CHAPTER 3

Dopamine Receptor Agonists for Protection and Repair in Parkinson's Disease: An Update

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Abstract: Dopamine agonists have been shown to possess neuroprotective properties in different *in vitro* and *in vivo* experimental Parkinson's disease models, because their capability to counteract- neuronal cell death. Here we update the molecular evidence underlying the wide pharmacological spectrum of dopamine agonists currently used for treating Parkinson's disease patients. In particular, the mechanism of action of different dopamine agonists does not always appear to be restricted to the stimulation of selective dopamine receptor subtypes since at least some of these drugs are endowed with antioxidant, antiapoptotic or neurotrophic properties. These activities are molecule-specific and may contribute to the clinical efficacy of these drugs for the treatment of chronic and progressive neurodegenerative diseases in which oxidative injury and/or protein misfolding and aggregation exert a primary role. However, despite increasing number of experimental results confirm their neuroprotective effects, further studies are needed to definitively confirm dopamine agonists as disease-modifying agents.

Keywords: Alzheimer's disease, amyolid fibril, amyotrophic lateral sclerosis, apomorphine, bromocryptine, disease-modifying therapy, dopamine, dopamine receptor agonists, Free radicals, neurodegeneration, neurogenesis, neuroimaginig, neuroprotection, oxidative stress, Parkinson's disease, pergolide, pramipexole, protein aggregation, ropinirole, rotigotine, α-synuclein.

INTRODUCTION

Parkinson's disease (PD) is the most common neurodegenerative movement disorder. Approximately 2% of the population older than 65 years suffer from this slowly progressive neurodegenerative disease. More than 90% of PD cases are

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sporadic. The primary cause of the disorder is the progressive loss of the pigmentated DAergic neurons in the substantia nigra *pars compacta* (SNpc) accompanied by the appearance of intracytoplasmic inclusions known as Lewy bodies, containing aggregated alpha synuclein. Despite significant progress has been made in controlling the symptoms of the disease, a neuroprotective or disease-modifying therapy that can slow or stop disease progression has not yet been obtained. To date, the etiopathogenesis of nigral DAergic neuron loss in PD is unknown. However, the presence of ongoing oxidative stress as the result of inefficacious antioxidant defence mechanisms and generation of radical oxygen species (ROS) in the SNpc of the parkinsonian brain are considered to be important pathogenic mechanisms [1-3]. It should be noted that part of these free radicals are inevitably produced by DA metabolism in the brain either by enzymes through the action of monoamine oxidase-B or by auto-oxidation [4]. Other sources of increased radical production may be endogenous neurotoxins occurring in the brain like tetrahydroisoquinolines or exogenously administered neurotoxins like the widely used herbicide paraquat which have similar neurochemical properties like the well-known neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [5-7]. Moreover, it has been suggested that PD could be associated with excitotoxicity and apoptosis [8] and with a loss of neurotrophic factors [9, 10]. Finally, protein aggregation and misfolding have emerged as important mechanisms not only in PD, with α-synuclein aggregating Lewy bodies, but also in many other neurodegenerative disorders, including as Alzheimer's disease (AD). Therefore, an effective anti-parkinsonian therapy should interfere with the overall mechanisms that finally lead to the progressive DAergic death in the CSN.

Levodopa combined with a peripheral DOPA-decarboxylase inhibitor and a COMT inhibitor is considered the therapy of choice for PD. Levodopa is nearly always effective, but has a high incidence of adverse effects with long term use, including response fluctuations (on/off phenomena) and dyskinesias. More recently DAergic agonists, acting directly at the receptor level, would be able to decrease the incidence of these motor complications [2, 3]. However it seems that these molecules are also endowed with the ability to modify the disease progression protecting neuron from degeneration.

Here we update the knowledge about the potential effects of dopaminergic agonists in restoring the impaired DA function as well as in preventing neurodegenerative processes because of thier additional pharmacological properties.

NEUROPROTECTIVE AND NEURORESTORATIVE PROPERTIES OF DA AGONISTS

The potential of DA agonists in inhibit intracellular death pathways and/or stimulate neuron regeneration may be a consequence of specific DA receptor stimulation or be completely independent from them. These additional pharmacological effects may enlarge the pharmacological spectrum of different DA agonists, contribute to slow down the progression of the neurodegenerative process, also involving sites of action distal from the nigro-striatal areas.

