REVIEW

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KNS-760704 [(6R)-4,5,6,7-tetrahydro-N6-propyl-2, 6-benzothiazole-diamine dihydrochloride monohydrate] for the Treatment of Amyotrophic Lateral Sclerosis

Valentin K. Gribkoff and Michael E. Bozik

Knopp Neurosciences, Inc., Pittsburgh, PA, USA

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Correspondence
Valentin K. Gribkoff, Ph.D., Knopp Neurosciences, Inc., 2100 Wharton Street, Suite 615, Pittsburgh, PA 15203, USA.
Tel.: +1 412 735 0231;
Fax: +1 412 488 8487; E-mail: valentin.gribkoff@knoppneurosciences.com

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Developing effective treatments for chronic neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) has proven extremely difficult. ALS is universally fatal, characterized by progressive weakness due to the degeneration of upper and lower motor neurons, and leads eventually to respiratory failure which is the usual cause of death. Only a single treatment has been approved, the modestly effective nonspecific neuroprotectant Rilutek® (riluzole; 2-amino-6-(trifluoromethoxy)benzothiazole). KNS-760704 [(6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine dihydrochloride, RPPX], a synthetic amino-benzothiazole with demonstrated activity in maintaining mitochondrial function, is being developed as a treatment for ALS. It has proven to be effective in multiple *in vitro* and *in vivo* assays of neuroprotection, including the G93A-SOD1 mutant mouse model; however, its specific mechanism of action remains unknown. The potential of KNS-760604 as a treatment for ALS was first suggested by studies showing that its optical enantiomer, Mirapex® [(6S)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine; pramipexole dihydrochloride; PPX], a high-affinity agonist at dopamine D2, D3, and D4 receptors, exhibits important neuroprotective properties independent of its dopamine receptor agonism. In cell-based assays, both RPPX and PPX reduce the production of reactive oxygen species (ROS), attenuate the activation of apoptotic pathways, and increase cell survival in response to a variety of neurotoxins. However, PPX has limited utility as a clinical neuroprotective agent because the drug concentrations required for neuroprotection would likely produce unacceptable dopaminergic side effects. RPPX, on the other hand, while possessing the same neuroprotective potential as PPX, is a much lower-affinity dopamine receptor agonist and may therefore be more useful in the treatment of ALS. This review will examine the data supporting the hypothesis that the RPPX may have therapeutic potential for the treatment of neurodegenerative disorders including ALS. In addition, we will briefly review recent preclinical data in support of RPPX, and discuss the current status of its clinical development.

Introduction

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease, Charcot's sclerosis) is a rapidly progressing neurodegenerative disorder characterized by the specific, targeted death of motor neurons, resulting in eventual paralysis and death. As the disease progresses, the patient loses upper and lower motor neuron function, accompanied by spasticity, muscle degeneration, and paralysis, including the loss of respiratory function that is the usual cause of death [1–3]. The disease does spare some motor neurons, preserving function to some degree in those muscles

controlling eye movement and bladder control, although there is some debate about whether selective motor neuron sparing occurs [4]. Clinically significant dementia is uncommon, but there is increasing recognition that mild cognitive impairment frequently accompanies the disease and the recently identified TDP-43 proteinopathy suggests a potential pathogenetic link between ALS and frontotemporal lobar degeneration [5–7]. While rare, ALS is one of the most common of the chronic neurodegenerative disorders. There are approximately 30,000 patients in the United States, with about 5000 new patients diagnosed each year. It affects adults, predominantly in the 5th to 7th decades of life, with a small preponderance in males, and the median survival time is 3–5 years from symptom onset and approximately 1.5 years from definitive diagnosis [3,8–10].

