Pharmacological Therapy in Parkinson's Disease: Focus on Neuroprotection

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Although the number of available therapeutic approaches in Parkinson's disease (PD) is steadily increasing the search for effective neuroprotective agent is continuing. Such research is directed at influencing the key steps in the pathomechanism: the mitochondrial dysfunction, the oxidative stress, the neuroinflammatory processes and the final common apoptotic pathway. Earlier-developed symptomatic therapies were implicated to be neuroprotective, and promising novel disease modifying approaches were brought into the focus of interest. The current review presents a survey of our current knowledge relating to the pathomechanism of PD and discusses the putative neuroprotective therapy.

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Introduction

After the original description of the clinical picture by James Parkinson in 1817 [1], the first breakthrough in the therapy of Parkinson's diseases' (PD) was the introduction of L-dopa in the 1960s [2]. L-dopa led to significant improvements in the standard of life of individuals with PD, as measured in the activity of daily living, employability and survival, and is still the most potent mode of treatment, but a number of issues remain unresolved. (1) L-dopa treatment was associated with adverse motor and nonmotor complications. (2) Whereas various drugs have been suggested as potential neuroprotectants, none of the currently applied forms of therapy has been proven to have a clear disease-modifying, curative benefit [3]. Rather than focusing on the evidence-based therapeutic interventions, the present review will offer an overview of the recent developments that might become future therapeutic avenues. We have chosen to base our review to reflect current knowledge relating to the pathomechanism of PD.

Parkinson's Disease

Clinical Features

The incidence of PD has been calculated to be 326.3 per 100,000 person-years [4]. The incidence rate increases with age in both men and women from 0.3 per 1000 person-years in subjects aged 55 to 65 years to 4.4 per 1000 person-years in those aged 85 years or above [5]. The age-adjusted relative risk in men as compared with women has been reported 2.13 in one study [4] in contrast with no significant difference in another cohort [5].

The disease is defined by distinct clinical features, such as bradykinesia, resting tremor, rigidity, and postural instability. Besides the motor symptoms a cognitive impairment develops [6–11]. In the early stages of the disease, the symptoms are mild and often missed by the patient and family members, becoming evident only retrospectively. The early symptoms start asymmetrically, usually with deterioration in dexterity of fine movements. Relatives and colleagues may notice general slowing-down, changes in facial expression, and an impaired arm swing. The disease becomes bilateral and then progresses slowly

during a period of years. Eventually, the patients become chair- and bed-bound.

Pathological Findings

Pathologically, the disease is characterized by degeneration of the dopaminergic cells of the substantia nigra pars compacta and the appearance of cytoplasmatic inclusion bodies, the Lewy neurites, and Lewy bodies [12,13]. The major components of the Lewy bodies are alpha-synuclein, ubiquitin, phosphorylated neurofilaments, parkin, components of the ubiquitin proteasomal system, molecular chaperones, and lipids [14]. Various nondopaminergic structures are affected by the disease, including the cholinergic neurons of the nucleus basalis Meynert, the noradrenergic neurons of the locus coeruleus, the serotonergic neurons of the raphe nucleus, the cortical, brainstem, and spinal cord neurons and the peripheral autonomic nervous system. It has recently been suggested that there is a gradual caudo-rostral progression of the pathological changes, starting from the neurons of the nuclei of the autonomic nervous system, progressing through the lower brainstem, reaching the basal ganglia and finally involving the cortical neurons too [15]. Nonetheless, reviews of earlier studies [16] and more recent pathological studies [17] have demonstrated that a significant proportion of patients do not follow the proposed pathway, and the validity of the hypothesis has been questioned [14,16].

Pathogenesis

It is currently considered that the pathogenesis of PD involves several major interacting pathways (Figure 1). A mitochondrial dysfunction of various origins was first shown to lead to the development of oxidative stress and a cell energy insufficiency. Similarly, immune mechanisms identified in PD (see below) cause oxidative dam-

age, but were also postulated to give rise to apoptosis through more direct mechanisms. Another important pathway is related to the abnormal aggregation of proteins. Glutamate excitotoxicity has additionally been implicated in striatal cell death in PD. The various pathological processes subsequently coincide into a common pathway of apoptosis.

Oxidative Stress

Oxidative stress, a key concept of PD pathogenesis, reflects a disturbed balance between the generation of reactive oxygen species (ROS) and antioxidative mechanisms. The major source of ROS is in the mitochondria, as by-product of oxidative phosphorylation [18]. The free oxygen radicals generated by the mitochondria are converted to H_2O_2 , spontaneously, or by superoxide dismutase, and in turn this is either reduced to water by glutathione peroxidase or converted to hydroxyl radicals in the Fenton reaction, catalyzed by iron.

The ROS bring about oxidative damage of proteins, lipids, and DNA [19]. An imbalance between ROS generation and antioxidative mechanisms in PD has been demonstrated by a decreased nigral level of the antioxidant glutathione [20], reduced mRNA expressions of Cu/Zn-superoxide dismutase and glutathione peroxidase [21], and an increased concentration of iron (Fe^{3+}) [22]. Increased iron concentrations were reported in the substantia nigra of severe cases of PD [23,24] but not in milder cases. The iron deposition was localized to the neuromelanin granules of the substantia nigra pars compacta [25] that is a strong iron chelator. *In vivo* MRI studies indicated reduced T2 relaxation times that was related also to the increased iron content of the substantia nigra [26,27]. Dopaminergic cells are prone to oxidative damage as the metabolism of dopamine by monoamine oxidase produces H_2O_2 [28]. Dopamine can also be metabolized to reactive dopamine quinol which has been shown to be neurotoxic through the covalent modification of proteins [29].

