

Implications of co-Morbidity for Etiology and Treatment of Neurodegenerative Diseases with Multifunctional Neuroprotective-Neurorescue Drugs; Ladostigil

MOUSSA B.H. YODIM^{*}, TAMAR AMIT, ORIT BAR-AM, ORLY WEINREB and MARA YOGEV-FALACH

Technion-Rappaport Family Faculty of Medicine, Eve Topf and NPF Centers for Neurodegenerative Diseases, Department of Pharmacology, Haifa, Israel. Youdim@tx.technion.ac.il

(Submitted 10 October 2005; Revised 22 March 2006; In final form 22 March 2006)

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 several multifunctional drugs. These include ladostigil (TV3326) [(N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate], which combines the pharmacophore-neuroprotective effects of rasagiline, a selective monoamine oxidase (MAO)-B inhibitor, with the cholinesterase (ChE) inhibitory activity of rivastigmine or iron chelating moiety such as M30. In the case of M30 the pharmacophore of brain permeable iron chelator VK-28 plus the MAO inhibitor-neuroprotective propargylamine moiety of rasagiline are combined in a single molecule as a potential treatment for Alzheimer's disease, Lewy body disease, and Parkinson's disease with dementia. Here, we discuss the activities of ladostigil in terms of its cholinesterase cognitive enhancing potential, antiParkinson, antidepressant, neuroprotection and APP (amyloid precursor protein) processing potential. One major attribute of ladostigil is its neuroprotective activity in neuronal cell cultures and *in vivo*. Employing an apoptotic model of neuroblastoma SK-N-SH cells, the molecular mechanism of its neuroprotective activity has been determined. The current studies show that ladostigil significantly decreased apoptosis via

inhibition of the cleavage and prevention of caspase-3 activation through a mechanism related to regulation of the Bcl-2 family proteins, resulting in reduced levels of Bad and Bax and induced levels of Bcl-2. In addition, ladostigil elevated the levels of pPKC(pan). We have also followed the regulation of APP processing and found that ladostigil markedly decreased apoptotic-induced levels of holo-APP, as well as stimulated the release of the non-amyloidogenic soluble APP (sAPP α) into the conditioned medium via an established protein kinase C-MAPkinase dependent pathway. Similar to ladostigil, its S-isomer, TV3279, which is a ChE inhibitor lacking MAO inhibitory activity, exerted similar neuroprotective properties and APP processing, suggesting that the mode of action is independent of MAO inhibition. These effects were shown to reside in the propargylamine moiety. These findings indicate that the dual actions of the anti-apoptotic-neuroprotective activity and the ability to modulate APP processing, could make ladostigil a potentially valuable drug for the treatment of Alzheimer's disease.

Keywords: Ladostigil; Rasagiline; M30; Amyloid precursor protein; α -secretase; Acetylcholinesterase inhibitor; Monoamine oxidase A and B inhibitors; Neuroprotection; Parkinson's disease; Alzheimer's disease; Depressive illness

Abbreviations

$\Delta\Psi_m$, mitochondrial membrane potential;
AChE, acetylcholinesterase;
AChEIs, acetylcholinesterase inhibitors;
AD, Alzheimer's disease;
APP, amyloid protein precursor;
sAPP α , soluble amyloid precursor protein;
ChE, cholinesterase;
ERK, extracellular signal-regulated kinase;
MAO, monoamine oxidase;
MAPK, mitogen-activated protein kinase;
PKC, protein kinase C;
PD, Parkinson's disease;
PT, permeability transition pore;
SOD, superoxide dismutase

INTRODUCTION

In Parkinson's disease (PD) behavioural symptoms such as anxiety, depression and psychosis are common, and dementia occurs in about 90% of patients. These symptoms can be more disabling than the motor dysfunction, and they negatively impact on the quality of life caregiver, being more frequently associated with nursing home placement. Depression can be treated with antidepressants, and selective serotonin reuptake inhibitors are widely used, but there is still need for controlled clinical trials. Management of L-dopa induced psychosis in PD is complex and includes elimination of identifiable risk factors, reduction of polypharmacy, and administration of atypical neuroleptics, which can alleviate psychotic symptoms without worsening motor functions. Cholinesterase (ChE) inhibitors may prove additional benefit in demented PD patients, and recent evidence suggests that ChE inhibitors such as rivastigmine may be effective in the treatment of dementia associated with PD (Oxman, 1996; Tanberg *et al.*, 1996; Newman, 1999; McDonald *et al.*, 2003; Shih *et al.*, 2004; Leentjens, 2004; Veazey *et al.*, 2005). As life expectancy continues to increase over time, dementia is becoming an increasingly more common problem and a major cause of disability in older persons. It is now more important than ever to identify and manage common causes of dementia, given variations in disease course, treatments and the possibility for modification of risk factors. Dementia with Lewy bodies (DLB) is a dementia syndrome characterized by progressive cognitive decline, with fluctu-

ating cognition, recurrent detailed and well-formed hallucinations, and parkinsonism.

Gene expression and proteomic profiling studies on Parkinsonian and Alzheimer brains are indicating that neurodegeneration in these disorders are the results of the cascade of several events, where each of which can cause death of neurons, possibility by an apoptotic process (Mandel *et al.*, 2003; Grunblatt *et al.*, 2004; Kastel *et al.*, 2005; Emilsson *et al.*, 2006; Jee *et al.*, 2006). Since present treatments involve drugs developed to act at a single component in the pathology of these diseases, the new drug treatment has been suggested to be either polypharmacology or development of multifunctional drug with various targets for CNS, possessing both neuroprotective activity and potentiating the neurotransmitter functional activity associated with the disorder.

Polypharmacology for treatment of neurodegenerative disorders has an inherent side effect, especially in older patients that cannot be easily managed. Recent studies on drug development for neuropsychiatric disorders have indicated that multifunctional drugs with different CNS targets may be a more viable approach, since such drugs would generate fewer less toxic metabolites (Venkatachalam *et al.*, 2004; Umehara *et al.*, 2005; Youdim and Buccafusco, 2005; Di Stefano *et al.*, 2006; Fry *et al.*, 2006; Yamada *et al.*, 2006). Recently we have taken such an approach and have developed several novel multifunctional drugs for the treatment of PD, Alzheimer's disease (AD) and Lewy body disease (LBD) co-morbidity with depressive illness. These include ladostigil (Weinstock *et al.*, 2000a,b; Sterling *et al.*, 2002; Youdim and Buccafusco, 2005) and multifunctional iron chelator-monoamine oxidase (MAO) inhibitor M30 series (Gal *et al.*, 2005; Zheng *et al.*, 2005a,b). Our novel multifunctional drug ladostigil (TV3326) [(N-propargyl-(3R) aminoindan-5yl)-ethyl methyl carbamate], is a bifunctional drug that was developed from the pharmacophore of rasagiline, to possess several CNS targets, with carbamate ChE- and propargyl monoamine oxidase- (MAO-) inhibitory activities (Sterling *et al.*, 2002), and to possess many of the neuroprotective actions of the selective MAO-B-inhibitor rasagiline (N-propargyl-(1R)-aminoindan) (Azilect) (Weinstock *et al.*, 2000b; Youdim *et al.*, 2001 a,c; Sterling *et al.*, 2002; Maruyama *et al.*, 2003; Youdim and Buccafusco, 2005; Youdim *et al.*, 2005) (FIG. 1).

