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Parkinson’s disease (PD), first described by Dr. James Parkinson in 1817 as paralysis agitans, or the *shaking palsy* in his famous monograph “An Essay on the Shaking Palsy” (Parkinson, 1817). PD is a neurodegenerative disorder of SN, an area in the basal ganglia which controls voluntary movements. After Alzheimer’s, PD is the second most common neurodegenerative disease. According to the United Nations, at least four million people worldwide suffer from PD (Bornebroek et al., 2007; de Lau and Breteler, 2006). It is estimated that the prevalence and incident rates of PD in Europe is approximately 108 to 257/100,000 and 11 to 19/100,000 per year, respectively. In the older age groups (i.e. > 60 years) the rates of prevalence and incidence are much higher: 1280 to 1500/100,000 and 346/100,000, respectively (Von Campenhausen et al., 2005). It is expected that prevalence of PD and the anticipated socio-economical burden related to this disease will increase in the next few decades as a result of both increased longevity and multiplication of effective therapies (Lang and Lozano, 1998a; 1998b).

**AN ESSAY ON THE SHAKING PALSY.**

**CHAPTER I.**

**DEFINITION—HISTORY—ILLUSTRATIVE CASES.**

SHAKING PALSY. (*Paralysis Agitans.*)

Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace; the sense and intellects being uninjured.

Fig. 1. James Parkinson’s essay on shaking Palsy

PD occurs when certain neurons of the brain, mostly in the SN die or become impaired. The symptoms of PD result from the loss of these
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dopamine (DA)-secreting (dopaminergic) cells and subsequent loss of melanin, secreted by the same cells, in the pars compacta region of the SN (also known as black substance). This leads to inhibition of the direct pathway of movement and activation of the indirect pathway of movement. Since the direct pathway facilitates movement and the indirect pathway inhibits movement, the loss of these cells leads to a hypokinetic movement disorder. The lack of DA results in an excessive inhibition of the thalamus, leading to hypokinesia (Lang and Lozano, 1998a; 1998b).

MAJOR SYMPTOMS OF THE PARKINSON'S DISEASE

PD affects mainly the motor system and its cardinal symptoms are tremor, rigidity, akinesia, postural abnormalities, and gait impairment (Lang and Lozano, 1998a; 1998b; Shulman et al., 2011). In addition to the motor symptoms, mental disorders like depression or psychosis, and autonomic and gastrointestinal dysfunction may occur; all of which considerably impair the quality of life of the patients (Ferrer, 2011, Martinez-Martin et al., 2011).

Fig. 2. Postural instability and rigidity observed in an PD patient
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Tremor in PD has a number of characteristics that make it easy to differentiate from other causes of tremor: it is slow with frequency of 4 to 6 Hz and affects asymmetrically upper and lower limbs (Deuschl et al., 1998; Hallett, 1998).

Rigidity or increased stiffness of the muscles, frequently associated with cog-wheeling, is a plastic, lead-pipe form of hypertonia that affects many muscles of the limbs, trunk and responsible for the typical stooped posture of the PD patients.

Akinesia (lack of movement) is perhaps the most disabling symptom of PD that includes many features such as delayed motor initiation and slow performance of voluntary movements (bradykinesia), insufficiency of motion (hypokinesia), difficulty in reaching a target with a single continuous movement, rapid fatigue with repetitive movements, inability to execute simultaneous actions and inability to execute sequential actions (Zaidel et al., 2009).

Gait can also be altered in PD. Akinesia is particularly obvious during gait where it is responsible for the short, shuffling steps, reduced arm swing, hesitations in start, turning-around and sometimes leading to freezing phenomena (Zaidel et al., 2009).

Various combinations of PD symptoms, their complexity, severity, location and variability over the time in a particular patient generate a significant functional disability that increases as the disease progresses (Hoehn and Yahr, 2001). Apart from the complex and constantly evolving pattern of these motor changes, there is considerable interpersonal heterogeneity of PD, making comparisons between individual patients a difficult task. Therefore, measuring the effect of therapeutic interventions on the symptoms of PD is a major challenge.

Thenon-motor symptoms include disorders of mood, behavior, thinking, and sensation (Chaudhuri et al., 2006; Ferrer, 2011). The symptoms of PD are only evident when loss of at least 50% of the dopaminergic neurons in the SNpc occurs, leading to an over 80% reduction in DA levels in the striatum (Ilgin et al., 1999; Deumens et al., 2002). DA functions as a neurotransmitter, playing important roles in behavior and cognition, motor activity, motivation and reward,
regulation of milk production, sleep, mood, attention, and learning (Bjorklund and Dunnett, 2007). The average onset age for the disease is 55 years and the risk increases dramatically with age from 20/100,000 overall to 120/100,000 at age 70. In the United States alone, it is estimated that 40,000 new patients are diagnosed with PD each year, and over 1 million Americans are affected (Fischer, 1999).

**EPIDEMIOLOGY OF PD**

PD is the second most common neurodegenerative disorder, after Alzheimer's disease (AD) (Schapira, 1999a; de Lau and Breteler, 2006). This disease is found in all ethnic groups, but with geographical differences of prevalence. Early onset of sporadic PD is rare, with about 4% of patients developing clinical signs of this disease before an age of 50 years (Van Den Eeden et al., 2003). Approximately 1–2% of the population over 65 years suffers from PD. This figure increases to 3% to 5% in people 85 years and older (Fahn, 2003). As PD is mainly an illness of later life, it is more common in developed countries where people live longer. Age is the most common risk factor that contributes to the incidence of developing PD. The incidence rates increase rapidly over 60 years of age, with only 4% of PD patients developing the disease when they are under the age of 50 years (Van Den Eeden et al., 2003). Age-standardized incidence rates of PD in population-based studies in European countries and the USA range from 8.6 to 19.0 per 100,000 inhabitants when strict diagnostic criteria of PD are applied (Twelves et al., 2003). Gender is also a factor that contributes to PD incidence rates. For men, the incidence rate is 19 per 100,000, which are 91% higher than that of women, whose incidence is 9.9 per 100,000. The possible neuroprotective effects of oestrogen have been speculated to play a role in this finding, but this hypothesis remains controversial (Saunders-Pullman, 2003). Based on race/ethnicity, the highest incidence rates are recorded for Hispanic populations (16.6), followed by non-Hispanic white (13.6), Asian (11.3), and Black (10.2 per 100,000) populations, suggesting that race/ethnicity also affects the incidence of PD (Van Den Eeden et al., 2003). However, there are substantial variations in reported incidence rates, most probably due to...
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methodological differences between studies, in particular differences in case ascertainment and use of diagnostic criteria.

**PATHOPHYSIOLOGY OF PD**

The pathophysiology of PD involves the degeneration of dopaminergic neurons in the nigro-striatal pathway i.e. dopaminergic neurons present in the striatum and the SNpc. Due to the degeneration of these dopaminergic neurons, there is an imbalance between the dopaminergic and cholinergic neurotransmitters which under normal physiological conditions remains in a balanced state i.e. levels of DA (an inhibitory neurotransmitter) decreases while the acetylcholine (an excitatory neurotransmitter) level increases (Fig. 3.). This imbalance results in the loss of inhibitory mechanisms over the excitatory mechanism and causes the motor dysfunction in the form of tremor (shaky movements), rigidity, etc.

![Acetylcholine](image1.png) ![Dopamine](image2.png)

**Fig. 3. Imbalance between dopamine and acetylcholine, the main cause of motor dysfunction**

The pathological hallmarks of PD are progressive loss of nigrostriatal dopaminergic neurons in the SN with resultant depletion of DA, and the presence of Lewy bodies and Lewy neurites, cytoplasmic granular and filamentous inclusions composed largely of α-synuclein and ubiquitin, in the remaining neurons (Dauer and Przedborski, 2003; Forno, 1996; Goldman et al., 1983). The dopaminergic neurons in the SNpc project to the striatum and their loss leads to alterations in the activity of the neural circuits within the basal ganglia that regulates movement (Fig. 4.) (Chinta and Andersen, 2005). SNpc neuronal cell death in PD seems to follow a “dyingback” pattern (Dauer and Przedborski, 2003). The terminal loss in the striatum precedes and is more robust than that of the SNpc cell body (Bernheimer et al., 1973; Herkenham et
al., 1991), suggesting that the striatal dopaminergic neuronal terminals are primary target of the degenerative process.

Lewy bodies were first described by Frederic Lewy in 1912 (Holdorff, 2002) and their complexity remained unsolved until relatively recently. Lewy bodies are eosinophilic cytoplasmic inclusions that consist of a dense core surrounded by a halo of 10-nm wide radiating fibrils, of which the primary structural component is $\alpha$-synuclein (Forno, 1996; Spillantini et al., 1998). Increasing evidence indicates that abnormalities in $\alpha$-synuclein lead to Lewy body formation (Spillantini et al., 1998). In addition to dopaminergic neuronal cell
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loss and Lewy body formation, microglia-associated inflammation is another characteristic feature of PD pathology. Increased reactive amoeboid microglia has been found in the SN of PD patients in postmortem studies (McGeer and McGeer, 1998; Hirsch et al., 2003). Activated microglia produce a wide range of proinflammatory mediators, including cytokines: tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β), IL-6, and interferon-gamma (INF-γ) (Mogi et al., 1994; Hunot and Hirsch, 2009) and inflammation-inducible enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) (Knott et al., 2000). Despite diverse etiologies of PD, increasing evidence suggests that convergent pathogenic mechanisms involving oxidative stress and protein aggregation exist, leading to dopaminergic neuronal cell death (Dauer and Przedborski, 2003). Additionally, there is increasing evidence that microglia-associated inflammation may be another determinant factor for the degeneration of nigral dopaminergic neurons (Whitton, 2007).

ETIOLOGY OF PD

The cause of PD is still largely unknown. Several etiologies such as trauma and head injury (Factor and Weiner, 1991), infection (Poskanzer and Schwab, 1963), rural lifestyle (Jankovic et al., 1990), environmental factors (Logroscino, 2005), and genetic factors (de Lau and Breteler, 2006) have been proposed. In most of the sporadic cases of PD, generally various environmental factors play a key role in the disease etiology. The main impetus for the environmental causation theory of PD arose from the identification of the chemical compound MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) that was associated with evident parkinsonism in four young drug users after using a Demerol derivative intravenously (Langston et al., 1983; 1984). The chemical has a consistently similar effect in laboratory animals (McGeer and McGeer, 2008; Gupta et al., 2009).

In addition to MPTP, other environmental factors including pesticide rotenone, herbicide paraquat and fungicide maneb have been implicated as risk factors in PD (Betarbet et al., 2000). These chemical compounds share a similar structure and have been reported to interact with mitochondrial enzyme complexes especially, complex I (Dawson and Dawson, 2003). Mitochondrial dysfunction has been associated with the pathogenesis of PD (Schapira,
Less than 10% of PD cases are familial and due to genetic defects. However, the discovery of PD genes is particularly significant because it allows for the generation of transgenic models to specific forms of the disease. Furthermore, phenotypic similarity between the genetic and sporadic forms of the disease impliess that they share important pathogenic mechanisms. Therefore, studying PD-related genes will provide important insights into the convergent pathobiologic pathway of PD (Maguire-Zeiss and Federoff, 2003). Increasing evidence suggests that sporadic cases of PD actually result from complex interactions between environmental and genetic factors. The common susceptibility genes that have been hypothesized to contribute to the risk of sporadic PD include genes involved in DA metabolism, mitochondrial metabolism, cholesterol metabolism, xenobiotic metabolism, and detoxification (Tan et al., 2000; Benmoyal-Segal and Soreq, 2006).

The causes of the dopaminergic neuronal degeneration may be the insult of environmental toxins such as MPTP, rotenone etc or the neuroinflammation induced by bacteria (LPS, lipopolysaccharide)/viruses etc. Once these toxins enter the dopaminergic neurons in the nigro-striatal pathway, they are thought to activate the glial cells to induce the gene expression of COX-2 and iNOS, which further result in the synthesis and release of prostaglandins (PGs) and NO. These toxins also induce the expression of transcription factors such as nuclear factor-kappa B (NF-κB) and release of proinflammatory cytokines such as TNF-α, ILs, which further induces the gene expression of COX and iNOS. In neurons, they also result in the mitochondrial dysfunction and hence, ATP depletion which leads to the neuronal cell death. Mitochondrial dysfunction leads to the generation of reactive oxygen species such as super oxide anions, which combines with the released nitric oxide to form a potent free radical “peroxynitrite”. All these events produces a vicious cycle of events and ultimately lead to the dopaminergic neuronal death and hence the pathogenesis of PD.

**PATHOGENESIS OF PD**

Despite diverse etiologies of PD, increasing evidence suggests that convergent pathogenic mechanisms involving oxidative stress and protein aggregation exist, leading to dopaminergic neuronal cell death (Dauer and
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Przedborski, 2003; Maguire-Zeiss and Federoff, 2003). Additionally, there is increasing evidence that microglia-associated inflammation may be another determinant factor for the degeneration of nigral dopaminergic neurons (Whitton, 2007).

a) Deregulation of neurotransmission

The degeneration of dopaminergic neurons results in alterations in the GABAergic, glutamatergic, serotonergic and norepinephrinergic systems, which further leads to various motor and non-motor defects. Dopaminergic neuronal degeneration in SN which normally inhibits the output of GABAergic neurons (mostly express D2 receptors) in the corpus striatum becomes overactive. This in turn can activate subthalamic nucleus (STN). The overactivation of STN, caused by nigral dopaminergic neurons loss, leads to an excessive inhibitory output from the globus pallidus pars interna and SN pars reticulata to the cortex and brainstem motor system, via thalamus, causing the manifestation of bradykinesia, tremor and rigidity (Fig. 5) (Lang and Obeso, 2004).
Fig. 5. Normal functional anatomy and pathological functional anatomy of the basal ganglia. The arrows point to the direction of the different nerve tracts and the colors indicate the neurotransmitters involved at each level. The widths of the tracts are proportional to the strength of the signal. Positive signs at the end of a nerve tract indicate excitatory impulses and negative signs indicate inhibitory impulses. (http://www.mdvu.org/library/disease/pd/par_path.html)

b) Oxidative Stress

The brain is only 2–3% of the total body mass, but it consumes 20% of body oxygen (Mariani et al., 2005). Neuronal cells are particularly susceptible to oxidative damage due to high levels of polyunsaturated fatty acids in their membranes and relatively low activity of endogenous antioxidant enzymes. Many cellular reactions utilize molecular oxygen for catalysis and energy production. These reactions in turn produce reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl radicals and in the presence of NO, reactive nitrogen species (RNS) such as peroxynitrite and nitro-tyrosyl radicals are also produced (Tieu et al., 2003). Oxidative stress in cells either due to excessive production of ROS or insufficient antioxidant defense (particularly in the elderly) can damage cellular proteins, lipids and DNA or activate apoptotic pathways (Betarbet et al., 2005; Mariani et al., 2005). Reduced glutathione, a very important antioxidant defense enzyme in the brain, is markedly decreased in the SN of PD patients (Schulz et al., 2000).
Evidence for the implication of oxidative stress in PD pathogenesis is overwhelming (Jenner and Olanow, 1998). In PD, ROS in the affected SN generally arises from three sources, including DA metabolism, dysfunction of mitochondria and microglial phagocytosis. DA metabolism has been associated with increased levels of ROS. The degradation of DA by monoamine oxidase to produce hydrogen peroxide (H$_2$O$_2$) causes increased formation of oxidized glutathione (GSH), suggesting the occurrence of oxidative stress and impairment of a major antioxidant system (Spina and Cohen, 1988).

