Research Article

The Anticholinesterase Phenserine and Its Enantiomer Posiphen as 5′Untranslated-Region-Directed Translation Blockers of the Parkinson’s Alpha Synuclein Expression

Sohan Mikkilineni, Ippolita Cantuti-Castelvetri, Catherine M. Cahill, Amelie Balliedier, Nigel H. Greig, and Jack T. Rogers

1 Neurochemistry Laboratory, Massachusetts General Hospital (East), CNY2, 149, 13th Street, Charlestown, MA 02129, USA
2 MIND, Massachusetts General Hospital, Charlestown, MA 02129, USA
3 Drug Design and Development Section, Laboratory of Neurosciences, Intramural Research Program, National Institute on Aging, Baltimore, MD 21224, USA

Correspondence should be addressed to Jack T. Rogers, jrogers@partners.org

Received 1 July 2011; Accepted 29 February 2012

1. Introduction

Parkinson disease (PD) is a slowly progressive neurodegenerative disease affecting up to 3% of the population over the age of 65 years. Clinically, it is characterized by a set of motor symptoms and nonmotor symptoms that can include dementia. Motor symptoms include rigidity, postural instability, bradykinesia, and resting tremor [1, 2]. The core pathological feature correlating with most of these motor symptoms is a loss of dopaminergic neurons from the substantia nigra pars compacta (SNC). Pathological inclusions, known as Levy bodies, are found within some of the remaining dopaminergic neurons [3]. The destruction of the dopaminergic neurons at onset is at least 70% and, at the end stage of the disease the loss of dopamine (DA) neurons, can exceed 95% [3]. Therapy for the symptoms of PD is based primarily on replacement of dopaminergic function and can be remarkably effective in alleviating motor symptoms for a number of years [1, 4]. Additional nonmotor symptoms can be present and include depression, anxiety, sensory abnormalities, anosmia, sleep, and autonomic disorders, in addition to dementia [1, 5–8].

Particular nonmotor symptoms, like sleep disturbances [9], loss of smell [6, 10], and depression, can occur well before the presence of detectable motor symptoms [5]. The presence of psychotic symptoms, which can affect up to 40% of the patients, is often associated with anti-parkinsonian
treatment and is dose dependent [2, 7]. By contrast, the neuropsychiatric symptoms of PD, like depression and dementia, are more common at the later stages of the disease [6, 8, 11, 12]. These neuropsychiatric symptoms may be correlated directly to the progression of PD itself as in PD dementia or PDD, to the presence of Dementia with Lewy Bodies (DLB) or to the presence of comorbidities like Alzheimer’s Disease (AD) [2]. The incidence of dementia in PD is six times higher than in age-matched controls; in all forms of dementia associated with PD, the use of cholinesterase inhibitors may be beneficial [2, 13–17].

The etiology of PD is still elusive [18]. A variety of genetic factors have been linked to the etiology of familial PD [19]. The first was the protein alpha-synuclein (α-syn), an abundant brain protein that appears to be involved in vesicle trafficking and participates in the regulation of DA release [20]. The first reports to implicate α-syn in PD described mutations in the protein, which cause autosomal dominant forms of the disease [21, 22]. Subsequent studies revealed that such mutations were exceedingly rare but also that aggregates of α-syn could be found in all cases of familial and sporadic PD [23]. It is important to recognize that the genetic mechanisms identified to date are individually rare and, collectively, represent only a small fraction of the cases of PD observed by practitioners. This hence leaves the vast majority of PD unexplained at present.

Events associated with the inflammatory cascade [24] as well as with iron metabolism [25, 26] and translational control of gene expression have been modeled to be associated with PD and LBD [27]. There are also reported examples of disrupted signaling events such as occuring in response to inflammation cytokines: for example, mutations to the signaling kinase LRRK-2 may activate inflammatory events in this neurodegenerative disease [24, 28], perhaps affecting novel signalling pathways [24]. Certainly, increased iron in the individual dopaminergic neurons of the substantia nigra (SN) has been reported to be closely associated with the pathogenesis of PD [29], and antioxidants have been tested as a means to alleviate the severity of PD [30]. DLB brains exhibit lowered SNCA mRNA but higher insoluble protein, suggesting misregulation of SNCA mRNA translation additional to its clearance by chaperones [31], although reduced autophagy may provide the explanation. Translational control of α-syn may be governed, at least in part, by a uniquely configured 5′untranslated region (5′UTR) of its transcript, which encodes a homology with the known APP and ferritin iron-responsive elements [32–35].