Figure 1 Interaction of pathomechanical pathways in PD.

Mitochondrial Dysfunction

The primary role of mitochondria is the energy supply of the cell through oxidative phosphorylation. In the double-membrane organization of the mitochondrion, four distinct compartments can be defined, each having unique functional role: the outer membrane, the intermembrane space, the inner membrane, and the matrix. The elements of the mitochondrial electron transport system are all located on the inner membrane: complex I (nicotinamide adenine dinucleotide ubiquinone oxi-reductase), complex II (succinate ubiquinone

oxi-reductase), complex III (ubiquinone/cytochrome c reductase), complex IV (cytochrome oxidase), and complex V (ATP synthase). Two mobile electron carriers are located in the intermembrane space and in the inner membrane are the cytochrome c and the coenzyme Q (CoQ; ubiquinone). As the electrons are transported down the respiratory chain (Complex I-V), the proton concentration in the intermembrane space increases and a strong electrochemical gradient is established across the inner membrane (mitochondrial transmembrane potential). The energy produced by equalization of this gradient through complex V is used for ATP synthesis (Figure 2—inset). Although this process is efficient a small percentage of the electrons may reduce oxygen prematurely and produce ROS.

The first evidence of the key role of a mitochondrial dysfunction emerged from the observation that the 1-methyl-4-phenylpyridinium ion (MPP⁺), an active metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; a contaminant of opiate drugs), which is an inhibitor of mitochondrial complex I, causes dopaminergic cell death and rapid-onset parkinsonism [30,31]. Consecutively, a deficient function of the complexes of the electron transport system was demonstrated in the platelets and skeletal muscle of PD patients [32–34]. The background of such a complex I deficiency seems to be linked to the mitochondrial DNA, as indicated by neuroblastoma cells with mitochondria derived from the platelets of PD patients [35,36]. Knowledge of the mitochondrial electron transport system deficiency led to the development of promising, novel therapeutic interventions, such as near-infrared light therapy, which was suggested to modulate the activity of complex IV [37], and mitochondrial gene therapy was also postulated as a possible future therapeutic avenue [38].

Whatever the reason for and the specific mechanism of the mitochondrial dysfunction, it was hypothesized to cause neurodegeneration via one of the following pathways: (1) oxidative stress (see above), (2) energy depletion (that itself may cause cell death by activating K-ATP channels [39], by compromising the proteasomal activity [40] or altering the neural amino acid levels [41]), and (3) the promotion of apoptosis (see below).

Neuroinflammation

The role of neuroinflammation in the pathogenesis of PD was suggested by several early findings and also by recent studies. Microglial activation and astrocytic reactions were described in the substantia nigra of PD patients post-mortem [42–44], though it is not yet clear if this is the cause or a result of the progressing disease. It has also

been reported that CD4⁺ and CD8⁺ T cells are present in the mouse model of PD close to vessels and dopaminergic neurons [45]. In a series of papers, Mogi and coworkers reported increased levels of tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), IL-6, epidermal growth factor, transforming growth factor- α , transforming growth factor- β 1, β 2-microglobulin, and IL-2 in the brain of PD patients [46–52]. In the serum of human patients increased levels of IL-2, TNF- α , IL-6, RANTES, and antibodies against dopamine-oxidized proteins were found [53–56]. Similar changes were observed in the cerebrospinal fluid [51,57]. The contribution of the microglial response to the PD pathogenesis was demonstrated in *in vivo* imaging studies [58,59]. Parallel changes in microglial activation and the corresponding dopaminergic terminal loss in the affected nigrostriatal pathway in early PD were revealed by means of PET. An association between polymorphisms and mutations of various neuroinflammation related genes and PD was suggested [60–66].

The exact mechanism of the neuroinflammation-related neurotoxicity is not yet fully understood, but several studies have suggested that, besides the direct apoptosis-inducing effect of cytokines, it is related to the oxidative burst arising from the activated microglia [67–71].

Protein Aggregation

Alpha-synuclein is the major component of the Lewy bodies in sporadic PD. Overproduction and reduced clearing of the protein by lysosomes and proteasomes may play a key role in PD pathogenesis. Elevated levels of alpha-synuclein may result in aggregation and the formation of toxic oligomers that may interfere with the lysosome and proteasome functions [72] creating a vicious circle [73]. Under physiological conditions alpha-synuclein exists as α -helical conformation, whereas under pathological conditions it can refold into a β -sheet-rich conformation that can furnish toxic oligomers and amyloid fibrils. These protein aggregates can in turn promote the misfolding of wild-type proteins. This hypothetical mechanism, suggested to be similar to a prion-like mechanism [74], was confirmed by the demonstration that, some 10 years after implantation, embryonic dopaminergic cells displayed PD pathology [75,76]. Also Desplats and colleagues found that over-expressed synuclein could be transferred to healthy cells that will express pathological changes [77]. This mechanism would also be in accordance with the observation by Braak that the appearance of alpha-synuclein follows a predictable sequential spread throughout the nervous system [15].