Likewise, the metabolism of DA might be responsible for the high basal levels of oxidative stress in SN (Jenner, 2003). Mitochondria dysfunction is a major source of oxidative stress. Several environmental toxins implicated in PD cause Parkinsonism by inhibiting complex I or III of the mitochondrial electron-transport chain. Additionally, many of the genes associated with PD also implicate mitochondria in disease pathogenesis. So far, mutations or polymorphisms in some PD causative genes have been identified to increase mitochondria dysfunction-associated oxidative stress: α-synuclein, parkin, DJ-1, and PINK1 (Canet-Aviles et al., 2004; Zhou et al., 2011).

Evidence suggests that α-synuclein function in lipid metabolism and the mutation of α-synuclein results in significant disruption to normal mitochondrial membrane phospholipid composition (Ellis et al., 2005). Intriguing work has highlighted a protective role for Parkin within mitochondria. Knockout studies have shown that reduced levels of Parkin are involved in mitochondrial oxidative phosphorylation (Greene et al., 2003), although the precise mechanisms for the protective function of Parkin in mitochondria are still not clear. DJ-1 localizes to mitochondria (Zhang et al., 2005) and contains many residues that are readily oxidized. It has been suggested that DJ-1 protects neurons from oxidative stress by acting as a redox dependent chaperone (Zhou et al., 2011). PINK1 localizes to mitochondrial membranes and contains a catalytic serine-threonine kinase domain (Hatano et al., 2004). Overexpression of PINK1 protects cells from mitochondrial depolarization and apoptosis, and expression of pathogenic mutations of PINK1 does not protect against apoptosis-inducing agents, implying a mitochondrial protective effect of PINK1 (Abou-Sleiman et al., 2006; Hatano et al., 2004).
Activated microglia is a robust source of extracellular ROS in the central nervous system (CNS). As professional phagocytes, microglia help maintain CNS homeostasis by engulfing cell debris or other abnormal substances. In the course of their activities, microglia produce ROS by activating membrane-bound NADPH oxidase. NADPH oxidase is the major source of ROS following a range of initiating activators (Medow et al., 2011).

Also relevant is the fact that the iron levels, which in a non-pathological situation are significantly higher in SN than in other brain regions, further increase in SN of PD patients (Ben-Shachar et al., 1991). Nigral dopaminergic neurons are more susceptible to oxidative stress, since dopamine can be easily auto-oxidized into toxic dopamine-quinone species, superoxide radicals and hydrogen peroxide (Graham, 1978).

c) Endogenously formed DA-O-Quinones as neurotoxic species

It is established that DA is oxidized sequentially in SNpc neurons to DA-o-quinone, leukoaminochrome, aminochrome, 5,6-dihydroxyindole, indole-5,6-quinone which finally polymerizes to form neuromelanin (Costa et al., 1992). Moreover, 5-cysteylnidopamine, a metabolite of DA-quinone, is formed at an accelerated rate as the SNpc becomes depigmented and is elevated in the caudate of schizophrenics (Fornstedt and Carlsson, 1989). Detoxification of O-quinone normally occurs by conjugation with glutathione in a reaction catalyzed by...
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by glutathione-S-transferases (Segura-Aguilar et al., 1997), but an increased frequency of deletion of this gene has been observed in the brain of schizophrenics (Harada et al., 2001).

The DA concentration is dependent on the reuptake of extracellular DA released during neurotransmission from monoaminergic vesicles and on DA synthesis catalyzed by tyrosine hydroxylase (Fig. 7.). Normally, free cytosolic DA is transported into monoaminergic vesicles by VMAT-2 to be used for neurotransmission. Inside of monoaminergic vesicles, DA hydroxyl groups are hardly protonated, and a high concentration of DA is stored without risk of oxidation or polymerization to form neuromelanin. Free cytosolic DA is removed by mono amino oxidase (MAO). Under certain conditions, DA oxidation occurs and DA o-quinone is formed. However, at physiological pH, DA o-quinone is a transient metabolite since the amino chain is immediately cyclized to form aminochrome (Fig. 7.).

Fig. 7. Dopamine metabolism in dopaminergic neurons

Aminochrome can participate in four different reactions: (i) polymerization with other aminochrome-containing molecules including proteins, lipids, and metals (ii) formation of adducts with alpha-synuclein, inducing and stabilizing the formation of neurotoxic protofibrils (iii) one-electron reduction by
flavoenzymes to form the leuko-aminochrome o-semiquinone radical, which can auto oxidize in the presence of oxygen to generate redox cycling between aminochrome and this radical; and (iv) two electron reduction, a reaction catalyzed by DT-diaphorase, to form leuko-aminochrome.

Oxidation of DA in the presence of oxygen will give rise to the formation of O$_2^-$ and H$_2$O$_2$, which can interact with iron. The enzyme xanthine oxidase (XO) is also thought to be a source of O$_2^-$ and participate in reactions in which iron ions are involved, leading to the generation of more damaging OH species. O$_2^-$ has an extremely short half-life (Halliwell, 1989) and rapidly undergoes dismutation to H$_2$O$_2$ and then undergoes a Fenton-type reaction in the presence of iron to yield cytotoxic OH (Ben-Shachar et al., 1991). ROS have been implicated in dopaminergic toxicity caused by MPTP (Chiuheh et al., 1993), 6-OHDA (Cohen and Heikkila, 1974), iron (Youdim et al., 1989), ischemia/reperfusion injury (Obata et al., 1994), and stroke (Mohanakumar et al., 1994). Intracellular, as well as extracellular DA has been found to autoxidize to cytotoxic products such as quinine and oxygen free radicals. The effects of MPP$^+$ the toxic metabolite of dopaminergic neurotoxin MPTP, on the formation of ·OH in the extracellular fluid of caudate nucleus are shown in Fig. 8.

![Diagram of dopamine autooxidation via Fenton's reaction in the basal ganglia](image-url)

a) xanthine + O$_2$ \( \xrightarrow{\text{XO}} \) O$_2^+$ + H$_2$O$_2$ + urea

b) For the Fenton reaction : H$_2$O$_2$ \( \longrightarrow \) ·OH + OH$^-$

Fig. 8. Dopamine autooxidation via Fenton’s reaction in the basal ganglia (Adapted from Obata et al., 2001)
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Excessive autoxidation of DA leads to accumulation of both cytotoxic quinones and ROS, which might overwhelm cellular antioxidant defense mechanisms, resulting in degeneration of dopaminergic neurons (Obata et al., 2001). The highest iron levels are found in pallidum not in SN and iron is associated to neuromelanin also in normal subjects (Zecca et al., 1996). In SN, H and L-ferritins are the major storage molecules for iron in glia, while in neurons most of iron is bound to neuromelanin (Zecca et al., 2001).

d) Mitochondrial Dysfunction

Mitochondria are the intracellular organelles responsible for the supply of ATP; they are semiautonomous, they contain their own DNA and protein synthesizing machinery, although most of the proteins that reside in the mitochondria are nuclear gene products (Fig. 9.) (Attardi and Schatz, 1988). Defects in the electron transport chain within the mitochondria are major factors contributing to the production of free radicals. It has been described that mitochondrial function is altered in the course of aging and also in PD, particularly an alteration in the mitochondrial respiratory chain complex I (Beal, 2003).

Fig. 9. Diverse cellular pathways modulated by mitochondrial dysfunction that culminate in cell death

Many studies have shown that mitochondrial dysfunction is implicated in the pathogenesis of PD (Orth and Schapira, 2002). Two consistently identified biochemical abnormalities in PD SN are mitochondrial complex I deficiency and increase in free radical production (Beal, 2003; Dawson and Dawson, 2003).
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The complex I defect in idiopathic PD is of particular interest, since inhibitors of this complex cause nigral cell death in humans and in animals. Parkinsonism was induced in humans by MPP+, a metabolite of MPTP (Langston et al., 1983; Langston et al., 1999), and more recently, the pesticide rotenone was shown to induce neurodegenerative changes similar to PD in rats (Betarbet et al., 2000), both molecules being complex I inhibitors (Fig. 10). Co-enzyme Q10 (CoQ10), which improves electron respiratory chain function and scavenges free radicals, has been described to exist at a lower concentration in patients with PD (Shults et al., 1997), probably due to the complex I defect observed in these patients (Schapira et al., 1990). Complex I deficiency and free radical damage are inter-related, since a defect in complex I causes an increase in the release of superoxide ions from the respiratory chain and, in turn, free radicals increase leads to an impaired activity of respiratory chain proteins. Much of the cellular damage caused by rotenone, a dopaminergic neurotoxin in rodents seems to be mediated not by ATP depletion but by generation of free radicals (Betarbet et al., 2000).

Fig. 10. Schematic diagram of the mitochondrial electron transport chain (Adapted from Betarbet et al., 2002)

e) Protein Aggregation

The presence of Lewy bodies is a defining feature of PD. However, the mechanisms underlying Lewy body formation are still elusive. Intracellular
aggregation of α-synuclein appears to play a key role in the generation of Lewy bodies. Recent studies have shed light on the cause of α-synuclein aggregation and its relevance to the pathogenesis of PD (Choi et al., 2011; Eller and Williams, 2011; Greenbaum et al., 2005). The accumulation of aggregated α-synuclein is determined by a dynamic equilibrium between production and degradation. Several factors have been reported to enhance α-synuclein misfolding and aggregation, including protein overexpression or mutation, chemical modification, and molecular interaction. α-synuclein has a high self-aggregation propensity; overexpression or mutation is known to accelerate this aggregation process (Greenbaum et al., 2005; Winner et al., 2011). Chemical modification of α-synuclein such as oxidation, nitration, phosphorylation, and dopamine adduction, are also likely to have direct effects on normal conformation of this protein, thus affecting its aggregation propensity (Winner et al., 2011).

Previous studies have shown that exposure to oxidative and nitrosative species stabilizes α-synuclein filaments and this stabilization may be due to dityrosine cross-linking. Nitrated or phosphorylated α-synuclein has been observed in synucleinopathy lesions (Giasson et al., 2000; Waxman and Giasson, 2010). DA facilitates α-synuclein to form soluble oligomers but inhibits fibrillation, suggesting the toxic species is represented by a soluble oligomer and not the insoluble fibril (Hasegawa et al., 2006). Cellular α-synuclein is available for physical interactions with other molecules such as lipid, tubulin, synphilin-1 or metals; these interactions sometimes result in alterations in protein conformation and increase the aggregation propensity of α-synuclein (Burke et al., 2008; Uversky, 2008). The degradation systems for α-synuclein are mainly the ubiquitin-proteasome system (UPS) and the autophage-lysosomal system. Failure in these systems leads to the accumulation of misfolded α-synuclein. It is therefore notable that two causative genes of PD, parkin and ubiquitin c-terminal hydrolase (UCH)-L1, are directly related to UPS (Duan et al., 2006; Imai et al., 2000).

Numerous findings suggest that α-synuclein gains pathogenic functions through forming higher order quaternary structures to damage specific subcellular targets, such as mitochondria, golgi and lysosome. In a
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study, overexpression of α-synuclein in a hypothalamic neuronal cell line results in formation of α-synuclein inclusions, leading to mitochondrial alterations accompanied by increased levels of free radicals and decreased secretion of gonadotropin-releasing hormone (Hsu et al., 2000). Increased free radicals would in turn trigger α-synuclein aggregation, initiating a vicious circle of oxidative stress and α-synuclein aggregation. In addition, α-synuclein aggregation has been associated with Golgi fragmentation and lysosomal dysfunction, which lead to cell death (Gosavi et al., 2002; Rockenstein et al., 2005). Although, it is well accepted that α-synuclein aggregation is implicated in PD pathogenesis, it still remains to be determined which aggregated species, including β-sheet rich oligomers, protofibrils and stable amyloid fibrils, are the most neurotoxic. Increasing evidence favors a toxic misfolded intermediate protofibril scenario and suggests that pore-like protofibrils are responsible for the cellular impairment by causing membrane permeabilization (Volles and Lansbury, 2007).

f) Inflammation

Microglia-associated inflammation is a pathological hallmark of PD. The inflammatory process (activation/proliferation of microglia and secretion of proinflammatory cytokines and free radicals) could be an event secondary to the neurodegenerative process and in turn exacerbate the progression of cell death. However, under certain circumstances, inflammation could be a primary event that leads to the neurodegenerative process. Since dopaminergic neurons in the SN are relatively sensitive to “ROS” (Jackson-Lewis and Smeine, 2005) and that there is a large population of microglia in the SN in comparison to other CNS regions (Block et al., 2007), inflammation may be one of causative factors in inducing neurodegeneration. Researchers have demonstrated that intranigral injection of LPS produced microglial activation and subsequent progressive degeneration of the dopaminergic neurons, suggesting microglia-mediated inflammation underlies the neuronal cell death in the SN (Kacimiet al., 2011; Reisenauer et al., 2011).

Activated microglia exert their neurotoxic effects by releasing proinflammatory cytokines such as TNF-α, IL-1β, IL-6 and IFN-γ, free radicals including ROS and NO, as well as inflammatory mediators such as
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PGE₂, leading to nigral cell damage and death (Fig. 11.). Elevated levels of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 in the striatum of PD brains have been demonstrated (Mogi et al., 1994; Muller et al., 1998). Activated glial cells expressing pro-inflammatory cytokines, such as TNF-α, IL-1β and IFN-γ as well as iNOS have been reported in the SN in PD (Hunot et al., 1997; Hunot et al., 1999). Furthermore, enhanced expression of IL-1, IL-6 and TNF-α has also been shown in the cerebrospinal fluid as well as in the basal ganglia of PD patients (Mogi et al., 2007; Nagatsu et al., 2000). TNF-α and IL-1β are robust activators of NF-κB and contribute to neuronal cell death by triggering apoptotic transduction pathway (Nagatsu et al., 2000; Hailer et al., 2005).

Fig. 11. Upregulation of neuroinflammatory mechanisms in microglia and dopaminergic neurons following neuroinflammatory insult

These inflammatory cytokines, along with factors released from the dying dopaminergic cells, seems to amplify and sustain the neuroinflammation as well as further consequent immune responses leading to a potentially lethal descent into irreversible destruction of nigral dopaminergic neurons (Orr et al., 2002). It has been shown that caspase-11, an apoptotic factor, mediates MPTP-induced nigrostriatal dopaminergic degeneration, and is involved in both
MPTP and LPS-induced selective dopaminergic neurotoxicity (Furuya et al., 2004). Furthermore, 6-OHDA-lesioned rats have been demonstrated the increased levels of TNF-α in both SN and striatum (Mogi et al., 1999).

Additionally, TNF-α, IL-1β, and INF-γ induce the expression of iNOS (Hirsch et al., 2003), presumably mediated by a low-affinity IgE receptor CD23, which is expressed exclusively on glial cells in the SN of PD patients (Hunot et al., 1999). iNOS is responsible for NO production, contributing to neuronal toxicity (Dawson et al., 1993). Collectively, cytokine/CD23-dependent activation of iNOS in microglia may be involved in the cascade of events leading to dopaminergic neuronal degeneration (Hunot and Flavell, 2001). Moreover, TNF-α and IL-1β can upregulate COX-2, resulting in the production of PGE₂ and induction of an intraneuronal toxic effect directly on dopaminergic neurons (Mosley et al., 2006). Another neurotoxic effect may arise from the ROS production, mediated by the membrane-bound NADPH oxidase (Gao et al., 2003). ROS such as superoxide can react with NO to form the highly toxic peroxynitrite ONOO⁻, which causes nitration of tyrosine residues on cellular proteins, leading to both structural and functional alterations (Surendran and Rajasankar, 2010).

NEUROINFLAMMATION IN NEURODEGENERATIVE DISEASES

Alzheimer’s, Huntington’s and Parkinson’s disease are the examples of neurodegenerative diseases that are becoming more prevalent in today’s population. While the etiology of these diseases may differ, there is a common defining characteristic in which neuroinflammation is most common among these neurodegenerative diseases. Elevated levels of proinflammatory cytokines, upregulation of iNOS, COX-2 and activated microglia has been observed in PD patients in the SN and striatum (Whitton, 2007). However, neuroinflammation in these disorders were previously viewed as an epiphenomenon, where damaged neurons are able to induce proinflammatory response via glia cells (Farooqui and Farooqui, 2011; Skaper, 2007).

