)
Upper respiratory tract infection	30 (5.6%)	16 (6.4%)
Back pain	20 (3.8%)	3 (1.2%)
Peripheral oedema	13 (2.4%)	3 (1.2%)
Muscle spasm	9 (1.7%)	3 (1.2%)
Pain in extremities	9 (1.7%)	3 (1.2%)
Tremor	6 (1.1%)	0
Arthralgia	6 (1.1%)	5 (2.0%)
Nasopharyngitis	6 (1.1%)	5 (2.0%)
Epileptic seizures	4 (0.8%)	1 (0.4%)
Injury	4 (0.8%)	3 (1.2%)

Analysis is based Schwid et al. 1997; Goodman et al. 2007; Goodman et al. 2008; Goodman et al. 2009 and Goodman et al. 2010.

transient potassium current I_A involves two kinds of effects. In the presence of aminopyridine, time to peak current during a depolarization is shortened and the initial rate of fall of current is hastened. This in part indicates a blocking effect involving open I_A channels [Thompson, 1982].

Kocsis and colleagues examined the different effects of 4-AP in ventral and dorsal roots from rats. They found that in ventral roots the action potential is broadened on 4-AP treatment. The effect on the dorsal roots was that the neurophysiological response to a single stimulus was increased. Hence, sensory fibres are hyper excitable when exposed to 4-AP [Kocsis *et al.* 1986].

Targ and colleagues confirmed these findings in demyelinated sciatic rat nerve. They showed that 4-AP could overcome conduction block, broaden the action potential, give rise to multiple spikes after a single stimulus, but also to some extent result in spontaneous impulse activity and multiple spike discharge [Targ and Kocsis, 1986].

Using the voltage-clamp technique on single fibres from frogs, Schauf showed that 4-AP exerts its effect on the internodal part of demyelinated axons [Schauf, 1987].

Bowe and colleagues corroborated the findings of Kocsis and Targ and concluded that application of 4-AP to demyelinated rat sensory fibres can lead to repetitive firing and furthermore to burst activity. In addition, they demonstrated that sensitivity diminishes with age [Bowe *et al.* 1987].

In experimental chronic contusion injuries in cat spinal cord, Blight also found that 4-AP produced bursts of action potentials in response to a single stimulus and spontaneous firing. Also 4-AP, to some extent, alleviated temperature-dependent conduction block, which is in line with the findings of Bostock and colleagues described above. In addition, it was noticed that some axons seemed sensitive to the effects of 4-AP while others did not [Blight, 1989].

In a guinea pig model, Tibbs and colleagues found a slow, but unambiguous increase in Ca^{2+} uptake following the addition of 1 mM 4-AP [Tibbs *et al.* 1989].

In a mouse neuroblastoma cell culture, Hirsh and Quandt showed a concentration-dependent block of whole cell potassium currents. They also showed that the curve of the fractional block breaks around 0.8 at a concentration of

approximately 3 mM. In addition, it was shown that the point of 4-AP action is intracellular using both inside-out and outside-out techniques [Hirsh and Quandt, 1993].

In a guinea pig model of spinal cord contusion, Shi and colleagues confirmed the enhancement of action potential in chronically injured mammalian white matter by 4-AP. Furthermore, they demonstrated a paradoxical suppression of conduction in normal and injured spinal tracts with concentrations of 4-AP above 1 mM. In a subsequent study they found a 4-AP induced improvement of conduction in acutely injured spinal cords. The effect in acute injuries required a higher concentration than in chronically injured cords (100 μM as opposed to 10 μM) [Shi and Blight, 1997; Shi *et al.* 1997].

In a rat model, Smith and colleagues did not find a consistent effect of 4-AP in restoring conduction through demyelinating lesions induced by ethidium bromide. However, they did report prominent effects on synaptic transmission and an increase in skeletal muscle twitch tension [Smith *et al.* 2000].