The causes of ALS are largely unknown, with ~90% of patients having no known hereditary disease etiology (sporadic ALS, sALS). The remaining patients demonstrate evidence of genetic linkage (familial ALS, fALS); currently four major chromosomal loci, termed ALS1, ALS3, ALS6, and ALS7, have been associated with typical disease presentations [1,11–14]. Additional loci have been associated with atypical forms of ALS, including slow-progressing juvenile forms and ALS with concurrent dementia. ALS1 is the most frequently occurring of the known loci, accounting for about 20% of fALS patients, and reflects mutations in the gene responsible for the enzyme copper/zinc (Cu/Zn) superoxide dismutase-1 (SOD1) [15,16]. The other major loci associated with typical ALS code for genes with unknown function, and currently provide little in the way of insights for drug development. Recently, several studies have implicated TAR DNA binding protein 43 (TDP-43) in both frontotemporal lobar degeneration with ubiquitinated inclusions and ALS, including sALS [6,7,17–20]. In one of these studies it was observed that the TDP-43 inclusions were found in sALS and in some cases of SOD1-negative fALS, but were absent in ALS1 [17]. These results, while still preliminary (e.g., there is not yet an animal model demonstrating that mutated TDP-43 results in ALS-like disease), may suggest a promising future disease target.

SOD1, on the other hand, has been definitively linked to one form of fALS. SOD1 is a ubiquitous enzyme, usually found in the cytosol, that catalyzes the conversion (dismutation) of superoxide (O_2^-) to water and hydrogen peroxide and is therefore a major antioxidant mechanism [21]. However, the mutation of this gene in ALS1 apparently contributes to the disease in a complex way not predicated on the loss of SOD function, but rather primarily from emergent toxicity, possibly associated with protein aggregates of the mutant enzyme [22–30]. These discoveries have yielded transgenic mouse models of ALS1-linked fALS [31–33] that are currently employed in the search for new therapeutic agents in ALS; however, their utility may be limited because the mutations in this gene are responsible for only ~2% of ALS patients. Should a potential drug be specific for some aspect of mutant SOD1 toxicity, as opposed to a final common pathway of motor neuron death common to all forms of ALS, including sALS, its utility would (by definition) be limited to that small percentage of the patient population.

Despite these limitations, the identification and characterization of the ALS1 SOD1 mutations responsible for some cases of ALS holds promise for future drug development in the disease, and may provide some insights into more general mechanisms of ALS disease etiology. In general, however, the current picture with regard to disease mechanisms remains elusive and speculative [1,34–36]. Putative causal mechanisms must account for the pathognomonic features of ALS, including its targeting of motor neurons, its characteristic progression, and its largely sporadic etiology. One current focus in the search for common mechanisms of motor neuron death across ALS (and other forms of neurodegeneration) is the mitochondrion, the organelle responsible for supplying cellular energy [37–42] and whose dysfunction may contribute significantly to the pathology underlying the major chronic neurodegenerative disorders.

Mitochondrial Dysfunction and Neurodegeneration

Mitochondria are membrane-bound organelles present in eukaryotic cells, and are the major source of cellular energy. They consist of an outer mitochondrial membrane (OMM), an inner mitochondrial membrane (IMM), an intermembrane space, and a mitochondrial matrix bounded by the extensively folded IMM, which forms the characteristic mitochondrial "cristae" easily observed in electron micrographs [43–45]. As the site of oxidative phosphorylation, they funnel electrons harvested from catabolism and transferred to molecular acceptors (nicotinamide nucleotides, NADH, or flavin nucleotides, $FADH_2$) to oxygen via an electron transport chain, eventually resulting in the reduction of molecular oxygen to water. The electron transport chain is located on the IMM and consists of four complexes (complex I–IV) responsible for the redox reactions accomplishing various steps in electron transfer [42–44,46–49]. The final component, complex IV, is a cytochrome c oxidase, which transfers electrons from cytochrome c to oxygen, and simultaneously transfers protons across the IMM. The transport of protons from the matrix to the intermembrane space creates a potential (electrical) difference and a pH gradient across the IMM. This pH and electrical gradient

creates an electronegative environment in the matrix (proton motive force). The IMM is highly impermeable to protons, and they are transferred into the matrix by the action of ATP synthase, thereby coupling the production of cellular energy (ATP) to cellular catabolism. To accomplish these functions, mitochondria possess a small, maternally-inherited genome that encodes 13 protein components of the respiratory chain, and additional tRNAs and rRNAs involved in intramitochondrial protein synthesis [50,51]. The protein constituents of mitochondria are supplemented significantly by the specific, targeted transfer of proteins from elsewhere in the cell [52].