Antioxidant Activity

Increased oxidative stress is thought to be involved in nigral cell death, that is characteristic of PD. This oxidative stress may be further exacerbated by levodopa therapy. These mechanisms have been proven *in vitro* and in animal models, but their relevance in humans remains speculative [2, 3].

Most DA agonists have demonstrated protective properties in cell culture against a range of toxins, including DA, 6-hydroxydopamine (6-OH-DA), 1-metyl-4-phenylpyridinium (MPP^+) and hydroxy peroxide [2, 3, 11]. DA agonists also protect against toxin action *in vivo*, as shown in rodents receiving intrastriatal injection of 6-OH-DA or MPP^+. As reported in details in the following chapters, at cellular level, independent groups have demonstrated decreased free radical production and an amelioration of DA neuronal loss following DA agonist treatment.

Stimulation of Neurogenesis

The neurotransmitter and its receptors appear early during ontogenesis and affect cell proliferation in the embryonic germinal zones [12, 13]. Interestingly, the regulation of neural stem cells by dopamine persists in the restricted brain areas where neurogenesis occurs in adulthood, particularly in the subventricular zone (SVZ) within the lateral wall of the lateral ventricles and in the subgranular zone of the hippocampus. Ongoing adult neurogenesis is currently believed to be an important form of neural plasticity, enabling organisms to adapt to environmental changes and possibly influencing learning and memory throughout life. On the other hand promotion of adult neurogenesis may offer a potential approach for replacing neurons or neuritic networks that degenerate or lose function during aging or in neuropathological settings, including Parkinson's disease. This therapeuthic strategy is particularly intriguing since in post-mortem brains of individuals with Parkinson's disease the numbers of neural precursor cells in

neurogenic regions are dramatically reduced [14]. In a very elegant study Hoglinger and co-authors [14] provided experimental evidence that the highly proliferative precursors cells located in the adult murine subependymal zone lining the lateral ventricle receive dopaminergic afferents. This innervation appears functionally relevant. Transient and bilateral dopaminergic denervation by administration of MPTP resulted in transient and bilateral decrease in the subependymal zone proliferation. Additionally, ablation of mesencephalic dopaminergic neurons of adult rats by stereotaxic injection of 6-OHDA into the nigrostriatal pathway resulted in the unilateral dopaminergic denervation of the subependymal zone as well as in the marked reduction in the number of proliferating precursors. Conversely, cell proliferation was restored by L-DOPA chronic infusion. Similar results were obtained in the hippocampal neurogenic region, which has been shown to receive a dopaminergic from the ventral tegmental area. More recently, the existence of a topographically organized projection from the SNpc to the SVZ was also demonstrated in primates [15], with the anteromedial SNpc projecting to the anteroventral SVZ and the posterolateral SNpc to the posterodorsal SVZ.

The effects on precursor cell proliferation elicited by dopamine are likely to be mediated by D2-like receptors, since D2 and D3 receptors are expressed on neural stem/progenitors cells. As recently summarized by Joyce and Millan [16], different D3 receptor-preferring agonists augment mitogenesis in the SVZ. In particular, Van Kapen and associates demonstrated induction of neurogenesis leading to the regeneration of DAergic pathways, suggesting that this effect may participate to the restoration of DAergic nigrostriatal pathway and locomotor activity in rat model of PD [17, 18]. However, species-specific differences of D3 receptors in regulating neurogenesis have been reported [19, 20]. A two-fold induction of cell proliferation in the SVZ and rostral migratory stream of the adult rats was demonstrated following icv administration of the dopamine D(3) receptor agonist, 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) for 2 weeks [17]. The same drug had no effect on mice [20, 21]. More robust are the data on the involvement of D2 receptors in regulating neurogenesis, based on both the use of D2-null mice and D2 agonists/antagonists [22].