α-Synuclein is, in fact, the central ~15 kDa protein implicated in the pathogenesis of neurodegenerative α-synucleinopathies [36], including PD—the most prevalent movement disorder in humans. Other α-synucleinopathies include DLB, Lewy body variant of Alzheimer’s disease (AD), and multiple system atrophy. In these disorders, α-syn undergoes a conformational change and oligomerization, causing a toxic gain of function. Neurodegenerative deposition of α-syn aggregates occurs, most commonly in Lewy bodies. Lewy bodies are rare in PD patients, present only in the surviving neurons. α-syn aggregates are in dystrophic neurites. Glial cytoplasmic inclusions are in multiple systems atrophy [37]. Pathogenic pathways of α-synuclein and amyloid-β (Aβ) converge via interaction of these two amyloidogenic proteins, which coprecipitate into β-pleated oligomers and insoluble fibrils [38–40], although Aβ and α-syn rarely colocalize in brain amyloid deposits [41]. There is certainly also overlap between the 5′UTRs of the SNCA and amyloid precursor protein (APP) transcripts, another similarity that provides a further link between PD and AD [27, 33–35].

Such overlap in the 5′UTRs of SNCA and APP mRNAs is consistent with our and other groups’ recent demonstrations that both are key players in iron metabolism and α-syn may be translationally controlled by cellular iron levels as was demonstrated for APP expression [35, 42]. In this regard, APP is a copper-dependent iron export ferroxidase [43], and α-syn a Cu-binding ferrireductase [44, 45]. These similarities, together with the utility of anticholinesterases in AD and PD, provided our rationale to test whether known anticholinesterases that are beneficial and Aβ lowering [46] would, likewise, provide anti-α-syn activity in neural cells.

The most common therapy for PD is L-dopa that is routinely used to overcome the archetypical problems of tremor. As disease progresses, however, clinically available anticholinesterases have been increasingly empirically used, based in part on an putative impairment of the cholinergic system in developing PD [47].

Therefore, our study investigated the effectiveness of the cholinesterase inhibitor phenserine and its noncholinergic (+) chiral enantiomer, posiphen in lowering α-synuclein expression in neural cell lines. Both agents have been shown to effectively lower APP synthesis rate both in neuronal cultures and animals via translational regulation mediated at the level of the APP mRNA 5′UTR. As both agents have been developed through to clinical studies and appear well tolerated in AD, if effective in reducing α-syn levels, they hold translational promise as potential therapeutics for PD.

Furthermore, as posiphen can be effectively dosed in rodents and humans in far greater amounts than phenserine, thereby generating high levels of its primary metabolites, the three most prominent primary metabolic products of posiphen were also characterized. As a model to examine the α-syn-lowering efficacy of these agents, we assessed the cellular therapeutic impact of these screened SNCA 5′UTR-directed translation blockers to reduce α-synuclein expression in neural cells lines and subsequently also in the primary neurons from the PAC/Tg(SNCA) genomic human SNCA mouse model of PD (Figures 2 and 6) [48].

1.1. Hypothesis/Model. Our model to be tested is shown in Figure 7. The overarching hypothesis is that the drug phenserine (anticholinesterase) and its enantiomer are known translation blockers of the amyloid precursor protein of Alzheimer’s disease (AD) and, as such, have been tested in clinical trials for their antiamyloid efficacy and potential to improve cognition. We noted that the RNA target in the 5′UTR of APP mRNA was similar to that found in the alpha-synuclein transcript. Therefore, we decided to see if posiphen and phenserine might block α-synuclein,
the central culprit protein in PD. To achieve this end, we conducted Western Blot experiments firstly with SH-SY5Y dopaminergic neuroblastoma cells and then with primary E18 neurons from a genome model of PD. Our rationale for conducting this study was to determine if posiphen and phenserine are two 5’UTR-directed drugs that would reduce alpha-synuclein expression to provide therapeutic benefit to Parkinson’s disease patients.

