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Research Article

4-aminopyridine – the new old drug for the treatment of neurodegenerative diseases

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Abstract

In this review are described the preclinical and clinical pharmacological data as well as new therapeutic indications for the use of 4-aminopyridine. 4-aminopyridine is a potassium (K^+) channel blocker that has a long history and various application areas. It is a chemical agent developed in 1963 as a bird poison. The first approval for clinical application of 4-aminopyridine was in 70's in Bulgaria, since anesthetists in that country have confirmed its effect as reversal agent for nondepolarizing myorelaxants. The Bulgarian pharmaceutical company Sopharma commersialized 4-aminopyridine under the trade name Pymadin. Since then 4-aminopyridine was extensively studied and in 2010 is approved in the USA for the treatment of walking disabilities in patients with multiple sclerosis. In recent years, data from clinical trials indicated that K-channel blockade may prove to be an appropriate strategy to overcome disturbances in nerve impulses conduction associated with demyelination of the central nervous system.

Keywords

4-aminopyridine, multiple sclerosis, neurodegenerative diseases

Introduction

Multiple sclerosis (MS) is the most frequent cause of neurological deficit in young adults (Solari et al. 2002). It is a chronic inflammatory neurodegenerative disease of the central nervous system (CNS) characterized by demyelination which can cause axonal conduction block (Judge and Bever 2006). The symptomatology is heterogeneous and includes paraesthesia, palsy, optic neuritis, diplopia, vertigo and bladder disturbances (Compston and Coles 2008; Smith and McDonald 1999). There is no effective cure for MS, but there are available therapies approved for reduction of the symptoms and progression of the disease (https://www.mayoclinic.org/diseases-conditions/multiple-sclerosis/diagnosis-treatment/drc-20350274).

One of the strategies to overcome axonal conduction deficit is a potassium channel blockade. Apprvoved drug with this mechanism of action is 4-aminopyridine (4-AP, INN fampridine, USAN dalfampridine) for treatment of walking impairment in patient with MS. Besides this pharmacological action, 4-AP also enables an enhancement of neuro-neuronal or neuromuscular transmission

in normally myelinated neurons. These pharmacological properties have encouraged extensive investigation of its therapeutic potential for symptom menagment of disorders of neuromuscular transmission and in demyelinating diseases (Kim et al. 1980; Smith et al. 2000). Here we describe history of a preclinical and clinical pharmacological data of 4-aminopyridine.

History

Some of the pharmacological effects of aminopyridines have been known for many years, but only since Bulgarian pharmacologists and anesthetists, based on their experimental and clinical studies, recommended the use of 4-aminopyridine hydrochloride (Pymadin, Sopharma, Bulgaria) for facilitation of neuromuscular transmission. These clinical data made possible the application of 4-AP as an antidote of non-depolarizing neuromuscular blocking agents (Mitzov 1967; Paskov et al. 1986). Since then many studies have been carried out in order to clarify the pharmacological profile and to determine the areas of clinical administration of this compound.

As a result of his experimental work, Bulgarian pharmacologist Mitzov (1967) reported that

4-AP show hypertensive, breathing excitement and anti-curare effects. 4-AP has a direct excitatory effect on the respiratory and vasomotor centers. The hypertensive action is a result of its influence on vasomotor center and sympathetic ganglion. It has been found, that 4-AP cannot cause release of catecholamines from the adrenal medulla and does not possess anticholinesterase activity (Mitzov 1967).

4-aminopyridine has been introduced for the first time in the pharmaceutical industry in Bulgaria in the middle of 70s under the trade names Pymadin and Nivalin P – combination drug containing galantamine and 4-AP (Angelova 2013). In the USA, Ampyra (USAN dalfampridine, Acorda Therapeutics, Inc.) was approved by the U.S. Food and Drug Administration (FDA) in 2010 as a treatment for improvement of walking in adult patients with MS-related walking difficulties. This effect is demonstrated by an increase in walking speed. FDA approval is based on safety and efficacy data from 56 clinical trials involving more than 2,000 people, over 1000 of whom were diagnosed with MS.

