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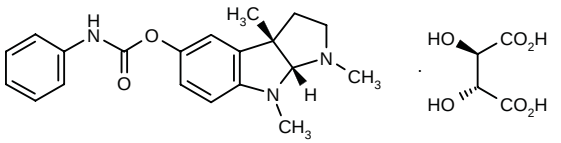
Phenserine Tartrate

Treatment of Alzheimer's Disease

(–)-5-*O*-(*N*-Phenylcarbamoyl)eseroline L-tartrate

N-Phenylcarbamic acid (3a*S*,8a*R*)-1,3a,8-trimethyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-*b*]indol-5-yl ester L-tartrate



$C_{20}H_{23}N_3O_2.C_4H_6O_6$

Mol wt: 487.5061

CAS: 156910-61-1

CAS: 101246-66-6 (as free base)

CAS: 159652-53-6 (as racemate)

EN: 204755

Abstract

Alzheimer's disease (AD) is a progressive and ultimately fatal disorder predominantly affecting the elderly. AD has no known cure and investigation continues for a treatment and preventative therapy. To date, several products have emerged with a variety of mechanisms of action. Because a reduced number of cholinergic markers and cells have been found in the brains of AD patients, researchers have hypothesized that the symptoms of AD may be due to a central cholinergic deficiency. Thus, cholinergic therapy is considered an attractive treatment for AD. One of the most effective types of cholinergic therapy is inhibition of the enzyme responsible for hydrolysis of acetylcholinesterase (AChE), which results in an increase in acetylcholine, a neurotransmitter involved in memory and cognition. Phenserine tartrate, a phenylcarbamate of physostigmine, is one such compound that has been shown to potently and selectively inhibit AChE. Phenserine is less toxic than physostigmine and tacrine and has been demonstrated to increase memory and learning in rats. The compound is more brain-selective than other agents and more rapidly cleared from the blood, suggesting an improved safety profile. Moreover, phenserine has also been shown to inhibit formation of β-amyloid precursor protein, suggesting the possibility of slowing progression of AD. Phenserine has been shown to be safe and well tolerated in phase I and II trials as a treatment for AD, and phase III development is planned for the near future.

Synthesis

Phenserine can be obtained by several ways:

1) Hydrolysis of physostigmine (I) with either NaOMe in ethanol (1, 2) or NaOBu in refluxing butanol (3) (better yield) gives eseroline (II), which is condensed with phenyl isocyanate (III) by means of NaOMe in benzene (1, 2), Na in benzene (4) or Na in ether/benzene (5). Scheme 1.

2) Condensation of 5-methoxy-1,3-dimethyl-2,3-dihydro-1*H*-indol-2-one (IV) with 2-chloro-*N*,*N*-dimethylethylamine (V) by means of $NaNH_2$ in toluene gives 3-[2-(dimethylamino)ethyl]-5-methoxy-1,3-dimethyl-2,3-dihydro-1*H*-indol-2-one (VI), which is reduced with Vitride (sodium bis(2-methoxyethoxy)aluminum dihydride) in toluene to yield the secondary alcohol (VII). Optical resolution of (VII) by means of (+)-2,3-di-*O*-(*p*-toluoyl)tartaric acid provides the (2*S*,3*S*)-diastereomer (VIII), which by reaction with methyl iodide in ethyl ether affords the ammonium salt (IX). Cyclization of compound (IX) by means of methylamine in acetonitrile provides esermethole (X), the methyl ether of eseroline, which is demethylated by means of BBr_3 in dichloromethane to give eseroline (II). Finally, eseroline is condensed with phenyl isocyanate (VIII) by means of Na in ethyl ether (6). Scheme 2.

3) Condensation of 5-methoxy-1,3-dimethyl-2,3-dihydro-1*H*-indol-2-one (IV) with 2-chloroacetonitrile (XI) by means of a chiral phase transfer catalyst gives 2-(5-methoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1*H*-indol-3-y)-acetonitrile, which is separated into enantiomers by chromatography. Reductocyclization of the (*S*)-enantiomer (XII) by means of Vitride affords (3a*S*)-N^1-noresermethole (XIII), which is submitted to a reductive methylation to provide esermethole (X). *O*-demethylation of (X) by means of BBr_3 or $AlCl_3$ gives eseroline (II), which is finally condensed with phenyl isocyanate (7). Scheme 3.

Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders leading to dementia. It