Due to their critical function in cell energy production, mitochondria have been implicated in a large number of diseases, including neurodegenerative disorders [37–39,53–56]. Of particular interest are the pathological production of highly reactive and toxic oxygen species, and the induction of apoptotic processes [47]. At several points in the electron transport chain, small amounts of superoxide and peroxide are produced, which can result in the production of the reactive oxygen species (ROS) hydrogen peroxide and hydroxyl radical [57,58]. While highly damaging if left unchecked, ROS are normally eliminated by SOD and other endogenous antioxidants. Breakdown of normal mechanisms of ROS removal have been hypothesized to result in a number of diseases, including acute and chronic neurodegenerative disorders [39,40,59,60].

This brief overview of mitochondrial function suggests several potential pathological mechanisms of disease. Any disruption of electron transport or its sequelae, such as reduced ATP production, will result in cellular compromise, particularly during oxidative stress. An overproduction of ROS, or a breakdown in mechanisms for their scavenging, can result in damage to cellular components. Another major potential pathway of mitochondrial disease is the activation of cell death pathways (apoptosis) via a breakdown in the permeability of the mitochondrial membrane and the release of normally impermeable or tightly regulated intramitochondrial components. This latter mechanism is generally referred to as mitochondrial permeability transition (mPT). This process is voltage-dependent, can be elicited by oxidative stress and calcium (Ca^{2+}) entry into the matrix, and appears to depend on the activation of an incompletely characterized protein complex involving principally the IMM, but eventually affecting the OMM as well [61,62]. This protein complex, known as the mPT pore, permits indiscriminant proton entry into the matrix, ultimately resulting in swelling of the IMM, disruption of the OMM, and a relatively nonspecific increase in permeability that prominently includes diffusion of cytochrome c into the cytosol [61,63,64]. Cytosolic cytochrome c activates the caspase system leading to apoptotic cell death [42,47,48,59,64,65]. Opening of this extremely high-conductance mPT "pore" can be recorded using patch clamp techniques [66–68], and can be inhibited by pharmacological agents such as cyclosporin A and related compounds [67,69]. It is currently unclear if mPT or the mPT pore has a normal, nonpathological function, but it has become an important target in the search for treatments of mitochondrial dysfunction, including the characterization of the mitochondrial effects of the (6S)- and (6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazolediamine enantiomers, Mirapex® and KNS-760704 (see below).

Neurons are highly metabolically demanding cells, and the number, structure, and localization of mitochondria in neurons reflect these high energy requirements. In addition to "normal" cellular processes, neurons and other electrically excitable cells utilize the tightly regulated and electronegative transmembrane potential to set the stage for characteristic events (such as action potentials and neurotransmitter mobilization, release, and reception) that allow for intercellular communication. While the opening and closing of voltage-gated ion channels underlying phenomena such as the action potential do not require energy *per se*, the restoration and maintenance of the ionic balances that contribute to the neuronal membrane potential and the high levels of synthesis of neurotransmitters represent major and to some degree unique neuronal energy requirements. The energy requirements of neurons are therefore high, but also quite variable, and depend both on cell type and functional requirements at any given moment. These energy requirements underlie the impressive sensitivity of neurons to acute hypoxia [70], and make them vulnerable to trauma and the acute degenerative processes following strokes, underscoring the role of mitochondria in acute neurodegeneration [71,72]. Mitochondria were once considered highly efficient organelles unlikely to be involved in the etiology of common chronic diseases. Increasingly, however, it has been recognized that these organelles are complicated, fragile, and susceptible to many known and unknown functional disruptions. This has been particularly true in the study of neurodegenerative disorders. In addition to their response to acute hypoxia, mitochondrial dysfunction has now been suggested to underlie or contribute to essentially all of the major neurodegenerative disorders, including chronic illnesses such as Alzheimer's disease, Parkinson's disease (PD), ALS, and Huntington's disease [37,39,40,54,55].

