REVIEW

Explaining ADAGIO: A Critical Review of the Biological Basis for the Clinical Effects of Rasagiline

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ABSTRACT: The ADAGIO study demonstrated a symptomatic benefit for rasagiline in early Parkinson's disease (PD) and suggested a disease-modifying effect. Evidence indicates that mitochondrial dysfunction plays a role in the pathogenesis of PD and that this may be the site of effect for rasagiline. In this systematic review, evidence for the role of mitochondria in the pathogenesis of PD are reviewed in light of other proposed mechanisms of neuronal degeneration and the actions of rasagiline and its component parts, namely propargylamine and the metabolite, aminoindan. Evidence for the role of mitochondria in the pathogenesis and treatment of PD are reviewed in light of other proposed mechanisms of neuronal degeneration and clinical actions of rasagiline. Monoamine oxidase B (MAO-B) located in the outer mitochondrial membrane controls dopamine metabolism in early PD, and this is the likely location for the symptomatic action of rasagiline. Accumulating evidence indicates that mitochondrial impairment contributes to dopaminergic neuronal loss in PD, either directly or through other mechanisms such as oxidative stress or protein misfolding. Further rasagiline affects numerous mitochondrial mechanisms that prevent apoptotic cell death including prevention of opening of the mitochondrial transition pore, decreased release of cytochrome C, alterations in pro-antiapoptotic genes and proteins, and the nuclear translocation of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Thus, the functional neuroprotective actions of rasagiline may not be dependent on MAO-B inhibition, but rather may involve actions of the propargylamine moiety and the aminoindan metabolite. An accumulating body of literature indicates a mitochondrial site of action for rasagiline and highlights the neuroprotective action of the drug, providing strong biological plausibility for disease-modifying effects of the drug such as those observed in ADAGIO.

Key Words: rasagiline; mitochondria; Parkinson's disease; neuroprotection

Rasagiline (N-propargyl-1-(R)-aminoindan) is a potent and irreversible inhibitor of monoamine oxidase type B (MAO-B)[1] and has demonstrated efficacy as monotherapy in early Parkinson's disease (PD)[2] or as an adjunct to L-dopa therapy in more advanced disease.[3,4] The potential of rasagiline to modify the progression of PD was examined in the ADAGIO delayed-start study, which was designed to separate the symptomatic effects of rasagiline from a potential disease-modifying effect.[5–7] At the approved 1-mg daily dose, those patients treated with rasagiline met all 3 hierarchical endpoints that would be expected from a drug with disease-modifying effects,[5] including a slower rate of Unified Parkinson's Disease Rating Scale (UPDRS) deterioration in those subjects who were on rasagiline during the first 9 months of the (early-start group) compared to those in the group who received placebo during the first 9 months of the study (delayed-start group).[6] The 2-mg dose did not meet the criteria for disease-modification. There is accumulating data suggesting that this is likely due to

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the limits of the clinical scale used in the study, rather than a lack of drug action.[6,8–10] Interestingly, very recent *post hoc* analysis has now identified improvements in activities of daily living (ADL subscore of the UPDRS) as a key contributor to the actions of rasagiline at the approved daily dose in early PD.[10]

The results of ADAGIO highlight the importance of understanding the mechanism through which rasagiline might exert a disease-modifying effect. How these link to the pathogenesis of PD are important questions, but they have not been reviewed in the context of the ADAGIO study. A key change leading to neuronal loss in PD occurs at the level of the mitochondria, which is also the location of MAO-B. This review explores the possibility that the effects of rasagiline suggested by the ADAGIO study relate to reduced dopaminergic degeneration through effects on mitochondrial function.

MAO-B: Symptomatic Effects of Rasagiline

Importantly for the actions of rasagiline, MAO-A and MAO-B are located in the outer membrane of the mitochondrial wall and both are equally involved in the oxidative deamination of dopamine.[11] Under normal physiological conditions in the striatum, synaptic dopamine levels are tightly regulated by its active reuptake into presynaptic terminals where MAO-A is predominantly located and serves to regulate dopamine's actions through oxidative metabolism. In contrast, with neuronal degeneration, the role of reuptake diminishes, and the MAO-B located in glial cells that surround dopaminergic synapses becomes the predominant site for dopamine metabolism.[12] This inhibition of MAO-B then prolongs the availability and activity of endogenous dopamine in the striatum.[13] The concept of the switch from presynaptic metabolism of dopamine by MAO-A to glial metabolism by MAO-B has important implications for the symptomatic treatment of PD. The increased prominence of MAO-B in dopamine metabolism in the denervated striatum implies that MAO-B inhibition directly "targets" the parkinsonian state. It might also suggest that MAO-B inhibitors are less likely to affect dopaminergic function in other brain regions in PD where innervation is relatively intact, such as the limbic and cortical areas. This might serve to explain the low incidence of psychotic reactions and compulsive behaviors seen on rasagiline monotherapy.

