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The neuroprotective mechanism of action of the multimodal drug ladostigil

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1. ABSTRACT

The recent therapeutic approach in which drug candidates are designed to possess diverse pharmacological properties and act on multiple targets has stimulated the development of the multimodal drug, ladostigil (TV3326) ((N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate). Ladostigil combines neuroprotective effects with monoamine oxidase -A and -B and cholinesterase inhibitory activities in a single molecule, as a potential treatment for Alzheimer's disease (AD) and Lewy Body disease. Preclinical studies show that ladostigil has antidepressant and anti-AD activities and the clinical development is planned for these dementias. In this review, we discuss the multimodal effects of ladostigil in terms of neuroprotective molecular mechanism *in vivo* and *in vitro*, which include the amyloid precursor protein processing; activation of protein kinase C and mitogen-activated protein kinase pathways; regulation of the Bcl-2 family members; inhibition of cell death markers and up-regulation of neurotrophic factors. Altogether, these scientific findings make ladostigil a potentially valuable drug for the treatment of AD.

2. INTRODUCTION

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease in the elderly population and it has been estimated that about 5% of adults over 65 years is affected by this disease (1). Its predominant clinical manifestation is the progressive memory deterioration and other changes in brain function, including disordered behavior and impairment in language, comprehension and visual-spatial skills (2). The neuropathology of AD is characterized by several features, including extracellular deposition of amyloid beta peptide (Aβ)-containing plaques in the cerebral cortical regions, accompanied by the presence of intracellular neurofibrillary tangles and a progressive loss of basal forebrain cholinergic neurons leading to reductions in cholinergic markers, such as acetylcholine levels, choline acetyltransferase (ChAT) and muscarinic and nicotinic acetylcholine receptor binding (3, 4). Additionally, there is accumulating evidence that many cytotoxic signals in the AD brain can initiate apoptotic processes, including oxidative stress (OS), inflammation and accumulation of iron at the sites of neurodegeneration (5-7). Significant reduction also occurs in serotoninergic

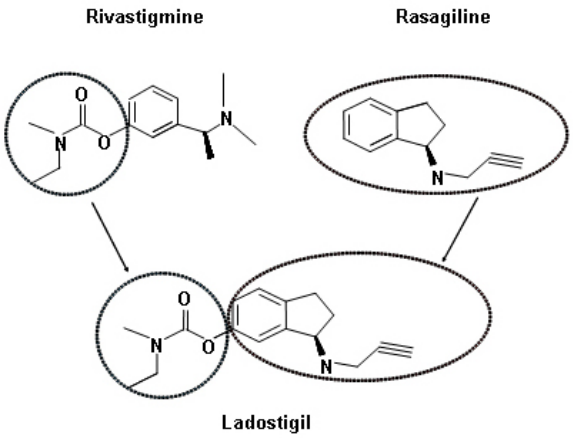

Figure 1. The chemical structures of the multifunctional-anti-Alzheimer drug, the R-enantiomer, ladostigil (TV3326) ((N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate) which has been synthesized with a carbamate cholinesterase inhibitory moiety of rivastigmine in the aminoindan structure of the selective MAO-B inhibitor, rasagiline.

and noradrenergic transmission, which might explain the relatively high incidence of depression found in AD patients (8, 9). Thus, it seems likely reasonable to conclude that AD therapy will require multiple drug therapy to address the varied pathological aspects of the disease.

Currently, numerous clinical trials have demonstrated the safety and efficacy of acetyl cholinesterase inhibitors (AChEIs) in the treatment of AD. Yet, their benefits in AD are likely to be more complex than simply replacement of lost acetylcholine (10-13). As reviewed recently, there is growing preclinical evidence that AChEIs block some of the fundamental neurodegenerative processes involved in AD (14). AChEIs, such as tacrine, donepezil, galantamine, huperazine A and ganstigmine were reported to protect neurons from death in various cell culture models of neurodegenerative diseases (14). In addition, there is evidence that several cholinesterase inhibitors (ChEIs) also affect various neuropathological markers of AD and modulate the cleavage of the non-amyloidogenic amyloid precursor protein (APP) processing (13). Therefore, it is suggested that AChEIs possessing properties of neuroprotection and/or APP processing might be more beneficial than those that only inhibit acetyl cholinesterase (AChE) to treat AD and in particular to prevent the pathogenesis of AD.