In pharmaceutical markets outside the United States, fampridine (4-AP) is commercialized by Biogen Idec, who received the licenced rights for development of this drug from Acorda Therapeutics, Inc. In July 2011 Biogen Idec received conditional approval from the European Medicinal Agency for 4-AP under the trade name Fampyra (10 mg prolonged-release fampridine tablets) indicated for the improvement in walking in adult patients with MS who experience walking disabilities (Expanded Disability Status Scale 4–7) (http://ir.acorda.com/investors/investor-news/investor-news-details/2011/Acorda-Therapeutics-Statement-on-European-Union-Approval-of-FAMPYRA-/default.aspx).

Chemical properties

4-Aminopyridine is a member of a family of mono-amino and di-amino derivatives of pyridine. The IUPAC name of 4-aminopyridine is pyridine-4-amine; molecular mass 94.12 g/mol; molecular formula C5H4N–NH2. It is a white to off-white non-hydroscopic powder, practically soluble in water. No evidence of polymorphism has been found. 4-AP has no stereochemical centers. Its pKa is 9.17 (indicating that this compound is protonated free base) and its logP is 0.76 and the pH of its solution (50 mg/ml in water) is 11. 4-AP can be manufactured by heating pyridine with sodium amide in N,N-dimethylaniline at 180 °C (https://pubchem.ncbi.nlm.nih.gov/compound/4-aminopyridine; https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@rn+504-24-5:@odhsdb@/cgi-bin/sis/search2/f?./temp/~11hFvG:1@).

Pharmacodynamic

4-aminopyridine is a lipid-soluble compound which easily crosses the blood-brain barrier (Bever and Judge 2009; Blight and Henney 2009), while e.g. 3,4-diaminopyridine is water soluble and, therefore, unable to pass the intact blood-brain barrier (Judge and Bever 2006). Mechanism of action of 4-AP is connected to a selective blockade of potassium channels and also to influence different processes in CNS: increases neuronal excitability (Buckle and Haas 1982), enhances the release of neurotransmitters dopamine, noradrenaline, acetylcholine and glutamate (Damsma et al. 1988), restores pacemaking in the cerebellum (Strupp et al. 2008), relieves conduction block in demyelinated axons and potentiates synaptic and neuromuscular transmission (Goodman and Stone 2013). This wide range of effects are mediated through voltage-dependent potassium channels (K*v* channels) that are important for action potential generation, for regulation of neuronal excitability and critically involved in generation of synchronized network oscillations in cortex, basal ganglia and thalamus (Lesage 2003). Myelination of the neurons provide increased conduction speed of electrical signals over large distances and thereby improved cognitive function (Fields 2008). During action potential propagation in a normal myelinated axon, sodium (Na^+) channels that are find in high density at the nodes of Ranvier open transiently, causing the action potential to jump from one node of Ranvier to the next (saltatory conduction). The internodal part of the axon is covered by myelin and contains a higher density of potassium (K^+) than sodium channels (Waxman 1996). Myelin sheat protect K^+ channels from activation during the passage of an action potential. In demyelinated axons the internodal membrane and its ion channels become exposed to larger electrical transient during the action potential. Under these conditions, leakage of ions through the K^+ channels can lead to action potential conduction block. 4-aminopyridine at low concentration may prolong nerve action potentials by blocking these exposed channels and inhibiting repo-

larisation, subsequently improving axon potential conduction (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

K^+ channel blockade by 4-AP has also other important physiological consequences. Prolonged action current results in a larger than normal Ca2+ influx at presynaptic terminals (Paskov et al. 1986). This leads to increased transmitter release from end terminals and enhanced neuro-neuronal or neuromuscular transmission (Kim et al. 1980). The findings of Kim et al. (1980) showed that 4-AP produced dose-dependent increase 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 (Agoston et al. 1984). The effect on neurotransmission may also underlie some of the observed clinical side effects of 4-AP including epileptiform seizure activity (Hayes 2004).

The K^+ channel blockade by 4-AP has potent immunomodulatory properties that may have relevance to autoimmune disease-mediated axonal demyelination. 4-AP blocks K^+ channels in T lymphocytes and modifies their proliferative and effector cell functions (Judge et al. 1997). In the rat autoimmune encephalomyelitis model of MS, blockade of K^+ channels has been shown to delay the hypersensitivity response to myelin basic protein and improve the symptoms of the disease (Devaux et al. 2004).