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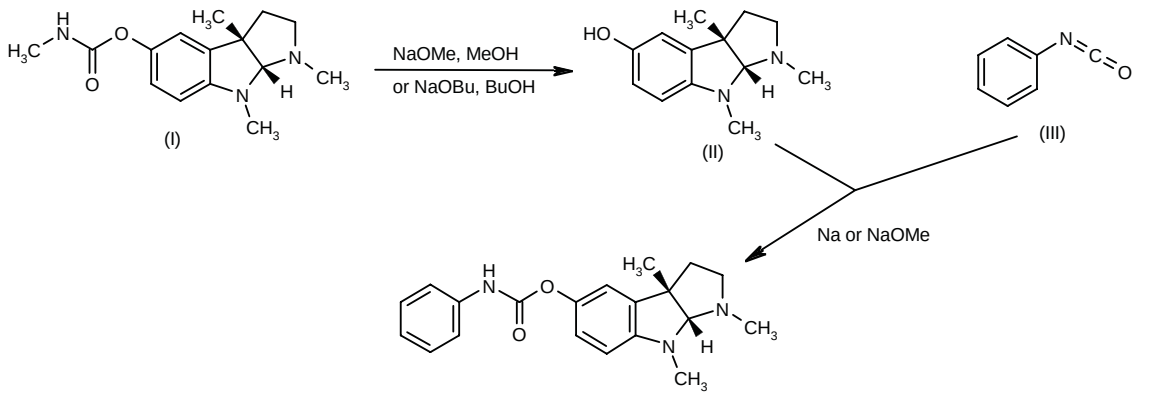
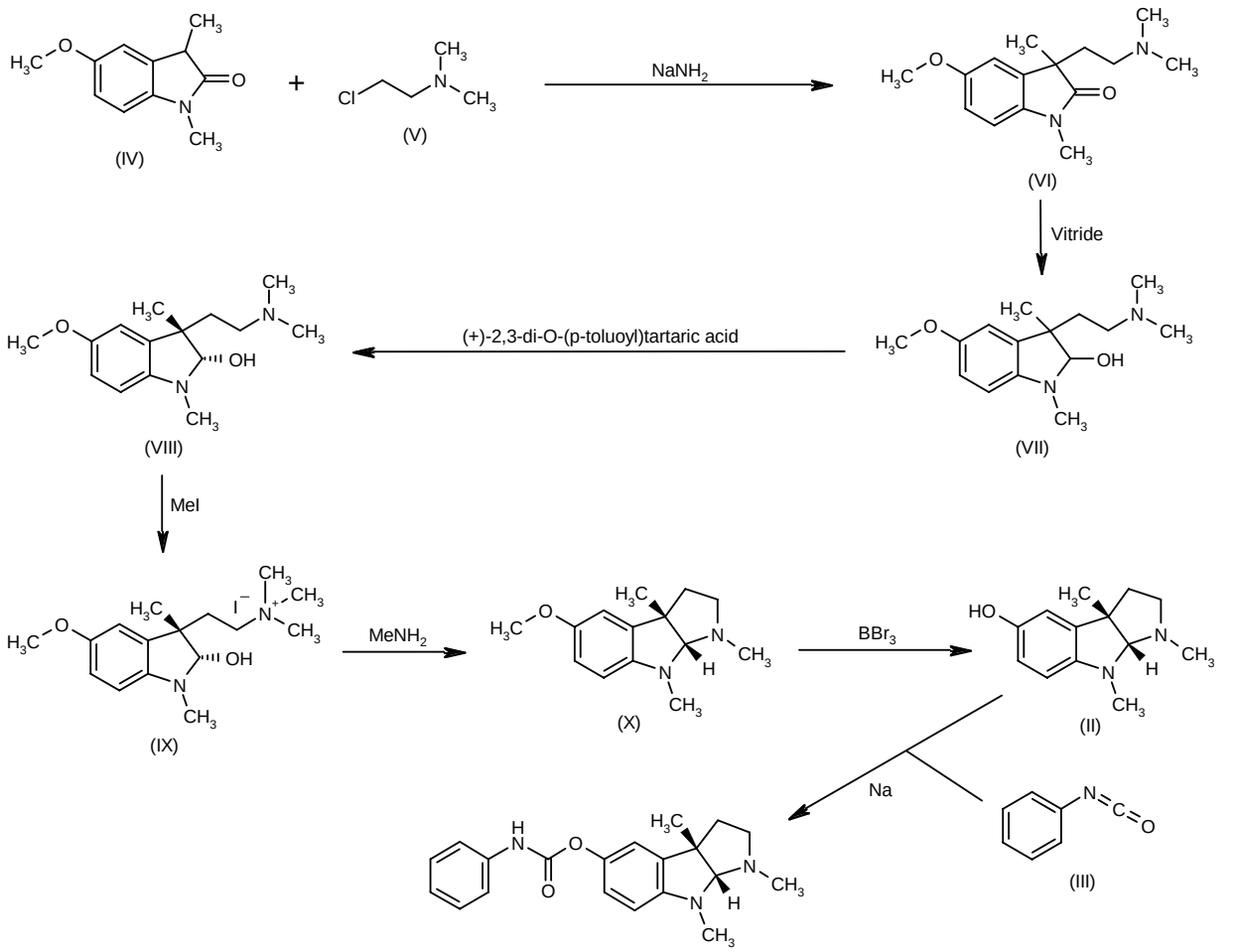
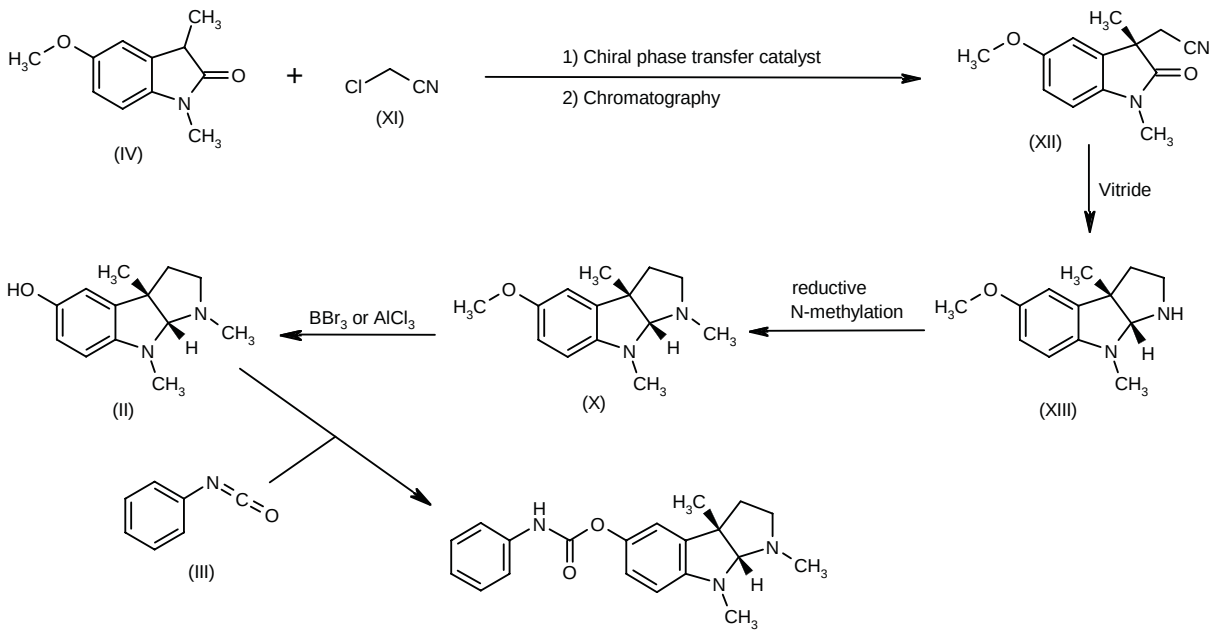
Scheme 1: Synthesis of Phenserine



