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Neuroprotection by Rasagiline: A New Therapeutic Approach to Parkinson's Disease?

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ABSTRACT

Neuronal death in Parkinson's disease (PD) may originate from the reciprocal interactions of a restricted number of conditions, such as mitochondrial defects, oxidative stress and protein mishandling, which would favor a state of apoptotic cell death in the nigrostriatal pathway. The search for pharmacological treatments able to counteract the nigrostriatal degeneration, possibly by interfering with these phenomena, has recently raised considerable interest in rasagiline [R(+)-N-propargyl-1-aminoindan], a potent, selective, and irreversible inhibitor of monoamine oxidase B (MAO-B). Rasagiline, like selegiline, is a propargylamine, but is ~10 times more potent. Unlike selegiline, rasagiline is not metabolized to amphetamine and/or methamphetamine and is devoid of sympathomimetic activity. Numerous experimental studies, conducted both *in vitro* and *in vivo*, have shown that rasagiline possesses significant protective properties on neuronal populations. The pro-survival effects of the drug appear to be linked to its propargyl moiety, rather than to the inhibitory effect on MAO-B. Rasagiline's major metabolite, aminoindan — which possesses intrinsic neuroprotective activity — may also contribute to the beneficial effects of the parent compound.

Rasagiline has been recently evaluated in early PD patients, with results that are consistent with slowing the progression of the disease. Therefore, the neuroprotective activity shown by the drug under experimental conditions may be reflected in the clinic, thus providing new perspectives for the treatment of PD.

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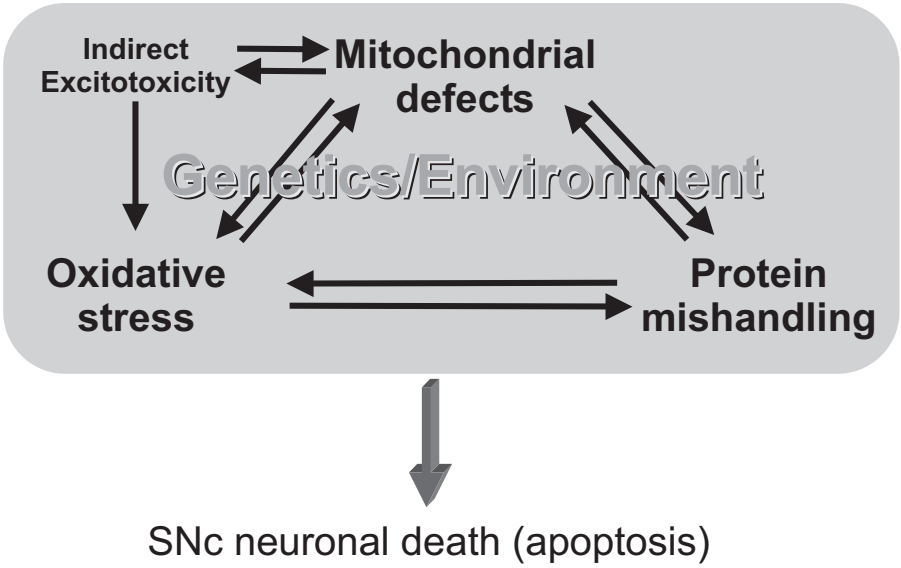

Fig. 1. Schematic overview of the pathogenic hypotheses of Parkinson's disease: interplay of the main mechanisms potentially involved, superimposed over a background where genetic and/or environmental factors may also intervene. SNc, Substantia nigra pars compacta.

PARKINSON'S DISEASE: AN OVERVIEW OF PATHOGENIC HYPOTHESES

Parkinson's disease (PD) is a neurodegenerative disorder in which the ability to control voluntary movements is gradually lost, as a consequence of profound changes in the functional organization of the basal ganglia nuclei. The pathological hallmark of PD is the progressive degeneration of melanin-containing dopaminergic neurons of the substantia nigra pars compacta (SNc). Cell loss in the SNc causes severe deficits in dopaminergic stimulation of the corpus striatum — the target of SNc neuron projections — which triggers a cascade of functional modifications in the basal ganglia circuitry, resulting in the typical motor symptoms of the disease (bradykinesia, tremor, rigidity) (14,26).