In a guinea pig model exposing spinal cord to stretch injury, Jensen and colleagues found that 4-AP could increase the action potential amplitude by 100% at a concentration of 100 μM. At 1μM concentration it was increased by 43%. They also found a concomitant increase of refractoriness [Jensen and Shi, 2003].

In an *in vitro* expression of hERG (human ether-a-go-go-related gene) in human embryonic kidney 293 cells, Renganathan and colleagues demonstrated a concentration-dependent inhibition of potassium currents, which might give rise to prolongation of the QT interval, which is recognized as a surrogate marker for proarrhythmic risk. The calculated IC_{50} (concentration resulting in 50% inhibition) was approximately four orders of magnitude higher than therapeutic plasma concentrations [Renganathan *et al.* 2009].

Finally, Thomas and colleagues studied the effect of 4-AP on canine cardiac purkinje fibres. They found that 4-AP at a concentration of 500 μM significantly increased action potential duration, though only examined in five specimens [Thomas *et al.* 2010].

In spite of limitations to experimental animal studies, such as different models employed, they consistently provide evidence of the mechanisms of 4-AP. It is shown that 4-AP can improve impulse conduction through a demyelinated lesion. Also, it seems that 4-AP can alleviate temperature-dependent conduction block and improve skeletal muscle twitch tension. Furthermore, it is shown that sensory fibres are hyper excitable when exposed to 4-AP, and that therapeutic doses of 4-AP do not block cardiac potassium channels.

Clinical trials with paraclinical endpoints

In 70 subjects with MS (43 women and 27 men), Van Diemen and colleagues examined visual evoked potentials (VEPs) and eye movement registration (EMR). VEPs showed a significant decrease in latency and increase in amplitude on the left eye. No possible explanation as to why there was a significant decrease on the left eye and not on the right eye was offered. They also found a significant improvement in smooth pursuit gain and maximum speed for both adduction and abduction of both eyes [van Diemen *et al.* 1993b].

Fujihara and Miyoshi recorded motor evoked potentials (MEPs) in upper and lower extremities in six patients with MS with clinically stable spastic paraparesis before and after intravenous administration of 4-AP. The study showed a significant increase in mean MEP amplitude in both upper and lower extremities and a decrease in the variability of onset latencies in the lower extremities. The variability of onset latencies in the upper extremities did not differ [Fujihara and Miyoshi, 1998].

In summary, the few existing studies with paraclinical endpoints might suggest that improved impulse conduction in the visual and motor tracts can be induced by 4-AP.

Clinical trials with clinical endpoints

In total, 17 trials with clinical endpoints were identified. Twelve studies [Bever *et al.* 1994; Davis *et al.* 1990; Jones *et al.* 1983; Polman *et al.* 1994a, 1994b; Romani *et al.* 2004; Rossini *et al.* 2001; Smits *et al.* 1994; Stefoski *et al.* 1987; Stefoski *et al.* 1991; Van Diemen *et al.* 1992, 1993a] tested immediate-release 4-aminopyridine and five [Goodman *et al.* 2007, 2008, 2009, 2010; Schwid *et al.* 1997] tested sustained-release 4-aminopyridine. Some studies [Bever *et al.* 1994; Davis *et al.* 1990; Jones *et al.* 1983; Polman *et al.* 1994a; Stefoski *et al.* 1987, 1991; Van Diemen *et al.* 1992, 1993a] included both clinical and paraclinical

endpoints. An overview of the included clinical trials can be found in Table 1.

