

CHAPTER 2.

LITERATURE REVIEW

2.1. NEURODEGENERATIVE DISEASES

Neurodegenerative disorders are a heterogeneous group of diseases of the nervous system that have different aetiologies. Many are hereditary, some are secondary to toxic or metabolic processes and others result from infections (Fahn & Przedborski, 2000). Due to the widespread prevalence of neurodegenerative diseases, they represent significant medical, social and financial burdens on the society (Alexi *et al.*, 2000).

The entire process of neurodegeneration is still not fully understood however, it is believed to be part of the normal development of the nervous system. An excess of neuronal cells in the nervous system triggers a competitive survival process and only those neurons that are functionally and spatially correct survive (Cowan *et al.*, 1984). During the normal development of the nervous system, neuronal loss plays an important role (Oppenheim, 1991). Under normal conditions, the majority of the surviving neurons stay viable and functional throughout the lifetime of an individual (Mattson, 2006). Weak neurons that do not survive the competition die through an intrinsic cell suicide program known as apoptosis (Holbrook *et al.*, 1996).

Neurodegeneration in Parkinson's disease (PD) and Alzheimer's diseases (AD) occurs by a complex process which consists of several pathways and cascades and ultimately leads to the death of neuronal cells in certain areas of the brain, depending on the disorder (Youdim & Bakhle, 2006). This neuronal loss often involves apoptosis. Although apoptosis during development is a beneficial process, its occurrence in the mature brain is harmful, and leads to a decrease in the number of functional neurons, which cannot be replenished by cell division (Holbrook *et al.*, 1996). A combination of factors is believed to be responsible for the occurrence of these diseases:

- Aging is considered the most important risk factor of neurodegenerative disorders such as PD and AD. The prevalence of neurodegenerative disorders is rapidly increasing as average lifespan increases (Mattson, 2006). Normal aging is defined as aging without disease. Many aged people do not exhibit symptoms of a disease and lead normal lives, but nonetheless display pathological changes that are characteristic of AD and PD (Giorgio *et al.*, 2007).

- Oxidative stress plays an important role in the pathogenesis of major neurodegenerative diseases. Several defense and repair mechanisms have evolved to deal with oxidative stress and oxidative damage (Riederer *et al.*, 1989). However, when the reactive oxygen species (ROS) production overwhelms the endogenous antioxidant systems, they can potentially damage various types of macromolecules, such as lipids, proteins, carbohydrates and DNA (Halliwell & Gutteridge, 1985). In PD and AD, these defense mechanisms are seriously comprised. The activities of various antioxidant defense molecules are reduced in the brains of PD and AD patients. The antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and glutathione reductase (GSHRd), display reduced activities in the affected brain regions in AD (Zemlan *et al.*, 1989; Pappolla *et al.*, 1992). PD is also characterized by a reduction in amounts of the thiol-reducing agent glutathione (GSH) in the substantia nigra (SN), (Perry *et al.*, 1982; Spina & Cohen, 1989), and the magnitude of depletion correlates with the severity of the disease. Interestingly, other human pathologies such as cancer and cardiovascular disease have also been linked to elevated levels of ROS (Holbrook *et al.*, 1996). The increased level of oxidative stress in the brain is believed to be critical for the initiation and progression of neurodegeneration (Youdim & Bakhle, 2006).

Neurodegenerative diseases are characterized by abnormalities of specific regions of the brain and specific populations of neurons (Jenner & Olanow, 1998; Riederer *et al.*, 2004). The death of hippocampal and cortical neurons is responsible for the symptoms of AD, while the death of nigrostriatal dopaminergic neurons leads to PD (Holbrook *et al.*, 1996). The brain is believed to be particularly susceptible to the damaging effects of ROS because of its high metabolic rate and reduced capacity for cellular regeneration. In PD and AD, evidence of ROS damage has been reported within the specific brain region that is affected (Jenner & Olanow, 1998; Riederer *et al.*, 2004). For example, markers for lipid peroxidation have been identified in dying hippocampal and cortical neurons in patients with AD (Butterfield *et al.*, 2002). Also, markers for the oxidation of α -synuclein, a protein found in the Lewy bodies (LBs) (Zecca *et al.*, 2004), have been reported in PD patients (Good *et al.*, 1998).

Although promising leads have arisen for the treatment of neurodegenerative disorders, no therapy has been shown to halt or slow disease progression. The pathogenesis of PD occurs by complex mechanisms which are poorly understood. Numerous treatment strategies have been investigated and among these are monoamine oxidase (MAO) inhibitors which have been used

successfully to treat some of the symptoms of PD. The enzyme MAO-B is believed to play a role in the degenerative process and is considered to be a drug target for the symptomatic as well as protective treatment of PD.

2.2. PARKINSON'S DISEASE

2.2.1. General background

Idiopathic PD is a progressive, central nervous system neurodegenerative disorder, characterized by the selective degeneration of dopaminergic neurons in the substantia nigra (SN) (Alexi *et al.*, 2000; Lees *et al.*, 2009). It is currently regarded as the most common neurodegenerative disorder of the aging brain after AD and affects approximately 1% of the population older than 60 years. There is a worldwide increase in the disease prevalence due to the increasing age of human populations. PD etiology remains unknown and the nature of the pathological process underlying degeneration in PD still has to be resolved. Its pathogenesis may be understood as a multifactorial cascade of deleterious factors (Fahn & Przedborski, 2000). The incidence of the disease rises steeply with age from 17.4 in 100 000 in persons between 50 and 59 years of age, to 93.1 in 100 000 in persons between 70 and 79 years, with a lifetime risk of developing the disease of 1.5% (Bower *et al.*, 1999; de Rijk *et al.*, 1995). The median age of onset of the disease is 60 years and the mean duration of the disease from diagnosis to death is about 15 years, with a mortality ratio of 2 to 1 (Katzenschlager *et al.*, 2008).

2.2.1.1. Neurochemical and neuropathological features

PD usually involves the pigmented neuronal systems of particularly the zona compacta of the SN, which forms part of the dopaminergic nigrostriatal pathway (Dauer & Przedborski, 2003). This pathway consists of dopaminergic neurons whose cell bodies are located in the SN pars compacta, with the axons and nerve terminals projecting to the striatum (Dauer & Przedborski, 2003). However, the neuropathology of PD is not only restricted to the nigrostriatal pathway and histological abnormalities have also been found in many other dopaminergic and non-dopaminergic neurons (Agid *et al.*, 1999). A definitive neuropathological diagnosis of PD however, requires loss of dopaminergic neurons in the SN, gliosis and the presence of LBs, in the few remaining SN dopaminergic neurons (Dauer & Przedborski, 2003). PD is clinically characterized by symptoms such as muscle rigidity, resting tremors (Standaert & Young, 2000), loss of facial expression, hypophonia, diminished blinking, akinesia and postural instability (Ballard *et al.*, 1985; Fahn & Przedborski, 2000). The motor disabilities

characterizing PD are primarily due to the reduction of striatal dopamine content caused by the death of dopaminergic neurons in the SN pars compacta (Jenner & Olanow, 1998). These neurons play a role in basal ganglia control of motor and affective behaviour (Agid *et al.*, 1999). Once the dopamine neuronal cell death reaches the critical level of 85-90%, the neurological symptoms of PD appear (Riederer *et al.*, 2004; Jenner & Olanow, 2006).

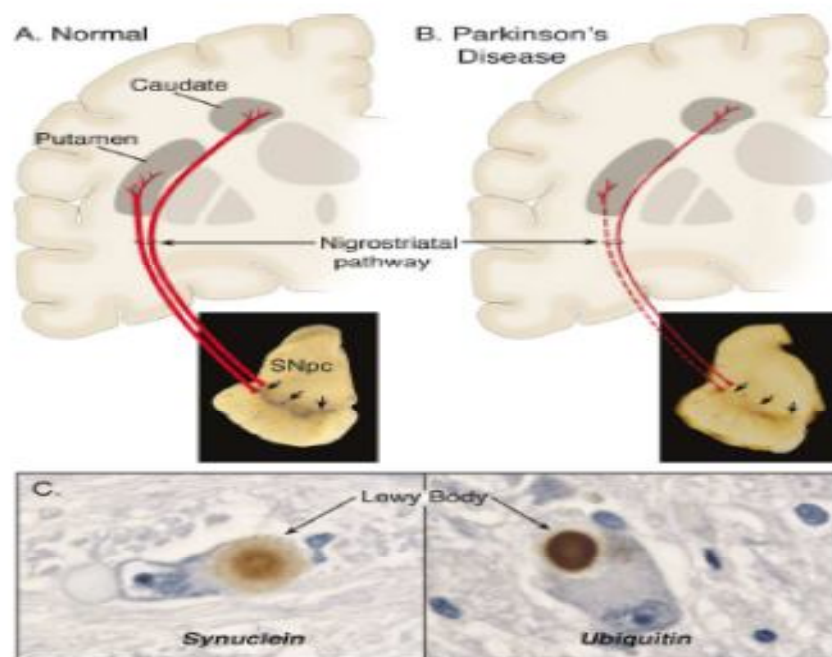


Figure 6: Neuropathology of PD showing (A) a normal nigrostriatal pathway, (B) a diseased nigrostriatal pathway with depigmentation of the SNpc as the nigrostriatal pathway degenerates and (C) immunohistochemical labelling of intraneuronal inclusions (LBs), in a SNpc dopaminergic neuron (Dauer & Przedborski, 2003).

2.2.1.2. Etiology

There are three hypotheses regarding the etiology of PD, but none have been proven. Aging of the CNS, genetic and environmental factors have been implicated in the cause of the disease (Dauer & Przedborski, 2003). One of the most explored hypotheses is that PD may occur by exposure to environmental agents or endogenous toxins, resulting in the acceleration of the normal age related decline in the number of SN dopamine containing neurons.

2.2.1.2.1. Age

Ageing is the major risk factor of PD, although 10% of patients with the disease are younger than 45 years of age. PD is one of the most common neurodegenerative diseases of the elderly. The incidence seems to decrease in the ninth decade of life (Taylor *et al.*, 2005), which could likely be related to under-diagnosis of elderly people of that age. Morphological and biochemical studies have demonstrated a progressive decline in the dopaminergic system with increasing age (Jenner & Olanow, 2006). It has been noted that an increase in age is associated with a non uniform decrease in the total number of neuronal cells in the brain. For example, only a small amount of neurons are lost from the hypothalamus, while there is a greater loss of nerve terminals in the SN (Dauer & Przedborski, 2003).

The role that aging plays in the pathogenesis of PD is still unclear, although striatal dopamine is lost as the degeneration of the nigrostriatal neurons occur (Gilgun-Sherki *et al.*, 2001). Aging in the central nervous system has been associated with elevated levels of mutation of DNA defects in mitochondrial respiration and increased oxidative damage. It is thought that oxidative injury might directly cause aging by oxidatively damaging macromolecules such as DNA, lipids and proteins (Gilgun-Sherki, *et al.*, 2001). The inappropriate activation of apoptosis may be caused by higher levels of free radicals that are produced as a normal part of cell metabolism (Hirsch *et al.*, 1999).

2.2.1.2.2. Genetics

The contribution of genetic factors to the pathogenesis of PD is increasingly being recognized (Jenner & Olanow, 2006). Although PD is often regarded as a sporadic disorder, there are much rarer early-onset familial forms which are due to a dominant gene mutation (Tanner, 2003).

Parkin

The aggregation of cytotoxic proteins due to defect in protein handling by the ubiquitin proteasome system (UPS), has also been linked to several mutations in genes and are associated with the early onset of PD (Lucking *et al.*, 2000). In the ubiquitylation cycle, the gene, parkin, is vital, since its protein product has ubiquitin ligase activity (Shimura *et al.*, 2000; Mata *et al.*, 2004). The UPS plays a key role in cellular quality control and in defense mechanisms. Parkin is an E3 ubiquitin ligase, involved in targeting misfolded proteins for degradation, and mutations of parkin found in genetic forms of PD disrupt its E3 ubiquitin ligase activity (Imai *et*

et al., 2000; Shimura *et al.*, 2000). The first parkin mutations were identified in 1998, and they were found to lead to reduced activity of the parkin protein product. It was suggested that neurodegeneration is probably due to a loss of function of parkin (Kitada *et al.*, 1998).

α -Synuclein

α -Synuclein belongs to a family of highly conserved small proteins, that include β and γ -synuclein. It is expressed in most tissues, but is predominately found in the synaptic terminals in the CNS (Maroteaux *et al.*, 1988). Mutations in α -synuclein have been implicated in PD (Prasad *et al.*, 1999; Chu & Kordower, 2006) where it has been found to be a major component of LBs. Recent studies have demonstrated a linear increase in α -synuclein protein with age, a pattern which correlates with a decrease in nigrostriatal neurons (Chu & Kordower, 2006). The age-related increase in α -synuclein at a level that exceeds the capacity of the proteasomes to clear them, leads to proteolytic stress, which may lead to neuronal death (Olanow *et al.*, 2006).

Leucine rich repeat kinase 2 (LRRK-2)

The most common genetic cause of PD to date is mutation in the gene LRRK2 (Healy *et al.*, 2008) which causes about 2% of all cases of PD (Deng *et al.*, 2005; Kachergus *et al.*, 2005). The LRRK-2 gene codes for a large protein, known as dardarin, which contains a serine/threonine kinase domain and a GTPase domain. Six pathogenic mutations in *LRRK-2* have been reported. The most common of these is the Gly-2019-Ser mutation, which has a worldwide frequency of 1% in sporadic cases and about 4% in patients with hereditary parkinsonism (Healy *et al.*, 2008).

Studies revealed that in historically isolated populations (Ozelius *et al.*, 2006; Hulihan *et al.*, 2008) up to 40% of hereditary PD cases observed may be caused by mutation in the *LRRK-2* gene (Lesage *et al.*, 2005, Lesage *et al.*, 2006). In North African Arabs, Ashkenazi Jews and in Portuguese people, almost a third of all patients diagnosed with parkinsonism have an *LRRK-2* mutation (Healy *et al.*, 2008). The clinical presentation closely resembles sporadic PD, but patients tend to have a slightly more benign course and are less likely to develop dementia (Schapira *et al.*, 1989).

2.2.1.2.3. Environmental factors

To date, the cause of PD has not been discovered, but it is believed that environmental factors may be linked to the onset of the disease. For example, 1-methyl-4-phenyl-1,2,3,6-

tetrahydropyridine (MPTP), a small molecule causes parkinsonism in humans (Ballard *et al.*, 1985) as well as in cats and in several rodents (Przedborski & Vila, 2001). In rodents, only specific strains of mice are susceptible to MPTP neurotoxicity (Inoue *et al.*, 1999). MPTP is a thermal breakdown product of a meperidine-like narcotic analgesic that was used as a synthetic heroin. The continuous exposure to pollutants from farms, well water and industries have also been reported to play a role in the onset of the disease (Tanner, 2003). Interestingly, caffeine and cigarette smoking have been linked with reduced risk of developing PD (Alam *et al.*, 2004; Hernan *et al.*, 2001).

2.2.1.3. Pathogenesis

The pathogenesis of PD has been linked to oxidative-mediated events, including increased MAO activity and ROS generation (Jenner, 2003).

2.2.1.3.1. Reactive oxygen species

Although oxygen is vital for life, paradoxically, by-products of its metabolism produce ROS, which are highly toxic to cells. Due to its bi-radical nature, oxygen readily accepts unpaired electrons (Jenner, 2003) to yield a series of partially reduced species collectively known as ROS. These include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals ($HO^\cdot$), peroxy radicals ($ROO^\cdot$), alkoxy radicals ($RO^\cdot$), and nitric oxide (NO). Oxygen may also be completely reduced to water (Beal, 2000). Most of the superoxide radicals are formed in the mitochondrial and microsomal electron transport chains. Except for cytochrome oxidase, all other elements of the mitochondrial respiratory chain (for example ubiquinone) may transfer an electron directly to oxygen (Hemnani & Parihar, 1998). Also, superoxide can be generated by autooxidation of semiquinones on the internal mitochondrial membrane.

Cells have endogenous antioxidant systems to counteract excessive ROS. These include superoxide dismutase (SOD), catalase, ascorbic acid and glutathione (GSH), amongst others (Riederer *et al.*, 1989). However, when ROS production overwhelms the endogenous antioxidant systems, they can potentially damage various types of macromolecules, such as lipids, proteins, carbohydrates and DNA (Halliwell & Gutteridge, 1985). In PD and AD, these defense mechanisms are compromised, and the activities of the various antioxidant defense molecules that would normally protect against the injurious effects of ROS, are reduced. The antioxidant enzymes SOD, GSHPx and GSHRd, for example, display reduced activities in affected brain regions in AD (Zemlan *et al.*, 1989; Pappolla *et al.*, 1992). PD in turn, is

characterized by reduced amounts of the antioxidant GSH in the SN (Perry *et al.*, 1982; Spina & Cohen 1989). The magnitude of depletion correlates to the severity of the disease.

2.2.1.3.2. MAO-B activity

MAO-B is of therapeutic importance in PD, since this enzyme catalyzes the oxidation of dopamine in the brain. The oxidative deamination of biogenic amines, including dopamine and phenylethylamine (PEA) by MAO-B, produces hydrogen peroxide (H_2O_2) as a by-product (Youdim *et al.*, 2004). MAO-B activity also increases with age, which results in an increased level of dopamine metabolism and the production of higher levels of ROS, via H_2O_2 formation. This age-related increase in brain MAO-B levels may thus contribute to the neuropathology associated with PD and may explain the increased prevalence of the disease in aged individuals (Soong *et al.*, 1992). Because MAO-B is predominantly located in the glial cells (Youdim *et al.*, 2004), the increase of activity with age may be attributed to glial cell proliferation (Novaroli *et al.*, 2006).

2.2.2. Symptomatic Treatment

Although current therapies for PD significantly improve the quality of life, there is no cure for PD. Current medication only provide relief from the symptoms, and do not treat underlying dopaminergic neuron degeneration (Lees *et al.*, 2009). Another limitation of current PD medications is their sometimes disabling side effects (Alexi *et al.*, 2000). Treatment of PD is divided into three categories: (1) protective or preventative therapies are those that slow or prevent further neurodegeneration, (2) symptomatic therapies and (3) restorative or regenerative therapies are aimed at promoting neuronal survival and function (Alexi *et al.*, 2000). To date, no treatment has been shown to be “neuroprotective”.

Dopaminergic Therapy

2.2.2.1. Levodopa

In the symptomatic treatment of PD, the first-line treatment is based on dopamine replacement therapy, which restores appropriate concentration of dopamine in the synaptic cleft of dopaminergic neurons. This is usually achieved by administration of the dopamine precursor, levodopa (L-DOPA), a precursor to dopamine, which enters the brain via a carrier-mediated transport system, where it is converted to dopamine by the enzyme L-aromatic amino acid decarboxylase (L-AAAD) (Jenner & Olanow, 1998). It is one of the oldest and most effective therapies for the symptomatic treatment of PD and still remains an important therapy for PD.

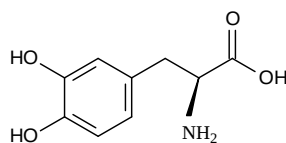


Figure 7: Structure of levodopa.