The *primum movens* of the degenerative process underlying PD remains unknown. In fact, no single causative factor has yet been identified for the sporadic form of the disease, which represents the vast majority of PD cases. Indeed, due to the multi-factorial nature of the disease, the process leading to nigral cell death is likely to originate from the reciprocal interactions of a number of unfavorable conditions (Fig. 1). These include mitochondrial defects (impaired activity of complex I, in particular), enhanced formation of reactive oxygen species leading to oxidative damage, and aberrant protein aggregation (77,78). The latter may be linked to the reduced efficiency of a mechanism specifically devoted to the intracellular degradation of altered proteins, the ubiquitin-proteasome system, whose involvement in PD pathogenesis has been recently pointed out (61,71). The combined actions of these phenomena may disrupt the physiological dynamics of apoptosis within the SNc, thus triggering uncontrolled neuronal death (18,39). An additional role may also be played by the glutamatatergic hyperactivity developing at the subthalamo-ni-

gral level, as a consequence of the striatal dopaminergic denervation, which would sustain the progression of the disease with a mechanism known as "indirect excitotoxicity" (8,15,29).

Defective regulation of apoptosis may, indeed, play a central role in the process of nigral cell loss (36,86). As opposed to necrosis — a passive mechanism of cell death associated with organelle disruption, cell swelling and membrane rupture — apoptosis is an active process, triggered by the activation of specific cellular mechanisms and evolving through a series of defined steps (6,88). Apoptosis can be triggered by numerous factors, some of which have been implicated in PD pathogenesis, such as impaired activity of mitochondrial enzyme complex I (31), excessive stimulation of glutamate receptors (58,98) or proteasomal impairment (25,42,45). Signs of apoptotic cell death (5,57,59), increased levels of apoptosis effectors caspase-3 and caspase-8 (32,34,60,85), of pro-apoptotic protein Bax (33,84), along with upregulation of anti-apoptotic protein Bcl-2 (50,62), have been detected in the SNc of PD patients. Pro-apoptotic changes have also been reported in peripheral cells of PD patients (13,56). The role of apoptosis in PD pathogenesis is further supported by the fact that the two toxins most frequently used to replicate the PD-related nigrostriatal damage in experimental animals, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA), cause nigral degeneration by triggering the apoptotic cascade (16,20,37). Apoptosis also plays a central role in two additional animal models of PD, recently described, which are based on the chronic, systemic administration of complex I inhibitor rotenone (11) or proteasome inhibitors lactacystin and epoxomycin (55). In these models, the nigrostriatal lesion is associated with the formation of cytoplasmic inclusions in the SNc, immunoreactive for proteins such as ubiquitin and α-synuclein, which also characterize the typical inclusions found in PD patients (Lewy bodies).

NEUROPROTECTION AS A THERAPEUTIC STRATEGY: THE ROLE OF MAO-B INHIBITORS

Although nearly four decades have passed since its introduction into clinical practice (19), replacement of deficient dopamine with its direct precursor, L-3,4-dihydroxyphenylalanine (L-DOPA), is still the most effective and commonly prescribed treatment for PD. However, long-term treatment with L-DOPA is associated with several well-known motor complications that are extremely discomforting for the patient. These include "on-off" fluctuations, freezing episodes, lack of responsiveness ("wearing off") and, above all, abnormal, uncontrollable movements, known as dyskinesias (64). More importantly, the use of L-DOPA aims at relieving PD motor symptoms by replacing the deficient neurotransmitter, without interfering with the pathogenic mechanisms of the disease.