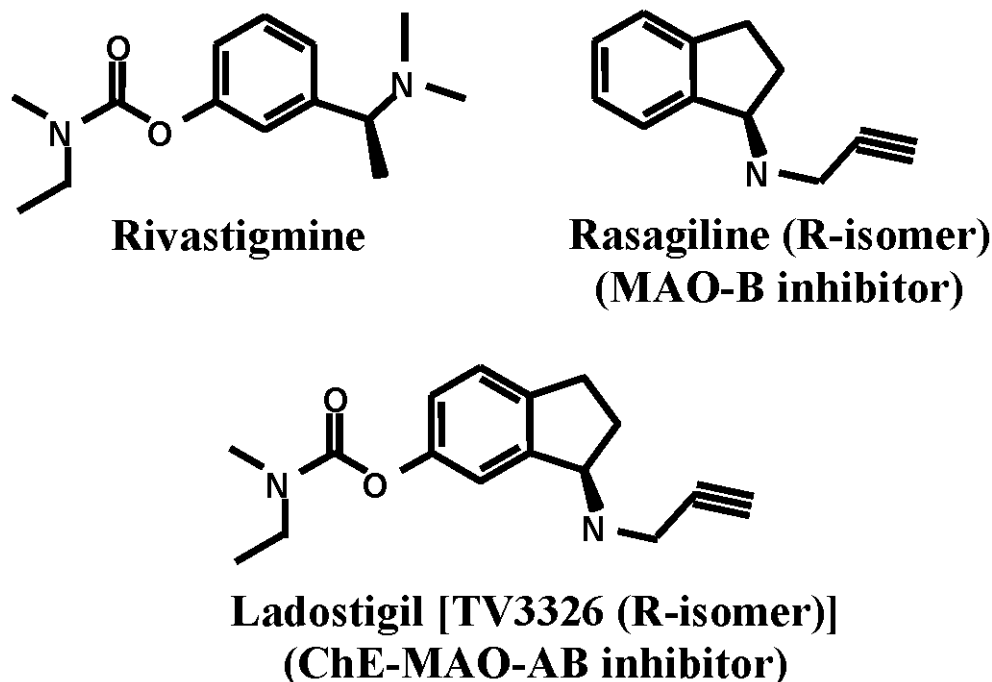


FIGURE 1 Structures of cholinesterase inhibitor, rivastigmine; selective MAO-B inhibitor, rasagiline and multifunctional neuroprotective cholinesterase-brain selective MAO-AB inhibitor, ladostigil (TV3326).

MULTIFUNCTIONAL ANTI ALZHEIMER DRUG, LADOSTIGIL, WITH VARIOUS CNS TARGETS

The neuroprotective activity profile of ladostigil is similar to that of rasagiline and include its ability to prevent the fall in the mitochondrial membrane potential ($\Delta\Psi_m$) and cytotoxicity in SH-SY5Y and PC12 cells in response to oxidative stress induced by N-methyl-R-sasolinol, peroxyxynitrite, glucose-oxygen deprivation and serum deprivation (Wadia *et al.*, 1998; Weinstock *et al.*, 2000b; Maruyama *et al.*, 2003). It also possesses neuroprotective activity *in vivo*, significantly reducing hippocampal cell damage induced by global ischemia in gerbils, and the cerebral oedema induced in mice by closed head injury (Weinstock *et al.*, 2000a; Youdim *et al.*, 2001b; Youdim and Weinstock, 2002b). In addition, we have recently described that ladostigil stimulated the release of the neurotrophic/neuroprotective, non-amyloidogenic-soluble amyloid precursor protein (sAPP α) via activation of protein kinase C (PKC) and mitogen-activated protein kinase- (MAPK-) pathways (Yogev-Falach *et al.*, 2002), and reduced the levels of APP in mouse

hippocampus (Bar-Am *et al.*, 2004). Our studies have described the dual effects of ladostigil and its S-enantiomer, TV3279, in terms of neuroprotection and APP processing, using the effective model of long-term culture to induce apoptosis of human SK-N-SH neuroblastoma cells (Yang *et al.*, 2002). Furthermore, studies on structure-activity relationship were conducted with rasagiline, ladostigil and their S-optical isomer derivatives, to assess whether the propargyl (FIG. 2) moiety is essential for the neuroprotective mechanism. Indeed, removal of the propargyl moiety from these drugs results in the loss of neuroprotective-antiapoptotic activity as seen with aminoindan, hydroxyaminoindan and aminoindan-5-yl-ethyl methyl carbamate (Akao *et al.*, 2002; Maruyama *et al.*, 2002; 2003; Yi *et al.*, 2006). More recently, we have shown that propargylamine itself has the same neuroprotective activity, with the same molecular mechanism and similar potency, but without MAO inhibitory activity (Bar Am *et al.*, 2005).

CHOLINESTERASE INHIBITION

In vitro ladostigil, unlike rivastigmine, causes a slowly developing inhibition of both acetyl-

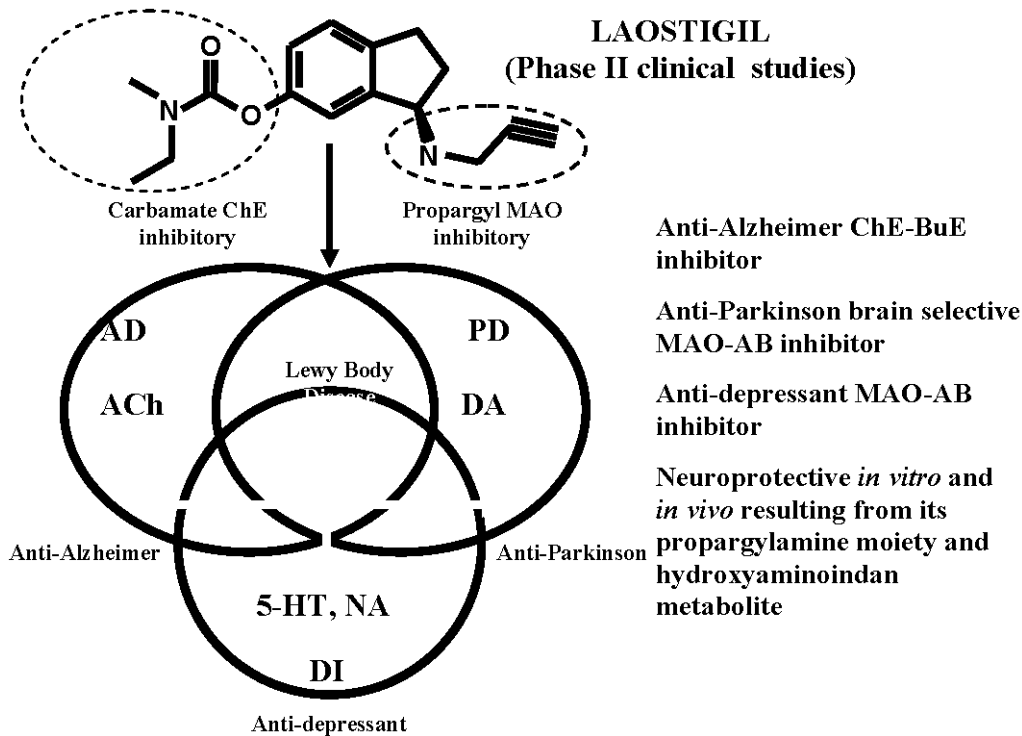


FIGURE 2 Neuropharmacological actions of ladostigil. **AD**, Alzheimer's disease; **DI**, depressive illness; **PD**, Parkinson's disease; **5-HT**, serotonin; **AChE**, acetylcholine; **DA**, dopamine, **NA** noradrenaline.

cholinesterase (AChE) and butyrylcholinesterase (BuChE), but it is about 100 times more potent against BuChE than AChE in rat brain preparations. This is considered to be an advantage over an AChE-selective inhibitor, because the levels of BuChE do not decline like those of AChE in the brains of patients with AD; and its inhibition can contribute to the maintenance of acetylcholine (ACh) levels in the synaptic gap (Giacobini, 2001; Pepeu unpublished data). Ladostigil also inhibits both MAO-A and MAO-B, thereby increasing brain dopamine. This leads to enhanced functional activity, since selective MAO-A or selective MAO-B inhibitors individually do not increase brain levels of dopamine as much as non-selective inhibitors (Youdim and Riederer, 1993). Oral ladostigil inhibits rat cortical ChE (comprised of about 90% AChE and 10% BuChE) by 20-80% at doses ranging from 9 to 200 mg/kg (Weinstock *et al.*, 2000b). Ladostigil (12-26 mg/kg) causes a dose-related antagonism of the scopolamine-induced spatial memory deficits in rats, similar to rivastigmine and galantamine. This indicates that ladostigil is able to increase brain ACh levels sufficiently to compete with scopolamine for

muscarinic receptors participating in memory formation or retention (Weinstock *et al.*, 2000b).