The rapid metabolism of levodopa, both peripherally and centrally, hampers its therapeutic potential. Levodopa is typically combined with inhibitors of L-AAAD, such as benserazide and carbidopa (Jankovic & Marsden, 1993), or with catechol-O-methyltransferase (COMT) inhibitors, such as entacapone and tolcapone, and sometimes with MAO-B inhibitors, such as (R)-deprenyl (Drucharch & Muiswinkel, 2000). Carbidopa and benserazide prevent the peripheral conversion of levodopa to dopamine. These combinations increase levodopa bioavailability to the brain and reduce the peripheral adverse effects of dopamine.

Long-term levodopa therapy is associated with a high incidence of motor complications called dyskinesias (involuntary movements) (Jankovic, 2001; Jankovic, 2005). These complications (Chalmers-Redman & Tatton, 1996) can be as disabling as the parkinsonian symptoms themselves (Marsden *et al.*, 1982). Many proposals have been put forward to account for these side effects but none have been definitively proven. The occurrence of levodopa-induced motor complications remains a major obstacle to the proper management of PD patients. However, dopamine agonist drugs are also effective in treating the early symptoms of PD, but may also provoke identical dyskinetic movements, although with lower incidence (Tolosa & Marin, 1997; Marsden *et al.*, 1982).

2.2.2.2. Dopamine agonists

Dopamine agonists mimic the function of dopamine in the brain by directly stimulating dopamine receptors. They are frequently used alone to treat early PD or in combination with levodopa to treat advanced PD. Levodopa still remains the most effective agent for the symptomatic treatment of PD (Olanow *et al.*, 2004). In order to extend its efficacy and decrease motor complications, levodopa may be administered with a dopamine agonist or an L-AAAD inhibitor. With longer half-lives than levodopa, dopamine agonists provide more sustained enhancement of dopaminergic function and delays the levodopa induced motor complications (Jankovic, 2005).

Among the dopamine agonists are bromocriptine, pergolide, carbagoline, talipexole and apomorphine (Lida *et al.*, 1999). Several new dopamine agonists are being studied to potentially join ropinirole and pramipexole, which are already on the market (Silverdale *et al.*, 2003; Lida *et al.*, 1999). They have been introduced as early monotherapy for PD, because their use is associated with a much lower incidence of motor side effects (Bracco *et al.*, 2004; Rascol *et al.*, 2000). One of these, pramipexole, has progressed to Phase III trials. Another lisuride, which is in the form of a skin patch, is already marketed in Europe (Sommer & Stacy, 2008). Interestingly, dopamine receptor agonists may be considered potentially “neuroprotective” as they act at D₂ autoreceptors found on dopaminergic SN terminals, to suppress dopamine release and thus reduce oxidative stress (Olanow *et al.*, 2004).

2.2.2.3. Carbidopa and benseraside

Carbidopa is an L-AAAD inhibitor used in the treatment of PD (Sommer & Stacy, 2008). Carbidopa prevents the metabolism of levodopa in the peripheral tissues, making more levodopa available to partition into the brain. It also increases levodopa blood levels. Carbidopa is always used in combination with levodopa, as it does not have any anti-parkinsonian activity when used alone (Lees *et al.*, 2009).

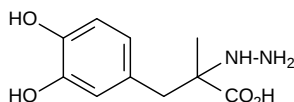


Figure 8: Structure of carbidopa.

Benseraside is also a peripherally-acting inhibitor of L-AAAD (Shen *et al.*, 2003). Since benseraside is unable to cross the blood-brain barrier it only prevents the decarboxylation of levodopa in the peripheral tissues.

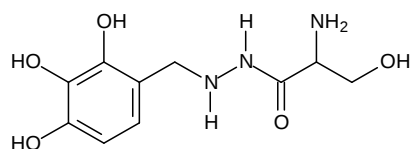


Figure 9: Structure of benseraside.

Similar to carbidopa, benseraside has little therapeutic effect on its own and is always given in combination with levodopa (Ryan & Slevin, 2006). Since benseraside prevents the peripheral

conversion of levodopa to dopamine, the adverse effects caused by peripheral dopamine, such as nausea and arrhythmia are minimized.

2.2.2.4. COMT inhibitors

COMT inhibitors and MAO-B inhibitors are drugs that reduce the metabolism of dopamine and thus increase the amount of dopamine in neurons. These inhibitors also prolong the beneficial effect of levodopa. COMT inhibitors that are currently available include entacapone and tolcapone (Sommer & Stacy, 2008; Bonifácio *et al.*, 2007). COMT inhibitors are used as an adjunct to L-AAAD inhibitors and they reduce the methylation of the catechol hydroxyl groups of dopamine.

2.2.2.5. MAO-B Inhibitors

The design of treatment strategies for PD are currently aimed at drugs that target the mechanism of neuronal cell death and delay or even halt the progression of this disease. Such drugs may represent therapy for PD. MAO-B inhibitors are thought to possess neuroprotective properties and are an important approach in the treatment of PD (Youdim & Bakhle, 2006). MAO-B is considered to be one of dopamine's major metabolising enzymes. Inhibition of MAO-B blocks the metabolism of dopamine and enhances both the endogenous dopamine levels and dopamine produced from exogenously administered levodopa (Foley *et al.*, 2000; Yamada & Yasuhara, 2004). MAO-B inhibitors may also increase the levels of phenylethylamine which leads to the release of dopamine and noradrenaline in the CNS. The inhibition of dopamine degradation by MAO-B inhibitors combined with supplementation of dopamine by levodopa has been shown to be successful in the treatment of PD patients (Palhagen *et al.*, 2006). It is important to note that selective inhibition of either MAO-A or -B, will not change the levels of dopamine drastically in the human striatum (Riederer & Youdim, 1986). When combined with levodopa, inhibitors of MAO-A and MAO-B however potentiate dopamine levels derived from levodopa (Youdim & Bakhle, 2006).

Mechanism-based inhibitors of MAO-A and-B have been used clinically and can act as antidepressants or neuroprotective drugs. Inhibition of MAO-A increases brain levels of noradrenaline and serotonin, which are generally low in depressive patients. MAO-A inhibitors are therefore used in the treatment of depression (Riederer & Youdim, 1986). As mentioned above, selective inhibition of MAO-B decreases the deamination of dopamine thus conserving the depleted supply of dopamine in the brain. MAO-B inhibitors may also act as

neuroprotective agents (Youdim & Bakle, 2006; Hubàlek, *et al.*, 2005) by reducing the production of hydrogen peroxide (H_2O_2) generated during amine oxidation. A highly active free radical, the hydroxyl radical is formed from H_2O_2 (Jenner, 2003) via the Fenton reaction. This radical can damage nucleic acids, proteins and membrane lipids, thus contributing to neuronal degeneration (Nicotra *et al.*, 2004). MAO-B inhibitors may thus decrease oxidative stress in healthy dopaminergic neurons, by decreasing H_2O_2 production (Burke *et al.*, 2004). The aldehyde product of MAO-catalyzed dopamine oxidation is also potentially neurotoxic. The formation of aldehydes may be prevented by treatment with MAO inhibitors (Burke, 2003).

2.2.2.6. (R)-Deprenyl, lazabemide and rasagiline

Interest in selective inhibitors of MAO-B has increased in the last years due to their therapeutic roles in age related neurodegenerative diseases such as PD (Foley *et al.*, 2000; Nicotra *et al.*, 2004). The discovery that MAO-B inhibitors can act both as a symptomatic and neuroprotective agents established their role in the treatment of PD (Youdim & Bahkle, 2006).

(R)-Deprenyl, is a highly potent, irreversible MAO-B inhibitor (LeWitt & Taylor, 2008; Youdim & Green 1975) and is structurally related to phenylethylamine. Low doses of this compound inhibit the oxidative deamination of dopamine, phenylethylamine and benzylamine but not of adrenaline or 5-HT. However, at higher doses its selectivity is lost (Youdim & Green, 1975). For a MAO inhibitor to be effective in PD, it must raise levels of dopamine at its receptor sites in the striatum. Using microdialysis techniques in rat striatum, chronic (but not acute) treatment with rasagiline and (R)-deprenyl, increased by a similar extent, dopamine levels in the microdialysate (Youdim, 1988; Finberg *et al.*, 1998). (R)-Deprenyl is effective both as an adjuvant to levodopa and as monotherapy (Golbe, 1989) and slows the rate of degeneration of dopaminergic neurons. It is therefore widely used as a neuroprotective treatment in PD (Youdim & Bakhle, 2006).

The limitations of (R)-deprenyl however, are that it is a propargyl amphetamine derivative that undergoes extensive metabolism to amphetamine metabolites (Mahmood, 1997; Riederer *et al.*, 2004), which are potentially neurotoxic and associated with adverse cardiovascular and psychiatric effects (Churchyard *et al.*, 1997). (R)-deprenyl has also been shown to lose selectivity for MAO-B at higher doses, resulting in the dangerous “cheese reaction” (Golbe, 1989). However, recent developments have produced a new orally disintegrating tablet (ODT) formulation of (R)-deprenyl which dissolves on contact with saliva and is absorbed mostly in the

buccal cavity (Seager, 1998). This pregastric absorption minimizes the first pass metabolism of the drug, increases its bioavailability and greatly reduces metabolite concentrations when compared with the conventional formulation (Clarke *et al.*, 2003). The return of enzyme activity, following treatment with inactivators such as (R)-deprenyl requires *de novo* synthesis of the MAO-B protein and safety considerations associated with the metabolites of such irreversible inhibitors still remain (Riederer *et al.*, 2004).

Lazabemide

Lazabemide differs from (R)-deprenyl in several properties: it is a reversible inhibitor of MAO that has greater selectivity for the type B enzyme *versus* type A and undergoes rapid clearance after discontinuation. Furthermore, lazabemide is not a propargylamine compound and is therefore not metabolized to amphetamine (LeWitt *et al.*, 1993). Initial studies with lazabemide in untreated PD subjects, revealed that its symptomatic effects were similar to those of (R)-deprenyl and it is also believed to possess unique antioxidant properties (Mason *et al.*, 2000). Although lazabemide provides only a small improvement in the symptoms of PD, it delayed the need for levodopa in 51% of PD subjects (LeWitt *et al.*, 1993).

Rasagiline

Like (R)-deprenyl, rasagiline is a selective irreversible MAO-B inhibitor used in the treatment of PD (Youdim & Bakhle, 2006). However, it is not an amphetamine derivative and undergoes first-pass metabolism to the inactive non-toxic metabolite aminoindan. Therefore, the amphetamine-like adverse effects associated with (R)-deprenyl treatment are eliminated. Rasagiline has an approximate bioavailability of 36% and *in vivo* studies have shown that rasagiline is up to ten times more active than (R)-deprenyl as a neuronal survival agent (Mandel *et al.*, 2003; Maruyama *et al.*, 2000).

Generally, the propargylamine neuroprotective agents are anti-apoptotic (Abu Raya *et al.*, 2002; Bonneh-Barkay *et al.*, 2005). Structure-activity relationship studies with derivatives and metabolites of rasagiline have shown that their neuroprotective and neurorescue property resides in their propargyl moiety (Mandel *et al.*, 2003; Mandel *et al.*, 2005). Several other propargylamine derivatives have been known to be effective neuroprotective agents and the MAO-A inhibitor, clorgyline (De Girolamo *et al.*, 2001) and (R)-deprenyl (Magyar & Szende, 2004) are examples of such compounds. Rasagiline offers both symptomatic and neuroprotective effect, both in animal models and several clinical trials (Youdim *et al.*, 2003) and

is believed to alter the course of PD. It was demonstrated that it is safe, well tolerated and delays the progression of the disabilities, especially motor function, associated with PD (LeWitt & Taylor, 2008; Yacoubain & Standaert, 2009).

Non-Dopaminergic Targets

2.2.2.7. Anticholinergic drugs

Although the primary agents used for the pharmacological treatment of PD are those which directly or indirectly act as dopamine agonists, the first drugs used for treatment, were those with anticholinergic activity (Standaert & Young, 1995). Synthetic anticholinergic drugs remained the mainstay of drug treatment for PD until the arrival of levodopa and amantadine (Sweeney, 1995). Although levodopa and other centrally acting dopaminergic agonists have replaced anticholinergic drugs, they still have application in treatment of PD. Although the mechanism of action is still unknown, it is believed that anticholinergic drugs can correct the relative imbalance between the dopaminergic and cholinergic neurological pathways that occur in less advanced forms of PD. This occurs by reducing neurotransmission mediated by neostriatal acetylcholine (Sweeney, 1995; Standaert & Young, 1995).

Anticholinergic drugs may also alleviate some of the symptoms of PD, in particular the involuntary resting tremor. Anticholinergic drugs are used as monotherapy early in the course of the disease and in combination with levodopa in more advanced PD (Sweeney, 1995; Standaert & Young, 1995). Common anticholinergics include trihexphenidyl, procyclidine, benztropine, biperiden, diphenhydramine and orphenadrine (figure 10) (Sweeney, 1995). Anticholinergic drugs have been associated with unfavourable side effects, especially in older patients. Their use has declined drastically in recent years because they may induce delirium, aggravate dementia, cause constipation and toxic megacolon. They may also induce severe weight loss and retention of urine in men with prostatism and may cause the onset of narrow angle glaucoma (Cooper *et al.*, 1992).

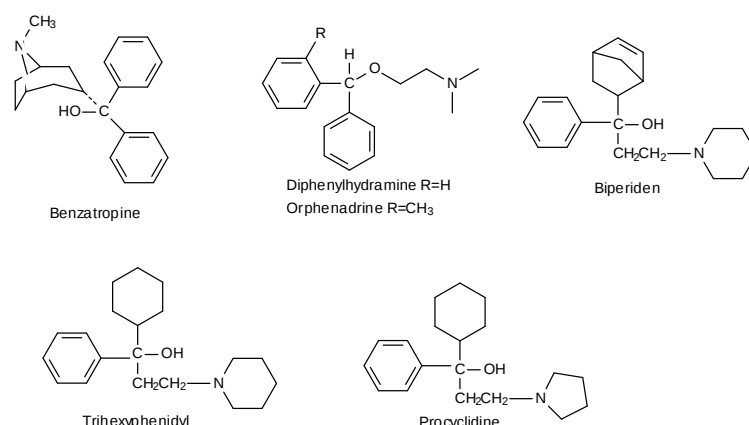


Figure 10: Structures of anticholinergic drugs used in the treatment of PD.

2.2.2.8. Adenosine A_{2A} receptor antagonists

The search for alternative or adjunctive approaches that can modulate basal ganglia motor circuitry is still ongoing. The most studied non-dopaminergic target for the treatment of PD is the adenosine A_{2A} receptor (Schwartzschild *et al.*, 2006; Jenner, 2003). A_{2A} receptors are selectively localized in the basal ganglia and functions at the indirect output pathway, where they control gamma-aminobutyric acid (GABA) and acetylcholine release. A_{2A} receptors are particularly relevant to PD because their expression and concentration in the brain is restricted to the striatum (Svenningsson *et al.*, 1999). Studies have shown that blockade of A_{2A} receptors may be neuroprotective, since antagonists of A_{2A} receptors protect against excitotoxic and ischemic neuronal injury (Phillis, 1995). A_{2A} antagonists also cause a reduction in the loss of striatal dopaminergic terminals and nigral dopaminergic neurons in MPTP-treated animals (Xu *et al.*, 2005). One selective A_{2A} antagonist, KW-6002, has entered clinical trials for the treatment of PD (Xu *et al.*, 2005)

2.2.2.9. NMDA receptors

Receptors in the brain such as *N*-Methyl-D-aspartate (NMDA) and metabotropic glutamate have been targeted as potential therapeutic approaches for PD. Excessive NMDA receptor activation has been implicated in the pathogenesis of chronic neurodegenerative diseases, such as PD and AD (Korczyn & Nussbaum, 2002). Glutamate can act as an excitotoxin contributing to neuronal damage (Lange *et al.*, 1993; Lange & Riederer, 1994). Based on this, a rationale for PD neuroprotection, is to block glutamate neurotransmission in the SN.

The only anti-glutamatergic drugs approved for the treatment of PD are the non competitive

NMDA receptor antagonists, 1-amino adamantane, adamantine (an antiviral drug), and memantine. These drugs act at the phencyclidine (PCP) binding site (Kornhuber *et al.*, 1989). They are believed to have moderate anti-kinetic efficacy and neuroprotective effects, although they carry the risk of psychotic side effects (Riederer *et al.*, 1991). For this reason, glutamate agonists are offered as a combination therapy with levodopa, and may allow for a reduction of the levodopa dose and prevent or postpone the side effects of long term levodopa administration (Marsden & Parkes, 1976).

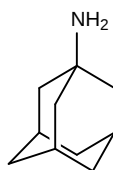


Figure 11: Structure of amantadine.

Amantadine is an antiviral drug used for the treatment of flu, and was recently found to be an NMDA receptor antagonist. It was first discovered to be effective in the treatment PD in the 1960s, following its introduction as a prophylaxis for influenza. Amantadine is usually administered in combination with levodopa (Parkes *et al.*, 1974). In high doses, amantadine can also act as an anti-dyskinetic drug (Verhagen Metman *et al.*, 1998). Another compound, riluzole, a member of the benzothiazole class, is currently used for the treatment of amyotrophic lateral sclerosis (ALS) (Korczyn & Nussbaum, 2002). Riluzole acts by blocking the presynaptic release of glutamate. Riluzole offers neuroprotection in various experimental models of neuronal injury involving excitotoxic mechanisms (Dessi *et al.*, 1993).

Other Non-Dopaminergic Drugs

2.2.2.10. Zonisamide

Zonisamide is an antiepileptic drug, efficient in treating refractory epilepsy (Sobieszek *et al.*, 2003). It is a sulfonamide derivative that is now being considered for the treatment of PD (Pedro & Beunavventura, 2009). Zonisamide inhibits voltage-dependent sodium (Hashimoto *et al.*, 2003) and calcium channels of the T-type (Suzuki *et al.*, 1992). Although the mechanism by which zonisamide may improve PD symptoms is unclear (Pedro & Beunavventura, 2009), zonisamide may function by increasing dopaminergic and serotonergic (5-HT) transmission (Okada *et al.*, 1995). Okada and colleagues (1999), demonstrated that zonisamide inhibited MAO-B, without the affecting activity of type-A (MAO-A) of this enzyme.

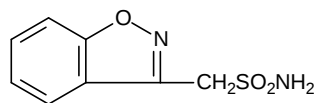


Figure 12: Chemical structure of zonisamide.

In addition, zonisamide scavenges hydroxyl and NO radicals in a dose-dependent manner (Mori *et al.*, 1998a) which may result in the protection of neurons.

2.2.2.11. α -Synuclein-directed therapies

Although its mechanism of neurodegeneration in PD is not well understood, α -synuclein appears to play an important role (Haywood & Staveley, 2004). Disruption of α -synuclein aggregation has been the focus of research, to develop novel therapies against PD. α -Synuclein aggregation can be reduced at several levels: 1) by reducing α -synuclein protein production; 2) by increasing α -synuclein clearance; 3) by preventing or reducing chemical modifications that can promote aggregated species; or 4) by directly interfering with aggregation.