Scheme 2: Synthesis of Phenserine



Scheme 3: Synthesis of Phenserine



accounts for about one-half to two-thirds of all cases. AD is a progressive and ultimately fatal disorder that predominantly affects the elderly. It affects parts of the brain that control memory, thought and language. Currently, the number of individuals worldwide suffering from AD has been estimated to be 15 million and this number includes approximately 4 million in the U.S. alone, where 360,000 new cases are reported each year. Until a cure or preventative therapy is discovered, it is estimated that 45 million people will be afflicted by the disorder worldwide by 2050. The prevalence of the disease doubles every 5 years after the age of 65. For example, in the U.S. the prevalence of AD among individuals aged 65 or older is 8% while the rate is 30% among those aged 85 or older (8, 9).

AD is classified according to age onset (before or after age 60) and mode of occurrence (familial or sporadic). Late-onset AD is the most common type, typically occurring after age 60 although degenerative processes in the brain may be evident as early as 20 years prior to emergence of symptoms. Symptoms of AD include gradual memory loss, reduced ability to perform routine tasks, disorientation regarding space and time, difficulties in learning, loss of language skills, impaired judgement, rapid mood changes and alterations in personality. It appears that AD disrupts normal thinking and memory via a blockade of signaling between neurons. The disease generally begins with mild cognitive impairment as the neurons in the part of the brain that controls memory (*i.e.*, hippocampus-entorhinal complex) are destroyed, resulting in short-term memory loss and difficulties in performing easy, routine tasks. Later, the disease attacks the cerebral cortex resulting in a reduction in language skills and alterations in personality and the ability to make judgements. Finally, other brain areas are affected and eventually atrophied so that the patient ultimately becomes helpless, incontinent, bedridden and unresponsive to the outside world (8).

AD is characterized neuropathologically by abnormal protein deposits in the brain and cerebral vasculature which include extracellular amyloid plaques or so-called senile plaques consisting mainly of amyloid β-protein (Aβ) and intracellular neurofibrillary tangles which are made up of tau protein. Aβ plays a crucial role in the pathogenesis of AD. It is the amyloidogenic 39-42 residue fragment which results from the cleavage of β-amyloid precursor protein (APP). Another important pathogenic component of AD is the inflammation which accompanies amyloid accumulation and several inflammatory molecules produced by neurons, astrocytes and microglia can be localized in AD lesions. These molecules include complement proteins, inflammatory cytokines, acute-phase reactants, proteases and protease inhibitors, and many exhibit neurotoxic effects, thus also contributing to the progressive neuronal loss manifesting in AD (8, 10, 11).

The actual causes of AD are unknown although genetic disposition and environmental factors (*e.g.*, exposure to aluminum, zinc, lead and other metals) have been implicated in development of the disorder. Strong evidence exists pointing toward a genetic component. Apolipoprotein E allele ε4 (ApoE-ε4) on chromosome 19 is associated with an increase in neurofibrillary pathology

and is linked to an enhanced risk of late-onset (sporadic) AD; risk increases with the number of copies of the allele present. However, this mutation is not solely responsible for the development of AD. Another gene linked to late-onset AD is A2M. This gene encodes the plasma protein α_2-macroglobulin (αM2) which binds soluble Aβ and mediates its degradation. Other mutations linked to the disorder include mutations in presenilin-1 (PS1) and presenilin-2 (PS2) genes on chromosome 14.1 and the APP genes on chromosome 21 (8).

The search for a cure and preventative therapy for AD continues with a variety of products having different mechanism of action (*e.g.*, antiinflammatory, hormonal replacement and Aβ-targeted therapies among others) emerging. One type of treatment that is particularly attractive is cholinergic therapy. The brains of AD patients have a reduced number of cholinergic cells and cholinergic markers (*i.e.*, reductions in presynaptic cholinergic receptors and levels of acetylcholine [ACh] and choline acetyltransferase [ChAT]) and researchers have hypothesized that a central cholinergic deficiency causes the symptoms of AD. Cholinergic therapy includes 3 approaches: enhancement of ACh production; interference with ACh degradation through inhibition of acetylcholinesterase (AChE); and cholinergic muscarinic agonissm at postsynaptic brain neurons. To date, the most effective cholinergic therapy is inhibiting the enzyme responsible for hydrolysis of ACh. ACh is a neurotransmitter implicated in memory and cognition, and therefore increasing ACh levels would be extremely beneficial in patients with AD (8).

