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Promises of novel multi-target neuroprotective and neurorestorative drugs for Parkinson's disease

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SUMMARY

The cascade of neurotoxic events involved in neuronal degeneration suggests that it is naive to think mono-target drugs can induce disease modification by slowing the process of neurodegeneration in Parkinson's disease (PD). Employing the pharmacophore of rasagiline (N-propargyl-1-R-aminoindan), we have developed a series of novel multi-target neuroprotective drugs, including: (A) drugs [ladostigil, TV-3326 (N-propargyl-3R-aminoindan-5yl)-ethyl methylcarbamate)] with both cholinesterase-butyrylesterase (Ch-BuE) and brain-selective monamine oxidase-AB (MAO-AB) inhibitory activities and (B) iron chelator-radical scavenging drugs (M30) possessing brain-selective MAO-AB inhibitor activity and the neuroprotective-neurorescue propargylamine moiety of rasagiline. This was considered to be valid since brain MAO and iron increase in PD and aging, which could lead to oxidative stress-dependent neurodegeneration. The multi-target iron chelator, M30, has all the properties of ladostigil, but is not an acetylcholinesterase (CHE) inhibitor. However, M30 has both neuroprotective and neurorestorative activities for nigrostriatal dopamine neurons in post-lesion MPTP, lactacystin and 6-hydroxydopamine animal models of PD. The neurorestorative activity has been identified as being related to the ability of the drug to activate hypoxia-inducible factor (HIF) by inhibiting prolyl-4-hydroxylase. M30 regulates cell cycle arrest and induces the neurotrophins brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), erythropoietin (EPO), as well as glia-derived neurotrophic factor (GDNF). These unique multiple actions of M30 make it potentially useful as a disease modifying drug for the treatment of PD.

Abbreviations

AD:	Alzheimer's disease
BDNF:	brain-derived neurotrophic factor
CHE:	acetylcholinesterase
CNS:	central nervous system
DLB:	dementia with Lewy bodies
EPO:	erythropoietin
ERK:	extracellular-signal-regulated kinase
GDNF:	glia-derived neurotrophic factor
GLUT:	glucose transporter
GPx:	glutathione peroxidase
GSK-3β:	glycogen synthase kinase-3β
HIF:	hypoxia-inducible factor
HO-1:	heme oxygenase-1
iNOS:	inducible nitric oxide synthase
MAO:	monoamine oxidase
MAPK:	mitogen-activated protein kinase
MEK:	mitogen-activated protein kinase kinase
PD:	Parkinson's disease
PKB/Akt:	protein kinase B
PKC:	protein kinase C
SOD-1:	superoxide dismutase-1
TfR:	transferrin receptor
VEGF:	vascular endothelial growth factor

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1. Neuroprotective activity of antiparkinson multi-target drugs, ladostigil and M30

It is doubtful whether a mono-target drug would possess disease modifying activity in progressive neurodegenerative diseases, since these disorders have a complex pathology and molecular cascade of events that results in neurodegeneration. Thus, novel therapeutic approaches for the treatment of neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and dementia with Lewy bodies (DLB) comprise drug candidates designed specifically to act on multiple central nervous system (CNS) targets. These drugs possess activity against decline in cognition,

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extrapyramidal symptoms and depression, which are common features of progressive neurodegenerative disorders [1–5].

The second most common form of dementia is DLB, which is characterised by the presence of intracytoplasmic, eosinophilic, neuronal inclusion bodies in the neocortex, limbic areas and subcortical nuclei. These are thought to be responsible for the neuronal damage and give rise to the cognitive deficits, extrapyramidal symptoms and behavioural abnormalities characteristic of the disorder. Depressive symptoms occur in a large proportion of subjects with PD and DLB [6]. This has been attributed to alteration of neurotransmission and possible degeneration of noradrenaline and serotonin neurons innervating the limbic system [7].

Although there is still no definitive consensus on the etiology of PD, clear evidence exists for defects in mitochondrial function [8], dysregulation of brain iron [5], inflammatory responses and abnormalities of energy metabolism in these conditions. Significant decreases in the oxidative metabolism of glucose are evident in cortical and nigral areas at relatively early stages of PD [9] together with a reduction in the number of glucose transporters. There is evidence of a decrease in the activity of catalase and an increase of monoamine oxidase (MAO)-B in glial cells [10]. These enzymatic changes could contribute to oxidative stress through the formation of higher levels of H_2O_2 from the reaction of MAO. A failure in mitochondrial complex I can exacerbate the oxidative stress. In each of these conditions, damage from free radicals has been shown to cause lipid and protein peroxidation. The resulting toxic products contribute to neuronal death.