Potential methods to reduce α -synuclein protein production include the use of small molecules that interfere with the transcription of α -synuclein (Haywood & Staveley, 2004). Another approach is to increase the clearance of α -synuclein (Haywood & Staveley, 2004; Lo Bianco *et al.*, 2004; Yamada *et al.*, 2005). For example, reduced, insoluble α -synuclein aggregates (Klucken *et al.*, 2004), was observed when chaperone function is promoted since chaperone proteins such as Hsp70, promotes α -synuclein clearance. Also, the compound, geldanamycin, reduces α -synuclein aggregation *in vitro* by increasing Hsp70 levels (McLean *et al.*, 2004). In addition, vaccine-based therapies are now been pursued as potential strategies for increasing α -synuclein clearance (Masliah *et al.*, 2005). α -Synuclein transgenic mice vaccinated against α -synuclein showed decreased α -synuclein accumulation (Masliah *et al.*, 2005). Since oxidative modification and phosphorylation of α -synuclein promotes aggregation, antioxidant therapies and kinase inhibitors could also help reduce α -synuclein aggregation (Chen & Feany, 2005; Smith *et al.*, 2005).

2.2.2.12. Kinase inhibitors

Pathogenic mutation of LRRK-2 leads to familial PD (Healy *et al.*, 2008), and is associated with increased kinase activity (West *et al.*, 2005; West *et al.*, 2007). This makes the kinase activity of LRRK-2 an important target for neuroprotective therapy (Greggio *et al.*, 2006). Kinases are

generally good targets for small molecule therapies and certain therapies in other diseases are based on inhibition of kinase activity. At this point, however, the endogenous substrates for LRRK-2 are unknown, making it difficult to develop LRRK-2 kinase inhibitors (Greggio *et al.*, 2006).

2.2.3 DRUGS FOR NEUROPROTECTION

2.2.3.1. Dopaminergic drugs: Pramipexole and Ropinirole

Several dopaminergic drugs may possess neuroprotective effects despite initially being considered for their symptomatic actions in PD. Early indications of the potential beneficial effects of dopaminergic agonists on neurons was observed from a study in rats, in which daily oral intake of pergolide lessened age-related attrition of SN neurons (Felton *et al.*, 1992).

Pramipexole

Pramipexole is a non-ergot dopamine agonist that may also act as an antioxidant (Cassarino *et al.*, 1998). Pramipexole is a D₂ receptor agonist and also binds to D₃ and D₄ receptors. The interaction with these receptors, stimulate dopamine activity in the nerves of the striatum and SN (Vu *et al.*, 2000).

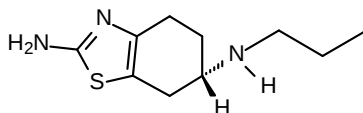


Figure 13: Structure of pramipexole.

Studies with pramipexole have demonstrated a number of potentially protective actions against:

- (1) oxidative stress, where it scavenges reactive oxygen species both *in vivo* and *in vitro* (Cassarino *et al.*, 1998.; Ferger *et al.*, 2000).
- (2) the influence of various experimental toxins on dopaminergic neurons, including methamphetamine, 3-acetylpyridine, 6-hydroxydopamine (6-OHDA) (Vu *et al.*, 2000).

Pramipexole also protects against MPTP toxicity in non-human primates (Iravani *et al.*, 2006), since it inhibits the mitochondrial permeability transition produced by this neurotoxin (Cassarino *et al.*, 1998). Pramipexole inhibits lipid peroxidation and reduction of SN injury in C57BL/6 mice treated with MPTP (Zou *et al.*, 2000). This compound has also demonstrated a protective effect (Kitamura *et al.*, 1998) in clinical studies. Results from the SPECT studies, conducted by the Parkinson Study Group (2002), confirmed this attribute. Subjects treated with pramipexole

demonstrated less decline in striatal dopamine transporter binding compared with those assigned to levodopa treatment.

Ropinirole

Ropinirole, like pramipexole, is a potent non-ergoline dopamine agonist with similar neuroprotective actions (Lida *et al.*, 1999). It acts as a non-ergoline D₂, D₃, and D₄ dopamine receptor agonist with highest affinity for D₃ receptors (Eden *et al.*, 1991). Ropinirole is effective both as early monotherapy and as an adjunct to the treatment with levodopa.

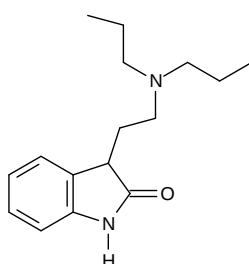


Figure 14: Structure of ropinirole.

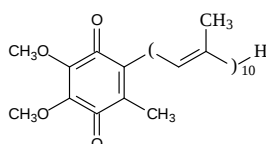
In animal models, ropinirole enhances mechanisms against oxidative stress and exerts a protective action against 6-OHDA-induced loss of nigrostriatal dopaminergic projections in mice (Rakshi *et al.*, 2002). Also, in clinical studies, ropinirole showed evidence for a reduction in disease progression compared to levodopa, suggesting a disease-modifying effect (Tanaka *et al.*, 2001). The similarity in action between pramipexole and ropinirole support the possibility of a “class effect” conferred by dopaminergic agonists but not seen with levodopa. Such results imply that dopaminergic agonists, either on the basis of dopaminergic stimulation or other properties, can act to mediate recovery of dopaminergic nigrostriatal neurons.

2.2.3.2. Antioxidant therapy

α-Tocopherol or (Vitamin E) is a chain-breaking antioxidant, that enters into lipid-soluble cellular regions such as biological membranes and acts by quenching oxyradical species. Although several compounds with antioxidant properties have been considered for clinical investigations, only α-tocopherol has undergone testing. One such study, the DATATOP trial (Parkinson Study Group, 1989), found no evidence for deficiency of α-tocopherol in PD, and severe deficiency states do not lead to parkinsonism. It is however, strongly believed that this naturally occurring antioxidant may offer a safe and promising option for testing the hypothesis of oxidative stress in the pathogenesis of PD (Parkinson Study Group, 1993).

2.2.3.3. Mitochondrial energy enhancement drugs: Coenzyme Q10 and creatinine

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a lipid-soluble quinone composed of a redox active quinone ring and hydrophobic tail (figure 15). In humans, it contains 10 isoprenoid units in the tail, whereas the rodent form, coenzyme Q9 (CoQ9), has nine isoprenoid units (Beal, 1999; Bonakdar & Guarnieri, 2005). It is a metabolic supplement and a cofactor in the electron transport chain in mitochondria. CoQ10 has recently gained attention for its potential role in the treatment of neurodegenerative diseases, since it possesses potent antioxidant actions (Beal, 1999). Studies of *in vitro* models of neuronal toxicity and animal models of neurodegenerative disorders have demonstrated potential neuroprotective effects of CoQ10 and have provided evidence for its potential in averting the progression of PD (Shults *et al.*, 1999).



A recent study (Shults *et al.*, 2002) examined the efficacy of CoQ10 in PD patients for a period of 16 months. Results showed slight symptomatic improvements in patients treated with CoQ10 compared to placebo.

Creatine is naturally synthesized in the human body from amino acids, primarily in the kidney and liver, and is transported in the blood for use by muscles. About 95% of the body's total creatine content is located in skeletal muscle (Matthews *et al.*, 1999). Creatine was discovered

in the 1800s as an organic constituent of meat. In the 1970s, Soviet scientists reported that oral creatine supplements may improve athletic performance during brief intense activities such as sprints (Balsom *et al.*, 1994). Mitochondrial dysfunction plays a major role in the pathogenesis of PD and studies have demonstrated that creatine may improve the function of mitochondria (Matthews *et al.*, 1999).

Creatine may also act as an antioxidant by preventing damage from compounds that are harmful to brain cells (Lawler *et al.*, 2002). The augmentation of brain creatine concentration is another pharmacological strategy for targeting defective mitochondrial respiration (Robert *et al.*, 2008). Several clinical trials with creatine show promising results. For example, in mice treated with MPTP, supplementing their diet with creatine for 2 weeks before administration of the neurotoxin, led to reduced damage of dopaminergic SN neurons (Matthews *et al.*, 1999). Creatine improves patient mood and leads to a smaller dose increase of dopaminergic therapy but has no effect on the symptomatic improvement of the disease (Bender *et al.*, 2006).

2.2.3.4. Anti-inflammatory drugs

Non steroidal anti-inflammatory drugs (NSAID) are a heterogeneous group of compounds which share many pharmacological properties and side effects. They are the main drugs used as analgesics and antipyretics to reduce the unwanted consequences of inflammation, by inhibiting cyclooxygenase (COX), an enzyme that catalyzes the formation of prostaglandins (PGs) (Asanuma & Miyazaki, 2006). NSAIDs, together with steroidal anti-inflammatory drugs (SAIDs) are capable of halting or suspending inflammatory process progression and many of these anti-inflammatory drugs are already in common use for other indications.

Neuroinflammation may aggravate the course of PD (Hald & Lotharius, 2005; Marchetti & Abbracchio, 2005), and has long been of concern as increased microglia activation and production of cytokines are associated with PD (Tansey *et al.*, 2007; McGeer & McGeer, 2007). Results of neurotoxin models of PD and corroborating findings, obtained in transgenic animal models and epidemiological studies, strongly support the hypothesis that this neurodegenerative mechanism is not purely neuronal, as was previously thought (McGeer & McGeer, 2004). In fact, postmortem examinations showed that neuronal degeneration in PD is associated with massive gliosis, due to a population of activated glial cells, the microglia (Teismann *et al.*, 2003). Such evidence has been confirmed in MPTP-induced parkinsonism in monkeys (McGeer *et al.*, 2003), and humans (Langston *et al.*, 1999). These new findings

unfortunately do not allow early diagnosis of the disease, because the neuroinflammatory process is silent and unnoticed, due to the absence of pain fibres in the brain.

Several studies in animal PD models have demonstrated that certain NSAIDs, such as aspirin, ibuprofen and indomethacin have neuroprotective qualities and can reduce dopaminergic cell death (Esposito *et al.*, 2007). Initial studies by Chen and co-workers (2003) showed that the risk of developing PD was lowered by 45% due to the use of NSAID. A follow-up study by the same group however, showed that only ibuprofen had this neuroprotective effect (Chen *et al.*, 2005).

2.2.3.5. Anti-apoptotic drugs: Minocycline, TCH346 and CEP-1347

Several lines of evidence have pointed to the activation of apoptosis as a possible mechanism of neurodegeneration in PD. On this basis, the use for anti-apoptotic drugs in PD may be merited.

Minocycline

Minocycline, a second generation tetracycline, long used as an antimicrobial agent, has anti-inflammatory effects independent of its antimicrobial activity (Amin *et al.*, 1996). As a means to slow disease progression, anti-inflammatory agents, including NSAIDs and minocycline, have been pursued as potential disease-modifying treatments for PD. Clinically well tolerated and completely absorbed when taken orally, this drug has an excellent brain tissue penetration (Aronson, 1980). Minocycline has been studied extensively and has been shown to be neuroprotective against a wide variety of toxic insults both *in vitro* and *in vivo*. Minocycline blocks microglial activation which is a prominent feature in the brain of PD patients and may also have anti-apoptotic activity in culture (Tikka & Koistinaho, 2001; Tikka *et al.*, 2001). In animal models, particularly rodents, minocycline protects against dopaminergic cell loss in both the MPTP (Du *et al.*, 2001; Wu *et al.*, 2002) and 6-OHDA models (He *et al.*, 2001; Du *et al.*, 2001). Despite these attributes of minocycline, preclinical results still need to prove that it is a potential neuroprotective agent for the treatment of PD (Diguët *et al.*, 2004).

TCH346

TCH346 is a novel compound, developed because of its shared structural similarities with (R)-deprenyl. However, it is not an MAO-B inhibitor, and unlike (R)-deprenyl is not metabolized to amphetamine metabolites. TCH346 is an anti-apoptotic drug that inhibits the glycolytic enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key step in age-induced neuronal

apoptosis (Ishitani *et al.*, 1996a; Ishitani *et al.*, 1996b). Cell culture studies with PC12 and human neuroblastoma cell lines showed increased survival with this agent (Kragten *et al.*, 1998).

In both 6-OHDA and MPTP animal models, TCH346 reduced dopaminergic cell loss (Andringa *et al.*, 2000; Andringa 2003). For instance, in rhesus monkeys exposed to MPTP, near-complete protection against the development of motor impairment was achieved, when TCH346 administration was started 2 hours after the second MPTP infusion, and continued for 2 weeks (Andringa *et al.*, 2000). However, clinical studies, involving 301 patients over 12 to 18 months failed to show any clinical significant effect (Olanow *et al.*, 2006).

CEP-1347

CEP-1347 is another anti-apoptotic agent and is a semisynthetic derivative of a bacterial fermentation product termed K252, which acts as an inhibitor of mixed lineage kinase-3. This enzyme is a major component of the c-Jun-mediated terminal kinase signaling pathway, which is involved in apoptotic death of neurons. Neurons undergoing apoptosis can be rescued by factors that decrease c-Jun N-terminal kinase (JNK) activity. For this reason, CEP-1347 may rescue motor neurons undergoing apoptosis in PD (Maroney *et al.*, 1998). Initial preclinical studies showed promising results (Saporito *et al.*, 1999; Mathiasen *et al.*, 2004). For example, mice and monkeys exposed to MPTP have showed enhanced survival of SN neurons when treated with this compound (Maroney *et al.*, 1998).

2.2.3.6. Trophic factors

Trophic factors are proteins that support and protect subpopulations of cells. A number of them have been reported to act on dopaminergic neurons *in vitro* and *in vivo*, making them potential therapeutic candidates for PD. Trophic factors may enhance dopaminergic survival (Herzog *et al.*, 2007) regardless of the mechanism of cell death. Glial-cell-line-derived neurotrophic factor (GDNF) possesses potent neurotrophic effects in dopaminergic neurons and is neuroprotective in animal models (Herzog *et al.*, 2007; Kordower *et al.*, 2000). Although this approach of cell repair looks promising, one serious limitation is that the administration of the growth-factor is by continuous intraputaminial infusion (Herzog *et al.*, 2007).

2.2.3.7. Adenosine A_{2A} antagonists

The non-selective adenosine receptor antagonist, caffeine, reduces the incidence of PD in men

(Ross *et al.*, 2000).

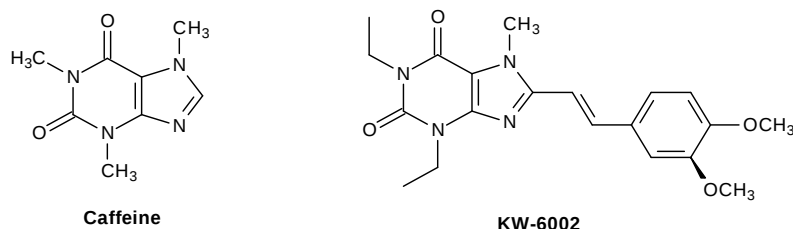


Figure 16: Structures of caffeine and KW-6002.

Epidemiological studies have revealed the existence of an inverse relationship between the consumption of caffeine and the risk of developing PD, especially in Japanese-American men (Ross *et al.*, 2000; Ascherio *et al.*, 2001). The risk of developing PD was shown to be five times higher among non caffeine drinkers than among casual drinkers (Ascherio *et al.*, 2001). Interestingly, also in women, caffeine use was linked to a reduced risk of PD, but only among those who have not taken hormone-replacement therapy (Ascherio *et al.*, 2003). Since pretreatment with A_{2A} antagonists and not A_1 antagonists (Chen *et al.*, 2002) also reduces MPTP-induced nigrostriatal lesions significantly, its protective effect is probably associated with the interaction with adenosine A_{2A} receptors (Chen *et al.*, 2001; Schwarzschild *et al.*, 2006).

Istradefylline, commonly known as (KW-6002) is a xanthine-based antagonist of the adenosine A_{2A} receptor. This drug is capable of selectively blocking adenosine A_{2A} receptors and is considered a promising drug for the treatment of PD (Xu *et al.*, 2005; Bara-Jimenez *et al.*, 2003). KW-6002 improves the recovery of MPTP-lesioned animals (Ikeda *et al.*, 2002; Jenner, 2003). Specifically in monkeys, the co-administration of KW-6002 with daily apomorphine injections, acts prophylactically to prevent dyskinesia onset (Chase *et al.*, 2003). KW-6002 also safely prolongs the efficacy half-time of levodopa, as it potentiates the antiparkinsonian response to low-dose levodopa, with fewer dyskinesias than that produced by optimal-dose levodopa alone (Chase *et al.*, 2003). Although this drug progressed to Phase III trials, it failed to prove effective. Four other potential adenosine receptor antagonists Fipamezole (JP-1730), SCH-420814, BIIA-014 and LU AA4707 are in early phases of development (Sommer & Stacy 2008). It is believed these drugs may extend both the duration and quality of antiparkinsonian action of levodopa.

2.2.3.8. Recent advances and future strategies in the treatment of PD

The present strategy for the treatment of neurodegenerative diseases, such as PD and AD, includes the use of MAO-B and cholinesterase inhibitors which have a single mechanism of action. Due to the multifactorial nature of these diseases, with no one factor being solely responsible for the symptoms and pathogenesis, this strategy offers only incomplete benefit to patients and does little to alter the course of the disease (Grunblatt *et al.*, 2004). More recent drug development strategies involve multifunctional drugs with dual or multiple inhibition targets such as cholinesterase, monoamine oxidase, nitric oxide synthase and iron chelation (Youdim & Buccafesco, 2005). These bi- or multifunctional compounds may provide greater symptomatic efficacy and may act as improved potential disease modifying drugs (Youdim & Buccafesco, 2005).

2.2.4. MECHANISM OF NEURODEGENERATION

An understanding of the mechanisms underlying the pathogenesis of PD is critical for the design of therapies. Several mechanisms have been implicated in the disease process (Yacoubain & Standaert, 2009; Alexi *et al.*, 2000) which may act separately or cooperatively. These mechanisms include oxidative stress, metabolic compromise (such as mitochondrial dysfunction), protein aggregation and misfolding, inflammation, excitotoxicity and apoptosis (Green & Greenmayre, 1996). It is thought that no single mechanism is the primary cause of all cases of PD (Yacoubain & Standaert, 2009). Furthermore, studies have shown that dopaminergic degeneration is only a part of the neurodegeneration observed in PD (Green & Greenmayre, 1996).

2.2.4.1. Oxidative stress and mitochondrial dysfunction

Mitochondria are responsible for the synthesis of ATP in neurons via a complex system involving the tricarboxylic acid cycle and the electron transport chain (Wallace, 2005). The tricarboxylic acid cycle generates NADH and FADH₂, which donates electrons to complex I or complex II. The electrons are transferred to complex III, then to complex IV, and finally to O₂. ATP is produced primarily from the redox energy released during this electron transfer process. This generates the electrochemical gradient of H⁺ across the inner membrane (Wallace, 2005). The possibility, however, exists that at complex I and complex III, the transported electrons (up to 2%) may react with molecular oxygen and yield superoxide anion (Boveris *et al.*, 1972). Superoxide may be converted into other reactive oxygen species (ROS), such as hydrogen peroxide and the highly reactive hydroxyl radical. Another consequence of superoxide production is the formation

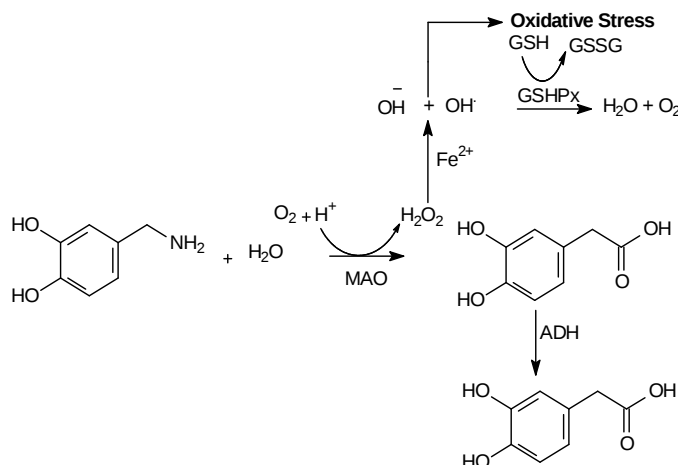
of highly toxic peroxynitrite (ONOO). This species is formed when nitric oxide free radical ($\text{NO}\cdot$) reacts with the superoxide radical. Peroxynitrite is slowly converted to nitrotyrosines when it reacts with tyrosine in proteins. Increased nitrotyrosine levels have been recorded in human brain tissues of patients with neurodegenerative diseases (Boveris *et al.*, 1972). The excess of reactive free radicals results from an overproduction of reactive species or a failure of cell buffering mechanisms that normally limit their accumulation and result in what is commonly known as “oxidative stress”.