Phenserine tartrate, a phenylcarbamate of physostigmine first prepared in 1916 (4), is one such AChE inhibitor that has been shown to potently and selectively inhibit AChE (greater than 50-fold selectivity over butyrylcholinesterase [BChE]). When compared to other marketed agents for AD, phenserine is more brain-selective and is more rapidly cleared from the blood. It has a brain-to-blood ratio of 10:1. Thus, phenserine can result in maximum therapeutic effects in improving memory without severe and bothersome side effects. In fact, phenserine was shown to be less toxic than both physostigmine and tacrine. Moreover, in addition to its potent action on AChE, phenserine has also been shown to inhibit APP formation, suggesting the possibility of slowing the progression of AD. Phenserine was selected for further development as a treatment for AD.

Pharmacological Actions

A number of *in vitro* studies have compared the ability of phenserine to inhibit AChE and BChE with (–)-physostigmine and other carbamate analogues. Although phenserine was not as potent as (–)-physostigmine against electric eel AChE (IC_{50} = 350 ± 90 *vs.* 61 ± 187 nM), it was more selective than the latter agent for AChE as compared to human plasma BChE (1300 ± 400 *vs.* 14 ± 6 nM). Comparable inhibitory activity was observed for phenserine (IC_{50} = 24 ± 6 nM) and (–)-physostigmine (IC_{50} = 27.9 ± 2.4 nM) against a human erythrocyte AChE preparation. Phenserine was more selective for and more potently inhibited AChE from human brain frontal cortex (IC_{50} = 36 ± 3 nM) and caudate nucleus (IC_{50} = 21 ± 1 nM) as compared to BChE from human brain frontal cortex (IC_{50} = 2500 ± 1100 nM) (12-15). The *in vitro* inhibitory activity of phenserine against a several AChE and BChE preparations is summarized in Table I.

Phenserine was also effective in inhibiting AChE *in vivo*. In rats, a single i.v. dose (1 mg/kg) resulted in steady-state inhibition of plasma AChE (76.5% within 5 min of dosing and over 50% for at least 8 h postdosing; half-life > 8.25 h). At 240 min, AChE in cerebrospinal fluid was inhibited by more than 90% as compared to 60% observed for plasma AChE. In contrast, BChE was inhibited by less than 30%. In addition, phenserine (2 and 4 mg/kg i.p.) enhanced ACh levels in the striatum 193 ± 38 and 344 ± 111%, respectively, at 10 min postdosing; these levels remained significantly increased (187 ± 23 and 208 ± 15% at 90 min, respectively) as compared to pretreatment levels (16, 17).

The kinetics of inhibition of human erythrocyte AChE and rat brain AChE were evaluated in 2 studies. In the first study, phenserine (0.025-0.40 μM) was shown to dose-dependently inhibit human erythrocyte AChE with an IC_{50} value of 0.0453 μM. The K_m for the hydrolysis of the substrate, acetylthiocholine iodide, and the V_{max} in this preparation were determined to be 0.124 mM and 0.980 μmol/min/mg protein, respectively. Phenserine-induced inhibition of AChE was concluded to be noncompetitive, with a K_i value estimated to be 0.048 μM (16). In the second study, the inhibitory activity of phenserine was compared with tolserine against rat brain membrane-bound AChE. Both agents inhibited AChE in a dose-dependent manner (IC_{50} = 411 and 195 nM, respectively). The K_m for hydrolysis of acetylthiocholine iodide and the V_{max} were 0.037 mM and 0.038 μmol/min/mg protein, respectively. It was concluded that inhibition for both agents was of a mixed type in this preparation and the K_i values were estimated to be 311.3 nM for phenserine and 250.2 nM for tolserine (18, 19).

The efficacy of phenserine in improving cognition *in vivo* has been demonstrated in both young and elderly rats. Administration of phenserine (1.5, 3, 4, 5, 7.5 or 10 mg/kg i.p. 60 min before maze training) to young rats (3 months old) was shown to attenuate scopolamine (0.75 mg/kg 30 min before training)-induced learning impairment in a shock-motivated T-maze. All doses of phenserine except for 7.5 mg/kg improved error performance, run time, shock frequency and shock duration. The 7.5 and 10 mg/kg doses were associated with side effects such as chewing, grooming and shakes and animals treated with the 10 mg/kg became very aggressive (16). Treatment with phenserine (0.25, 0.5 or 0.75 mg/kg i.p. 24 after training) also attenuated learning impairment in the shock-motivated 14-unit T-maze induced by the NMDA receptor antagonist, 3-(±)2-carboxypiperzin-4-yl) propyl phosphonic acid (CPP; 9 mg/kg i.p. 24 h after training). Although all phenserine doses were effective, the

Table I: In vitro activity of phenserine tartrate (from Prous Science Integrity®).

Activity	Material	Parameter	Value	Ref.
AChE affinity	Brain, rat	K_d	0.311 μM	19
AChE inhibition	Electrophorus electricus	IC_{50}	0.350 ± 0.090 μM	12
AChE inhibition	Brain, rat	K_i	89.2 nM	19
AChE affinity	Brain, rat	IC_{50}	0.411 μM	19
BChE inhibition	Plasma, human	IC_{50}	1.30 ± 0.400 μM	12
AChE inhibition	Striatum, human	IC_{50}	0.021 ± 0.001 μM	13
AChE inhibition	Cortex, human	IC_{50}	0.036 ± 0.003 μM	13
AChE inhibition	Erythrocytes, human	IC_{50}	0.024 ± 0.006 μM	14
AChE inhibition	Erythrocytes, human	IC_{50}	0.024 ± 0.006 μM	13
AChE inhibition	Plasma, human	IC_{50}	0.024 ± 0.006 μM	33
BChE inhibition	Erythrocytes, human	IC_{50}	1.56 ± 0.045 μM	33
BChE inhibition	Plasma, human	IC_{50}	1.30 ± 0.085 μM	14
AChE inhibition	Electric organ, Torpedo	IC_{50}	0.350 ± 0.090 μM	13
BChE inhibition	Plasma, human	IC_{50}	1.30 ± 0.400 μM	13
BChE inhibition	Cortex, human	IC_{50}	2.50 ± 1.10 μM	13
AChE inhibition	Erythrocytes, human	IC_{50}	0.045 μM	18
AChE inhibition	Erythrocytes, human	K_i	48 nM	18

lowest dose was the most potent in significantly decreasing the number of errors as compared to control CPP-treated rats (20).