Ladostigil is not an MAO inhibitor *in vitro*, and an acute oral dose of 120 mg/kg of ladostigil is necessary to produce 50% inhibition of MAO-A and MAO-B in the brain of rats. However, once-daily administration of a lower dose of ladostigil (26 mg/kg), for 2 weeks, inhibits brain MAO-B by 71% and MAO-A by 66% in mice; and by more than 85% in rat and rabbit brains - with very little or no effect on these enzymes in the liver and intestine (Weinstock *et al.*, 2000a; 2002c). At these doses ladostigil shows increased MAO inhibitory activity in brain, with some selectivity for MAO-B. Treatment of rats with ladostigil for 2 months results in brain MAO inhibition of more than 90%, without any appreciable effect on the intestinal or liver enzymes in rats and mice. Furthermore, in rabbits, ladostigil (26 mg/kg) inhibited brain MAO-AB by more than 90% after only 2 weeks of daily administration but did not inhibit intestinal MAO-A, which comprises over 80% of the total MAO in this tissue (Weinstock *et al.*, 2002a). In view of the fact that ladostigil does not inhibit MAO-A or -B *in vitro*, it is likely that in

the brain it acts as a prodrug and the brain-selective effect of the drug results from the local formation of a more active metabolite. Several such metabolites have recently been identified in the blood of rats and monkeys after oral administration of ladostigil. One of these is produced by hydrolysis of the carbamate moiety of ladostigil by ChE to yield 6-hydroxyrasagiline, which has MAO inhibitory activity similar to the antiparkinson drug, rasagiline (Azilect) (Youdim *et al.*, 2005). The MAO inhibitory potency of hydroxyrasagiline is 500-600 times that of ladostigil, with a potency of 0.46 and 0.35 mM for MAO-A and MAO-B, respectively. It is mostly likely that adequate concentration of these and other MAO-inhibitory metabolites accumulate in brain to levels sufficient to adequately inhibit MAOs - leading to increased levels of dopamine, serotonin and noradrenaline, to account for its respective anti-Parkinson and antidepressant activities in the MPTP- (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) model of PD, and forced swim test for depression.

CHOLINERGIC AND AMINERGIC NEUROTRANSMISSION

We determined the effects of ladostigil and its optical S-isomer on aminergic neurotransmitter levels and motor behavioral activity in naive and in L-dopa- or L-tryptophan-treated rats (Sagi *et al.*, 2005). Chronic treatment of rats with ladostigil (52 mg/kg, for 21 days) inhibited hippocampal and striatal MAO-A and MAO-B activities by >90%. This ladostigil treatment also increased striatal levels of dopamine and serotonin, and inhibited striatal ChE activity by approximately 50%. Chronic TV3279 (26 mg/kg, for 21 days) similarly inhibited approximately 50% of striatal ChE activity, but did not affect MAO activity or amine levels. In sharp contrast to the inductive effect of the MAO A/B inhibitor, tranlycypromine (TCP), on stereotyped hyperactivity in response to L-dopa (50 mg/kg) or L-tryptophan (100 mg/kg), ladostigil completely inhibited these behavioral hyperactivity syndromes. Accordingly, acute rivastigmine (2 mg/kg) and chronic TV3279 abolished the ability of TCP to initiate L-dopa-induced hyperactivity, while scopolamine (0.5 mg/kg) reversed the inhibitory effect of chronic ladostigil on L-dopa-induced hyperactivity, suggesting that ladostigil may attenuate hyperloco-

motor activity by activating central cholinergic muscarinic receptors. Finally, while chronic ladostigil administration to naive rats resulted in preserved spontaneous motor behavior, acute treatment with ladostigil decreased motor performance, compared to control animals. In contrast, chronic as well as acute treatments with TV3279 reduced spontaneous motor activity. Thus, the aminergic potentiation by ladostigil may counteract its cholinergic inhibitory effect on spontaneous motor behavior. Our results suggest that potentiation of both aminergic and cholinergic transmission systems by ladostigil contributes equally to motor behavior performance, which is substantially impaired in comorbidity of dementia with Parkinsonism including DLB.

LIMITED POTENTIATION CARDIOVASCULAR RESPONSE TO TYRAMINE BY LADOSTIGIL

One major side effect of the non-selective MAO inhibitor antidepressants, tranlycypromine and phenelzine, is their potentiation of the cardiovascular effects of sympathomimetic tyramine, present in ingested foods (cheese reaction) - consequent to the inability of liver and other organs to inactivate tyramine (Youdim and Weinstock 2004). The lack of (inhibitory) effect of ladostigil on MAO-A in liver and intestine, suggested that ladostigil would have less interaction with tyramine-containing foods or beverages versus nonselective MAO inhibitors such as iproniazid and tranlycypromine. It was found that after chronic treatment of rabbits with ladostigil, tyramine increased blood pressure by 30 mm Hg only at doses exceeding 30 mg/kg, compared to about 60-70 mg/kg in untreated animals (Weinstock *et al.*, 2002b). In human subjects this amount is equivalent to about 900 mg (2 kilos of camember cheese), which is much more than would be present in any reasonable amount of tyramine-containing food or beverage (Da Prada *et al.*, 1987). By contrast, a 30-mm Hg increase in blood pressure was achieved with only 2 mg/kg of tyramine in rabbits treated with tranlycypromine (20 mg/kg), which inhibited brain MAO-A and MAO-B to a similar extent as ladostigil, but caused more than 90% inhibition of intestinal MAO (Weinstock *et al.*, 2002b).

Antidepressant Activity of Ladostigil

A significant percentage of parkinsonian and Alzheimer subjects have predisposition to depressive illness, which has been attributed to the loss of serotonin by raphe neurons and noradrenaline by locus coeruleus neurons (Oxman, 1996; Tanberg *et al.*, 1996; Newman, 1999; McDonald *et al.*, 2003; Leentjens, 2004; Shih *et al.*, 2004; Veazey *et al.*, 2005). Thus, the management of the psychiatric (mental dysfunction) aspect of these neurodegenerative disorders is receiving prominence. MAO inhibitors were the first antidepressants to be discovered. MAO inhibitory activity is associated with increased brain levels of noradrenaline and serotonin, since MAO normally metabolizes the neurotransmitters. A test in rats for potential antidepressant drugs is the forced swim or "Porsolt test", in which rats are confined on two consecutive days to a narrow cylinder with water for 15 and 5 min, respectively. After initial attempts at swimming and struggling, rats develop a form of "learned helplessness" - indicated by immobility, which is manifested more than 70% of the time, following exposure on the second day. Pretreatment with antidepressant drugs, including MAO-AB-inhibitors (tranylcypromine), MAO-A-inhibitors (clorgyline, moclobemide) [but not MAO-B-inhibitors], tricyclic antidepressants (*e.g.*, amitriptyline), selective serotonin reuptake inhibitors (*e.g.*, fluoxetine) and others, reduces the period of this immobilization (Porsolt *et al.*, 1979; Borsini and Meli, 1988). Ladostigil at a dose of 26 mg/kg/day for 2 weeks, or 52 mg/kg for 1 week, inhibited rat brain MAO-A and MAO-B by more than 75%, resulting in increased brain levels of serotonin, noradrenaline, and dopamine. In these rats there was a significant reduction in the time of immobility, similar to the effect of chronic treatment with amitriptyline (10 mg/kg/day) or the reversible MAO-A inhibitor, meclobemide (20mg/kg/day (Weinstock *et al.*, 2002c). In rats and mice, given ladostigil, there is a significant increase in the brain levels of serotonin, noradrenaline and dopamine, attributable to its *in vivo* inhibition of brain MAO-AB, thereby accounting for its antidepressant action similar to that of other MAO-AB and MAO-A inhibitors (Weinstock *et al.*, 2002b).

