Targeting Adenosine Signaling in Parkinson’s Disease: From Pharmacological to Non-pharmacological Approaches

Luiza R. Nazario, Rosane S. da Silva and Carla D. Bonan*

Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular; Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil

Parkinson’s disease (PD) is one of the most prevalent neurodegenerative disease displaying negative impacts on both the health and social ability of patients and considerable economical costs. The classical anti-parkinsonian drugs based in dopaminergic replacement are the standard treatment, but several motor side effects emerge during long-term use. This mini-review presents the rationale to several efforts from pre-clinical and clinical studies using adenosine receptor antagonists as a non-dopaminergic therapy. As several studies have indicated that the monotherapy with adenosine receptor antagonists reaches limited efficacy, the usage as a co-adjuvant appeared to be a promising strategy. The formulation of multi-targeted drugs, using adenosine receptor antagonists and other neurotransmitter systems than the dopaminergic one as targets, have been receiving attention since Parkinson’s disease presents a complex biological impact. While pharmacological approaches to cure or ameliorate the conditions of PD are the leading strategy in this area, emerging positive aspects have arisen from non-pharmacological approaches and adenosine function inhibition appears to improve both strategies.

Keywords: adenosine, A2AAR, dopaminergic system, neurodegeneration, Parkinson disease

GENERAL ASPECTS OF PARKINSON’S DISEASE

Parkinson’s disease (PD) is the second most prevalent chronic neurodegenerative disease, affecting more than 1% of the elderly population, with diagnostic confirmation occurring when the loss of dopaminergic neurons in the striatum is close to 80% (de Rijk et al., 2000). PD is also diagnosed in people less than 40 years old, named early-onset PD (Crosiers et al., 2011). PD is associated with the formation of Lewy bodies and neurites (Braak et al., 2003), mainly composed of aggregated forms of α-synuclein (Spillantini et al., 1998). The loss of dopaminergic neurons causes a reduction in the release of dopamine, leading to motor symptoms such as bradykinesia, rigidity, imbalance and tremor (Jankovic, 2008). PD presents in sporadic and familial forms. The risk factors involved in the development of PD are both genetic and environmental (Mortimer et al., 2012; Noyce et al., 2012; Van der Mark et al., 2012; Pezzoli and Cereda, 2013). The familial form, with specific genetic targets, represents less than 10% of PD cases (Dawson and Dawson, 2010). The genetic aspects of the disease are linked to mutations in several genes related to a multitude of cellular mechanisms, such as protein aggregation, protein and membrane trafficking, lysosomal autophagy, immune response, synaptic function, endocytosis, inflammation, and metabolic pathways (Redenšek et al., 2017).
The genes SNCA (PARK1), UCHL1 (PARK5), LRRK2 (PARK8), GIGYF2 (PARK11), OMI/HTRA2 (PARK13), VPS35 (PARK17), and EIF4G1 (PARK18) result in autosomal dominant PD, and PRKN (PARK2), DJ-1 (PARK7), ATP13A2 (PARK9), PLA2G6 (PARK14), FBX07 (PARK15), DJNC6 (PARK19), and SNCJ1 (PARK20) causes autosomal recessive PD (Lautier et al., 2008; Di Fonzo et al., 2009; Klein and Westenberger, 2012; Deng et al., 2015; Bartonikova et al., 2016; Miki et al., 2017; Scott et al., 2017). The gene contribution from other loci (PARK 3, 10, 12, and 16) is under investigation (Dawson and Dawson, 2010). However, a putative causative mutation in the gene that encodes the A1 adenosine receptor, located in the locus PARK16, has been related to susceptibility to PD (Jaberi et al., 2016). Among the environmental contributors to PD development are occupational exposure of pesticides, such as Rotenone and Paraquat, infection by Helicobacter and HCV, low body weight and sedentary lifestyle (McCarthy et al., 2004; Villar-Cheda et al., 2009; Golabi et al., 2017; Sharma and Lewis, 2017; Shen et al., 2017).

THE RELATIONSHIP OF ADENOSINE AND DOPAMINE SIGNALING

Adenosine affects dopaminergic signaling through receptor heteromer formations and shared intracellular pathways. Adenosine is a neuromodulator that acts through the A1 (A1AR) and A3 (A3AR) inhibitory adenosine receptors and A2A (A2AR) and A2B (A2BR) excitatory adenosine receptors (Ralevic and Burnstock, 1998). D1 (D1DR) and D2 (D2DR) dopamine receptors are found co-localized with A2AAR and A1AR, mGluR5 and NMDA (Hillion et al., 2002; Lee et al., 2002; Baggio et al., 2016). The dopamine-adenosine receptor heteromers are constituted mainly of D1DR/A1AR and D2DR/A2AR, displaying antagonistic properties. A1AR agonist decreases the binding potential of dopamine to D1DR, and reduces the D1DR-induced cAMP production, while A1AR antagonists activate D1DR increasing cAMP levels (Ferré et al., 1998). A3AR activation appears to have some influence on dopamine release and vesicular transport, while no functional impacts have been registered in dopamine receptors (Golembiowska and Zylewska, 1998; Björklund et al., 2008; Shen et al., 2011).