Stimulation of Neurotrophic Factors

One of the most convincing evidence for the role of neutrophic factors in PD is the study of Elsworth *et al.* [23]. They found that implantation of adeno-associated virus type 2 encoding gial derived neurotrophic factor (AAV2-GDNF) in the normal monkey caudate nucleus induced overexpression of GDNF, that

persisted for at least 6 months after injection. In a 6-month within-animal controlled study, AAV2-GDNF enhanced the survival of fetal dopamine neurons by 4-fold, and increased the outgrowth of grafted fetal dopamine neurons by almost 3-fold in the caudate nucleus of MPTP-treated monkeys, compared with control grafts in the other caudate nucleus. GDNF is a potent neurotrophic factor that is crucial to the development, survival, and outgrowth of DA neurons [24, 25]. GDNF is highly expressed in the developing rat striatum, yet its concentration is relatively low in the adult brain [26-28]. Several studies in the 6-OH-DA lesioned striatum of rats have demonstrated improvements in survival and outgrowth of grafted fetal DA neurons when central injections of GDNF have been administered, or when GDNF overexpressing cells have been co-grafted to the striatum [29-35]. Other than GDNF, brain-derived neurotrophic factor (BDNF) is one of the major trophic factors for DAergic neurons [36-38]. Transplantation of modified fibroblasts or astrocytes that express BDNF into either the striatum or the midbrain attenuates 6-OH-DA-induced losses of nigrostriatal neurons [39, 40]. Also, BDNF can modulate dopaminergic neurotransmission in nigrostriatal neurons, as shown by elevated rotational behavior and increased turnover of dopamine in the striatum [41]. BDNF can promote functional recovery from 6-OHDA lesions following expression in striatal cells from an AAV vector [41]. These studies support the relevance of neurotrophic factors for a neurorestorative effect expected in the treatment of PD. As reported in the following chapters, some DA agonists have been found to stimulate either BDNF or GDNF in experimental cell cultures. These pharmacological effects may be useful to improve treatment for PD.

Anti-Fibrillary Activity

Several data suggest anti-fibrillary effects of DA agonists. The aggregation of β-amyloid peptide (Aβ) and alpha-synuclein in the brain has been implicated as a critical step in the development of AD and LBD, respectively. Thus, in addition to antioxidant strategies, increasing evidence points to the possibility of achieving neuroprotection by DA agonists through inhibition of fibril formation [42]. DAergic agents were indeed found to dose-dependently inhibit generation of, as well as destabilize preformed, β-amyloid fibrils [43]. Using fluorescence spectroscopy with thioflavin S, electron microscopy, and atomic force microscopy, the effects of selegiline, DA, pergolide, bromocriptine, and trihexyphenidyl on alpha-synuclein fibrils formation have been recently studied. All molecules except for trihexyphenidyl, dose-dependently inhibited the formation of alpha-synuclein fibrils. Moreover, these molecules dose-dependently destabilized preformed alpha-synuclein fibrils [44]. The anti-fibrillary actions

elicited by the DA agonists appear to be independent of DA receptor stimulation and detectable in cell-free systems.

2. EXPERIMENTAL EVIDENCE FOR NEURORESCUE ACTIVITY

Apomorphine

Apomorphine, a non-ergoline DA receptor agonist, is a short-acting and non-selective DA D1/D2 receptor agonist used for treating PD since many decades. Subcutaneous intermittent injections or continuous infusions of apomorphine have been proposed for the management of sudden, unexpected and refractory levodopa-induced "off" states in fluctuating PD [45]. The original evidence for neuroprotective and antioxidant effects of apomorphine, and other DA receptor agonists, is from Youdim's group [46]. Rational for their pivotal studies was the relevance of ROS generation in the neurotoxicity associated with PD [1, 4]. Since compounds with a catechol structure have metal chelating properties and can act as reducing agents [47], it was attractive the idea that apomorphine may inhibit metal-catalyzed free radical processes and act as a radical scavenger. Studies on the effect of apomorphine on lipid peroxidation and protein carbonyl formation after ascorbate/iron-induced free radical formation in rat brain mitochondrial fractions clearly demonstrated the antioxidant properties of apomorphine in brain tissue [46, 48]. It has later been shown that apomorphine exhibited neuroprotection against DA depletion in 6-OH-DA lesioned-rats [49] and MPTP-treated mice [50]. Furthermore, continuous subcutaneous infusion of apomorphine was found to rescue striatal DAergic terminals and increase the tyrosine hydroxylase and DA-transporter immunoreactivity against toxicity induced by MPTP in mice and enhanced the number of tyrosine hydroxylase-positive cells in the ventral tegmental area in partially 6-OH-DA-lesioned rats [51, 52]. It has also been observed that apomorphine increased the survival of cultured mesencephalic DAergic cells [53] suggesting trophic effects of apomorphine either *in vivo* or *in vitro*. The neuroprotective effect of apomorphine was further supported by the result of different pharmacological properties including antioxidant activity, potent iron chelating action, inhibition of lipid peroxidation, induction of neurotrophic factors and anti-inflammatory effects [46, 50, 54-56]. Specific brain gene expression changes have been reported in the chronic MPTP model in the late stage of degeneration, employing cDNA expression array, which indicate a domino cascade of events involved in in oxidative-stress, inflammatory processes AND signal transduction and glutamate toxicity that finally lead to neuronal cell death [57].