Anti Parkinson Activity of Ladostigil

The neurotransmitter dopamine is a substrate for both MAO-A and MAO-B (Yang and Neff, 1974). Whereas rat and mouse brains contain about equal proportions of these enzymes, the extrapyramidal region of the human has about four times more MAO-B than MAO-A (Collins *et al.*, 1970; O'Carroll *et al.*, 1983). This was one reason why Birkmayer *et al.* (1975; 1977) introduced the MAO-B inhibitor selegiline as therapy in PD subjects - to potentiate the pharmacological actions of L-dopa. The potential role of extrapyramidal MAO-B in the pathology of PD was strengthened by the discovery that extrapyramidal symptoms were produced by the neurotoxin MPTP, which itself is inert but is metabolized by MAO-B in striatal microglia to the toxic metabolite, MPP⁺ (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridinium ion) (Snyder and D'Amato, 1986). The latter is then taken up by striatal dopamine nerve endings, resulting in their degeneration by intraneuronal oxidative stress (Wu *et al.*, 2000). MAO-B inhibitors protect against MPTP neurotoxicity by preventing its conversion to MPP⁺ (Heikkila *et al.*, 1985; Wu *et al.*, 2000). However, they may also reduce the neuronal damage produced by MPP⁺ by an action independent of MAO-B blockade (Le *et al.*, 1997; Wu *et al.*, 2000). Because ladostigil has protective actions against oxidative stress in addition to inhibiting MAO-B, we tested its effect in the MPTP mice model of PD to see if, like other propargylamine-containing compounds, it could prevent degeneration of nigrostriatal dopamine neurons. It was found that chronic, once-daily administration of ladostigil (26 mg/kg) for 14 days inhibited brain MAO-A and MAO-B by 70%, preventing MPTP-induced depletion of striatal dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Because TV-3326 also inhibited MAO-A, it increased brain levels of serotonin and noradrenaline. These findings suggest that like selegiline, TV-3326 may have therapeutic value in treating the extrapyramidal symptoms in AD and DLB.

Neuroprotection

Accumulating evidence suggests that apoptosis may contribute to neuronal death in a variety of neurodegenerative diseases, such as AD and PD (Jellinger 2000; Yuan and Yankner 2000). But by no

means has this been established. Nevertheless, it is essential to investigate the mechanisms underlying the prevention of neuronal cell death related with such destructive diseases in the central nervous system. Apoptotic signaling is a multi-step pathway initiated by opening a mitochondrial mega-channel, called the permeability transition pore (PT), followed by decline in $\Delta\Psi_m$, resulting in the release of cytochrome C and apoptosis-induced factors. The PT is regulated by anti-apoptotic proteins Bcl-2 and Bcl-xL and pro apoptotic Bax (which disrupts the $\Delta\Psi_m$) (Tsujimoto and Shimizu, 2000). The latter effect of Bax activates cysteine-dependent, aspartate-specific protease (caspases) that act as effectors and executioners of apoptosis. Activation of the cysteine protease caspase-3 appears to be a key event in the execution of apoptosis in the central nervous system (Tsujimoto and Shimizu, 2000).

A series of drugs have been proposed as candidates for neuroprotection trials (Ravina *et al.*, 2003). Among them, rasagiline and selegiline (Youdim *et al.*, 2001a; Finberg *et al.*, 1996; Ebadi *et al.*, 2002; Parkinson Study Group 2002; Sharma *et al.*, 2003) and related propargylamines exert neuroprotective effects against a variety of insults *in vitro* and *in vivo* (Finberg *et al.*, 1998; Abu Raya *et al.*, 1999; Huang *et al.*, 1999; Maruyama *et al.*, 2000b,c; Tatton *et al.*, 2002; Sagi *et al.*, 2003; Youdim, 2003; Youdim *et al.*, 2005). It has been suggested that rasagiline suppresses apoptotic death by stabilizing the $\Delta\Psi_m$ and preventing activation of the following apoptotic processes: activation of caspase-3; nuclear translocation of glyceraldehyde-3-phosphate dehydrogenase; and nucleosomal DNA fragmentation (Akao *et al.*, 2002; Youdim and Weinstock 2002b; Maruyama *et al.*, 2003). Moreover, rasagiline has been shown to cause up-regulation of putative anti-apoptotic and antioxidative proteins, such as Bcl-2, Bcl-xL, superoxide dismutase (SOD), catalase, and glutathione peroxidase (Carrillo *et al.*, 2000; Ketani *et al.*, 2000; Youdim and Weinstock 2002b; Youdim *et al.*, 2005). Recently, we reported that rasagiline reduced apoptosis via regulation of Bcl-2 family members associated with the pro-survival PKC isoforms, PKC α and PKC ϵ (Weinreb *et al.*, 2004) and nuclear NF- κ B transcription factor, which is activated by extracellular signal-regulated protein kinase (ERK) cascade (Maruyama *et al.*, 2004).

Molecular Mechanism of Ladostigil: Neuroprotective, Neurorescue and Amyloid Precursor Protein Processing Activities

Numerous clinical trials have demonstrated the safety and efficacy of AChE inhibitors (AChEIs) in the treatment of AD. Yet, their benefits in AD are likely to be more complex than simply preventing the loss of acetylcholine (Francis *et al.*, 1999; DeKosky *et al.*, 2002; Giacobini 2004; Racchi *et al.*, 2004). As reviewed recently, there is growing preclinical evidence that AChEIs block some of the fundamental neurodegenerative processes that are involved in AD (Francis *et al.*, 2005). AChEIs, such as, tacrine, donepezil, galantamine, huperazine A and ganstigmine were reported to protect neurons from death in various apoptotic cell culture models of neurodegenerative diseases (Francis *et al.*, 2005). In addition, it is now clear that several ChEIs also promote non-amyloidogenic APP processing (Racchi *et al.*, 2004). Therefore, it is suggested that AChEIs possessing properties of anti-apoptosis or APP processing might be more beneficial in treating AD or preventing the pathogenesis of AD, than those AChEIs that simply inhibit AChE.

The bifunctional anti-AD drug, ladostigil, was developed by us in an attempt to combine the pharmacophore neuroprotective properties of the anti-PD MAO-B inhibitor drug, rasagiline, with the ChE inhibitory moiety of the anti AD drug rivastigmine (Weinstock *et al.*, 2000a,b; Youdim and Weinstock, 2002a; Youdim and Buccafusco, 2005). We have assessed the dual effects of ladostigil in terms of neuroprotection and APP processing, using an apoptotic model of neuroblastoma SK-N-SH cells (Yogev-Falach *et al.*, 2006). This study has demonstrated that ladostigil provides substantial protection from induction of apoptosis caused by long-term cultured human neuroblastoma SK-N-SH cells, which exhibit a neuronal phenotype and have multiple neurochemical markers (Biedler *et al.*, 1973; 1978). This apoptotic model has been reported to be a reliable cytotoxic system for screening potential neuroprotective agents, since it mimics certain aspects of neurodegeneration, including nutrient deprivation, failure to remove metabolic waste, energy depletion, and oxygen insufficiency (Ba *et al.*, 2003). Although the exact cellular mechanism of this model is not yet fully understood, the observation of severe cell viability

loss, a strong apoptotic signal and the involvement of PKC regulation imply that cell death in this model could involve oxidative stress and mitochondrial dysfunction (Ba *et al.*, 2003).