The heteromerization of D2DR/A2AR is one of the most studied receptors interaction. A2AAR agonists reduce the in vitro affinity of the D2DR agonist through an increase in D2DR Kd without affecting receptor density (Ferré et al., 1991). In vivo studies confirmed these findings since the administration of A2AAR antagonist increased the effects of the D2DR agonist in the rat striatum and basal ganglia, while the action of A2AAR agonists was opposite (Hillefors-Berglund et al., 1995; Strömberg et al., 2000). This heteromerization was confirmed through co-immunoprecipitation, fluorescence resonance energy, bioluminescence resonance energy transfer and ex vivo proximity ligation studies (Hillion et al., 2002; Canals et al., 2003; Trifilieff et al., 2011; Fernández-Dueñas et al., 2015). Studies with PET in the human brain showed the increased binding of a D2DR antagonist, after the administration of caffeine, a nonselective antagonist of adenosine receptors (Volkow et al., 2015).

The interaction between adenosinergic and dopaminergic receptors has been described as intramembranous, involving direct interaction between receptors, or the modulation of G-proteins and the consequent influence on cAMP-dependent proteins (Fuxe et al., 1998; Ferré et al., 2001; Hillion et al., 2002; Fredholm and Svenningsson, 2003). The administration of D2DR antagonists can reduce the cAMP production by A2AR and the D2 agonist administration induces increase in cAMP levels by A2AAR (Vortherms and Watts, 2004; Botsakis et al., 2010). A2AAR stimulation, in vitro, causes the phosphorylation and activation of DARPP-32, which can be inhibited by D2DR activation (Nishi et al., 1997). A2AAR antagonists increase D2DR-dependent regulation of c-fos, which is more intense when dopaminergic neurodegeneration is presented (Pollack and Fink, 1995; Svenningsson et al., 1999). Compelling evidence for the impairment of D2DR/A2AAR oligomers in the striatum of rats was obtained in experimental Parkinsonism induced by 6-hydroxydopamine (6-OHDA) (Fernández-Dueñas et al., 2015). The ventral striatopallidal GABA pathway appears to be a target of mGluR5/D2DR/A2AAR interactions. The co-administration of A2AAR and mGluR5 agonist enhances GABA release compared with mGluR5 agonist alone, and this effect decreases with the administration of D2DR agonists (Díaz-Cabiale et al., 2002). In addition, D2DR/A2AAR controls NMDA-mediated excitation in neurons from the nucleus accumbens through a direct protein–protein interaction (Azad et al., 2009).

SUPPORT FOR THE A2AAR ANTAGONISM HYPOTHESIS FROM ANIMAL STUDIES

The co-expression of D2DR/A2AAR receptors and their close functional and structural association in the striatopallidal GABAergic neurons reveals sites for therapeutic intervention and has received attention in the last three decades (Fink et al., 1992; Kase, 2001; Kelsey et al., 2009). The non-specific blockade of adenosine receptors by methylxanthines produces contralateral rotations in animals with dopaminergic lesions induced by 6-OHDA, since contralateral rotations have been related to an indirect stimulation of dopamine receptors in the lesioned area (Watanabe et al., 1981; Herrara-Marschitz et al., 1988).

During the late 1990s and early 2000s, exciting results from animal models of Parkinsonism indicated that A2AAR antagonism improves motor activity by reducing the postsynaptic effects of dopamine depletion. Caffeine neuroprotection against 1-methyl-4-phenyl-1,2,3,6-tetrahydroxyphenylalanine (MPTP)-induced lesion showed to be especially dependent on A2AAR from the striatal neurons, but not exclusively (Chen et al., 2001; Xu et al., 2016).
A2AAR antagonist KW6002 (Istradefylline) was shown to be powerful enough to increase locomotion activity and potentiate dopaminergic agonist motor effects in MPTP- and 6-OHDA-lesioned animals (Kanda et al., 1999, 2000; Grondin et al., 1999; Koga et al., 2000; Bibbiani et al., 2003). The anti-parkinsonian effects of KW6002 and similar drugs, such as KW17837, appear to be dose-dependent, effective in the postsynapse and beyond the direct effect on the dopaminergic system, and act on glutamatergic/gabaergic neurotransmission and monoamine oxidase activity (Bibbiani et al., 2003; Petzer et al., 2003; Tanganelli et al., 2004; Orru et al., 2011). MSX-3, a water-soluble precursor of the highly specific A2AAR antagonist MSX-2, which exhibits greater potency for A2AAR than KW6002, appeared to be a candidate of monotherapy since it alleviates the symptomatic parkinsonian locomotor deficiency in a genetic model of dopaminergic degeneration (Yang et al., 2007; Marcellino et al., 2010).

While some studies advocated that A2AAR antagonism, as a monotherapy, could reach a mildly lower or similar efficacy of L-DOPA treatment without inducing dyskinesia (Grondin et al., 1999, Pinna et al., 2007), the promisor effect of these drugs appeared to be when co-administered with L-DOPA, simultaneously inhibiting A2AAR and activating D2 DR. A2AAR-knockout animals demonstrated weak and transitory rotational sensitization and no sensitized grooming as a response to L-DOPA (Fredduzzi et al., 2002). The blockade of adenosine receptors by caffeine promoted additive or synergistic interactions with L-DOPA (Yu et al., 2006), whereas the co-administration of specific A2AAR antagonists, such as KW6002, ST1535, and L-DOPA, potentiated the anti-parkinsonian effect of L-DOPA without exacerbating dyskinesia (Kanda et al., 2000; Koga et al., 2000; Bibbiani et al., 2003; Matsuya et al., 2007; Tronci et al., 2007). However, some studies using several A2AAR antagonists, such as SCH4123-48, BIB014 (Vipanendat), KW6002 and caffeine, when administered concomitantly and chronically with L-DOPA, failed to avoid dyskinesia (Jones et al., 2013).