Numerous data has challenged this idea and are indicative that neuroinflammation may play a more prominent role in the onset in addition to disease progression (Bartels and Leenders, 2007; Lofrumento et al., 2010; Lull
and Block, 2010). In the CNS, glial cells, in addition to providing support to neuronal function, serve to respond to stress and insults by transiently upregulating inflammatory processes. Under normal circumstances, these responses are kept in check by other endogenous anti-inflammatory and neuroprotective mechanisms (Choi et al., 2009; Skaper 2007). In the diseased brain however, the dysregulation of the glial cells, in a self perpetuating manner (Choi et al., 2009; Hunter et al., 2007), inevitably promotes severe and chronic neuroinflammation that could lead to degeneration of the neurons which is now widely touted as the neuroinflammation hypothesis.

**CELLS INVOLVED IN NEUROINFLAMMATION**

a) **Microglia**

Microglia is generally found throughout the CNS and plays an integral part of the immune defense. These cells account for approximately 20% of the total glial population (Gehrmann et al., 1995) and in the adult mice, they predominate in the grey matter with the highest concentrations being found in the hippocampus, olfactory telencephalon, basal ganglia and SN (Block and Hong, 2007). They have a mesodermal origin and belong to the monocyte macrophage lineage. Under normal conditions, the resting microglia, with its ramified structure, is able to move and survey the environment to detect for any changes in the surrounding area, thus acting as the CNS first line of immune defense (Gao and Hong, 2008). In the event of an immunogenic stimuli or injury, the microglia is activated and functions similar to a macrophage. It was postulated that the activated microglia could be functionally discerned into two states, namely the phagocytic phenotype (innate activation) or an antigen presenting phenotype (adaptive activation) that could ultimately determine the range of cytokines that are produced (Halliday and Stevens, 2011). The activation of the microglia is accompanied by a significant morphology change (ameboid shape where the cells undergo shortening of cellular processes and enlargement of the soma). These activated microglia phagocytose the cellular debris or foreign materials. At the same time, they produce chemokines to attract more microglia, cytokines and factors that promotes microglia proliferation (Halliday and Stevens, 2011). Furthermore, the activated microglia
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Tightly regulated neuroinflammation is beneficial for recovery under certain circumstances. For instance, microglia has been shown to stimulate myelin repair, eliminate toxic proteins and avert neurodegeneration (Gao and Hong, 2008). However the problem arises when regulations of these inflammatory processes are derailed. Under such conditions, the activated microglia produce significantly large amount of cytotoxic factors such as superoxide (O$_2^-$), NO and TNF-α (Block and Hong, 2007). This excessive, uncontrolled inflammation, that induce an increase in cytotoxic factors, if left unchecked, could produce considerable damage to neighboring healthy tissue.

b) Astrocytes

Astrocytes were long believed to be structural cells as they make up to about 50% of human brain volume (Margakis and Rothstein, 2006). However in recent years, astrocytes have been shown to serve many housekeeping functions, including maintenance of the extracellular environment and stabilization of cell-cell communications in the CNS. Astrocytes are characterized by its star-shaped cells, which are important for amino acid, nutrient and ion metabolism in the brain, coupling of neuronal activity and cerebral blood flow and modulation of excitatory synaptic transmission (Barreto et al., 2011; Margakis and Rothstein, 2006). In the diseased state such as in multiple sclerosis and AD, activated astrocytes, are believed to facilitate leukocyte recruitment to the CNS by increasing leukocyte adhesion molecules and chemokine production (Moynagh, 2005). It is difficult to understand the contribution of astrocytes in inducing chronic neuroinflammation as it is functionally entangled with other cell types. However, there are evidences from genetic mutations in astrocytes able to mimic certain neurodegenerative diseases. In cells expressing the familial AD persenilin-1 mutation, calcium disturbances were found to occur at lower ATP and glutamate concentrations than in wild-type astrocytes, supporting the idea that the change in calcium signaling between astrocytes could ultimately contribute to dysfunction of neurons in a diseased state (Halliday and Stevens, 2011; Margakis and Rothstein, 2006). It has been shown that LPS stimulates astrocytes to produce
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PGs, complements C3, and cytokines (Li et al., 2011; Rappold and Tieu, 2010). These observations suggest that astrocytes may play an important role during immunological response as it shares many important functional characteristics with macrophages.

The hallmark of brain inflammation, also designated as neuroinflammation, is the activation of glial cells, mainly microglia. An intense glial (astrocytes and microglia) activation was observed in SN of PD patients (Hunot et al., 1997; Hunot et al., 1999; McGeer et al., 1988) and in animal models of PD (Hirsch and Hunot, 2009). A large number of studies have postulated that microglia and astrocytes play a key role in progressive degeneration of DA neurons in PD (Halliday and Stevens, 2011; Li et al., 2011). Although, these cells can have a neuroprotective function by the secretion of neurotrophic factors and elimination of cellular debris and/or pathogens, activated microglia can also trigger neuronal damage via release of pro-inflammatory and neurotoxic factors (Gonzalez-Scarano and Baltuch, 1999; Li et al., 2011). It is known that inflammatory or neurotoxic factors activate astrocytes released by reactive microglia and/or injured neurons. Reactive astrocytes secrete, primarily, neurotrophic factors, but they can also produce inflammatory and neurotoxic factors similar to those secreted by activated microglia (Gonzalez-Scarano and Baltuch, 1999). Therefore, both glial cells, depending on the degree of activation, can influence the fate of the injured neurons. In PD brain, the dying DA neurons and the alteration in the neurochemical environment can trigger microglia and astrocyte activation (Block and Hong, 2007; Litteljohn et al., 2010).

The NO in conjugation with COX is known to increase the production of proinflammatory PGs and exert highly toxic effects in neurons. Further, inflammatory stimuli and ROS activates NF-κβ in microglial cells, oligodendrocytes and neurons to promote the transcription of inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α), apoptosis-promoting factors (p53, Bax), COX-2 and iNOS (O'Neill and Kaltschmidt, 1997). Thus, inflammatory cytokines activate NO production via NF-κβ activation or direct promotion of iNOS transcription to lead to a cytotoxic cycle. Similarly, other studies have indicated the altered expression of pro-apoptotic genes, increased levels of p53 gene,
IFN-γ, suggesting apoptosis and neuroinflammation in the Parkinsonian brains (Mogi et al., 1994; Mogi et al., 2007). Aspirin and sodium salicylate, two NSAIDs are known to inhibit LPS and/or cytokine-induced NF-κβ activation and iNOS mRNA expression at therapeutic concentrations, and consequently reduce NO production (Aeberhard et al., 1995; Frantz and O'Neill, 1995).

A new strategy of anti-inflammatory drug therapy includes the dual inhibition of COX and LOX enzymes (Bishnoi et al., 2005; Li et al., 2008). Recently, Li and colleagues (Li et al., 2008) have shown that phenidone (a dual inhibitor of COX/LOX) was superior in attenuating the LPS-induced neurotoxicity as compared to either COX- or LOX-inhibitor given alone. Dual inhibition of COX/LOX may, therefore provide a novel therapeutic strategy to overcome neuroinflammation in neurodegenerative disorders. Earlier, in an in-vitro experiment, Klegeris and McGeer (2002) demonstrated that a combination of COX and 5-LOX inhibitors had greater therapeutic potential in protecting against mononuclear phagocyte neurotoxicity than single inhibitor of either class.

Better understanding of neuroinflammatory process triggered by environmental toxins such as 6-OHDA, MPTP, rotenone, LPS, etc., to elicit the degeneration of nigrostriatal dopaminergic neurons could provide the deep insight into initiating mechanisms and those that aid in the disease progression and the anti-inflammatory therapies to abandon them.

NEUROINFLAMMATION AND NEURITE OUTGROWTH

Activation of microglia has also been shown to induce cell death at high concentrations of endotoxins such as LPS and advanced glycation endproducts (AGEs) in-vitro (Münch et al., 2003). Although it is known that activated microglia is able to produce various cytotoxic factors. However, the exact mechanism through which these reactive glial cells induce neuronal death is not completely understood. At a sublethal dose of LPS or AGEs, they were reported to induce activation of microglia that can lead to a reduction of neurite outgrowth (Münch et al., 2003). More specifically, TNF-α has been shown to reduce neurite outgrowth and branching in the hippocampal neurons via small GTPase Rho proteins (Neumann et al., 2002). The reduction of neurite
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outgrowth during a mild inflammation (with an absence of T cell amplified systemic inflammation) with factors secreted by the activated microglia could interfere with the cytoskeleton reorganization. The change in synaptic reorganization has shown to induce learning and memory deficits even in the absence of cell death (Baxter et al., 1996). The reduction of neurite outgrowth has been linked to NO and NO-derived products. NO can directly regulate actin reorganization in the neurites, by inducing signaling cascades involved in growth cone collapse and through regulation of gene transcription (Münch et al. 2003).

**NEUROINFLAMMATION AND OXIDATIVE STRESS GENERATION**

Oxidative stress is a prevalent feature in numerous neurodegeneration diseases even though the source of ROS is still debatable (Block and Hong, 2007). In the microglia, the ROS production is catalyzed by the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme complex that converts $O_2$ to $O_2^-$. Distributed in both the cell membrane and membrane of organelles, the ROS generated under normal conditions has some beneficial functions as ROS generation plays a vital role in host defense. ROS are involved in cell defense against pathogens, but also in reversible regulatory processes in most cells and tissues (Bedard and Kraus, 2007). Hence, like the proinflammatory cytokines, the beneficial or detrimental effect of ROS lies on a fine balance.

In normal aging humans, the level of ROS increases with age as predicted by the “free-radical theory of aging” (Dröge, 2002; Gil Del Valle, 2011) and this increase in ROS levels is usually accompanied by a decline in cognitive and motor functions although not associated with a significant loss of neurons (Dröge and Shipper, 2007). Furthermore, a decrease in antioxidant enzymes and concentrations of small-molecular-weight antioxidants in blood and tissue cells, also induce an age-dependent elevation in the proportion of ROS and free radicals that are normally being removed (Gil Del Valle, 2011). The involvement of NADPH oxidases in aging has been linked to the increased level of ROS in the CNS (Krause, 2007). More interestingly neural damage induced by extracellular secretion of ROS has been shown to be mediated by NADPH oxidase through the activation of microglia (Qin et al. 2006). These
oxidative conditions are able to induce irreversible damage to proteins, lipids, carbohydrates and nucleic acids.

In AD and PD patients, NADPH oxidases were reported to be upregulated in the CNS (Block and Hong, 2007). In addition to the reduction in the concentrations of antioxidants present in the system, most patients suffering from AD and PD also experience an increase in ROS production, further uncoupling the redox balance in the CNS. The excessive ROS in the system could ultimately trigger the mitochondrial apoptosis pathway, inducing a mitochondrial dysfunction by the release of cytochrome-c into the cytoplasm (Ng, 2008). Thus, during chronic neuroinflammation, the increased ROS production induces cognitive deficits as well as is able to trigger the apoptotic pathway that culminates neuronal death. The generation of ROS, is reported to act as a common signaling mechanism for phagocytes where the gangliosides activate microglia through protein kinase-C and NADPH oxidase (Min et al. 2004). Furthermore, changes in the morphology and proliferation of microglia (microgliosis) are regulated by H$_2$O$_2$ produced from NADPH oxidase (Lull and Block, 2010). In return, higher levels of ROS in the intracellular positively regulate the inflammatory response where an increased production of pro-inflammatory response is able to affect the cell survival by increasing lipid peroxidation and protein nitration (Levesque et al., 2010). Hence, it seems that the catalytic events of NADPH oxidase in the activated microglia are essential contributors of oxidative stress and inflammation that in extreme conditions could lead to neuronal damage and ultimately affect cognitive ability.

NEUROINFLAMMATION AND NEUROGENESIS

Neuroinflammation has also been shown to induce a blockade in neurogenesis (Monje et al., 2003). Neurogenesis refers to the birth of new neurons that occur within the CNS. In the hippocampus, neurogenesis continues throughout life and its amount correlates closely with the hippocampal functions of learning and memory (Monje et al., 2003). Any disruption to the environment of these proliferating neural stem or progenitor could lead to a disruption of neurogenesis and ultimately cognitive deficits. In patients receiving therapeutic cranial radiation therapy, a decline in cognitive function has been reported due to ablation of cell proliferation in the CNS.
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(Monje and Palmer, 2003). LPS administration has reported to induce an increase in central pro-inflammatory cytokine production, which decreased the hippocampal neurogenesis (Monje et al., 2003). This disruption of neurogenesis by LPS was also shown to be able to induce spatial learning and memory deficits task (Wu et al., 2007). The direct mechanism as to how neuroinflammation is able to induce a disruption to neurogenesis has yet to be fully elucidated. However, it is hypothesized that inflammatory cytokines such as IL-6 and TNF-α indirectly inhibit cell proliferation and neurogenesis in the dentate gyrus by increasing the levels of circulating glucocorticoids via centrally stimulating the hypothalamic-pituitary adrenal (HPA) stress axis (Vallières et al., 2002). It was suggested that glucocorticoids could affect cell proliferation by directly repressing the transcription of cyclin-D1, a common cell-cycle regulator that controls G1-S phase transition, by binding to the promoter and affecting the β-catenin/TCF pathway (Boku et al., 2009).

An increase in COX-2 expression in the granular cell layer and blood vessels, areas that are known to be neurogenic in the dentate gyrus was observed after LPS treatment. The involvement of COX-2 was associated with a decrease in newborn cell survival but not cell differentiation where the number of 5-bromo-2-deoxyuridine (BrdU) labeled cells decreased significantly after LPS treatment (Bastos et al., 2008). COX-2 may modulate neurogenesis in the dentate gyrus through the generation of PGs such as PGE₂ and PGD₂ that induces apoptosis in a variety of cell types (Bastos et al., 2008). However, the involvement in COX-2 in reducing cell proliferation is still under investigation as other studies have reported that the reduction of the number of newborn neurons were associated with neuronal differentiation rather than neuronal proliferation. Inflammatory mediators such as IL-6, TNF-α and IL-18 were reported to induce an increase in glial differentiation (Liu et al. 2010). This suggests the complexity of the effect of neuroinflammation in neurogenesis.

CURRENT STATUS OF ANTI-INFLAMMATORY DRUGS

Inflammation is a complex biological process mediated by variety of mediators. Of these mediators, PGs arising from the COX cascade have been extensively studied over the years. The outcome of this has been the basis of the use of NSAIDs in inflammatory disorders (Furst, 1999). In 1990s, the
breakthrough discovery that COX exists in two different isoforms namely COX-1 and COX-2 attracted the renewed interest to understand the role of two isoforms in the regulation of PG synthesis and their biological action in normal and pathophysiological conditions (Vane, 1998). A third isoforms COX-3 has been discovered with its skeptic expression in humans (Burdan et al., 2006; Kis et al., 2005). Subsequently, COX hypothesis was proposed, which suggested that COX-2 isoform was induced mainly during inflammation whereas, constitutive COX-1 has housekeeping properties of maintaining gastric mucosa, kidney functions, etc. The beneficial effects of anti-inflammatory drugs are ascribed to due to their COX-2 inhibitory action, while the undesirable gastrointestinal side effects are due to inhibition of COX-1 enzyme (Bombardier et al., 2000). This gave rise to the discovery of selective COX-2 inhibitors known as “coxibs”, rofecoxib, celecoxib, etoricoxib, parecoxib, etc. (Warner and Mitchell, 2004). Coxibs, because of their gastroprotective properties became most widely used medications worldwide until recent reports led to their withdrawal due to certain cardiovascular complications. This led to set back on the development of selective COX-2 inhibitors.