Mitochondria and the Pathogenesis of PD

Mitochondria play an essential role in normal cell function and in the control of the activity of key organelles and cellular processes involving energy production and utilization through the electron transport chain and Krebs cycle. They determine continuing cell survival but are the major source of cellular free radical formation. In PD, there is a 30% to 40% decrease in the activity of mitochondrial complex I activity in substantia nigra[14–16] and reduced activity of α-ketoglutarate dehydrogenase, a key Krebs cycle enzyme.[17,18] Changes in complex I appear specific to PD and are not found in related disorders, such as multiple system atrophy (MSA) or progressive supranuclear palsy (PSP), they do not occur in other brain regions outside of the basal ganglia but can be demonstrated in platelets and muscle.[19] The alterations in complex I activity appear encoded since the construction of cybrids using mitochondrial DNA (mtDNA) from platelets in PD showed transfer of the deficiency accompanied by evidence of oxidative stress.[20] Subsequently, a variety of deletions in mtDNA have been uncovered in PD but examination of the mitochondrial genome has not revealed a common fault.[21] However, functional defects in mitochondria associated with oxidative damage occur with aging and in PD, with imaging studies demonstrating mitochondrial dysfunction in early and advanced PD.[22]

More recently, specific gene mutations associated with familial PD (including PINK1, DJ-1, alpha-synuclein, LRRK2, and parkin) have been shown to be either mitochondrial proteins or are associated with mitochondrial dependent cell death.[23] Mitochondrial impairment was shown in fibroblasts from skin biopsies from patients with the G2019S mutation in LRRK2.[24] A genomewide association study has also shown that genes controlling cellular bioenergetics which are expressed in response to the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) are underexpressed in PD.[25] Through an interaction with the Parkin interacting substrate (PARIS),[26] activation of PGC-1α increases expression of nuclear-encoded subunits of the mitochondrial respiratory chain, and blocks the dopaminergic neuron loss induced by mutant alpha-synuclein or rotenone.[25,27] This interconnection with alpha-synuclein is an important one, as this protein, along with mitochondrial dysfunction, appears to play a significant role in the pathological process that underlies PD.

Mitochondria and Rasagiline

Symptomatic Efficacy of Rasagiline

The results of ADAGIO showed that 1 mg of rasagiline reduces total-UPDRS scores by -3.01 ± 0.43 units versus placebo, reflecting its symptomatic efficacy.[6] The MAO-B inhibitory properties of rasagiline are attributed to irreversible covalent binding of its propargyl moiety to the flavin adenine dinucleotide (FAD) moiety of the enzyme located in the outer

mitochondrial wall. Rasagiline is highly selective for MAO-B compared to MAO-A,[28] but it does inhibit of MAO-A at concentrations above those usually produced by the approved daily dosage. By binding to the FAD moiety, rasagiline prevents the access of dopamine to MAO-B, thereby inhibiting oxidative deamination to 3,4-dihydroxphenlyacetic acid (DOPAC), and raising the levels of dopamine.[1] The irreversible binding of rasagiline to MAO-B and its inactivation implies a continuous sustained effect on endogenous dopamine levels, consistent with the tonic stimulation of dopamine receptors that occurs under normal physiological conditions.[29] In addition, MAO-B inhibition with rasagiline increases the availability of phenylethylamine, which can enhance striatal dopamine release.[30]

Disease-Modifying Effects of Rasagiline

Effects at the mitochondrial level may also be responsible for a disease-modifying effect in PD. Mitochondria play a key role to apoptosis, a mechanism of cell death proposed in PD. A fall in mitochondrial membrane potential leads to increased outer wall permeability and the opening of the permeability transition pore, and swelling of the mitochondria. Subsequent release of cytochrome c initiates the caspase cascades leading to cell death with accompanying decreases in levels of antiapoptotic and increases in the levels of proapoptotic proteins.[31] A disease-modifying drug that interferes with apoptotic cell death would be expected to modify these processes or markers of their effect.