In the specific case of ALS, several lines of evidence suggest a common mitochondrial mechanism, although as yet no single defect is proven to initiate the disease, except in the case of SOD1 fALS. Even in this case, as

discussed above, it is apparently not the loss of SOD1 function that leads to the disease, but rather a gain in toxic function. Recent evidence has shown that the mutant dismutase-negative SOD1 has a lower repulsive charge, and can form insoluble disulfide-bond linked aggregates that correlate with the onset of a disease-like phenotype in transgenic mice [73–76]. These aggregated proteins are located at least in part in the mitochondria, while the usual localization of SOD1 is predominately cytosolic. The aggregated protein can apparently recruit wild-type SOD1 into the aggregates in double-transgenic (mutant SOD-1 and wt- SOD-1) mice, associating with the IMM and causing damage to mitochondrial cristae [76]. Protein aggregation in mitochondria can lead to swelling and other mitochondrial malformations, conceivably leading to opening of the mPT pore and cell death, although mutant SOD1 may also have other toxic effects, such as peroxynitrite production [77]. Some of these findings parallel some of the earliest observations concerning disease mechanisms in ALS, which arose from ultrastructural studies of mitochondria in muscle and spinal neurons in ALS patients. For example, neuronal mitochondria in these patients showed swelling and vacuolization consistent with mitochondrial injury and similar to the mitochondrial damage observed in transgenic SOD1 mutant mouse motorneurons [78,79]. While there is evidence that certain SOD1 mutants may damage mitochondria, causing or contributing to ALS symptoms, the relationship between mitochondria and sporadic ALS (sALS) is less extensive. There have been many reports of abnormalities in mitochondria from sALS patients, including alterations in the processing and or levels of mitochondrial electron transport chain constituents [80,81]. Other abnormalities observed in sporadic ALS patients have included mitochondrial DNA deletions and reduced ATP levels [82]. Data implicating mitochondrial mechanisms in ALS-induced neuronal degeneration suggest a number of possible targets for the discovery of new and effective treatments for ALS.

Benzothiazoles and Neuroprotection

Benzothiazoles are structurally uncomplicated molecules with a broad range of biologic activity. Simple synthetic substitutions to the benzothiazole nucleus (Fig. 1A)have yielded compounds possessing diverse therapeutic potential as anti-infective, anti-inflammatory, and antitumor agents [83–85]. In the central nervous system, benzothiazoles have demonstrated neuroprotective properties in both acute and chronic models of neurodegeneration [86,87]. They have also shown utility as *in vivo* labels of brain amyloid in patients with Alzheimer's disease and have been shown to prevent protein

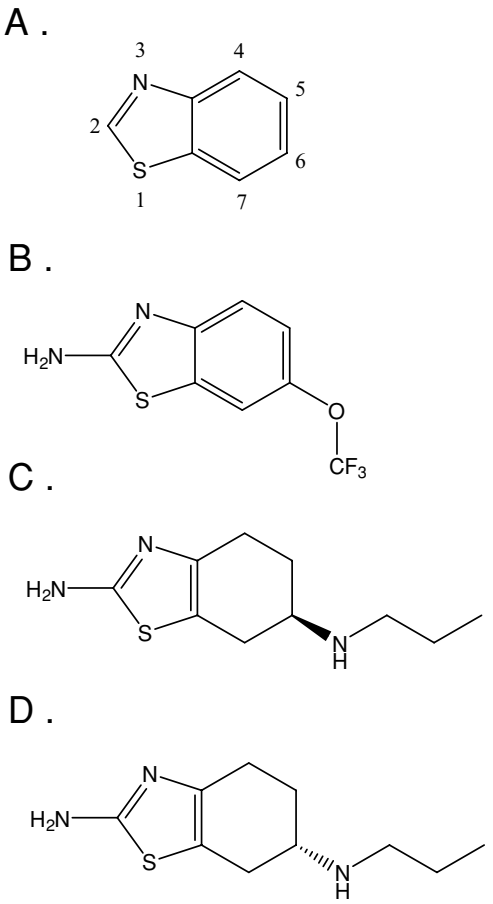

Figure 1 Structures of the benzothiazole core (A), Rilutek® (B), RPPX (C), and Mirapex® (D).

aggregation of huntingtin in models of Huntington's disease [88,89]. Currently, the only medicine approved for use in ALS is a benzothiazole, Rilutek® (riluzole; 2-amino-6-trifluoromethoxy-benzothiazole; Fig 1B.). This compound is a low-potency and nonspecific modulator of many pharmaceutical targets, including excitatory amino acid receptors, ion channels (sodium, calcium, and potassium), and protein aggregates [90–99], and its mechanism-of-action clearly remains speculative. Nevertheless, this compound is the only clinically-effective drug developed to date for the treatment of ALS, producing a small, but significant effect increase in survival without a significant improvement in measures of motor function. A recent review of clinical trials with Rilutek® demonstrated that the increase in mean survival time for ALS patients varied from 3 months for a pooled patient sample from trials where patients received 100 mg of the drug, to 1.7 months for a pooled sample of patients receiving various dose levels [100]. Considering the severity of the disease and the modest efficacy of Rilutek®, it

is clear that ALS remains a pressing unmet medical need. The remainder of this review will focus on a drug currently entering clinical trials for the treatment of ALS, KNS-760704.