The mechanism behind the effects of A2AAR antagonists alone or as co-adjuvant drugs appears to beyond actions on dopaminergic system (Fuxe et al., 2009; Maggio et al., 2009; Figure 1). The A2AAR exerts its neuronal activity in the striatum in a manner that is partially independent of D2Rs (Chen et al., 2001). Actually, KW6002 decreases the neuronal activity of the striatopallidal indirect pathway in the absence of D2R-mediated signaling (Aoyama et al., 2000). Dopaminergic neurodegeneration induced by transgenic mutant human α-synuclein is prevented in mice lacking the A2AAR reinforcing the potential of shared downstream pathways (Ferraro et al., 2012). However, the adenylyl cyclase activity did not differ in a genetic model of PD, suggesting that coupling to G-proteins of dopaminergic and adenosinergic receptors should be a target (Botsakis et al., 2010). Regional differences appear in the anti-parkinsonian ability of A2AAR antagonism, since caffeine given at or before MPTP exposure blocks the nigral neurodegenerative process without restoring the striatal nerve terminal neurochemical features (Sonsalla et al., 2012). Motor sensitization developed in unilaterally 6-OHDA-lesioned rats submitted to L-DOPA has been associated with an overexpression of the GABA-synthesizing enzyme glutamic acid decarboxylase, dynorphin, and enkephalin mRNAs in the striatal effenter indirect pathway (Fink et al., 1992; Tronci et al., 2007). The impact of A2AAR antagonism over enkephalin content seems to promote motor recovery in D2DR-knockout animals, but did not promote changes in the preproenkephalin mRNA in a 6-OHDA model (Fink et al., 1992; Aoyama et al., 2000). The functional relation of D2DR/A2AAR in striatal medium spiny neurons appears to receive contributions of cholinergic signaling with consequences for the anti-tremor benefits of A2AAR antagonists (Simola et al., 2006; Tozzi et al., 2011; Salamone et al., 2013). The existence of A2AAR/mGluR heteromers and shared intracellular cascades steps, such as the stimulation of DARPP32 phosphorylation, increase in CAMP levels and elevated c-fos expression, provides clues to the possible contribution of glutamatergic and adenosinergic signaling to the beneficial effects of adenosine receptor antagonism (Nash and Brotchie, 2000; Kachroo et al., 2005). Effects resembling akinesia in 6-OHDA-lesioned rats were fully reversed by either a single treatment of an A2AAR antagonist or an mGluR antagonist at higher doses, or by a combined treatment with ineffective doses of each compound (Coccurello et al., 2004). Increased A2AAR mRNA levels, decreased DARPP-32 phosphorylation and increased phosphorylation of ERK1/2 appeared in 6-OHDA-lesioned rats that display L-DOPA motor sensitization (Tomiyama et al., 2004; Song et al., 2009). This altered downstream signaling pathway is recovered by CSC (8-(3-chlorostryryl) caffeine), an A2AAR antagonist (Song et al., 2009). Amelioration of motor response by A2AAR antagonism seems to be accompanied by the rescue of dopamine, dopamine metabolites, glutamate, and GABA striatal levels as well as the reversal of astroglial and microglial activation and antioxidant properties with beneficial outcomes on cognition (Aguiar et al., 2008; Golembiowska et al., 2013; Uchida et al., 2014).

Prodrugs such as DP-L-A2AANT were designed to conjugate the beneficial effects against dopaminergic degeneration obtained by the combined action of dopamine and A2AAR antagonists in central nervous system (Dalpiaz et al., 2012). In addition to the potential dual action on adenosinergic and dopaminergic systems, the complimentary action on glutamatergic and adenosinergic systems appeared as prospective targets for dual anti-parkinsonian approaches. The combination of A2AAR antagonists and NR2B or mGluR antagonists has demonstrated attractive effects on motor activity with potential in the treatment of PD (Michel et al., 2014, 2015; Beggiate et al., 2016). A2AAR–CB1–D2 DR-receptor-heteromer has been suggested as a component of motor alterations associated with dyskinesia and a possible target of multi-targeted drugs (Bonaventura et al., 2014; Pinna et al., 2014). The effects of caffeine-derived compounds over A2AAR and that of monoamine oxidase B have revealed that these proteins are targets for synergistic action with benefits on dopaminergic degeneration (Petzer and Petzer, 2015). Sulphanilphthalimides are also presented as a dual-targeted-direct compound acting in A1AR and monoamine oxidase B (Van der Walt et al., 2015). The association of L-dopa, serotonin 5-HT1A/1B receptor agonist and A2AAR antagonist...
also demonstrated a promissory strategy in 6-OHDA-lesioned rats exhibiting prevented or reduced dyskinetic-like behavior without impairing motor activity (Pinna et al., 2016).