These considerations have prompted extensive investigation, in recent years, to identify new pharmacological agents with the potential to modify the course of the disease, due to intrinsic neuroprotective properties. Indeed, various attempts have been made, in the past two decades, with compounds belonging to various classes of drugs (antioxidants, bioenergetics, dopamine agonists, glutamate antagonists, neurotrophic factors). The rationale behind the use of these drugs varied according to their diverse mechanisms of action, ranging from the blockade of a neurotoxic process directly damaging SNc neurons to the supplementation of trophic or other vitality factors required for the survival of nigral neurons, or to the inhibition of later steps in the cell death process, related to the

activation of the apoptotic cascade (47). Most of these compounds have shown neuroprotective potential in experimental models of PD, and various studies with neurotrophin-like compounds or bioenergetic supplements (such as coenzyme Q10 or creatine) are currently under way in PD patients (47). However, there remains a lack of significant correlative clinical studies to confirm disease modification in PD patients.

A class of compounds that has particularly attracted the interest of neuroscientists is that of the inhibitors of monoamine oxidase (MAO) B, due to the potential role of this enzyme in the pathogenesis of the nigrostriatal damage underlying PD (see below). MAO is an important enzyme primarily involved in neurotransmission within the central nervous system. The enzyme, which is present in two forms (MAO-A and MAO-B), is mainly responsible (both peripherally and centrally) for the oxidative deamination of monoaminergic neurotransmitters, such as norepinephrine, serotonin and dopamine. Norepinephrine and serotonin are preferential substrates for MAO-A, while dopamine is efficiently metabolized by both forms (94). In the brain, MAO-B is the predominant form and is found in relative abundance within the human basal ganglia nuclei (75,97). In the 1970s, the theoretical possibility of potentiating dopaminergic transmission by inhibiting dopamine metabolism raised great interest in the potential use of non-selective MAO inhibitors, originally developed to treat depression, for PD therapy. With the use of non-selective agents, however, came the risk of hypertensive crisis caused by the peripheral inhibition of MAO-A and the subsequent potentiation of cardiovascular tyramine effects with the ingestion of high tyramine-containing foods, such as aged cheese and red wine. In order to avoid this reaction, known as the "cheese reaction," selective inhibitors of MAO-B were developed. The availability of a selective, irreversible MAO-B inhibitor, such as selegiline (L-deprenyl; a propargyl-derived compound, structurally related to pargyline and clorgyline), prompted numerous studies on the efficacy of this pharmacological approach in PD therapy (75,97).

The neuroprotective potential of this approach was subsequently suggested by studies on PD pathogenesis, which indicated that oxidative stress may play a substantial role in SNc degeneration. The SNc is exposed to higher levels of oxidative stress relative to other areas of the brain, for reasons which include autooxidation of melanin, presence of high levels of iron, and, more importantly for PD, oxidative catabolism of dopamine by MAO-B. These factors contribute to a high rate of reactive oxygen species formation, which appears to be enhanced in PD patients (40,41). The elucidation of the underlying mechanism of the neurotoxic effects of MPTP, which requires oxidative deamination by MAO-B to transform into the active toxin 1-methyl-4-phenylpyridinium (MPP^+) (49), lent further support to the hypothesis that MAO-B inhibition may protect nigral neurons from exogenous agents requiring this enzymatic step to become toxic. These concepts formed the basis for the scientific rationale and design of the *DATATOP* (**D**eprenyl **A**nd **T**ocopherol **A**ntioxidative **T**herapy **O**f **P**arkinsonism) study, in which the efficacy of selegiline and vitamin E supplementation were tested (66), as well as in subsequent studies (47). In general, the results of these studies showed an initial symptomatic efficacy of selegiline compared to placebo (while vitamin E proved ineffective). However, it was also shown that the effect of selegiline disappeared with washout and after the first year of treatment. Indeed, the notion that selegiline is metabolized into L-methamphetamine (74) subsequently led various authors to suggest that transformation of the drug into such a neurotoxic molecule may actually interfere with selegiline's neuroprotective activity (2,4,65).

A NEW COMPOUND ON THE SCENE: RASAGILINE

Pharmacodynamic/Pharmacokinetic Profile

More recently, emerging data stimulated great interest in a potent second-generation, irreversible MAO-B inhibitor, rasagiline [R(+)-N-propargyl-1-aminoindan]. Rasagiline is structurally related to selegiline, but is considerably more potent; unlike selegiline, rasagiline is not metabolized to amphetamine and/or methamphetamine, and is thus devoid of sympathomimetic activity (24,93).