### 2. Materials and Methods

#### 2.1. Materials.**

Dulbecco’s modified essential medium (DMEM, catalog no. 12-614Q); FBS (catalog no. 14-503E), L-glutamine (catalog no. 17-605E), penicillin/streptomycin (catalog no. 17-602E); phenol red free media (phenol red-free DMEM with 4.5 g/L D-glucose (catalog no. 12-917F)) were purchased from Lonza (Portsmouth, NH). Trypsin/EDTA (catalog no. 25-053-CL) was purchased from Cellgro. Steady-Glo (catalog no. E2250) was purchased from Promega (Madison, WI). For the Western Blot secondary screen, penicillin/streptomycin was acquired from Bio-Whittaker (Walkersville, MD), mouse monoclonal anti-alpha-synuclein was purchased from BD Transduction Laboratories, and anti-beta-actin was acquired from Chemicon, Inc. We routinely use 384-well plates (catalog no. 3570), which were purchased from Corning and Falcon TC flasks (catalog no. 353112) were purchased from Becton Dickinson (Waltham, MA). SH-SY5Y cells were purchased from the ATCC, Manassas, VA.

#### 2.2. Cell Culture.**

Transfected SH-SY5Y neuroblastoma cells were grown to confluency in 35 mL complete DMEM with 4.5 g/L D-glucose supplemented with 10% FBS, 200 μM L-glutamine, 100 μM penicillin/streptomycin, and 200 μg/mL geneticin in a T175 TC flask in a TC incubator (37°C, 95% humidity, 5% CO₂) (doubling time = 24 h). Untransfected SH-SY5Y counterparts were grown in the absence of geneticin. Cells were harvested by washing the monolayer quickly with 5 mL trypsin/EDTA (1X), aspirating, then adding 5 mL trypsin/EDTA and incubating for 5 min at 37°C, 95% humidity, 5% CO₂, after which cells were collected into phosphate-buffered saline and centrifuged into a pellet for storage at −70°C.

Primary cortical neurons from wild-type mice and from the PAC-Tg(SNCA(wt) human SNCA genomic mice [48] were cultured as outlined by the method of Ray et al., 2009 [49]. Briefly, we recovered the embryonic day 15–18 pups after sacrificing pregnant females, separated out the brain, and removed the meninges and blood vessels. We then dissected out the cortices and placed them in separate eppendorf tubes containing 500 μL of HBSS without Ca²⁺/Mg²⁺ salts supplemented with 1 mM sodium pyruvate and 10 mM HEPES, pH 7.4. On ice, individual cells were isolated by titurating 10 times using a glass pasture pipette with the tip barely fire polished. We adjusted the volume to 1.5 mL, by adding 1 mL of HBSS with Ca²⁺/Mg²⁺ salts + Na pyruvate + HEPES, restoring the divalent cations by adding HBSS so that the nondispersed tissue could settle for 5 min, on ice. In the tissue culture laminar hood, we transferred the supernatant into a new 15 mL tube and centrifuged for 1 min at 900 rpm, 4°C. We gently resuspended the pellet in 2 mL of HBSS with Ca²⁺/Mg²⁺ salts + Na pyruvate + HEPES and took an aliquot for counting (2 mL for approx. 5 embryos). We then plated ~ 1 × 10⁵ cells/well of a 24-well or 2 × 10⁷/in 12-well plates. Each set of plates were coated with poly D-lysine containing poly L-lysine coverslips for micro immunocytochemical confirmation of neuronal integrity.

#### 2.3. Bioinformatics Methods.

The α-synuclein RNA sequences were selected using the NCBI Gene search and the Ensembl database (see [34]). Since the 5’ UTRs were of primary interest, the coding sequences (CDS) were mostly disregarded, apart from the initiating AUG. As we reported [34], the splice junction between two exons occurred at a CAGUGU site 25 = nucleotides from the AUG and that this pattern was conserved among the other species investigated. Thus, in order to study a balanced sequence, 25 nucleotides before the splice junction from the first exon were used to create 50 nucleotide RNA sequences (mouse and rat had 52 nt sequences due to insertions).