Arachidonic acid (AA) is also a substrate for another enzyme namely 5-lipoxygenase (5-LOX). The 5-LOX pathway is involved in the generation of leukotrienes (LTs) which mediate their action by acting on LT receptors. The LTs thus formed are responsible for the vascular permeability changes during acute inflammation. The inhibition of COX enzyme favors AA metabolism towards LOX pathway and therefore, treatment with NSAIDs increases the formation of LTs possibly leading to gastric damage (Funk, 2001). Co-medication of 5-LOX inhibitors or LT receptor antagonists with NSAIDs has been shown to be beneficial not only in relieving pain and inflammation but also in preventing or reducing NSAID-induced gastric damage (Martel-Pelletier et al., 2003). In the following page a brief review of COX and LOX pathways, their metabolites and their role in pathophysiology to pain and inflammation has been described. Newer strategy in the development of dual inhibitors as therapeutic approach has also been discussed.
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THE COX PATHWAY

COX pathway involves oxygenation of AA to prostanoids such as prostaglandins, prostacyclin and thromboxanes (Vane and Botting, 1998) (Fig. 12). COX, the enzyme that catalyzes a rate limiting formation of PGs was located in 1976 and cloned in 1988 (Smith et al., 2000). It possesses two distinct catalytic activities: a COX activity which catalyzes the transformation of AA to PGG$_2$ by reaction with two molecules of oxygen, and a peroxidase activity which reduces 15S-hydroperoxide PGG$_2$ to 15S-alcohol PGH$_2$. Following this, several isomerases catalyze the transformation of PGH$_2$ into different PGs (PGD$_2$, PGE$_2$, PGF$_{2\alpha}$). Prostacyclin synthase catalyzes the conversion of PGH$_2$ into PGI$_2$, and thromboxane synthase catalyzes the transformation of PGH$_2$ into thromboxane A$_2$ (TXA$_2$), respectively (Smith et al., 2000).

Fig. 12. Representative pathway of arachidonic acid metabolism and biosynthesis of prostaglandins
ISOFORMS OF COX

In the early 1990s, it was shown that the COX enzyme existed in two isoforms: COX-1 and COX-2 (Smith et al., 2000). Recently a third isoform, COX-3, has been discovered (Davies et al., 2004; Willoughby et al., 2000); however, its expression has not been proven in humans at present. Thus, COX-1 is classified as a constitutive enzyme responsible for basal physiological functions, whereas COX-2 has been classified as an inducible enzyme, which is primarily involved in inflammation (Kulkarni and Dhir, 2009; Smith et al., 2000). Similar to COX-1 and COX-2, COX-3 isoform is considered to be associated with PG-induced pain and inflammatory processes. However, the initial speculation that acetaminophen (paracetamol) acted through inhibition of the COX-3 isoform (Kis et al., 2005) has now been questioned (Hinz et al., 2008).

COX is expressed in the neurons and the glial cells of the brain. The first research group to localize COX-1 in neurons and glial cells in monkey brain were Tsubokuro and coworkers (1991), while Yamagata and colleagues first described the basal COX-2 expression in rat brain (Yamagata et al., 1993). In brain tissue, very less detectable levels of COX-2 mRNA are seen under normal conditions. However, the highest level of COX expression is observed in olfactory bulbs, followed by midbrain and hypothalamus, with the lowest levels being in hippocampus (Kulkarni and Dhir, 2009).

Immunocytochemical analysis of mixed cortical cell cultures reveals that COX-2 expression is restricted to neurons, whereas COX-1 is expressed in both neurons and astrocytes (Hewett et al., 2000). COX-1 is primarily expressed in the dorsolateral tegmentum, the dentate gyrus, the superior colliculus, distinct hypothalamic regions, the hippocampus, the raphe nuclei and the nucleus of the solitary tract (Norton et al., 1996).

PHARMACOLOGICAL AGENTS THAT INHIBIT COX PATHWAY

NSAIDs bind to the active site of COX enzyme which catalyzes the transformation of AA to PGs (Vane and Botting, 2003). Broadly, NSAIDs are classified as COX-1 or COX-2 selective based on their selectivity to COX isoforms (Kulkarni et al., 2000, Kulkarni and Dhir, 2009) (Table 1). The
classical NSAIDs like aspirin, indomethacin, ibuprofen and naproxen have no enzyme selectivity and inhibit both COX-1 and COX-2 with similar potency. According to COX hypothesis, these drugs exert anti-inflammatory action by blocking COX-2 activity and at the same time produce unwanted side effects, mainly GI disturbance and renal toxicity due to the inhibition of COX-1 activity (Smith et al., 2000).

Subsequently, selective COX-2 inhibitors were developed that promised to have a similar anti-inflammatory, antipyretic and analgesic activities as that of classical NSAIDs, but without GI complications (Kulkarni et al., 2000). The first type of drugs that were developed included compounds like meloxicam, nimesulide, and etodolac, which has preferential COX-2 inhibiting property. These compounds have a partisan effect of COX-2, although they are devoid of COX-1 inhibitory activity (Kulkarni et al., 2000). The favorable safety profile of these compounds over classical NSAIDs has been observed in few clinical studies (Bombardier et al., 2000; Silverstein et al., 2000).

<table>
<thead>
<tr>
<th>Class</th>
<th>Properties</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>NSAIDs that inhibit both COX-1 and COX-2 completely with little selectivity</td>
<td>Aspirin, Ibuprofen, Diclofenac, Naproxen, Indomethacin, Piroxicam</td>
</tr>
<tr>
<td>Group 2</td>
<td>NSAIDs that inhibit COX-2 with a 5-50 fold selectivity</td>
<td>Celecoxib, Etodolac, Meloxicam, Nimesulide</td>
</tr>
<tr>
<td>Group 3</td>
<td>NSAIDs that inhibit COX-2 with a &gt;50 fold selectivity</td>
<td>Rofecoxib, NS-398</td>
</tr>
<tr>
<td>Group 4</td>
<td>NSAIDs that are weak inhibitors of both isoforms</td>
<td>5-Aminosalicylic acid, Sodium salicylate, Nabumetone, Sulfasalazine</td>
</tr>
</tbody>
</table>
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The second type of COX-2 inhibitors developed, celecoxib and rofecoxib, popularly known as “coxibs” has selective COX-2 inhibitory action with little or no COX-1 inhibitory activity (Fig. 13.). The first generation of coxibs was approved by USFDA in 1999 for clinical use (Talley et al., 1999; Warner and Mitchell, 2004). The clinical trials (Phase III) showed that celecoxib and rofecoxib were significantly better than placebo but as effective as ibuprofen, diclofenac or naproxen with lower incidence of ulcers (Bombardier et al., 2000). Following these results, a number of COX-2 selective agents termed as second generation coxibs were approved for clinical use. These included valdecoxib, parecoxib (Talley et al., 1999), etoricoxib (Riendeau et al., 2001), lumiracoxib (Ding and Jones, 2002). However, the use of selective COX-2 inhibitors has come under scrutiny with alarming reports of cardiovascular and GI side effects (http://www.fda.gov/cder).

![Fig. 13. Structure of various selective COX-2 inhibitors](image)

**COX AS THERAPEUTIC TARGET**

a) Gastrointestinal tract – Inhumans and other species, it has been shown that COX-1 but not COX-2 is expressed constitutively throughout the GI tract (Karman et al., 1996). PGs such as PGE\(_2\) and PGI\(_2\) produced by COX-1 are known to exhibit cytoprotective effects on the GI mucosa by reducing gastric acid secretion by parietal cells in the stomach, increase mucosal blood flow, and stimulate the release of viscous mucus. Selective COX-2 inhibitors are efficient anti-inflammatory agents with less GI toxicity due to their selective inhibition of COX-2 and sparing action on COX-1. However, there are reports of constitutive COX-2 expression in healthy human and rabbit GI mucosa (Zimmermann et al., 1998). It has also been reported that during the
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Glulcer formation process, COX-2 may be induced and that it could play a role in the GI healing (Mizuno et al., 1997). Clinical trials have indicated short term GI safety benefits of selective COX-2 inhibitors compared to traditional NSAID therapy (Bombardier et al., 2000; Schnitzer et al., 2004). However, long term studies are limited and inconclusive.

b) Kidney – PGs regulate vascular tone and normal blood flow thereby maintaining renal function (Katori and Majima, 2000). Studies using animal models of renal diseases, and patients with congestive heart failure, liver cirrhosis or renal insufficiency have shown that PGE2 was primarily responsible for maintaining normal kidney function (Vane and Botting, 1998). In humans, COX-1 is constitutively expressed in the vasculature, the collecting ducts and the loop of Henle, whereas low levels of COX-2 are expressed constitutively in the macula densa, epithelial cells lining the ascending loop of Henle and medullary interstitial cells of the renal papillae (Vane, 1998; Katori and Majima, 2000). The COX-2 enzyme is involved in normal renal development and COX-2 deficient mice develop severe nephropathy (Dinchuk et al., 1995; Morham et al., 1995). Studies have shown that NSAID-induced sodium retention in healthy and elderly patients is mediated by the inhibition of COX-2, whereas a decreased glomerular filtration rate is associated with inhibition of COX-1. These studies confirm that both COX isoforms are involved in renal physiology (Ye et al., 2006). Earlier studies have indicated, among a group of current selective COX-2 inhibitors, that rofecoxib is associated with increased renal and arrhythmia risks (Zhang et al., 2006; Harris and Breyer, 2006).

c) Cardiovascular system – It is well known that the COX-1 isoform is constitutively expressed in platelets and is responsible for the formation of pro-aggregatory TxA2. In contrast, the synthesis of anti-aggregatory PGI2 in endothelial cells is primarily catalyzed by COX-2 (McAdam et al., 1999). Aspirin acts as an irreversible inhibitor of COX-1 in platelets by acetylating the Ser530 residue. This leads to blocking of TxA2 synthesis resulting in a reduced risk of thrombosis. COX-mediated vascular control has been demonstrated in COX-1 and COX-2 knock out animal models. Mice deficient in COX-2 die within 48 hours after birth with a patent ductus arteriosus. Similarly, mice deficient in both isoforms of COX die within 12 hours of birth due to a similar condition (Loftin et al., 2001). Reports demonstrated certain cautions
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regarding the use of COX-2 inhibitors in patients due to risk of cardiovascular morbidity such as myocardial infarction (Mukhejree et al., 2001). Although selective COX-2 inhibitors have no effect on TxA2 production, by decreasing PGI2 production, selective COX-2 inhibitors may tip the natural balance between prothrombotic TxA2 and anti-inflammatory PGI2 that could potentially increase the possibility of atherothrombotic cardiovascular event. In addition, there are other indications of protective role for PGE2 and PGI2 derived from the COX-2 pathway pertaining to oxidative damage (Mitchell and Warner, 2006). Accordingly, both COX-1 and COX-2 derived PGs appear to have a profound role in the regulation of vascular homeostasis. The VIGOR trial for rofecoxib showed increased risk of cardiovascular events compared to naproxen (Bombardier et al., 2000). However, the CLASS trial for celecoxib and the TARGET trial for lumiracoxib did not indicate an increased risk of cardiovascular events (Bombardier et al., 2000; Silverstein et al., 2000; Schnitzer et al., 2004). While the debate continues on cardiovascular risks associated with COX-2 inhibitors, indications are clear that COX-2 selective inhibitors as a class are associated with cardiovascular risks (Mitchell and Warner, 2006). Cheng and coworkers (2006) investigated the mechanisms by which COX-2 inhibitors increase the risk of myocardial infarction. Their studies demonstrated that selective inhibition, knockout, or mutation of COX-2, or deletion of the receptor for COX-2 derived PGI2, was shown to accelerate thrombogenesis and elevate blood pressure in mice. These responses were attenuated by COX-1 knock down, which mimics the beneficial effects of low-dose aspirin. In addition, these authors suggested that inhibition of microsomal PGE synthase-1 (mPGES-1) may exhibit efficient anti-inflammatory activity with no adverse cardiovascular events (Cheng et al., 2006).

d) Cancer – Several reports have shown that traditional NSAIDs exhibit anticancer activities. Sulindac and indomethacin exhibited protective effects against colorectal cancer (Waddell and Loughry, 1983). After the discovery of the COX-2 isoform, several studies have shown that COX-2 is expressed at high levels in a wide variety of cancer tissues, such as colon, breast, prostate, and pancreas, and appears to control many cellular processes. Selective COX-2 inhibitors have been extensively studied in the treatment and prevention of a variety of cancers (Hahn et al., 2010; Kanaoka et al., 2007; Li et al., 2011).
anticancer activity exhibited by NSAIDs and selective COX-2 inhibitors could be associated with multiple COX-dependent and COX-independent pathways (Bundschuer et al., 2010; Zhu et al., 2002). The selective COX-2 inhibitor celecoxib induces apoptosis in human prostate cancer cell lines (PC-3) expressing COX-2 by blocking antiapoptotic kinase Akt activation, and the antiangiogenic activity of COX-2 inhibitors may constitute another mechanism to prevent tumor growth (Pang et al., 2007). The clinical efficacy of COX-2 inhibitors in preventing the recurrence of colon polyps was investigated but was stopped early because of an increase in adverse cardiovascular events (Bresalier et al., 2005). These findings have put a shadow of doubt on the long term use of selective COX-2 inhibitors in chemoprevention.

e) Central nervous system – The use of NSAIDs has been associated with a delay in the onset of AD and PD (Breitner et al., 1995; Asanuma et al., 2003; Esposito et al., 2007; Etminan et al., 2008). Since these diseases are associated with inflammatory conditions in brain, the protective effect provided by NSAIDs is consistent with their anti-inflammatory activity. Initially, selective COX-2 inhibitors were touted as a potential therapy to treat AD since long term treatment using NSAIDs leads to GI toxicity (Sandson and Felician, 1998). Since PD progression has an inflammatory pathology, studies on mice deficient with COX-2 exhibited resistance in animal models of PD (Feng et al., 2002; Teismann et al., 2003). These results showed that COX-2 plays an important role in animal models of dopaminergic neuron degeneration (Feng et al., 2002). Pzedborwski and coworkers described the pathological role of COX-2 in the development of PD where they examined the role of increased levels of COX-2 in generating a toxic DA quinonespecies which was responsible for dopaminergic neuronal degeneration. Studies have shown that the selective COX-2 inhibitor paracoxib, rofecoxib, valdecoxib exhibits neuroprotective activity in animal models of PD (Aguirre et al., 2008; Gupta et al., 2009; Gupta et al., 2011). Moreover, COX-2 has been reported to mediate microglial activation and secondary dopaminergic cell death in mouse MPTP model of PD (Vijitruth et al., 2006). Other studies have shown the beneficial effect of COX-inhibitors in various neuronal disorders such as epilepsy, depression, drug-addiction, and stress related pathologies (Naidu and Kulkarni, 2002; Dhir et al., 2007; Akula et al., 2008).
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However, the long term cardiovascular and hepatic toxicities associated with selective COX-2 inhibitor therapy makes their application in CNS disorders questionable (Brophy, 2007; Teoh and Farrell, 2003; Traversa et al., 2003).

Recent evidences indicate that, beside COX derived arachidonic acid metabolites, a number of other mediators are involved in producing and maintaining inflammation. Most NSAIDs inhibit COX activity without decreasing the generation of LTs via LOX pathway. This newer concept of 5-LOX in pathological conditions with prospects of its inhibition is being considered by researchers. The development in this aspect is discussed below:

THE LOX PATHWAY

Lipoxygenase (LOX) are a family of structurally related non-heme iron containing dioxygenases that catalyze the addition of molecular O₂ to polyunsaturated fatty acids with a (2,2)-1,4-pentadiene structural unit to give an unsaturated fatty acid hydroperoxide. The reaction is stereo and regio- specific. LOX may tightly control a reaction with molecular O₂ or otherwise forms mixed products and release free radicals (Brash, 1999). The major biochemical steps catalyzed by LOX are summarized in Fig. 14.