Neuroprotective properties of rasagiline occur in a range of in vitro systems related to dopaminergic cell death and the effects uncovered mimic those needed to prevent apoptosis, which may play a role in PD (Fig. 1). Using dopaminergic neuroblastoma SH-SY5Y and pheochromocytoma PC12 cells subjected to toxic insults inducing apoptotic cell death, it was shown that rasagiline stabilizes the mitochondrial membrane potential,[32,33] prevents mitochondrial swelling, and prevents release of cytochrome c and caspase activation.[33,34] Mitochondrial stress leads to increased cytoplasmic levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and its conversion from a dimer to tetramer, which then translocates to the nucleus where it interferes with the transcriptional upregulation of antiapoptotic molecules such as Bcl2 and Bcl-xl. By inhibiting its S-nitrosylation,[35,36] rasagiline prevents the nuclear accumulation of GAPDH,[37–39] leading to upregulation of Bcl2 and Bcl-xl and downregulation of the proapoptotic proteins Bad and Bax, and so maintaining mitochondrial membrane potential, and preventing apoptosis.[34,39] Importantly, the effects of rasagiline are also associated with increased levels of the neurotrophic factors, brain-derived neurotrophic factor (BDNF) and glial cell–derived neurotrophic factor (GDNF)[40] that preserve dopaminergic neuron function, implying that this drug might have multiple ''neuroprotective'' actions.

The important next question is whether any of these *in vitro* neuroprotective actions of rasagiline translate into functional models of PD. In the 6-hydroxydopamine (6-OHDA)-lesioned rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primate, rasagiline treatment resulted in increased cell survival accompanied by improved motor function.[41,42] The effects in the MPTP-treated primate are not surprising, as inhibition of MAO-B will prevent the conversion of MPTP to MPP^+, but the effects against 6-OHDA cannot be accounted for in this way. In any case, the effects in MPTP-treated primates illustrate that the functional nature of the inhibition of MAO-B by rasagiline is effective in blocking the toxic actions of potential environmental causes of PD.

Rasagiline is also effective in 2 other models that have relevance to PD. Proteasomal inhibition in the substantia nigra may be important in PD, leading to mishandling and misfolding of proteins and to Lewy body formation.[43] The proteasomal inhibitor lactacystin leads to apoptotic dopaminergic cell loss when injected directly into the substantia nigra. Importantly, rasagiline prevents the loss of dopaminergic cells when either administered prior to or following lactacystin injection and improved motor function.[44] Similarly, in mice overexpressing mutant α-synuclein and treated with the complex II inhibitor, 3-nitropropionic acid, to induce a model of MSA, rasagiline again prevented both nigral and striatal neuronal loss while preserving motor function.[45]

Does MAO-B Inhibition Explain the Neuroprotective Effects of Rasagiline?

The primary action of rasagiline as an MAO-B inhibitor would seem the obvious explanation for its neuroprotective effects. However, the S-isomer of rasagiline (TVP1022) lacks significant MAO-B activity, but is also neuroprotective in the same models of PD.[32,46–48] Moreover, the S-isomer stabilizes mitochondrial membrane potential and alters other markers of mitochondrial induced apoptosis.[32] In addition, the *in vitro* neuroprotective action of rasagiline studies were undertaken in dopaminergic cell lines that do not contain MAO-B[49] and the concentrations of rasagiline required were lower than those required for MAO-B inhibition.[50]

This raises the question of alternative actions of rasagiline. However, there have not been any published investigations of other potential mitochondrial binding sites where rasagiline could act to stabilize the mitochondrial membrane potential and prevent the cascade of events leading to apoptotic or other forms

