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Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness

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A few years after the foundation of the British Pharmacological Society, monoamine oxidase (MAO) was recognized as an enzyme of crucial interest to pharmacologists because it catalyzed the major inactivation pathway for the catecholamine neurotransmitters, noradrenaline, adrenaline and dopamine (and, later, 5-hydroxytryptamine, as well). Within the next decade, the therapeutic value of inhibitors of MAO in the treatment of depressive illness was established. Although this first clinical use exposed serious side effects, pharmacological interest in, and investigation of, MAO continued, resulting in the characterization of two isoforms, MAO-A and -B, and isoform-selective inhibitors. Selective inhibitors of MAO-B have found a therapeutic role in the treatment of Parkinson's disease and further developments have provided reversible inhibitors of MAO-A, which offer antidepressant activity without the serious side effects of the earlier inhibitors. Clinical observation and subsequent pharmacological analysis have also generated the concept of neuroprotection, reflecting the possibility of slowing, halting and maybe reversing, neurodegeneration in Parkinson's or Alzheimer's diseases. Increased levels of oxidative stress in the brain may be critical for the initiation and progress of neurodegeneration and selective inhibition of brain MAO could contribute importantly to lowering such stress. There are complex interactions between free iron levels in brain and MAO, which may have practical outcomes for depressive disorders. These aspects of MAO and its inhibition and some indication of how this important area of pharmacology and therapeutics might develop in the future are summarized in this review.
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Keywords: Monoamine oxidase A and B; selegiline; rasagiline; moclobemide; antidepressants; Parkinson's disease; Alzheimer's disease; oxidative stress; iron; neuroprotection

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; ChE, cholinesterase; DA, dopamine; DLB, Lewy Body disease; DOPAC, dihydroxyphenylacetic acid; FAD, flavin adenine dinucleotide; GPO, glutathione peroxidase; GSH, reduced glutathione; 5-HIAA, 5-hydroxyindole acetic acid; 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; IRE, iron responsive element; MAO, monoamine oxidase; MPP^+, *N*-methyl-4-phenyl-dihydropyridine; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PKC, protein kinase C; RIMA, reversible monoamine oxidase A inhibitor; sAPPα, soluble amyloid precursor protein alpha; SSRI, serotonin selective reuptake inhibitor

Introduction

The story of monoamine oxidase (MAO) and its inhibitors is particularly fascinating because of the way in which this subject has gone in and out of favour with scientists and clinicians over the past 75 years. The early work of characterising the enzyme, identifying substrates, localizing activity in the body and describing its functions built up to its initial success with MAO inhibitors as the first and for some time the only treatment, for depressive illness. Then, the incidence of side effects led to a search for other treatments not based on MAO, and scientific interest in MAO disappeared except for a few persistent enthusiasts. Now, after several years in the wilderness, MAO and a new generation of inhibitors are again in the forefront of research and clinical activity. This review will trace these developments in MAO research and, where possible, explain the successes and failures of a topic in which many members of the BPS have been involved.

Early work

It begins in 1928 with Mary Hare-Bernheim describing an enzyme catalyzing the oxidative deamination of tyramine, which she called tyramine oxidase. A few years later, Hugh Blaschko found that tyramine oxidase, noradrenaline oxidase and aliphatic amine oxidase were the same enzyme, capable of metabolizing primary, secondary and tertiary amines. This enzyme did not metabolize diamines (such as histamine) and it was Zeller who eventually named it mitochondrial monoamine oxidase (MAO; EC1.4.3.4) (Youdim *et al*., 1988; 2005). Blaschko's interest in MAO started when he came to England in the 1930s and, because of his work on the biosynthesis of catecholamines, he was fully aware of the pharmacological

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significance of an enzyme inactivating substrates such as noradrenaline, adrenaline, tyramine and dopamine. Indeed, this enzyme was to be one of his primary interests throughout his long and productive research career.

However, the next significant development occurred by chance. Prompted by observations of patients undergoing treatment for tuberculosis, experiments showed that an anti-tuberculosis drug, isoniazid, was also a potent inhibitor of MAO. A related compound, iproniazid, became the first MAO inhibitor to be used successfully in the treatment of depressive illness. It was also the first antidepressant and one of the first psychotropic drugs used therapeutically. In the late 1950s and 1960s, iproniazid and other MAO inhibitors demonstrated remarkable antidepressant action but their clinical value was seriously compromised by side effects. Iproniazid itself caused liver toxicity, associated with its hydrazine structure. Although this problem was resolved by the development of other, non-hydrazine, MAO inhibitors, these, notably tranylcypromine, induced another important side effect, the 'cheese reaction' (Youdim *et al*., 1988).

The cheese reaction (Figure 1) is induced by tyramine and other indirectly acting sympathomimetic amines present in food (most commonly in certain cheeses, hence the name) and fermented drink, such as beer and wine. Under normal circumstances, such dietary amines are extensively metabolized by MAO in the gut wall and in the liver and they are thus prevented from entering the systemic circulation. In the presence of a MAO inhibitor, this protective system is inactivated and tyramine or other monoamines present in ingested food are not metabolized and enter the circulation. From here they have access to, and induce a significant release of noradrenaline from, peripheral adrenergic neurons (Finberg *et al*., 1981; Finberg & Tenne, 1982). The consequence of this release is a severe hypertensive response which, in some cases, can be fatal. These serious side effects stimulated a search for antidepressants that were not MAO inhibitors and to their eventual replacement by the uptake inhibitors, the tricyclic antidepressants, and more recently the serotonin selective re-uptake inhibitors (SSRI) such as Prozac.

During this first period of clinical use of the MAO inhibitors, research into the basic science of MAO showed it to be located on the outer mitochondrial membrane and to be a flavo-protein, with FAD as the cofactor (Figure 2). Much later this cofactor was identified as the site at which irreversible inhibitors of MAO, such as pargyline and rasagiline, are covalently linked (Youdim *et al*., 2005). The reaction mechanism of MAO involves oxidative deamination of primary, secondary and tertiary amines to the corresponding aldehyde and free amine, with the generation of hydrogen peroxide (Figure 3). The aldehyde is rapidly metabolized by aldehyde dehydrogenase to acidic metabolites. It is these acidic metabolites (5-hydroxyindole acetic acid (5-HIAA) from 5-hydroxytryptamine (5-HT, serotonin) or dihydroxy-phenyl-acetic acid (DOPAC) from dopamine) that are commonly used as the measure of MAO activity *in vitro* or *in vivo*. Recently, gene profiling of post-mortem samples of substantia nigra from Parkinson's disease (PD) patients disclosed a deficiency in aldehyde dehydrogenase that could allow a build-up of neurotoxic aldehydes derived from dopamine by MAO (Grunblatt *et al*., 2004).