In spite of the extremity of the apoptotic model employed, highly significant neuroprotection was evident upon treatment with ladostigil, including inhibition of caspase-3 activation induced by the apoptotic conditions, induction of Bcl-2, reduction of Bad and Bax protein levels, and stimulation of PKC phosphorylation. Indeed, our finding that ladostigil significantly induced PKC phosphorylation, complements previous studies showing the crucial role of PKC activation in cell survival/death signals that protect against cell death. Thus, we have recently reported that activation/regulation of PKC in association with the Bcl-2 protein family, promotes neuronal survival by rasagiline and its propargyl moiety (Weinreb *et al.*, 2004). Studies using the apoptotic model of long-term SK-N-SH culture demonstrated that AChE expression simultaneously increased with its aggregation in the nuclei of apoptotic cells (Yang *et al.*, 2002). There is increasing evidence that AChE might be involved in apoptosis. 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 (Calderon *et al.*, 1998). AChE was also found to have additional effects that could result in an increased expression of APP in glia cells (von Bernhardi *et al.*, 2003). In this context, increased expression in generation of β -amyloid (A β) peptides may play a central role in the amyloidogenesis process in AD (Mills and Reiner, 1999). Thus, the observed reduction of holo-APP protein levels and induced sAPP α after treatment with ladostigil could be of clinical value towards accelerating non-amyloidogenic APP processing, thereby reducing the possibility of generation of toxic A β .

In support of this, our previous study showed that treatment with ladostigil markedly decreased the levels of cell-associated, holo-APP in mouse hippocampus, which indicates that APP processing can also be regulated by ladostigil under *in vivo* conditions (Bar-Am *et al.*, 2004). Earlier reports have shown the ability of another ChEI, phenserine, to reduce APP in cell culture and *in vivo*. In addition, phenserine reduced the levels of

secreted A β (Shaw *et al.*, 2001). The regulation of APP by phenserine was suggested to occur via a translational mechanism, independent of cholinergic activity (Shaw *et al.*, 2001).

Several ChEIs, such as tacrine (Chong and Suh, 1996), physostigmine (Lahiri and Farlow, 1996), metrifonate (Racchi *et al.*, 2001), ganstigmine (Mazzucchelli *et al.*, 2003) and donepezil (Zimmermann *et al.*, 2004) increased sAPP α release in cell culture. However, the observations that phenserine (Shaw *et al.*, 2001) and tacrine, at high concentration (Lahiri and Farlow, 1996), decreased sAPP α release suggest that the regulation of APP processing by ChEIs is not simply associated with AChE inhibition. It is likely that several different mechanisms are in operation. For example, ChEIs increased PKC levels *in vitro* (Pakaski and Kasa, 2003) and attenuated the A β ₁₋₄₀-induced down-regulation of PKC in rats (Zhang *et al.*, 2004). Moreover, donepezil promoted the trafficking of α -secretase to the membrane, thus enhancing α -secretase activity (Zimmermann *et al.*, 2004). Accordingly we have recently shown that PKC and MAPK pathways may be involved in the enhancement of sAPP α release by ladostigil (Yogev-Falach *et al.*, 2002). However, the effect dose not appear to result from its ChE inhibitory activity, since rasagiline and N-propargylamine, which do not inhibit ChE, were also able to induce PKC and ERK activation and promote sAPP α release (Yogev-Falach *et al.*, 2003).

Similar to ladostigil, its S-isomer, TV3279, which is a ChEI, but lacks MAO inhibitory activity, exerted pronounced neuroprotective properties and APP processing, suggesting that the mode of action is independent of MAO inhibition. These results are consistent with the current and previous data, providing clear evidence that the neuroprotection by ladostigil and rasagiline does not depend on inhibition of MAO-B, but rather is associated with some intrinsic pharmacological action of the propargyl moiety in these compounds, to increase activity of mitochondria cell survival proteins (Maruyama *et al.*, 2000a,b; Akao *et al.*, 2002). More recently, N-propargylamine itself has been demonstrated to have a neuroprotective function, with similar potency to that of rasagiline (Weinreb *et al.*, 2004; Yi *et al.*, 2005). It is now apparent that the anti-apoptotic function of N-propargylamine, similar to ladostigil is mediated by PKC-MAPK-

dependent activation associated with Bcl-2 family members and mitochondrial membrane stabilization (Maruyama *et al.*, 2003; Weinreb *et al.*, 2004; Yi *et al.*, 2005). In addition, we have recently found that the MAO-A and MAO-B inhibitory potency of N-propargylamine was significantly lower than its ability to induce neuroprotection, further establishing that MAO inhibition is not a pre-requisite for neuroprotection by propargyl-related compounds (Youdim *et al.*, 2001b,c; Yi *et al.*, 2005; Zheng *et al.*, 2005).

CONCLUSIONS

The multifunctional "dirty" drug, ladostigil, was designed to possess the neuroprotective activity established for rasagiline and address the therapeutic requirements needed to delay the progression of neurodegenerative diseases (Parkinson's disease with dementia; Lewy Body disease, and dementia with extrapyramidal symptoms) with features of dementia, behavioural abnormalities, depression and extrapyramidal symptoms (FIG. 2). Ladostigil inhibits AChE and BuChE, shows significant prevention of scopolamine-induced memory impairment in rats, and has a larger therapeutic ratio than other ChE inhibitors currently in use for the treatment of AD, including rivastigmine (Weinstock *et al.*, 2000a,b). A unique feature of this drug is its ability to produce brain-selective inhibition of both MAO-A and MAO-B in rats, rabbits and monkeys and increase brain levels of dopamine, serotonin and noradrenaline. This property enables the drug to exert anti-Parkinson activity in an MPTP model of the disease, and possess antidepressant activity like that of amitriptyline and moclobemide without causing any clinically significant potentiation of the pressor response to oral tyramine. It is thought that inhibition of both cholinesterases and MAO-A and MAO-B will have a greater advantage and pharmacological action as compared to drugs that inhibit only one form of the isozyme. Because the neurodegeneration in AD and PD is associated with oxidative stress and impairment of mitochondrial function, an additional advantage of ladostigil is its ability to reduce apoptosis and the fall in mitochondrial membrane potential resulting from oxidative stress in neuronal cell cultures. Ladostigil has also been shown to stimulate the processing of APP to the neuroprotective soluble APP α , thereby reducing

the likelihood of its processing to toxic A β peptide, a key player in the progression of AD. These latter actions probably result from direct activation of protein kinase C and do not involve either ChE or MAO inhibition. These unique multiple properties of ladostigil make it a potentially useful drug for the treatment of dementia with Parkinsonian-like symptoms (Lewy body disease) and depression.

Acknowledgement

We would like to thank Teva Pharmaceutical Co. (Netanya, Israel), Technion-Research and Development (Haifa, Israel), and Golding Ressearch Fund for Parkinson's disease for their generous support of this work. This work is in partial fulfillment of MYF's Ph.D. thesis.

References

- Abu Raya S, E Blaugrund, V Trembovler, E Shilderman-Bloch, E Shoami and P Lazarovici (1999) Rasagiline, a monoamine oxidase-B inhibitor, protects NGF-differentiated PC-12 cells against oxygen-glucose deprivation. *J. Neurosci. Res.* **1**, 456-463.
- Akao Y, W Maruyama, S Shimizu, H Yi, Y Nakagawa, M Shamoto-Nagai, MBH Youdim, Y Tsujimoto and M Naoi (2002) Mitochondrial permeability transition mediates apoptosis induced by N-methyl(R)salsolinol, an endogenous neurotoxin, and is inhibited by Bcl-2 and rasagiline, N-propargyl-1(R)-aminoindan. *J. Neurochem.* **82**, 913-923.
- Ba F, PK Pang and CG Benishin (2003) The establishment of a reliable cytotoxic system with SK-N-SH neuroblastoma cell culture. *J. Neurosci. Meth.* **123**, 11-22.
- Bar-Am O, M Yogev-Falach, T Amit, Y Sagi and MBH Youdim (2004) Regulation of protein kinase C by the anti-parkinson drug, MAO-B inhibitor, rasagiline and its derivatives, *in vivo*. *J. Neurochem.* **89**, 1119-1125.
- Bar-Am O, O Weinreb, T Amit and MB Youdim (2005) Regulation of Bcl-2 family proteins, neurotrophic factors, and APP processing in the neurorescue activity of propargylamine. *FASEB J.* **19**(13), 1899-1901.
- Biedler JL, L Helson and BA Spengler (1973) Morphology and growth, tumorigenicity, and cytogenetics of human neuroblastoma cells in continuous culture. *Cancer Res.* **33**, 2643-2652.
- Biedler JL, S Roffler-Tarlov, M Schachner and LS Freedman (1978) Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. *Cancer Res.* **38**, 3751-3757.
- Birkmayer W, P Riederer, MB Youdim and W Linauer (1975) The potentiation of the anti-kinetic effect after L-dopa treatment by an inhibitor of MAO-B, deprenyl. *J. Neural Transm.* **36**, 303-326.
- Birkmayer W, P Riederer, L Ambrozi and MBH Youdim (1977) Implications of combined treatment with 'Madopar' and L-deprenyl in Parkinson's disease. A long-term study. *Lancet*