Ex vivo studies demonstrated that rasagiline is significantly more potent than selegiline against brain MAO-B activity. The doses required for selegiline to reach a clinically relevant degree of MAO-B inhibition in the brain (>90%) were ten times those required for rasagiline to produce the same effect. In vivo, rasagiline retains its selectivity for MAO-B inhibition in peripheral tissues such as the liver and small intestine (93).

In both healthy humans and PD patients, rasagiline 0.5 mg/day to 10 mg/day, administered as a single dose, is well tolerated and displays a dose-dependent inhibition of platelet MAO-B. After washout, platelet MAO-B inhibition remains evident for 7 days, while baseline values are restored after 2 weeks (72,87). Repeated administration of rasagiline is characterized by a dose-dependent reduction in the time required to reach maximum MAO-B inhibition, with almost complete inhibition being obtained after 2 days of treatment with the 10 mg dose. Repeated administration of rasagiline is also characterized by an increase in the drug's bioavailability. At steady state, values of both maximum plasma concentration and area under the plasma concentration-time curve are 2–3 times higher than the corresponding values observed after a single administration (87). In healthy male volunteers that underwent L-[11C]deprenyl PET scanning, rasagiline was shown to specifically and irreversibly bind to human brain MAO-B (27). Rasagiline-induced inhibition of MAO-B is likely to be associated with modifications in the central dopaminergic tone, as suggested by the increased release of dopamine detected in the striata of rats chronically treated with the drug (44).

Neuroprotective Activity

Numerous experimental studies, carried out both *in vitro* and *in vivo*, have shown that rasagiline possesses neuroprotective properties. These properties, which are unrelated to the inhibitory effect on MAO-B, have been substantiated by clinical evidence, suggesting that rasagiline may, indeed, affect the progression of the neurodegenerative process: a pivotal study, using the randomized delayed start design in early PD patients (TEMPO), has demonstrated that early treatment with rasagiline monotherapy is more beneficial compared to the treatment delayed by 6 months (69) (see below).

Experimental studies

In vitro, the drug was shown to counteract the pro-apoptotic effects of various toxins, such as *N*-methyl-*R*-salsolinol, 6-OHDA and peroxynitrite (52,54). Rasagiline also increased survival in additional models using insults such as oxygen and glucose deprivation in differentiated PC12 cells (1,2). Subsequent studies have shown that rasagiline can prevent cell death by interfering with various steps of the apoptotic cascade, including activation of caspase 3 and poly (ADP-ribose) polymerase-1 (PARP-1), translocation of

glyceraldehyde-3-phosphate dehydrogenase (GADPH) and nucleosomal DNA fragmentation; the drug is also able to induce the expression of anti-apoptotic proteins Bcl-2 and Bcl-xL, while downregulating pro-apoptotic Bad and Bax proteins (4,7,51,90,92,96). Mitochondria, in particular, seem to represent a key cellular target for the neuroprotective activity of rasagiline. These intracellular organelles play an important role in the intrinsic mechanisms of apoptosis (92), particularly in the initial phase, when changes in mitochondrial membrane permeability, resulting from pro-apoptotic stimuli, lead to opening of the mitochondrial permeability transition pore (MPTp) complex. This is followed by massive swelling of mitochondria and decline in mitochondrial membrane potential, associated with release of cytochrome *c*, a powerful signal for the activation of the final steps of the apoptotic process (17,30,46,91). Thus, in human dopamine-derived neuroblastoma (SHSY5Y) cells, rasagiline prevents the swelling of mitochondria and loss of mitochondrial membrane potential elicited by the pro-apoptotic toxin *N*-methyl-*R*-salsolinol (3,63). This outcome is closely related to the effects that rasagiline exerts on the anti-apoptotic and proapoptotic members of the Bcl-2-Bax family, which influence the MPTp in opposite ways. In fact, antiapoptotic proteins whose expression is induced by rasagiline, such as Bcl-2 or Bcl-xL, stabilize the MPTp; on the contrary, pro-apoptotic proteins that are downregulated by the drug (such as Bax or Bad) promote MPTp opening and the resulting collapse of mitochondrial membrane potential (9,10,83). An additional mechanism underlying the pro-survival activity of rasagiline may be represented by the drug-induced activation of protein kinase C (PKC) (7,90,92), which exerts a protective effect on neuronal cells (21,48). Rasagiline also interacts with growth factor-related cellular mechanisms: the drug increases the expression of glial cell line-derived neurotrophic factor (GDNF) in SH-SY5Y cells, through the activation of the nuclear transcription factor NF-kB (53).