In other areas of the CNS, such as the cortex, 4-AP enhances action potential generation and regulates repetitive firing of cortical pyramidal cells stimulating the release of glutamate and acetylcholine (Golding et al. 1999). In addition, 4-AP induces robust oscillation in pyramidal neurons and at high concentrations has the potential to induce seizures. The epileptic potential of 4-AP has been connected in part to action at the level of interneurons possibly by inhibiting GABA receptors (Traub et al. 1995).

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 (Kirchoff et al. 1992). Either one of these properties may underlie the paresthesias or other sensory changes evident in clinical trials in patients with MS or spinal cord injury (SCI) (Hayes 2004).

Preclinical pharmacology and toxicology

Toxicology

Single dose toxicity

Results of the safety pharmacological studies indicated that fampridine (4-AP) had an acceptable pharmacological safety profile in animals. The toxicological data for fampridine (4-AP) was obtained from oral single-dose toxicity studies in rats, rabbits and dogs and repeat-dose toxicity studies up to three months in mice, six months in rats and 12 months in dogs.

In a single dose toxicity study, approximate median lethal oral doses (LD50) ranged between 14 mg/kg for male rats and 22 mg/kg for female rats. The LD50 value raised to 40 mg/kg (both sexes) when the once daily drug administration was instead separated into 4 sub-doses given every 6 hours. In rabbits, the median lethal dose was 23 mg/kg for both sexes. In dogs, no toxicities were obvious at total daily doses of up to 5 mg/kg four times a day. In general, death occurred short after administration (on the day of dosing). It was concluded that findings of the single dose toxicity studies are probably related to the short half-life of fampridine (4-AP) and suggested that toxicity is related to peak plasma levels rather than overall exposure.

In the repeated dose studies in rats and dogs, the results revealed CNS associated disturbances including tremor, convulsions, trembling, ataxia, decreased activity, weakness, hypersalivation, dilated pupils, increased respiratory rate and laboured breathing. Gait abnormalities and hyper-excitability were also observed. These signs were associated with the pharmacology of fampridine (4-AP); they were rapid in onset and seemed to attenuate during continued dosing as well as to reverse in surviving animals after discontinuation of treatment. The urogenital tract was identified as a target for fampridine (4-AP) toxicity in rats leading to cause of death in some cases.

Fampridine (4-AP) was tested for genotoxic potential in standard *in vitro* (AMES test, mouse lymphoma assay) and cytogenetic *in vivo* tests in mice and rats. All tests were performed in compliance with GLP and there was no evidence for genotoxic potential. The cancerogenic potential of fampridine (4-AP) was tested in two long-term studies over two years in mice and rats. There were no significant differences in clinical parameters between control and treatment groups in either of the studies. Differences between treatment and control groups were only observed in female rats manifested in a slight increase in benign uterine polyps. There was no other evidence for a treatment-related neoplastic changes in mice or in rats (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Published data from *in vitro* study with porcine granulosa cells (PGC-2 cell line) revealed that 4-AP (2mM) has the potentail to inhibit basal and follicle stimulating hormone-stimulated progesterone production by granulosa cells. Exposure to 4-AP is also associated with decreased synthesis of estradiol by PGC-2 cell line. These changes in steroide hormone profiles could contribute to dysmenorrhoea and infertility (Grinsted et al. 1989; Reame 1992). In the embryo-foetal development studies on rats and rabbits, no eveidence for embryotoxicity or developmental anomalies has been found. In a study in which rats were dosed orally from gestation day 7 to day 21 of lactation, decreased weight gain (at dose 9 mg/kg/day) in postnatal has been observed. In the rabbit study a possible small treatment-related increase in resorption, pre/post implantation loss was seen, which could indicate that success of pregnancy outcome is reduced (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Preclinical pharmacology

The pharmacokinetic profile of fampridine (4-AP) was evaluated *in vitro* as well as *in vivo* (in rats and dogs). The toxicokinetic parameters have been determined in mice, rats, rabbits and dogs. The primary routes of administration in rat and dog were intravenous and oral. Overall, the ADME (absorption, distribution, metabolism, excretion) properties of fampridine were similar across species examined including humans.