The ClustalX2 graphical program was used to align the RNA sequences to identify any evolutionary conservation between species. For alpha synuclein, the AUG start region of the CDS was a reference point for sequence alignments, allowing a comparison of the sequences in both exons around the splice junction, due to high local conservation fidelity. Secondary sequences were then generated by the University of Vienna’s RNAfold webserver (with standard settings) and were annotated using the RNAfold command-line software. The RNAfold server provided the most probable secondary structure based on minimum-free energy calculations.

We calculated the alignment homology by comparing any species’ RNA sequence (as listed) against the Homo sapiens sequence on each side of the splice junction. We also calculated the homology between the core L- and H-ferritin IREs with that of alpha synuclein and APP mRNAs. Only nucleotides that matched respective to the Homo sapiens sequence were scored a point; we determined the percent homology on each side by totaling the points scored and dividing by the total number of nucleotides on that side. The results from each side were then compared to illuminate the difference in conservation across the splice junction.

#### 2.4. Constructs.**

The SNCA-5’UTR-pGL3 construct was generated from the pGL3 expression vector (Promega). In this case, a PCR fragment encoding 48 base α-synuclein 5’UTR was cloned between the Hind-III and Nco1 sites in front of the luciferase gene in PGL3. Transiently transfected cells expressed either pGL3 or the SNCA-5’UTR-pGL3 construct. Stably transfected neural cell lines were generated via neomycin selection after cotransfection with the RSV2-neomycin plasmid to express the SNCA-5’UTR-pGL3 construct (H2A cells) or pGL3 (control cells).

#### 2.5. Stable-Transfection-Based Screen of a Library of Natural Product Inhibitors of α-Synuclein Translation.**

A 720 compound natural products, including added phenserine and...
Figure 1: Continued.
After 24 hr transfection

2.6. Transient Transfection Assays. which scored as a hit or contradictory in the luciferase assay.

′ arrangement of splice sites and exhibited an AGU triloop, whereas, in lower vertebrates, this AGU motif was located in the stem regions of these transcripts. Shown are the red lettering at the apex of the H-ferritin IRE [59] where the analogous AGA from the APP IRE is depicted [50]. The human several species were predicted to be folded, as described in the Materials and Methods section, and the pseudotriloop AGU is depicted in addition of 25 uL Steady-Glo reagent (Promega), plates were vortexed for 30 sec and 35 minutes later luminescence read on an Infinite F2000 plate reader (Tecan) [34, 50].

2.8. Western Blot Assay. Human SH-SY5Y cells and primary cortical neurons (E18 fetal cells) were exposed to increasing concentrations of phenserine and posiphen for 48 hours. Cytoplasmic protein lysates were prepared by homogenizing the cells in midRIPA buffer (25 mM Tris pH 7.4, 1% NP40, 0.5% sodium deoxycholate, 15 mM NaCl, protease inhibitors, RNase inhibitor, and 10 μM DTT). Western Blotting for alpha-synuclein was performed using mouse monoclonal anti-alpha-synuclein (BD Transduction Laboratories) and anti-beta-actin (Chemicon). The blots were developed using chemiluminescence (PIERCE) and visualized with a Phospholimer (BioRad, Hercules, CA), and bands were quantified using QuantityOne software (BioRad).

3. Results and Discussion

In Figure 1, we performed a full bioinformatic analysis of the SNCA 5′UTR demonstrating by computer-mediated predictions [50] that the 5′UTR of the SNCA transcript folds into a unique RNA stem loop resembling an iron-responsive element (IRE) RNA structure that is related to, but distinct from, the H-ferritin and APP 5′ UTR-specific IREs [33]. We are currently testing the capacity of intracellular iron chelation with desferrioxamine to repress neural α-synuclein translation acting via the IRE in the 5′ UTR of its transcript, as we reported for the APP and ferritin mRNAs [42, 51].