The LOXs are classified according to the position in which they oxidize arachidonic acid. Although three mammalian oxygenases, 5-LOX, 12-LOX, and 15-LOX exist, the most biologically important is the 5-LOX. It is principally found in cells of myeloid origin, such as polymorphonuclear leukocytes, macrophages, eosinophils, mast cells, monocytes, basophils, and B lymphocytes that are involved in inflammatory and immune reactions. 5-LOX has been purified from several sources and in each case activity is dependent on Ca²⁺ and ATP, a feature that distinguishes the enzyme from other LOXs. It catalyses the initial step in LT biosynthesis, converting arachidonic acid to the stereospecific molecule, (5S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid to form 5-HPETE, or 5-hydroperoxy eicosatetraenoic acid (Fig. 14.)
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**ARACHIDONIC ACID**

- 5-HETE
- 5-HPETE

**Lipoxygenase**

- **SLipoxygenase**
  - (membrane bound)
  - 5-Lipoxygenase (cytoplasmic)

**Fig. 14. Lipoxygenase pathway for the biosynthesis of leukotrienes**

It also catalyses the stereospecific removal of a hydrogen with radical migration and the formation of the unstable epoxide, LTA₄. The conversion of LTA₄ to the potent chemo-attractant, LTB₄, is catalyzed by the enzyme LTA₄ hydrolase, while the conjugation of LTA₄ with reduced glutathione to form LTC₄ is catalyzed by the enzyme LTC₄ synthase. The glutamyltranspeptidase enzyme catalyses the conversion of LTC₄ to LTD₄ through the conversion of the conjugated tripeptide, glutathione, to the conjugated dipeptide, cysteinylglycine. LTD₄ is biologically more potent than LTC₄. The enzyme LTD₄ dipetidase converts LTD₄ to LTE₄. LTC₄, LTD₄, and LTE₄ are also called peptidoleukotrienes, or cysteinyl-LTs (CysLTs).
5-LOX is normally present in the cytoplasm, but in response to elevation of intracellular Ca\(^{2+}\), the enzyme translocates to the cell membrane and links to a transmembrane protein, FLAP (five-LOX activating protein) (Dixon et al., 1990). The enzyme arachidonate-12-LOX catalyzes the transformation of arachidonic acid into 12-hydroperoxyeicosatetraenoic acid (12-HPETE). This enzyme has been found in human platelets, human erythroleukemia cells, and endothelial cells of human umbilical vein. The enzyme arachidonate-15-LOX catalyzes the biosynthesis of 15-hydroxyeicosatetraenoic acid (15-HETE), that is subsequently converted to lipoxins by neutrophils.

LTs are potent mediators of inflammation. Over a range of different concentrations, LTB\(_4\) causes: neutrophil, eosinophil, lymphocyte and monocyte chemotaxis; neutrophil and eosinophil aggregation; the induction of neutrophil degranulation and lysosomal enzyme release; the induction of neutrophil–endothelial cell adhesion; the modulation of pain induced by inflammatory reactions; and the modulation of certain immune responses. These responses may be mediated, in part, through the generation of cell cytokines: it has been described that the activation of NF-κB responsible for the expression of pro-inflammatory cytokines, such as TNF-α, and of adhesion molecules requires reactive O\(_2\) intermediates produced by the 5-LOX cascade (Wallace et al., 1993). LTs have been implicated as important mediators of chronic inflammation and joint destruction in experimental models of rheumatoid arthritis (Brahn, 1991); blood and synovial fluid levels of LTB\(_4\) are elevated in patients with rheumatoid arthritis (Grespan et al., 2008). LTB\(_4\) is the most obvious candidate for the inflammatory lesions created by neutrophil observed in the colonic and small bowel mucosa in ulcerative colitis and Crohn’s disease (Bertolini et al., 2001). In fact, human colonic epithelial cells are capable of synthesizing LTs, and LTB\(_4\) levels are elevated in colonic mucosa biopsies removed from patients with these two disorders. Finally, in animal models of immune-mediated glomerulonephritis, LTB\(_4\) has been associated with neutrophil infiltration and degranulation in the glomeruli (Bertolini et al., 2001).

The CysLTs have powerful spasmogenic actions, especially in airway smooth muscle and in the vasculature, and are released during asthmatic attacks, inflammation, rheumatoid arthritis, and hypersensitivity reactions.
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These mediators are at least 100 times more potent than histamine as bronchoconstrictors in man when administered by aerosol (McMillan and Walker, 1992). The vasoconstriction in the gastric mucosa causes an important reduction of the blood flow and accounts for the ulcerogenic effect of CysLTs (Guslandi, 1987). Several experimental data support the postulate that COX inhibition by NSAIDs, besides causing a reduction in the synthesis of vasodilatory and gastroprotective PGs, diverts arachidonate to the 5-LOX pathway, thus increasing the formation of LTs and CysLTs (Rainsford, 1987; 1993; 2007). This causes vasoconstriction of the gastric mucosa and increases the formation of free radicals from the peroxidative cleavage of hydroxyeicosatetraenoic acids (Wallace et al., 1993) with further mucosal injury. Indeed, gastric and intestinal mucosal lesions by NSAIDs are prevented by the concurrent administration of 5-LOX inhibitors (Steinhilber, 1999).

**LEUKOTRIENE AS THERAPEUTIC TARGET**

**(a) Allergic disease** – Since the original description of the slow reacting substances of anaphylaxis (mixture of LTC₄, LTD₄ and LTE₄), the LTs have been demonstrated to play an important role in allergic diseases of airway. Early studies reported presence of LTC₄, LTD₄, LTE₄ in bronchoalveolar lavage fluids (Zaitzu et al., 1998), plasmas (Shiratsuki et al., 1990) or urine (Taylor, 1994) of patients with antigen-induced asthma. Patients with rhinitis exposed to dry or cold air showed accumulation of LTs in nasal lavage fluids (Leval et al., 2002). Zouboulis and coworkers (2003) reported the potential involvement of LTB₄ in the development of acne where it induced the cell proliferation, differentiation and apoptosis of sebaceous glands in skin.

**(b) Nociception** – Unlike PGs, the role of LTs has been less extensively studied in terms of their role in pain and hyperalgesia. LTs are known to sensitize sensory neurons and also activate extracellular signal-regulated secondary responses. LTB₄ challenge by different routes results in significant hyperalgesia (Levine et al., 1984). The potential involvement of CysLTs in nociception has been reinstated in animal models of nociception (Jain et al., 2001). However, targeting LTs synthesis or their action for the relief of pain is still in infancy and requires an elaborative understanding.
(c) Inflammation – LTs are potent mediators of inflammation. LTB\(_4\) is a potent stimulator of leukocyte activation and adhesion of these cells to vascular endothelium elicits chemokinetic and chemotactic responses. LTB\(_4\) has been implicated in pathogenesis of certain inflammatory diseases (rheumatoid arthritis, ulcerative colitis) where in it stimulates the production and release of proinflammatory cytokines from macrophages, lymphocytes or synovial membrane (Rainsford et al., 1996; Silva et al., 2010). CysLTs also contribute to inflammation by provoking vasoconstriction and increase in the permeability of post capillary venules. This allows leakage and migration of fluid and proteins into inflammatory site (Wallace and Ma, 2001). However, further experiments are needed to establish the relative role of LTs in various inflammatory conditions.

(d) Gastrointestinal system– Experimental trials have demonstrated the participation of LTs in GI damage. LTs induce microvascular injury, gastric vessel vasoconstriction, and promote breakdown of mucosal barrier. In GI tract, LTs stimulate the secretion of gastric acid, and cause the release of of IL-1, and proinflammatory cytokines from gastric cells (Martel-Pelletier et al., 2003). Colon tissues from animals and patients with ulcerative colitis showed presence of LTs and the use of 5-LOX inhibitors ameliorated the intestinal inflammation (Xu et al., 2009).

(e) Cancer – Over expression of LOX enzymes and enhanced production of LTs has been demonstrated in several types of cancer. Both 5-LOX and platelet type 12-LOX enzyme are substantially expressed in human pancreatic cancer cell lines (Tong et al., 2005). The use of 5-LOX selective inhibitors inhibited lung cancer proliferation in-vivo and suppressed mouse skin carcinogenesis (Oi et al., 2010; Schroeder et al., 2007; Shureiqi and Lippman, 2001) suggesting that LOX pathway could be promising therapeutic target in cancer treatment.

(f) Neurodegeneration and aging – Similar to COX, LOX enzyme too has a pivotal role in physiological functioning and inflammation of cerebral vasculatory system and inflammatory processes (Zhu et al., 2010). Evidences suggest that 5-LOX expression may be enhanced during a stimuli-evoked neurodegeneration. 5-LOX has been reported to be expressed in the brains of animals exposed to reperfusion (Zhang et al., 2003) or excitotoxic brain injury.
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(Phillis and O’Regan, 2003). 5-LOX has been demonstrated to significantly contribute to membrane lipid peroxidation, cytotoxicity and neurodegeneration (Manev et al., 2000). Although there are various experimental evidences that postulate the role of 5-LOX pathway and LTs in neuronal disorders and age-related pathological changes, no clinical study has yet been reported in this regard.

**PHARMACOLOGICAL AGENTS THAT INHIBIT LT SYNTHESIS**

In view of their pathophysiological properties, interventions with the biosynthesis or the action of LTs propose a therapeutic benefit in a variety of allergic and inflammatory diseases. Anti-LT therapy applies two basic pharmacological strategies: suppression of the biosynthesis of LTs and the use of LT receptor antagonists. Suppression of LT synthesis can be achieved (I) by inhibition of phospholipases releasing the precursor arachidonic acid, (II) direct inhibition of 5-LOX and (III) inhibition of FLAP.

The various compounds that possess LOX inhibiting property have been summarized in Table 2.
## Table 2. Various compounds inhibiting the LOX pathway

<table>
<thead>
<tr>
<th>Compound</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-861</td>
<td>Potent, selective and orally active 5-LOX inhibitor, shown to enhance and accelerate fracture healing, increase Ca^{2+} concentration in MDCK cells, and is used to probe fatty acid metabolism and LT production.</td>
</tr>
<tr>
<td>Auranofin</td>
<td>A gold(I)-phosphine thiolate small molecule. Blocks the release of pro-inflammatory mediators from basophiles and mast cells. Inhibits mitochondrial thioredoxin reductase and 5-LOX. Quenches reactive singlet oxygen species</td>
</tr>
<tr>
<td>Baicalein</td>
<td>A flavone that inhibits Ca^{2+} uptake, certain types of LOX, LT biosynthesis and the release of lysosomal enzymes. It is a potent anti-inflammatory agent.</td>
</tr>
<tr>
<td>BW A4C</td>
<td>Selective 5-LOX inhibitor, inhibits synthesis of LTB_{4}</td>
</tr>
<tr>
<td>BW B70C</td>
<td>A selective 5-LOX inhibitor. Reported to decrease 12-HETE formation, LTC_{4} synthesis</td>
</tr>
<tr>
<td>Caffeic Acid</td>
<td>Inhibits 5-LOX (IC_{50}=3.7 μM). At 2.2 μM, caffeic acid selectively inhibits 15-LOX.</td>
</tr>
<tr>
<td>Esculetin</td>
<td>A phenolic compound that acts as a 5-LOX inhibitor. Reported to display anti-inflammatory and antioxidant properties, as well as induce cell cycle arrest and apoptosis</td>
</tr>
<tr>
<td>L-655,238</td>
<td>Potent and selective inhibitor of 5-LOX-activating protein (FLAP).</td>
</tr>
<tr>
<td>MK-886 sodium salt</td>
<td>A potent and selective inhibitor of 5-LOX-activating protein (FLAP). Binds to FLAP with high affinity and prevents the activation of 5-LOX.</td>
</tr>
<tr>
<td>Zileuton</td>
<td>Orally active 5-LOX inhibitor that inhibits LTB_{4} synthesis. Inhibits antigen-induced contraction of tracheal strips in-vitro and shows antiasthmatic activity in-vivo.</td>
</tr>
</tbody>
</table>
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DUAL INHIBITORS OF COX AND 5-LOX

An interesting paper published in 1996 by Nickerson-Nutter and Medvedeff observed that combination of LT synthesis and naproxen, a non-selective COX inhibitor, produced a significant reduction in the collagen-arthritis in mouse. No compound alone produced any inhibition of arthritis (Nickerson-Nutter and Medvedeff, 1996). This observation was the beginning of new era in the therapeutic approach i.e. use of inhibitors of COX and 5-LOX in the management of inflammatory disorders. Various sites of NSAID action in the arachidonic acid pathway has been schematically represented in Fig. 15.

Fig. 15. Various sites for NSAIDs action in the arachidonic acid pathway

Both conventional NSAIDs and selective COX-2 inhibitors primarily exert their effect by reducing the production of PGs that are induced during an inflammatory event. In recent years, it has been clarified that along with PGs, other key lipid mediators, LTs and LXs complimentarily participate in the development and persistence of inflammatory processes. Since LTs are non-responsive to the effect of NSAIDs, it is suggested that simultaneous dual inhibition of COX and 5-LOX pathways might have synergistic effects and
archive optimalwider spectrum of anti-inflammatory effect (Martel-Pelletier et al., 2003). Studies have demonstrated that the presence of COX (COX-1/2 alone or combined) inhibition, LTs (LTB₄ and CysLTs) generated via a shunt pathway induced chemotaxis and local vasoconstriction that enhances the local blood flow to gastric mucosa (Wallace et al., 1990). Thus the dual inhibitors of COX and 5-LOX may have two theoretical advantages of stronger anti-inflammatory and a protective effect on GI mucosa.

In addition to this, COX and LOX dual inhibition does not block the 12-LOX or 15-LOX pathways, which contribute to the formation of anti-inflammatory LXs (Martel-Pelletier et al., 2003). Apart from these two advantages the dual inhibition approach of inhibiting COX-1/2 and 5-LOX might also provide additional cardioprotection. Platelets and leukocytes on endothelial cells constitute an early mechanism of vascular inflammatory damage and consequent vessel occlusion. Various dual COX/LOX inhibitors have been summarized in Table 3.

**Table 3. Dual COX/LOX inhibitors used in experimental practice**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Property</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>Inhibits NF-κB, JNK, 5-LO, COX-2, and blocks amyloid peptide induced expression of TNF-α, IL-1β, MCP-1, IL-8, and CCR5.</td>
</tr>
<tr>
<td>ETYA</td>
<td>A competitive inhibitor of arachidonic acid metabolism and secretory response of isolated rat pancreatic islets to arginine and glucose.</td>
</tr>
<tr>
<td>Phenidone</td>
<td>Inhibits both LOX and COX pathways, the synthesis of Fos-related antigen protein, and is described as an anti-inflammatory and anti-oxidant compound.</td>
</tr>
<tr>
<td>Licofelone</td>
<td>One of the most promising and potent dual inhibitor of COX and 5-LOX enzyme</td>
</tr>
</tbody>
</table>


Inagaki and colleagues (2000) found a novel anti-arthritic agent, S-2474 which displayed a dual inhibition of COX/5-LOX with good selectivity toward COX-2 inhibition, like celecoxib. In rats, it exerted excellent anti-inflammatory activity without ulcerogenic effects and showed cytokine-modulating properties in THP-1 cells (Inagaki et al., 2000). Another compound, ER-34122, 5-[(1,5-bis(4-methoxyphenyl)pyrazol-3-yl)dimethoxymethyl]-2-chlorobenzamide, is an orally active dual inhibitor of COX/5-LOX, and has shown an enhanced anti-inflammatory effect in mice in vivo when compared with indomethacin (Horizoe et al., 1998). These promising data have been further confirmed by the finding that ER-34122 suppresses polymorphonuclear neutrophil (PMN) infiltration, subsynovial soft tissue oedema, and multiplication of synovial lining cells in the early stage of arthritis in MRL/lpr mice (Horizoe et al., 1998). Tepoxalin, another dual inhibitor compound, significantly inhibited gastric LTB4 synthesis in rats and markedly suppressed PG synthesis at a site of peripheral inflammation (Wallace et al., 1993). Tepoxalin also inhibited IL-2, IL-6, and TNF-α production with an IC50 of 10–12 mM (Ritchie et al., 1995). However, tepoxalin was discontinued in clinical Phase II (Inagaki et al., 2000).