The effects of fampridine (4-AP) on CNS was evaluated with an *in vivo* study analysing EEG changes in Sprague-Dawley rats. The safety pharmacological effects of fampridine were investigated at *i.v.* doses of 0.5, 1, 2 and 4 mg/kg in comparison to saline. Blood samples were collected at different timepoints and EEG activity was recorded at baseline and post-injection every 13 minutes for 3 hours. Administration of fampridine significantly changed EEG activity with a threshold concentration of 109–135 ng/mL, which correspondence with an increased risk of seizures reported in humans at fampridine plasma concentrations greater than 100 ng/mL (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

The effects of fampridine (4-AP) on the cardiovascular system, in particular the arrhythmic risk, were evaluated in a battery of *in vivo* and *in vitro* studies. The *in vitro* assays were conducted on HERG-expressing HEK293 cells (Renganathan et al. 2009) and dog Purkinje fibres (Thomas et al. 2010). The *in vivo* study was performed in Beagle dogs (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section). Both *in vitro* studies were representative for a potential of fampridine to extend the QT interval, although only at concentrations exceeding levels clinically relevant (at least 2000 times higher). No changes on the ECG were evident in dogs *in vivo* when fampridine was analysed at concentrations more than 50 times higher than the maximal determined in healthy subjects. Results based on the cardiovascular safety pharmacology studies, considered occurrence of QT prolongation after fampridine treatment rather not to appear. This lack of significant arrhythmic risk at therapeutic levels correspond with absence of arrhythmic effects of fampridine (4-AP) in toxicity studies in dogs.

Clinical data

Effects on neuromuscular transmission

4-aminopyridine was introduced in Bulgaria in early 70's as a reversal agent for nondepolarising neuro-muscular blocking drugs by the team of the Bulgarian pharmacologist Dimitar Paskov. In their early clinical studies 4-AP (20 mg *i.v.*) was shown to reverse partial neuromuscular blockade induced by tubocurarine (Paskov et al. 1986). After extensive clinical investigations 4-aminopyridine received official approval in Bulgaria and has become a standard reversal agent in clinical anaesthesia in that country.

The first clinical studies with 4-aminopyridine in Western Europe were started 10 years later, in 1980. These initial studies by Agoston confirmed the findings, reported by the team of Bulgarian investigators Paskov and Stoyanov (Paskov et al. 1986). Experimental studies have shown that aminopyridines are effective antagonists of the block produced by (+)- tubocurarine, gallamine and pancuronium. It was found that 4-aminopyridine does not antagonise neuromuscular block produced by the depolarising muscle relaxants, decamethonium or suxamethonium (Paskov et al. 1986).

In addition, the antagonistic effect of 4-aminopyridine as would be expected is greatly potentiated by small doses of anticholinesterase drugs. The Bulgarian anaesthetists often add 4-aminopyridine to the anticholinesterase drug galantamine (Paskov et al. 1986) or applied combination drug Nivalin P (Sopharma, Bulgaria) (Angelova 2013). Miller et al. (1979) have made a through study of the interactions between 4-aminopyridine and neostigmine or pyridostigmine.

It is found that 4-aminopyridine is devoid of anticholinesterase activity in any dose that might be administered *in vivo*. A wide range of studies including mechanical recording, electrophysiological analysis, collection and assay of acetylcholine, and electron microscope studies have demonstrated that the facilitatory action of 4-aminopyridine, and derivatives of it, on neuromuscular transmission is the result of a prejunctional effect on the nerve endings through which the evoked release of acetylcholine is increased (Paskov et al. 1986).

4-AP in the treatment of multiple sclerosis

Despite many investigations and theories, the exact etiology and pathogenesis of MS still remains not fully elucidated. One of the most widely accepted hypothesis is that MS is an inflammatory disease controlled by T cell-mediated autoimmune reaction against the myelin sheet which predominantly affects the white matter. It is also found that exposure of paranodal K^+ channels contributes to axonal dysfunction (Waxman 1982). MS produce electrophysiological indicators of central conduction deficits, e.g. long latency visual evoked potentials (Davis et al. 1990) or motor evoked potentials (Fujihara and Miyoshi 1998) and tends to be responsive to environmental factors or interventions, such as cooling, known to modify ion channel kinetics (Davis 1970).