Similar neuroprotective effects have been obtained with the S-isomer of rasagiline (TVP 1022), which is 1000 times less active as a MAO inhibitor than rasagiline (95); this finding is particularly interesting, because it demonstrates that the cytoprotective efficacy of rasagiline is not due to MAO-B inhibition, but may result from the intrinsic anti-apoptotic properties of the propargylamine moiety of the drug.

Neuroprotection by rasagiline has been substantially confirmed *in vivo*. In animal models of PD, in particular, rasagiline counteracted the experimentally-induced nigrostriatal damage (12,35,43). In monkeys, rasagiline (10 mg/kg, s.c.) prevents the neurodegenerative effect of MPTP, when given prior to the toxin (43). Recent evidence also shows that, in rodents, chronic administration of rasagiline effectively counteracts the progressive degeneration of nigrostriatal neurons caused by intrastriatal injection of 6-OHDA (12), which confirms the potent activity previously shown by rasagiline against this neurotoxin in SH-SY5Y cells (52). In this case, rasagiline administration was started immediately after the toxin injection. Either of the two doses used (0.8 or 2.5 mg/kg/day, s.c.) displayed similar efficacy (12).

Rasagiline has also demonstrated neuroprotective activity in models not directly related to PD, such as experimental ischemia induced by middle cerebral artery occlusion in rats (80), closed head injury in mice (38), spontaneously hypertensive rats (22,23) and transgenic model (mouse) of familial amyotrophic lateral sclerosis (89).

Interestingly, rasagiline's main metabolite, 1-(R)-aminoindan, is not an amphetamine derivative, does not possess MAO inhibitory activity, but appears to contribute to the neuroprotective activity of the parent compound. Aminoindan has shown beneficial activity in animal models of motor and cognitive dysfunctions, such as haloperidol-induced

catalepsy and α-methyl-p-tyrosine induced hypokinesia, in rats (79). Further experimental studies suggest that aminoindan may have intrinsic neuroprotective activity. For example, in cultured PC-12 cells deprived of serum and nerve growth factor (NGF) — a condition resulting in apoptotic cell death — pretreatment with aminoindan grants a degree of protection similar to that obtained with rasagiline (4). On the contrary, the major metabolite of selegiline, L-methamphetamine, exerts a cytotoxic effect, which counteracts the neuroprotective activity of the parent compound (2,4). This observation would explain the greater neuroprotective potential of rasagiline over selegiline and apparent failure of this latter compound as a disease modifying drug for PD.

Clinical Studies

In the past five years, evidence has accumulated on the efficacy of rasagiline in the treatment of PD, either as monotherapy or adjunct therapy to L-DOPA. In 2000, Rabey et al. reported significant efficacy of rasagiline mesylate, as add-on therapy to L-DOPA, in a double-blind, randomized, placebo-controlled, parallel-group 12-week study conducted in 70 patients (72). The clinical improvement was particularly evident in fluctuating patients, at all three doses of rasagiline used (0.5, 1, and 2 mg/day), as expressed by a decrease in total Unified Parkinson's Disease Rating Scale (UPDRS) score with respect to the placebo group. An additional Phase II study focused on the potential efficacy of rasagiline monotherapy in early PD patients (81). In this 10-week, double-blind, placebo-controlled trial, 56 PD patients were randomly assigned to rasagiline mesylate (1, 2, or 4 mg/day) or placebo. Consistent improvements in total UPDRS score were reported in patients receiving rasagiline, while frequency and types of adverse events did not differ from those observed in the placebo group.