KNS-760704 and Mirapex®: Evidence for Neuroprotection Independent of Dopamine Receptor Interaction

KNS-760704 (Fig. 1C) is an amino-benzothiazole that, like Rilutek®, has been demonstrated to be neuroprotective in multiple *in vitro* and *in vivo* assays but whose specific mechanism of action remains unknown. Its potential as a treatment for ALS was first suggested by studies of its optical enantiomer, Mirapex® (pramipexole dihydrochloride; PPX; (6S)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazole-diamine; Fig. 1D) in a wide range of neuroprotective assays. PPX is a nonergot dopamine receptor agonist discovered in the 1980s as a synthetic analogue of the dopamine "autoreceptor" agonist (R)-(–)-apomorphine [101]. It was subsequently characterized as a high-affinity agonist at dopamine D_2, D_3, and D_4 receptors [102,103] and approved in 1997 for the treatment of PD and for restless legs syndrome (RLS) in 2006.

Like ALS, PD is a neurodegenerative disorder that has been linked to mitochondrial dysfunction [104,105]. Defects in the electron transport chain and associated increases in ROS contribute to the neurodegeneration characteristic of cells in the substantia nigra (SN) [106]. Hall et al. [107] evaluated the neuroprotective potential of PPX in attenuating the postischemic degeneration of dopaminergic neurons in the SN using a gerbil bilateral carotid occlusion model of brain ischemia. When dosed twice daily for 28 days, the compound reduced cell loss in the SN, but not the hippocampus, relative to control. Although PPX reduced dopamine turnover in SN cells, the reduction was insufficient to account for its observed neuroprotective effects, suggesting that a more likely mechanism of PPX neuroprotection might be through a direct antioxidant action.

In this light, further studies of PPX focused on establishing the compound's potential as a neuroprotectant in nondopaminergic neurons, in determining the degree to which dopamine receptor agonism contributed to its ability to protect neurons, and in delineating the interaction of the molecule with mitochondrial mechanisms thought to be related to neuronal degeneration. Sethy et al. [108] found that in rats treated with the nicotinamide antagonist neurotoxin 3-acetylpyridine (3-AP), PPX administration for up to 3 h post-3-AP treatment significantly reduced toxin-related reductions in ATP levels in cerebellum, protected neurons in the inferior olivary nucleus, and improved motor coordination measures. PPX also reduced the accumulation of cytochrome c (and α-synuclein, a presynaptic protein found in aggregated form in PD) in response to administration of the neurotoxin methylpyridinium ion (MPP^+) in SH-SY5Y cells [109], and has been shown to increase the amount of antiapoptotic protein Bcl-2 in the dendritic processes of hippocampal and cortical neurons following multi-day administration to rats [109–111]. Bcl-2 has been shown to inhibit the release of cyctochrome c under a number of proapoptotic conditions, so these results were further evidence of a neuroprotective potential of the compound.

Cassarino et al. [112] examined the production of ROS in cultured SH-SY5Y neuroblastoma cells *in vitro* and in rat striatum *in vivo* (via microdialysis) in response to treatment with MPP^+. In these systems, PPX significantly reduced the concentration of oxygen radicals, albeit nonpotently. Most interesting, they also used an indirect measure of mitochondrial swelling to estimate the effects of PPX on mPT. In these experiments, using mitochondria from liver cells, PPX was able to reduce mPT induced by calcium and phosphate in a concentration-dependent manner. Cyclosporine A, a known blocker of mPT and the mPT pore, was fully effective at a concentration of 25 μM, as was PPX at a concentration of 100 μM. In later experiments, Sayeed et al. [68] looked directly at the interaction of PPX with the mPT pore using patch clamp recording in mitoplasts prepared from liver cells. The compound blocked opening of the pore in a concentration-dependent manner with an apparent IC_{50} of 500 nM (compared with an IC_{50} of 26 nM for cyclosporine A).