- 1, 439-443.
- Borsini F and A Meli (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology* **94**, 147-160.
- Calderon FH, R von Bernhardt, G De Ferrari, S Luza, R Aldunate and NC Inestrosa (1998) Toxic effects of acetylcholinesterase on neuronal and glial-like cells *in vitro*. *Mol. Psychiatry* **3**, 247-255.
- Carrillo MC, C Minami, K Kitani, W Maruyama, K Ohashi, T Yamamoto, M Naoi, S Kanai and MB Youdim (2000) Enhancing effect of rasagiline on superoxide dismutase and catalase activities in the dopaminergic system in the rat. *Life Sci.* **67**(5), 577-585.
- Chong YH and YH Suh (1996) Amyloidogenic processing of Alzheimer's amyloid precursor protein *in vitro* and its modulation by metal ions and tacrine. *Life Sci.* **59**, 545-557.
- Collins GG, M Sandler, ED Williams and MBH Youdim (1970) Multiple forms of human brain mitochondrial monoamine oxidase. *Nature* **225**, 817- 820.
- Coyle JT, DL Price and MR DeLong (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science* **219**, 1184-1190.
- Da Prada M, G Zurcher, I Wuthrich and WE Haefely (1987) On tyramine, food, beverages and the reversible MAO inhibitor moclobemide. *J. Neural Transm.* **26** (Suppl.), 31-56.
- DeKosky ST, MD Ikonomovic, SD Styren, L Beckett, S Wisniewski, DA Bennett, EJ Cochran, JH Kordower and EJ Mufson (2002) Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. *Ann. Neurol.* **51**, 145-155.
- Di Stefano A, P Sozio, A Cocco, A Iannitelli, E Santucci, M Costa, L Pecci, C Nasuit, F Cantalamessa and F Pinnen (2006). L-dopa- and dopamine-(R)-alpha-lipoic acid C conjugates as multifunctional codrugs with antioxidant properties. *J. Med. Chem.* **49**(4), 1486-1493.
- Ebadi M, S Sharma, S Shavali and H El Refaey (2002) Neuroprotective actions of selegiline. *J. Neurosci. Res.* **67**(3), 285-289.
- Emilsson L, P Saetre and E Jazin (2006) Alzheimer's disease: mRNA expression profiles of multiple patients show alterations of genes involved with calcium signaling. *Neurobiol. Dis.* **21**(3), 618-625.
- Finberg JP, I Lamensdorf, JW Commissiong and MBH Youdim (1996) Pharmacology and neuroprotective properties of rasagiline. *J. Neural Transm. Suppl.* **48**, 95-101.
- Finberg JP, T Takeshima, JM Johnston and JW Commissiong (1998) Increased survival of dopaminergic neurons by rasagiline, a monoamine oxidase B inhibitor. *Neuroreport* **9**, 703-707.
- Francis PT, AM Palmer, M Snape and GK Wilcock (1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. *J. Neurol. Neurosurg. Psychiatry* **66**, 137-147.
- Francis PT, A Nordberg and SE Arnold (2005) A preclinical view of cholinesterase inhibitors in neuroprotection: do they provide more than symptomatic benefits in Alzheimer's disease? *Trends Pharmacol. Sci.* **26**, 104-111.
- Fry FH, AL Holme, NM Giles, GI Giles, C Collins, K Holt, S Pariagh, T Gelbrich, MB Hursthouse, NJ Gutowski and C Jacob (2005) Multifunctional redox catalysts as selective enhancers of oxidative stress. *Org. Biomol. Chem.* **3**(14), 2579-2587.
- Gal S, H Zheng, M Fridkin and MB Youdim (2005) Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases. *In vivo* selective brain monoamine oxidase inhibition and prevention of MPTP-induced striatal dopamine depletion. *J. Neurochem.* **95**(1), 79-88.
- Giacobini E (2004) Cholinesterase inhibitors: new roles and therapeutic alternatives. *Pharmacol. Res.* **50**, 433-440.
- Heikkila RE, RC Duvoisin, JP Finberg and MB Youdim (1985) Prevention of MPTP-induced neurotoxicity by AGN-1133 and AGN-1135, selective inhibitors of monoamine oxidase-B. *Eur. J. Pharmacol.* **116**, 313- 317.
- Huang W, Y Chen, E Shohami and M Weinstock (1999) Neuroprotective effect of rasagiline, a selective monoamine oxidase-B inhibitor, against closed head injury in the mouse. *Eur. J. Pharmacol.* **366**, 127-135.
- Jee SW, JS Cho, JH Oh, SB Shim, DY Hwang, SH Lee, YS Song, SH Lee and YK Kim (2005) cDNA microarray-based analysis of differentially expressed genes in transgenic brains expressing NSE-controlled APPsw. *Int. J. Mol. Med.* **16**(4), 547-552.
- Jellinger KA (2000) Cell death mechanisms in Parkinson's disease. *J. Neural Transm.* **107**, 1-29.
- Katsel PL, KL Davis and V Haroutunian (2005) Large-scale microarray studies of gene expression in multiple regions of the brain in schizophrenia and Alzheimer's disease. *Int. Rev. Neurobiol.* **63**, 41-82.
- Kitani K, C Minami, W Maruyama, S Kanai, GO Ivy and MC Carrillo (2000) Common properties for propargylamines of enhancing superoxide dismutase and catalase activities in the dopaminergic system in the rat: implications for the life prolonging effect of (-)deprenyl. *J. Neural Transm. Suppl.* **60**, 139-156.
- Lahiri DK and MR Farlow (1996) Differential effect of tacrine and physostigmine on the secretion of the beta-amyloid precursor protein in cell lines. *J. Mol. Neurosci.* **7**, 41-49.
- Le W, J Jankovic, W Xie, R Kong and SH Appel (1997) (-)Deprenyl protection of 1-methyl-4 phenylpyridium ion (MPP⁺)-induced apoptosis independent of MAO-B inhibition. *Neurosci. Lett.* **224**, 197-200.
- Leentjens AF (2004) Depression in Parkinson's disease: conceptual issues and clinical challenges. *J. Geriatr. Psychiatry Neurol.* **17**(3), 120-126.
- Maruyama W, MBH Youdim and M Naoi (2000a) Antiapoptotic function of N-propargylamine-1(R)- and (S)-aminoindan, rasagiline and TV1022. *Ann. NY Acad. Sci.* **939**, 320-329.
- Maruyama W, Y Akao, MBH Youdim and M Naoi (2000b) Neurotoxins induce apoptosis in dopamine neurons: protection by N-propargylamine-1(R)- and (S)-aminoindan, rasagiline and TV1022. *J. Neural Transm. Suppl.*, 171-186.
- Maruyama W, Y Akao, MBH Youdim and M Naoi (2000c) Neurotoxin induced apoptosis in dopamine neurons: protection by propargylamine derivatives, rasagiline and TV 1022, In: *Advances in Research on Neurodegeneration*, Vol. 8 (Riederer P, DB Calne, R Horowski, Y Mizuno, CW Olanow, W Poewe and MBH Youdim, Eds.) (SpringerVerlag: Wien, New York).
- Maruyama W, Y Akao, MC Carrillo, K Kitani, MB Youdim