Recently, a multifunctional compound, ladostigil (TV3326) (N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate) (Figure 1) has been synthesized, with a carbamate cholinesterase inhibitory moiety of rivastigmine (15) in the aminoindan structure of the monoamine oxidase (MAO)-B inhibitor, anti-Parkinson drug, rasagiline (N-propargyl-(1R)-aminoindan) (16-18). Ladostigil has an inhibitory mechanism of acetyl and butyrylcholinesterase (BuChE) with a longer duration of action than rivastigmine (19), as well as an inhibitory activity of MAO and many of the neuroprotective actions of rasagiline (20-23), such as prevention of the fall in the mitochondrial potential and cytotoxicity in human neuroblastoma SH-SY5Y and rat PC12 cells in response to OS-induced by nitric oxide donor (NO), 3-morpholinosydnonimine (SIN-1) or glucose oxygen deprivation (20, 23, 24).

Several novel molecular targets are described to be involved in the substantial neuroprotective mechanism of action of ladostigil *in vitro* and *in vivo*, including regulation of APP processing (25, 26), activation of protein kinase C (PKC) and mitogen-activated protein kinase-(MAPK-) pathways (25, 26) and modulation of cell survival genes and proteins (27). In addition, ladostigil was demonstrated to reduce neuronal death in various cellular models of apoptosis (25, 27), as well as *in vivo* brain damage models, such as reducing mouse cerebral edema induced by closed head injury (21). The S-isomer of ladostigil, TV3279 has no MAO-A or- B inhibitory activity (28). However, both drugs have been shown to possess an anti-AD activity in preventing scopolamine-induced deficit in spatial learning (16). Thus, the brain selective MAO-A and B inhibitory activity of ladostigil accounts only for its neuroprotective-anti-PD activity in preventing N-methyl-4-phenyl-1,2,3,6 –tetrahydropyridine (MPTP)-induced nigrostriatal dopaminergic neurodegeneration in mice (29).

To date, various AChEIs have been demonstrated to produce significant symptomatic improvement in cognitive performance and behavioral abnormalities that occur in AD and the related disorders, Vascular and Lewy bodies dementias (30-32). Indeed, ladostigil is shown to improve cognitive deficits in aged monkeys (33), exhibits a novel potential antidepressant activity in rat models of anxiety and depression (19, 34, 35) and prevents memory deficits induced by scopolamine (28) or streptozotocin (STZ) in rat models of AD (36). Currently, the available anti-AD medications, which include AChEIs or N-methyl-D-aspartate (NMDA)-receptor antagonists use for the treatment of moderate to severe Alzheimer dementia cases, are efficient to produce modest symptomatic improvements in some of the patients, but not to cure or stop the disease progression (37, 38). In order to fill in this gap, the multifunctional drug ladostigil was designed to target various underlying pathogenic mechanisms of AD, expected to have a disease-modifying effect and thus, to slow or even block the onset progression of AD. Indeed, ladostigil is a promising novel neuroprotective therapeutic compound (presently is in Phase IIa studies in man), which produces neuroprotective and antidepressant-like activities. In this review, we discuss the scientific evidence for the potential use of ladostigil and its effect on molecular pathways and cellular processes that are considered to be involved in AD.