Bromocryptine

Bromocryptine is the first DA receptor agonist that has been approved for anti-parkinsonian therapy since 1974 [58]. It was first used as adjunctive therapy to levodopa in patients experiencing motor fluctuations and later it was recommended as monotherapy in the early stage of the disease [59]. From the pharmacological point of view, bromocryptine was the first DA receptor agonist endowed with D2 receptor specificity to be described. Bromocryptine has been shown to protect mice and DAergic cells against 6-OH-DA and MPTP, and levodopa-induced cell loss, respectively; it also attenuated DA depletion in mouse striatum in response to methamphetamine [60-62]. The neuroprotective effect of bromocryptine was dependent on both its action as a D2 receptor agonist and its antioxidant capacity. In this context, it has been reported that bromocryptine is able to scavenge hydroxyl and superoxide radicals *in vitro* [61, 63] and to inhibit hydroxyl radical formation and lipid peroxidation *in vivo* [61]. There is evidence that excitotoxic mechanisms contribute to the pathogenesis of PD and that glutamate signaling could be an important mechanism for the death of dopaminergic neurons and trigger the induction of programmed cell death [64]. Bromocryptine has been shown to exert a protective effect against glutamate-induced cytotoxicity in primary cultures of rat cortical or mesencephalic neurons [65] The neuroprotective effect was mediated *via* D2 receptors, because it was attenuated by domperidone, a D2 DAergic receptor antagonist.

Pergolide

Pergolide is a synthetic ergoline derivate that has been indicated in monotherapy as an efficacious treatment in patients with early stage PD. Several studies demonstated the neuroprotective effect of pergolide, observed either *in vivo* or *in vitro* [66-68]. The neuroprotective effect of pergolide has been shown to be mediated by free radical scavenging activity particularly hydroxyl radicals and nitric oxide and by decreasing phospholipid peroxidation [69, 70] suppressing apoptotic pathways through inhibiting of NF-κB nuclear translocation [71] and stabilizing the mitochondrial function [68]. Data obtained in our laboratory demonstrated that pergolide protected SH-SY5Y neuroblastoma cells from cell death induced by H_2O_2 [72]. Unfortunately, peroglide therapy was associated with valvular heart fibrosis; for this reason it was withdrawn from sale in the United States and the European Medicines Agency has added new warnings and controindications to the product information [73-75].

Ropinirole

Ropinirole is a non-ergoline DA receptor agonist that exhibits a high affinity for the D2 and D3 receptor subtypes but little or no affinity for the D1 receptor [76, 77]. Symptomatically, it was reported that ropinirole was as effective as bromocriptine in reducing motor complications and decreasing levodopa dose without increasing adverse events including dyskinesia [78]. Also, ropinirole monotherapy was effective in treating resting tremor in early PD [79], in reducing periodic leg movements and in improving sleep efficiency in patients with restless legs syndrome [76, 80]. These positive effects of ropinirole in PD are believed to be due to stimulation of the post-synaptic DA D2-type receptor [77]. In experimental models for PD, it has been found that ropinirole reversed the motor and behavioral deficits induced by MPTP in marmosets [77] and showed neuroprotective effect against 6-OH-DA in mice [78]. Activation of glutathion and glutathion-regulating enzymes such as glutathione peroxidase, glutathione reductase and glutathione transferase as well as activation of catalase and superoxide dismutase were principal neuroprotective mechanisms mediated by ropinirole [81, 82].