- and M Naoi (2002) Neuroprotection by propargylamines in Parkinson's disease: suppression of apoptosis and induction of prosurvival genes. *Neurotoxicol. Teratol.* **24**(5), 675-682.
- Maruyama W, M Weinstock, MB Youdim, M Nagai and M Naoi (2003) Anti-apoptotic action of anti-Alzheimer drug, TV3326 [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, a novel cholinesterase- monoamine oxidase inhibitor. *Neurosci. Lett.* **341**, 233-236.
- Maruyama W, A Nitta, M Shamoto-Nagai, Y Hirata, Y Akao, MBH Youdim, S Furukawa, T Nabeshima and M Naoi (2004) N-Propargyl-1-(R)-aminoindan, rasagiline, increases glial cell line-derived neurotrophic factor (GDNF) in neuroblastoma SH-SY5Y cells through activation of NF-kappaB transcription factor. *Neurochem. Int.* **44**, 393-400.
- Mazzucchelli M, E Porrello, G Villetti, C Pietra, S Govoni and M Racchi (2003) Characterization of the effect of ganstigmine (CHF2819) on amyloid precursor protein metabolism in SH-SY5Y neuroblastoma cells. *J. Neural Transm.* **110**, 935-947.
- Mills J and PB Reiner (1999) Regulation of amyloid precursor protein cleavage. *J. Neurochem.* **72**, 443-460.
- McDonald WM, IH Richard and MR DeLong (2003) Prevalence, etiology, and treatment of depression in Parkinson's disease. *Biol. Psychiatry* **54**(3), 363-375.
- O'Carroll AM, CJ Fowler, JP Phillips, I Tobbia and KF Tipton (1983) The deamination of dopamine by human brain monoamine oxidase. Specificity for the two enzyme forms in seven brain regions. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **322**, 198-202.
- Pakaski M and P Kasa (2003) Role of acetylcholinesterase inhibitors in the metabolism of amyloid precursor protein. *Curr. Drug Targets CNS Neurol. Disord.* **2**, 163-171.
- Parkinson Study Group (2002) A controlled trial of rasagiline in early Parkinson disease: the TEMPO Study. *Arch. Neurol.* **59**, 1937-1943.
- Porsolt RD, A Bertin, N Blavet, M Deniel and M Jalfre (1979) Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur. J. Pharmacol.* **57**, 201-210.
- Racchi M, M Sironi, A Caprera, G Konig and S Govoni (2001) Short and long-term effect of acetylcholinesterase inhibition on the expression and metabolism of the amyloid precursor protein. *Mol. Psychiatry* **6**, 520-528.
- Racchi M, M Mazzucchelli, E Porrello, C Lanni and S Govoni (2004) Acetylcholinesterase inhibitors: novel activities of old molecules. *Pharmacol. Res.* **50**, 441-451.
- Ravina BM, SC Fagan, RG Hart, CA Hovinga, DD Murphy, TM Dawson and JR Marler (2003) Neuroprotective agents for clinical trials in Parkinson's disease: a systematic assessment. *Neurology* **60**, 1234-1240.
- Sagi Y, O Weinreb, M Weinstock and MBH Youdim (2003) Neuroprotective and neurorescue properties of rasagiline and TV3326 in MPTP model of Parkinson's disease. *Neural Plas.* **8**, 197-198.
- Sagi Y, N Drigues and MB Youdim (2005) The neurochemical and behavioral effects of the novel cholinesterase-monoamine oxidase inhibitor, ladostigil, in response to L-dopa and L-tryptophan, in rats. *Br. J. Pharmacol.* **146**(4), 553-560.
- Sharma SK, EC Carlson and M Ebadi (2003) Neuroprotective actions of selegiline in inhibiting 1-methyl, 4-phenyl, pyridinium ion (MPP⁺)-induced apoptosis in SK-N-SH neurons. *J. Neurocytol.* **32**(4), 329-343.
- Shaw KT, T Utsuki, J Rogers, QS Yu, K Sambamurti, A Brossi, YW Ge, DK Lahiri and NH Greig (2001) Phenserine regulates translation of beta-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. *Proc. Natl. Acad. Sci. USA* **98**, 7605-7610.
- Shih RA, PL Belmonte and PP Zandi (2004) A review of the evidence from family, twin and adoption studies for a genetic contribution to adult psychiatric disorders. *Int. Rev. Psychiatry* **16**(4), 260-283.
- Snyder SH and RJ D'Amato (1986) MPTP: a neurotoxin relevant to the pathophysiology of Parkinson's disease. The 1985 George C. Cotzias lecture. *Neurology* **36**, 250-258.
- Sterling J, Y Herzig, T Goren, N Finkelstein, D Lerner, W Goldenberg, I Miskolczi, S Molnar, F Rantal, T Tamas, G Toth, A Zagyva, A Zekany, G Lavian, A Gross, R Friedman, M Razin, W Huang, B Kraus, M Chorev, MBH Youdim and M Weinstock (2002) Novel dual inhibitors of AChE and MAO derived from hydroxy aminoindan and phenethylamine as potential treatment for Alzheimer's disease. *J. Med. Chem.* **45**, 5260-5279.
- Tandberg E, JP Larsen, D Aarsland and JL Cummings (1996) The occurrence of depression in Parkinson's disease. A community-based study. *Arch. Neurol.* **53**, 175-179.
- Tatton WG, RM Chalmers-Redman, WJ Ju, M Mammen, GW Carlile, AW Pong and NA Tatton (2002) Propargylamines induce antiapoptotic new protein synthesis in serum- and nerve growth factor (NGF)-withdrawn, NGF-differentiated PC-12 cells. *J. Pharmacol. Exp. Ther.* **301**, 753-764.
- Tsujimoto Y and S Shimizu (2000) Bcl-2 family: life-or-death switch. *FEBS Lett.* **466**, 6-10.
- Umehara T, K Fukuda, F Nishikawa, M Kohara, T Hasegawa and S Nishikawa (2005) Rational design of dual-functional aptamers that inhibit the protease and helicase activities of HCV NS3. *J. Biochem. (Tokyo)* **137**(3), 339-347.
- Veazey C, SO Aki, KF Cook, EC Lai and ME Kunik (2005) Prevalence and treatment of depression in Parkinson's disease. *J. Neuropsychiatry Clin. Neurosci.* **17**(3), 310-323.
- Venkatachalam TK, PA Goodman, S Qazi, O D'Cruz and FM Uckun (2004) Rational drug design of multifunctional phosphoramidate substituted nucleoside analogs. *Curr. Pharm. Des.* **10**(15), 1713-1726.
- von Bernhardt R, G Ramirez, GV De Ferrari and NC Inestrosa (2003) Acetylcholinesterase induces the expression of the beta-amyloid precursor protein in glia and activates glial cells in culture. *Neurobiol. Dis.* **14**, 447-457.
- Wadia JS, RME Chalmers-Redman, WJH Ju, GW Carlile, JL Phillips, AD Fraser and WG Tatton (1998) Mitochondrial membrane potential and nuclear changes in apoptosis caused by serum and nerve growth factor withdrawal: time course and modification by (-)-deprenyl. *J. Neurosci.* **18**, 932-947.
- Weinreb O, O Bar-Am, T Amit, O Chillag-Talmor and MBH Youdim (2004) Neuroprotection via pro-survival protein kinase C isoforms associated with Bcl-2 family members. *FASEB J.* **18**, 1471-1473.
- Weinstock M, T Goren and MBH Youdim (2000a) Development of a novel neuroprotective drug (TV3326) for the treatment of Alzheimer's disease, with cholinesterase and monoamine