The evidence levels were rated from Ib to IIc. The ratings were obtained because all trials were intervention trials with placebo control. However, some trials were open label [Jones *et al.* 1983; Polman *et al.* 1994b] or single blinded [Stefoski *et al.* 1987]. A substantial amount of the included trials have relatively small sample sizes ranging from 8 to 36 [Bever *et al.* 1994; Davis *et al.* 1990; Goodman *et al.* 2007; Jones *et al.* 1983; Polman *et al.* 1994a, 1994b; Schwid *et al.* 1997; Smits *et al.* 1994; Stefoski *et al.* 1987, 1991]. Nonetheless all studies point in the direction of a positive clinical effect of 4-AP/SR-AP with the exception of Smits and colleagues [Smits *et al.* 1994]. Seven trials had a Jadad score less than 3 [Davis *et al.* 1990; Jones *et al.* 1983; Polman *et al.* 1994a, 1994b; Rossini *et al.* 2001; Stefoski *et al.* 1987; Stefoski *et al.* 1991] and 10 trials had a Jadad score of at least 3 [Bever *et al.* 1994; Goodman *et al.* 2007, 2008, 2009, 2010; Goodman and Stone, 2012; Romani *et al.* 2004; Schwid *et al.* 1997; Smits *et al.* 1994; van Diemen *et al.* 1992, 1993b]. The average Jadad score was 3.34.

Immediate-release 4-aminopyridine

Jones and colleagues examined two groups of patients with MS in an open-label placebo-controlled trial. Group one comprised five patients with visual Uthoff's phenomenon. They demonstrated an overall improvement in visual measurements (luminance threshold and temporal resolution). In two subjects, scotoma disappeared after 4-AP treatment. Group two consisted of five patients with a clinically stable spinal form of MS. Four had optic atrophy. There was no change in Expanded Disability Status Scale (EDSS) but a trend towards improvement in visual measurements. One subject reported improvement in walking capacity [Jones *et al.* 1983].

Stefoski and colleagues also performed an open-label placebo-controlled trial with 12 patients with MS and five healthy controls. They used intravenous 4-AP or saline placebo. They demonstrated a significant effect on motor, visual and oculomotor functioning. The clinical evaluation was blinded. One subject demonstrated a clear gait improvement that was proven to be reversible. The quality of the gait improvement was not described [Stefoski *et al.* 1987].

In 1990 the first randomized, double-blind, placebo-controlled trial was performed by Davis and colleagues. They found improved motor function in nine out of 13 subjects, vision in 11 out of 13 and oculomotor function in one out of two. Furthermore, VEP latencies improved [Davis *et al.* 1990].

Stefoski and colleagues showed improvement in motor function, in terms of strength and coordination, improving gait and critical flicker fusion and VEPs. Oculomotor function was tested in three subjects, all improving on 4-AP [Stefoski *et al.* 1991]. In the same subjects Van Diemen and colleagues demonstrated a significant improvement in EDSS, reflecting an improvement in the Pyramidal Functional Score. Improvement in VEPs, smooth pursuit gain and adduction peak velocities was also demonstrated [van Diemen *et al.* 1992]. In a later publication based on the same data they compared intravenous and oral administration. Here a significant effect on smooth pursuit gain in the intravenous phase was found. In the oral phase, 37% showed improvement in smooth pursuit gain, with the largest effects observed in subjects with a high serum concentration of 4-AP. Side effects were more frequent and severe in the intravenous phase [Van Diemen *et al.* 1993a].

Polman and colleagues performed an open-label extension to the 1992 Van Diemen trial offering participants continuation of the 4-AP treatment. The study also included a group consisting of subjects recruited for *de novo* treatment with 4-AP. In total, 31 subjects were treated for up to 32 months. Improvement in ambulation and fatigue was found in 13, visual function in five and cognitive function in four [Polman *et al.* 1994b].

In addition, Polman and colleagues treated 24 participants (14 nonresponders and 10 responders to 4-AP) with 4-AP and DAP in a double-blind, randomized, crossover design. They demonstrated that 4-AP is superior to DAP in the treatment of patients with MS [Polman *et al.* 1994a].

Bever and colleagues included eight subjects with objective deficits of visual acuity and lower extremity motor strength and tone. Subjects were randomized to placebo, high-concentration 4-AP (60-100 ng/ml) or low-concentration 4-AP (30–59 ng/ml) with subsequent crossover. They demonstrated a significant improvement in contrast sensitivity and a trend towards improvement in

flicker fusion frequency and VEP latencies. Lower extremity muscle strength was tested both manually and quantitatively, and there was a significant improvement in subjects with high serum concentrations of 4-AP. Quantitative testing showed small nonsignificant changes and there were no changes in EDSS and ambulation [Bever *et al.* 1994].