In myelinated axons, the blockade of fast, voltage-gated K^+ channels has little effect on the action potential and nodal conduction (Bostock et al. 1981). Demyelination alters the structural and functional relationships of voltage-gated ion channels along the axonal membrane and also lead to exposure of K^+ channels which impairs the action potential generation and conduction. These conduction deficits contribute to the neurological deficits experienced by SCI and MS patients (Waxman 1998). 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.

K-channel blockade by 4-AP has other important physiological consequences like increased neurotransmitter release from presynaptic terminals which resulted in increased transmitter release from end terminals and enhanced neuro-neuronal or neuromuscular transmission (Kim et al. 1980). K-channel blockade has been investigated as a novel targeted immunomodulatory approach to the management of neuroinflammatory demyelinating diseases (Wulff et al. 2003).

Many of the early clinical applications of 4-AP used intravenous administration or oral gelatin capsules containing the powder form of 4-AP triturated with lactose or microcrystalline cellulose. All these immediate release (IR) formulations are characterized with short time-to-peak serum concentration and biological half-life. More recently, a prolonged release (PR) matrix tablet form of 4-AP has been developed (Fampyra PR tablets), and is approved for improvement in walking in patients with MS. The PR formulation, with its longer half-life and lower peak serum levels (Hayes et al. 2003), 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.

Clinical trials

The approval of fampridine (4-AP) was based on the results of three randomized, double blind, placebo-controlled, parallel-group clinical trials (MS-F202, MS-F203, MS-F204). The study MS-F202 was a dose comparison study (10, 15, 20 mg twice daily) and there were also two pivotal studies MS-F203 and MS-F204 (10 mg twice) for evaluation of fampridine efficacy and safety. In all studies the concentration of fampridine was measured at each study visit in order to evaluate a plasma level-response relationship. The efficacy of the drug was based on the Timed 25 Foot Walk test (T25FW). In this test a patient was requested to walk as quickly as he/she can safely, from one end to the other end of a clearly marked, unobstructed, 25-foot course. The time in seconds was recorded. After a rest period of maximum 5 minutes the test was repeated again. The walking speed for a particular study visit was the average of the walking speeds of the 2 trials performed. If one of the two trials could not be fulfilled then the walking speed for that visit was to be the walking speed from the completed trial (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Pharmacokinetic properties in humans

In a study from 2004 Hayes reviewed pharmacokinetic, experimental and clinical studies on 4-AP and one randomized, controlled trial on prolonged-release (PR) 4-AP, and concluded that PR 4-AP most likely yields fewer side effects and more robust clinical gains than 4-AP. In 2007 the second trial on PR 4-AP was published (Goodman et al. 2008) and the overall conclusion of subsequent reviews (Bever and Judge 2009; Espejo and Montalban 2012) is that PR 4-AP has a clinically meaningful beneficial effect on walking speed and muscle strength of the lower extremities. These latest reviews further conclude that PR 4-AP is generally well tolerated, most adverse events being mild to moderate.

Absorption

Pharmacokinetic data indicated that after oral administration fampridine is rapidly and completely absorbed from the gastrointestinal tract. There was no data for absolute bioavailability of fampridine prolonged-release tablets, but relative bioavailability (as compared to an aqueous oral solution) is 95%. The prolonged-release formulation of fampridine tablet has a delay in the absorption of the drug manifested by slower rise to a lower peak concentration without any effect on the extent of absorption.

There is a small reduction, approximately 2–7% (10 mg dose), in the area under the plasma concentration-time curve (AUC0-∞) of fampridine when taken with food. This small reduction in AUC is not expected to cause a reduction in the therapeutic efficacy. However, Cmax increases by 15–23%. Because there is a clear relationship between Cmax and dose related adverse reactions, it is recommended to take fampridine PR tablets without food (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#product-information-section).

Distribution

Fampridine (4-AP) is a lipid-soluble drug which readily crosses the blood-brain barrier. Fampridine is characterized by low binding to plasma proteins (bound fraction varied between 3–7% in human plasma). Volume of distribution of fampridine is approximately 2.6 L/kg. Fampridine is not a substrate for P-glycoprotein (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#product-information-section).