Subsequent work with PPX examined the contribution of dopamine agonism to observed effects on mitochondria and neuroprotection. Several lines of evidence have demonstrated that PPX exerts its effects, at least in part, by mechanisms that are independent of its dopamine receptor affinity. For example, in pheochromocytoma (PC12) cells, pretreatment with PPX (1–100 μM) resulted in protection against H_2O_2-induced cell death [113], an effect that was not blocked by several dopamine receptor antagonists. In a similar study, PPX (5–20 μM) was shown to protect cultured dopaminergic mesencephalic-derived (MES) 23.5 cells from dopamine, 6-OH-dopamine, and H_2O_2-induced cytotoxicity [114], and these effects were not blocked by the dopamine D_2 antagonist raclopride or the D_3 antagonist U99194A. Ramirez et al. [115] reported that PPX was neuroprotective in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated male C57BL/6 mice in which the D_3 receptor gene was deleted (D_3 –/– mice) although the

dopamine sparing effect in the knockout mice was not as robust as in mice expressing the wildtype gene (D_3 +/+ mice). Similarly, the significant neuroprotective effects of PPX in this model were attenuated but not eliminated by the coadministration of the D_3 receptor antagonist A-437203.

KNS-760704: Exploiting the Neuroprotective Potential of Pramipexole

KNS-760704 is a >99.95% chirally pure formulation of (6R)-4,5,6,7-tetrahydro-N6-propyl-2,6-benzothiazolediamine dihydrochloride and, as such, is devoid of any of the dopamine agonist effects that might be attributed to trace contamination by the S(–) enantiomer, PPX. RPPX, which refers generally to the R(+)-enantiomer of pramipexole dihydrochloride without regard to a specific formulation, had been anecdotally reported to have modestly lower affinity for D_2 and D_3 receptors. Our recent work demonstrates that KNS-760704 is in fact a very low-affinity agonist (as much as 1000-fold lower affinity) at these receptors (Gribkoff, unpublished data). While PPX is a potent and effective treatment for PD, its utility as a clinical neuroprotective agent is limited because the drug concentrations required for neuroprotection would likely produce unacceptable dopaminergic side effects such as hypotension and seizures. KNS-760704, with its low dopamine receptor affinity, thus has a unique and potentially more useful profile than PPX for the treatment of neurodegenerative diseases such as ALS. This potential, however, is predicated on its having neuroprotective properties equipotent with PPX.

Given the magnitude of stereo-specific receptor affinity difference between the enantiomers, a number of studies used both PPX and RPPX to test whether dopamine receptor affinity is a critical requirement for pramipexole neuroprotection and to evaluate the relative neuroprotective potency of the enatiomers. Abramova et al. [116] examined the ability of PPX and RPPX to modulate the activation of caspases and the retention of calcein in response to MPP^+ application to SH-SY5Y cells. Both enantiomers were equipotent and effective at blocking these components of the mitochondrial-induced cell death pathways, and in the case of calcein retention, appeared to be maximally effective at concentrations below 1 μM for both PPX and RPPX. Gu et al. [117] showed in SHSY-5Y cells exposed to 5 mM MPP^+ that PPX and RPPX in concentrations from 1 to 100 μM produced similar, dose-dependent reductions in cytochrome c release and preserved mitochondrial membrane potential at 10 μM. Using nondopaminergic JK cells, they also showed that both PPX and RPPX (10 μM) significantly increased cell survival following exposure to 5mM MPP^+.