3. LADOSTIGIL TARGETING AMYLOID PRECURSOR PROTEIN PROCESSING

Much evidence suggests that the accumulation of Aβ in AD may play a pivotal role, thus a bulk of studies is focused on possible drug intervention along the amyloid

pathways in AD (39-41). In this context, recent findings demonstrating that ladostigil markedly suppressed holo-APP protein levels and elevated soluble-APP alpha (sAPPα) in different cellular model systems, can be of clinical value towards accelerating non-amyloidogenic APP processing, thereby reducing the possibility of generation of the toxic Aβ (27, 42). Consistent with this, previous study showed that treatment with ladostigil clearly decreased the levels of cell-associated, holo-APP in the mice hippocampus, which indicates that APP expression, can also be regulated by ladostigil under *in vivo* conditions (26). Moreover, the observation that ladostigil did not alter APP mRNA levels, may suggest that the decrease in APP protein levels can be attributed to suppression of APP translation. Earlier reports have shown the ability of another ChEI, phenserine, to reduce the levels of APP and secreted Aβ (43). In a recent study, selective BuChE inhibition was found to reduce APP and Aβ levels *in vitro* and *in vivo* (44). The mechanism underlying these effects may involve both cholinergic and noncholinergic actions regulating APP synthesis and processing, as was characterized with phenserine, which reduced APP levels via lowering the translational efficiency of APP mRNA (43). Regulation of APP processing by ladostigil was demonstrated to involve PKC- and MAPK- dependent pathways (25). However, the effect does not appear to result from ChE inhibition activity, since rasagiline and N-propargylamine were also able to induce PKC and ERK activation and promote sAPPα release (45). Several ChEIs, such as tacrine (46), physostigmine (47), metrifonate (48), ganstigmine (49) and donepezil (50) increased sAPPα release in cell culture. However, the observations that phenserine (43) and tacrine, at high concentration (51), decreased sAPPα release suggest that the regulation of APP processing by ChEIs is not simply associated to AChE inhibition. It is likely that several different mechanisms are in operation. For example, ChEIs increased PKC levels *in vitro* (52) and attenuated the $A\beta_{1-40}$ -induced down- regulation of PKC in rats (53). Moreover, donepezil promoted the trafficking of α-secretase to the membrane, thus enhancing α-secretase activity (50).

Similar to ladostigil, its S-isomer, TV3279, which is a ChEI but lacks of MAO inhibitory activity, exerts pronounced neuroprotective properties and APP processing, and thus suggesting that the mode of action is independent of MAO inhibition (27). These results are consistent with previous data, providing clear evidence that the neuroprotective effect of ladostigil, as well as of the anti-Parkinson drug, rasagiline, does not depend on inhibition of MAO, but rather is associated with some intrinsic pharmacological action of the propargyl moiety on the mitochondrial cell survival proteins (54-57). Recently, N-propargylamine was found to inhibit MAO activity significantly lesser than its abilities to induce neuroprotective, anti- apoptotic activities and regulate of APP processing (57, 58), further establishing that MAO inhibition is not pre-requisite for the neuroprotective activity of ladostigil.