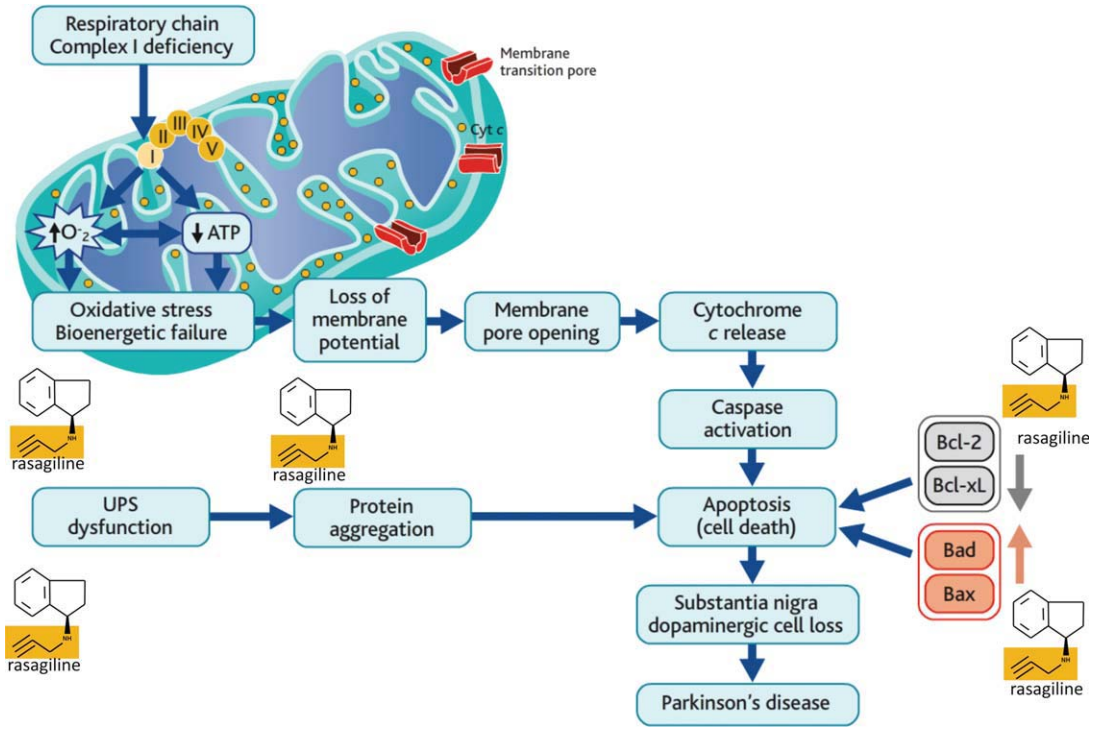

FIG. 1. Antiapoptotic function of rasagiline.

of cell death.[50] Where these effects take place is also unclear. MAO-B is largely localized to glial cells surrounding dopaminergic synapses, but the neuroprotective effects of rasagiline are evident against direct toxic insults to dopaminergic cells. *In vivo*, the effect of rasagiline could be indirect, with an action on glia leading to the release of protective factors, such as GDNF and BDNF. But this would not explain direct protective effects at the neuronal level. The effect of rasagiline could involve an action on mitochondria located in dopaminergic terminals but this has not been investigated. However, there is evidence pointing toward an involvement of neuronally located MAO-A. In many studies, N-methyl-(R)-salsolinol was used to disrupt mitochondrial function. It binds to MAO-A and induces apoptosis that is prevented by small interfering RNA (siRNA) against MAO-A.[51] Similarly, usage of siRNA to suppress MAO-A inhibits induction of Bcl-2 and Bcl-xl by rasagiline.[52] In addition, overexpression of MAO-B in SHSY-5Y cells decreases the rasagiline induced increase in Bcl-2,[50] although this may simply reflect that the concentration of rasagiline was too low to block MAO-B activity. The results of these *in vitro* studies should be interpreted with caution, as the concentrations of rasagiline used are higher than found in man. Although inhibition of MAO-A in the clinical use of rasagiline is modest,[28] MAO-A activity may also be relevant to the neuroprotective effects of rasagiline in experimental studies, and this needs to be investigated further.

A Role for the Propargylamine Moiety and the 1-Amino-Indan Metabolite in Neuroprotection

The potential disease-modifying properties of rasagiline arose from its chemical structure which includes an indan ring and a propargylamine moiety.[1] Propargylamines inhibit apoptosis both in *in vitro* and *in vivo* models of PD[53] and structure-activity relationship studies showed that a propargylamine group and a hydrophobic group with an appropriate interatomic distance as found in rasagiline were required for neuroprotection.[49,54] N-propargylamine exerts its effects on apoptosis through mitochondrial mechanisms, including the downregulation of Bax and Bad proteins, the induction of Bcl-2 and Bcl-xL proteins, the induction of GDNF and BDNF, and stimulation of protein kinase C phosphorylation.[38,49,54] However, it is clear that presence of a propargylamine moiety alone would not fully explain a clinical disease-modifying effect.