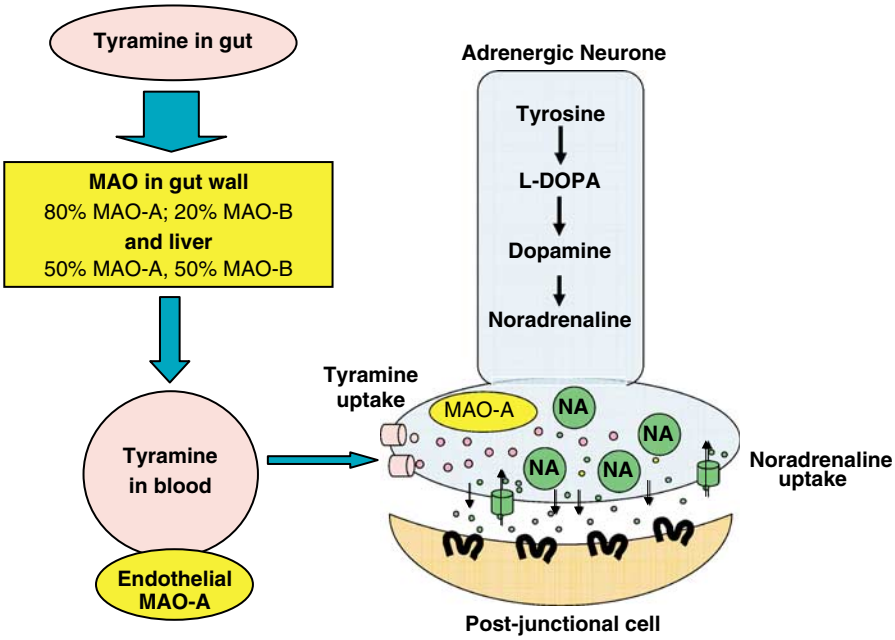

Figure 1 The 'cheese reaction' – potentiation of cardiovascular effects of tyramine (or other indirectly acting sympathomimetic amines) by irreversible inhibitors of MAO. Normally, dietary tyramine suffers extensive 'first pass' inactivation by the MAO isoforms in gut wall and then in the liver. The tyramine that survives to enter the systemic circulation is further attenuated by the MAO in vascular endothelial cells and lung (Bakhle, 1990). At the adrenergic neurone, uptake of tyramine initiates the release of noradrenaline, which accounts for the sympathomimetic effects of tyramine. *Irreversible* inhibition of MAO-A, the predominant isoform in the periphery, allows greatly increased amounts of tyramine to enter the systemic circulation and, from there, adrenergic neurons, consequently increasing noradrenaline release and effect. By contrast, *reversible* inhibitors of MAO-A (RIMAs) are displaced from the enzyme by tyramine which is then metabolized normally by the enzyme. Thus circulating tyramine never attains the high levels resulting from irreversible inhibition of MAO.

A crucial finding at this time (the late 1960s) was that MAO was not a single enzyme but could exist in at least two forms that had different pH optima and sensitivity to heat inactivation. These isoforms had two other differences that were of great pharmacological significance – substrate and inhibitor specificity. The type A MAO was defined as being inhibited by clorgyline and metabolizing noradrenaline and 5-HT, whereas type B MAO was resistant to clorgyline and preferred benzylamine as substrate (Johnston, 1968). Tyramine and dopamine were equally well metabolized by both forms of the enzyme (Youdim *et al*., 2005). This definition of MAO-A and -B was soon followed by another important finding, that the isoforms were differently distributed in mammalian brain, with for instance, greater MAO-B activity in basal ganglia (Collins *et al*., 1970). These findings suggested that by mapping the distribution of human brain MAO isoforms, combined with isoform selective inhibitors, it should be possible to develop treatments based on MAO inhibition for the treatment of depression and other neuropsychiatric diseases, without the dangerous side effects inherent with older, non-selective, MAO inhibitors (Youdim *et al*., 1972). However, although the selective MAO-A inhibitor, clorgyline, increased brain levels of noradrenaline and 5-HT and showed antidepressant activity in a series of clinical trials, the consistently observed cheese reaction forced the abandonment of this inhibitor as an antidepressant.

By the early 1970s, it was clear that the cheese reaction would always prevent selective or non-selective inhibitors of MAO-A from being clinically acceptable as antidepressants and that in turn discouraged basic research on MAO. But what about MAO-B? One strong disincentive to study MAO-B was that neither noradrenaline nor 5-HT was metabolized by this isoform and the consensus of pharmacological opinion and knowledge, at that time, was that these two monoamines were the neurotransmitters of real importance in the brain. Another disincentive was that the uptake inhibitors (the tricyclic antidepressants) were giving good clinical results without cheese reactions and, consequently, research effort had been transferred to these inhibitors and to the many new 5-HT receptors being identified in the CNS.

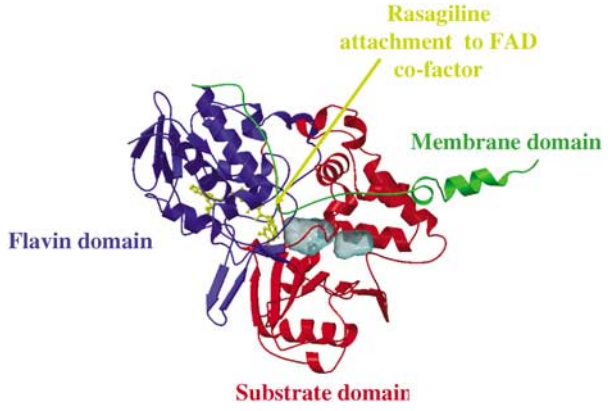

Figure 2 The crystal structure of human MAO-B. There are three functionally distinct domains, as shown. In red, the substrate domain contains two 'cavities' shown in cyan. The outer space is the entrance cavity leading to the inner space, substrate binding cavity, closer to the flavin cofactor. The flavin-binding domain is shown in blue with the FAD molecule in yellow. In green, the C-terminal helical region which attaches the protein to the mitochondrial membrane. Rasagiline covalently links to the flavin *via* its propargylamine group (yellow arrow) and the indan ring then extends into the substrate-binding cavity, blocking access for substrate.