Licofelone or ML3000 ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1H-pyrrolizine-5-yl]-acetic acid) is one of the most promising and potent dual inhibitor of COX and 5-LOX enzyme. Originally discovered by Merck GmbH and developed by EuroAlliance (a consortium of Alfa Wassermann SpA, Lacer SA and Merckle), is presently under clinical investigation for the treatment of osteoarthritis (Ding and Ciuttini, 2003). In in-vitro studies, licofelone has been reported to suppress both 5-LOX (with an IC50 of 0.18 μM) and COX-2 (with an IC50 of 0.21 μM) (Singh et al., 2006).

Licofelone has good oral bioavailability and peak plasma levels reaches at 3-4 hrs of ingestion. It has a long half-life (t1/2 ~ 11 hr) with highest accumulation in liver, kidney, heart and intestine (Deigner et al., 1995). Licofelone is well tolerated, no toxic symptoms were observed with a single dose of licofelone (300 mg/kg p.o. or 100 mg/kg i.p.) in mice. Licofelone has shown anti-inflammatory, analgesic, and antiasthmatic effects in several experimental models and does not cause any gastrointestinal damage (Rotondo...
et al., 2002). The anti-inflammatory action of this drug has been shown in animal models of osteoarthritis (Jovanovic et al., 2001; Pelletier et al., 2005), and it is currently being evaluated in phase III clinical trials in this disorder. Moreover, antithrombotic effects of licofelone due to inhibition of COX-1-mediated platelet function have been reported in mice and rat models (Tries et al., 2002) and in human platelets (Rotondo et al., 2004).

Licofelone, compared with classic NSAIDs, have been found to possess a unique ability to inhibit leukocyte rolling and adhesion to endothelium (Ulbrich et al, 2005). Its analgesic, anti-inflammatory, and antiplatelet properties are present at doses, which are safe for gastrointestinal tract (Cicero et al., 2005).

The results from a randomized trial in healthy human volunteers indicate that licofelone has a potential gastrointestinal safety advantage over conventional NSAIDs because 200 or 400 mg b.i.d. licofelone was associated with a lower incidence of ulcers compared with 500 mg b.i.d. naproxen (Bias et al., 2004). Consequently, since licofelone shares the anti-inflammatory effect and gastric safety of COX-2 inhibitors (Lehmann and Beglinger, 2005) but also inhibits COX-1-mediated platelet function, thereby avoiding the prothrombotic state, this drug may have a better cardiovascular profile than COX-2 inhibitors.

NEUROINFLAMMATION AND PD

Various studies have demonstrated the role of neuroinflammation in the pathophysiology of Parkinson’s disease, a disorder of the motor system, characterized by progressive and selective neuro-degeneration of dopaminergic system in the nigrostriatal region of the brain (Mosley et al., 2006; Bartels and Leenders, 2007). Neuroinflammation is known to activate microglia which further leads to increased expression of NOS and COX expression by NF-kB, a key transcription factor in the inflammatory pathway (Fig. 16.) (Hunot et al., 1997; Ghosh et al., 2007; Watanabe et al., 2008). Striatal microglial and astroglial activation has been observed in experimental models of Parkinson’s disease, (Kim and Joh, 2006; Yu et al., 2008).

Many aspects involving inflammatory process in the dopaminergic cell loss include COX and subsequent formation of prostaglandins which plays a
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crucial role and represent as the potential sites for attenuating the progression of PD. Earlier, NSAIDs have been proven beneficial in exhibiting neuroprotective effects in PD as supported by various experimental and epidemiological studies (Aubin et al., 1998; Tisemann et al., 2003; Hernan et al., 2006; Esposito et al., 2007). In the light of above evidences, various anti-inflammatory strategies suggested for their protective effects in PD are summarized under the following subheadings:

A. Inhibition of glial cell activation and pro-inflammatory cytokines.
B. Inhibition of iNOS, nitric oxide and peroxynitrite.
C. Inhibition of COX activation.
D. Dual Inhibition of COX/LOX activation.
E. Inhibition of mitochondrial dysfunction.

![Fig. 16. Putative role of inflammatory changes in Parkinsonism (Adapted from Bracia et al., 2003).](image)

A. Inhibition of glial cell activation and pro-inflammatory cytokines.

During the pre-clinical examination of the PD brain samples, it is indicated that there is presence of activated microglia as well as increased levels of transcription factors (NF-κB), pro-inflammatory cytokines such as TNF-
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c, IL-1β, IL-6 etc (Fig. 16.). So, the first strategy to prevent neuroinflammation is to target or attenuate the activation of microglia along with the inhibition of pro-inflammatory cytokines. If these processes are halted, the further cascade of inflammatory pathway can be prevented.

McCoy et al. (2006) observed that nigral administration of XENP345, a recombinant dominant negative TNF-inhibitor attenuated the neurotoxin and endotoxin-induced death of nigral dopaminergic neurons. Similarly, systemic administration of sodium hydrosulphide significantly attenuated the microglial activation in SN as well as accumulation of proinflammatory cytokines (Hu et al., 2011). In another study, prothrombin kringe-2 has shown to induce death of mesencephalic dopaminergic neurons via action of microglia in in-vivo and in-vitro studies (Kim et al., 2010).

Anti-inflammatory drugs such as pioglitazone, a PPAR-γ agonist, and the tetracycline derivative minocycline have been shown to reduce glial activation and protect the SN in an animal model of the PD (Hirsch et al., 2003; Thomas and Le, 2004). Therefore, inhibition of the glial reaction and the inflammatory processes may thus represent a therapeutic target to reduce neuronal degeneration in PD (Table 4).

<table>
<thead>
<tr>
<th>Table 4: Effect of anti-inflammatory neuroprotective agents in PD models</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Minocycline</td>
</tr>
<tr>
<td>6-OHDA</td>
</tr>
<tr>
<td>LPS</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Drug</th>
<th>Model</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIP</td>
<td>MPTP</td>
<td>↓ microglial activation, IL-1β, TNF-α, iNOS Reduced SN DA neuron loss and ST DA depletion</td>
</tr>
<tr>
<td>6-OHDA</td>
<td></td>
<td>Reduced SN DA neuron loss and motor activities</td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>MPTP</td>
<td>↓ microglial activation, iNOS Reduced SN DA neuron loss</td>
</tr>
<tr>
<td>Naloxone</td>
<td>LPS</td>
<td>↓ microglial activation Reduced SN DA neuron loss</td>
</tr>
<tr>
<td>Dextromethorphan</td>
<td>MPTP</td>
<td>↓ microglial activation</td>
</tr>
</tbody>
</table>

(Liu et al., 2006)

**B. Inhibition of iNOS, NO and peroxynitrite.**

The next strategy is to inhibit the expression of iNOS and further prevent the release of NO. Since, during the neuroinflammation, activated glial cell induces the increased expression of iNOS via activation of transcription factor, NF-κB, leading to the release of NO which combines with O₂⁻ anions forming a very potent free radical "peroxynitrite" which results in the degeneration dopaminergic neurons and neuronal death. Therefore, by inhibiting the iNOS we can prevent the formation of NO/peroxynitrite and further halt the process of neuronal degeneration. In experimental studies, selective iNOS inhibitors have demonstrated potential protective effect in attenuating PD-like symptoms in animals (Broom et al., 2011; Dehmer et al., 2004; Kim et al., 2010). Broom and colleagues (2011) demonstrated that selective iNOS inhibitor GW274150 afforded neuroprotection against 6-OHDA model of PD. Similarly, 7-nitroindazole, a selective nNOS inhibitor, demonstrated its antioxidant property in attenuating MPTP-induced neurotoxicity in experimental animals (Royland et al., 1999; Thomas et al., 2008; Watanabe et al., 2008).

**C. Inhibition of COX activation.**

In experimental models of PD, anti-inflammatory drugs especially NSAIDs such as sodium salicylate, parecoxib etc. have been studied to evaluate their neuroprotective effects in-vivo (Aguirre et al., 2008). MPTP-
induced Parkinsonism is a widely used animal model to evaluate antiparkinson activity of drugs. Glucocorticoids such as dexamethasone also showed neuroprotective effects against MPTP and intranigral administration of LPS (Kurkowska-Jastrzebska et al., 2004). Various researchers have studied the neuroprotective potential of minocycline, a semi-synthetic tetracycline derivative, and have exhibited significant protection against dopaminergic neuronal loss in different models of PD that include MPTP, 6-OHDA and LPS-induced neuroinflammation (Du et al., 2001; He et al., 2001; Tomas-Camardiel et al., 2004). In these models, minocycline afforded neuroprotection by attenuating the cell loss pathways in dopaminergic neurons.

In transgenic model of COX-2 deficient mice, researchers have demonstrated that MPTP produces less dopaminergic neuronal degeneration as compared to normal mice. It is therefore, understood that there may be certain genes coding for inflammation present in the dopaminergic neurons, which becomes inactive in COX-2 deficient mice and thus exhibi.ts neuroprotection (Feng et al., 2002). Therefore, it is speculated that inhibiting COX-isoenzyme may be a useful tool/target in preventing the neuronal loss in Parkinson's disease. This speculation has been substantiated by the fact that inhibition of either COX-1 or COX-2 isoenzyme has neuroprotective potential in various models of neuronal injury (Aubin et al., 1998; Tiesmann et al., 2003; Agruire et al., 2008).

D. Dual Inhibition of COX/LOX activation.

Since conventional NSAIDs are dual inhibitors of COX-1 and COX-2, and they have some drawbacks, including gastrointestinal tract damage, kidney failure, and overproduction of LTs (Leone et al., 2007). Selective COX-2 inhibitors were developed to overcome the adverse side-effects, but clinical studies suggested their side-effects on as gastrointestinal system, increased systemic blood pressure, cardiovascular disease and hypersensitivity (de Gaetano et al., 2003). In order to avoid these potential side-effects, new drugs have been targeted for dual inhibition of COX and LOX, which interferes with the biosynthesis of both PGs and LTs. Licofelone, a new dual inhibitor of COX and 5-LOX, was shown to have anti-inflammatory and analgesic activity comparable to that of conventional NSAIDs and COX-2 inhibitors but with an
improved gastrointestinal safety profile (Laufer et al., 1994; Bias et al., 2004). Moreover, combinatory inhibition of COX and LOX achieves a more potent neuroprotection than single inhibitors of either class (Klegeris and McGeer, 2002; Singh et al., 2007; Gupta et al., 2010). Li and colleagues have demonstrated that phenidone, a dual COX/LOX-inhibitor was superior in attenuating LPS-induced dopaminergic neurodegeneration and microglia activation as compared to either single COX or LOX-inhibitor (Li et al., 2008). Therefore, dual inhibition of COX and LOX offers a promising target for developing novel anti-inflammatory drugs and may provide a novel therapeutic approach of neuroinflammatory diseases.

E. Inhibition of mitochondrial dysfunction.

Various studies have demonstrated the possible role of oxidative damage and mitochondrial dysfunction in PD (Betarbet et al., 2000; Gupta et al., 2010). Therefore, agents that inhibit mitochondrial dysfunction may be used as of the probable drug targets to afford neuroprotection in neurodegenerative diseases such as PD. Recent advances in the dissection of the complex cellular pathways that influence mitochondrial structure and function have exposed new potential therapeutic targets. The peroxisome-proliferator-activated receptor gamma coactivator (PGC)-1α is a transcriptional coactivator that regulates mitochondrial biogenesis and energy metabolism, thereby aiding in the maintenance of energy homeostasis. Resveratrol, a polyphenolic compound abundant in grape skins, induced the expression of genes involved in mitochondrial biogenesis and oxidative phosphorylation by activating NAD dependent deacetylase sirtuin-1 (SIRT1) and PGC-1α (Lagouge et al., 2006). Although the effects of resveratrol in PD are unclear, the agent seems to protect against MPTP-induced dopaminergic neuron loss in mice (Lu et al., 2008).

Researchers have demonstrated that mitochondrial ATP-sensitive K [mitoK(ATP)] channels may provide a convergent target that could integrate different neurotoxic mechanisms of mitochondrial function (Busija et al., 2004; Costa and Garlid, 2008). In primary mesencephalic cultures and neuron-enriched cultures, treatment with the mitoK(ATP) channel blocker 5-hydroxydecanoate, inhibited the dopaminergic degeneration induced by low
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doses of 6-OHDA (Rodriguez-Pallares et al., 2009). Similarly, Glycogen synthase kinase-3β (GSK3β) has emerged as a major therapeutic target due to its involvement in several neurodegenerative diseases, including PD. The existence of GSK3β in mitochondria suggests that it has access to substrates resident in this organelle, which could include proteins of the intrinsic apoptosis pathway sequestered in healthy mitochondria, as well as mitochondrial metabolic proteins. King and coworkers have demonstrated that unregulated mitochondrial GSK3β activity mimicked complex-I inhibitors such as MPTP and rotenone (King et al., 2001). Therefore, GSK3β-mediated impairment in complex-I activity could be a possible target in attenuating mitochondrial dysfunction.

Another agent that has been reported to attenuate mitochondrial inhibition is CoQ10, a cofactor of mitochondrial uncoupling proteins (Echtay et al., 2000, 2002). Activation of these proteins reduces mitochondrial-free radical generation. CoQ10 induces mitochondrial uncoupling in the SN of primates, and this is associated with marked neuroprotection against MPTP toxicity (Ohnishiet al., 2008). Rasagiline is a MAO-B inhibitor demonstrated multiple effects on mitochondrial function, including stabilization of the mitochondrial membrane potential (Klivenyi et al., 2006). Reactive nitrogen species (RNS) such as NO and its metabolite peroxynitrite may inhibit complex-I activity via several different mechanisms including S-nitrosylation, nitration, and protein thiol formation. Studies using various cell and animal PD models have demonstrated that selective mitochondrial complex-I inhibition in dopaminergic cells may be due to both NO-mediated S-nitrosylation and nitration of complex-I sub-units (Singh et al., 2010; Chinta and Anderson, 2011). Strategies to modulate mitochondrial NO levels will therefore likely be a promising approach to enhance mitochondrial function and protect dopaminergic neurons against oxidative or nitrosative insult.
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ANIMAL MODELS FOR PD

The key features of PD are progressive neurodegeneration of dopaminergic neurons in the SN and motor symptoms such as tremors and bradykinesia. During the last few decades, many investigators have aimed to generate animal models that recapitulate at least some of the cardinal symptoms of PD. Two approaches, based on either genetic manipulation or neurotoxins, are being used to develop a PD model in animals.

GENETIC MODELS

a) α-synuclein

A lot of attention has been focused on the α-synuclein gene, because α-synuclein is a major component of Lewy body, and amino acid mutations have been identified as causative in dominantly or recessively inherited familial PD (Polymeropoulos et al., 1997). The first transgenic mouse model that over-expressed wild type human α-synuclein showed α-synuclein positive neuronal inclusions in the SN, neocortex, and hippocampus. Despite the degeneration of nerve terminals in the striatum, no loss of DA neurons has been observed in the SN of these mice (Hsu et al., 2000). To determine the effect of α-synuclein defects in dopaminergic neurons, wild type, A53T, and A30P α-synuclein mutant mice were used to target α-synuclein overexpression or mutation in cells expressing tyrosine hydroxylase (TH). Mice with a single gene mutation failed to recapitulate DA cell loss and did not display synuclein pathology. Mice with double mutations (A30P/A53T) in the human form of α-synuclein exhibited a progressive loss of dopaminergic neurons in the SN with decreased motor activity. However, the pathological relevance is controversial since the double mutation of α-synuclein protein has not been found in human familial PD (Thiruchelvam et al., 2004).