Figure 2 Apoptotic processes in PD. Extrinsic pathway initiated from the cell surface receptors (e.g., Fas) and proceeding through a cascade of events, in which caspases are involved. The activation of the intrinsic pathway begins with the permeabilization of the mitochondrial outer membrane, which leads to the release of key mitochondrial proteins (such as cytochrome c, Smacs, AIF, etc.). Members of the Bcl2 family of proteins

are important modulators of pro- and antiapoptotic mechanisms. The inset shows the structure of the mitochondrion and the respiratory chain (JNK, c-Jun N-terminal kinase; Smacs, secondary mitochondria-derived activator caspases; AIF, apoptosis inducing factor; Apaf-1, apoptotic peptidase activating factor 1).

Exitotoxicity and Calcium Homeostasis

A further key process implicated in PD pathogenesis is glutamate exitotoxicity. Glutamate is the major excitatory neurotransmitter of the nervous system that acts on NMDA (N-methyl-D-aspartate), AMPA (α -amino-3-hydroxy-5-methylisoxazole propionic acid) and metabotropic receptors (mGlu1-mGlu8) [78,79]. Besides its key role in neurotransmission by activating NMDA and AMPA receptors and voltage-gated ion channels, glutamate causes a massive influx of extracellular calcium and also its release from the endoplasmic reticulum, initiating neurotoxic processes. The increased intracellular calcium concentration may influence apoptosis-related factors such as the Bcl-2 family proteins [80], and endoplasmic reticulum-related protein processing and folding [81]. Moreover, calcium enters the mitochondria and under normal circumstances potentiates oxidative phosphorylation to meet the increased metabolic demands of restoring the calcium homeostasis [82]. In the event of an enhanced calcium influx, it may ultimately compromise the mitochondrial energy production, further increasing the mitochondrial calcium concentration and leading to depolarization and ultimately to opening of the mitochondrial permeability transition pore and commencing apoptosis [83].

Dopaminergic cells of the substantia nigra pars compacta are critically sensitive to glutamate exitotoxicity as a reduced dopaminergic activity leads to an enhanced compensatory recursive glutamatergic input [84]. Further, in order to maintain the constant striatal dopamine concentration, dopaminergic cells have a pace-maker activity generated through ion channels [85,86] that increase the intracellular calcium level [83], thereby increasing the susceptibility of these cells.

Apoptosis

Apoptosis, programmed cell death, is a cascade of biochemical events leading to characteristic morphological changes and consequently to cell death [87] (Figure 2). The apoptotic cascade is initiated by two pathways (extrinsic and intrinsic) that converge to the same final effector mechanisms.

The extrinsic pathway is activated through cell surface death-receptors belonging in the TNF receptor superfamily; activation of the caspase cascade that in turn will activate the effectors, the death substrates [88]. It has been proposed that inflammatory cytokines released from the activated immune cells and microglia found in the brain of PD patients [42–45] may induce apoptosis directly through TNF cell surface receptors [89]. However, there are conflicting results about the importance of the TNF receptor-mediated processes in animal

models [90–94]. Another receptor involved in the initiation of the extrinsic pathway is Fas (also belonging in the TNF receptor superfamily), activated by the Fas-ligand (Fas-L: expressed mainly by activated T cells); this has been shown to be overexpressed in PD patients and also in MPTP-treated mice [95,96]. However, contradictory results have also been published regarding the role of the Fas-L/Fas/caspase-8 pathway in PD [97,98]. The c-Jun N-terminal kinase (JNK) have been identified a key transcriptional modulator of the Fas/Fas-L system and is a crucial component of the neurodegeneration [99–102]. Nonetheless, inhibition of the JNK signal transduction pathway upstream at the level of mixed lineage kinases by GEP-1347 in a prospective clinical trial did not prove to be effective [103].

The pivotal event in the intrinsic or mitochondrial pathway is the increased permeabilization of the mitochondrial outer membrane. This either occurs by opening of the mitochondrial permeability transition pore or is mediated by the direct action of proapoptotic proteins, the Bcl-2 family members Bax and Bak [104]. Once the outer membrane is permeable, key mitochondrial proapoptotic proteins are released to the cytosol, such as cytochrome c, AIF, and Smacs. Cytochrome c binds to Apaf-1 and forms a protein complex known as apoptosome that in turn activates procaspase-9 to initiate an enzymatic reaction cascade leading to the execution of apoptosis in cells [105]. The key regulator of the mitochondrial permeability and the release of the proapoptogenic proteins are the proteins belonging in the Bcl-2 family [106].

Etiology of PD

Although epidemiology studies have suggested several environmental factors as risk factors for PD, no single etiological factor has yet been conclusively established. MPTP, a contaminant of synthetic opiates, is the first link between environmental factors and the disease. Rural living [107,108], well-water [108,109], pesticides, and herbicides [109,110] (not necessarily independently) have been implicated as risk factors for PD [111,112].