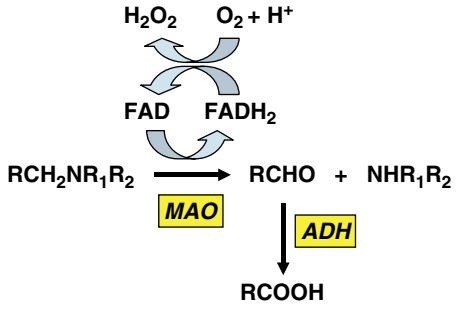

Figure 3 Reaction pathway of monoamine metabolism by oxidative deamination by mitochondrial MAO. The primary product of MAO acting on a monoamine is the corresponding aldehyde, usually rapidly further oxidized by aldehyde dehydrogenase (ADH) to a carboxylic acid, which is the final excreted metabolite. Note also that the FAD-$FADH_2$ cycle generates hydrogen peroxide which itself requires inactivation by catalase or, in the brain, glutathione peroxidase (see also Figure 7).

MAO-B and l-deprenyl

Despite this general lack of interest, Knoll & Magyar (1972) persisted in their study of another irreversible MAO inhibitor, l-deprenyl, derived from propargylamine, and which selectively inhibited MAO-B. This compound, at low doses, inhibited the

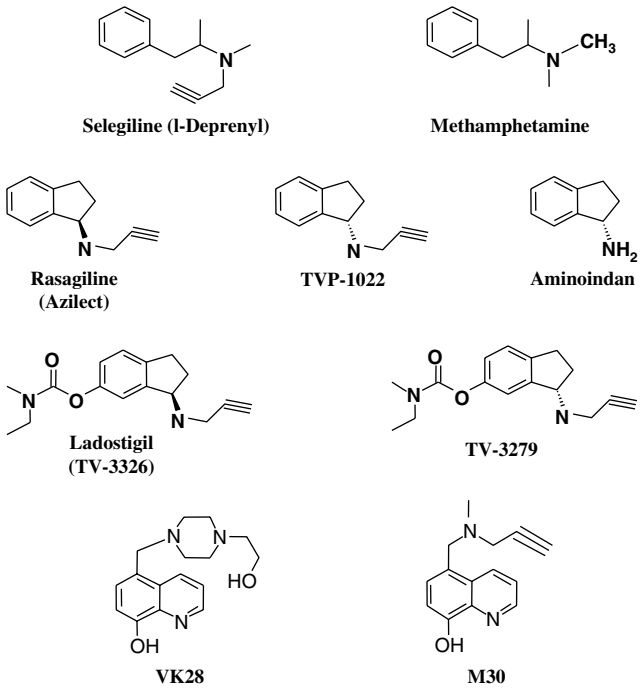

Figure 4 Structures of MAO inhibitors. In the top row, the structural similarity between selegiline/l-deprenyl and methamphetamine is shown. Below are the aminoindan series of propargylamine compounds such as rasagiline. Next, the bi-functional MAO and cholinesterase inhibitors (ladostigil) and lastly, the iron chelator-MAO inhibitors.

oxidative deamination of dopamine, phenylethylamine and benzylamine but not that of noradrenaline or 5-HT, but at higher doses that selectivity was lost. l-Deprenyl (Figure 4) was evaluated as an antidepressant, devoid of the cheese reaction, but without much success. Little attention was paid to its possible clinical usefulness in PD, already known to be due to a deficiency in dopamine, and this inhibitor was used for the next few years mainly as an experimental tool to explore the function of MAO-B.

The experimental findings with this inhibitor (Green & Youdim, 1975; Green *et al*., 1977) were the basis of the eventual use of l-deprenyl in PD (Birkmayer *et al*., 1975). Peter Riederer was looking for a MAO inhibitor, free from the cheese reaction, to use as an adjunct in PD patients already treated with L-DOPA, and the obvious choice was l-deprenyl because the basal ganglia from human brain had predominantly MAO-B (Collins *et al*., 1970; Youdim *et al*., 1972). The first clinical trial in 47 patients was successful and, more significantly, none of the patients showed a cheese reaction (Birkmayer *et al*., 1975). Several other trials followed and l-deprenyl soon became an accepted part of therapy for PD in Europe, although patients in the United States had to wait for another 15 years before the FDA licensed the drug and gave it a new name in the process, selegiline. (In this review, l-deprenyl and selegiline are used interchangeably, the choice being determined largely by the name used in the references cited.)

l-Deprenyl and neuroprotection

By 1980, l-deprenyl plus L-DOPA was well established as therapy in PD and data from a long-term study of this treatment accumulated. A preliminary analysis of clinical response and survival of these patients indicated that those receiving l-deprenyl plus L-DOPA had a better survival rate than those treated with L-DOPA alone (Birkmayer *et al*., 1985). These results suggested that l-deprenyl was slowing the rate of degeneration in dopaminergic neurons, an effect referred to as 'neuroprotection'. No such effect had been observed or discussed previously, with any other treatment for PD. Some clues as to the possible mechanism of neuroprotection came from studies with *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) which caused PD-like effects in humans. This compound had been inadvertently synthesized by a drug addict as an impurity in his preparation of meperidine and after using it, he had developed PD-like symptoms while still in his twenties. This neurotoxin was also selectively toxic to the dopaminergic neurones in the substantia nigra in animals and induced PD-like neurodegeneration in animal models. This model proved to be most valuable in studies of the development and progress of this disease and hence of neuroprotection. Pretreatment with non-selective MAO inhibitors protected the nigrostriatal neurons in mice against the damage caused by MPTP, but of the selective inhibitors, only l-deprenyl, not clorgyline, was protective (Heikkila *et al*., 1984). This was compatible with the neurotoxin being metabolized selectively by MAO-B to the active toxin, MPP+. Interestingly, similar results were obtained with another MAO-B substrate, phenylethylamine (Melamed & Youdim, 1985) and attributed to competition at the active site of MAO-B, thus preventing oxidation of MPTP to the toxic metabolite, MPP+. This combination of an agent inducing PD-like neurodegeneration and a means to prevent it in animal models raised hopes that a similar cause and its prevention might be found in humans. Regrettably, no MPTP-like neurotoxin has been identified either as an endogenous factor or in the environment and, although we have a possible mechanism, we still have not established the cause of PD.