In 2002, the Parkinson Study Group published the results of a 26-week, placebo-controlled investigation of rasagiline treatment in patients with early PD (68). The study — termed TEMPO (Rasagiline Mesylate [**T**VP-1012] in **E**arly **M**onotherapy for **P**D **O**utpatients) — was a double-blind, placebo-controlled, delayed-start clinical trial that included over 400 subjects. Patients were not taking L-DOPA and rasagiline was administered at 1 or 2 mg once daily. Results showed that, compared to placebo (in which UPDRS scores deteriorated), both doses of rasagiline stabilized UPDRS scores, particularly for the activities of daily living and motor subscales, as well as a quality of life measure (PDQUALIF ratings). The idea that rasagiline may, indeed, modify the progression of the disease was suggested by the second part of the TEMPO study, designed to assess whether earlier initiation of rasagiline treatment resulted in better clinical response than delayed initiation (69). In this study, subjects were randomized to receive rasagiline — 1 or 2 mg/day — for 12 months or placebo for 6 months, followed by rasagiline — 2 mg/day — for 6 months. The results showed that the beneficial effect of rasagiline was more pronounced in patients treated with the drug from the start, as opposed to those for whom there was a 6-month delay in treatment initiation. This finding cannot be explained entirely by symptomatic activity and suggests that rasagiline may have an effect on disease progression. Since all subjects were receiving the active drug in the second part of the study, it is highly unlikely that the differences observed at the final visit were due to symptomatic effects of

rasagiline, which, at this point of the study, were presumably balanced in the two patient groups (69).

More recently, the Parkinson Study Group has published the results of another study (PRESTO: **P**arkinson's **R**asagiline: **E**fficacy & **S**afety in the **T**reatment of "**O**FF"), conducted in over 450 PD patients treated with L-DOPA and affected by motor fluctuations. Rasagiline proved beneficial compared to placebo, inducing an amelioration of motor fluctuations — expressed by a significant reduction of the total daily "off" time — and PD symptoms (70). The efficacy of rasagiline in counteracting motor fluctuations, when used as adjunct therapy to L-DOPA, was confirmed by another very recent study, termed LARGO (**L**asting effect in **A**djunct therapy with **R**asagiline **G**iven **O**nce daily) (73). In this trial (n = 687), the study design included a comparator arm, in which patients were receiving entacapone, a catecholamine-*O*-methyltransferase (COMT) inhibitor, known as an effective add-on therapy for motor fluctuations (67,76). The LARGO study showed that rasagiline effectively reduced the time spent in the "off" state, while increasing the "on" time, and that the magnitude of these effects was similar to that of entacapone. Moreover, two ancillary studies of LARGO demonstrated that rasagiline, but not entacapone, also improved freezing of gate (28) and motor symptoms in the "off" state (82).

Side Effects

Safety data have been collected throughout the clinical development of rasagiline. Monotherapy with rasagiline as well as adjunct therapy to L-DOPA is highly tolerable, as demonstrated by the low patient drop-out rates in the clinical studies. Overall, adverse events rates were low, and the frequencies of adverse events in the rasagiline treatment groups were similar to those in the placebo groups (68,70,73). The most frequent adverse events observed with rasagiline alone were headache and arthalgia. When rasagiline was used as an adjunct to L-DOPA, the frequent side effects were dyskinesia and accidental injury.

CONCLUSIONS

The search for a treatment able to counteract the nigrostriatal degeneration of PD has yielded numerous reports on the efficacy of various classes of drugs that acted through different mechanisms in experimental models of PD. When tested in clinical trials, however, the majority of these compounds failed to demonstrate efficacy. Considerable experimental evidence has accumulated in support of rasagiline's neuroprotective efficacy, showing that the drug increases neuronal survival and that this effect is related to the anti-apoptotic properties of its propargyl moiety. Results from one clinical trial, recently conducted in PD patients, showed that the use of rasagiline — as monotherapy in early patients — may slow the progression of the disease. Therefore, the neuroprotective activity shown by the drug under experimental conditions might translate into a pharmacological tool for clinical practice, opening new perspectives in the treatment of PD.

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