**SUPPORT FOR THE A<sub>2A</sub>AR ANTAGONISM HYPOTHESIS FROM CLINICAL TESTS**

The A<sub>2A</sub>AR biding sites and mRNA levels in PD patients with dyskinesia are increased in striatopallidal pathway neurons in relation to healthy patients (Martinez-Mir et al., 1991; Calon et al., 2004). These data, in association with the experimental benefits of A<sub>2A</sub>AR antagonists in dopaminergic degenerative diseases increased the enthusiasm regarding non-dopaminergic drug development. Table 1 updates the clinical trials assigned in the EUA and European Union using adenosine receptor antagonists. Istradefylline had long-term tolerability and safety, including as an adjuvant therapy to levodopa (Hauser et al., 2003; Stacy et al., 2008). In 2008, US Food and Drug Administration issued a non-approvable letter to the use of Istradefylline in humans based in the concern if the efficacy findings support clinical utility of Istradefylline in patients with PD. However, Kyowa Hakko Kirin has received approval for the use of Istradefylline as adjunctive therapy in Japan (Dungo and Deeks, 2013; Mizuno et al., 2013). After the additional data request, a 12-week randomized study to evaluate oral Istradefylline in subjects with moderate to severe PD ended with disappointing results, since Istradefylline did not change the off time per day (NCT01968031). However, a clinical trial is currently open (NCT02610231). Preladenant was evaluated as monotherapy to patients with early PD since it reduced the mean daily off time in a phase II study; however, no evidence has supported its efficacy in phase III studies (Hauser, 2011; Stocchi et al., 2017). BIIB014 and SCH900800 also failed to prove efficacy in clinical trials, while Tozadenant showed a mean daily off time reduction accompanied by adverse events of dyskinesia, nausea, and dizziness (Hauser et al., 2014). A safety and efficacy study of Tozadenant to treat end of dose wearing off in PD patients using L-DOPA is currently open (NCT02453386). Multiple epidemiological studies indicate that caffeine is able to prevent PD development (Ross et al., 2000; Ascherio et al., 2001). In a pilot study of caffeine for daytime sleepiness in PD, there was evident benefit on the motor manifestations of disease with no adverse effects (Postuma et al., 2012). Recently, a clinical trial has aimed to evaluate the efficacy of caffeine
| Drug | Sponsor | Identifier number (year) | Parkinson's disease patient condition | Outcome measures (dose tested) | Phase | Status | Results |
|------|---------|-------------------------|----------------------------------------|-------------------------------|-------|--------|---------|
| Istradefylline (KW6002) | Kyowa Hakko Kirin Co., Ltd | NCT02810231* (2015) | Moderate to severe disease | Safety and tolerability (20 or 40 mg oral daily) | III | Active – not recruiting – | |
| | | NCT01968031* (2013) NCT01968031* (2013) | Moderate to severe disease | Efficacy and safety (20 or 40 mg daily) | II | Completed | No change in the OFF time |
| | | 2013-022254-70** (2014) | Moderate to severe disease | Efficacy and safety (20 or 40 mg daily) | II | Completed | No change in the OFF time |
| | | NCT00957203* (2009) | Advanced disease treated with levodopa | Long-term safety and efficacy (20 or 40 mg daily) | II | Completed | Reduction in daily OFF time |
| | | NCT00955526* (2009) | Levodopa-treated | Efficacy in reducing the mean total hours of awake time per day spent in the OFF state (20 or 40 mg daily) | II | Completed | Reduction in daily OFF time |
| | | NCT00456704* (2007) | Advanced disease treated with levodopa/carbidopa | Safety and efficacy compared with placebo in subjects with OFF-time (20 and 60 mg daily) | II | Completed | Significant reduction in OFF time, and was well tolerated as adjunctive treatment to levodopa |
| | | NCT00456586* (2007) | Advanced disease treated with levodopa/carbidopa | Safety and efficacy compared with placebo in subjects with OFF phenomena (40 mg daily) | II | Completed | Istradefylline was safe, well tolerated, and effective at improving end-of-dose wearing |
| | | NCT00455507* (2007) | Advanced disease treated with levodopa | Efficacy for reducing the mean total hours of awake time per day spent in the OFF state (20 or 40 mg daily) | II | Completed | Istradefylline was well tolerated as adjunctive therapy to levodopa for subjects with Parkinson's disease |
| | | 2004-002844-93** (2005) | Motor response complications on levodopa therapy | Long-term tolerability and safety (20 or 40 mg daily) | II | Completed | |
| | | NCT00250393* (2005) | Not specified | Change in Unified Parkinson's Disease Rating Scale (UPDRS) Part-III (Motor examination) (40 mg daily) | II | Completed | |
| | | NCT00203957* (2005) | Motor response complications on levodopa | Confirmation of long term tolerability and safety (20 or 40 mg daily) | II | Completed | |
| | | NCT00199420* (2005) | Advanced disease treated with levodopa | Percentage of OFF time (10, 20 or 40 mg daily) | II | Completed | |
| | | NCT00199407* (2005) | Advanced disease treated with levodopa | Efficacy for reducing the percentage of OFF time (20 mg daily) | II | Completed | |
| | | NCT00199394* (2005) | Advanced disease treated with levodopa | Percentage of awake time spent in the OFF state (40 mg daily) | II | Completed | |
| Drug | Sponsor | Identifier number (year) | Parkinson's disease patient condition | Outcome measures (dose tested) | Phase | Status | Results |
|------|---------|--------------------------|---------------------------------------|---------------------------------|-------|--------|---------|
|      |         | NCT00199381* (2005)      | Patients who have recently completed one year of treatment with istradefylline | Long-term tolerability and safety (20 or 40 mg daily) | III    | Completed | The sponsor decided to terminate the study early (not for safety reasons) |
|      |         | NCT00199368* (2005)      | Patients with motor response complications on levodopa therapy. Who have completed prior istradefylline studies | Safety Study (20 or 40 mg daily) | II     | Completed |
|      |         | NCT00199355* (2005)      | Advanced disease treated with levodopa/DCl. | OFF time (20 or 40 mg daily) |        |          |
|      | NINDS   | NCT00006337* (2000)      | Not specified | Effects on symptoms and dyskinesias | II     | Completed |
|      | SCH900800 | Merck Sharp & Dohme Corp. | NCT01500707* (2011)                      | Moderate to severe disease treated with levodopa | Pharmacokinetics of SCH 900800 (20 mg daily) | I  | Study withdrawn | - |
|      | Preladenant (SCH 420814) | Merck Sharp & Dohme Corp. | NCT01294800* (2011)                      | Moderate to severe disease experiencing motor fluctuations and receiving levodopa | Efficacy on "off" time (2, 5, 10 mg twice/day) | II | Completed | Change from baseline in mean "Off" time |
|      |         | NCT01227265* (2010)      | Moderate to severe disease | Efficacy and safety (2-5 mg twice/day) |        | Completed | Not superior to placebo in reducing off time from baseline |
|      |         | NCT01155479* (2010)      | Early Parkinson's disease | Efficacy and safety (2, 5, 10 mg twice/day) |        | Completed | Change from baseline in motor impairments and disability |
|      |         | 2009-015161-31** (2010)  | Moderate to severe disease | Efficacy and safety (2, 5, 10 mg twice/day) |        | Completed |
|      |         | 2009-015162-57** (2010)  | Moderate to severe disease | Extension study (2, 5, 10 mg twice/day) |        | Study withdrawn | Lack of efficacy in the parent studies. |
|      |         | NCT01155466* (2010)      | Moderate to severe disease | Stability in levodopa dose (2, 5, 10 mg twice/day) |        | Completed | No change from baseline in mean "Off" Time |
|      |         | 2009-013552-72** (2010)  | Early Parkinson's disease | Dose-range-finding efficacy and safety (2, 5, or 10 mg twice/day) |        | Completed | No statistically significant or clinically meaningful difference vs. placebo |
|      |         | NCT01215227* (2010)      | Moderate to severe disease | Long-term safety and tolerability from patients of NCT01155466 and NCT01227265 (2, 5, 10 mg twice/day) |        |          | Terminated early due to the lack of efficacy in the parent studies NCT1155466 and NCT01227265 |
|      |         | NCT00845000* (2009)      | Levodopa treated | Effects on the dyskinesia and antiparkinsonian actions of a levodopa infusion (1.0 or 100 mg daily) | I     | Completed | |