Nevertheless, these studies with MPTP and l-deprenyl have contributed greatly to the concept of neuroprotection, as distinct from treatment of the symptoms of neurodegeneration. An analogy may be made with rheumatic disease. Here, ibuprofen and similar anti-inflammatory agents alleviate the pain, oedema, stiffness and other consequences of the disease without affecting its progression. Disease modifying agents such as corticosteroids, methotrexate or leflunomide actually modify the underlying disease process and are meant to slow the progression of the disease. Neuroprotective agents would prevent the progression of PD and perhaps other neurodegenerative diseases, not just control the symptoms of neuronal loss. Such agents might also counteract the effect of exposure to the causative agent(s) when they are finally identified, slowing or even preventing critical damage to the neurones. Neuroprotection is now a major concern in the study of neurodegenerative diseases and we shall return to a different aspect of this topic later in the review.

Development of rasagiline (azilect) and related MAO-B inhibitors

In 1978, AGN 1133, one of a series of MAO inhibitors which were aminoindan propargylamine derivatives and had been originally developed as anti-hypertensive drugs, was found to be a potent but non-selective, irreversible MAO inhibitor, whereas its *N*-demethylated derivative AGN 1135 was a potent selective inhibitor of MAO-B (Finberg *et al*., 1981). A major disadvantage of the original selective MAO-B inhibitor, l-deprenyl, was its sympathomimetic actions, probably related to its chemical structure. It is an amphetamine derivative (Figure 4) and is metabolized *in vivo* almost completely to methamphetamine compounds with sympathomimetic activity (Blandini, 2005). One advantage of AGN 1135, therefore, was that it was not an amphetamine derivative and showed no sympathomimetic or other physiological activities (Finberg & Youdim, 1985). Because of the aminoindan ring structure, AGN 1135 is a mixture of two isomers and the R(+) enantiomer of AGN 1135, now called rasagiline (Figure 4), was nearly three orders of magnitude more potent than the S(−) enantiomer, TVP1022 (Figure 4), in inhibiting MAO-B (Youdim *et al*., 2001; 2005).

For a MAO inhibitor to be effective in PD, it must raise levels of dopamine at its receptor sites in the striatum. Because metabolism of dopamine is largely carried out by MAO-A in rodent brain, it was not clear that inhibition of MAO-B *per se* would enhance dopamine levels adequately. Using microdialysis techniques in rat striatum, chronic (but not acute) treatment with rasagiline and selegiline was shown to increase, by a similar extent, dopamine levels in the microdialysate. This effect was explained by an increase in endogenous levels of β-phenylethylamine, which is a MAO-B selective substrate. In primates, which have a larger proportion of MAO-B in the brain than rodents, extracellular dopamine levels in striatum were studied following local infusion of L-DOPA via the

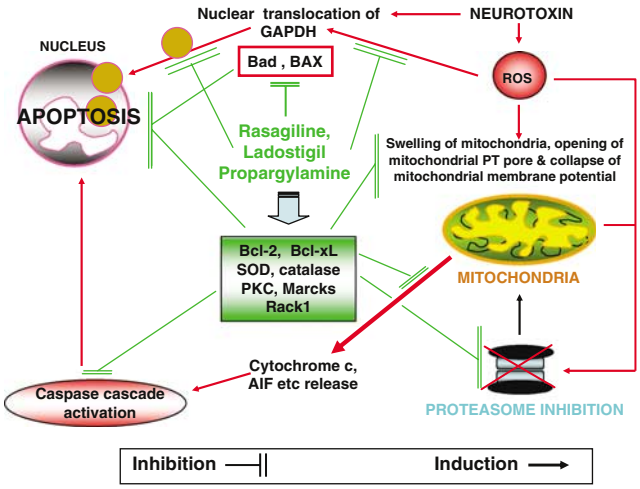

Figure 5 The interactions of irreversible, propargylamine-based, MAO-B inhibitors with apoptotic pathways. There are both direct effects such as inhibition of GAPDH translocation to the nucleus and indirect effects, *via* Bcl and Bax, etc. The overall outcome of these many interactions is a strong anti-apoptotic effect, independent of MAO inhibition.

microdialysis probe. Rasagiline, administered systemically, enhanced dopamine levels in microdialysate following L-DOPA (Finberg *et al*., 1998). This new MAO-B inhibitor appeared to have the appropriate activities to alleviate the symptoms of PD.

As for neuroprotection, rasagiline possesses activity superior to that of selegiline in neuronal cultures (Mandel *et al*., 2005). The mechanism of this effect has been related to activation of the anti-apoptotic pathways involving prevention of collapse of the mitochondrial membrane potential by activation of Bcl-2 and Bcl-Xl and downregulation of Bad and Bax, at the level of the mitochondria (Youdim *et al*., 2005). Furthermore, the Bcl-2-dependent neuroprotective activity was itself dependent on the regulation and translocation of PKC-dependent MAP kinase (Mandel *et al*., 2005) (Figure 5). Structure activity studies with rasagiline have clearly shown that the neuroprotective activity of rasagiline is dependent on its propargyl moiety and that the S-enantiomer of rasagiline, TVP1022, and propargylamine itself both exhibit similar activity (Mandel *et al*., 2005). A very interesting development was that all three compounds are anti-apoptotic in cultures of cardiac myocytes implying a possible use in cardiovascular medicine as well (Youdim & Buccafusco, 2005).