An important component of the actions of rasagiline is the activity of its metabolite, 1-amino-indan (AI).[55] In rodent models of PD, AI enhances striatal dopamine transmission and improves motor function.[56] These effects were independent of MAO inhibition (although AI is a weak inhibitor of MAO-B[57]) and were dependent on the presence of dopaminergic terminals. But so far, no interaction of AI was found with more than 100 potential neuronal and enzymatic targets.[56] Interestingly, in *in vivo* microdialysis studies, AI increased synaptic concentrations of dopamine and

serotonin but not noradrenaline.[56] However, in a cytotoxic human neuroblastoma SK-N-SH cell model of high-density culture-induced neuronal death, AI significantly reduced the S139 phosphorylation level of the apoptotic-associated γ-H2A.X protein, decreased the levels of cleaved caspases-3 and -9, while increasing the levels of phosphorylated protein kinase C (PKC) as well as Bcl-2 and Bcl-xL.[58] In the same series of experiments, AI was also shown to protect rat pheochromocytoma PC-12 cells against 6-OHDA neurotoxicity. This leads to the conclusion that AI may contribute to the neuroprotective activity of rasagiline.[58]

Alternative mechanisms: Interplay Between Mitochondrial Proteasomal Dysfunction and Alpha-Synuclein Aggregation

Protein aggregation leading to Lewy body formation is a key pathological feature of PD and alpha-synuclein is a major component of Lewy bodies and Lewy neurites. Under normal conditions, unwanted intracytoplasmic proteins are normally cleared by the ubiquitin-proteasome system (UPS) and lysosomes.[59] Postmortem studies show impaired proteasomal function in the substantia nigra of PD patients,[43,60] and cell culture studies show an association between mitochondrial dysfunction and proteasomal impairment, and the combination consistently enhances dopaminergic cell damage and death.[61–64] For example, proteasomal function is impaired due to a reduction of mitochondrial dependent adenosine triphosphate (ATP) synthesis.[65] Conversely, proteasome inhibition by lactacystin in cells expressing mutant alpha-synuclein increases mitochondria-dependent apoptotic cell death[63] and rasagiline affords protection against lactacystin-induced neurodegeneration.[44] Although the mechanisms for rasagiline's effects in this model are unknown, improved mitochondrial functioning results in improved proteasomal function.

It is well known that mitochondrial dysfunction in PD leads to increased oxidative stress and that oxidative stress also causes further alpha-synuclein aggregation and further impairs proteasomal ubiquitination.[66–69] Rasagiline does not directly scavenge free radicals, but in SH-SY5Y cell lines, it acts to stabilize mitochondrial membrane potential against the collapse induced by peroxynitrite generated from SIN-1.[32,70] Interestingly, rasagiline protects against cell death induced by the combination of free radicals generated by paraquat in either wild-type or A53T mutant alpha-synuclein overexpression. This protection was associated with a reduction in caspase 3 activation, a reduction in superoxide generation, and a trend to ameliorate the fall in mitochondrial membrane potential. In addition, rasagiline induced an increase in cellular glutathione levels.[71] Clearly, there are a number of potential links between mitochondrial function and alpha-synuclein misfolding, and this connection merits further research.

The Difficult Question of the Differences Between Rasagiline and Selegiline

The difference between rasagiline and selegiline has been much debated. Both are MAO-B inhibitors, both contain a propargylamine moiety, and both have similar preclinical evidence for the alteration of mitochondrial function by non-MAO-B mediated mechanisms.[72] From an evidence-based perspective, it is difficult to compare the outcome of the clinical trials that have underpinned the potential for disease-modification because of the different designs. There is no doubt that the interpretation of the clinical investigations with selegiline were initially marred by the finding of a symptomatic effect of the drug in early PD.[73] Although the general impression as to whether or not selegiline is disease-modifying remains controversial, there are still reports of a long-term effect of selegiline altering disease outcome.[74] From the outset, the ADAGIO delayed-start study was designed to overcome the effect of a confounding symptomatic effect of rasagiline and as such it represents the only clinical trial that can purport to show a disease-modifying effect for PD.[6] Indeed, the recent failure of pramipexole to produce a similar outcome in the almost identically designed PROUD study[75] could be interpreted as showing that rasagiline has unique characteristics leading to disease-modification.