- oxidase inhibitory activities. *Drug Dev. Res.* **50**, 216-222.
- Weinstock M, C Bejar, RH Wang, T Poltyrev, A Gross, JPM Finberg and MBH Youdim (2000b) TV3326, a novel neuroprotective drug with cholinesterase and monoamine oxidase inhibitory activities for the treatment of Alzheimer's disease. *J. Neural Transm.* **60** (Suppl.), 157-169.
- Weinstock M, T Goren and MBH Youdim (2000c) Development of a novel neuroprotective drug (TV3326) for the treatment of Alzheimer's disease, with cholinesterase and monoamine oxidase inhibitory activities. *Drug Dev. Res.* **50**, 216-222.
- Weinstock M, N Kirschbaum-Slager, P Lazarovici, C Bejar, MBH Youdim and S Shoham (2001) Neuroprotective effects of novel cholinesterase inhibitors derived from rasagiline as potential anti-Alzheimer drugs. *Ann. NY Acad. Sci.* **939**, 148-162.
- Weinstock M, E Gorodetsky, RH Wang, A Fross, O Weinreb and MBH Youdim (2002a) Limited potentiation of blood pressure response to oral tyramine by brain-selective monoamine oxidase A-B inhibitor, TV-3326 in conscious rabbits. *Neuropharmacol.* **43**, 999-1005.
- Weinstock M, T Poltyrev, C Bejar, Y Sagi and MBH Youdim (2002b) TV3326, a novel cholinesterase and MAO inhibitor for Alzheimer's disease with co-morbidity of Parkinson's disease and depression, In: *Mapping the Progress of Alzheimer's and Parkinson's Disease* (Mizuno Y, A Fisher and I Hanin, Eds.) (Kluwer Academic/Plenum Publishers:New York), pp 199-204.
- Weinstock M, T Poltyrev, C Bejar and MBH Youdim (2002c) Effect of TV3326, a novel monoamine-oxidase-cholinesterase inhibitor, in rat models of anxiety and depression. *Psychopharmacology* **160**, 318-324.
- Wu RM, RC Chen and CC Chiueh (2000) Effect of MAO-B inhibitors on MPP⁺ toxicity *in vivo*. *Ann. NY Acad. Sci.* **899**, 255-261.
- Yamada Y, Y Miura, A Sakaki, T Yoshida and K Kobayashi (2006) Design of multifunctional peptides expressing both antimicrobial activity and shiga toxin neutralization activity. *Bioorg. Med. Chem.* **14**(1), 77-82.
- Yang HY and NH Neff (1974) The monoamine oxidases of brain: selective inhibition with drugs and the consequences for the metabolism of the biogenic amines. *J. Pharmacol. Exp. Ther.* **189**, 733-740.
- Yang CS, HY He and XJ Zhang (2002) Increased expression of intranuclear AChE involved in apoptosis of SK-N-SH cells. *Neurosci. Res.* **42**, 261-268.
- Yi H, W Maruyama, Y Akao, K Chen, K Iwasa, J Shih, MBH Youdim and M Naoi (2005) The N-propargylamine moiety of rasagiline has neuroprotective activity via mitochondrial membrane stabilization and induction of Bcl-2. *J. Neural Transm.* **112**, 21-32.
- Yogev-Falach M, O Bar-Am, T Amit, O Weinreb and MBH Youdim (2006) <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=16935943&query_hl=1&itool=pubmed_docsum> A multifunctional, neuroprotective drug, ladostigil (TV3326), regulates holo-APP translation and processing. *FASEB. J.* **20**(12), 2177-2179. Epub 2006 Aug 25.
- Yogev-Falach M, T Amit, O Bar-Am, Y Sagi, M Weinstock and MBH Youdim (2002) The involvement of mitogen-activated protein (MAP) kinase in the regulation of amyloid precursor protein processing by novel cholinesterase inhibitors derived from rasagiline. *FASEB J.* **16**, 1674-1676.
- Yogev-Falach M, T Amit, O Bar-AM and MBH Youdim (2003) The importance of propargylamine moiety in the anti-Parkinson drug rasagiline and its derivatives for MAPK-dependent amyloid precursor protein processing. *FASEB J.* **17**, 2325-2327.
- Youdim MBH (2003) Rasagiline: an anti-parkinson drug with neuroprotective activity. *Exp. Rev. Neurother.* **3**, 737-749.
- Youdim MB and JJ Buccafusco (2005) Multi-functional drugs for various CNS targets in the treatment of neurodegenerative disorders. *Trends Pharmacol. Sci.* **26**, 27-35.
- Youdim MB and P Riederer (1993) Dopamine metabolism and neurotransmission in primate brain in relationship to monoamine oxidase A and B inhibition. *J. Neural Transm. Gen. Sect.* **91**(2-3), 181-195.
- Youdim MBH and M Weinstock (2002a) Novel neuroprotective anti-Alzheimer drugs with antidepressant activity derived from the anti-parkinson drug, rasagiline. *Mech. Ageing Dev.* **123**, 1081-1086.
- Youdim MBH and M Weinstock (2002b) Molecular basis of neuroprotective activities of rasagiline and the anti Alzheimer drug, TV3326, [(N-Propargyl-(3R)Aminoindan-5-YL)-ethyl-methyl carbamate]. *Cell. Mol. Neurobiol.* **21**, 555-573.
- Youdim MB and M Weinstock (2004) Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology* **25**(1-2), 243-250.
- Youdim MBH, A Gross and JPM Finberg (2001a) Rasagiline [N-Propargyl-1R(+)-aminoindant], a selective and potent inhibitor of mitochondrial monoamine oxidase B. *Br. J. Pharmacol.* **132**, 500-506.
- Youdim MBH, E Grunblatt, Y Levites and S Mandel (2001b) *Drugs to Prevent Cell Death in Parkinson's Disease: Neuroprotection Against Oxidative Stress and Inflammatory Gene Expressions* (Calne D and S Calne, Eds.) (Lippincott Williams & Wilkins:Philadelphia), Vol. 86, pp 115-125.
- Youdim MBH, A Wadia, NA Tatton and M Weinstock (2001c) The anti-Parkinson drug rasagiline and its cholinesterase inhibitor derivatives exert neuroprotection unrelated to MAO inhibition in cell culture and *in vivo*. *Ann. NY Acad. Sci.* **939**, 450-458.
- Youdim MB, W Maruyama and M Naoi (2005) Neuropharmacological, neuroprotective and amyloid precursor processing properties of selective MAO-B inhibitor antiparkinsonian drug, rasagiline. *Drugs Today (Barc.)* **41**(6), 369-391.
- Yuan J and BA Yankner (2000) Apoptosis in the nervous system. *Nature* **407**, 802-809.
- Zhang HY, H Yan and XC Tang (2004) Huperzine A enhances the level of secretory amyloid precursor protein and protein kinase C-alpha in intracerebro ventricular beta-amyloid-(1-40) infused rats and human embryonic kidney 293 Swedish mutant cells. *Neurosci. Lett.* **360**, 21-24.
- Zheng H, LM Weiner, O Bar-Am, S Epsztejn, ZI Cabantchik, A Warshawsky, MB Youdim and M Fridkin (2005a) Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. *Bioorg. Med. Chem.* **13**(3), 773-783.
- Zheng H, S Gal, LM Weiner, O Bar-Am, A Warshawsky, M Fridkin and MBH Youdim (2005b) Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases: *in vitro* studies on antioxidant activity, prevention of lipid peroxide formation and monoamine oxidase inhibition. *J. Neurochem.* **95**(1), 68-78.
- Zimmermann M, F Gardoni, E Marcello, F Colciaghi, B Borroni, A Padovani, F Cattabeni and M Di Luca (2004) Acetylcholinesterase inhibitors increase ADAM10 activity by promoting its trafficking in neuroblastoma cell lines. *J. Neurochem.* **90**, 1489-1499.