The neuroprotective activity of rasagiline was demonstrable, as a disease modifying effect, in PD patients. Clinical trials showed that patients who started rasagiline treatment earlier experienced less functional decline, as assessed by total UPDRS scores, than those who delayed treatment for 6 months (Blandini, 2005). This effect was present after a 1-year evaluation period and was also seen at long-term (5.6 years) follow-up. Such findings support the claim that the clinical benefits of rasagiline are not entirely symptomatic in nature and may reflect, in addition, a neuroprotective effect. To establish such effects definitively, assessment of patients for many more years will be needed. Nevertheless, as a result of a series of clinical trials showing the efficacy and safety of rasagiline in PD patients, both as monotherapy and in conjunction with L-DOPA; this drug is now available for clinical use in Europe and Israel and approval from the FDA is expected soon. On this occasion, there was little delay between the two sides of the Atlantic, in recognizing the value of selective inhibition of MAO-B in PD.

Selective reversible MAO-A inhibitors in Parkinson's disease and depression

The studies with selegiline and rasagline provided encouragement for others to continue with the development of selective, but reversible, inhibitors of MAO-A, lacking the cheese reaction. In rodents, MAO-A is present in the extraneuronal compartment and within the dopaminergic terminals, where it is involved in the metabolism of both intraneuronal and released dopamine, respectively (Figure 6). The intraneuronal enzyme ensures a low level of the neurotransmitter monoamines within the neuron (Youdim *et al*., 1988). However, little attention had been paid to MAO-A inhibition as a practical means of controlling dopamine levels in brain, even though it had clearly been established that dopamine is as well metabolized by MAO-A, as by MAO-B, and that the striatum contains MAO-A (Green *et al*., 1977). This was partly because MAO-A inhibition was known to induce the cheese reaction and partly because there was little evidence of the effect of MAO-A inhibition on dopamine levels in humans. In brains obtained at autopsy from patients after treatment with either selegiline or clorgyline, the increase in dopamine was not as marked as the increase in phenylethylamine, noradrenaline and 5-HT (Youdim *et al*., 1972; Riederer & Youdim, 1986). Neither selegline, rasagline or clorgyline alter dopamine levels in rat brain. These results clearly indicated that when one isoform of MAO was fully inhibited, the other isoform would metabolize dopamine adequately. Thus with *selective* inhibition of MAO-A *or* -B, the level of dopamine will not change drastically in the human striatum (Riederer & Youdim, 1986), in contrast to those of the monoamines that are substrates for only one isoform.

This situation was profoundly changed with the advent of the *reversible* MAO-A inhibitors (RIMA) such as moclobemide (Haefely *et al*., 1992), which did not provoke the cheese reaction. This is because reversibility allows competition and so ingested tyramine (or other dietary amine) is able to displace the inhibitor from the enzyme and be metabolized in the normal way, in the gut and liver. It had also become possible to show dynamic changes in striatal dopamine by microdialysis studies in rodents and these showed a clearly increased release of dopamine after moclobemide or clorgyline or rasagiline (Haefely *et al*., 1992). Thus although selective inhibition of MAO-A or-B did not affect the *steady state level* of dopamine in the brain, such inhibition did affect its *release*. Such action could explain the anti-symptomatic effects of these drugs which was being observed in PD patients.

The first clinical studies of moclobemide in PD were as an addition to therapy with L-DOPA and dopaminergic agonists. Here and in other studies (Youdim & Weinstock, 2004), moclobemide had a mild symptomatic benefit, mostly on motor functions. This inhibitor was also safe and effective in patients treated with L-DOPA and a peripheral decarboxylase inhibitor. The relatively weak anti-PD effect may be a simple matter of dose; the daily dose in early trials was 450 mg but now up to 900 mg has been used without side effects and a

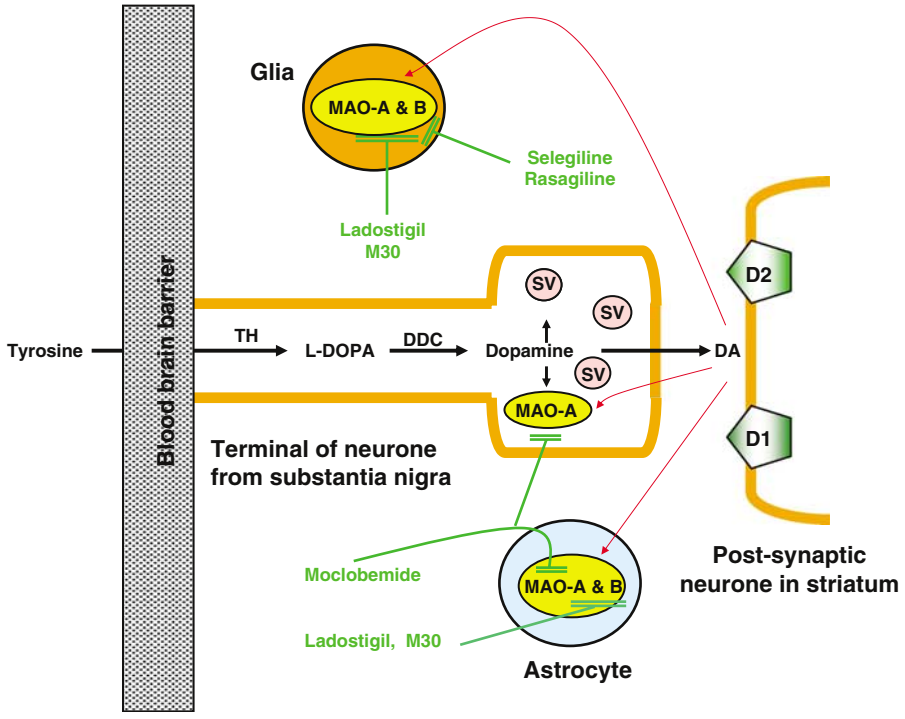

Figure 6 Pathways of dopamine synthesis in dopaminergic neurons and metabolism by MAO-A and -B in the brain. Tyrosine passes through the blood–brain barrier and is hydroxylated by tyrosine hydroxylase (TH) to DOPA and then decarboxylated by DOPA decarboxylase (DDC) to dopamine (DA) within the neuron. Dopamine is taken up into synaptic vesicles (SV) or metabolized by MAO-A in neuronal mitochondria. After release from the terminal, extracellular dopamine is cleared by uptake into astrocytes and glia also containing MAO-A and MAO-B. Selective inhibition of one MAO isoform allows the other to metabolize dopamine effectively and does not alter the steady state levels of striatal dopamine. On the other hand, non-selective inhibition of both isoforms induces highly significant increase in striatal dopamine and in other brain regions. D1 and D2, dopamine receptors.

better clinical response. However, another more fundamental cause could be the reversibility of the inhibitor. Microdialysis studies showed that moclobemide can be displaced from its binding site on MAO-A by dopamine (Colzi *et al*., 1993) and dis-inhibition of the enzyme would result. This would allow dopamine to be metabolized and decrease the amounts available for binding to receptors. Clinical evaluation of other reversible selective MAO-A inhibitors such as brofaromine (Davidson, 2003) and befloxatone (Bottlaender *et al*., 2003), both with greater affinity for MAO-A, will help to clarify this point.