4. THE INVOLVEMENT OF CELL SURVIVAL AND SIGNALING PATHWAYS IN LADOSTIGIL NEUROPROTECTIVE ACTIVITY

Studies using various cellular apoptotic models demonstrated that AChE expression simultaneously increased with its aggregation in the nuclei of apoptotic cells (59). Indeed, there is increasing evidence that AChE might be involved in apoptosis (60). Transfection with AChE leads to an increase of apoptosis in retinal cells and highly purified AChE has been shown to have toxic effects, both in neuronal- and glial-like cell lines via the apoptotic mechanism (61). In this context, various AChEIs exhibited neuroprotection properties regulating the expression levels of antiapoptotic and proapoptic genes and proteins (62, 63). In extremely neurotoxic model of human neuroblastoma SK-N-SH, ladostigil was recently reported to have a significant neuroprotective activity, including inhibition of caspase-3 activation, induction of Bcl-2 and reduction of Bad and Bax gene and protein expression (27). These findings are consistent with previous studies demonstrating that rasagiline, as well as its propargyl moiety promote neuronal survival, mediated by PKC-, MAPK- dependent activation associated with Bcl-2 family members (57, 58) and mitochondrial membrane stabilization (23, 64). Ladostigil induced as well, stimulatory effects on PKC and MAPK cascades (25), promoting the phosphorylation of p44 and p42 MAPK, which was abolished by specific inhibitors of MAPK activation (25). This may be implicated in the intriguing prospect that suggests the involvement of ERK activation in the central nervous system in mammalian synaptic plasticity and learning (65). Thus, the effect on ERK activation may be attributed in the context of previous reports indicating that ladostigil is a novel therapeutic drug for the treatment of dementia co-morbid with depression. It has been demonstrating that ladostigil selectively reverses the behavioral and neurochemical effects induced by prenatal stress (35), has antagonistic effect on scopolamine-induced impairments in spatial memory (28) and prevents memory deficits induced by intracerebroventricular injection of STZ in rats (36, 66). These findings are allied with the elevation of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) mRNA expression (67) and activation of PKC-MAPK signaling pathways by ladostigil (25), since previous studies demonstrated that neurotrophic factors play critical roles in the development and function of neurons and may serve as regulatory factors for synaptic transmission, learning and memory (68, 69). Thus, it has been proposed that several neurodegenerative disorders, such as PD, AD and Huntington's disease are linked to a lack of trophic factor support in those neurons and brain areas associated with these diseases (68-70). Additionally, upon ligand-receptor binding, BDNF and GDNF stimulate intracellular signaling pathways involved in differentiation and survival, including phospholipase C-γ, phosphatidylinositol 3-kinase (PI3K) and MAPK (68, 71, 72). Thus, elevation of neurotrophic factors by ladostigil, which may possibly initiate respective cell signaling cascades, might suggest an involvement of neurotrophic factors in the neuroprotective mechanism of action of

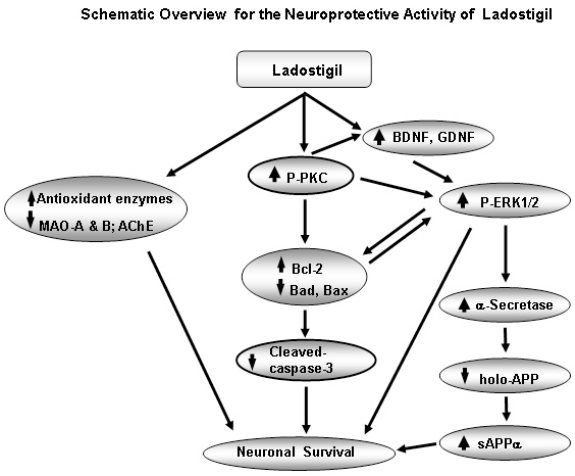

Figure 2. Schematic overview demonstrating protein and gene targets involved in the neuroprotective activity of ladostigil, with respect to the pathological features described for AD, such as extracellular deposition of Aβ, marked cholinergic cortical afferent dysfunction, lack of trophic factor support and cytotoxic signals that can initiate cell death processes and OS at those neurons and brain areas associated with this disease.

ladostigil. Furthermore, ladostigil was recently described to up-regulate the brain-specific isoform of the synaptotagmin (Syn) family, Syn IV in old rat hippocampus (73, 74). The current hypothesis of Syn IV function states that its up-regulation is correlated with a neuroprotective-like activity resulting in neurotransmitter release and thus, it may be suggested that this pathway is associated with the neuroprotective mechanism of action of ladostigil.