Phenserine also displayed marked activity in ameliorating learning in aged rats (21-22 months). Chronic treatment of aged rats with phenserine (1-3 mg/kg i.p. for 5 days) significantly reduced the number of errors made in the shock-motivated 14-unit T-maze as compared to controls. Although minimal motoric side effects such as fine tremor were observed in a few rats treated with the high (3 mg/kg) dose, no side effects were associated with doses of 1-2 mg/kg. Other performance variables such as run time, shock frequency and duration were also decreased in phenserine-treated rats although the effects were less consistent than those observed for the reduction in errors (21).

In addition to its potent action in inhibiting the acetylcholinergic system resulting in cognition enhancement, phenserine has been shown in a number of studies to inhibit APP formation. The effects of phenserine (5 or 50 μM for 16 h) on intra- and extracellular APP and Aβ production in human neuroblastoma cells lines (SK-N-SH, SH-5Y-5Y) and human astrocytoma cells (U373) were examined. Treatment with the agent markedly decreased intra- and extracellular APP protein levels in both neuroblastoma and astrocytoma cells in a time- and dose-dependent manner. In addition, secreted Aβ was also significantly decreased in neuroblastoma cells by 14% and 34% at 8 and 16 h, respectively. APP synthesis was found to be significantly reduced with phenserine treatment (about 50%). However, treatment with the agent had no effect on APP mRNA levels in astrocytoma cells. Because phenserine reduced APP protein expression without altering message levels, it was concluded that the agent modulates APP expression at a posttranscriptional level (22-24).

A further study *in vitro* has elucidated a novel mechanism of action by which phenserine modulates APP processing. This study used human astrocytoma cells (U373) transfected with 5' UTR-CAT which contains 90 nucleotides of the 5'-untranslated region (UTR) of APP mRNA in front of the reported chloramphenicol acetyl transferase gene (CAT). The 5'-UTR of APP contains an important IL-1 responsive element and the TGF responsive box (CAGA box). Treatment of cells with phenserine (25 g/ml for 44 h) decreased CAT activity fused to the 5'-UTR of APP; CAT activity of the CAT reporter fused to a control vector was unaffected. In contrast, treatment of transfected cells with TGFβ1 (5 ng/ml) increased CAT activity. From these results, it was suggested that phenserine controls translation of APP mRNA via a putative IL-1 responsive element and/or TGF element (23).

Phenserine was shown to reduce APP *in vivo*. The efficacy of phenserine (2.5 mg/kg s.c. b.i.d. for 7 days) in reducing lesion-induced increases in secreted APP was demonstrated in rats with scopolamine-induced lesions of the nucleus basalis of Meynert. Phenserine treatment significantly reduced levels of secreted APP in the cerebrospinal fluid (CSF) of lesioned rats. Further analysis of brains showed that phenserine, an acetyl-selective cholinesterase inhibitor, was 70-fold more selective for cortical AChE than BChE (IC_{50} = 22.2 ± 1.4 *vs.* 1552 ± 272 nM). In contrast, the nonspecific cholinesterase inhibitor diisopropyl fluorophosphate (DFP; 1.5 mg/kg s.c.) which is 9-fold more selective for BChE, potently reduced both brain cortical AChE and BChE (IC_{50} = 38 ± 1.5 and 4.3 ± 2.6 nM, respectively), but did not alter APP levels in CSF of lesioned rats (25).

Pharmacokinetics

The pharmacokinetics of phenserine (1 mg/kg i.v.) were determined in rats. The C_{max} of the agent following a single dose was 117 ± 19 ng/ml at 5 min and the agent

was rapidly cleared from plasma ($t_{1/2}$ = 12.6 min). Levels of phenserine were significantly 10-fold higher in the brain (peak = 1246 ± 101 ng/g at 5 min) than in plasma and similarly decreased rapidly, with a $t_{1/2}$ of 8.5 min. The volume of distribution and clearance values obtained were 5516 ml/kg and 303 ml/min/kg, respectively (17).

Phenserine was shown to be significantly less toxic than physostigmine *in vivo* in rats. The maximum tolerated dose (MTD) of physostigmine was 0.5 mg/kg i.p. while doses of 15 mg/kg phenserine were tolerated. In experiments in which rats were administered phenserine at doses 5 times those shown to enhance cognition (1 and 5 mg/kg i.p.) for 28 consecutive days, no toxicity was reported after examination of blood parameters, markers of renal and hepatic function and histological analysis of brain, kidney and liver (26).

The safety and pharmacokinetics of single-dose phenserine tartate (5-20 mg p.o.) have been evaluated in a phase I, blinded, placebo-controlled, dose escalation trial involving 32 healthy elderly subjects (55-80 years). Doses of 5 and 10 mg were well tolerated. The MTD was determined to be 10 mg. No serious adverse events were reported. The most common adverse events were headache and vomiting with an increase in severity and incidence seen at the 20 mg dose level. Although the C_{max} and $AUC_{0\text{-}24\ h}$ values increased with dose, neither were dose-proportional. The C_{max} and mean peak inhibition (I_{max}) of AChE for subjects administered 10 mg were 1.95 ng/ml at 1.5 h and 26 % at 1.75 h, respectively. The half-life of AChE inhibition was 11 h (27).