A more obvious application of MAO-A inhibition is to treat depression. The two monoamines implicated in depressive illness are noradrenaline and 5-HT, both substrates for MAO-A and the antidepressant effects of MAO-A inhibition with the earlier, non-selective, irreversible inhibitors (clorgyline, tranylcypromine) had been already established. The major disadvantage was the incidence of the cheese reaction with those early inhibitors. Because the selective reversible inhibitors did not provoke this reaction, moclobemide was first assessed as an antidepressant and found to be effective, improving vigilance, psychomotor speed and long-term memory (Bonnet, 2003). There have also been reports of improvement in memory and choice reaction time in elderly patients. In other studies with non-depressed elderly people and in healthy young volunteers, moclobemide had no significant effects on cognitive functions. Overall, moclobemide appears to be safe and devoid of major side effects, although it is considered as a mild antidepressant, better tolerated by older patients (Bonnet, 2003). In the treatment of therapy-resistant depression, MAO-inhibitors provide an important option and a combination of reversible MAO-A (such as moclobemide) and reversible MAO-B (such as lazabemide) inhibitors may be worth considering. However, combination of MAO-inhibitors (even selective ones) with uptake inhibitors, either the tricyclic antidepressants or, especially, the serotonin-selective reuptake inhibitors (SSRI), should be avoided as such combinations may provoke the 'serotonin syndrome', a serious adverse reaction (Boyer & Shannon, 2005).

The antidepressant action of moclobemide is also a useful and beneficial side effect in PD patients, a significant proportion (40–60%) of whom exhibit signs of depression. This is not surprising bearing in mind that, apart from the deficit in striatal dopamine, significant reductions of noradrenaline in the locus coeruleus and of 5-HT in the raphe nucleus have consistently been reported in PD brain samples (Yamamoto, 2001). The link has been strengthened by recent genetic studies demonstrating that the polymorphism in the 5-HT-transporter gene observed in endogenous depression is identical to that in those PD patients who have severe and frequent episodes of depression (Gingrich, 2002). Also the 5-HT-transporter gene-linked polymorphic region, but not the MAO-A gene promoter-associated polymorphism, may be a risk factor for depression in PD patients. Interestingly, neither polymorphism increases the risk for development of PD itself. It was therefore reasonable to assess this MAO-A inhibitor in PD patients with depression. These studies showed moclobemide to exert useful antidepressant actions and, importantly,

Table 1 Some MAO inhibitors used and under development in the treatment of depression and of PD

	Inhibitor selectivity	*Mode of action*
Antidepressant		
Iproniazid	A + B	Irreversible
Phenelzine	A + B	Irreversible
Isocarboxazid	A + B	Irreversible
Tranylcypromine	A + B	Irreversible
Nialamide	A + B	Irreversible
Clorgyline	A	Irreversible
Moclobemide	A	Reversible
Brofaromine	A	Reversible
Under development		
Ladostigil	A + B (brain selective)	Irreversible
M30	A + B (brain selective)	Irreversible
Befloxatone	A	Reversible
Anti-Parkinsons'		
Selegiline (Deprenyl)	B	Irreversible
Rasagiline (Azilect, Agilect)	B	Irreversible
Lazabemide	B	Reversible
Under development		
M30	A + B	Irreversible
Ladostigil	A + B	Irreversible

to be well tolerated with standard PD therapies (Youdim & Weinstock, 2004). Furthermore, in PD patients who were not depressed, moclobemide did not significantly influence cognitive measures of mood.

Selective MAO-B inhibitors in depressive illness

Selegiline (l-deprenyl) was originally evaluated as an antidepressant but the results of these early studies were unimpressive and probably related to the high doses used. Selegiline loses its selectivity for MAO-B and will also inhibit MAO-A, at high doses. Several other reversible and irreversible selective inhibitors of MAO-B have been studied but it is unlikely that any of these (Table 1) will be used as antidepressants. Nevertheless, a new mode of treatment is being assessed, involving combinations of reversible MAO-A and MAO-B inhibitors or its pharmacological equivalent, a non-selective reversible inhibitor of MAO. It is not immediately clear why inhibiting MAO-B should influence a dysfunction that appears to be mediated primarily by substrates for MAO-A, but one possible mechanism is an indirect modulation of 5-HT action by dopamine. In rats, the behavioural response to increased levels of brain 5-HT formed from tryptophan or 5-hydroxytryptophan (5-HTP) was dopamine dependent (Green & Youdim, 1975; Green *et al*., 1977) and inhibition of dopamine biosynthesis blocked the responses to these 5-HT precursors. Such an interaction may underlie the increased response, in bipolar depression, to the combination of l-deprenyl and 5-HTP, relative to either agent given alone (Mendlewicz & Youdim, 1983). A non-selective reversible MAO inhibitor could produce an equivalent increase in brain levels of dopamine and 5-HT.

Bifunctional cholinesterase and MAO inhibitors for dementia in Parkinson's disease and Alzheimer's disease

Dementia is recognized as a common feature of PD and such patients are classified as having diffuse Lewy Body disease (DLB) from the post-mortem findings of characteristic lesions in the brain. DLB patients on L-DOPA therapy for their extrapyramidal disorders responded cognitively to cholinesterase (ChE) inhibitors, used primarily in Alzheimer's Disease (AD), without loss of response to the anti-PD drugs (Youdim & Buccafusco, 2005). These observations initiated a search for a single bifunctional compound combining pharmacophores for MAO-B inhibition and for ChE inhibition as a treatment for DLB patients. A series of compounds based on the selegiline and rasagiline pharmacophores and incorporating a carbamate moiety were synthesized and tested as inhibitors of these enzymes (Mandel *et al*., 2005).