Genetic alterations have been identified as the cause of PD in familial cases (Table 1), which account for ~5–10% of all PD cases.

The first mutation identified as linked to PD was in the gene of alpha-synuclein, the major component of Lewy bodies [113]. It was observed that the overproduction and reduced clearing of the protein may play a key role in PD. Increased levels of alpha synuclein result in aggregation and the formation of toxic oligomers that may interfere with the lysosome and proteasome functions, creating a vicious circle [114]. Hence, interventions against the

Table 1 Genetic background of Parkinson's disease

Protein/Gene	Locus	Putative physiological and pathogenetic role	Key references
Synuclein	Chr 4	Pathological oligomer formation	[113–118]
PARK-1	4q21–23		
Parkin	Chr 6	Ubiquitin-protein ligase	[119–124]
PARK-2	6q23–27	By ubiquitination catalyze protein degradation	
UCH-L1	Chr 4	Ubiquitin C terminal hydrolase	[125–129]
PARK-5	4q14–15	Generates monomeric ubiquitin by cleaving polymeric ubiquitin to monomers and from the ubiquitylated peptides	
PINK-1	Chr 1	A serine-threonine kinase	[130–134]
PARK-6	1p35–36	Regulator of mitochondrial functions	
DJ1	Chr 1	Redox-sensitive modulator of antiapoptotic pathways	[135, 136]
PARK-7	1p36		
LRRK2	Chr 12	GTP-ase and protein kinase	[137–142]
PARK-8	12p11	Modulator of apoptotic processes	

processing, misfolding and aggregation of alpha-synuclein and those augmenting the clearing processes are promising for the development of novel therapeutic methods [143–150].

The most frequent mutation among PD patients with recessive inheritance [119] is PARK-2. Parkin is a ubiquitin-protein ligase located at the outer mitochondrial membrane [120]. By catalyzing the ubiquitination of many proteins, it facilitates their degradation by the proteasome [151–154] and this effect is linked to the attenuation of JNK and p38 mitogen-activated protein kinase and the subsequent cleavage of caspase-3. The above results certainly justify the delivery of parkin gene to the midbrain dopaminergic cells of patients carrying this mutation. Preclinical animal studies are already under way [155,156], but several issues must be resolved before this new treatment modality can be applied in clinical trials [155].

Ubiquitin C-terminal hydrolase (UCH-L1) was first linked to PD by an autosomal dominant point mutation (I93M) in two siblings with a strong family history, but incomplete penetrance [125]. The possibility was also raised that a single nucleotide polymorphism (S18Y) is linked to a reduced susceptibility to PD [126–128], though contradictory results were also published recently [157]. UCH-L1 is a neuronal enzyme that catalyzes the hydrolysis of C-terminal ubiquitin esters and amides [129]. The major function of the enzyme is the generation of monomeric ubiquitin by cleaving polymeric ubiquitin to monomers and from the ubiquitylated peptides that are the main products of proteasomal degradation of polyubiquitinated proteins [129]. It has been proposed that there is a fine balance between the concentration-dependent alpha-synuclein aggregation, the ubiquitination of alpha-synuclein by parkin for proteasomal degradation and the subsequent recycling of ubiquitin by

UCH-L1 [158]. A disturbance of this balance at any point may give rise to a shift to pathological changes, and hence the suggested key roles of mutations in these genes in the pathogenesis of PD.

Ubiquitinated protein, PTEN-induced putative kinase 1 (PINK1) [130] is a nuclearly encoded protein localized to the intermembrane space of the mitochondrion [131,132]. The protein exerts serine-threonine kinase activity and is also capable of autophosphorylation, which may have an important regulatory role. The role of PINK1 in the pathogenesis of PD may be related to defective oxidative phosphorylation in consequence of changes in the phosphorylation of key mitochondrial proteins of the respiratory complexes [159]. Alternatively, failing physiological phosphorylation triggers a signaling cascade that opens the mitochondrial transition pores and apoptosis commences [160,161].

Although the physiological role of the highly conserved protein DJ-1 is not yet clear, it is very likely that it is a transcriptional factor and redox-sensitive modulator of the antioxidative and antiapoptotic pathways [162, 135, 163]. As a potential therapeutic alternative, DJ-1 transferred either by the gene or by the recombinant protein itself reduces MPTP or 6-hydroxy-dopamine-induced (6-OHDA) neurotoxicity [164,165]. Compounds that prevent oxidation of DJ-1 also successfully prevent oxidative stress-induced dopaminergic cell death in the rat [166].

LRRK2/dardarin, a member of the ROCO protein family is characterized by GTP-ase and protein kinase activity [137]. Mutations markedly augment the kinase activity [138,139] or change the GTP-ase activity, which in turn influences the kinase activity [139]. The kinase activity seems to be crucial in the development of LRRK2-related apoptotic processes mediated by cytochrome c and caspase-3 [167–169], but domains without kinase activity

have also been demonstrated to be important for the initiation of apoptosis [170]. From a possible therapeutic aspect, heat shock protein 90 has been shown to connect to LRRK2 *in vivo* [171] and inhibition of the chaperone activity of this protein disrupts this association and leads to the proteasomal degradation of LRRK2 [172].