(Continued)
| Drug | Sponsor | Identifier number (year) | Parkinson's disease patient condition | Outcome measures (dose tested) | Phase | Status | Results |
|------|---------|-------------------------|--------------------------------------|--------------------------------|-------|--------|---------|
| Tozadenant (SYN115) | BioTe Therapies Inc. | NCT03051607* 2014-005630-60 ** (2017) | Experiencing end of dose "Wearing-Off" | Efficacy and safety as adjunctive therapy to levodopa (60 mg oral daily) | III | Active | - |
| | | NCT01283594* (2011) | Motor fluctuations on levodopa | Safety and efficacy as an adjunct to levodopa (60, 120, 180, 240 mg twice/day) | II/III | Completed | Tozadenant (120 or 180 mg) was generally well tolerated and was effective at reducing off-time. |
| BIIB04 | Oxford BioMedica | NCT00627588* (2008) | Early Parkinson's disease | Safety, efficacy and dose evaluation | II/II | Completed | - |
| Caffeine | McGill University Health Center | NCT01738178* (2012) | Not specified | Motor effects of caffeine persist (or even magnify) helps reduce dose of other PD meds and/or prevents their side effects (200 mg daily) | III | Completed | - |
| | Ron Postuma | NCT01190735* (2010) | Not specified | Optimal caffeine dose with maximal motor benefit and the least amount of undesirable adverse effects (100-200 mg twice/day) | III | Completed | - |
| | | NCT00459420* (2007) | Not specified | Effect on sleepiness and motor symptoms (100-200 mg daily) | II/III | Completed | No significant benefit on excessive daytime sleepiness |

*ClinicalTrials.gov **EU Clinical Trials Register.
for motor and non-motor aspects of disease (NCT01738178). Nowadays, changing the dose and frequency of daily drug taking had no benefits in the use of adenosine receptor antagonists as a monotherapy or as an adjuvant of current Parkinsonism treatment.

**ASSOCIATION OF A2A AR ANTAGONISM AND NON-PHARMACOLOGICAL APPROACHES**

Non-pharmacological approaches are strategies to combine, reinforce and complement the pharmacological options for the management and prevention of PD (Figure 1). Dance, treadmill and aquatic exercises feasibility to PD management have been evaluated in clinical trials with benefits to life quality, based in cognitive and motor features (Picelli et al., 2016; Carroll et al., 2017; Shanahan et al., 2017). Recently, it was demonstrated that treadmill exercises induce brain activation in PD (Maidan et al., 2017). These benefits have been reproduced in animal models of PD suggesting that physical exercise prevents the development of L-DOPA-induced dyskinesia and its association with hyperphosphorylation of DARPP-32, c-Fos expression and increased brain-derived neurotrophic factor (BDNF) levels (Gyárfás et al., 2010; Aguiar et al., 2013; Shin et al., 2017). Studies with wheel running rats revealed that A2A and A2A AR expression is reduced in the striatum, reinforcing the idea that physical exercise is able to promote neuroplasticity and neuroprotection to brain regions related to motor control, probably through the reduction of antagonistic adenosine effects over dopamine signaling (Clark et al., 2014).