In PD, evidence of oxidative damage to proteins, lipids and nucleic acids has been found in the SN of patients with PD (Alam *et al.*, 1997). Also, the activity of complex I is reduced in the SN of PD patients (Schapira *et al.*, 1989), while inhibitors of complex I, such as MPP^+ and rotenone, have been shown to cause parkinsonian syndromes in animals (Betarbet *et al.*, 2000). It is suggested that a reduction in complex I activity results in increased ROS production and consequent oxidative damage. A reduction in complex I in PD may also result in ATP depletion, which leads to the accumulation of intracellular Ca^{2+} in the mitochondria instead of the cytoplasm. This triggers a variety of down stream neurotoxic processes such as activation of Ca^{2+} dependent proteases and lipases, which in turn disrupt their normal functions (Betarbet *et al.*, 2000). These events may be contributors to the pathogenesis of PD.

2.2.4.2. The role of iron and oxidative stress

Iron may lead to neurodegeneration via two mechanisms: (1) by the accumulation of iron in specific brain regions and (2) as a result of defects in iron metabolism and/or homeostasis. Increased iron levels have been observed in the SN of PD patients (Reiderer *et al.*, 1989). This promotes free radical damage, particularly in the presence of neuromelanin (Zecca *et al.*, 2004). The role of iron in neurodegeneration is dependent on the metabolism of monoamines by MAO, which is a major source of hydrogen peroxide (H_2O_2) in the brain (Gutteridge, 1986). In the PD brain, GSH levels are low, (Riederer *et al.*, 1989) which results in the accumulation of H_2O_2 . Increased levels of H_2O_2 is available for the Fenton reaction, which uses iron as the ferrous ion Fe^{2+} , to produce the highly active free radical, the hydroxyl radical, from H_2O_2 . The hydroxyl radical depletes cellular antioxidants and damages lipids, proteins and DNA. With increasing age, brain iron and brain MAO activity increases (Riederer *et al.*, 1989). Since both of these are components of the Fenton reaction, the potential for hydroxyl radical generation increases with age (Mandel *et al.*, 2005). The inhibition of MAO in PD patients not only increases dopamine

levels, but may at the same time decrease H_2O_2 production and the potential for hydroxyl radical formation and the consequent oxidative stress.



Scheme 1: The Fenton reaction: ADH: aldehyde dehydrogenase; GSH: glutathione; GSHPx: glutathione peroxidase; GSSG: oxidized glutathione.

2.2.4.3. Protein aggregation and misfolding

Protein aggregation and misfolding plays a role in several neurodegenerative disorders including PD, AD and Huntington's disease (Alexi *et al.*, 2000). In PD, the aggregation of α -synuclein may be caused by the mutation of the α -synuclein gene which is found in families with autosomal dominant PD (Polymeropoulos *et al.*, 1997; Zarranz *et al.*, 2004). Although mutations in α -synuclein account for only a small proportion of inherited PD cases, α -synuclein is recognised as the major component of LBs found in sporadic PD (Irizarry *et al.*, 1998). Evidence such as gene duplication (Singleton *et al.*, 2003), point mutations (Narhi *et al.*, 1999), overexpression (Masliah *et al.*, 2005) and oxidative damage of α -synuclein (Souza *et al.*, 2000) are thought to promote the aggregation of α -synuclein, which may in turn play a role in PD pathogenesis.

Mutations in the parkin gene (Imai *et al.*, 2000; Shimura *et al.*, 2000) may negatively affect the clearance of misfolded proteins (McNaught *et al.*, 2002). This in turn promotes aggregation of damaged proteins (Kitada *et al.*, 1998). Interestingly, native α -synuclein is not a substrate for parkin although modified forms may be (Shimura *et al.*, 2001). Also, brains from patients with parkin-associated PD do not usually contain LBs (Mori *et al.*, 1998b; Farrer *et al.*, 2001).

2.2.4.4. Neuroinflammation

Generally, the first line of defence against injury and infection is inflammation. However, an

excessive inflammatory response can result in additional injury (Wyss-Coray & Mucke, 2002). It is thought that inflammation may play a role in the neurodegeneration associated with PD (McGeer & McGeer, 2004). The brain appears to be particularly susceptible to destructive inflammatory processes, because brain cells are unable to divide and recover from injury (Carson & Sutcliffe, 1999). The characteristic feature of brain inflammation is the activation of microglia, particularly microglia which may be transformed into a macrophage-like phenotype once activated (Tansey *et al.*, 2007; McGeer & McGeer, 2007). Among these toxic products produced by microglia, reactive free radicals have been shown to contribute substantially to the oxidative damage in PD. Activation of microglia is present in the SN and striatum from postmortem PD brains (Teismann *et al.*, 2003) and in PD animal models (McGeer *et al.*, 2003). Pro-inflammatory cytokines, such as IL-1 β , IL-6 and TNF- α , are also elevated in the CSF and basal ganglia in PD patients (Mogi *et al.*, 1994a; Mogi *et al.*, 1994b). Also implicated in PD pathogenesis are elevated levels of complement proteins (Yamada *et al.*, 1992).

2.2.4.5. Excitotoxicity

Oxidative stress damage which occurs as a result of glutamate neurotoxicity is known as "excitotoxicity". Glutamate is the primary excitatory transmitter in the mammalian central nervous system and principally responsible for the excitotoxic process (Alexi *et al.*, 2000). Dopaminergic neurons in the SN have high levels of glutamate receptors and receive glutamatergic innervation from the subthalamic nucleus and cortex. As SN neurons are lost, disinhibition of the subthalamic nucleus may occur, with the excessive release of glutamate (Rodriguez *et al.*, 1998). Excessive glutamate receptor stimulation results in intracellular calcium overload and a cascade of events leading to neural cell death (Mody & MacDonald, 1995). Calcium influx produced by glutamate receptor stimulation may also lead to the activation of nitric oxide synthase and eventually to the generation of peroxynitrite (Dawson & Dawson, 1996).

Recent studies have shown that NMDA receptor antagonists have neuroprotective effects in animal models (Turski *et al.*, 1991; Brouillet & Beal 1993). Anti-glutamatergic drugs approved for the treatment of PD include 1-amino adamantane, amantadine (an antiviral drug), and memantine (Kornhuber *et al.*, 1994). It has also been proposed that calcium channel blockers may reduce calcium influx during excitotoxicity (Surmeier, 2007). High levels of intracellular calcium exert a large energetic burden on neuronal cells, since intracellular calcium has to be sequestered regularly into the endoplasmic reticulum and mitochondria to prevent the activation

of cell death (Surmeier, 2007).

2.2.4.6. Apoptosis

Apoptosis, or programmed cell death, is a process of deliberate suicide that involves a series of biochemical events, leading to a characteristic cell morphology and death (Holbrook *et al.*, 1996). The apoptotic process disposes of cell corpses and fragments and it is thought to participate in neural development (Holbrook *et al.*, 1996). The decision for apoptosis can either come from the cell itself, the surrounding tissue or from a cell that is part of the immune system.

Apoptosis most probably represents an end-stage process in PD neurodegeneration. In PD, apoptosis is believed to be triggered by oxidative stress, protein aggregation (Tatton, 2000), excitotoxicity or inflammatory processes (Hirsch *et al.*, 1999). Apoptotic cell death has also been observed in PD animal models (Blum *et al.*, 2001; Jellinger, 2001; Mattson, 2006). The well known MAO-B inhibitor (R)-deprenyl, which is used in PD, has been shown to be neuroprotective, possibly by inhibiting apoptosis (Youdim & Bahkle, 2006). Although the initial cause for neurodegeneration in PD is still unknown, compounds that act as inhibitors of apoptosis may act as potential neuroprotective agents. Recently, two different compounds TCH346 and CEP-1347 that inhibit apoptotic signalling have been tested in human PD trials (Olanow *et al.*, 2006).

2.2.4.7. Loss of trophic factors

Several studies have shown that neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Howells *et al.*, 2000), glial-derived neurotrophic factor (GDNF) (Chauhan *et al.*, 2001), and nerve growth factor (NGF) (Mogi *et al.*, 1999), are reduced in the SN of PD patients. These factors have been proposed as a potential neuroprotective therapy in PD and may stimulate growth of dopaminergic neurons (Hefti *et al.*, 1989).

Table 5: Summary of mechanisms of PD pathogenesis and targets for therapy
(Yacoubain & Standaert, 2009).

PD pathogenic mechanism	Targets for neuroprotection
Oxidative stress and mitochondrial dysfunction	Inhibitors of dopamine metabolism (MAO inhibitors, dopamine receptor agonists) Electron transport enhancers (CoQ10) Other antioxidants (vitamin E, uric acid) Glutathione promoters (selenium)
Protein aggregation and misfolding	Inhibitors of α -synuclein aggregation Agents that reduce α -synuclein protein levels Enhancers of parkin function Enhancers of UCH-L1 function Enhancers of proteosomal or lysosomal pathways
Neuroinflammation	NSAIDs, statins, minocycline
Excitotoxicity	NMDA receptor antagonists Calcium channel antagonists
Apoptosis and cell death pathways	Anti-apoptotic agents
Loss of trophic factors	Neurotrophic factors (GDNF, neurturin)

2.3. MONOAMINE OXIDASE

2.3.1. General background and tissue distribution

MAO is an integral protein of the mitochondria and is a flavin-adenine dinucleotide (FAD)-containing enzyme (Johnston, 1968). It is located in the outer membrane mitochondria in neuronal, glia and other cells (Johnston, 1968; Youdim & Bahkle, 2006).

Structure and composition of MAO

MAO-A is composed of 527 amino acids while MAO-B is composed of 520 amino acids. Both isoforms are covalently bound to the mitochondrial outer membrane through a C-terminal transmembrane polypeptide segment (Binda *et al.*, 2006) and contain the peptide sequence Ser-Gly-Gly-Cys-Tyr near the active site to which is attached the FAD cofactor, the only redox cofactor that is absolutely required for catalysis. This FAD cofactor is covalently bonded to the cysteine residue through a thio-ether linkage between a cysteinyl residue and the 8- α CH₃ group of the isalloxazine ring (Binda *et al.*, 2006). In MAO-B, the cysteine lineage is to Cys-397 while in

MAO-A, Cys-406 is the residue involved. Both isoforms of MAO are approximately 60-kDa. Human MAO-A and MAO-B share approximately 70% sequence identity, suggesting that they may be derived from a common gene (Johnston, 1968). This explains the overlapping specificities in substrates and inhibitors. Although the active site of the two isoforms of MAO share 93% homology, MAO-B has two distinct active site cavities (Binda *et al.*, 2002) while MAO-A has only a single active site cavity (De Colibus *et al.*, 2005).

Distribution of MAO-A and MAO-B

As mentioned, MAO-A and -B are encoded by different genes and are expressed in a tissue-specific manner. MAO-A and MAO-B are tightly bound to the mitochondrial outer membrane and a small proportion of the enzymes are associated with the microsomal fraction. In mammals, MAO activity has been identified in all cell types with the exception of erythrocytes (Berry *et al.*, 1994). Also, MAO-A and -B are distributed differently in the mammalian brain (Youdim, 1988), with greater MAO-B activity and density in the basal ganglia (Collins *et al.*, 1970). During development, MAO-A appears before MAO-B, with the level of the latter increasing in the brain after birth (Nicotra *et al.*, 2004).

Studies have shown that dopamine is equally well metabolized by both MAO types in the human brain (O'Carroll *et al.*, 1983). It is known that the peripheral organs of humans contain MAO. For example, the skeletal muscles have MAO-A levels comparable to MAO levels in the liver, while MAO-B levels are twice that found in the liver (Glover & Sandler, 1986). The stomach is very rich in both forms of MAO (Johnston, 1968). Within neurons, MAO-A is present in the cell bodies of all catecholaminergic neurons (Westlund *et al.*, 1988). MAO-A is the only isozyme present in neuronal cell bodies of the dopaminergic projections from the SN (Westlund *et al.*, 1988). In contrast, MAO-B is present in serotonergic regions of the hypothalamus and in astrocytes (Westlund *et al.*, 1988.; O'Carroll *et al.*, 1983).

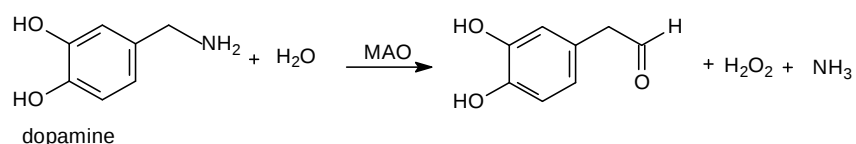
Using ¹¹C-labelled irreversible inhibitors of MAO-A and MAO-B, the distribution of MAO-A and MAO-B in the human brain was determined (Fowler *et al.*, 1987). These studies showed that the concentration of MAO-B in the SN is three-fold higher than that of MAO-A (Azzaro *et al.*, 1985). Nigral MAO-B is located primarily in the glial cells (Denney *et al.*, 1982), and is generally in excess in the tissues in which it occurs. It is necessary to inhibit at least 80% of the enzyme to achieve a pharmacological effect (Green *et al.*, 1977).

2.3.2. Biological function of MAO-B

The oxidation of biogenic amines to the corresponding imines are catalyzed by either the quinoprotein class of enzymes (usually primary amines) (Hartmann & McIntyre, 1997) or the flavin-containing amine oxidases (primary, secondary or tertiary amines). MAO plays an essential role in the oxidative deamination of biogenic and food-derived amines, both in the central nervous system and in peripheral tissues (Glover & Sandler, 1986).

The principal role of MAO-B is to degrade foreign amines and thus limit their access to synaptic vesicles (O'Carroll *et al.*, 1983). MAO-B may have a unique protective function by acting as a metabolic barrier in the microvessels of the blood–brain barrier (BBB). Studies to determine the contributions of MAO-A and MAO-B to the metabolism of neurotransmitters have mainly focused on DA and serotonin (5-HT). Dopamine is oxidized by either MAO-A or MAO-B, but is thought to be a preferential MAO-B substrate in the human brain (Youdim & Bahkle, 2006). The presence of two isozymes of MAO in the CNS suggests that while one form of MAO plays a principal role in the metabolism of a particular amine, in the event of inhibition, the other isozyme can take over its function (Garrick & Murphy, 1980). Experiments have revealed that the metabolism of dopamine occurs mainly by MAO-B present in glial cells (Oreland *et al.*, 1983). It is reported that 75% of dopamine metabolism in human brain homogenates is catalyzed by MAO-B and similar results was obtained using guinea-pig (Azzaro *et al.*, 1985) and pig (Stenström *et al.*, 1987).

As illustrated in scheme 2, dopamine is oxidized by MAO-B, to the corresponding imine product which is rapidly hydrolyzed to dopaldehyde (Olanow, 1990). The glial metabolism of dopamine by MAO-B only becomes very important when the dopamine reuptake pathway is inhibited or impaired (Liccione & Azzaro, 1988).



Scheme 2: The MAO catalyzed oxidation of dopamine.

2.3.3 Substrate and inhibitor specificities

The classification of MAO into two isoenzyme forms, MAO-A and MAO-B, was first suggested by Johnston (Johnston, 1968). This was done on the basis of substrate preference and inhibitor

selectivities. MAO-A preferentially metabolizes serotonin, noradrenaline and adrenaline and is selectively and irreversibly inhibited by low concentrations of clorgyline. MAO-B preferentially metabolizes exogenous amines such as phenethylamine and is selectively inhibited by low concentrations of (R)-deprenyl (Johnston, 1968). Dopamine is considered to be a mixed substrate for both MAO forms but is preferentially oxidized by MAO-B in the human brain (Garrick & Murphy, 1980). Kynuramine is also considered a mixed substrate for both forms of human MAO-A and MAO-B.

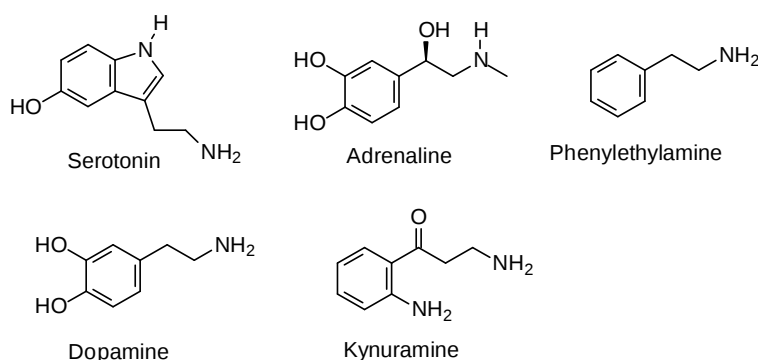


Figure 17: Examples of human MAO substrates.

2.3.4. Biological function of MAO-A

In the human central nervous system (CNS), MAO-A metabolizes serotonin, noradrenaline and dopamine (Youdim & Bahkle, 2006). It is the principal isoform in the intestinal tract, where it metabolizes dietary amines such as tyramine (Youdim, 1988). The metabolism of noradrenaline occurs by MAO-A upon re-uptake into the neurons (Westlund *et al.*, 1988) while the metabolism of serotonin is mediated by MAO-A in the glial cells (Fagervall & Ross, 1986; Liccione & Azzaro, 1988). Serotonin cell bodies contain only MAO-B and not MAO-A. This suggests that glial cells have an important role in metabolizing serotonin. Indeed, inhibitors of MAO-A, but not MAO-B have been shown to increase brain serotonin levels and also have antidepressant activity (Jouvet, 1969). Since MAO-A is present in dopaminergic neurons in humans, MAO-A is responsible for the metabolism of dopamine which has been transported back into the neuronal cells. However, MAO-A catalyzed metabolism of dopamine only becomes important when MAO-B is inhibited. MAO-A in extra- and intraneuronal compartments is essential for maintaining the concentrations of serotonin, noradrenaline and dopamine within their respective neurons (Westlund *et al.*, 1988).

2.3.5. The cheese reaction

Nonselective, irreversible inhibitors of MAO such as tranylcypromine, phenelzine and iproniazid have been used in the treatment of depression (Youdim & Weinstock, 2004). Peripheral inhibition of MAO-A may potentiate the sympathomimetic action of indirectly acting amines (e.g. tyramine). This results in a hypertensive crisis, the so-called “cheese effect” (Yamada & Yasuhara, 2004) since certain cheeses contain high amounts of tyramine. This “cheese reaction” occurs when tyramine (Da Prada *et al.*, 1988), which is normally metabolized by intestinal MAO-A, enters the systemic circulation (Youdim *et al.*, 2006). Tyramine may potentiate sympathetic cardiovascular activity by causing an abnormal release of noradrenaline from peripheral adrenergic neurons which may result in a severe hypertensive response which can be fatal (Hasan *et al.*, 1988).