In clinical trials ropinirole reduced the long-term decline of striatal fluorodopa uptake compared to levodopa therapy indicating a preserving effect on terminal function of DAergic neurons [83, 90]. Previous *in vitro* study showed that ropinirole can promote the differentiation and survival of DAergic neurons, and it can upregulate the expression and secretion of GDNF and BDNF [84]. Considering the potential effects of ropinirole in neuroprotection, it has been suggested that ropinirole may have an anti-apoptotic effect through interfering with MAP kinase pathway and caspase-dependent pathway. Recent studies showed that ropinirole has neuroprotective effect against rotenone-induced apoptosis in both SH-SY5Y cells and primary mesencephalic cultures [85]. Exposure to rotenone significantly activated p-JNK, p-P38 and p-c-Jun in SH-SY5Y cells. Activation of JNK and P38 was responsible for the inhibition of the anti-apoptotic protein Bcl-2, and the induction of phosphorylation of c-Jun, a nuclear transcription factor and a known target of JNK, which further promotes the release of cytochrome c from the mitochondria to the cytoplasm and it leads to caspase-9 activation [86-89]. Pretreatment with ropinirole inhibits p-JNK, p-P38 and p-c-Jun expression, indicating that ropinirole may act at early stage of apoptosis. These effects appear to be mediated by neurotrophic factors GDNF and BDNF, since both of them were found to increase in the primary mesencephalic cultures after treatment with ropinirole and pramipexole [84, 91]. Ropinirole also increased GDNF in mouse astrocytes taken from whole brain [92] The anti-

apoptotic effect of ropinirole can be suppressed by D2 and D3 receptor antagonists sulpiride and nafadotride. Nafadotride exhibited greater effect in blocking ropinirole mediated anti-apoptosis, suggesting that D3 receptor may play a significant role.

Pramipexole

Pramipexole (PPX), a non-ergot DA agonist, has been successfully applied to the treatment of early and severe PD. It appears efficacy in the reduction of motor fluctuations and, in association with levodopa, it allows a reduction of the dose of the latter. Furthermore, pramipexole is able to improve the affective symptomatology. Pramipexole exhibits an 8-fold higher affinity for D3 than D2 little or no affinity for the D1 receptor. The neuroprotective effects elicited by this drug have been associated with different mechanism, such as antioxidant effects, mitochondrial stabilization or induction of the antiapoptotic Bcl-2 family. In particular, Le et al. [93] reported that pramipexole protected DAergic MES 23.5 cell line against DA, 6-OH-DA and hydrogen peroxide-induced cytotoxicity possibly through antioxidant effects, and such neuroprotection was independent from DA receptor stimulation not being prevented by selective D2 or D3 antagonists. Similar results were obtained by Fujta et al. [94] and Uberti *et al.* [95], who demonstrated that pramipexole inhibited generation of H_2O_2-induced reactive oxygen species in PC12 cells and SH-SY5Y neuroblastoma cells, respectively. In a recent study we showed that, in retinoic acid differentiated SH-SY5Y cells, PPX preventing cell death inhibiting mithocondrial reactive oxygen and that these ability are held by both S (-) and R (+) enantiomers [96].

In a search for an appropriate cell model for studying neuroprotection, Presgraves *et al.* [97] characterized differentiation conditions of the SH-SY5Y neuroblastoma cell line for phenotypic markers of DAergic cells, Cells were differentiated with retinoic acid (RA), 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and RA followed by TPA (RA/TPA). Interestingly, RA/TPA differentiated cells exhibited 3-fold and 6-fold higher levels, respectively, of DA D2 and D3 receptors than undifferentiated or RA-differentiated cells. Pretreatment with pramipexole was protective against MPP+ in the RA/TPA differentiated cells but not in undifferentiated or RA differentiated cells. The neuroprotective effect of pramipexole was concentration-dependent and DA D2/D3 receptor dependent. In contrast, protection by pramipexole against DA was not DA receptor dependent.

An additional mechanisms underlying protection by D2/D3 receptor agonist has been suggested to involve neurotrophic factors. To verify this hypothesis, the D3

receptor preferring agonists S32504 [(+)-*trans*-3,4,4*a*,5,6,10*b*-hexahydro-9-carbamoyl-4-propyl-2*H*-naphth[1,2-*b*]-1,4-oxazine] and pramipexole were utilized in a terminally differentiated neuroblastoma SH-SY5Y cell line exhibiting a DAergic phenotype [98]. The cytotoxic effects of MPP^+ were stereospecifically antagonized by S32504 and by pramipexole, but not by their inactive stereoisomers, R(+) pramipexole and S32601, respectively. Neuroprotective effects afforded by S32504 and pramipexole were specifically antagonized by the selective D3 antagonists S33084, U99194A, and SB269652, and by the D2/D3 antagonist raclopride. The preferential D2 receptor antagonist LY741626 was ineffective as the D1 antagonist SCH23390.