Smits and colleagues examined the effect of 4-AP on cognitive function in 20 subjects with doses up to 40 mg/day. Outcomes included 10/36 Spatial Recall Test (SRT), Paced Auditory Serial Addition Test (PASAT), Symbol Digit Modalities Test (SDMT), Word List Generation (WLG) and Verbal Memory Test (VMT). No significant changes were found, but there where trends toward improvement in 10/36 SRT and PASAT [Smits *et al.* 1994].

In a 1-year trial, Rossini and colleagues demonstrated improvement in fatigue in a group receiving a high-concentration treatment (>30 ng/ml) compared with a group receiving a low concentration (≤30 ng/ml). There was no significant improvement when comparing the intervention group as a total to the placebo group [Rossini *et al.* 2001].

Romani and colleagues performed a trial comparing the effects of 4-AP and fluoxetine on fatigue, depression and motor function. There was an improvement in fatigue and depression in both groups, the improvement for depression being more pronounced in the fluoxetine group. Motor function, evaluated as maximal voluntary contraction of thumb adduction, improved in the 4-AP group [Romani *et al.* 2004].

Sustained-release 4-aminopyridine (fampridine)

Schwid and colleagues performed the first trial using SR-AP in 1997. Ten subjects with MS were included and treated with 17.5 mg SR-AP or placebo tablets twice a day for 1 week. A 1-week crossover followed a 1-week washout. Muscle strength was evaluated both manually and quantitatively, including shoulder abductors, elbow flexors and extensors, knee flexors and extensors, and ankle dorsiflexors. A significant effect on walking speed was demonstrated. Also, a trend towards improvement of muscle strength was seen, while a less clear trend towards improvement in grip strength was noted [Schwid *et al.* 1997].

Goodman and colleagues performed a dose-ranging trial on SR-AP in 2007. Thirty-six subjects with MS were included, of whom 25 received SR-AP in 5 mg increments up to 40 mg twice a day while 11 received placebo. In the prospectively planned analysis change in walking speed, measured by the Timed 25 Foot Walk Test (T25FW), did not reach significance. A *post hoc* analysis of data from the T25FW converted into walking speed (ft/s), reached significance. A significant change on lower extremity manual muscle testing (LEMMT) was also demonstrated. Both the SR-AP and placebo groups demonstrated improvement in self-reported fatigue [Goodman *et al.* 2007].

Goodman and colleagues also performed a dose comparison trial [Goodman *et al.* 2008]. Forty-seven subjects received placebo twice a day, 51 subjects received 10 mg SR-AP twice a day, 50 subjects received 15 mg SR-AP twice a day and 57 subjects received 20 mg SR-AP twice a day. Prospectively they planned to evaluate efficacy and safety through Multiple Sclerosis Functional Composite [consisting of T25FW, PASAT and 9 Hole Peg Test (9-HPT)] [Cutter *et al.* 1999], LEMMT, 12-Item MS Walking Scale (MSWS-12) and spasticity. In the prospective analysis of T25FW there was no significant change between any of the treatment groups and placebo. A *post hoc* analysis using consistent improvement in walking speed as a criterion was then performed. The *post hoc* analysis showed a response rate in the placebo group of 8.5% compared with 35.3%, 36.0% and 38.6% in the 10, 15 and 20 mg SR-AP twice a day groups, respectively. The difference was significant for all three groups individually and for the pooled SR-AP data compared with placebo. Muscle strength improved significantly in the 10 and 15 mg SR-AP twice a day groups compared with placebo. This was not the case for the 20 mg SR-AP twice a day group. No significant changes were demonstrated in 9-HPT, PASAT, spasticity or MSWS-12 [Goodman *et al.* 2008].