The first conditional transgenic mouse in which the expression of α-synuclein was regulated by the tetracycline system was established by Nuber et al. (2008). These mice showed a modest loss of dopaminergic neurons in the SN without any synuclein-positive inclusions. In addition, they showed reduced neurogenesis and neurodegeneration in the hippocampus without fibrillary inclusions. When α-synuclein gene expression is turned off, the progression of
PD-like phenotypes is alleviated, but not reversed, indicating that continuous expression of the α-synuclein gene is necessary for disease progression (Nuber et al., 2008). Unlike the mouse model, α-synuclein overexpression in Drosophila showed a stronger PD-like phenotype. Over-expression of mutant (A53T and A30P) or wild type α-synuclein in flies resulted in a progressive loss of dopaminergic neurons in the dorsomedial clusters and correlated with a loss of climbing ability. In addition, α-synuclein positive inclusions were observed in dopaminergic neurons (Drobyshova et al., 2008).

**b) Leucine-rich repeat kinase (LRRK) 2**

The leucine-rich repeat kinase (LRRK) 2 gene contains 51 exons and encodes a large protein composed of 2527 amino acids. RNA expression occurs predominantly in the basal ganglia and the hippocampus. The protein encoded by the LRRK2 gene includes several independent domains including a leucine-rich repeat (LRR) domain, a kinase domain, a Roc GTPase domain and a C-terminal WD40 domain (Mata et al., 2006). Mutations in LRRK2 have been recognized in approximately 7% of familial PD cases as well as 1-3% of idiopathic PD cases (Nichols et al., 2005). So far, at least 20 polymorphic loci in the LRRK2 gene have been identified and linked to familial PD. Among them, the G2019S missense mutation is the most common, accounting for up to 6% of familial cases in Europe (Nichols et al., 2005). Unlike other PD-associated genes, the LRRK2 G2019 mutation gives rise to late-onset PD which has a clinically similar phenotype to idiopathic PD. However, the exact mechanism by which the LRRK2 mutation induces the disease is unknown (Ng et al., 2009). In Drosophila models, over expression of either wild type or G2019S mutated LRRK2 causes adult-onset dopaminergic neuron degeneration, accompanied by decreased motor activity that is alleviated by L-DOPA treatment. In contrast to this, transgenic flies that overexpress R144C mutated LRRK showed no difference in the number of DA neurons (Lee et al., 2007).

**c) Parkin**

Mutations in parkin, a component of E3 ubiquitin ligase encoded by the PARK2 gene is identified as the cause of autosomal recessive juvenile Parkinsonism (ARJP) in humans (Kitada et al., 1998). It is assumed that the
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loss of Parkin function may impair the ubiquitin-proteasome system and subsequently cause the neurotoxic accumulation of its substrates. Indeed, many substrates of Parkin such as CDCrel (septin 5), Cyclin E, and far upstream element-binding protein-1 (FBP-1) were found accumulated in the brain of parkin associated PD patients, even though the DA neuron specific substrates have not been reported (Nakamura and Lipton, 2010). Parkin null mutant mice have been generated by several independent groups. However, none of these transgenic mice exhibited typical PD pathologies. In addition, the accumulation of Parkin substrates was not detected in the brain (Nakamura and Lipton, 2010). Moreover, the neurodegenerative phenotype of parkin mutants is more severe when combined with loss-of-function mutations of glutathione S-transferase S1 (GstS1), an important detoxification enzyme that reduces oxidative stress (Kitada et al., 1998; Whitworth et al., 2005). A subsequent study also revealed that overexpression of GstS1 suppresses DA neurodegeneration in the parkin mutant flies, suggesting that overexpression of GstS1 has potential therapeutic value in the treatment of PD (Whitworth et al., 2005).

NEUROTOXIN MODELS

Before the advent of genetic models, the majority of experimental PD studies relied on toxin based models. Toxin-based models recapitulate nigrostriatal neurodegeneration, however the degeneration process is often acute compared to idiopathic PD, which requires decades to develop in humans. The most common neurotoxins used to induce dopaminergic neurodegeneration include MPTP, 6-hydroxydopamine (6-OHDA), rotenone, paraquat, and maneb. A common feature of these neurotoxins is that they all affect mitochondria, either by inhibiting mitochondrial complex I or complex III, resulting in the production of ROS. Additionally, they also exert their neurotoxic effect by inducing microglia-mediated inflammation by microglial activating agent, LPS which is now well established as an effective initiator of SN dopaminergic neurodegeneration. The various animal models employed for the screening and evaluations of anti-parkinson activity of drugs are discussed as follows:
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a. Drug-induced catatonia

Antipsychotic drugs like perphenazine, haloperidol etc produces Parkinson-like symptoms as observed in different animal models (Kulkarni et al., 1980; Naidu and Kulkarni, 2002; Singh and Kulkarni, 2002). These drugs block the dopamine D2-receptors, results in the imbalance of dopamine-acetylcholine system and produce Parkinson-like symptoms in animals. Reserpine also produces catalepsy by inhibiting the uptake of catecholamines in the storage vesicles and hence depleting the dopamine levels in the brain (Singh et al., 2003).

b. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

MPTP a lipophillic molecule that produces Parkinson-like symptoms readily crosses blood-brain barrier and metabolizes to active metabolite MPP⁺ by monoamine oxidase B (MAO-B) that exerts its Parkinson like-effect in vivo (Langston et al., 1983). MPP⁺ accumulates in dopaminergic neurons and inhibits mitochondrial complex I, thus producing reactive oxygen species which further deteriorates the condition of ATP deficient dying neurons (Dauer and Przedborski, 2003).

c. 6-Hydroxydopamine (OHDA)

6-Hydroxydopamine (OHDA) is the first chemical known to have neurotoxic effects on the catecholaminergic pathway and produce experimental Parkinson in animals (Archer et al., 1988; Luthman et al., 1989). 6-OHDA is a hydroxylated analogue of the natural neurotransmitter dopamine (Blum et al., 2001). Upon entering neurons, 6-OHDA accumulates in the cytosol and induces cell death by inhibiting mitochondrial complex I and the production of ROS (Betarbet et al., 2002), which has a similar effect to MPTP. In addition, 6-OHDA is easily oxidized and herein contributes to oxidative stress by directly taking part in free radical forming reactions. Unlike MPTP, systemically administered 6-OHDA fails to cross the blood brain barrier. Thus, 6-OHDA has to be injected stereotactically into the brain. Although the formation of α-synuclein-positive Lewy bodies does not occur in 6-OHDA models (Dauer & Przedborski, 2003; Schober, 2004), 6-OHDA drastically increases α-synuclein immunoreactivity and triggers thioflavin T-positive deposits in SN neurons.
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(Alves Da Costa et al., 2002). 6-OHDA has been shown to trigger two neurochemical alterations that directly impact α-synuclein physiology. First, 6-OHDA prevents α-synuclein degradation by directly inhibiting cellular proteasomal activity, likely due to its pro-oxidant activity (Hirschet al., 2003). Second, 6-OHDA triggers α-synuclein aggregation by oxidizing α-synuclein. It has been demonstrated that oxidized α-synuclein is more prone to aggregation (Ostrerova-Golts et al., 2000) and aggregated proteins significantly inhibit the cellular proteasomal machinery (Bence et al., 2001). The toxic effects of 6-OHDA are in part mediated through the activation of microglia. Direct administration of 6-OHDA into the SN of mice activates microglia and increases the number of activated microglia in the SN with the subsequent loss of dopaminergic neurons (He et al., 2001). Moreover, 6-OHDA-lesioned rats have been demonstrated to display increased levels of TNFa in both SN and striatum (Mogi et al., 1999). Activation of microglia is probably the consequence of neurodegeneration caused by 6-OHDA injury.

Like dopamine, 6-hydroxydopamine is oxidized to generate free radical (such as superoxide anion and hydroxyl radicals via Fenton's reaction) and quinines. Numerous species are sensitive to 6-OHDA, including mice, cats, dogs, monkeys and rats, and a quantifiable motor deficit is present without LB pathology (Hirsch et al., 2003).

d. Rotenone

Rotenone is a naturally occurring complex ketone and commonly used as a pesticide/herbicide. Rotenone has been considered a potential environmental risk factor for the development of PD (Betarbet et al., 2000). Being extremely lipophilic, rotenone easily crosses the blood-brain barrier, rapidly enters into the brain, freely crosses cellular membranes independently of any transporters (unlike MPP+ and 6-OHDA), and accumulates in subcellular organelles, such as mitochondria (Talpade et al., 2000), where it induces oxidative stress by inhibiting complex I of the electron transport chain (Schuler and Casida, 2001). Chronic administration of rotenone induces pathology that fully recapitulates the typical features of PD: nigro-striatal neurodegeneration, beginning in the nerve terminals and progressing retrogradely to the cell bodies; formation of cytoplasmic inclusion resembling LBs; and the
development of parkinsonian behavior, including hypokinesia and rigidity in rats (Greenamyre et al., 1999; Betarbet et al., 2000). The rotenone-induced degeneration of dopaminergic neurons may not solely result from an impairment of neuronal mitochondrial complex I activity but also may involve the activation of microglia (Gao et al., 2003). An in-vitro study has shown that rotenone-induced microglial activation occurs prior to apparent neurodegeneration (Gao et al., 2003), suggesting rotenone, rather than factors from damaged neurons, is the primary trigger for microglial activation. Although complex-I in the mitochondria is evenly distributed throughout cells in the brain, only the dopamine neurons in the SN selectively degenerate with exposure to rotenone (Betarbet et al., 2000; Choi et al., 2008). This suggests that the dopamine neurons in the SN are particularly sensitive to rotenone (Betarbet et al., 2000).

In the rat, in addition to inducing the loss of SN DA neurons, rotenone induces formation of Lewy body, which has not been noted in either the 6-OHDA or MPTP models. Chronic, intravenous administration of rotenone produces PD-like motor symptoms, such as bradykinesia, postural instability, and tremors. These symptoms improve with administration of L-dopa or dopamine agonist treatments (Betarbet et al., 2000).

However, the rotenone model does pose several disadvantages. First, PD-like pathology induced by rotenone is variable, depending on the protocol of administration of rotenone. Second, the association between PD-like motor symptoms and the loss of dopaminergic neurons has not been fully confirmed in the rotenone model. These motor changes may also be caused by the abnormalities arising in the cardiac system, stomach, and liver that are seen in the rotenone model (Inden et al., 2011).

e. Paraquat and maneb

Paraquat is another candidate herbicide that is linked to PD. Paraquat is capable of crossing the blood-brain barrier, but slowly, inefficiently, and to a limited extent (Corasaniti et al., 1998). Systemic subchronic exposure to paraquat elicits a significant dopaminergic cell loss and some decrease in striatal dopamine nerve terminal density accompanied with a neurobehavioral
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syndrome characterized by reduced ambulatory activity (Brooks et al., 1999). The neurotoxicity of paraquat mainly arises from the induction of oxidative stress by inhibiting complex I of the electron-transport chain and the activation of cholinergic and glutamatergic transmission (Corasaniti et al., 1998). Moreover, paraquat is a potent redox cycler itself, being readily converted to a free radical, which results in subsequent ROS production (Yumino et al., 2002). In addition, paraquat administration leads to a significant increase in brain levels of α-synuclein and is accompanied by the accumulation of α-synuclein-containing intraneuronal aggregates (Manning-Bog et al., 2002). Finally paraquat is able to activate microglia and as a result predisposes dopaminergic cells to degeneration (Purisai et al., 2007). The fungicide maneb is largely used in agricultural regions for the control of field crop pathologies. Much less is known about the mechanisms of maneb neurotoxicity. Maneb is assumed to be able to cross the blood-brain barrier (Thiruchelvam et al., 2002). Chronic exposure of humans to maneb has been linked to the development of parkinsonism (Costello et al., 2009; Liet al., 2005). Moreover, the acute exposure of maneb in experimental animal models decreases locomotor activity (Morato et al., 1989).

Although the overall toxic effects of paraquat or maneb are not as prominent as those of MPTP or rotenone, neurotoxicity is dramatically enhanced when these agricultural chemicals are applied in mixture. Indeed, maneb appears to interact synergistically with paraquat in a mouse model, with treated animals being characterized by reduced motor activity and increased damage at the level of both striatal terminals and nigral cell bodies (Thiruchelvam et al., 2000). This creates a basis for a multiple-hit environmental model for PD in which exposure to a single chemical may be insufficient to induce disease, whereas exposure to multiple neurotoxicants may ensure neuropathological changes (Thiruchelvam et al., 2000).

f. Lipopolysaccharide-induced Parkinson’s model

LPS is a Gram-negative bacterial endotoxin that potently activates microglial cells by interacting with microglial membrane receptors such as Toll-like receptor 4 (TLR4) (Kacimiet al., 2011). Engagement of TLR4 induces nuclear localization of the transcription factor nuclear factor κB (NFκB) and
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subsequent activation of genes in proinflammatory pathways (Orr et al., 2002). LPS, a potent inflammogen, activates microglial cells and produces selective neurotoxicity to dopaminergic neurons, whereas GABAergic or serotonergic neurons are not affected (Gao et al., 2003). Microglial activation by LPS plays a crucial role in the dopaminergic neuronal damage via release of plethora of proinflammatory agents, reactive oxygen species and reactive nitrogen species (Meng et al., 2008). Therefore, it is observed that LPS in itself is non-neurotoxic but it modulates its effects via activation of glial cells (Arai et al., 2004). Moreover, neurotoxins such as 6-OHDA, MPTP or rotenone synergistically enhance the neurotoxicity when combined with LPS. It has been shown that an intranigral injection of LPS causes extensive microglial activation and degeneration of DA neurons in both the SN and striatum (Herrera et al., 2000). The long-lasting damage to the dopaminergic neurons after a single insult implies that a transient exposure to LPS may initiate a sequence of events leading to apparently permanent cell loss, mimicking the progressive neurodegeneration in PD (Herrera et al., 2000). LPS has been used prior to the treatment of neurotoxin paraquat to demonstrate a role of microglia in initiating paraquat-induced neurodegeneration (Purisai et al., 2006).
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CURRENT THERAPEUTIC OPTIONS IN PD AND THEIR LIMITATIONS

The aim of current antiparkinson therapies is to restore or compensate for the diminished endogenous dopamine that are lost as the result of the pathophysiology of PD. Antiparkinson therapies act through one of several mechanisms; some replenish, or mimic the action of naturally occurring DA; while some prevent the breakdown of DA peripherally and within the CNS; and some act on the non-dopaminergic systems that influence movement, preventing uncontrolled movements (Fig. 17.).