The safety and pharmacokinetics of multiple-dose phenserine (5 and 10 mg p.o. once or twice daily for 6 days) were evaluated in a randomized, double-blind, placebo-controlled, phase I trial involving 32 healthy elderly volunteers (55-80 years). Phenserine was safe and well tolerated with no dose-limiting toxicity or serious adverse events observed. The most frequent adverse event was headache which was not dose-related and occurred with similar frequency in phenserine- and placebo-treated groups. The C_{max} increased with dose and was similar for subjects treated once or twice daily. The I_{max} of AChE was about 15% and 30% for the 5 and 10 mg groups, respectively, with no difference seen between once- and twice-daily dosing (28).

Clinical Studies

The efficacy and safety of a 12-week oral phenserine treatment (5 mg b.i.d. for 2 weeks followed by 10 mg b.i.d. for 10 weeks) were examined in a randomized, double-blind, placebo-controlled, phase II trial involving 72 patients with mild to moderate AD; the trial included a 4-week washout period after which patients were reassessed. A significant increase in cognitive performance was observed in phenserine-treated patients according to scores from the Cambridge Neuropsychological Test Automated Battery (CANTAB) Paired Associative Learning (PAL) task. This task, which is sensitive to short-term memory changes, revealed that while placebo-treated patients' scores declined by 6.8 points, those treated with phenserine improved by 9.7 points. A significant improvement was also observed with phenserine treatment in the maze assessment (seconds to complete) of the extended ADAS-cog. Overall ADAS-cog scores after 12 weeks of treatment improved by 2.57 points in the phenserine groups as compared to only 0.72 points on placebo. After the 4-week washout period, ADAS-cog scores returned to baseline in those patients previously treated with phenserine, indicating a reversal of treatment effects (29).

In the above study, phenserine was well tolerated. Of the phenserine-treated patients, 63% reported 1 or more adverse events as compared to 50% of placebo patients. Three phenserine-treated patients and 1 placebo-treated patient withdrew for dizziness. Only 1 serious adverse event resulting in withdrawal was reported but was unrelated to treatment. The most common adverse event reported was dizziness seen in 4.2% and 17% of the patients in the phenserine groups treated with b.i.d. doses of 5 and 10 mg, respectively; with the exception of the 1 patient who withdrew for dizziness, this adverse event was not reported in other placebo-treated patients. Nausea was reported in 4.2% and 8.5% of patients treated with 5 and 10 mg b.i.d. phenserine, respectively, as compared to 0% with placebo. In addition, 4 (8.5%), 3 (6.4%) and 3 (6.4%) patients in the 10 mg b.i.d. group developed peripheral edema, skin disorder and sinus bradycardia, respectively. No patients on placebo experienced peripheral edema or skin disorder, although the rate for sinus bradycardia was 8.3%. It was concluded that the adverse events observed were consistent with those for other marketed anticholinesterase agents although the incidence was less (30).

Phenserine continues to undergo phase II trials as a treatment for AD and phase III development is planned for the near future (31, 32).

Source

Axonyx, Inc. (US).

References

1. Marta, M., Castellano, C., Oliverio, A., Pavone, F., Pagella, P.G., Brufani, M., Pomponi, M. *New analogs of physostigmine: Alternative drugs for Alzheimer's disease?* Life Sci 1988, 43: 1921-8.

2. Pomponi, M., Pavone, F., Oliverio, A., Marta, M., Castellano, C., Brufani, M. (Consiglio Nazionale delle Ricerche). *Physostigmine derivs. with acetylcholinesterase inhibition properties, and the relative production process.* EP 0154864, EP 0354594.

3. Yu, Q.-S., Schönenberger, B., Brossi, A. *Reactions of (–)-physostigmine and (–)-N-methylphysostigmine in refluxing butanol and at high temperature: Facile preparation of (–)-eseroline.* Heterocycles 1987, 26: 1271-5.

4. Polonovski, M. *Study on the alkaloids of the Calabar bean (V). Action of phenyl isocyanate: Phenylic homologs of eserine and genserine.* Bull Soc Chim Fr Mém 1916, 19: 46-59.

5. Brossi, A., Brzostowska, M., Rapoport, S., Greig, N., He, X.-S. (US Department of Health & Human Services). *Substd. phenserines as specific inhibitors of acetylcholinesterase.* EP 0606366, JP 1995503703, US 5171750, WO 9306105.

6. Pei, X.-F., Greig, N.H., Flippen-Anderson, J.L., Bi, S., Brossi, A. *125. Total synthesis of racemic and optically active compounds related to physostigmine and ring-C heteroanalogues from 3-[2'-(dimethylamino)ethyl]-2,3-dihydro-5-methoxy-1,3-dimethyl-1H-indol-2-ol.* Helv Chim Acta 1994, 77: 1412-22.

7. Brossi, A., Pei, X.-F., Greig, N.H. *Phenserine, a novel anticholinesterase related to physostigmine: Total synthesis and biological properties.* Aust J Chem 1996, 49: 171-81.

8. Prous Science Drug R&D Backgrounders: *Alzheimer's disease (online publication).* Updated December 20, 2002.

9. Albert, M.S., Drachman, D.A. *Alzheimer's disease: What is it, how many people have it, and why do we need to know?* Neurology 2000, 55: 166-8.