In a subsequent phase III trial, responder status was the primary outcome. Subjects with MS entered a 2-week single-blind placebo-controlled run in, taking one tablet every 12 h. After the run in subjects were randomized to SR-AP 10 mg twice a day or placebo tablets twice a day at a ratio of 3:1. A total of 229 were assigned to SR-AP and 72 to placebo. Response to treatment was defined as faster walking speed for at least three of the

four visits on treatment than the maximum speed for any of the visits off treatment. Thirty-five percent met this criterion and the increase in walking speed was on average 25.2%. The change in the nonresponder group was 7.5% and in the placebo group 4.7%. The increase in the responder group reached significance compared with both the nonresponder and the placebo group. There was no significant difference between the latter two. There was also a significant change in lower extremity muscle strength, measured by LEMMT, in the SR-AP group compared with the placebo group. There was a significant difference in MSWS-12 score between the responder group and the nonresponder group. Finally, there was a trend towards improvement in spasticity [Goodman *et al.* 2009].

The results described above were confirmed in a second phase III trial. The treatment design was similar to the above apart from a randomization ratio of 1:1. In this trial 42.9% of subjects in the SR-AP group met the responder criterion compared with 9.3% in the placebo group. The average increase in walking speed in the responder group was 24.7%. Again there was a significant difference in MSWS-12 scores between the responder group and the nonresponder group. Lower extremity muscle strength improved significantly in the responder group compared with the placebo group. The difference in muscle strength between the nonresponder group and the placebo group did not reach significance [Goodman *et al.* 2010].

Subsequently, the data from the first phase III trial were analyzed to determine the minimally important clinical difference in terms of walking speed measured by T25FW. Anchor- and distribution-based approaches were applied. In the anchor-based calculation Clinicians Global Impression was used as anchor. In the distribution-based approach 2.77 × standard error of the mean or 0.50 standard deviation units were used. Anchored to the CGI term 'minimally improved' subjects had a mean improvement of 0.36 ft/s or a 17.2% relative change in walking speed from a baseline value of 2.1 ft/s. Using the distribution-based approach the minimally important clinical difference was estimated to be 0.35–0.37 ft/s [Coleman *et al.* 2012].

In summary, clinical trials show improvement in walking speed and muscle strength in the lower extremities measured by LEMMT. In addition, several studies suggest improvement in visual and oculomotor function and possibly also in cognition, fatigue and spasticity.

Side effects

Side effects in the trials using immediate release 4-AP are listed in Table 2. Analysis is based on references 41–48 and 51. In total, 340 participants entered these trials; 300 received 4-AP and 217 received placebo and 20 participants received fluoxetine. In the 4-AP group, 288 instances (0.96 per participant) of side effects were reported as opposed to 22 instances (0.1 per participant) in the placebo group. Five participants experienced side effects to fluoxetine [Romani *et al.* 2004]. The most common side effects to 4-AP were paraesthesia, which was seen in 104 cases (34.7%) and dizziness in 101 cases (33.7%). Most side effects were reported to be mild, though one case of encephalopathy [Bever *et al.* 1994], three cases of generalized tonic clonic seizures [Bever *et al.* 1994; Polman *et al.* 1994b] and one case of hepatitis [Polman *et al.* 1994b] were reported. Encephalopathy was seen in a subject with a serum concentration of 4-AP of 114 ng/ml and one of the cases of epileptic seizure in a subject with a serum concentration of 104 ng/ml. The second case of epileptic seizure was seen in a subject treated with 5 mg capsules of 4-AP and the third case in a subject treated with 20 mg/day. The subject suffering from hepatitis received 30 mg 4-AP in three daily doses. Furthermore, disorientation was reported, though the exact number of cases was not clear [Jones *et al.* 1983].

Fifty-nine cases of paraesthesia were seen in subjects treated with 4-AP intravenously, which was also the case with 19 incidences of dizziness and the three cases of vascular pain reported by Fujihara and Miyoshi [Fujihara and Miyoshi, 1998].