These observations furnish important clues concerning the pathomechanism of PD and have been utilized as starting points for the development of several drugs. However, a recent large-scale study did not find significant difference in the pairwise concordance of parkinsonism in mono- and dizygotic twin brothers older than 50 years. The authors concluded that genetic factors do not play an important role in sporadic PD when the disease begins after 50 years of age, which is the situation in the majority of cases [173]. However, genetic factors appear to be important when disease begins before age 50 years.

Neuroprotection in PD

L-Dopa

L-Dopa was introduced into PD therapy in the late 1960s, and is still the most effective symptomatic therapy. Essentially, all PD patients, but especially those with early PD benefit from L-Dopa therapy [174]. L-Dopa is usually coadministered with a peripheral decarboxylase inhibitor (carbidopa or benserazide) to prevent peripheral conversion to dopamine, thereby stabilizing the bioavailability of dopamine and reducing the peripheral side effects. To reduce the elimination of L-Dopa further, it may be coadministered with catechol-O-methyl transferase (COMT) inhibitor entacapone and tolcapone [175–178].

L-Dopa is the direct biosynthetic precursor of dopamine, is actively transported across the blood–brain barrier and is transformed to dopamine by aromatic-L-aminoacid decarboxylase. One site of the decarboxylation is in the remaining dopaminergic neurons, but serotonergic, noradrenergic, and “D” neurons and glial cells have also been shown to take up L-Dopa and transform it to dopamine [179–181]. The importance of decarboxylation in the nondopaminergic neurons and glia is not yet clear. Dopamine produced in nondopaminergic cells may be taken up by dopamine transporters in dopaminergic neuron terminals and packed into dopamine-containing synaptic vesicles by vesicular monoamine transporter 2 (VMAT2). A reduced level of VMAT2 has been demonstrated to lead to dopamine-mediated neurodegeneration [182], and gain-of-function haplotypes that display increased transcriptional activity have been found to be protective against PD in women [183]. Cytosolic dopamine accumulation itself has been suggested to be

neurotoxic through the generation of ROS [184]. The Earlier versus Later Levodopa (ELLDOPA) study, which examined the question of whether the L-Dopa therapy hastens the disease course [185], did not reveal detrimental effect of L-dopa therapy, and after 2 weeks of wash-out patients on L-Dopa therapy still exhibited beneficial effects. However, the wash-out time is much longer, and interpretation of the results of the imaging substudy of ELLDOPA led to the possible neuroprotective effect of L-Dopa remaining in question.

Dopamine Agonists

Dopamine agonists, which were introduced into the therapy of PD in the 1970s have several advantages over L-Dopa. As they do not need metabolic modification, their effect is not dependent on the surviving dopaminergic cell pool. Their effect does not depend on the dopamine metabolism, and consequently is not related to the possible toxic effect of dopamine. The derivation drugs selectively acting only on certain types of dopamine receptor might afford a possibility to reduce the dopaminergic adverse effects.

Dopamine agonists such as bromocriptine (a potent D2 agonist and mild D1 receptor antagonist), carbergolide (a long-acting D2 agonist), pergolide (a D1, D2, and somewhat selective D3 receptor agonist), and ropinirole (a nonergot dopamine agonist) have been shown to exert free radical-scavenging action and to protect against MPTP, dopamine, 6-OHDA, and nitric oxide toxicity both *in vitro* and *in vivo* animal models [186–192]. The antioxidant effects of these drugs are related to their molecular structure (a hydroxylated benzyl ring), downregulation of dopamine turnover (partly a D2 receptor-dependent effect) [193,194], and also the upregulation of protective scavenging enzymes (e.g., they upregulate the expressions of glutathione-related enzymes) [190,194].

It has been suggested that apoptogenic cytochrome c released from the mitochondria induces an apoptotic process through Apaf-1, caspase-9, and caspase-3 that is later involved in the pathogenesis of PD [195]. Cytochrome c, a heme protein that is an essential component of the mitochondrial electron transport system, has also been denoted as a stimulator of alpha-synuclein aggregation [196]. The dopamine agonists talipexole and pramipexole have been shown to possess neuroprotective effects in MPTP-treated mice [197], 6-OHDA-treated rats [198], invertebrate flatworms [199], the human neuroblastoma cell line SH-SY5Y [200], and nonhuman primates [201], the effect being related to inhibition of the release of cytochrome c and aggregation of alpha-synuclein [202]. It was also demonstrated that this neuroprotective effect of pramipexole is not dependent on dopamine receptors or

either antioxidative effect of the drug [203], but may be related to protection against glutamate excitotoxicity by reduction of the intracellular dopamine content [204]. However, evidence has been provided for at least a partial dopaminergic effect [205].

Recent results have indicated that dopamine agonists may exert their neuroprotective effect through growth factor-like properties. Pramipexole and ropinirole acted on dopaminergic neurons by modulating the production of endogenous brain-derived (BDNF) and glial cell line-derived neurotrophic factors [206] that were shown to mediate neuroprotection [207]. Several lines of evidence suggest that BDNF plays a substantial role in preventing the neuronal degeneration seen in PD. Reduced BDNF mRNA expression was found in the substantia nigra of PD patients [208]. In alpha-synuclein-transfected glioma cells, the expression of BDNF was induced, but not by the A30P and A53T-mutated alpha-synuclein gene [209]. It was also demonstrated that inhibition of BDNF expression by antisense oligonucleotide infusion causes the loss of nigral dopaminergic neurons [210]. The Val66Met polymorphism of BDNF gene was proposed to be a susceptibility factor for PD [211,212], but the experimental findings are not clear-cut [213,214].