In a recent study, Danzeisen et al. [118] examined the uptake of radiolabeled PPX ([^{3}H]PPX) into neurons and neuronal mitochondria, the antioxidant properties of both PPX and RPPX, the effects of the compounds on glutamate-induced cell death, and the effects of PPX and RPPX on survival time in SOD1 (G93A) mutant mice. Uptake of [^{3}H]PPX (and presumably RPPX, although this was not measured) into both neurons and neuronal mitochondria was confirmed, and intramitochondrial uptake was shown to be energy-dependent and reduced by a reduction in mitochondrial membrane potential. Both enantiomers were found to have equipotent (although not potent) antioxidant potential against H_2O_2 and superoxide, and somewhat more potent and equal effects on the release of nitric oxide in response to the nitric oxide donor (*Z*)-1-[2-(2-aminoethyl)-*N*-(2-ammonioethyl)amino] diazen-1-ium-1,2-diolate (DETA). They were also protective against the cytotoxic effects of glutamate in cultured H-22 hippocampal neuroblastoma cells. These cells are killed following application of glutamate by the generation of ROS, rather than by a receptor-mediated excitotoxic cascade, and both enantiomers were very effective, although not potent, neuroprotectants with EC_{50}s in the range of 370 μM for PPX to 190 μM for RPPX. In control mice, both PPX and RPPX were shown to enter brain with a brain-to-plasma ratio in excess of 6. Following oral dosing at 100 mg/kg commencing at 45 days of age in G93A-SOD1 mutant mice, RPPX was protective, resulting in a modest but significant 7-day increase in survival time. PPX was dosed at 3 mg/kg in the G93A-SOD1 mutant mice, presumably because of intolerable dopaminergic side effects at higher doses. Even at this much lower dose, PPX administration was associated with a significant increase in wheel-running behavior attributed to dopaminergic activation. No significant difference was observed between control (vehicle) group survival and that of the PPX group at this low dose.

Testing the Hypothesis that RPPX may Provide Benefit in ALS

This paper is not intended to comprehensively review all preclinical data associated with PPX and RPPX, but rather to present the logic for initiating the clinical development of RPPX as a treatment for ALS. The data examined strongly suggest that PPX is neuroprotective, and may be accomplishing this via one or more mitochondrial mechanisms. They also indicate that these effects do not depend on dopamine receptor interaction, and that the

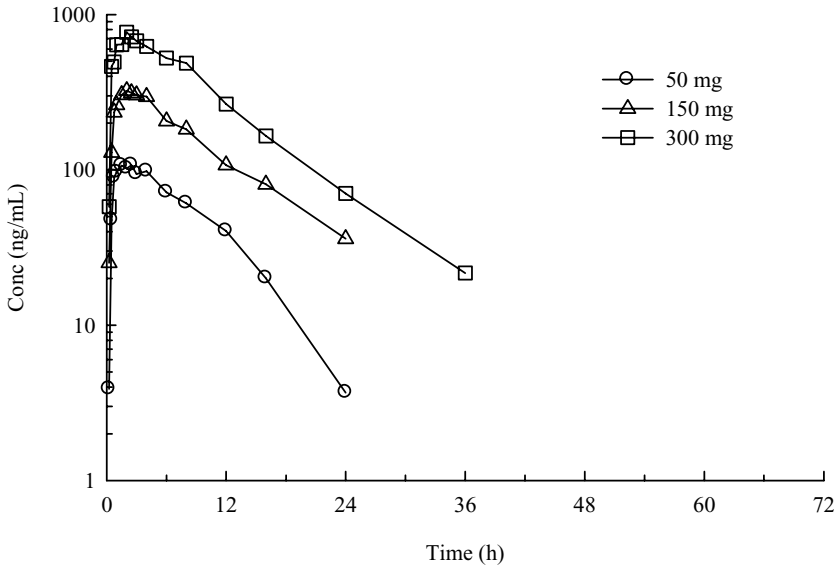

Figure 2 Mean human plasma concentrations of KNS-760704 after single oral administration of a 50, 150, or 300 mg dose to adult volunteers under fasting conditions. Note semi-logarithmic ordinate scale.

low-affinity enantiomer RPPX exerts equipotent cytoprotection in every modality measured to date. Given this, and the dose-limiting, dopamine related side-effects associated with Mirapex®, it follows that RPPX would be the more useful member of this enantiomer pair for clinical tests of effectiveness in ALS or any neurodegenerative disorder.

In support of future clinical trials, we have examined the physicochemical, pharmacokinetic, and toxicokinetic profiles of RPPX in preclinical studies. KNS-760704, the formulation of RPPX currently used in our clinical studies (see below), is a white crystalline solid with a molecular weight of 211.3 as the free base (formula weight of 302.3 as the dihydrochloride monohydrate actually used in the studies). It has chemical purity ≥99.99% and enantiomeric purity ≥99.95%. It has a melting point between 285 and 287°C, and is highly water soluble (>600 mg/mL). It is highly stable in solution in water and physiological buffer solutions, and is nonhygroscopic.