Three papers do not report the exact number of adverse events, but they report instances of disorientation, pain in the extremities, perioral paraesthesia, dizziness, gait imbalance, abdominal pain, vertigo, anxiety, pollachiuria and tachycardia [Jones *et al.* 1983; Rossini *et al.* 2001; Stefoski *et al.* 1987].

Side effects in the trials using slow release 4-AP are listed in Table 3. In total, 782 participants entered these trials. A total of 533 received SR-AP and 249 received placebo. In the SR-AP group,

614 instances (1.15 per participant) of side effects were reported as opposed to 166 instances (0.7 per participant) in the placebo group. The most common side effect was falls and balance disorder, which was seen in 20% of the SR-AP group and in 16% of the placebo group. Insomnia was more frequent in the SR-AP group (11.6%) than in the placebo group (4.0%). Also, dizziness (10.7% *versus* 4.8%), asthenia (9.8% *versus* 4.4%), headache (9.6% *versus* 4.0%), nausea (8.6% *versus* 2.8%), fatigue (6.4% *versus* 2.8%), urinary tract infection (13.5% *versus* 8.8%) and paraesthesia (5.6% *versus* 2.4%) were more frequent in the SR-AP group than in the placebo group. Upper respiratory tract infection was seen more frequently in the placebo group than in the SR-AP group (6.4% *versus* 5.6%). Also, arthralgia and nasopharyngitis were more frequent in the placebo group than in the SR-AP group (2% *versus* 1.1%). Most side effects were mild to moderate, though four cases of epileptic seizures were seen in the SR-AP group and one case in the placebo group. All cases in the SR-AP group were seen in patients receiving 25 mg SR-AP twice daily or more.

Schwid did not report the exact number of adverse events but they reported instances of nausea, dizziness, paraesthesia and insomnia [Schwid *et al.* 1997].

In summary, the frequency of side effects in trials applying 4-AP was 0.96 per participant in the 4-AP group and 0.1 in the placebo group (ratio 9.6) and in trials applying SR-AP it was 1.15 per participant in the SR-AP group and 0.7 per participant in the placebo group (ratio 1.64). Data show that intravenous administration of 4-AP gives rise to prominent side effects such as paraesthesia, dizziness and vascular pain.

Administered orally both 4-AP and SR-AP predominantly give rise to mild or moderate side effects, though 4-AP seems more prone to give rise to serious adverse events. Serious adverse effects to SR-AP, in the form of epileptic seizures, were reported, but only in dosages of 25 mg twice a day or higher, which is 2.5 times higher than the recommended therapeutic dose.

Discussion

This review identified 16 basic studies that reveal basic mechanisms underlying effects and side effects of 4-AP, 14 clinical studies that evaluate the effect of immediate release 4-AP and five clinical studies that assess the effect of sustained release 4-AP as a symptomatic treatment in patients with MS.

The underlying mechanisms of the compound are well described and understood, and the efficacy is clinically meaningful in symptomatic treatment of patients with MS. Effects are mainly seen on walking speed and muscle strength in the lower extremities and it is evident that slow release formulation minimizes the toxicity of the compound and thereby increases its usefulness.

Early experimental studies provide useful information on the mechanisms of 4-AP which, in part, can explain the effect of the compound. It is shown that 4-AP can improve impulse conduction through a demyelinated lesion by prolonging and broadening the action potential [Bostock *et al.* 1981; Bowe *et al.* 1987; Jensen and Shi, 2003; Kocsis *et al.* 1986; Sherratt *et al.* 1980; Shi and Blight, 1997; Shi *et al.* 1997; Targ and Kocsis, 1986; Thompson, 1982]. This can, in some instances, overcome conduction block resulting from demyelination. 4-AP most likely exerts its main effect in the central nervous system. This is supported by the fact that 4-AP, as opposed to DAP, can cross the intact blood–brain barrier [Bever and Judge, 2009; Blight and Henney, 2009] and also that superiority of 4-AP *versus* DAP in treating MS patients has been demonstrated [Polman *et al.* 1994a].