5. CONCLUSIONS AND PERSPECTIVES

The challenge of designing polypharmacological drugs has to link multiple *in vitro* activities to *in vivo* models and clinical settings (75, 76). Drug combinations, mixing target-acting compounds, provide a practical way to design specific polypharmacology. Nonetheless, developing combination of therapy raises complexity of drug-drug interaction, dosage ranging and metabolic shunt effects more than are derived from a single multifunctional drug (75, 76). A growing number of compounds have been specifically designed by conjugating two or more distinct pharmacophores, exhibiting safe dosage use and interaction with multiple target in molecular pathways, which are different from the un-conjugated pharmacophore (77, 78). The design of ladostigil was to address multiple central nervous system etiology in AD, which can have beneficial effects compared to its parental drugs, rasagiline and rivastigmine or to a combination therapy. For example, rivastigmine is neither MAO-A nor -B inhibitor, while ladostigil is an inhibitor of both MAO-A and -B (Figure 2), leading to an increase in brain dopamine and functional activities (16, 79).

In summary, the several targets and diverse pharmacological properties of ladostigil (Figure2) make this drug a potentially valuable for the clinical therapy for AD to delay further neurodegeneration of cholinergic neurons. Ladostigil possesses a neuroprotective activity and regulatory effect on holo-APP and sAPPα levels, hence reducing the possibility of generating the amyloidogenic pathway. The effect of ladostigil on post-transcriptional APP processing via the α-secretase pathway resulted from its stimulatory effects on PKC and MAPK cascades (Figure 2) (25, 27, 80). Additionally, the observation that the elevation of BDNF and GDNF expression by ladostigil is positively correlated with the activation of the signaling pathways of PKC-MAPK (Figure2), may suggest a linkage between neurotrophic factors and the neuroprotective mechanism of action of ladostigil, as described previously for rasagilne and its propargyl derivatives (57, 58). A different inspection of ladostigil drug activity is that chronic administration of ladostigil before and after STZ injection significantly reduced the alterations in microglia and astrocytes and prevented the increase in a marker of nitrative-oxidative stress, nitrotyrosine, and the development of episodic memory deficits (36). This report (36) suggests that these actions result from a combination of actions of ladostigil on neuronal and glial cells. The ability to inhibit ChE partially contributes to the effect on episodic memory, since ladostigil inhibits ChE (Figure 2) only in the cortex of STZ rat model of AD and no ChE inhibition occurs in the location of recognition, which depends on hippocampal cholinergic activity. Thus, the prevention of memory deficits resulted from distinct activities of those mentioned above, such as anti-oxidation (36). Indeed, previous study reported that ladostigil prevented apoptosis and attenuated OS in human neuroblastoma cells exposed to SIN-1, a donor of NO (23). Future studies are currently in progress, to elucidate the potential neuroprotective-antioxidant effect of ladostigil against OS-induced by hydrogen peroxide *in vitro*. Ladostigil was found to inhibit the Fenton reaction and reactive oxygen species generation and induce the activity and protein levels of several antioxidant enzymes, such as catalase, glutathione reductase, peroxiredoxin 1 and NADPH quinone 1 oxidoreductase (report in preparation). Improving our understanding of the multimodal molecular mechanism of ladostigil *in vitro* and *in vivo* (Figure 2) is of value for our knowledge of this drug potential in the clinical setting.

6. ACKNOWLEDGEMENT

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Abbreviations: AChEIs: acetylcholinesterase inhibitors, AD: Alzheimer's disease, Aβ; amyloid beta peptide, APP: amyloid precursor protein, BDNF: brain-derived neurotrophic factor, BuChE: butyrylcholinesterase, ChAT: choline acetyltransferase, ChEIs: cholinesterase inhibitors, ERK: extracellular signal-regulated kinase, GDNF: glial cell line-derived neurotrophic factor, MAO: monoamine oxidase, MAPK: mitogen-activated protein kinases, MPTP: N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, NMDA: N-methyl-D-aspartate, OS: oxidative stress, PD: Parkinson's disease, PI3K: phosphatidylinositol 3-kinase, PKC: protein kinase C, SIN-1: 3-morpholinosydnonimine, STZ: streptozotocin, Syn: synaptotagmin,

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