Biotransformation

Based on the results from the metabolite identification study, it can be concluded that limited metabolism of 4-AP occurs by liver enzymes in two-step process. The first step include hydroxylation of 4-AP to 3-hydroxy-4-AP, followed by conjugation of the 3-hydroxy-4-AP to 3-hydroxy-4- AP sulfate, with slower excretion of the sulfate conjugate relative to 4-AP and 3-hydroxy-4-AP (Caggiano and Blight 2013). No pharmacological activity was found for the fampridine metabolites against selected potassium channels *in vitro* (Caggiano et al. 2013). The 3-hydroxylation of fampridine (4-AP) to 3-hydroxy-4-aminopyridine by human liver microsomes most likely is catalyzed by Cytochrome P450 2E1 (CYP2E1). Study results confirm a direct inhibition of CYP2E1 by fampridine at 30 μM (approximately 12% inhibition) which is approximately 100 times the average plasma fampridine concentration measured for the 10 mg tablet. It is found that treatment of cultured human hepatocytes with fampridine had little or no effect on induction of CYP1A2, CYP2B6, CYP2C9,

CYP2C19, CYP2E1 or CYP3A4/5 enzyme activities (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Elimination

Fampyra is characterized by linear (dose-proportional) pharmacokinetics with a terminal elimination half-life of about 6 hours. The maximum plasma concentration (Cmax) and, to a smaller extent, area under the plasma concentration-time curve (AUC) increases in dose dependent manner. The major route of elimination for fampridine (4-AP) is renal excretion, with approximately 90% of the administered dose excreted inchanged in urine within 24 hours (Caggiano and Blight 2013). Renal clearance (CLR 370 ml/min) is substantially greater than glomerular filtration rate due to combined glomerular filtration and active excretion by the renal OCT2 transporter. Fampridine (4-AP) is eliminated mainly by the kidneys with active renal secretion accounting for about 60%. Organic Cation Transporter 2 (OCT2) is the transporter responsible for the active secretion of fampridine. Faecal excretion accounts for less than 1% of the administered dose.

There is no evidence of clinically relevant accumulation of fampridine (4-AP) taken at the recommended dose in patients with normal renal function. In patients with renal impairment accumulation occurs respective to the degree of impairment (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Pharmacokinetic data for special populations

Renal impairment: In patients with renal impairment (creatinine clearance [CrCl], ≤80 mL/min), mean peak plasma concentrations were 68%-101% higher and apparent clearance was 43%-73% lower relative to patients without impairment, excluding use of fampridine (4- AP) in patients with moderate (CrCl, 30–50 ml/min) or severe renal impairment (CrCl, <30 ml/min). These data suggest that excessive accumulation may occur with repeated dosing, which could preclude use of fampridine-PR in patients with severe renal impairment. Because fampridine has a narrow therapeutic range, it will also be advisable to monitor subjects with mild renal impairment for potential adverse effects (Cornblath et al. 2012).

Interaction with other medicinal products and other forms of interaction

Interaction studies have only been performed in adults. Simultaneous treatment with other medicinal products containing fampridine (4-aminopyridine, dalfampridine) is contraindicated.

Fampridine (4-AP) is eliminated mainly by the kidneys with active renal secretion accounting for about 60%. Organic Cation Transporter 2 (OCT2) is the transporter responsible for the active secretion of fampridine (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

The potential for drug interactions with dalfampridine (4-AP) was examined in clinical studies for 2 drugs that are prescribed to the patients with MS: baclofen and interferon beta-1b. Pharmacokinetic parameters, including AUC, Cmax, and Tmax for dalfampridine and baclofen, were unchanged by coadministration. The pharmacokinetic parameters AUC, Cmax, and Tmax of single and multiple doses of dalfampridine (IR formulation of 7.5 mg) were not affected by coadministration of interferon beta-1b (8 million U per dose subcutaneously) in 9 adult patients with MS (3 men and 6 women). However, the potential for effect of dalfampridine on the pharmacokinetic parameters of interferon beta-1b was not evaluated (Cornblath et al. 2012).