Deep Brain Stimulation (DBS) was approved by the FDA in 2002 as therapy for advanced PD (Suarez-Cedeno et al., 2017). From studies with animals, DBS appeared to have a neuroprotective effect against loss of dopaminergic neurons induced by classical dopaminergic neurotoxins (Maesawa et al., 2004). The use of A2A AR antagonism as an adjuvant of DBS in rodents suggests the potential to enhance the response in the treatment of parkinsonian symptoms, such as tremor (Collins-Praino et al., 2013). While clinical studies using transcranial direct current stimulation (tDCS) in PD suggest possible locomotor benefits, the biological mechanism is still under investigation (Benninger et al., 2011). In rodents, tDCS on the cerebral cortex promotes cognitive effects involving A1 AR, although the adenosinergic participation in tDCS responses of PD has not been evaluated (Márquez-Ruiz et al., 2012). Electroconvulsive therapy (ECT) has been proposed to be efficient for both motor and non-motor symptoms in PD with psychological problems (Nishioka et al., 2014; Calderón-Fajardo et al., 2015). The proposed mechanism for ECT includes the enhancement of dopaminergic transmission in the striatum and an increase in the levels of levodopa by disrupting the blood–brain barrier (Kennedy et al., 2003). The purinergic system appears to be influenced by ECT, since the action, metabolism and release of nucleotide and nucleoside are altered under ECT, but no correlation with PD was identified until now (Gleiter et al., 1989; Busnello et al., 2008; Sadek et al., 2011). A combination of drugs and non-pharmacological therapies could warrant new investigations into the preclinical and clinical studies, with hope for the amelioration and affects in PD prevention, management and treatment.

**CONCLUSIONS**

This review highlights the need to intensify research into adenosine signaling in the development of PD therapies. The interaction between adenosine and dopamine signaling has been extensively studied and contributed to knowledge of the role of non-dopaminergic neurotransmitters in the PD. As cholinergic, glutamatergic, GABAergic, cannabinergic and serotoninergic systems appear together with adenosinergic system in the myriad of pathways involved in the PD, appearing together with the possibility of improved results from dual or multi-targeted anti-parkinsonism approaches opened a new area of drug development. In addition, the association of pharmacological and non-pharmacological approaches brings new perspectives for a more effective treatment of PD and improved of quality of life for PD patients.

**AUTHOR CONTRIBUTIONS**

LN, Rds, and CB equally contributed to the definition of the scope and to the writing of the manuscript.

**FUNDING**

Rds is a Research Career Awardees of the CNPq/Brazil (Proc: 301599/2016-5). CB is a Research Career Awardees of the CNPq/Brazil (Proc 305035/2015-0).

**ACKNOWLEDGMENTS**

LN is a recipient of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)/PROEX fellowship.

**REFERENCES**

Aguiar, A. S. Jr., Moreira, E. L., Hoeller, A. A., Oliveira, P. A., Córdova, F. M., Glaser, V., et al. (2013). Exercise attenuates levodopa-induced dyskinesia in 6-hydroxydopamine-lesioned mice. Neuroscience 243, 46–53. doi: 10.1016/j.neuroscience.2013.03.039

Aguiar, L. M., Macêdo, D. S., Vasconcelos, S. M., Oliveira, A. A., de Sousa, F. C., and Viana, G. S. (2008). CSC, an adenosine A2A receptor antagonist and MAO B inhibitor, reverses behavior, monoamine neurotransmission, and amino acid alterations in the 6-OHDA-lesioned rats. Brain Res. 1191, 192–199. doi: 10.1016/j.brainres.2007.11.051

Aoyama, S., Kase, H., and Borrelli, E. (2000). Rescue of locomotor impairment in dopamine D2 receptor-deficient mice by an adenosine A2A receptor antagonist. J. Neurosci. 20, 5848–5852.

Ascherio, A., Zhang, S. M., Hernán, M. A., Kawachi, I., Colditz, G. A., Speizer, F. E., et al. (2001). Prospective study of caffeine consumption and risk of Parkinson’s disease in men and women. Ann. Neurol. 50, 56–63. doi: 10.1002/ana.1052
Collins-Praino, L. E., Paul, N. E., Ledgard, F., Podurgel, S. J., Kovner, R., Baqi, Y., et al. (2013). Deep brain stimulation of the subthalamic nucleus reverses oral tremor in pharmacological models of parkinsonism: interaction with the effects of adenosine A2A antagonism. Eur. J. Neurosci. 38, 2183–2191. doi: 10.1111/jen.12212

croisier, D., theuns, j., Cras, P., and van Broeckhoven, C. (2011). Parkinson disease: insights in clinical, genetic and pathological features of monogenic disease subtypes. J. Chem. Neuroanat. 42, 131–141. doi: 10.1016/j.jchemneu.2011.07.003

dalpiaz, A., cacchioni, B., Vicentini, C. B., Bortolotti, F., Spalluto, G., Federico, S., et al. (2012). A novel conjugated agent between dopamine and an A2A receptor antagonist as a potential anti-Parkinson multitarget approach. Mol. Pharm. 9, 591–604. doi: 10.1021/mp04089d