Previously, the RNA-directed anticholinesterase drug phenserine, together with its cholinergically inert chiral (+)
Figure 2: Posiphen and phenserine decrease both APP and α-synuclein levels dose-dependently in dopaminergic SH-SY5Y cells. Panel A: the 5′UTRs of both the APP and SNCA genes are 50% homologous with the IRE H-ferritin mRNA. Panel B: SH-SY5Y cells were treated with concentrations ranging from 0 to 10 μM phenserine and posiphen for 48 h. Harvested cell lysates were prepared. Quantitative Western Blotting established the anti-α-syn efficacy of posiphen and phenserine (IC50 < 5 μM); after standardization for β-actin (Densitometry of multiple lanes (n = 8) by ImageQuant). Cell viability was unaffected (measured by standardized ATP levels/cell (Tm (Cell-Titer-glo, Promega, Inc.)))).

enantiomer, posiphen, which are both in clinical development for AD, was shown to therapeutically limit brain Aβ levels in wild-type and AD mouse models [46]. This action was mediated, in whole or in part, by lowering the rate of synthesis of APP, from which Aβ is proteolytically cleaved. Here, we sought to demonstrate that phenserine and posiphen, likewise, blocked α-synuclein expression via their related 5′UTRs, encoding variant versions of the iron-responsive element RNA elements that potentially bind iron-regulatory proteins.

To elucidate whether our defined target would translate across species, we provide the results of an evolutionary evaluation of the conservation of IRE RNA stem loops in SNCA mRNA as shown in Figure 1. This is the RNA secondary structure sequence, together with the APP 5′UTR, that is targeted by phenserine and posiphen, as shown in Figures 2–4. The alpha-synuclein-specific IRE stem loop was formed at the splice junction of the first two exons in SNCA gene [35]. We also compared the predicted structure of the SNCA mRNA IRE with the canonical IREs in the H-ferritin and APP transcripts, which are transcribed from the single first exon of their genes confirming the uniqueness of translational repression of SNCA mRNA via its 5′UTR.

The anticholinesterase phenserine and its (+) enantiomer, posiphen, are proven APP 5′UTR mediated drugs with known pharmacokinetics in rodents and humans and identified target concentrations [46, 52]. Since we anticipated that both agents would be active in limiting α-synuclein translation via its 5′UTR, we had spiked an FDA library with posiphen and phenserine when we formerly screened against the SNCA 5′UTR RNA target [34]. Here, we confirm that both posiphen and phenserine repressed the SNCA 5′UTR-driven translation of a luciferase reporter in stable cells lines. Their capacity to inhibit SNCA mRNA translation is similar
to that of certain other defined FDA drug leads, including three glycosides and an immunosuppressant, mycophenolic acid (secondary Fe chelator), as we previously reported [34].

In Figure 2(a), the 5′UTRs of both APP and SNCA showed 40% homology to each other and also 50% homology to the IRE in H-ferritin mRNA. Multiple Western blot experiments were hence conducted to determine the impact of phenserine compared to posiphen to limit α-syn compared to APP expression. In this regard, SH-SY5Y cells were treated with both compounds for 48 hours with concentrations ranging from 0 to 10 μM. Viability studies determined that this range was well tolerated, in accordance with prior studies. In general and as shown in Figure 2(b), posiphen (in addition to but potentially slightly more potently than phenserine) decreased levels of α-syn in a dose-dependent manner in cultured neural cells (SH-SY5Y) as previously reported for APP. Whether this higher potency would translate to primary neurons and in vivo is a focus of future studies. In this paper, this was achieved with a preliminary determined IC₅₀ in the order of 5 μM, in the absence of toxicity.