In addition, animal studies provide a possible explanation of the pain in the extremities and back that can occur as side effects, as it has been demonstrated that sensory fibres are hyper excitable when exposed to 4-AP [Bowe *et al.* 1987; Kocsis *et al.* 1986; Targ and Kocsis, 1986]. Furthermore, experimental studies show that therapeutical doses of 4-AP are not cardiotoxic [Renganathan *et al.* 2009; Thomas *et al.* 2010].

There are considerable limitations to the experimental animal studies due to different models in terms of the species examined, different mechanisms of inducing demyelinating lesions and experiments conducted on fibres from peripheral nerves, roots and central nerves.

Regarding the clinical studies, it is a limitation that quality of studies was not an inclusion criterion in this review. This was chosen to make the review as comprehensive as possible. It is evident

that the quality of studies is higher in the later studies.

The effects shown in experimental studies are reflected in the clinical studies, which clearly show beneficial effects of 4-AP, both as an immediate release and a sustained release compound. The average improvement in walking speed of approximately 25% [Goodman *et al.* 2009, 2010] seems highly clinical relevant [Coleman *et al.* 2012]. The responder concept can be debated though because the degree of increase in walking speed is not a criterion for response. However, data show that the responder group and the nonresponder group are clearly separated, and that the improvement in the responder group is in line with that of the placebo group. In addition, clinical experience indicates that there might be an effect on visual and oculomotor function as well as on spasticity, cognition, fatigue and possibly on bowel and bladder function [Kachuck, 2009]. The possible effects on visual and oculomotor function are corroborated by paraclinical findings [Bever *et al.* 1994; Jones *et al.* 1983; Stefoski *et al.* 1987; van Diemen *et al.* 1992, 1993b]. However, it seems relevant to address these questions in future studies.

SR-AP seems safe in therapeutic doses. The reported side effects are predominantly mild to moderate. Epileptic seizures were only seen in doses of 25 mg twice a day or higher. Though the frequency of side effects was higher in the SR-AP group compared with the 4-AP group, so was the frequency of side effects in the placebo group in the trials investigating SR-AP. Hence SR-AP seems to have a more beneficial profile of side effects. In addition, the therapeutic effect of SR-AP is more stable than that of 4-AP and SR-AP is easy to use due to the twice-daily administration and the fact that it is possible to identify responders within a 2-week period.

Several questions remain unanswered. Should we expect an effect on visual and oculomotor function, spasticity, cognition, fatigue, upper extremity and possibly on bowel and bladder function? Or does 4-AP have a selective effect on the pyramidal tracts mainly on the long tracts to the lower extremities? If 4-AP has an effect on other systems, the EDSS range 4–7 must be questioned as a method for identifying candidates for Fampyra treatment. (Alkermes Pharma International Ltd., Monksland Athlone Co., Westmeath, Ireland)

Answers to these questions may be generated by clinical studies that target uniform symptoms for which meaningful and sensitive endpoints can be defined.

Thus, positive treatment effects on compromised dexterity, cognitive dysfunction, fatigue, Uthoffs phenomena [Blight, 1989; Bostock *et al.* 1981], urinary urgency and so on cannot be ruled out until proper studies have been conducted.

Unpublished data from our group suggest that 4-AP might have an effect on cognition as well as on walking speed, balance and coordination.

The effect on the muscle strength in the lower extremities might improve the outcome of exercise therapy because of better prerequisites for training. However, muscle strength was measured by LEMMT, which is a valid and reliable strength measure having an inherent problem on ceiling effect [Cuthbert and Goodheart, 2007].