dawson, T., and Dawson, V. L. (2010). The role of Parkin in Familial and Sporadic Parkinson’s Disease. Mov. Disord. 25, 532–539. doi: 10.1002/mds.22798

dseng, d., deng, x., yuan, l., Song, Z., Yang, Z., Xiong, W., et al. (2015). Genetic analysis of SNCA coding mutation in Chinese Han patients with Parkinson disease. Acta Neurol. Belg. 115, 267–271. doi: 10.1007/s13760-014-0347-2
de rijk, M. C., launer, L. J., Berger, K., Breteler, M. M., Dartigues, J. F., baldereschi, M., et al. (2000). Prevalence of Parkinson’s disease in Europe: a collaborative study of population-based cohorts. Neurology 54(11 Suppl. 5), S21–S23. doi: 10.1212/WNL.54.11.21A

diaz-cabale, Z., Vivó, M., Del Arco, A., O’connor, W. T., harte, M. K., müller, C. E., et al. (2002). Metabotropic glutamate mGlu5 receptor-mediated modulation of the ventral striopallidal GABA pathway in rats. Interactions with adenosine A2A and dopamine D2 receptors. Neurosci. Lett. 324, 154–158. doi: 10.1016/S0304-3900(02)00179-9

di Fonzo, A., Fabrizio, E., Thomas, A., Finciati, E., marconi, R., Tinazzi, M., et al. (2009). GIGYF2 mutations are not a frequent cause of familial Parkinson’s disease. Parkinsonism Relat. Disord. 15, 703–705. doi: 10.1016/j.parkreldis.2009.05.001

dungo, R., and Deeks, E. D. (2013). Istradefylline: first global approval. Drugs 73, 875–882. doi: 10.1007/s40265-013-0066-7

Fernández-duenas, V., Taura, J. J., Cottet, M., gómez-soler, M., López-cano, M., Ledent, C., et al. (2015). Untangling dopamine-adenosine receptor-receptor assembly in experimental parkinsonism in rats. Dis. Model. Mech. 8, 57–63. doi: 10.1242/dmm.018143

ferré, S., Popoli, P., Giménez-llorente, L., Rimondini, R., Müller, C. E., Strömbärg, L., et al. (2001). Adenosine/dopamine interaction: implications for the treatment of Parkinson’s disease. Parkinsonism Relat. Disord. 7, 235–241. doi: 10.1016/S1353-8020(00)00063-8

Ferré, S., Torvinen, M., Antoniou, K., Irenius, E., Civelí, O., Arenas, E., et al. (1998). Adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. J. Biol. Chem. 273, 4718–4724. doi: 10.1074/jbc.273.8.4718

Ferré, S., von Euler, G., Johansson, B., Fredholm, B. B., and Fuxe, K. (1991). Stimulation of high affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. Proc. Natl. Acad. Sci. U.S.A. 88, 7238–7241 doi: 10.1073/pnas.88.16.7238

Ferraro, L., Beggìato, S., Tomasini, M. C., Fuxe, K., Antonelli, T., and Tanganelli, S. (2012). A2A/D2 receptor heteromerization in a model of Parkinson’s disease. Focus on striatal aminoacidergic signaling. Brain Res. 1451–1461. doi: 10.1016/j.sjpneu.2013.04.044

Nazario et al. Adenosine Signaling in Dopaminergic Degeneration

Frontiers in Neuroscience | www.frontiersin.org 9 November 2017 | Volume 11 | Article 686
Michel, A., Downey, P., Van Damme, X., De Wolf, C., Schwarting, R., and Scheller, D. (2015). Behavioural Assessment of the A2a/NR2B combination in the unilateral 6-OHDA-lesioned rat model: a new method to examine the therapeutic potential of non-dopaminergic drugs. PLoS ONE 10:e0135949. doi: 10.1371/journal.pone.0135949

Miki, Y., Tanik, M., Mori, F., Kakita, A., Takahashi, H., and Wakabayashi, K. (2017). PLA2G6 accumulates in Lewy bodies in PARK14 and idiopathic Parkinson’s disease. Neurosci. Lett. 645, 40–45. doi: 10.1016/j.neulet.2017.02.027

Mizuno, Y., Kondo, T., and Japanese Istradefylline Study Group (2013). Adenosine A2A receptor antagonist istradefylline reduces daily OFF time in Parkinson’s disease. Mov. Disord. 28, 1138–1141. doi: 10.1002/mds.25418

Mortimer, J. A., Borenstein, A. R., and Nelson, L. M. (2012). Associations of solvents and risk of Parkinson disease. Neurology 70, 1174–1180. doi: 10.1212/WNL.0b013e3182698ced

Nash, J. E., and Brotchie, J. M. (2000). A common signaling pathway for striatal NMDA and adenosine A2A receptors: implications for the treatment of Parkinson’s disease. J. Neurosci. 20, 7782–7789.