Multiple transient transfections of SH-SY5Y cells were performed with the constructs that either translated luciferase driven by the 48 base SNCA 5′UTR (SNCA-5′UTR-pGL3) or the empty pGL3 expression vector (Figures 3 and 4). As shown (Figures 3 and 4), posiphen 50% repressed SNCA 5′UTR-conferred translation of a luciferase reporter transcript. In this regard, posiphen proved a highly selective inhibitor of SNCA 5′UTR-driven activity since this chirally pure compound inhibited SNCA 5′UTR-driven luciferase expression in H2A neural cells (i.e., SNCA 5′UTR-positive stably transfected neural cells). By contrast, phenserine and the known APP translation blocker, compound number 9 (included as a comparator), did not suppress
**Figure 4:** Selectivity of posiphen to inhibit translation driven by alpha-synuclein 5'UTR sequences: a second set of transient transfection experiments in which posiphen (10 μM) selectively inhibited alpha-synuclein 5'UTR-conferred luciferase expression in SH-SY5Y neural cells (SNCA 5'UTR-positive transfectants, \(N = 6\)). Confirming selectivity, posiphen increased luciferase expression in pGL3-transfected SH-SY5Y cells (\(\ast\)pGL3-SH-SY5Y serves as an experimental control since these cells were transfected with pGL3, which is the same as the H2A construct but lacks the alpha-synuclein 5'UTR).

**Figure 5:** Metabolic analogs of posiphen and their respective anticholinesterase activities [60]. Posiphen is devoid of anticholinesterase activity. However, its phase 1 metabolites, N8 demethylated, N1 demethylated, and di-demethylated N1, N8-bisnorposiphen showed ex vivo AChE and BChE inhibitory activity of clinical relevance [61, 62]. The compound N8-bisnorposiphen demonstrated no AChE activity. This activity has proven to be dose limiting in human safety studies.
SNCA 5′UTR conferred translation in H2A cells. Indeed, phenserine and compound number 9 elevated SNCA 5′UTR-conferred translation. These data support the mechanism-of-action of posiphen as a highly selective blocker of SNCA 5′UTR activity. However, phenserine—with the identical chemical structure but in a different three-dimensional (chiral) configuration—that has previously been shown to effectively inhibit translation driven by the APP 5′UTR clearly has different actions to posiphen at the SNCA 5′UTR. From this, we can deduce that the element of the SNCA 5′UTR targeted by posiphen has a stereospecific component. Additionally, since phenserine lowers α-syn levels (Figure 2(b)), further SNCA RNA sequences are likely involved in controlling this pathway of α-syn translational regulation.

Extending beyond transformed neuronal cell lines, primary neurons from wild-type mice and PAC SNCA transgenic mice (PAC-Tg SNCA) were evaluated for the capacity of posiphen to repress α-syn expression, as shown in Figure 6 (tested at 100 nM drug concentration). Posiphen proved not only active and well tolerated in SH-SY5Y cells but consistently reduced human α-synuclein expression in primary neurons (E18) at doses as low as 1 uM (75% reduction, not shown) without toxicity. This margin appeared to be greater than its capacity to lower APP production (20%) (utilized as a positive control) in these same cells (data not shown).
Following oral administration of posiphen to rodents, dogs, and human, the compound is subjected to metabolic processes and generates the same metabolic profile across these species. Specifically, via a phase 1 metabolism, posiphen undergoes N-demethylation in both the N1 and N8 positions to generate the respective primary metabolites, N1-norposiphen and N8-norposiphen (Figure 5). Each then undergoes further N-demethylation to generate the common metabolite, N1, N8-bisnorposiphen. Unlike phenserine, posiphen is devoid of cholinesterase inhibitory activity and, therefore, can be advantageously administered at higher clinical doses (in the order of 5, to 8-fold greater).

As shown in Figure 5, specific N-demethylated metabolites (in particular the N1-nor and N1,N8-bisnorposiphen) possess potentially clinically relevant IC$_{50}$ values to inhibit acetylcholinesterase (AChE). Such activity is less than phenserine and would be expected to have a slow onset in line with the time-dependent metabolism of posiphen to generate its metabolites. However, with regard to actions on α-synuclein expression, activity of the metabolites at this target, where posiphen is potent, could usefully increase and extend the drug’s in vivo efficacy. To elucidate this and aid planning for future animal studies, we characterized the relative capacities of posiphen’s metabolic analogs to impact α-syn expression (Figure 6).