BDNF potently protected against MPP^+-induced neurotoxicity. Antibody directed against BDNF concentration-dependently blocked both the neuroprotective effects of BDNF and those of pramipexole and S32504 against MPP^+. The protection afforded by BDNF was blocked by the P3K-AKT pathway inhibitor LY249002. Neuroprotective effects of pramipexole and S32504 against MPP(+) toxicity appear to be mediated by D3 receptors stimulation. Their actions also reflect downstream recruitment of BDNF and *via* a PK3-AKT pathway.

PPX has been recently shown to promote adult neurogenesis in SVZ in an acute rat Parkinson's disease model [99] and to increase the levels of brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor in a mouse model of PD induced by ubiquitin-proteasome system impairment [100] furthermore, the PPX-induced neurogenesis was better characterized by Merlo *et al.* [101]; the authors showed that PPX is able to favor expansion and neuronal differentiation of neural progenitors from the adult SVZ, by both D2 and D3 receptor-mediated mechanisms, which involves involves enhanced release of BDNF and subsequent activation of AKT. Finally, PPXwas demonstrated to inhibit the phosphorylation of α-synuclein with a mechanism independent of dopamine receptor activation, by inhibition of the ubiquitin proteasomal system. The phosphorylation of α-synuclein occurs in part at least through casein kinase 2, and PPX in turn reduces the phosphorylation of this enzyme, thereby inhibiting its activity [102].

Rotigotine

Rotigotine is a non-ergoline dopamine agonist developed for the once daily treatment of Parkinson's disease (PD) which provides a 24-hour continuous treatment through a transdermal delivery system (patch), to obtain the reduction of motor fluctuation. Rotigotine acts as a full agonist at dopamine receptors, with the

highest affinity for the D_3 receptor [103]**.** Rotigotine exerted its protective effects *via* dopamine receptor significantly reducing the production of superoxide radicals. In the acute MPTP mouse or progressive MPTP macaque model the agonist was shown to exert partial protective effects on dopaminergic nerve endings [104, 105]. Rotigotine treatment appears to protect dopaminergic neurons from cell death mediated by glutamate. In a very recent study, Oster *et al.* revealed the signalling pathways that are associated with rotigotine-induced neuroprotection against glutamate excitotoxicity, likely through the activation of the PI3K/Akt pathway followed by GSK-3β inactivation which was finally associated with an increased content of GSH [106].

3. PERSPECTIVES

Dopamine Receptor Agonists in Clinical Trials

The neuroprotective action of dopamine agonists has been demonstrated and repeatedly confirmed over the years by numerous studies *in vitro* and *in vivo*. However, some difficulties were encountered in translating these experimental results in a clinical benefit for the patients.

Two clinical trails investigated the neuroprotective potential of DA using imaging end-points; the CALM-PD was a multicenter randomized double-blind controlled clinical trial enrolling subjects with early PD, randomly assigned to receive pramipexole or levodopa. Using β-CIT SPECT scanning techniques, the study aimed to assess the uptake of radiolabeled β-CIT [107, 108]. In pramipexole-treated patients a reduction in β-CIT uptake was observed, suggesting a neuroportective role of the DA. Likely, the REAL-PET trial, which compared ropirinole with levodopa for the ^{18}F-dopa uptake in the putamen, showed less decrease in dopamine uptake in patient treated with ropinirole [109]. However, these studies are burdened by some important limitations, the most important the lack of a strong linkage to the clinical outcome, which did not differ significantly in DA treatment group compared with leovodopa treated patients [110, 111]. Confirming this limitation the PROUD study, recently designed to identify whether early *versus* delayed pramipexole initiation has clinical and neuroimaging benefits, has not reached the clinical endpoint [112].