2.3.6. MAO-A in depression

As mentioned, MAO-A oxidizes serotonin, a neurotransmitter that is involved in depression (Youdim *et al.*, 2006). The inhibition of MAO-A increases brain levels of serotonin which are generally low in depression patients. This leads to an antidepressant effect and is the main indication for reversible MAO-A inhibitors (Youdim & Bahkle, 2006).

2.3.7. The serotonin syndrome

Serotonin is synthesized from the amino acid L-tryptophan and over 90% of the serotonin in the body is found in enterochromaffin cells in the GI tract and in platelets (Johnston, 1968). Serotonin is either stored in vesicles or rapidly inactivated by MAO-A. Excess levels of serotonin may cause adverse effects collectively known as the “serotonin syndrome”. This results from abnormally high concentrations of serotonin which may be caused by serotonin reuptake inhibitors or MAO-A inhibitors that blocks the metabolism of serotonin (Youdim & Bahkle, 2006).

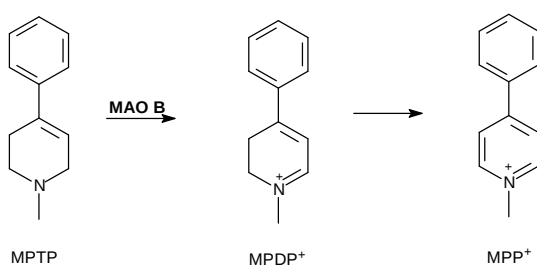
Serotonin syndrome may occur when combining MAO-A inhibitors with selective serotonin reuptake inhibitors or other serotonin-enhancing drugs (Rose *et al.*, 2000). MAO-A inhibitors, when used with catecholamines, may also induce acute hypertension (Youdim & Bahkle, 2006). Serotonin syndrome is characterized by the following symptoms: restlessness, hallucinations, loss of coordination, rapid heartbeat, sudden changes in blood pressure, increased body temperature, overactive reflexes, nausea, vomiting and diarrhoea (Youdim & Bahkle, 2006). These adverse side effects limit the use of MAO-A inhibitors in the treatment of depression.

2.3.8. The role of MAO-B in Parkinson's disease

The MAOs have been of interest since they catalyze the metabolism of a variety of catecholamine neurotransmitters. MAO-B is the predominant isoform in the human brain, and its main function is to metabolize dopamine and phenylethylamine. Phenylethylamine is a food-derived amine that stimulates dopamine release and inhibits its neuronal uptake (Youdim & Green, 1975). The oxidation of primary amine substrates such as dopamine, by MAO, results in the formation of aldehydes and H_2O_2 . This leads to the promotion of neurodegenerative diseases such as PD, since the MAO-B-catalyzed increased ROS production may contribute to an observed age-related increase in the incidence of mitochondrial damage in the brain, particularly in the SN (Soong *et al.*, 1992). MAO-B has been suggested to play a significant role in the neurotoxic processes associated with PD (Youdim & Green, 1975). Enhanced MAO-B activity is seen in the brains of people with PD, as a consequence of gliosis (Novaroli *et al.*, 2006). Increased levels of MAO-B have also been demonstrated in plaque-associated astrocytes of brains from AD patients (Saura *et al.*, 1994).

2.3.8.1. MAO-B and MPTP

MAO-B is also involved in the bioactivation of MPTP. MPTP is a proneurotoxin that undergoes MAO-B catalyzed oxidation to the unstable intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP^+) and finally to 1-methyl-4-phenylpyridine (MPP^+), the ultimate neurotoxic metabolite (Chiba *et al.*, 1984; Heikkila *et al.*, 1984), (shown in Scheme 3).



Scheme 3: The MAO-B catalyzed oxidation of MPTP to the corresponding 2,3-dihydropyridinium product MPDP^+ and the pyridinium species MPP^+ .

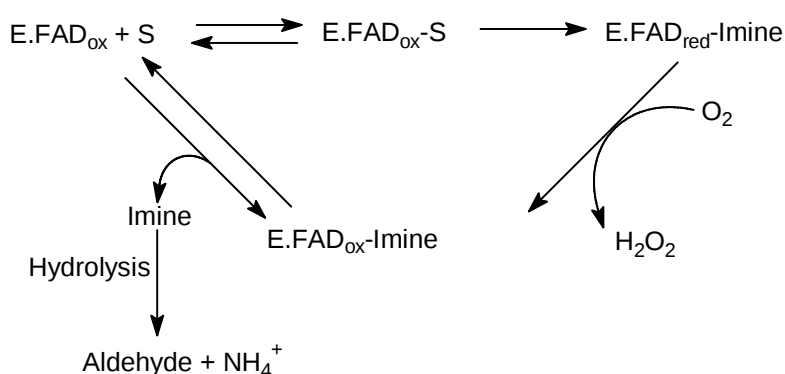
Studies have shown that inhibitors of MAO-B significantly reduce the neurotoxicity of MPTP in numerous animal models (Chiba *et al.*, 1984). Attempts to develop safer neuroprotective agents have focused on identifying compounds that protect against the degenerative processes associated with exposure to MPTP (Heikkila *et al.*, 1984).

2.3.8.2. MAO-B and the treatment of PD

The depletion of dopamine in the nigrostriatal pathway, as a result of the progressive deterioration of the dopaminergic neurons in the SN, is the main pathological feature of PD. A potential PD treatment strategy involves the administration of selective MAO-B inhibitors (Youdim & Bahkle, 2006). MAO-B inhibitors inhibit the deamination of dopamine and consequently conserve the depleted supply of dopamine in the brain and elevate dopamine levels derived from administered levodopa. This makes MAO-B inhibitors an option, either as monotherapy in early PD, or as adjunctive therapy in patients receiving levodopa treatment (Youdim *et al.*, 2006). In accordance with this view, inhibitors of MAO-B have been reported to enhance dopamine levels in the striatum of primates treated with levodopa, compared to central dopamine levels of animals treated with levodopa alone (Finberg *et al.*, 1998). MAO-B inhibitors also selectively and dramatically increase the level of phenylethylamine in the CNS (Youdim *et al.*, 2006).

2.3.8.3. Metabolism of dopamine

The primary product of MAO acting on an amine is an aldehyde. Dopamine is oxidized either by MAO-A or MAO-B, to the corresponding imine product which is rapidly hydrolyzed to dopaldehyde (Olanow, 1990). The MAO-B catalyzed oxidation of dopamine also yields as secondary product, hydrogen peroxide (H_2O_2). Inhibitors of MAO-B stoichiometrically decrease aldehyde and H_2O_2 production in the brain and since these species may be reactive (Youdim *et al.*, 2006). MAO-B inhibitors may thus offer neuroprotection.



Scheme 4: Scheme for the overall oxidative reaction catalyzed by the MAOs. Oxidation of the amine substrates leads to the reduction of FAD. The FAD is reoxidized by molecular oxygen to generate hydrogen peroxide. The imine product is hydrolyzed in a nonenzymatic process

2.3.8.4. MAO levels in the brain and aging

Many neuronal cells and their associated neurotransmitters and enzymes show age-related losses (Fowler *et al.*, 1980b). It has been established that MAO-B concentration and activity increases with age, while MAO-A activity remains unchanged (Fowler *et al.*, 1997). MAO-A activity is high at birth, rapidly decreases in the first year and then slowly stabilizes (Novaroli *et al.*, 2006). However, MAO-B activity stays unchanged throughout early childhood, and then increases with age until the 60th year of life (Delumeau *et al.*, 1994; Strolin-Benedetti & Dostert, 1989). Age-related increases in MAO-B are consistent with the localization of MAO-B within glial cells (Shih *et al.*, 1999). Reports that the numbers of glial cells increase with age in the normal human brain suggests this process may be responsible for the observed increase in MAO-B activity seen in PD (Barnham *et al.*, 2004; Youdim *et al.*, 2004).

2.3.8.5. The role of aldehyde dehydrogenase and glutathione peroxidase

The reaction mechanism of MAO involves oxidation of primary and secondary amines to yield the corresponding aldehyde with the generation of H_2O_2 . The aldehyde is rapidly metabolized by ADH to acidic metabolites (Youdim *et al.*, 2006). Neurotoxic aldehydes which are normally rapidly metabolized by ADH in the brain may accumulate in the parkinsonian SN (Riederer *et al.*, 1989). This finding was supported by an examination of SN samples from PD patients which indicated a deficiency in ADH that could allow a build-up of neurotoxic aldehydes derived from the catabolism of dopamine by MAO (Grunblatt *et al.*, 2004).

GSH plays a key role as an essential cellular antioxidant in the defense of neurons against oxidative damage induced by ROS (Skaper *et al.*, 1999). It is a tripeptide, composed of glutamate, cysteine and glycine. High intramolecular levels of GSH protect cells from ROS insults, by nonenzymatically reacting with ROS. GSH is also the substrate of GSHPx and is the general name of an enzyme family with peroxidase activity, whose main biological role is to protect an organism from oxidative damage (Skaper *et al.*, 1999). The biochemical function of GSHPx is to reduce free H_2O_2 to water. H_2O_2 , formed as a result of MAO catalysis is inactivated in the brain mainly by GSHPx using GSH as a substrate (Riederer *et al.*, 1989). The SN is protected from the MAO-B catalyzed production of H_2O_2 by high levels of GSH and GSHPx. 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.

2.3.9. Irreversible inhibitors of MAO-B

Irreversible inhibitors (inactivators or poison enzymes) are compounds that produce irreversible inhibition of the enzyme by forming stable covalent complexes. This blocks the access of the substrate to the active site (Rodwell & Kennely, 2000). The inhibition is not readily reversed and enzyme activity is not regained either by removing the remainder of the free inhibitor from the tissue or by increasing the substrate concentration.

2.3.9.1. (R)-Deprenyl

An example of an irreversible inhibitor is (R)-deprenyl, which is structurally related to phenylethylamine (shown in figure 18). (R)-Deprenyl is a highly potent and selective irreversible inhibitor of MAO-B and has been shown to inhibit the oxidative deamination of dopamine *in vivo* (Youdim & Green, 1975). It represents one of the drugs currently used for the treatment of PD. This compound also protects neurons from apoptosis in a variety of models (Youdim & Bakle, 2006). The mechanism of action of neuroprotection as well as inhibition of apoptosis still remains unknown.

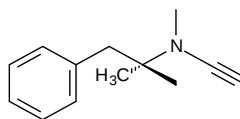


Figure 18: Structure of (R)-deprenyl.

(R)-Deprenyl is known to exert both symptomatic (Lees *et al.*, 1977; Djaldetti *et al.*, 2002) and neuroprotective effects in PD (Fernandez & Chen, 2007). It delays the need to initiate dopaminergic treatment in PD patients and has also been shown to postpone the emergence of symptoms that require the initiation of levodopa therapy in PD patients (LeWitt, 2004).

2.3.9.2. Pargyline

Pargyline is a non-selective MAO irreversible inhibitor which shows more inhibitory activity towards the B form of the enzyme (Youdim & Bakhle, 2006). The propargyl group of pargyline is thought to protect against neurodegeneration by preventing apoptosis. Metabolites of pargyline have also been found to possess MAO-B inhibitory activity. In rodents, N-propargylbenzylamine (NPB), a major metabolite of pargyline, is a potent inhibitor of MAO-B (Karoum, 1987). This metabolite, like (R)-deprenyl and pargyline, significantly increases urine and brain PEA, while only pargyline reduces serotonin metabolism. This finding suggests that the metabolism of pargyline to NPB contributes towards the MAO-B inhibitory effects of pargyline *in vivo* (Karoum, 1987).

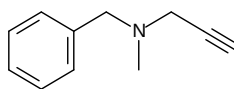


Figure 19: Structure of pargyline.

2.3.9.3. Rasagiline

Rasagiline is a selective irreversible MAO-B inhibitor used in early and late treatment of PD (Youdim *et al.*, 2003). Rasagiline exerts both symptomatic and neuroprotective effects. Similar to pargyline, the neuroprotective effects of rasagiline may be attributed to its propargyl moiety which is thought to prevent apoptosis (Fernandez & Chen, 2007).

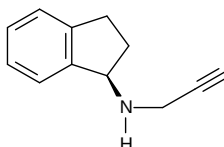


Figure 20: Structure of rasagiline.

In animal models of MPTP-induced parkinsonism, pretreatment with rasagiline inhibits the degeneration of dopaminergic nigral cells (Chen & Swope, 2005; Kupsch *et al.*, 2001). Rasagiline-mediated neuroprotection in the MPTP model may be directly attributed to inhibition of MAO-B, since MAO-B catalyzes the oxidation of MPTP to the ultimate neurotoxin MPP⁺. However, the finding that the S-enantiomer of rasagiline which is not an MAO-B inhibitor also exhibits this neuroprotective effect (Maruyama *et al.*, 2000; Youdim *et al.*, 1999), suggests that the mechanism by which rasagiline exerts its neuroprotective effects is not only dependent on MAO-B inhibition.

Interestingly, other propargylamine derivatives with protective pharmacological properties that are unrelated to MAO-B inhibition have also been shown to be neuroprotective in models of neurodegeneration. These compounds may function by inhibiting apoptosis (Mandel *et al.*, 2003, Carlile *et al.*, 2000). Rasagiline is known to enhance the release of dopamine in addition to retarding its catabolism and also increases the level of dopamine that is derived from levodopa in primates (Finberg *et al.*, 1998; Youdim *et al.*, 2003). This shows that rasagiline has the potential to alleviate the symptoms of PD.

2.3.9.4. *Ladostigil*

Ladostigil is a bifunctional compound which possesses both MAO-B and cholinesterase inhibition properties. Furthermore, ladostigil exhibits neuroprotective and antioxidant activities (Weinreb *et al.*, 2008). The combination of these activities makes ladostigil a potential drug for the treatment of AD (Sagi *et al.*, 2005).

Ladostigil is structurally related to rasagiline and is a nonselective inhibitor of MAO-A and -B. Ladostigil increased the central levels of noradrenaline, 5-HT and dopamine and also exhibits antidepressant effect in animal models (Sagi *et al.*, 2005). This provides a useful and beneficial side effect in PD patients, since PD patients also often exhibit signs of depression (Youdim & Bakhle, 2006).

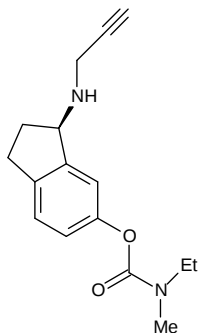


Figure 21: Structure of *ladostigil*.

Interestingly, chronic administration of ladostigil displayed tissue-selective action, as it inhibits irreversibly all MAO activity in the brain, while MAO-A is not inhibited in the gut and liver (Mandel *et al.*, 2005; Sagi *et al.*, 2005). In addition, ladostigil possesses potent anti-apoptotic activities, similar to those shown by rasagiline (Mandel *et al.*, 2005).

2.3.10. *Reversible inhibitors of MAO-B*

The development of selective and reversible MAO inhibitors is important for the treatment the symptoms of PD and also for the possibility of delaying neurodegeneration in PD (Youdim & Bakhle, 2006; Youdim *et al.*, 2006). Reversible inhibitors interact with enzymes mainly via hydrophobic interactions and hydrogen bonding (Binda *et al.*, 2003). This makes them therapeutically more desirable than inactivators, since enzyme activity can be regained relatively quickly following withdrawal of the reversible inhibitor. On the other hand, with irreversible inhibitors, following inactivation of the enzyme, activity can only be regained via *de*

novo synthesis of the enzyme protein (Thebault *et al.*, 2004). Several research groups are currently interested in discovering and characterizing new reversible inhibitors of MAO-B.

2.3.10.1. Lazabemide

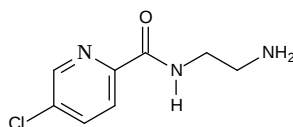


Figure 22: Structure of lazabemide.

Lazabemide is a short-acting, reversible MAO-B inhibitor, developed to treat PD and AD (Youdim & Finberg, 1991). Lazabemide also possesses antioxidant activity and is a potent inhibitor of membrane oxy-radical damage as a result of inhibiting membrane lipid peroxidation (Mason *et al.*, 2000). This finding makes it an exceptional drug to treat oxidative stress, one of the underlying causes of PD.

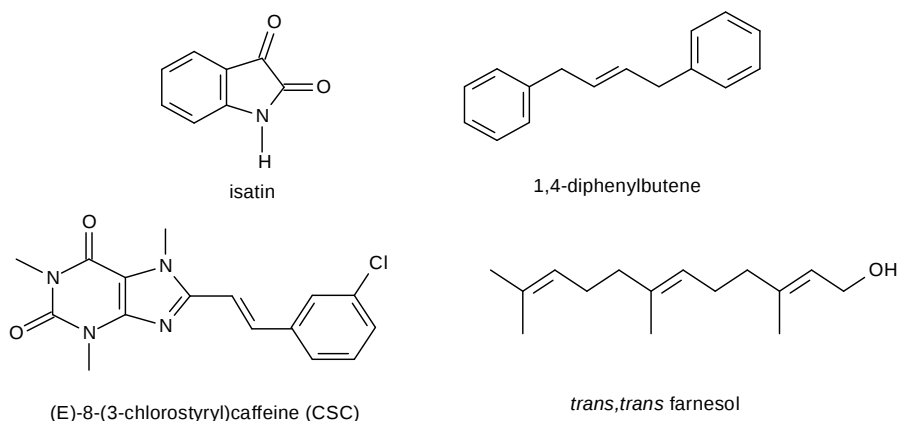


Figure 23: The structures of isatin, 1,4-diphenyl-2-butene, (E)-8-(3-chlorostyryl)caffeine and *trans,trans*-farnesol.

2.3.10.2. Isatin

Isatin, a small molecule, is a reversible endogenous MAO inhibitor (Glover *et al.*, 1988; Hamaue *et al.*, 1992). It is also found in the brain and inhibits human MAO-B with a K_i value of 3 μM (Hubàlek, *et al.*, 2005). Furthermore, isatin has a wide spectrum of biological properties: (a) a marker of stress and anxiety, (b) an anti-seizure agent and (c) an inhibitor of benzodiazepin receptors (Medvedev *et al.*, 1995). Isatin may play a role in the regulation of the brain levels of acetylcholine and dopamine as it significantly increases the levels of both transmitters in the rat striatum (Ogata *et al.*, 2003). In animal models of PD (Ogata *et al.*, 2003), isatin was found to improve akinesia and elevate dopamine levels. X-ray crystal structures of human recombinant MAO-B in complex with isatin have shown that isatin binds within the substrate cavity and is

stabilized via hydrogen bonding. The 2-oxo group and the pyrrole NH are hydrogen bonded to ordered water molecules present in the active site, whereas the 3-oxo group is not involved in any hydrogen bonding (Binda *et al.*, 2003).

2.3.10.3. 1,4-Diphenyl-2-butene

1,4-Diphenyl-2-butene is a contaminant of polystyrene microbridges used in protein crystallization and has been shown to be a moderately potent competitive inhibitor of human MAO-B with a K_i value of 0.7 μM (Hubàlek, *et al.*, 2005). Based on X-ray crystal structures, 1,4-diphenyl-2-butene was shown to bind to both the substrate and entrance cavities of MAO-B (Binda *et al.*, 2006). For this binding mode, the side chain of Ile-199 is rotated out of the active site cavity to accommodate the large inhibitor.