KNS-760704 did not significantly affect any of the major cytochrome P450 isoenzymes and was only moderately bound to human plasma proteins (40.3%). The compound enters the CNS efficiently, with brain-to-plasma ratios of about 5–15, depending on species and dose. KNS-760704 was negative for any mutagenic or genotoxic signal in a full battery of *in vitro* and *in vivo* assessments. The compound did not significantly affect the cardiac delayed rectifier (hERG) potassium current at or near concentrations of clinical relevance ($IC_{50} \sim 100$ μM for hERG inhibition *in vitro*) and in a good laboratory practices (GLP) study of cardiovascular safety in Gottingen minipigs, there were no adverse effects observed at the highest dose tested (75 mg/kg).

Acute and chronic toxicology studies have been completed in two species, rat and minipigs, and the results from these studies strongly support the ongoing development of KNS-760604 in ALS. The compound is rapidly and essentially completely absorbed when dosed orally and its half-life was in the range of 3–8 h across species. Both species achieved greater-than or equal-to dose-proportional peak plasma KNS-760704 levels and exposures in single and repeat-dose studies. The no-observed-adverse-effect-level (NOAEL) in rats of 100 mg/kg for males and ≥300 mg/kg in females after 6 months of dosing provides multiples of exposure of about 7 and 25, respectively, over the highest dose being evaluated in clinical studies (300 mg). In minipigs, the NOAEL of 50 mg/kg after 9 months of dosing provides exposure multiples of about 10 to 15 over the 300 mg dose in human subjects.

The clinical development of RPPX is being advanced on two fronts. RPPX has been evaluated in ALS subjects under research IND 60,948 held by James Bennett at the University of Virginia. In these open label studies, Bennett et al. have evaluated single and multiple doses of R-(+)-pramipexole in approximately 100 ALS subjects at a variety of dosages and durations of dosing up to a maximum daily dose of 100 mg TID. The drug has been safe and well-tolerated. In a futility study of 30 subjects with early sALS treated for 6 months with open-label RPPX at 10 mg TID following a 3-month lead-in phase, RPPX yielded a nonsignificant 13% reduction in the slope of decline on the ALSFRS-R. A subset of these patients evaluated at a dose of RPPX 20 mg TID for an average of 7.9 months showed a further, but still nonsignificant, reduction of 17% in the slope of decline on the ALSFRS-R compared with treatment at 10 mg TID [119].

Knopp Neurosciences, Inc. has licensed rights to the use of RPPX from the University of Virginia, and opened IND 75,959 for the evaluation of KNS-760704 in ALS. Randomized, placebo-controlled, double blind phase 1 safety studies of the drug in a total of 82 healthy adult subjects were completed in 2007. KNS-760704 has been safe and well-tolerated at single doses of 50, 150, and 300 mg and in twice-daily doses of 50, 100, and 150 mg for 4 1/2 days. It is highly orally bioavailable, ~90% renally excreted as unchanged drug and has demonstrated linear pharmacokinetics (Fig. 2). A phase 2A placebo-controlled clinical trial in ALS patients (www.clintrials.gov) was initiated in March 2008.

Conclusions

This review presented the logic for examining mitochondrial-targeted neuroprotective agents in ALS and other neurodegenerative disorders, and presented evidence that the D_2/D_3 dopamine receptor agonist and approved PD/RLS drug Mirapex® (PPX) is neuroprotective and interacts with several potentially important mitochondrial mechanisms. However, due to its very high affinity for dopamine receptors PPX is limited to low doses which may not fully exploit neuroprotective mechanisms. RPPX, which has significantly lower dopamine receptor affinity, has been shown to be tolerated at much higher doses, and therefore may be a more useful neuroprotective agent in chronic and acute neurodegenerative disorders, including ALS. Open-label studies in ALS patients with RPPX are being completed under IND 60,948, and placebo-controlled clinical trials in ALS patients with enantiomerically pure KNS-760704 are being initiated in 2008 under IND 75,959.

Conflict of Interest

The authors have no conflict of interest.

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