Dopamine Agonists in Neurodegenerative Disease

Because their antioxidant properties, DA has been proposed to be effective against other pathologies, beside PD, where oxidative stress is the main mechanism implicated in pathophysiology of the diseases. In fact, oxidative abnormalities

have been identified in several neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD). The treatment of patients affected by ALS with pramipexole reduced the systemic production of oxygen radicals, as demonstrated by measuring the levels of 2,3-dihydroxybenzoic acid (2,3-DHBA) in the blood, suggesting that pramipexole may interrupt free radical production in ALS [113]. However, in the phase 3 trial EMPOWER, dexpramipexole (the R (+) enantiomer of pramipexole) did not differ from placebo on any prespecified efficacy endpoint measurement [114]. An increasing amount of evidences suggest a central role of oxidative stress also in AD pathogenesis [115, 116]. Furthermore, many findings link free radical formation with excessive deposition of Aβ derived from the amyloidogenic processing of the larger amyloid precursor protein [117-122]. DA agonists were found to be active in preventing Aβ-induced both aggregation and ROS formation [22, 42-44, 95]. We studied the role of free radical in the neurotoxic events caused by different Aβ aggregation states, and investigated the neuroprotective effects of pramipexole in neuronal death induced by unaggregated, oligomeric and fibrils Aβ species [95]. Increasing evidences suggest that protofibrillar aggregates of Aβ recognized as diffuse plaques by neuropathological examination, are indeed the most toxic Aβ species. Oligomers of Aβ, rather than monomers or large fibrils, may form pores in the cell membrane, allowing influx of ions, that disrupt neuronal signaling and initiate cell death cascade [123, 124]. These data strongly support the hypothesis that each of the Aβ aggregation state possesses different biological and pathological functions. We challenged the neuronal SK-SH5Y cell line with $A\beta_{1-42}$ peptide in different states of aggregation. Contrary to unaggregated peptide, oligomers and fibrils caused generation of ROS and this effect was inhibited by pramipexole in a DA receptors independent manner. The action of pramipexole on Aβ activity is further supported by the data showing that pramipexole prevented the induction of caspase 3 activated by $A\beta_{25-35}$ [125]. These experimental data could acquire clinical significance in AD therapy; in fact, in a recent study, Koch *et al.* [126] demonstrated that dopamine agonists may restore the long term potentiation that is impaired or abolished in AD patients, thus providing novel implications for therapies based on dopaminergic stimulation.

CONCLUSION

The neuroprotective and neurorescue properties of dopamine agonists have been reconfirmed over the years in several *in vitro* and *in vivo* experimental models. These drugs are endowed with intrinsic and peculiar antioxidant, antiapoptotic,

neurotrophic and anti-fibril formation effects. These activities are molecule-specific and may represent additional pharmacological properties contributing to the clinical efficacy in PD and in the other neurodegenerative disease, in which oxidative stress and protein aggregation play a decisive role. However, an unequivocal disease-modifying effect of these molecules is difficult to prove, because some obstacles including the incomplete understanding of the mechanisms underlying neuronal death and the imprecise definition of doses and endpoints in clinical trials.

In conclusion, further studies to elucidate the PD pathogenesis and a better clinical trials design are needed to bridge the gap between the promising experimental results and the not yet optimal clinical efficacy of dopamine agonists.

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CONFLICT OF INTEREST

The authors confirm that this chapter contents have no conflict of interest.

DISCLOSURE

This is an updated report of the earlier review article by G. Ferrari Toninelli *et al.* "Dopamine receptor agonists for protection and repair in Parkinson's disease". Curr Top Med Chem 2008; 8(12):1089-99.

ABBREVIATIONS

6-OH-DA = 6-hydroxydopamine

AAV2 = Adeno-associated virus type 2

Aβ = β-amyloid peptide

BDNF = Brain derived neurotrophic factor

COMT = Cathecol-O-methyl transferase

DA = Dopamine

DOPA = 3,4-diidrossi-l-fenilalanina

GDNF = Glial derived neurothophic factor

LBD = Lewy bodies dementia

MPP^+ = 1-metyl-4-phenylpyridinium

MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

PD = Parkinson's disease

PI3K = Phosphatidylinositol 3 kinase

RA = Retinoic acid

ROS = Radical oxygen species

SNpc = Substantia nigra *pars compacta*

SVZ = Subventricular zone

TPA = 12-O-tetradecanoyl-phorbol-13-acetate

β-CIT = [^{123}I]2β-Carbomethoxy-3β–(4–iodophenil)tropane

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