2.3.10.4. (E)-8-(3-Chlorostyryl)caffeine

(E)-8-(3-Chlorostyryl)caffeine (CSC) is a potent and selective adenosine A_{2A} receptor antagonist (Müller *et al.*, 1997; Jacobson *et al.*, 1993) which has a high inhibition potency towards MAO-B. CSC was found to be an exceptionally potent MAO-B inhibitor with a K_i value of 0.128 μM for the inhibition of baboon liver MAO-B (Vlok *et al.*, 2006). In the MPTP mouse model, CSC was also shown to be neuroprotective. It is believed that this protection may be dependent in part on the ability of this compound to inhibit the MAO-B catalyzed bioactivation of MPTP as well as its ability to antagonize A_{2A} receptors (Chen *et al.*, 2002). Very little is known about the exact mode of binding of CSC to the active site of MAO-B, but due to its relatively large planar structure, it is suggested that this inhibitor also traverses both the entrance and substrate cavities of the enzyme. This dual mode of binding may explain the potent action of CSC as a MAO-B inhibitor (Vlok *et al.*, 2006).

2.3.10.5. Trans,trans-farnesol

Recent scientific investigations indicate that tobacco contains several secondary metabolites that may provide potential health benefits. *Trans,trans*-farnesol, a component of tobacco (Hubàlek, *et al.*, 2005), has been found to be a moderately potent competitive inhibitor of human MAO-B, with a K_i value of 2.3 μM . X-ray crystal structures of human recombinant MAO-B in complex with *trans,trans*-farnesol, have indicated that *trans,trans*-farnesol exhibits a dual binding mode and traverses both the entrance and substrate cavities of the enzyme (Hubàlek, *et al.*, 2005). The polar OH moiety is reported to be in close contact with the flavin, located in the substrate cavity, where it is stabilized via hydrogen bonding (Hubàlek *et al.*, 2005) while the

aliphatic chain extends to the entrance cavity. The “gate” separating the two cavities, the side chain of Ile-199, is shown to exhibit a different rotamer conformation that allows for the fusion of the two cavities in order to accommodate *trans,trans*-farnesol (Binda *et al.*, 2003). The potency of MAO-B inhibition by *trans,trans*-farnesol may possibly be explained by its dual mode of interaction.

2.3.10.6. Safinamide

Safinamide is the only reversible MAO-B inhibitor that is currently in Phase III clinical trials for the treatment of PD. Safinamide possesses a good safety margin and excellent bioavailability (Fariello, 2007). Besides being an inhibitor of MAO-B, safinamide also inhibits the uptake of dopamine and noradrenaline (Caccia *et al.*, 2006). Clinical studies in early PD patients have shown that safinamide improves the symptoms of PD (Stocchi *et al.*, 2004). Also, safinamide is associated with improvements in measures of cognitive function, including working memory (Stocchi *et al.*, 2004).

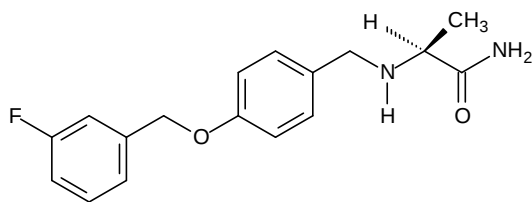


Figure 24: Structure of safinamide.

The crystal structures of human MAO-B, in complex with safinamide shows that it traverses both entrance and substrate cavities, making it a highly selective MAO-B inhibitor (Binda *et al.*, 2003) since the active site of human MAO-A is composed of only a single cavity (De Colibus *et al.*, 2005). The 3-fluorobenzoyloxy moiety is located in the entrance cavity space, whereas the amide is located in the substrate cavity and orientated towards the flavin cofactor (figure 25). The amide group of safinamide is engaged in two hydrogen bonds, one with Gln-206 and another with an ordered water molecule (Binda *et al.*, 2007). The crystal structures of human MAO-B, in complex with safinamide, reveal that it binds noncovalently to the enzyme. This represents a desirable property to minimize toxic side effects since *de novo* protein synthesis is not required for the recovery of enzymatic activity (Binda *et al.*, 2007).

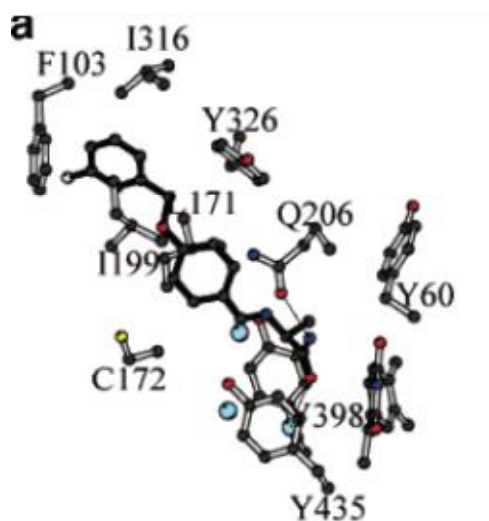


Figure 25: Illustration of safinamide bound to the MAO-B active site (Binda *et al.*, 2007).

2.3.11. Inhibitors of MAO-A

MAO inhibitors are used in the treatment of affective diseases such as depression (Youdim & Bahkle, 2006). Selective MAO-A inhibition in the CNS leads to increased brain levels of dopamine, noradrenaline and serotonin and is responsible for the antidepressant properties of these inhibitors. Earlier MAO-A inhibitors, such as iproniazid, were associated with acute liver toxicity, due to the hydrazine-containing structure of these inhibitors. This was overcome by the development of non-hydrazine inhibitors, such as tranylcypromine and pargyline. Although these reversible inhibitors can effectively block MAO-A in the CNS to obtain an antidepressant effect, inactivation of MAO-A in the gut wall may lead to the “cheese reaction” (Youdim & Weinstock, 2004). The “cheese reaction” greatly reduces the clinical use of MAO-A inhibitors. Since the gut predominately contains MAO-A, such problems do not occur with selective irreversible MAO-B inhibitors (Knoll, 2000; Youdim & Weinstock, 2004). An inhibitor such as moclobemide has been proven to be a new and safe reversible MAO-A inhibitor that is well tolerated in the treatment of depression and does not lead to the “cheese reaction” (Da Prada *et al.*, 1988; Da Prada *et al.*, 1990). Research into the discovery of new MAO-A inhibitors is ongoing. Some of the irreversible inhibitors discussed below include clorgyline, phenelzine and tranylcypromine which are still in clinical use.

2.3.11.1. Clorgyline

The most important use of MAO-A inhibitors is in the treatment of depression. Structurally related to pargyline, clorgyline is one of the earliest selective MAO-A irreversible inhibitors.

Clorgyline blocks the oxidation of noradrenaline and serotonin but not PEA (Youdim & Bahkle, 2006). The major disadvantage of clorgyline is the occurrence of the “cheese reaction”.

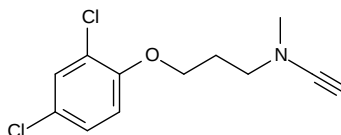


Figure 26: Structure of clorgyline.

2.3.11.2. Tranylcypromine and phenelzine

Both phenelzine and tranylcypromine are non-selective inhibitors of MAO-A and MAO-B (Youdim *et al.*, 2006). Phenelzine is an antidepressant/antipanic drug which also causes an elevation of brain levels of the amino acid neurotransmitter, gamma-aminobutyric acid (GABA) (Todd & Baker, 1995). Phenelzine in high doses was also found to be superior to imipramine in the treatment of major depression (Vallejo *et al.*, 1987).

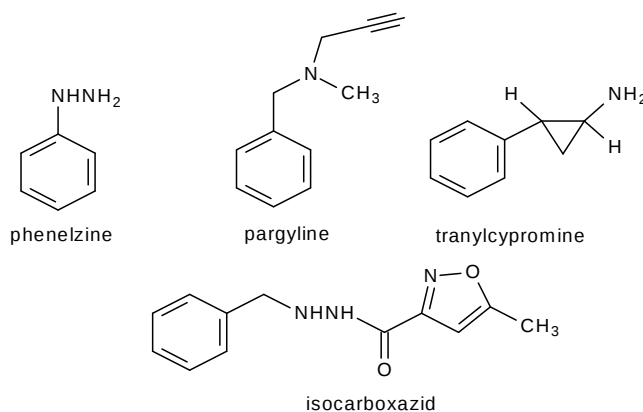


Figure 27: Structures of selected MAO-A inhibitors.

2.3.11.3. Moclobemide and brofaromine

Moclobemide is the first of a new generation of reversible inhibitors of MAO-A which does not potentiate the hypertensive actions of dietary tyramine (Youdim & Bahkle 2006; Haefely *et al.*, 1992). Moclobemide has been proven to be efficacious in the treatment of major depression (Heinze *et al.*, 1993), especially in elderly patients (Bonnet, 2003). Interestingly, in studies with non-depressed elderly people and in healthy young volunteers, moclobemide exhibits no significant effects on cognitive functions (Bonnet, 2003).

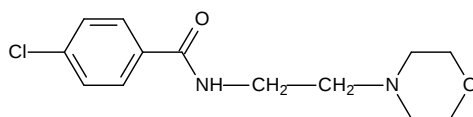


Figure 28: Structure of moclobemide.

The absence of the “cheese effect” with moclobemide is due to the fact that it is a competitive reversible inhibitor. This allows ingested tryamine or other dietary amines to be normally metabolized in the gut and liver. Moclobemide can be displaced from its binding site on MAO-A by tryamine (Colzi *et al.*, 1993) allowing tryamine to be metabolized normally. Interestingly, moclobemide may also increase dopamine release in the brain (Haefely *et al.*, 1992). This may explain the symptomatic effects of this drug in PD patients (Youdim & Weinstock, 2004).

Brofaromine is a reversible, selective inhibitor of MAO-A, and is used in the treatment of depression and anxiety (Lotufo-Neto *et al.*, 1999). Brofaromine also has serotonin reuptake inhibitory properties and this dual pharmacologic effects offer promise in the treatment of a wide spectrum of depressed patients. Brofaromine produces less severe anticholinergic side effects compared with standard drugs and is more effective than the tricyclic antidepressants and also better tolerated (Lotufo-Neto *et al.*, 1999).

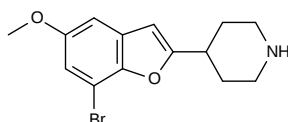


Figure 29: Structure of brofaromine.

2.3.11.4. Iproniazid

The discovery of iproniazid as an MAO inhibitor was by chance, when it was found out that an anti-tuberculosis drug isoniazid, also acted as an inhibitor of MAO (DiMartini, 1995). Isoniazid was used as an anti-depressant, but had to be discontinued due to side effects. A related compound, iproniazid became the first MAO inhibitor to be used successfully in the treatment of depressive illness. Like isoniazid, iproniazid was later shown to cause liver toxicity due to its hydrazine structure and hence removed from the market.

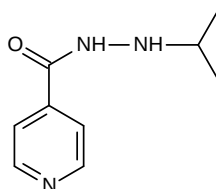


Figure 30: Structure of iproniazid.

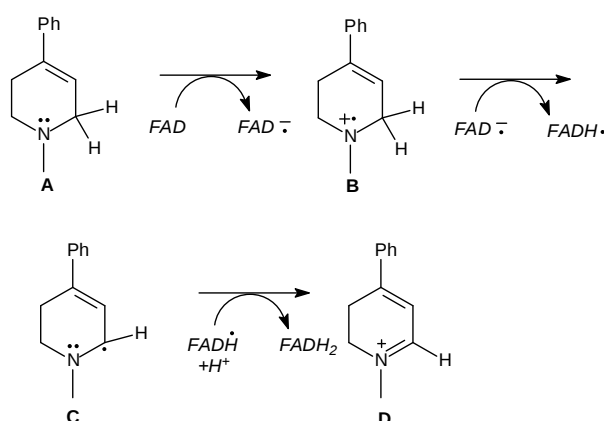
2.3.12. Mechanism of action of MAO-B

The detailed mechanism by which MAO-A and -B catalyze amine oxidation is still not well-defined and several mechanisms have been proposed. The mechanism of amine oxidation

catalyzed by the quinoprotein amine oxidases, is reasonably well understood and occurs through the formation of enzyme–substrate covalent adducts. Two catalytic mechanisms have been proposed for MAO catalysis: (a) the single electron transfer (SET) pathway and (b) the nucleophilic (polar) pathway (Miller & Edmonson, 1999; Walker & Edmonson, 1994).

2.3.12.1. The SET mechanism

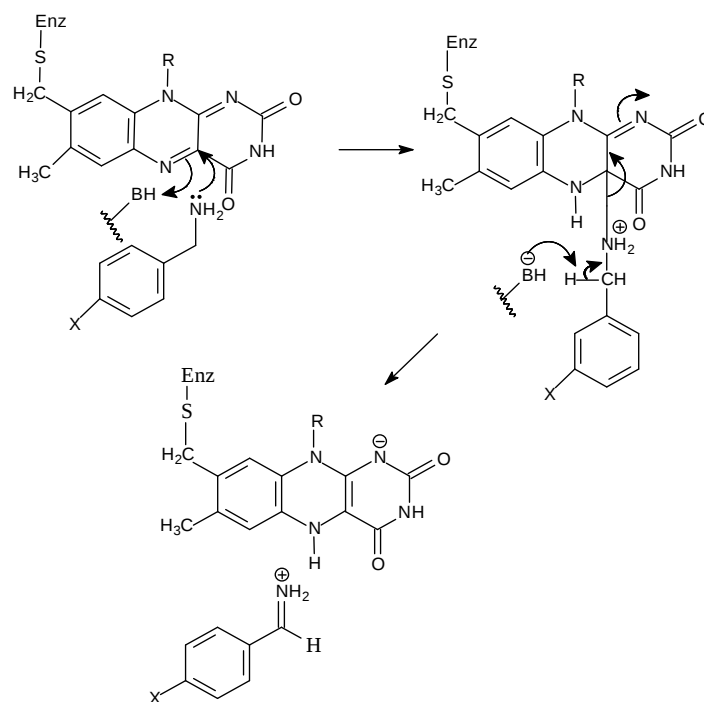
According to Silverman *et al.* (1980), the mechanism for the MAO catalyzed α -carbon oxidation of amines proceeds via a single electron transfer step (scheme 5), from the nitrogen lone pair of the substrate (**A**) to the oxidized flavin FAD to generate an aminyl radical cation (**B**) and the flavin semiquinone. α -Carbon deprotonation of **B** yields the α -amino radical (**C**). This α -amino radical transfers the second electron to the FAD semiquinone to give the reduced flavin and the iminium ion (**D**) (Silverman *et al.*, 1980).



Scheme 5: The proposed SET oxidation pathway for MAO catalysis as illustrated with MPTP as substrate (Silverman *et al.*, 1980).

2.3.12.2. The Polar nucleophilic pathway

MAO catalysis may occur via a polar nucleophilic mechanism which involves attack of the amine substrate at the FAD C-4a position to form a substrate–flavin adduct (scheme 6). This is followed by proton abstraction from the α -carbon of the substrate and may occur by an active site base in the enzyme active site. Formation of the imine product results from its elimination from the reduced flavin. The reactivity at the flavin C-4a atom is considered additional evidence for this catalytic mechanism. *In lieu* of active site base, the highly basic N5 atom of the flavin, which is generated following nucleophilic attack of the substrate, may also act as base for the deprotonation of the substrate α -carbon (Miller & Edmondson, 1999).



Scheme 6: The proposed polar nucleophilic mechanism for the MAO catalysed oxidation of benzylamine (Miller & Edmondson, 1999).

2.3.13. Three dimensional structure of MAO-B

In order to develop more effective and selective inhibitors, it is important to understand the inhibition and catalytic mechanism based on 3D protein structures. Binda *et al.* (2002) first determined the x-ray structure of human MAO-B at 3.0 Å and later improved the resolution to 1.7 Å. These structures include co-crystals with various inhibitors. The crystal structure shows that the enzyme is dimeric but not covalently linked and that it contains 520 amino acids (Binda *et al.*, 2002). The enzyme appears to be linked to the outer mitochondrial membrane via the C-terminal amino acids, 461–515, which forms a transmembrane helix.

The active site of the enzyme consists of two cavities, lined with mostly hydrophobic residues that connect the opening of the active site of the flavin containing binding site. The substrate binding cavity which is larger than the entrance cavity, has a volume of 420 Å³ (Binda *et al.*, 2002), with the FAD cofactor forming the back wall of the cavity. It is lined by a number of aromatic and aliphatic amino acids (Walker & Edmondson, 1994). In front of the FAD are two tyrosine residues, Tyr-398 and Tyr-435, which together with the FAD and Phe-343 at the top of the cavity, create an aromatic cage where catalysis takes place. The only hydrophobic patch in the substrate cavity is

the area defined by Tyr-60, Phe-343 and Tyr-398 (Binda *et al.*, 2002; Binda *et al.*, 2004). Amino acid residues which could function as active site acids or bases are not present in this cavity.

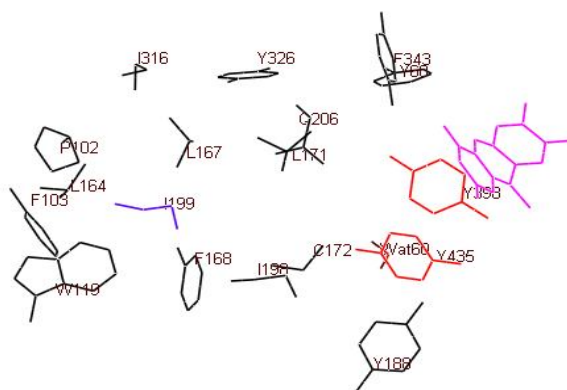


Figure 31: A model of the active site of human recombinant MAO-B. The residues Try-398 and Try-435 forming the aromatic cage are in red, Ile-199, the “gate” of the cavity is in blue and the FAD cofactor is in purple (Binda *et al.*, 2001).

The second cavity, the entrance cavity is a smaller hydrophobic cavity (volume of 290 Å³) and is lined with hydrophobic amino acids, Phe-103, Trp-119, Leu-164, Leu-167, Phe-168 and Ile-316. This creates a relatively lipophilic environment (Novaroli *et al.*, 2006). The side chains of residues, Tyr-326, Ile-199, Leu-171 and Phe-168 separate the two cavities (Binda *et al.*, 2001).

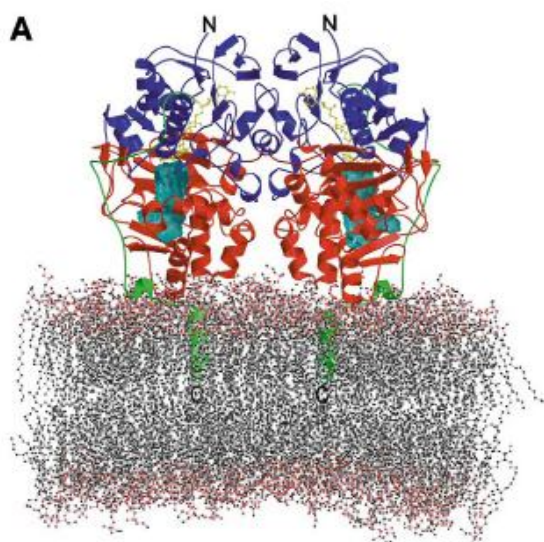


Figure 32: The crystal structure of human recombinant MAO-B (Binda *et al.*, 2001).