One of them, ladostigil [TV3326 [(*N*-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate] (Figure 4), was selected for further investigation. This compound is an inhibitor of both butyryl- and acetyl-cholinesterases and exhibits cognitive actions in animal models comparable to those of rivastigmine or galantamine, two ChE inhibitors already used in AD. Although ladostigil is a less potent ChE inhibitor than rivastigmine or galantamine, it has superior activity in the cognitive tests and less toxicity (Mandel *et al*., 2005; Sagi *et al*., 2005). Although ladostigil is structurally related to rasagiline, it does not inhibit either MAO-A or -B *in vitro* or acutely *in vivo*. However, after chronic treatment for 1–8 weeks with ladostigil, both isoforms of MAO in brain were inhibited with very little inhibition of the enzyme in gut or liver. This unexpected, tissue-selective, action allowed irreversible inhibition of all MAO activity in brain, with no cheese reaction, reflecting the lack of inhibition of the enzyme in gut and liver (Mandel *et al*., 2005; Sagi *et al*., 2005). As a non-selective MAO inhibitor, ladostigil increased levels of all three monoamines, noradrenaline, 5-HT and dopamine, in hippocampus and striatum of rats and mice and it showed antidepressant activity in animal models. Furthermore, like selective MAO-B inhibitors (selegiline, rasagiline and lazabemide) it prevented the striatal neurodegeneration and dopamine depletion induced by the neurotoxin, MPTP, in the mouse model of PD (Sagi *et al*., 2005). Because both isoforms of MAO are inhibited, ladostigil, unlike selegiline or rasagiline, markedly increased brain dopamine in MPTP-treated mice. In addition, ladostigil exhibited neuroprotective activity in cultures of neuronal cells. The compound also has anti-apoptotic activities identical to those established for rasagiline (Mandel *et al*., 2005). At present, neither the mechanism of tissue selectivity (brain vs gut and liver) nor of the difference between chronic and acute effects on MAO have been established. Both may be related to active metabolites of ladostigil produced in a tissue-selective manner. Elucidation of these mechanisms will clearly contribute to the development of similar compounds.

Ladostigil is now in phase II clinical studies for AD and DLB and eventually for PD. Although these compounds were designed for the treatment of dementia in a PD population, the wide range of activities actually expressed by ladostigil means that there are other groups of patients who might benefit, such as AD patients with depression.

Monoamine oxidase, iron and neurodegenerative disease

The activity of MAO is known to be influenced by levels of iron in animals and humans (Symes *et al*., 1969; Youdim *et al*., 1975). Furthermore, iron deficiency in rats was accompanied by behavioural defects along with the abnormal metabolism of monoamine neurotransmitters, particularly dopamine (Youdim & Green, 1975). At that time, the hypothesis that iron metabolism was related to CNS dysfunction and perhaps to the monoamine defect in PD, *via* effects on MAO, was not widely accepted. In fact, little attention was paid to iron metabolism and function in the brain, considering the crucial role iron has in many metabolic processes in the CNS. Over the subsequent years, more links between iron and CNS dysfunction have been uncovered. For instance, in many neurodegenerative diseases (PD, AD, amyotrophic lateral sclerosis, Huntington's disease, Friederich's ataxia and aceruloplasminemia), the sites of neuronal death in the brain are also sites at which iron accumulates (Zecca *et al*., 2004; Mandel *et al*., 2005). As well, several mutated genes have been linked to iron accumulation in the brain in aceruloplasminemia, Friedrich's ataxia and other neurodegenerative conditions (Zecca *et al*., 2004; Mandel *et al*., 2005).

The link between MAO, iron and neuronal damage appears to be an increase in oxidative stress. A normal product of monoamine oxidation by MAO is hydrogen peroxide (Figure 3). This is inactivated in the brain mainly by glutathione peroxidase which uses glutathione (GSH) as a cofactor. When brain GSH levels are low, as in PD (Riederer *et al*., 1989), hydrogen peroxide could accumulate and then be available for the Fenton reaction (Figure 7). In this reaction, iron, as the ferrous ion Fe^{2+}, generates a highly active free radical, the hydroxyl radical, from hydrogen peroxide. The hydroxyl radical depletes cellular anti-oxidants and reacts with and damages lipids, proteins and DNA. With increasing age, brain iron and brain MAO increase, thus increasing both components of the Fenton reaction and the potential for hydroxyl radical generation. Increased MAO activity is also seen in PD and AD patients (Mandel *et al*., 2005). Thus inhibition of MAO in these patients not only increases the monoamine levels available to activate membrane receptors but will, at the same time, decrease hydrogen peroxide production and the potential for hydroxyl radical formation and the consequent oxidative stress.

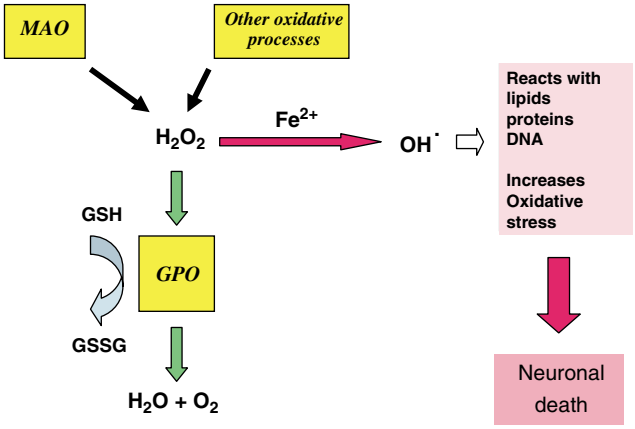

Figure 7 The mechanism of neurotoxicity induced by iron and hydrogen peroxide, *via* the Fenton reaction. Metabolism of monoamines by MAO is a major source of hydrogen peroxide (H_2O_2) in the brain. Normally the H_2O_2 is then inactivated by glutathione peroxidase (GPO) but it can be converted, chemically, by Fe^{2+} ions (Fenton reaction) into the highly reactive hydroxyl radical. This radical has widespread deleterious effects which can cause neuronal damage and death. When GSH levels are low and MAO and iron are increased (see text), the possibility of diversion of H_2O_2 *via* the Fenton reaction is correspondingly increased, with consequent increases in oxidative damage to neurons. Inhibition of MAO decreases the formation of H_2O_2 and iron chelation removes the Fe^{2+} ions, both decreasing the formation of hydroxyl radical and the levels of oxidative stress.