It is important that besides evidence from *in vitro* and animal studies, dopamine agonists have also been evaluated and their disease-modifying effects confirmed in clinical trials. The imaging surrogate marker of beta-CIT SPECT was utilized in the CALM-PD study [215] and fluoro-dopa PET the REAL-PET study [216]. The conclusion of both investigations was that the dopamine agonists pramipexole and ropinirole may have a neuroprotective effect. Whereas the use of imaging biomarkers is certainly justified, the exact relation to clinical measures must be further evaluated before use of such surrogate markers in clinical trials.

MAO-B Inhibitors

Monoamine oxidase (MAO) embedded in the mitochondrial outer membrane [217] is responsible for the degradation of dopamine. Within the central nervous system, the bulk of the enzyme is present as the B isoenzyme. As one of the key components of the oxidative degradation of dopamine and in the consequent oxidative stress, it has a central role in the pathogenesis of PD.

Selegiline, a selective and irreversible inhibitor of MAO-B, first synthesized by the Hungarian pharmacologist Ecseri [218] and characterized by Knoll and Magyar [219], improves parkinsonian symptoms and is approved worldwide for use as adjunctive therapy to L-Dopa. Rasagiline, an indane derivative, is a novel irreversible inhibitor of MAO-B. In contrast with selegiline, the

metabolites of rasagiline do not possess amphetamine-like effects and are not vasoactive either [220]. A metabolite of selegiline inhibits the neuroprotective effect of the drug, but no similar inhibition is observed in the case of rasagiline [221].

It is well known that MPTP toxicity can be avoided by the use of MAO-B inhibitors [222,223]. The metabolism of MPTP by MAO-B to MPP⁺ that generates free radicals is linked to the pathogenesis of PD [223,224]. Nevertheless, the neuroprotective effect of MAO-B inhibitors does not depend only on the enzyme-blocker properties of the drugs. The less potent enantiomers of selegiline and rasagiline still have a significant neuroprotective effect and the effect is also observed in the human dopaminergic cell line SH-SY5Y, which does not express MAO-B [225,226]. The antiapoptotic effect is related to inhibition of decrease in the mitochondrial membrane potential. The mitochondrial permeability transition pore that controls the initiation step of the apoptotic death process by reducing the mitochondrial membrane potential has a tertiary structure similar to that of MAO-B, which may be responsible for the neuroprotective effect of rasagiline. Alternatively, rasagiline has been observed to increase the expression of Bcl-2, a direct regulator of the mitochondrial permeability transition pore, and hence to have an antiapoptotic property [227,228]. Importantly, rasagiline exerts neurorestorative action when administered posttreatment after MPTP neurotoxicity. This effect is linked to activation of the pathway involving the neurotrophic tyrosine kinase receptor [229] a ligand of BDNF, the expression of which is also modulated by rasagiline [230,231].

A major metabolite of rasagiline, the 1-(R)-aminoindane was also shown to have neuroprotective effect in several *in vitro* and *in vivo* animal studies [232,233]. The mechanism of neuroprotectant effect of 1-(R)-aminoindane was related to modulation of apoptosis related proteins, such as decreasing the level of H2A.X, decreasing the cleavage and increasing the expression of caspase 9 and 3, increasing the level of antiapoptotic proteins Bcl-2 and Bcl-xl [232].

Furthermore, novel bifunctional MAO-B inhibitors possess also an iron chelating property that might be a key factor of the neuroprotectant feature of these drugs (HLA-20, M30) [234–237]. In these molecules, the propargylamid and MAO-B inhibitor moiety of rasagiline is combined with and iron chelating properties, consequently these drugs are able to block the iron-mediated production of free radicals in the Fenton reaction.

The neuroprotective effect of the MAO-B inhibitors selegiline and rasagiline has been the subject of several clinical trials. The Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) study [238]

revealed a beneficial effect of selegiline, but not of tocopherol, when the introduction of L-Dopa therapy was delayed. The Sinemet-Deprenyl-Parlodar (Sindepar) study indicated the superiority of selegiline as compared with placebo, as measured via the primary endpoint of the study: the change in UPDRS between the first and the final visit, after a 2-month of wash-out period following a 12-month treatment [239]. The recently reported initial results of the delayed start design Attenuation of Disease progression with Azilect Given Once-daily (ADAGIO) study also supported the neuroprotective effect of rasagiline [240,241].

Antiinflammatory Therapies

Nonsteroid antiinflammatory drugs (NSAID) and especially nonaspirin NSAIDs such as ibuprophen, have also been suggested to have a beneficial effect in PD [242,243], though the studies have not proved so far conclusive [244,245]. The mechanism by which NSAIDs exert their neuroprotective effect is still elusive. One obvious possibility is that they act by inhibiting the proinflammatory cyclooxygenase, but their ROS-scavenging effect may also be important [246].