In order to gain access to the substrate cavity where catalysis takes place, substrates and inhibitors must traverse the entrance cavity. Small molecule inhibitors such as isatin (figure 33), bind easily within the substrate cavity of the enzyme (Binda *et al.*, 2003, Binda *et al.*, 2004). However, large inhibitors such as 1,4-diphenyl-2-butene (figure 34), bind simultaneously to both the substrate cavity and the entrance cavity. For this binding mode to be possible, the side chain of Ile-199 is rotated out of the active site to allow for fusion of the two cavities (Binda *et al.*, 2003). This allows the inhibitor to span both the entrance and substrate cavities (Hubàlek *et al.*, 2005). The negatively charged membrane surface is expected to electrostatically guide the positively charged amine substrate to the active site of MAO-B. Studies have shown that the amine substrate must be deprotonated for binding and catalysis (Miller & Edmondson, 1999). The mechanism of deprotonation is still unknown.

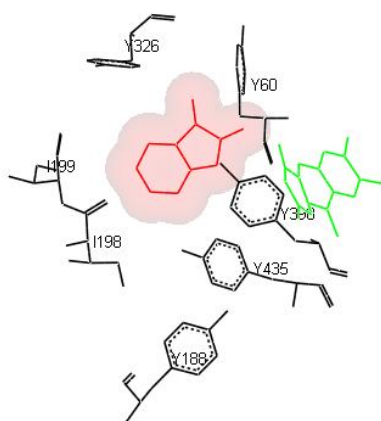


Figure 33: The active site of human recombinant MAO-B with isatin bound to the substrate cavity. Here, the side chain of Ile-199 is rotated into the “closed” position and the substrate and entrance cavities are separated (Hubàlek *et al.*, 2005).

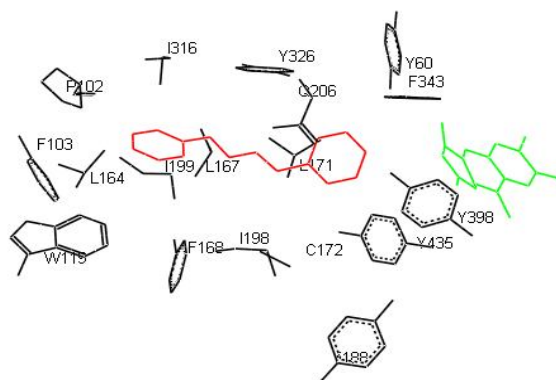


Figure 34: The active site of human recombinant MAO-B with 1,4-diphenyl-2-butene bound. The side chain of Ile-199 is rotated into the “open” position allowing for the substrate and entrance cavities to fuse (Binda *et al.*, 2006).

2.3.14. Three dimensional structure of MAO-A

The x-ray crystal structure of human MAO-A has only recently been studied (De Colibus *et al.*, 2005). The active site of human MAO-A consists of a single hydrophobic cavity of $550 \text{ \AA}^3$, which is smaller than that of human MAO-B ($700 \text{ \AA}^3$) (Ma *et al.*, 2004). Human MAO-A is unique in that it crystallizes as a monomer, while MAO-B crystallizes as a dimer. The change from the dimeric to the monomeric form is due to a Glu-151-Lys mutation, that is specific of human MAO-A (Andre's *et al.*, 2004). Like MAO-B, MAO-A also contains a C-terminal transmembrane helix which anchors the enzyme in the mitochondrial membrane (figure 35) (Ma *et al.*, 2004).

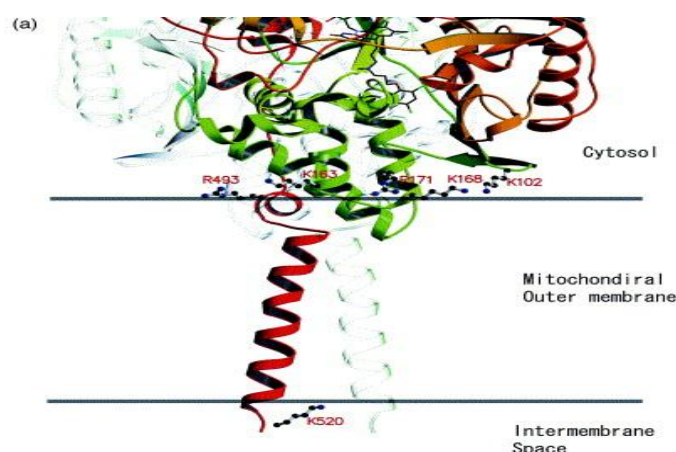


Figure 35: The membrane-binding regions of MAO-A. The C-terminal helix is thought to anchor in the mitochondrial membrane and positively charged residues are thought to interact with the membrane surface (Ma *et al.*, 2004).

Comparison of the MAO-A and MAO-B structures demonstrates that clorgyline is not able to fit into the inhibitor-binding pocket of MAO-B because binding is prevented by MAO-B Tyr-326 (De Colibus *et al.*, 2005). While the other smaller inhibitors seem to fit the binding pockets of both MAO-A and MAO-B, larger inhibitors such as 1,4-diphenyl-2-butene binds to MAO-B and is excluded from the pocket of MAO-A by Phe-208 (Ma *et al.*, 2004; De Colibus *et al.*, 2005). The X-ray crystal structure of MAO-A with clorgyline reveals that the inhibitor forms a covalent bond with FAD to inhibit MAO-A in an irreversible manner (figure 36) (De Colibus *et al.*, 2005). The covalent bond between clorgyline and FAD occurs at the N5 of the flavin. Two cysteine residues, Cys-321 and Cys-323, are located near the entrance of the catalytic site. The two chlorine atoms of clorgyline form hydrogen bonds with Cys-323 and Thr-326, respectively. Various types of hydrophobic interactions between clorgyline and the atoms of the protein stabilize the inhibitor binding. The residues involved in hydrophobic interactions include Ile-180, Phe-208, Glu-215, Cys-323, Ile-335, Tyr-407 and Tyr-444 (Ma *et al.*, 2004; De Colibus *et al.*, 2005).

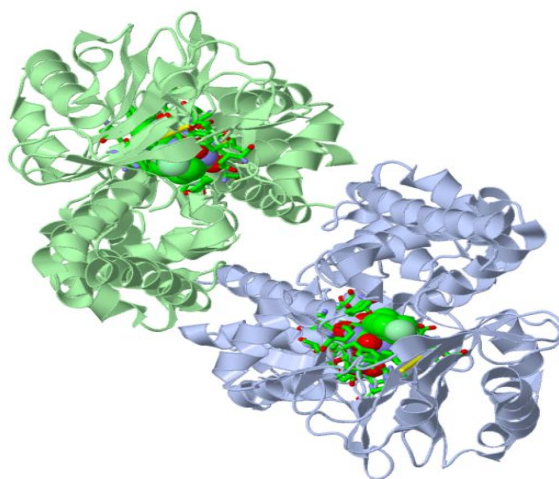


Figure 36: Three dimensional structure of recombinant human MAO-A in complex with clorgyline (De Colibus *et al.*, 2005).

Another x-ray structure shows human MAO-A in complex with the reversible MAO-A inhibitor harmine (figure 37) (Son *et al.*, 2008). The inhibitor in the active site of the enzyme interacts with several amino acid residues, namely Tyr-69, Asn-181, Phe-208, Val-210, Gln-215, Cys-323, Ile-325, Ile-335, Leu-337, Phe-352, Tyr-407, Tyr-444 and the FAD. Seven ordered water molecules are located in the active site. The inhibitor and the FAD are bridged through two water molecules by hydrogen bonds. The amide group of the Gln-215 side chain interacts tightly with harmine by π - π interaction, with an interplane distance of 3.4 Å. (Son *et al.*, 2008).

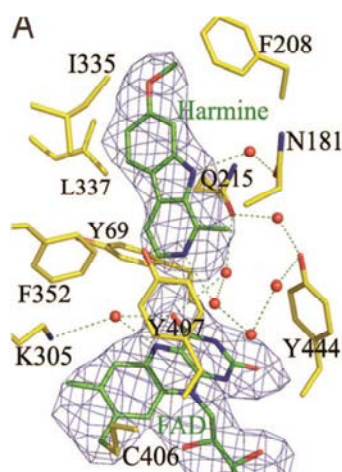


Figure 37: Illustration of the binding site of MAO-A complexed with the inhibitor, harmine. Amino acid residues are shown in yellow, and the FAD and harmine are shown in green. Dotted lines indicate hydrogen bonds (Son *et al.*, 2008).

Like all outer mitochondrial enzymes, it is believed that the C-terminal anchoring for this enzyme is important for its biological functions (De Colibus *et al.*, 2005). The results of experiments suggest that the flexibility of loop 108–118 is facilitated by the anchoring of the enzyme into the membrane and is essential for controlling substrate access to the active site (Son *et al.*, 2008).

2.3.15. *In vitro* determination of monoamine oxidase activity

MAO-A and -B generally catalyze the oxidative deamination of biogenic primary amines in the CNS and peripheral tissues. The reaction occurs as follows:



Scheme 7: Oxidation of amines by MAO

Secondary and tertiary monoamines do not form ammonia as product, but another amine, while some derivatives, such as MPTP, do not form either ammonia or an aldehyde (Singer *et al.*, 1988). The measurement of MAO activity is often based on directly measuring either the production of the product, aldehyde (or of the corresponding acid and alcohol), ammonia and H_2O_2 or the consumption of the substrate amine (RCH_2NH_2) or molecular oxygen (O_2) (Nicotra & Parvez, 1999). Less frequently, the enzyme activity is measured indirectly, which involves converting the enzyme catalyzed product into a more readily measured species. When the enzymatic activity is measured by the disappearance of the substrate, low substrate

concentration should be used to ensure that there is sufficient reduction of the concentration for reliable measurement (Nicotra & Parvez, 1999).

Measurements may be continuous or discontinuous and radiometric, fluorometric, spectrophotometric, chromatographic, polarographic and luminometric techniques may be used. A major problem in MAO activity determination, is that the majority of tissues co-express both MAO-A and MAO-B. However, exceptions are found in human placenta, which contains only MAO-A and platelets, and lymphocytes which contain only MAO-B (Youdim, 1988). The activities of MAO-A and -B in a particular tissue, which contains both forms of the enzyme, may be determined by inhibiting the MAO enzymes selectively with selective inhibitors such as clorgyline or (R)-deprenyl (Fowler *et al.*, 1978, Fowler *et al.*, 1980a).

2.3.15.1. Direct measurements

Direct measurements of MAO activity are often done *in vitro*. The direct products of amine oxidation have been determined via spectrophotometry, HPLC, fluorescence and polarographic analysis. Using the technique of polarography, which makes use of an oxygen sensitive electrode, oxygen consumption by the MAO-B catalytic cycle can be measured. Limitations of this technique are that it is unsuitable for the processing of a large number of samples (Averill-Bates *et al.*, 1993). Secondly, it requires a well controlled assay environment and is often insensitive and complicated due to the presence of other oxidizing enzymes. In another less frequently used method the rate of ammonia production can also be measured (Cotzias & Dole, 1951).

(1) Hydrogen peroxide

The detection of MAO-B generated hydrogen peroxide by measuring its absorbance spectrophotometrically at 230 nm has been reported. A disadvantage of this method is that many crude tissues may also contain other amine oxidases, such as as semi-carbazide-sensitive amine oxidases (SSAOs), which are capable of generating H₂O₂. In addition, the sensitivity at a wavelength of 230 nm is often lost due to interferences from the absorbance of most biological and synthetic compounds (Stevanato *et al.*, 1995).

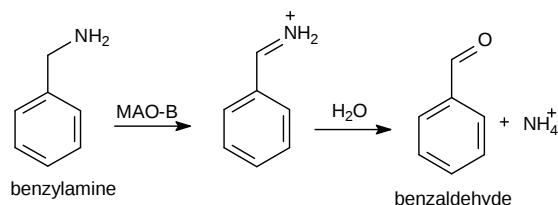
(2) Radiometric detection

Radiometric detection of the MAO-B catalyzed oxidation products from ¹⁴C-dopamine, ¹⁴C-tyramine and ³H-tyramine have also been used. This method involves the extraction of the

radiolabelled products of the enzymatic reaction into an organic solvent and measurement of radioactivity by a scintillation counter coupled to the HPLC system (Sim & Hsu 1990).

(3) Formation of aldehyde

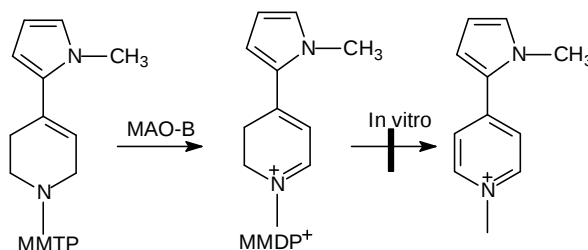
The aldehyde normally formed in the oxidative deamination of an amine by MAO may be directly measured. An example of this is the use of benzylamine as substrate. Benzylamine is converted to benzaldehyde via MAO-B. When using purified MAO-B as enzyme source, which is relatively free from background interference, the concentration of benzaldehyde ($\lambda_{\text{max}} = 250$ nm), may be measured spectrophotometrically (scheme 8). In contrast, background absorption in the near-UV wavelength range, when using crude enzyme preparations is too high to measure benzaldehyde concentrations by spectrophotometry. In this case benzaldehyde is often measured by HPLC-UV spectrophotometry (Vlok *et al.*, 2006).



Scheme 8: The MAO-B catalyzed oxidation of benzylamine to benzaldehyde.

(4) MMTP as substrate

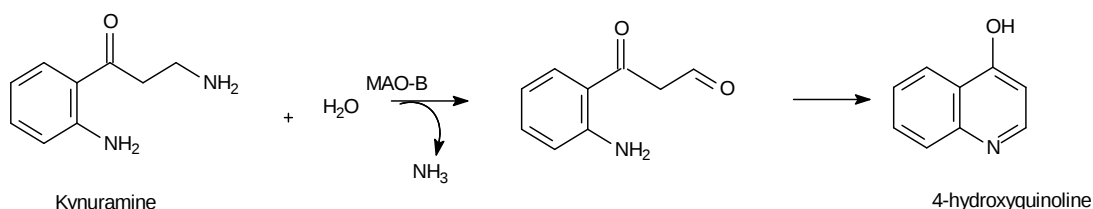
Another useful substrate for measuring MAO-A and MAO-B catalytic rates is 1-methyl-4-(1-methylpyrrol-2-yl)-1,2,3,6-tetrahydropyridine (MMTP). MMTP is oxidized by both MAO-A and -B to the corresponding dihydropyridinium metabolite, the 1-methyl-4-(1-methylpyrrol-2-yl)-2,3-dihydropyridinium (MMDP⁺) (Scheme 9) (Nimkar *et al.*, 1996). MMDP⁺ concentration is measured spectrophotometrically at 420 nm, a wavelength at which the substrate does not absorb light. Because of the favourable chromophoric characteristics and *in vitro* chemical stability of MMDP⁺, this assay is frequently used to measure activities of both MAO-A and -B.



Scheme 9: The MAO catalyzed oxidation of MMTP to the dihydropyridinium species, MMDP⁺.

(5) Kynuramine as substrate

A useful method of determining MAO activity is the use of kynuramine which is a non specific MAO substrate. Kynuramine absorbs ultraviolet light with a maximum absorbance at 366 nm (Weissbach *et al.*, 1960). The enzymatic deamination produces an aldehyde which, through an intramolecular condensation, leads to the formation of a quinoline derivative that shows no absorption at 366 nm (scheme 10), but instead the quinoline absorbs light maximally at 310 nm. Using spectrophotometry, the disappearance of kynuramine at 366 nm or appearance of quinoline may be measured. Since 4-hydroxyquinoline is also a fluorescent compound, while kynuramine does not exhibit fluorescence, the formation of 4-hydroxyquinoline may also be measured via fluorescence spectrophotometry at an excitation wavelength of 310 nm and an emission wavelength of 400 nm.



Scheme 10: The oxidation of kynuramine by MAO-B and subsequent cyclization to yield 4-hydroxyquinoline.

2.3.15.2. Indirect measurements

The H₂O₂ generated by MAO catalytic activity can be indirectly measured by using a horseradish peroxidase (HRP) coupled reaction system. In this reaction, H₂O₂ reacts in the presence of HRP, with the highly sensitive and stable probe, N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) (Zhou & Panchuk-Voloshina, 1997) which is a non-fluorescent compound. The spectral properties of the oxidation product of Amplex Red (resorufin), makes it suitable to determine its concentration via fluorescent spectrophotometry at an excitation wavelength of 560 nm and emission wavelength of 590 nm.

2.4. ENZYME KINETICS

In order to design new inhibitors of an enzyme, an understanding of enzyme catalysis is necessary. Enzymes often need coenzymes, which are smaller organic molecules or metallic cations, possessing special chemical reactivities or structural properties (Rodwell & Kennely, 2000).

2.4.1. Michaelis-Menten kinetics

Enzymes have catalytic sites to which a substrate (S) binds to form an enzyme-substrate complex (ES). The bound substrate is subsequently transformed into the product (P) and the free enzyme E, is regenerated (Silverman, 1996).



Scheme 11: Enzyme-catalyzed reaction. k_1 , k_{-1} and k_2 are the rate constants for the individual steps.

Unlike a first order reaction, where the rate of reaction is directly proportional to the substrate concentration, the rate of an enzyme catalyzed reaction initially increases with an increase in substrate concentration. At high [S], the enzyme is saturated with substrate and exists only in the ES form. At saturating conditions, the rate is no longer dependent on increased substrate concentration (Rodwell, 1993; Rodwell & Kennely, 2000). The maximum velocity ($V_{\max}$) is obtained when the entire enzyme exists as the enzyme-substrate complex. K_m , the Michaelis constant, is the substrate concentration at which the rate of the reaction velocity is half maximal ($V_{\max}/2$). K_m is determined experimentally by plotting the graph of reaction rate (V) versus concentration of substrate [S] (figure 38).

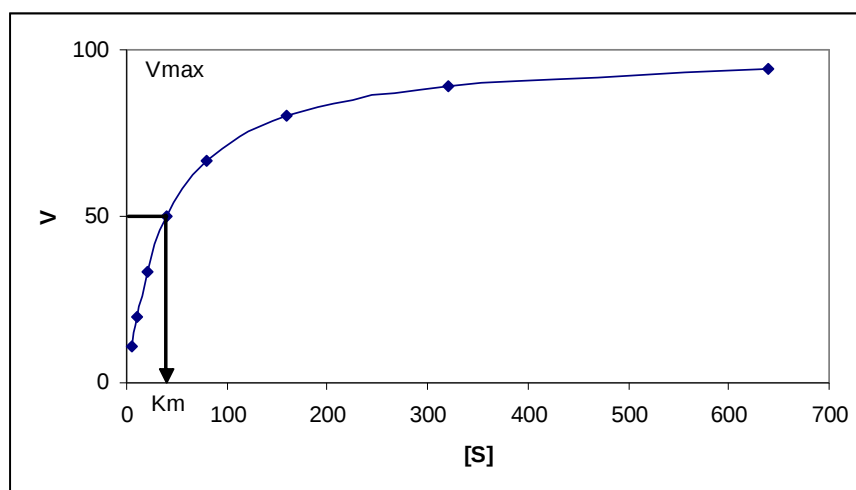


Figure 38: A graph of rate, V versus substrate concentration [S], illustrating the Michaelis-Menten behaviour of enzymes.