Fatigue is reported by as many as 75% at some point in the disease course [Krupp, 2006; Lerdal *et al.* 2007]. Impact of fatigue is correlated negatively to perceived health and as many as 68% assess fatigue as one of their worst problems [Flensner *et al.* 2008]. Nevertheless, it is still unresolved whether 4-AP/SR-AP has an effect on fatigue. A small effect on fatigue has been reported [Polman *et al.* 1994a, 1994b; Romani *et al.* 2004; Rossini *et al.* 2001] but Goodman and colleagues did not find a significant effect of SR-AP on fatigue in a large high-quality trial [Goodman *et al.* 2007].

Depression, pain, spasticity, bladder disturbances and other factors might contribute to fatigue [Braley and Chervin, 2010], but more vague primary mechanisms are also proposed.

Significantly increased tumour necrosis factor α (TNF α) mRNA expression, TNFα levels, and interferon γ levels have been found in fatigued patients compared with nonfatigued patients [Flachenecker *et al.* 2004; Heesen *et al.* 2006]. Endocrine disturbances have been proposed as well. Moreover, lower levels of dihydroepiandrosterone have been demonstrated in fatigued patients with MS compared with nonfatigued patients with MS [Tellez *et al.* 2006]. Neuronal dysfunction is also suggested as a cause by the finding of decreased glucose metabolism in the frontal cortex and basal ganglia of fatigued patients with MS [Roelcke *et al.* 1997]. Axonal damage might

also contribute to fatigue as magnetic resonance spectroscopy has demonstrated significant reductions in N-acetylaspartate/creatinine ratios in fatigued patients with MS [Tartaglia *et al.* 2004; Tellez *et al.* 2008]. Functional magnetic resonance imaging studies have also demonstrated increased activation in the cingulate gyri and left primary sensory cortex, suggesting compensatory reorganization and increased brain recruitment [Tartaglia *et al.* 2008]. Interestingly, when considering the effects of 4-AP/SR-AP, it has been suggested that patients with MS have higher energy expenditure than healthy controls when walking [Sandroff *et al.* 2012]. It seems likely that 4-AP/SR-AP can ameliorate fatigue on that basis and perhaps also if fatigue is driven by compensatory reorganization.

Identification of responders is another major area for future research. Response might depend on genetic polymorphism regarding the voltage-gated potassium channels or on the degree of demyelination as opposed to the degree of axonal degeneration due to demyelination. It is also possible that the explanation is a combination of the two. To address the question of responders/nonresponders it might be time to take the question from bed back to bench.

Conclusion

Experimental studies provide strong, though somewhat heterogeneous evidence of the mechanisms of action of 4-AP, which provides explanation of the effects and to some extent the side effects of the drug.

Clinical studies provide strong evidence for the effects and side effects of the drug in patients with MS.

Moreover, there is evidence that SR-AP is a safe and easily administered drug for treatment of walking disability in patients with MS in the 4–7 EDSS range. Administered in therapeutic doses, most side effects are transient and mild to moderate and SR-AP has a more beneficial side effect profile than 4-AP. A subset of patients, approximately 40%, respond to the drug and responders are easily identified over a 2-week test period. This is a great strength as it ensures that no non-responders are treated.

In conclusion there is a considerable consistency between the results in experimental and clinical studies. Experimental studies provide evidence for the mechanisms of 4-AP that can be translated into clinical studies providing evidence of the effect of 4-AP.

Furthermore, clinical studies provide evidence that SR-AP is more beneficial than 4-AP both in terms of clinical effect and side effects.

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Conflict of interest statement

HBJ has received travel grants and teaching honoraries from Biogen Idec, Novartis, Almirall and Merck Serono and serves as an advisory board member for Novartis and Almirall. UD has received research support, travel grants and teaching honoraries from Biogen Idec, Merck Serono and Sanofi Aventis. UD further serves as PI for the ongoing Biogen-sponsored ACTIMS study. MR has received travel grants and consultancy honoraries from Biogen Idec, Genzyme, TEVA and Novartis and serves as an advisory board member for Biogen Idec. ES has received unrestricted research grants and travel support from Biogen Idec, Merck Serono and Bayer Schering and travel grants from Novartis.

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