Minocycline a semisynthetic second-generation tetracycline has both antimicrobial and antiinflammatory effects. Minocycline has been found to be neuroprotective in a preclinical MPTP-induced PD model [247,248]. The neuroprotective effect is related to inhibition of the MPTP-induced glial inducible nitrogen monoxide synthase expression and inhibition of the phosphorylation of p38 mitogen-activated protein kinase [247]. A phase II clinical investigation (the Neuroprotective Exploratory Trials in Parkinson Disease, Futility Study 1 – NET-PD FS-1) did not rule out the potential value of the drug, and suggested further phase III trials [249,250].

There is also substantial interest in glatiramer acetate (Copolymer 1, Copaxone), which induces regulator T cells that secrete high amounts of antiinflammatory T-helper 2/3 cytokines [251,252]. It is also known that glatiramer acetate-specific T-helper cells can be stimulated to produce BDNF [251,253,254]. The neuroprotective effect has been demonstrated in preclinical animal studies [255–257]. Further studies are warranted to evaluate the effectiveness of Copaxone in PD.

Creatine

Creatine is a natural guanidine compound that plays a pivotal role in the cell energy metabolism. The creatine system serves as a spatial energy buffer between the mitochondria, the energetic centers of the cell, and the cytosolic energy-consuming processes. Mitochondrial crea-

tine kinase catalyzes the reversible transfer of a phosphate group from ATP, the high-energy product of the mitochondrial respiratory chain, to creatine, yielding high-energy phosphocreatinine.

In vitro and *in vivo* animal studies have indicated the neuroprotective effect of creatine [258]. One possible mechanism of creatine-induced neuroprotection is stabilization of the energy balance of the cells. Creatine treatment has been shown to increase the level of phosphocreatine [259,260], which is an important energy supplementation when the mitochondrial respiratory chain is deficient (e.g., complex I inhibition by MPTP). Another possible mechanism of neuroprotection might be inhibition of the mitochondrial permeability transition pore, the initial step of apoptosis. Creatine has been reported to stabilize the mitochondrial isoform of creatine kinase in an octomeric form that in turn can inhibit the mitochondrial permeability transition pore [261,262], but this was questioned by the report that creatine has a neuroprotective effect in MPTP-treated mice deficient in ubiquitous mitochondrial creatine kinase [263]. The neuroprotective effect of creatine has also been associated with direct antioxidant properties [264]. The phase II clinical trial NET-PD FS-1 did not exclude the potential of creatine in the treatment of PD [249,250].

Alpha-tocopherol

Tocopherol, one of the compounds in vitamin E is the main fat-soluble antioxidant in the organism. It is incorporated into cellular membranes and effectively inhibits the peroxidation of lipids by scavenging the chain-propagating peroxy radicals [265]. Tocopherol does not act in isolation, but as part of the antioxidant network, which also involves vitamin C, thiol antioxidants such as glutathione and lipoic acid.

Given that oxidative damage plays a central role in the pathogenesis of PD, antioxidants such as tocopherol are presumed to have a neuroprotective effect. MPTP toxicity was more pronounced in vitamin E-deficient mice and this effect was related to lipid peroxidation [266]. A dietary vitamin E deficiency itself causes a loss of tyrosine hydroxylase-immunopositive neurons in the substantia nigra of rats [267], presumably because of a defective antioxidative network. A number of studies have demonstrated neuroprotective effects of vitamin E [268,269]. On the other hand, vitamin E pretreatment does not attenuate MPTP striatal neurotoxicity [270,271] and a genetic vitamin E deficiency does not affect MPTP susceptibility [272].

Clinical studies have been carried out on the possible disease-modifying effect of alpha-tocopherol. An open-label study concluded that alpha-tocopherol and

ascorbic acid administered together deferred the initiation of L-Dopa by 2.5 years [273]. However, the multicenter, placebo-controlled clinical trial Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism (DATATOP) [238] did not reveal a positive disease-modifying effect of tocopherol.

Coenzyme Q

Coenzyme Q10 (CoQ10), also known as ubiquinone, is present in eukaryotic cells mainly in the mitochondria. It contains 1,4-benzoquinone and 10 isoprenal subunits. It exists in three different redox states (the fully oxidized ubiquinone, the free radical ubisemiquinone, and a fully reduced ubiquinol) and is an important component of the mitochondrial electron transport system. It transports electrons from complexes I and II to complex III of the electron transport chain. CoQ10 behaves as a free radical scavenger [274], this effect depending, at least partly, on the recycling of alpha-tocopherol [275]. It has been suggested that it acts in concert with dihydrolipoic acid, an organic antioxidant compound that has been also implicated as a potent neuroprotective agent [276,277].

CoQ10 exerts a neuroprotective effect in various *in vivo* models, for example, ischemia [278], malonate (an inhibitor of succinate dehydrogenase)-induced striatal degeneration [279], MPTP toxicity [280], and paraquat-induced oxidative stress [281]. This latter study is of especial importance because it involved use of the water-soluble formulation of CoQ10, which is thought to have significantly higher bioavailability [282].