10. Griffin, S.T. *Cytokines and Alzheimer's disease.* Neurobiol Aging 2000, 21(15): Abst 1305.

11. McGeer, P.L. *Innate immunity, autoxicity and degenerative neurologies.* Neurobiol Aging 2000, 21(15): Abst 1302.

12. Yu, Q.-S., Atack, J.R., Rapoport, S.I., Brossi, A. *Carbamate analogues of (–)-physostigmine: In vitro inhibition of acetyl- and butyrylcholinesterase.* FEBS Lett 1988, 234: 127-30.

13. Atack, J.R., Yu, Q.-S., Soncrant, T.T., Brossi, A., Rapoport, S.I. *Comparative inhibitory effects of various physostigmine analogs against acetyl- and butyrylcholinesterases.* J Pharmacol Exp Ther 1989, 249: 194-202.

14. Brzostowska, M., He, H.-S., Greig, N.H., Rapoport, S.I., Brossi, A. *Phenylcarbamates of (–)-eseroline, (–)-N1-noreseroline and (–)-physovenol: Selective inhibitors of acetyl and/or butyrylcholinesterase.* Med Chem Res 1992, 2: 238-46.

15. Yu, Q.-S., Greig, N.H., Holloway, H.W. *Syntheses and anticholinesterase activities of (3aS)-N1,N8-bisnorphenserine, (3aS)-N1,N8-bisnorphysostigmine, their antipodal isomers, and other potential metabolites of phenserine.* J Med Chem 1998, 41: 2371-9.

16. Iijima, S., Greig, N.H., Garofalo, P., Spangler, E.L., Heller, B., Brossi, A., Ingram, D.K. *Phenserine: A physostigmine derivative that is a long-acting inhibitor of cholinesterase and demonstrates a wide dose range for attenuating a scopolamine-induced learning impairment of rats in a 14-unit T-maze.* Psychopharmacology 1993, 112: 415-20.

17. Greig, N.H., De Micheli, E., Holloway, H.W. et al. *The experimental Alzheimer drug phenserine: Preclinical pharmacokinetics and pharmacodynamics.* Acta Neurol Scand 102(Suppl. 176): 74-84.

18. Al-Jafari, A., Kamal, M.A., Greig, N.H., Alhomida, A.S., Perry, E.R. *Kinetics of human erythrocyte acetylcholinesterase inhibition by a novel derivative of physostigmine: Phenserine.* Biochem Biophys Res Commun 1998, 248: 180-5.

19. Al-Jafari, A.A., Kamal, M.A., Alhomida, A.S., Greig, N.H. *Kinetics of rat brain acetylcholinesterase inhibition by two experimental Alzheimer's disease drugs, phenserine and tolserine.* J Biochem Mol Biol Biophys 2000, 4: 323-35.

20. Patel, N., Spangler, E.L., Greig, N.H., Yu, Q.-S., Ingram, D.K., Meyer, R.C. *Phenserine, a novel acetylcholinesterase inhibitor, attenuates impaired learning of rats in a 14-unit T-maze induced by blockade of the N-methyl-D-aspartate receptor.* NeuroReport 1998, 9: 171-6.

21. Ikari, H., Spangler, E.L., Greig, N.H., Pei, X.-F., Brossi, A., Speer, D., Patel, N., Ingram, D.K. *Maze learning in aged rats is enhanced by phenserine, a novel anticholinesterase.* NeuroReport 1995, 6: 481-4.

22. Lahiri, D.K., Farlow, M.R., Hintz, N., Utsuki, T., Greig, N.H. *Cholinesterase inhibitors, β-amyloid precursor protein and amyloid beta-peptides in Alzheimer's disease.* Acta Neurol Scand 2000, 102(Suppl. 176): 60-7.

23. Shaw, K.T.Y., Utsuki, T., Rogers, J., Yu, Q.-S., Sambamurti, K., Brossi, A., Ge, Y.-W., Lahiri, D.K., Greig, N.H. *Phenserine regulates translation of β-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development.* Proc Natl Acad Sci USA 2001, 98: 7605-10.

24. Lahiri, D.K., Utsuki, T., Shaw, K.T.Y., Ge, Y.-W., Sambamurti, K., Eder, P.S., Rogers, J.T., Farlow, M.R., Giordino, T., Greig, N.H. *Phenserine regulates translation of β-amyloid precursor protein message. A novel drug target for Alzheimer's disease.* In: Mapping the Progress of Alzheimer's and Parkinson's Disease, Y. Mizuno, A. Fisher, and I. Hanin (Eds.), Kluwer Academi/ Plenum Publishers, 2002, 211-5.

25. Haroutunian, V., Greig, N., Pei, X.-F., Utsuki, T., Gluck, R., Acevedo, L.D., Davis, K.L., Wallace, W.C. *Pharmacological modulation of Alzheimer's beta-amyloid precursor protein levels in the CSF of rats with forebrain cholinergic system lesions.* Mol Brain Res 1997, 46: 161-8.

26. Greig, N.H., Ingram, D.K., Pei, X.-F., Brossi, A., Holloway, H.W., Soncrant, T.T. *Phenserine, a new drug for Alzheimer's disease: Favorable toxicity profile.* Soc Neurosci Abst 1995, 21(Part 3): Abst 773.15.

27. Greig, N.H., Holloway, H.W., Brownell, L., Flanagan, D.R. Jr., Canfield, C.J., Burford, R.G., Bruinsma, G.B. *Anticholinesterase profile of phenserine in healthy elderly human subjects.* 8th Int Conf Alzheimer's Dis Relat Disord (July 20-25, Stockholm) 2002, Abst 328.