Figure 6 shows a Western Blot analysis representative of three experiments that compared the efficacy of posiphen with that of its three primary metabolites to limit α-synuclein expression ex vivo using E18 primary cortical neurons from human SNCA PAC mice. We consistently measured that N1-norposiphen (possessing AChE activity) proved most potent to limit α-synuclein expression (by 50%), and, likewise, APP expression was also reduced. As assessed at 100 nM in Figure 6, the other metabolites proved to be less active at these targets, indicating that relatively small structural changes (i.e., the loss of a methyl moiety) have significant impact. An assessment of dose-response for each metabolite is a focus of future studies, particularly within the achievable clinical range of the agents in human and in vivo range in animal models (Dr. Maria Maccecchini and Dr. Robert Nussbaum, personal communications).

4. Conclusions

We previously reported that phenserine, a (-)-physostigmine analogue and anticholinesterase that reached phase III clinical assessment for AD [53–55], inhibited APP translation though its 5’ UTR [56, 57]. Phenserine was found to be dose limiting consequent to its AChE inhibitory action, causing the classical cholinergic action of tremors in animal models and nausea in humans. Posiphen, the chirally pure (+) enantiomer of phenserine, by contrast possesses no direct anticholinesterase activity and, likewise, repressed neural
AD-specific APP translation via its 5′ UTR in mice. It has recently been developed (QR Pharma, Berwyn, PA) to the clinic as an APP synthesis inhibitor in AD to lower both brain Aβ generation as well as the levels of other toxic proteolytic products of APP. With a very different pharmacological and pharmacokinetic profile to phenserine, it has recently completed single-and multiple-dose escalating phase I clinical assessment in humans, appearing well-tolerated, and a proof of mechanism study, indicating target engagement.

In this paper, we demonstrated in human immortal neuronal cultures, and then ex vivo in primary cortical neurons from a human SNCA genomic mouse model, that posiphen acts as a safe 5′ UTR-directed inhibitor of toxic α-synuclein buildup. A final validation of the SNCA 5′ UTR target is to be achieved by unchanged β-synuclein (β-syn) and γ-synuclein (γ-syn) expression and is a focus of current studies. Importantly, we determined that key primary metabolites of posiphen, likewise, lower α-syn expression and may hence add to posiphen’s actions on this target in animal and human studies.

Of further interest, we established that phenserine similarly effectively reduced α-syn levels but achieves this via a different mechanism, compared to posiphen, potentially by interacting through other elements in the 5′UTR or even 3′UTR, and thereby highlighting the sensitivity of the target to small structure-activity changes. These studies additionally demonstrate how α-synuclein and Aβ pathomechanisms can converge via interaction of the two amyloidogenic proteins [38–40], although Aβ and α-syn rarely co-localize in amyloid deposits [41], but provide evidence that a drug targeting one disease may have therapeutic potential in another.

In this regard, we noted that there is 50% sequence similarity between the 5′ UTRs of the APP and SNCA genes. We therefore predicted overlap in the spectrum of drugs that would suppress APP mRNA translation through its 5′ UTR with those that suppress α-synuclein, highlighting in particular phenserine and posiphen (with preliminary IC50 values in the order of 5 μM and less). In future studies, we aim to characterize the anti-α-synuclein efficacy of posiphen analogs in addition to metabolic analogs and other compounds in primary neurons and establish the in vivo efficacy of the most effective SNCA 5′ UTR translation blockers.

Acknowledgments

The authors thank Prof. Robert Nussbaum and Dr. Yien-Ming Kuo (UCSF) for their PAC-Tg(SNCA) mice. This research was supported (i) for J. T. Rogers by the Michael J. Fox Foundation Novel Drug Discoveries Award, National Institute of Aging R01 AG20181, NINDS R21NS059434, Alzheimer’s Association Zenith Award 09–131352 and ISOA (ii) for N. H. Greig by the Intramural Research Program, National Institute on Aging, National Institutes of Health, (iii) for I. Cantuti-Caslevetri: the MGH/MIT Morris Udall Center of Excellence in PD Research (NIH NS38372) and the APDA Advanced Center for Parkinson Research at MGH, and (iv) for C. M. Cahill by NIH HD012437 29 S-1.

References


P. Davies, D. Moualla, and D. R. Brown, “α-synuclein is a cellular ferrireductase,” *PLoS ONE*, vol. 6, no. 1, Article ID e15814, 2011.