Another approach to the same goal is to remove the Fe^{2+} ions. Thus, the intraventricular injection of a well-known iron chelator, desferal, protected against lesions of nigrostriatal dopamine neurons induced by 6-hydroxydopamine or MPTP (Zecca *et al*., 2004; Mandel *et al*., 2005). Both these neurotoxins act after conversion, catalyzed by MAO, to an oxidized metabolite, a semiquinone or the radical ion, $MPP+$, respectively. The relatively weak effect of systemically administered desferal was attributed to its poor penetration into the brain and new chelating agents for iron which pass more readily through the blood brain barrier were therefore synthesized. One of these, VK 28, (Figure 4) showed good neuroprotection against MPTP and 6-hydroxydopamine lesions, but did not inhibit MAO, when given systemically (Mandel *et al*., 2005). A further development based on the parallel beneficial paths of MAO inhibition and iron chelation gave rise to compounds in which a propargylamine group was added to the hydroxyquinoline pharmacophore of VK-28. One of these derivatives, M30, (Figure 4) is a potent non-selective inhibitor of MAO-A and -B *in vitro* and *in vivo* (Gal *et al*., 2005; Zheng *et al*., 2005). More suprisingly, it showed tissue selectivity in that it inhibited MAO-A and -B in brain at doses that caused little inhibition of the enzymes in liver and gut. Thus, M30 increased brain dopamine, 5-HT and noradrenaline and prevented MPTP and kainate neurotoxicity in mice (Gal *et al*., 2005). It remains to be established if this compound has antidepressant activity and, crucially, if it induces the cheese reaction. M30 still retains iron chelating potency similar to that of desferal *in vitro* and exhibits neuroprotective and neurorescue activity, *in vitro* and *in vivo* (Gal *et al*., 2005; Zheng *et al*., 2005).

Both these iron chelating agents, M30 and VK-28, exhibit other pharmacological activities which could be significant in another neurodegenerative condition, AD, through their effects on the processing of amyloid precursor protein (APP). This protein which is the precursor of the neurotoxic Aβ-amyloid, has an iron responsive element (IRE) in its mRNA which allows cellular levels of iron to control translation, and hence synthesis of APP (Rogers *et al*., 2002). Consequently, the amounts of APP and Aβ amyloid formed in neuronal cell cultures and *in vivo*, were decreased by either VK28 or M 30 (Abramowich *et al*., unpublished experiments) In addition, these compounds activated the PKC-dependent α-secretase and increased the synthesis of a closely related protein, the soluble amyloid precursor protein alpha (sAPPα), which is neuroprotective and neurotrophic. These results suggest that there may be an alternative to the inhibitors of β-or γ-secretase for altering the processing of APP and that brain permeable iron chelating agents may offer a novel approach to the same beneficial goal, lower levels of Aβ amyloid.

Conclusion

More than 75 years after its first description, MAO has returned to the leading edge of research and clinical practice. Although the early work established the clinically valuable antidepressant effect of MAO inhibitors, their development was essentially brought to a halt by the apparently inescapable side effect of the cheese reaction. Only a few committed, maybe obsessive, researchers continued to study the then unimportant isoform, MAO-B. The discovery of a selective inhibitor of MAO-B (l-deprenyl/selegiline) and its logical application to the dopaminergic deficit in PD was a conceptual breakthrough, even though it took several years to be translated into clinical practice, where it is now firmly established. The next paradigm shift was the realization that reversible that is, competitive, inhibition, not isoform selectivity nor straightforward potency was the best means of avoiding the cheese reaction. That culminated in a series of RIMAs, still being assessed as antidepressant therapy.

Two further advances were serendipitous (as many in pharmacology) the discovery of tissue selectivity, as inhibition of brain vs liver enzyme, and the beneficial effect of isoform non-selective, but reversible, inhibitors. Both emerged from programmes aimed at quite different goals. We now have a full set of inhibitors – irreversible, isoform-selective or non-selective and reversible, selective or non-selective. Importantly, many are free of the cheese reaction and thus offer significant alternatives to existing therapy for depression, with or without PD or AD. With the range of inhibitors available today, the more pressing need is for adequate clinical evaluation rather than the discovery of new molecules.

In the course of these developments for the treatment of depression and PD, a new therapeutic possibility has been uncovered – neuroprotection. The concept of slowing the forward progress of and, maybe even halting, neurodegenerative disease is therapeutically very powerful and will ensure that it is followed up in clinical studies. The molecular mechanism of neuroprotection is not yet clear but the fact that either MAO inhibition or iron chelation achieves the same neuroprotective effect does suggest strongly that a reduction of local oxidative stress is a major component.

The study of MAO and its inhibitors has had a more general benefit in supporting the development of other enzyme inhibitors as therapeutic agents, with catechol-*O*-methyltransferase inhibitors (tolcapone and entacapone) for treatment of PD and cholinesterase inhibitors (tacrine, aricept, galantamine and rivastigmine) for AD. It has also revived interest in the design of bi-functional compounds. To attach a pharmacophore for cholinesterase inhibition to a MAO inhibitor and to retain both activities in the resultant compound is clearly a success but to do it again with a chelating agent is a real encouragement for this pharmacologically simple-minded approach. Maybe a 'three for the price of one' compound – an iron chelator with carbamate and propargylamine groups – is already being tested!

There are nevertheless many highly relevant questions still to be answered – is there a common pathway for neuroprotection that could be applied not only to PD but to AD and many other neurodegenerative diseases; what is the basis of the tissue selective inhibition of MAO; is there an endogenous analogue of MPTP, which would act as the causative agent of human PD? The answers to such questions and the clinical assessment of the compounds we already have will keep MAO and its inhibitors at the forefront of pharmacology for many more years to come.

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