A clinical trial evaluating the efficacy through measurement of the change in UPDRS score also highlighted a possible beneficial effect of CoQ10 in early PD [283], but no effect on the time to initiate symptomatic treatment. The NINDS NET-PD futility study did not reject CoQ10 as a potential agent and suggested phase III trials; however, the chosen futility threshold was questioned [284].

Tryptophan Metabolism in Neuroprotection

The essential amino acid tryptophan is metabolized in several alternative pathways, one of the most important of which is the kynurenine pathway [285], yielding the final product nicotinamide adenosine dinucleotide (NAD) (Figure 3).

Figure 3 The kynurenine pathway of the tryptophan metabolism.

The first stable metabolite of the pathway is kynurenine, which is transformed by kynurenine aminotransferase either to kynurenic acid or by kynurenine hydroxylase to 3-hydroxy kynurenine, which is further metabolized to quinolic acid, the precursor of NAD. These metabolites are usually referred to as neuroactive kynurenines [286,287]. Kynurenic acid is an antagonist of the strychnine-insensitive glycine-binding site of the NMDA receptor [288,289], a weak antagonist of AMPA and kainite receptors [290] and also an inhibitor of the $\alpha 7$ nicotinic receptor [291], which is involved in the presynaptic regulation of glutamate release. Importantly, the neuroinhibitory effect of kynurenic acid is concentration-dependent: in nanomolar concentrations, it facilitates field excitatory postsynaptic potentials [292]. 3-hydroxykynurenine and quinolic acid are both neurotoxic [293], and were shown to be the direct activator of NMDA receptors [294], modulate the release or reuptake inhibition of glutamate [295], be involved in lipid peroxidation [296] and the production of ROS [297].

The relation of the kynurenine pathway to PD has been demonstrated in several investigations. The level of kynurenine aminotransferase I (the enzyme that converts kynurenine to kynurenic acid) was found to be reduced after MPTP and 6-OHDA toxicity in the striatum of the rat [298,299]. MPP⁺ also reduces the level of kynurenic acid by inhibiting kynurenine aminotransferase II. In a human postmortem study, the concentration of both kynurenine and kynurenic acid proved to be reduced, in contrast with that of 3-hydroxykynurenine, which concentration was increased in several brain regions. Reduced level of kynurenine has been measured in serum of PD patients, which has been paralleled with reduced kynurenine aminotransferases activity [300]. The importance of these studies was speculated to lie in reduced NMDA antagonism, as a consequence of the reduced level of kynurenic acid or a shift in the tryptophan metabolism in the direction of 3-hydroxykynurenine and quinolone, both propagating neurotoxicity. In accordance with the former hypothesis, glutamate antagonists were shown to protect against MPP⁺ toxicity [301], signifying the importance of the glutamatergic excitotoxicity in the pathogenesis of PD. Importantly, the role of the non-NMDA, AMPA glutamate receptor too was demonstrated in kynurenic acid-related neuroprotection [302]. Another possible link between the kynurenine pathway and PD is the immune system. Interferon-gamma modulates indoleamine 2,3-dioxygenase, the enzyme that catalyzes the tryptophan-kynurenin transformation [303]. In PD patients, an amplified cellular immune activation was found to be paralleled by an increased kynurenine/tryptophan ratio [304].

From the above results, it is intuitive that a shift of the balance of the kynurenine pathway in the direction of kynurenic acid may have a potential neuroprotective effect [305]. The increase of endogenous kynurenic acid by nicotinylalanine, inhibiting kynureninase and kynurenine hydroxylase, protects against NMDA and quinolic acid toxicity [306]. Furthermore, Ro 61 8048, a kynurenine hydroxylase inhibitor, reduces the striatal glutamate level besides increasing the kynurenic acid concentration in the striatum, the cortex and the hippocampus [307]. A similar effect has been demonstrated in nonhuman primates [308].

Another option via which to modulate the kynurenine pathway in order to achieve neuroprotection is the use of kynurenine analogues. 7-Chlorokynurenate, a synthetic derivative of kynurenic acid and an antagonist of the glycine-binding site of the NMDA receptor, effectively protects against NMDA receptor-mediated toxicity [309,310]. The poor penetration of the drug can be overcome through the use of 4-chlorokynurenine a precursor of 7-chlorokynurenate, that readily crosses the blood-brain barrier and is converted enzymatically to the active metabolite [311,312].

Conclusions

The central mechanism of neurodegeneration shares common pathways in several neurodegenerative diseases, for example, PD, Huntington's disease, amyotrophic lateral sclerosis and Alzheimer's disease. Investigations into these processes have led to development of novel therapeutic approaches shown to be effective in *in vitro* studies and animal models. A small number of human investigations have also been carried out and positive results have been published, but no medication has yet proved unanimously neuroprotective. Further clinical trials are needed to unravel the potential neuroprotective effects of the above-cited drugs and approaches. On the other hand, the pathomechanism of PD is far from clear and additional investigations are needed to identify the possible pathways that could be targeted for therapeutic interventions.

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Author Contributions

Dr. Kincses and Dr. Vecsei both collaborated on the concept of the article. Drafting was carried out by Dr. Kincses.

Conflict of Interest

Both authors are employed by the Albert Szent-Györgyi Clinical Center, University of Szeged. Neither of the authors reports any conflict of interest.

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