Since tolterodine is known to block K^+ channels and increase the duration of action potential, the concomitant use of tolterodine with fampridine in one patient is associated with increased seizure risk (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

In 2001, Sopharma AD registered a 4-AP in Bulgaria by national authorization procedure (Annex to a marketing authorization № 3806/20.06.01) under the brand name Pymadin as s solution form for injection.

This medicinal product was approved for the following indications: in the complex therapy of Eaton-Lambert syndrome, MS, myasthenia gravis, Alzheimer's disease. For reversal of neuro- muscular blockade in patients with botulism, for treatment of verapamil intoxication. It can be used also for elimination of residual muscle paralysis as antagonist of nondepolarizing neuro- muscular blockers; in physiotherapy for ionophoresis in neuralgia and neuritis of traumatic, toxic and infectious nature (http://lekarstva-bg.eu/index.php?option=com_k2&view=item&id=4127%3A&tmpl=component&print=1&Itemid=2).

Reported side effects include: from CNS – headache, tremor, insomnia, increased excitability or rapid fatigue, increased muscle excitability to seizures. From cardiovascular system - increased arterial blood pressure, especially in combination with neostigmine.

Gastrointestinal system - nausea, vomiting and dry mouth. Described adverse reactions from skin are diaphoresis, rarely allergic reactions. According frequency, adverse reactions can be divided as: very common (≥ 1/10) – urinary tract infection, common (≥ 1/100 to < 1/10) – insomnia, anxiety, dizziness, headache, tremor and uncommon (≥1/1,000 to <1/100) – seizure, tachycardia, urticaria (https://www.ema.europa.eu/en/medicines/human/EPAR/fampyra#assessment-history-section).

Currently there are a lot of clinical trials with different status that investigated the application of 4-AP in varied diseases and conditions. Some of these trials are presented in Table 1 (https://clinicaltrials.gov/ct2/results?cond=&term=4-aminopyridine&cntry=&state=&city=&dist=).

Table 1. Clinical trials, evaluating effect of 4-AP in different disorders and diseases (https://clinicaltrials.gov/ct2/results?cond=&term=4-aminopyridine&cntry=&state=&city=&dist=).

Status	Study title	Condition	Interventions
Not yet recruiting 2019-2021	4-AP Treatment for Nerve Injury	Prostate cancer	Drug: 4-AP Other: Placebo
Completed 2002-2005	Assessment of Chronic Guillain-Barre Syndrome Improvement With Use of 4- AP	Guillain-Barre Syndrome	Drug: 4-AP
Completed 2012-2015	Short and Long Term Treatment With 4- AP in Ambulatory SMA Patients	Spinal Muscular Atrophy	Drug: 4-AP Other: Placebo
Completed 2016	Effect of Dalfampridine (4-AP) on Genioglossus Muscle Activity in Healthy Adults	Sleep Apnea, Obstructive	Drug: Placebo Drug: Dalfampridine
Completed 2012-2014	A Randomized Trial To Evaluate Ampyra for Gait Impairment in Parkinson's disease	Parkinson's disease	Drug: Ampyra first, then Placebo Drug: Placebo first, then Ampyra
Completed 2011-2013	Ampyra for Optic Neuritis in MS	Multiple Sclerosis, Optic Neuritis	Drug: Dalfampridine /Placebo Drug: Placebo/ Dalfampridine
Terminated 2015-2016	Fampridine Pregnancy Exposure Registry	Multiple Sclerosis, Pregnancy	Drug: Fampridine
Completed 2012-2017	Combination Therapy With Dalfampridine and Locomotor Training for Chronic, Motor Incomplete Spinal Cord Injury	Spinal Cord Injury	Drug: Dalfampridine Drug: Placebo

Conclusion

4-AP has a long history of approximately 50 years therapeutic application and clinical studies provide strong evidence for that PR form of 4-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 PR 4-AP has a more beneficial side effect profile than immediate-release formulations. The PR formulation, with its longer half-life and lower peak serum levels, 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. There are also many uncompleted clinical trials with 4-AP (e.g., Fampridine in MS Patients: A Cognition, Fatigue, Depression and Quality of Life Analysis) which may extend the thepapeutical indications of this drug. For the present no new therapeutic indications of 4-aminopyridine have been approved by regulatory agencies in USA and Europe.

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