This behaviour is described by the Michaelis-Menten equation (Rodwell & Kennely, 2000).

$$V = \frac{V_{\max} \times [S]}{K_m + [S]}$$

$$V = \frac{k_{\text{cat}} \times [E] [S]}{K_m + [S]}$$

Equation 1 and 2: *The reaction velocity V as a function of the substrate concentration [S] for an enzyme-catalyzed reaction.*

The turnover number (k_{cat}) represents the maximal catalytic activity of the enzyme and is the maximum number of molecules of substrate converted to product per active site per unit time (Segel, 1993). k_{cat} equals $V_{\max}$ divided by the total enzyme concentration [E]. The specificity constant (k_{cat}/K_m), provides a measure of how rapidly an enzyme can work at low substrate concentration [S]. It is useful for comparing the relative abilities of different compounds to serve as substrates for the same enzyme. The larger this number, the better the substrate (Segel, 1993).

2.4.2. The measurement of the kinetic parameters

The usual procedure for measuring the rate of an enzymatic reaction is to mix enzyme with substrate and observe the formation of product or disappearance of substrate as soon as possible after mixing, when the substrate concentration is still close to its initial value and the product concentration is small (Rodwell & Kennely, 2000).

2.4.2.1. K_m and $V_{\max}$ determinations

Due to the hyperbolic shape of V versus S plots, the approximate determination of $V_{\max}$ can only be done by extrapolation. To determine $V_{\max}$ and k_m , the Michealis–Menten equation is transformed into the equation for a straight line and a plot of $1/V$ versus $1/[S]$ is constructed (Equation 3).

$$\frac{1}{V} = \frac{K_m}{V_{\max} [S]} + \frac{1}{V_{\max}}$$

Equation 3: *The inverse of the Michealis-Menten equation.*

The y-axis intercept equals to $1/V_{\max}$ and the slope equals to $K_m/V_{\max}$. Such a plot is known as the Lineweaver–Burke double reciprocal plot and K_m and $V_{\max}$ can thus readily be obtained (figure 40) (Rodwell & Kennely, 2000).

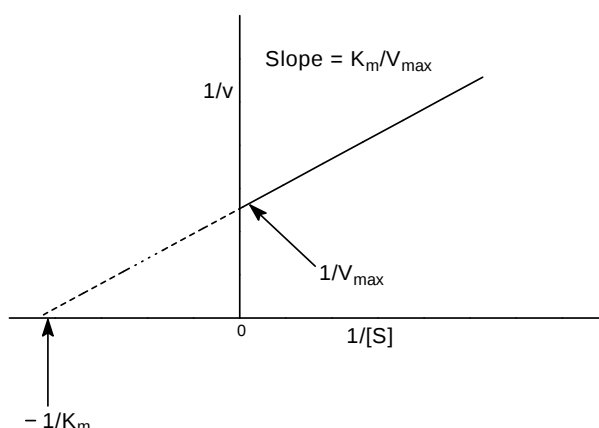
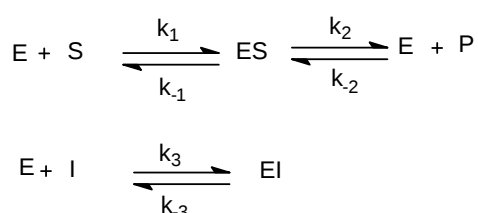


Figure 39: The Lineweaver-Burke double-reciprocal plot.

2.4.2.2. Competitive inhibition

Enzymes can be inhibited by compounds that interfere with the binding of the substrate. Enzyme inhibitors may be divided into two classes: reversible inhibitors, where removal of the inhibitor restores enzyme activity and irreversible inhibitors that permanently inactivate an enzyme (Silverman, 1996). Reversible inhibitors interact with the enzyme through noncovalent interactions. Reversible inhibitors among others include competitive, noncompetitive and uncompetitive inhibitors according to their effect on K_m and $V_{\max}$.



Scheme 12: Formation of enzyme complexes, where the inhibitor, *I* binds reversibly to the enzyme at the same site as the substrate.

A competitive inhibitor competes for the same binding site as the substrate (Rodwell & Kennely, 2000). A competitive inhibitor therefore decreases the number of free enzyme molecules available to bind to the substrate, and an increase in the concentration of the substrate can eliminate the effect of a competitive inhibitor. Competitive inhibition may be represented graphically by the Lineweaver-Burke plot (figure 40). The addition of a competitive inhibitor to an

enzyme catalyzed reaction increases the slope of the straight line, while the y-axis intercept remains unchanged. The intercept on the x-axis increases and becomes less negative. A competitive inhibitor raises the apparent K_m value of a substrate while V_{max} remains unchanged (Rodwell & Kennely, 2000).

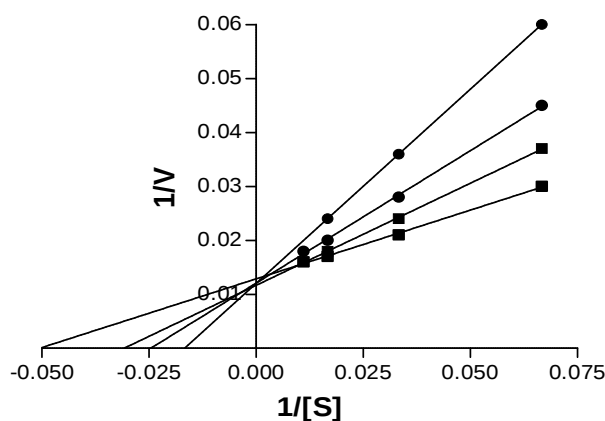


Figure 40: An example of a double reciprocal plot or Lineweaver-Burke plot in the presence of various concentrations of a competitive inhibitor.

2.4.2.3. IC_{50} and K_i determination

An IC_{50} value of an inhibitor is the concentration of inhibitor that produces 50% inhibition of the enzyme. IC_{50} values are determined by the characteristic plotting of the initial rate of oxidation versus the logarithm of the inhibitor concentration to obtain a sigmoidal dose–response curve (Kakkar *et al.*, 1999).

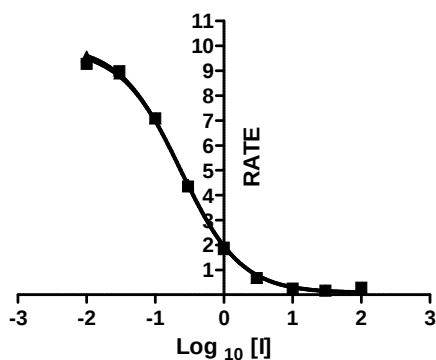


Figure 41: Plot of the rate of enzyme oxidation versus the logarithm of inhibitor concentration, $\text{Log} [I]$.

The K_i value for an inhibitor in turn can be determined from a plot in which the slopes of Lineweaver Burke plots are plotted versus the different inhibitor concentrations at which it has been constructed (figure 42). The K_i value is determined from the x-axis intercept (intercept = $-K_i$). In the presence of a concentration of inhibitor $[I]$ that is equal to K_i , the substrate concentration has to double to maintain the same original velocity compared to the velocity obtained in the absence of the inhibitor (Kakkar *et al.*, 1999).

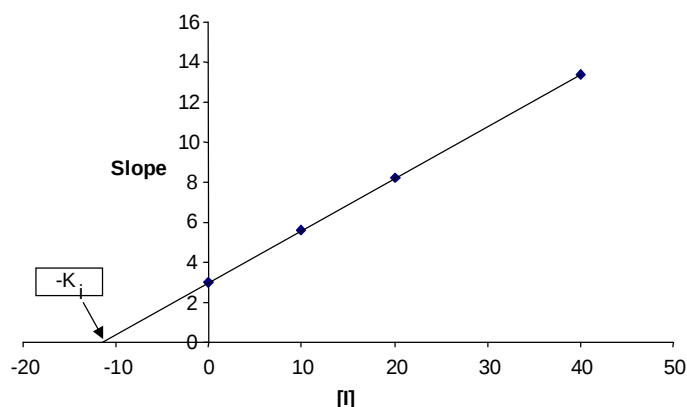


Figure 42: Secondary plot of the slopes from the double reciprocal plot versus inhibitor concentration.

The K_i value of a competitive inhibitor is used to describe the affinity of the inhibitor for the active site of the enzyme. In a series of competitive inhibitors, those with the lowest K_i values will cause the highest level of inhibition at a fixed concentration of inhibitor $[I]$. Generally, it is understood that if plasma or tissue concentrations of a competitive inhibitor are larger than K_i , the inhibition will be physiologically significant, but if lower than K_i , it becomes physiological insignificant (Kakkar *et al.*, 1999).

Also, K_i values may be calculated from the experimental IC_{50} values, according to the equation by Cheng & Prusoff:

$$K_i = IC_{50} / (1 + [S]/K_m)$$

Where, $[S]$ is the substrate concentration and K_m , the Michealis constant of the substrate (Cheng and Prusoff, 1973).

2.5 ANIMAL MODELS OF PARKINSON'S DISEASE

Animal models are a very important approach to study the pathogenesis and therapeutic strategies of human diseases. Many human disorders do not arise spontaneously in animals and have to be mimicked by neurotoxic agents. Most insights into PD pathogenesis come from

investigations performed in experimental models of PD, especially those produced by neurotoxins (Dauer & Przedborski, 2003; Bové *et al.*, 2005). The four most frequently used parkinsonian neurotoxins are 6-OHDA, MPTP, rotenone and paraquat (Forno *et al.*, 1993).

2.5.1 MPTP

2.5.1.1 General background

The neurotoxin, MPTP, is a by-product of the chemical synthesis of a meperidine analog 1-methyl-4-phenyl-4-propionpiperidine (MPPP) (Langston *et al.*, 1983). In the early 1980s, several drug users from Northern California developed acute akinesia following the intravenous injection of a street preparation of MPTP (Langston *et al.*, 1983). These symptoms were reversed by treatment with levodopa and an autopsy revealed destruction of the dopaminergic neurons in the SN of the brain (Williams, 1984). The discovery of the neurotoxic action of MPTP led to its use to create animal models of PD (Przedborski & Vila, 2001). The MPTP model of PD is regarded as the best experimental model of this neurodegenerative disease. It is now well established that MPTP produces an irreversible and severe parkinsonian syndrome when ingested by both humans and monkeys (Langston *et al.*, 1999; Forno *et al.*, 1993), which is characterized by all of the symptoms of PD (Kucheryants *et al.*, 1989).

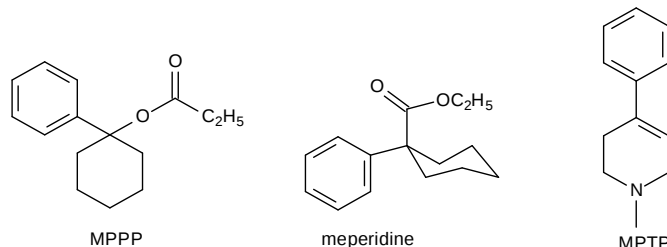


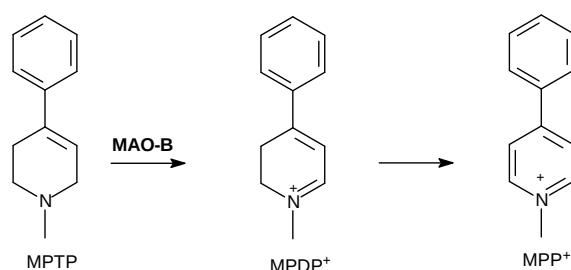
Figure 43: The structures of MPPP, meperidine, and MPTP.

Over the years, MPTP has been used in a number of different animal species, including mice (Dauer and Przedborski, 2003; Jenner, 2003). Interestingly in specific strains of rats, dopaminergic neurons are relatively resistant to MPTP-induced neurotoxicity (Inoue *et al.*, 1999).

2.5.1.2. Mechanism of action

The mechanism by which MPTP selectively destroys nigrostriatal neurons and induces a parkinsonian syndrome has been the subject of many studies (Nicklas *et al.*, 1985; Smeyne & Jackson-Lewis, 2005). The toxic effects of MPTP are mediated by the pyridinium species MPP^+ , which is a mitochondrial toxin. MAO-B has been identified as the principal catalyst responsible

for the metabolic activation of MPTP in the brains of mammals, including humans (scheme 3) (Chiba *et al.*, 1984). The transformation of MPTP into MPP^+ is a two-step process. First, MPTP undergoes a two-electron oxidation via MAO-B to generate $MPDP^+$. A second 2-electron oxidation generates MPP^+ (Chiba *et al.*, 1984; Markey *et al.*, 1984).

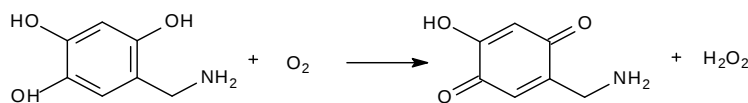


Scheme 3: The MAO-B-catalyzed oxidation of MPTP to the corresponding 2,3-dihydropyridinium product $MPDP^+$ and the pyridinium species MPP^+ .

MPTP is a lipophilic molecule, which is able to readily permeate lipid bilayer membranes and therefore crosses the blood-brain barrier (Markey *et al.*, 1984). Once in the brain, it is rapidly converted into MPP^+ (Ramsay & Singer, 1986). This process appears to take place mainly in MAO-B rich glial cells (Kitahama *et al.*, 1991). MPP^+ is actively accumulated in the mitochondria (Ramsay & Singer, 1986) where it blocks complex I of the mitochondrial respiratory chain. This results in the reduction of ATP synthesis, oxidative stress and eventual degeneration of nigrostriatal dopaminergic neurons (Singer *et al.*, 1988; Nicklas *et al.*, 1985).

2.5.2. Hydroxydopamine (6-OHDA)

6-OHDA is frequently used to create animal models of PD (Bové *et al.*, 2005). 6-OHDA possesses a high affinity for several catecholaminergic plasma membrane transporters, such as the dopamine (DAT) and norepinephrine transporters (NET) (Sauer & Oertel, 1994.; Ungerstedt, 1971). As a result, 6-OHDA can enter both dopaminergic and noradrenergic neurons and inflict damage to both the peripheral and the CNS of the catecholaminergic pathways (Cohen, 1984; Przedborski & Ischiropoulos, 2005). Since 6-OHDA cannot cross the blood-brain barrier, it must be administered by local stereotaxic injection into the SN or the striatum, to target the nigrostriatal dopaminergic pathway (Mandel & Randall, 1985). Following administration, 6-OHDA generates hydrogen peroxide (H_2O_2) (scheme 13) via the reduction of molecular oxygen (Dauer & Przedborski, 2003). One mole of 6-OHDA may produce several equivalents of O_2 by redox cycling chemistry (Przedborski & Ischiropoulos, 2005).



Scheme 13: The redox cycling of 6-OHDA produces H_2O_2 as a neurotoxin.

Unilateral 6-OHDA administration to experimental animals produces an asymmetric circling behaviour (Cenci *et al.*, 2002), which depends on the degree of the nigrostriatal lesion (Ungerstedt & Arbuthnott, 1970). Because the unilateral lesion can be quantitatively measured, this model offers a means to assess the antiparkinsonian properties of new drugs.

2.5.3. Rotenone

Rotenone is a member of a family of natural cytotoxic compounds, extracted from tropical plants (Talpade *et al.*, 2000). Rotenone is an inhibitor of complex 1 of the mitochondrial electron transport chain (Betarbet *et al.*, 2000). Studies have shown that oral delivery of rotenone causes little neurotoxicity in animals. Even chronic ingestion for 24 months at doses 30 times greater than those used to model PD by systemic infusion failed to cause any neurodegeneration (Betarbet *et al.*, 2000). To be useful in animal models, rotenone should be administered intravenously.

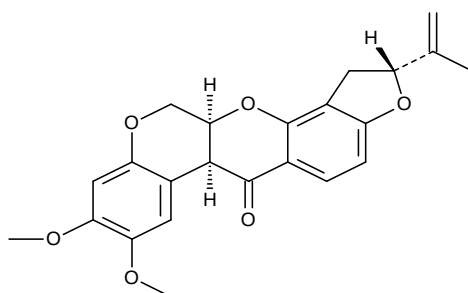


Figure 44: The chemical structure of rotenone.

Like MPTP, rotenone is highly lipophilic and thus readily gains access to all organs including the brain. The toxicity of rotenone occurs in the mitochondria since it binds at the same site as MPP^+ in complex 1. This impairs oxidative phosphorylation and induces oxidative damage (Betarbet *et al.*, 2000). Intravenous infusion of low doses of rotenone in rats leads to lesions of the striatum and the globus pallidus (Ferrante *et al.*, 1997). Interestingly, following rotenone administration, the remaining SN dopaminergic neurons contain proteinaceous inclusions similar to LBs in PD (Betarbet *et al.*, 2000).

2.5.4. Paraquat

Paraquat is a herbicide that destroys plant tissue by disrupting photosynthesis and by inducing oxidative stress. It has been implicated in PD, since it has structural similarity to the active toxic metabolite of MPTP, MPP^+ (Dauer & Przedborski, 2003).

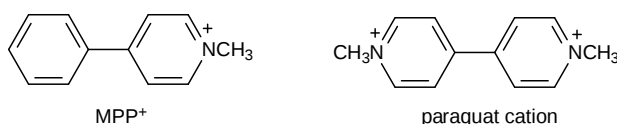
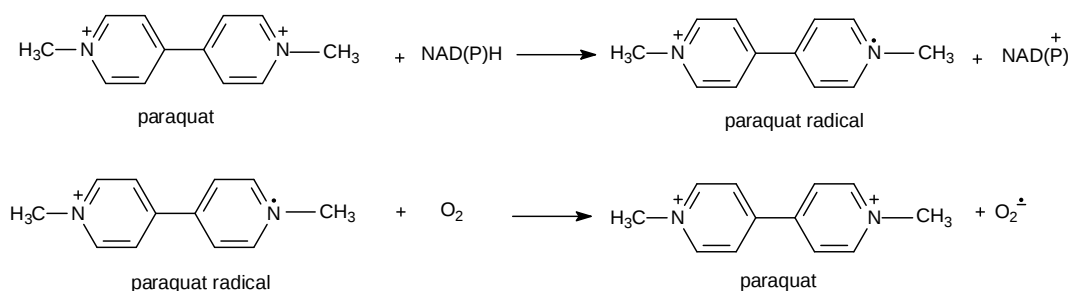


Figure 45: Comparison of the structure of MPP^+ and paraquat.

The toxicity of paraquat is mediated by redox cycling with a cellular diaphorase which results in the formation of superoxide radicals (Day *et al.*, 1999) (scheme 14).



Scheme 14: The redox cycling reaction of paraquat.

The systemic administration of paraquat to mice leads to dopaminergic neuron degeneration (Manning-Bog *et al.*, 2002). Despite its structural similarity with MPP^+ , paraquat is not a substrate of DAT and does not inhibit complex I of the brain mitochondria (Dauer & Przedborski, 2003). It therefore exerts selective dopaminergic toxicity in a manner that is unique from MPTP.

2.6. SUMMARY

Neurodegenerative disorders such as AD and PD are considered multifactorial disorders and as such several different strategies have been investigated as treatment options. None have so far proven to halt the progression of the disease. Reversible MAO-B inhibitors have been used successfully to treat PD as they offer both a symptomatic and a neuroprotective effect. Based on the important role of MAO-B inhibitors in PD and the potential advantages that reversible inhibitors may have over irreversible MAO-B inhibitors, the design and development of new reversible inhibitors of MAO-B is still ongoing.

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