28. Burford, R.G., Bruinsma, G.B., Brownell, L., Holloway, H.W., Greig, N.H., Flanagan, D.R. Jr. *A double-blind, randomized, sequential group, multiple oral dose study of the safety, tolerance and anticholinesterase profile of phenserine tartrate in healthy human subjects.* 8th Int Conf Alzheimer's Dis Relat Disord (July 20-25, Stockholm) 2002, Abst 331.

29. Kirby, L.C., Baumel, B., Eisner, L.S., Safirstein, B.E., Burford, R.G., Bruinsma, G.B. *The efficacy of phenserine tartrate following 12 weeks of treatment in Alzheimer's disease (AD) patients.* 8th Int Conf Alzheimer's Dis Relat Disord (July 20-25, Stockholm) 2002, Abst 333.

30. Kirby, L.C., Baumel, B., Eisner, L.S., Safirstein, B.E., Burford, R.G., Bruinsma, G.B. *The safety and side effect profile of phenserine tartrate following twelve (12) weeks of treatment in Alzheimer's disease (AD) patients.* 8th Int Conf Alzheimer's Dis Relat Disord (July 20-25, Stockholm) 2002, Abst 351.

31. *Axonyx - Investing in treatments for Alzheimer's disease and other cognitive disorders.* Axonyx Web Site November 2002.

32. *Axonyx announces phenserine clinical program for 2003.* Axonyx Press Release January 29, 2003.

33. Yu, Q.-S., Holloway, H.W., Utsuki, T., Brossi, A., Greig, N.H. *Synthesis of novel phenserine-based-selective inhibitors of butyrylcholinesterase for Alzheimer's disease.* J Med Chem 1999, 42: 1855-61.

Additional References

Haroutunian, V., Greig, N.H., Gluck, R., Fiber, E., Davis, K.L., Wallace, W.C. *Selective attenuation of lesion-induced increases in secreted β-APP by acetylcholinesterase inhibitors.* Soc Neurosci Abst 1995, 21(Part 1): Abst 208.1.

Lahiri, D.K., Farlow, M.R., Ge, Y.-W., Utsuki, T., Yu, Q.-S., Greig, N.H. *The acetylcholinesterase inhibitor phenserine regulates APP processing by a novel mechanism.* Neurology 2001, 56(8, Suppl. 3): Abst P05.066.

Lahiri, D.K., Ge, Y., Sambamurti, K., Utsuki, T., Greig, N.H., Farlow, M.R. *A novel target for phenserine drug in regulating the translation of APP message.* J Neurochem 2001, 78(Suppl. 1): Abst BP02-51.

Utsuki, T., Lahiri, D.K., Sambamurti, K., Shaw, K.T., Yu, Q.-S., Holloway, H.W., Soncrant, T.T., Greig, N.H. *The selective acetyl- and butyrylcholinesterase inhibitors phenserine and cymserine reduce the secretion of Alzheimer's β-amyloid precursor protein (βAPP) in vitro.* Soc Neurosci Abst 1999, 25(Part 1): Abst 239.1.

Lahiri, D.K., Farlow, M.R., Greig, N.H. *Anticholinesterases, phenserine, physostigmine and tacrine on the secretion of β-amyloid precursor protein in cell lines.* Mol Biol Cell 1996, 7(Suppl.): 490A.

Greig, N.H., Soncrant, T.T., Brossi, A., Ingram, D.K., Holloway, H.W., Yu, O.S., Farlow, M., Lahiri, D.K. *Phenserine reduces the secretion of β-amyloid precursor protein in cultured cells: Dual therapeutic approach for Alzheimer's disease (AD).* Soc Neurosci Abst 1996, 22(Part 3): Abst 651.4.

Shaw, K.T.Y., Utsuki, T., Rogers, J., Sambamurti, K., Yu, Q.-S., Soncrant, T.T., Lahiri, D.K., Greig, N.H. *Phenserine regulates translation of βAPP mRNA by a putative interleukin-1 responsive element, a new target for Alzheimer disease drug development.* Soc Neurosci Abst 2000, 26(Part 2): Abst 859.7.

Lahiri, D.K., Farlow, M.R., Ge, Y., Sambamurti, K., Utsuki, T., Greig, N.H. *Phenserine regulates translation of APP message via a 5'-untranslated region: Identification of a novel drug target for Alzheimer's disease.* 5th Int Conf Prog Alzheimer Parkinson Dis (March 31-April 5, Kyoto) 2001, Abst III-AD-46.

Ikari, H., Greig, N., Spangler, E., Brossi, A., Pei, X.-F., Soncrant, T., Ingram, D.K. *Performance of aged rats in a 14-unit T-maze is improved following chronic treatment with phenserine, a novel anticholinesterase.* Soc Neurosci Abst 1993, 19(Part 2): Abst 514.7.

Greig, N.H., De Micheli, E., Pei, X.-F., Brossi, A., Ingram, D.K., Soncrant, T.T. *Phenserine, a new drug candidate for the treatment of Alzheimer's disease: In vivo pharmacokinetics and pharmacodynamics.* Soc Neurosci Abst 1994, 20(Part 1): Abst 254.5.

Haroutunian, V., Ahlers, S.T., Greig, N., Acevedo, L.D., Utsuki, T., Gluck, R., Davis, K.L., Wallace, W.C. *Cholinergic pharmacology of secreted Alzheimer's β-amyloid precursor protein levels in the CSF of rats.* Soc Neurosci Abst 1997, 23(Part 2): Abst 636.17.

Greig, N.H., Pei, X.-F., Soncrant, T.T., Ingram, D.K., Brossi, A. *Phenserine and ring C hetero-analogs: Drug candidates for the treatment of Alzheimer's disease.* Med Res Rev 1995, 15: 3-31.