![Fig. 17. Mode of action of various antiparkinson therapies](image)

Although there are certain therapeutic interventions available for the treatment of PD, but these drugs have their own limitations and hence lead to their use with a word of caution. The classification of the current therapeutic options available for the PD which is summarized in Table 5.
### Classification of drugs

<table>
<thead>
<tr>
<th>Classification</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dopamine precursor</strong></td>
<td>Levodopa (L-DOPA)</td>
</tr>
<tr>
<td><strong>Peripheral DOPA decarboxylase inhibitor</strong></td>
<td>Carbidopa, Beneseride</td>
</tr>
<tr>
<td><strong>Selective dopamine receptor agonists</strong></td>
<td>Bromocriptine, Pergolide, Ropinirole, Pramipexole</td>
</tr>
<tr>
<td><strong>Monoamine oxidase (MAO-B) inhibitors</strong></td>
<td>Selegiline, Rasigaline</td>
</tr>
<tr>
<td><strong>Catechol-O-methyl transferase (COMT) inhibitors</strong></td>
<td>Entacapone, Tolcapone</td>
</tr>
<tr>
<td><strong>Anticholinergics</strong></td>
<td>Benztpoline, Trihexyphenidyl</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td>Amantadine</td>
</tr>
</tbody>
</table>

### Drugs Involved in Neurotransmission Improvement

Levodopa, the precursor of DA, improves the deficiency of DA in brain and is used in the pharmacological treatment of PD. But over a long period of time, patients on levodopa therapy develop tolerance whereby increased dose is required (Rajput et al., 2002). This results in the development of various side effects of L-DOPA therapy such as "on-off" phenomenon (Merims et al., 2003). However, more than 30 years after its discovery, levodopa remains the gold standard therapy for PD, and a large majority of patients who commence treatment with an alternative drug will eventually require the improved symptomatic control provided by levodopa therapy.
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**Fig. 18. Metabolism of L-DOPA**

*Peripheral DOPA decarboxylase inhibitor such as carbidopa is combined with levodopa to prevent its peripheral metabolism and results in the reduction of levodopa dose and attain higher plasma levels of the latter to reach brain (Aminoff, 2001).*

*Selective DA receptor agonists* such as bromocriptine, pergolide, ropinirole, pramipexole directly stimulate DA receptors for neuronal activity. DA agonists are an effective treatment for the motor features of early PD, and are used in combination with other therapies to control motor symptoms in patients with late PD (Rascol et al., 2000; Noyes et al., 2004). DA agonists can be beneficial either as monotherapy or as an adjunct to levodopa.

*Monoamine oxidase (MAO)-inhibitors* like selegiline, rasagline blocks MAO-B to reduce DA metabolism. MAO-B is important for the degradation of DA both endogenous and that produced by exogenous levodopa within dopaminergic neurons. When used as an adjunct to conventional levodopa MAO-B inhibitors, such as selegiline and rasagline, prevent the breakdown of dopamine, thus increasing the concentration of the neurotransmitter within the brain. Because of their mechanism of action and limited duration of effect, these agents are used as monotherapy only in the early stages of PD, when endogenous dopamine production is not markedly reduced. With disease...
progression, patients initiated on MAO-B inhibitor therapy will eventually require dopamine supplementation with levodopa (Tabakman et al., 2004; Blandini, 2005). However, MAO-B inhibitor regimens may be associated with hypertensive crises-the ‘cheese reaction’ - which occurs when tyramine-rich foods, beverages or dietary supplements enter the circulation and potentiate sympathetic cardiovascular complications (Youdim et al., 2006).

Catechol-O-methyl transferase (COMT)-inhibitors block the peripheral COMT activity to improve the pharmacokinetics of L-DOPA e.g. entacapone, tolcapone. COMT is the second major pathway in the breakdown of levodopa, a factor responsible for the short half-life of levodopa. Inhibition of this enzyme reduces the peripheral breakdown of levodopa and provides an extended duration of levodopa in plasma, which translates into more continuous delivery of levodopa to the brain, and thus, increases the amount of time when patients' PD symptoms are well controlled (Gordin et al., 2004). COMT inhibitors, have no inherent antiparkinson activity, thus, they are always given in combination with levodopa.

Anticholinergics such as benztropine, trihexyphenidyl block the acetylcholine receptors and decrease the Ach levels, thus prevents imbalance between DA and Ach levels.

Miscellaneous drugs such as Amantadine's exact mechanism of action remains unclear, but it is known to be a weak N-methyl-D-aspartic acid (NMDA) antagonist and an anticholinergic (Guttmann et al., 2003; Lozano et al., 1998). Amantadine can be prescribed alone, but can also be taken in combination with other PD therapies. Along with its antiparkinson effects, amantadine also has some anti-dyskinetic effect (Pahwa et al., 2006).

Antioxidant Therapies

Since there is increase in the oxidative stressors in brain of PD, modifying the diseased condition with antioxidant therapy can a suitable adjunct to the basic therapy. Various studies have focused that dietary intake of Vitamin E is associated with the reduced risk of developing PD (Zhang et al., 2002). Polyphenols such as curcumin, caffeic acid has to have neuroprotective
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potential for dopaminergic neurons against LPS-induced neurotoxicity (Yang et al., 2008) and in silico studies (Jagatha et al., 2008).

Newer drug therapies

Some of the newer therapeutic agents /combinations which are in the various stages of clinical trials have been enlisted in Table 6.

<table>
<thead>
<tr>
<th>Molecule (Sponsor)</th>
<th>Stage of Clinical Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPX066 (IMPAX Pharmaceutical)</td>
<td>Phase-III</td>
</tr>
<tr>
<td>Levodopa/Carbidopa Intestinal Gel (Solvay Pharmaceuticals, Inc.)</td>
<td>Phase-III</td>
</tr>
<tr>
<td>Glutamic acid decarboxylase Gene transfer (Neurologix. Inc.)</td>
<td>Phase-II</td>
</tr>
<tr>
<td>Coenzyme Q10 (National Institute of Neurological Disorders and Stroke)</td>
<td>Phase-III</td>
</tr>
<tr>
<td>S-Adenosyl-methionine (National Institute of Health)</td>
<td>Phase-II/III</td>
</tr>
<tr>
<td>ACP-103, Pimavanserin (ACADIA Pharmaceuticals Inc.)</td>
<td>Phase-III</td>
</tr>
</tbody>
</table>
MPTP AS ANIMAL MODEL OF PD

MPTP or its analogs (2-~methyl-MPTP, MPDP and MPP⁺) increase ROS formation because they cause a sustained DA efflux from the nigrostriatal nerve terminals, lasting for hours (Chiueh et al., 1992; 1993; Chiueh and Rauhala, 1998). The neurotoxic mechanism of MPP⁺ may be similar to but not identical to that of 6-OHDA (Lotharius et al., 1999). Most of the released DA is oxidized either enzymatically by MAO-A or non-enzymatically by iron complexes, leading to generation of cytotoxic species such as *OH, DA aldehyde, hydrogen peroxide, and semiquinone radicals inside and/or near the dopaminergic synapse in vivo (Fig. 19). In addition, MPP⁺ inhibits complex-I activities in mitochondrial preparations at high mM concentrations (Mizuno et al., 1988). The inhibition of complex-I may also lead to the generation of free radicals. It is not known whether the A9 DA neurons can concentrate enough MPP⁺ in-vivo to inhibit complex-I since the highest brain level of MPP⁺ is found in the locus caeruleus but not in the SN. Surprisingly, MPTP (1.5 mg/kg, i.v.) does not cause a significant decrease in norepinephrine. Thus, the contribution of the inhibition of complex-I on the generation of O₂ free radicals in the nigral neurons may be limited in the MPTP-induced Parkinsonian animal model. Finally, MPTP causes oxidative stress, DA depletion, and selective destruction of pigmented A9 neurons in humans and monkeys (Hirsch et al., 1988).

Surprisingly, owing to relatively low iron content in murine basal ganglia, MPTP does not cause significant nigral loss in rats and mice (Chiueh et al., 1992; 1993; Przedborski et al., 2000). In contrast to general belief, MPTP-induced DA depletion in rats and mice is reversible (Chiueh et al., 1992). Prevention of MPP⁺ uptake into nigral neurons may suppress MPTP’s neurotoxicity whereas blockade of vesicular transporters by MPP⁺ itself can augment dopaminergic toxicity because intracellular DA can no longer be protected and stored inside the synaptic vesicles (Gainetdinov et al., 1998). Exaggerated DA overflow may be harmful to neurons and other brain cells. Moreover, MPP⁺ increases the brain uptake of iron in the midbrain SN region of monkeys (Mochizuki et al., 1994). Therefore, MPTP-induced selective neurotoxicity of the pigmented A9 nigral neurons is mediated by DA-induced oxidative stress since the human A9 SN compacta neurons contain high iron.
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and DA levels (Chiueh et al., 1993; Chiueh and Rauhala, 1998). MPTP and iron complexes (ferrous citrate and hemoglobin) may accelerate and augment the life-long oxidative stress and thus shorten the degenerative period from decades to days in the midbrain nigral neurons.

MPTP easily crosses the BBB and is converted to its metabolites MPP⁺ by glial MAO-B. MPP⁺ is selectively taken in DA neurons throughout DAT and accumulated in the mitochondria, where it inhibits complex I of the mitochondrial electron transport chain. Inside neurons, MPP⁺ exerts its toxic effect by inhibiting the multienzyme complex I of the mitochondrial electron transport chain (ETC) resulting in ATP depletion and ROS production (Hasegawa et al., 1997; Fabre et al., 1999). Studies have shown that alternations in energy metabolism and generation of ROS occurred within hours of MPTP administration, days prior to the robust neuronal death. This suggests that initial energy failure and oxidative stress are not likely to directly kill most cells, but

Fig. 19 Schematic representation of the mechanisms of MPTP action in the nigrostriatal system.
rather trigger downstream cellular events that ultimately cause cell death (Jackson-Lewis et al., 1995; Mandir et al., 1999; Vila et al., 2001). MPTP appears to damage the nerve terminals of the striatum first and most severely (Jackson-Lewis et al., 1995), which follows the typical “dying back” pattern of neurodegeneration in PD (Dauer & Przedborski, 2003).

Chronic continuous MPTP administration rather than acute delivery can cause the accumulation of α-synuclein-immunopositive inclusions in the cytosol of SNpc dopaminergic neurons (Vila et al., 2000; Przedborski et al., 2000; Przedborski et al., 2004; Fornai et al., 2005). The formation of α-synuclein inclusions in the cell bodies of surviving neurons may be due to the up-regulation of α-synuclein at the mRNA and protein levels in response to MPTP intoxication (Vila et al., 2000), as a possible result of a cellular compensatory attempt to survive MPTP injury (Murphy et al., 2000). Alternatively, since α-synuclein is initially produced in the cell body and then rapidly transported to the nerve terminals (Withers et al., 1997) and as mentioned above MPTP damages terminals first, the accumulation of α-synuclein in the cell body possibly results from an impaired antegrade transport of the protein and accordantly gathering at the site of synthesis. Both acute and chronic MPTP treatment cause extensive microglial activation in the nigro-striatal region, characterized by increased expression of microglial cell markers and up-regulation of iNOS, and proinflammatory cytokines such as TNF-α, IL-1β and IL-6 (Kurkowska-Jastrzebska et al., 1999; Nagatsu et al., 2000; Vila et al., 2001). Although MPTP and its active metabolite MPP⁺ do not activate microglia (Gao et al., 2003), MPP⁺ damages dopaminergic neurons, resulting in release of some neuron-injury factors to promote microglial activation, which drives further dopaminergic neurotoxicity, resulting in a vicious, progressive and self-propelling cycle (Hunot and Hirsch, 2003; Block et al., 2007).

In primates, MPTP causes irreversible and severe Parkinsonian symptoms which are not distinguishable from those of idiopathic PD. These include the degeneration of dopaminergic neurons in the SN, Lewy body pathology, and motor symptoms which can be ameliorated with L-dopa or dopamine agonist (Langston et al., 1983). The main drawback of the primate MPTP model is that the disease progression is more rapid than that of...
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idiopathic PD, which develops over a few decades (Shimohama et al., 2003). Rodents show variable amounts of susceptibility to MPTP toxicity, which is dependent upon the species and strain of the animal (Hamre et al., 1999).

SUITABLE NEUROPROTECTIVE STRATEGIES

With respect to neurodegenerative disorders, neuroprotective therapy refers to interventions that are designed to preserve the integrity and function of vulnerable neurons to prevent the manifestation of the disease or to slow or halt the progressively disabling course of the disease. Degenerative diseases of the CNS frequently have a predilection for specific cell populations. In AD, many neuronal populations are involved but appears to affect cholinergic projections from the basal forebrain relatively early in its course (Arendt et al., 1983; Whitehouse et al., 1981). In contrast, PD has a more dramatic predilection for the dopaminergic neurons of the SNpc but also affects other monoamine cell populations and, to a lesser extent, other transmitter systems (Jellinger, 1989; Sian et al., 1999). To develop rational neuroprotective therapies for neurodegenerative disorders, it is first necessary to understand the mechanisms underlying the selective degeneration that characterize these disorders.

Despite many hypotheses the reasons for neuronal cell death and the selective vulnerability of specific neuronal cell populations are, as yet, unknown. Partial elucidation of the processes underlying the selective action of neurotoxic substances such as iron, 6-OHDA, glutamate, kainic acid, quinolinic acid or MPTP, has revealed possible molecular mechanisms for neurodegeneration (Gerlach et al., 1999). Hypotheses regarding the neurotoxic mechanisms of these substances have been the rationale for neuroprotective approaches (Table 7). These strategies have focused largely on oxidative mediated neuronal damage and the consequences of an increased rate of free-radical production. Although it has been possible in various experimental models of neurodegeneration to show aneuroprotective effect of active substances, such as selegiline and α-tocopherol (vitamin E), at the cellular, neurochemical and functional levels, it has not been possible to demonstrate an unequivocal neuroprotective effect in clinical studies (Broom et al., 2011; Itohe et al., 2006; Renet al., 2006).
Till now, therapeutic agents employed in the treatment of PD focuses only on the dopamine-acetylcholine pathway and there is no line of treatment which deals with the other associated symptoms of PD such as “neuroinflammation”. A re-examination of the potential use of agents with anti-inflammatory activity to lower the risks associated with developing PD may be in order and could afford significant protection in the management of PD.
Table 7: Possible molecular mechanisms for neurodegeneration and probable neuroprotective approaches in PD

<table>
<thead>
<tr>
<th>Molecular mechanism</th>
<th>Possible causative process</th>
<th>Probable Neuroprotective Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxidative stress</strong></td>
<td>- Metabolism of catecholamines</td>
<td>✓ Antioxidants</td>
</tr>
<tr>
<td></td>
<td>- Impaired free radical scavenging due to genetic predisposition</td>
<td>✓ MAO-inhibitors</td>
</tr>
<tr>
<td></td>
<td>- Inhibition of mitochondrial function</td>
<td>✓ NOS-inhibitors</td>
</tr>
<tr>
<td></td>
<td>- Altered iron metabolism</td>
<td>✓ DA agonists</td>
</tr>
<tr>
<td></td>
<td>- Activation of Glial cells</td>
<td>✓ Iron chelators</td>
</tr>
<tr>
<td>Disturbed iron metabolism</td>
<td>- Increased free redox-active iron</td>
<td>✓ Antiinflammatory drugs</td>
</tr>
<tr>
<td></td>
<td>- Defect in iron storage</td>
<td></td>
</tr>
<tr>
<td><strong>Mitochondrial dysfunction</strong></td>
<td>- MPTP like endogenous neurotoxins</td>
<td>✓ Coenzyme Q</td>
</tr>
<tr>
<td><strong>Excitotoxicity</strong></td>
<td>- Abnormal glutamate accumulation</td>
<td>✓ Glutamate antagonists</td>
</tr>
<tr>
<td></td>
<td>- Exogenous excitotoxins</td>
<td>✓ Calcium channel blockers</td>
</tr>
<tr>
<td>Disturbed Ca(^{2+}) homeostasis</td>
<td>- Cell membrane damage as a result of oxidative damage</td>
<td>✓ Calcium channel blockers</td>
</tr>
<tr>
<td></td>
<td>- Excitotoxicity</td>
<td></td>
</tr>
<tr>
<td><strong>Apoptosis</strong></td>
<td>- Lack of neurotrophic factors</td>
<td>✓ Neurotrophin substitution</td>
</tr>
<tr>
<td></td>
<td>- Death cytokines such as TNF</td>
<td>✓ Caspase/calpain/protease inhibitors</td>
</tr>
<tr>
<td></td>
<td>- Disturbed calcium homeostasis</td>
<td>✓ Calcium channel blockers</td>
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<tr>
<td></td>
<td>- Oxidative stress</td>
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<td>- Excitotoxicity</td>
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<td>- ATP depletion</td>
<td></td>
</tr>
</tbody>
</table>