In order to treat the cognitive, extrapyramidal and depressive symptoms of PD, we have developed two novel multi-target drugs. The first is ladostigil (N-propargyl-(3R)-aminoindan-5-yl-ethyl methyl carbamate, hemitartrate) (Fig. 1) in which the carbamate moiety of rivastigmine was introduced into the 6 position of the rasagiline molecule to provide inhibitory activity

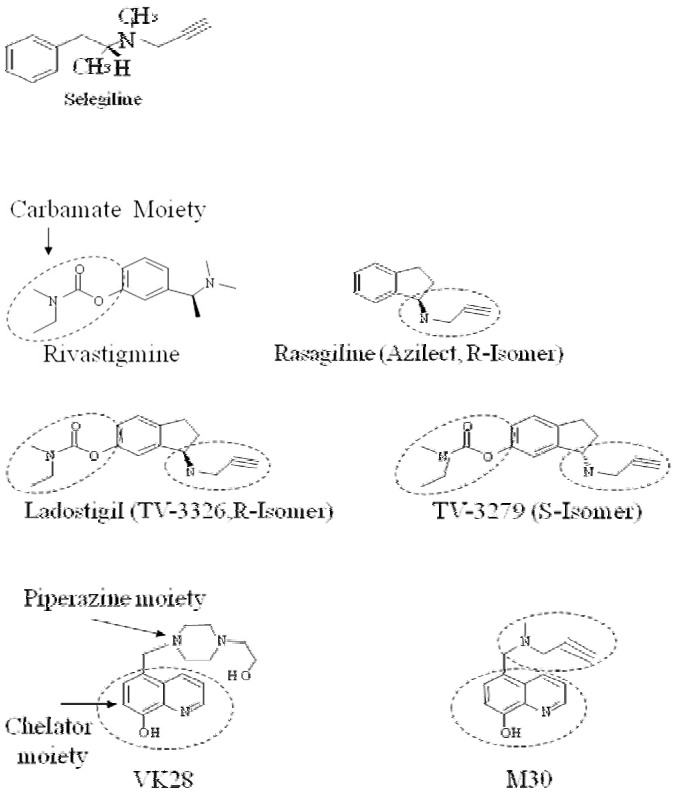

Fig. 1. The structures of propargylamine-possessing selegiline, rasagiline and multi-target drugs ladostigil, M30 and HLA20.

against acetylcholinesterase (CHE) [11] and the neuroprotective–neurorescue activity of rasagiline. This resulted in a reduction of some 5 orders of magnitude in MAO-B inhibitory activity *in vitro* compared with rasagiline. However, on repeated oral and intraperitoneal (IP) administration of ladostigil to rats and mice, brain-selective MAO-A and -B inhibition in the brain was seen at doses similar to those that inhibited CHE and these were much lower than would be expected from the poor *in vitro* activity [4,5,11]. The difference between the *in vitro* and *in vivo* MAO inhibitory activity has been attributed to the loss of the carbamate pseudoinhibitory moiety, with inhibition of CHE resulting in the accumulation of several propargylamine aminoindan metabolites, including hydroxy-rasagiline, in the brain that inhibit MAO-A and -B. In contrast, the second drug we have developed, the multi-target iron chelator M30 (Fig. 1), is a potent propargylamine-containing MAO-A and -B inhibitor *in vitro* and, similar to ladostigil, is brain selective for inhibition of MAO-A and -B *in vivo* [12,13] and has shown remarkable neuroprotective and neurorestorative activities in three classical animal models of PD, namely MPTP [12], lactacystin [14] and 6-hydroxydopamine (6-OHDA) [Kupershmidt et al., unpublished].

2. Neuroprotective and neurorestorative activities of ladostigil and M30

Ladostigil and M30 have many of the molecular mechanisms and neuroprotective actions of rasagiline in cultured neuronal cells and *in vivo* [4,5]. These include prevention of the fall in the mitochondrial potential and cytotoxicity in SY5Y and PC12 cells in response to oxidative stress induced by peroxynitrite or glucose oxygen deprivation [15]. Ladostigil also shows neuroprotective activity *in vivo*, significantly reducing hippocampal cell damage induced by global ischemia in gerbils and the cerebral edema induced in mice by closed head injury [15]. The neuroprotective effects against oxidative stress may enable ladostigil and M30 to delay the progression of PD and DLB. They also might provide symptomatic improvement of extrapyramidal symptoms by their ability to increase nigrostriatal dopamine transmission, since both drugs selectively inhibit MAO-A and -B and, unlike selective MAO-B inhibitors, increase brain levels of dopamine. They also may have effective antidepressant activity, as seen in the forced swim test animal model of depression, because of their brain MAO-A inhibition. M30 has been shown to possess a wide range of pharmacological activities, including pro-survival neurorescue effects, induction of neuronal differentiation and regulation of amyloid precursor protein and beta-amyloid levels. M30 also decreases apoptosis of SH-SY5Y neuroblastoma cells in a neurorescue, serum deprivation model via reduction of the pro-apoptotic proteins, Bad and Bax, and inhibition of the apoptosis-associated phosphorylated H2A.X protein (Ser 139) and caspase 3 activation. In addition, M30 induces the outgrowth of neurites, triggers cell cycle arrest in G(0)/G(1) phase and enhances the expression of growth associated protein-43 [4,5,10,16].

As a novel multifunctional brain permeable iron chelator, M30 possesses neuroprotective activities *in vitro* and *in vivo* against several insults applicable to various neurodegenerative diseases, such as AD, PD, and amyotrophic lateral sclerosis. Systemic chronic administration of M30 resulted in up-regulation of hypoxia-inducible factor (HIF)-1α protein levels in various brain regions (e.g. cortex, striatum, and hippocampus) and spinal cord of adult mice. Real-time RT-PCR revealed that M30 differentially induced HIF-1α-dependent target genes, including vascular endothelial growth factor (VEGF), erythropoietin (EPO), enolase-1, transferrin receptor (TfR), heme oxygenase-1 (HO-1), inducible nitric oxide synthase (iNOS), and glucose transporter (GLUT)-1. In addition,