At the preclinical level, there are also differences between the drugs. Rasagiline is more potent in the inhibition of MAO-B than selegiline and also in regard to its ability to reverse changes in mitochondrial function that lead to apoptosis. For example, *in vitro* studies in human neuroblastoma SH-SY5Y and glioblastoma 1242-MG cells show that rasagiline reduces mitochondrial-dependent apoptosis with more potency than selegiline.[70,76] In the MPTP-treated mouse, rasagiline exerts neuroprotective activity at far lower doses than selegiline. There are also examples in which rasagiline exerts a neurorestorative effect where selegiline has none. Again, in the MPTP-treated mouse, the administration of rasagiline following MPTP treatment restores midbrain dopamine neuronal function whereas selegiline does not.[77–79] Significantly, in the lactacystin-treated mouse, both rasagiline and selegiline prevent nigrostriatal damage and behavioral abnormalities when administered prior to lactacystin, but only rasagiline affords protection when the drugs were administered after lactacystin treatment.[44] This is potentially important if it translates in to the treatment of PD as a significant proportion of dopaminergic neurons are already lost at diagnosis. A further explanation for differences between rasagiline and

selegiline may lie in the components of each molecule. The propargylamine moiety, which both molecules possess, can alter apoptotic cell death but this alone may not be sufficient to produce a disease-modifying effect. The propargylamine, TCH346, was produced as a non-MAO-B inhibiting derivative based on the effects seen with selegiline, but it failed to show clinical neuroprotective effects at the doses employed.[80] Potentially, differences in metabolism may provide a fuller explanation. *In vivo*, selegiline is metabolized to amphetamine derivatives that inhibit the neuroprotective actions of both selegiline and desmethylselegiline. So, in theory, the neuroprotective effects seen *in vitro* may not translate in to a clinically relevant action because of metabolite formation. In contrast, rasagiline is metabolized to AI, which does possess neuroprotective effects even though these are not fully understood. Moreover, AI does not interfere with the neuroprotective effects of rasagiline or selegiline, whereas the selegiline metabolite, L-methamphetamine, inhibited both the effects of selegiline and rasagiline.[81]

This leads to the hypothesis that the overall activity of rasagiline may depend on a combination of its component parts rather than any individual portion of the molecule. The intact rasagiline molecule exerts neuroprotective effects, the propargylamine moiety shows a neuroprotective activity, and there is increasing evidence that AI possesses neuroprotective properties. In other words, rasagiline may exert greater effectiveness as a neuroprotective agent and have greater potential for disease-modification in PD because of the contributions made by its component parts, possibly acting through different mechanisms.[50]

Summary and Conclusions

This review was inspired by the results of the ADAGIO study, which was the first to use a delayed-start design to document a disease-modifying effect in PD. The results at the currently recommended dose were impressive and fully consistent with a disease-modifying effect. From the preclinical evidence for neuroprotective properties of rasagiline, a common theme emerges, indicating mitochondria as the site of action. Indeed, MAO-B inhibition located in the mitochondrial wall explains the symptomatic effects of rasagiline, and its potential disease-modifying properties can be explained through stabilization of mitochondrial function. Moreover, effects on mitochondria can also explain the effects of rasagiline on oxidative stress, excitotoxicity, and altered protein handling.[82] Precisely how these effects are mediated and the mitochondrial location through which rasagiline acts, remains to be determined. Importantly, the neuroprotective effects of rasagiline not only relate to the parent structure and the propargyl moiety but also its AI metabolite.

Before closing, it is worth mentioning that there could be considerable interplay between the symptomatic and disease-modifying actions of rasagiline. Preservation of dopaminergic neurons via a neuroprotective effect mediated through mitochondria would enhance endogenous dopamine levels in the striatum, further increasing the substrate for the symptomatic efficacy of rasagiline. Conversely, with the preservation of dopaminergic tone and the maintenance of normal or enhancement of physiological compensatory mechanisms through a symptomatic activity, disease progression would appear modified in the sense that compensatory mechanisms are being maintained or even enhanced. Even reduced breakdown of dopamine through MAO-B inhibition might reduce oxidative stress and so contribute to the overall disease-modifying effects of rasagiline.

What is now required is long-term experience with early treatment with rasagiline that will reveal its effectiveness on disease progression. However, collectively, the preclinical and clinical data for rasagiline are at least indicative of a disease-modifying effect and this must be considered when initiating therapy in early PD. ■

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