Nishi, A., Snyder, G. L., and Greengard, P. (1997). Bidirectional regulation of NMDA receptors via the NMDA receptor NR2B subunit. J. Neurosci. Res. 47, 47–55. doi: 10.1002/(SICI)1097-4571(19970115)47:1<47::AID-JNR3>3.0.CO;2-3

Nashio et al. Adenosine Signaling in Dopaminergic Degeneration

Nishio, A., Bakešová, J., Brugarolas, M., Quiroz, C., Beaumont, V., Goldberg, S. R., Pinna, A., Bonaventura, J., Farré, D., Sánchez, M., Simola, N., Mallol, J., Petzer, J. P., Steyn, S., Castagnoli, K. P., Chen, J. F., Schwarzschild, M. A., Van Ralevic, V., and Burnstock, G. (2011). Electroconvulsive therapy: a novel hypothesis for the involvement of purinergic signalling. Purinergic Signal. 7, 447–452. doi: 10.1007/s11296-011-9242-y

Salameh, J. D., Collins-Praino, I. E., Pardo, M., Podurgiel, S. J., Baqi, Y., Müller, C. E., et al. (2013). Conditional neural knockout of the adenosine A2A receptor and pharmacological A2A antagonism reduce pilocarpine-induced tremulous jaw movements: studies with a mouse model of parkinsonian tremor. Eur. Neuropsychopharmacol. 23, 972–977. doi: 10.1016/j.euroneuro.2012.08.004

Scott, L., Dawson, V. L., and Dawson, T. M. (2017). Trumping neurodegeneration: targeting common pathways regulated by autosomal recessive Parkinson’s disease genes. Exp. Neurol. 298(Pt B), 191–201. doi: 10.1016/j.expneurol.2017.04.008

Shanahan, J., Morris, M. E., Bhriain, O. N., Volpe, D., Lynch, T., and Clifford, A. M. (2017). Dancing for Parkinson Disease: a randomized trial of Irish set dancing compared with usual care. Arch. Phys. Med. Rehabil. 98, 1744–1751. doi: 10.1016/j.apmr.2017.02.017

Sharma, J. C., and Lewis, A. (2017). Weight in Parkinson’s Disease: phenotypical significance. Int. Rev. Neurobiol. 134, 891–919. doi: 10.1016/bs.irn.2017.04.011

Shen, H., Luo, Y., Yu, S. J., and Wang, Y. (2011). Enhanced neurodegeneration after a high dose of methamphetamine in adenosine A3 receptor null mutant mice. Neuroscience 194, 170–180. doi: 10.1016/j.neuroscience.2011.08.013

Shen, X., Yang, H., Wu, Y., Zhang, D., and Jiang, H. (2017). Association of Helicobacter pylori infection with Parkinson’s disease: a meta-analysis. Helicobacter 22:e12398. doi: 10.1111/hel.12398

Shin, H. K., Lee, S. W., and Choi, B. T. (2017). Modulation of neurogenesis via neurotrophic factors in acupuncture treatments for neurological diseases. Biochem Pharmacol. 141, 132–142. doi: 10.1016/j.bcp.2017.04.029

Simola, N., Fenu, S., Baraldi, P. G., Tabrizi, M. A., and Morelli, M. (2006). Dopamine and adenosine receptor interaction as basis for the treatment of Parkinson’s disease. J. Neurol. Sci. 248, 48–52. doi: 10.1016/j.jns.2005.05.038

Song, L., Kong, M., Ma, Y., Ba, M., and Liu, Z. (2009). Inhibitory effect of 8-(3-chloroestryl) caffeine on levodopa-induced motor fluctuation is associated with intracellular signaling pathway in 6-OHDA-lesioned rats. Brain Res. 1276, 171–179. doi: 10.1016/j.brainres.2009.04.028

Sonsalla, P. K., Wong, L. Y., Harris, S. L., Richardson, J. R., Khobayh, I., Li, W., et al. (2012). Delayed caffeine treatment prevents nigral dopamine neuron loss in a progressive rat model of Parkinson’s disease. Exp. Neurol. 234, 482–487. doi: 10.1016/j.expneurol.2012.01.022

Spiallitini, M. G., Crowther, R. A., Jak, S., Hasegawa, M., and Goodert, M. (1998). Alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson’s disease and dementia with Lewy bodies. Proc. Natl. Acad. Sci. U.S.A. 95, 6469–6473. doi: 10.1073/pnas.95.11.6469

Stacy, M., Silver, D., Mends, T., Sutton, J., Mori, A., Chakin, P., et al. (2008). A 12-week, placebo-controlled study (6002-US-006) of istradefylline in Parkinson’s disease. Neurology 70, 2233–2240. doi: 10.1212/WNL.0b013e31813833842.217217.17

Stocchi, F., Rascol, O., Hauser, R. A., Huyck, S., Tzontcheva, A., Capece, R., et al. (2017). Randomized trial of preladenant, given as monotherapy, in patients with early Parkinson disease. Neurology 88, 2198–2206. doi: 10.1212/WNL.0000000000004083

Strömbärg, L., Popoli, P., Müller, C. E., Ferré, S., and Fuxe, K. (2000). Electrophysiological and behavioural evidence for an antagonistic modulatory role of adenosine A2A receptors in dopamine D2 receptor regulation in the rat dopamine-denervated striatum. Eur. J. Neurosci. 12, 4033–4037. doi: 10.1046/j.1460-9586.2000.00288.x

Sueiras-Cedeno, G., Suscun, J., and Schiess, M. C. (2017). Earlier Intervention with Deep Brain Stimulation for Parkinson’s Disease. Parkinsonism. Dis. 2017:9358153. doi: 10.1155/2017/9358153

Svensningson, P., Fourreau, L., Bloch, B., Fredholm, B. B., Genon, F., and Le Moine, A. R. (1999). Opposite tonic modulation of dopamine and adenosine on c-fos gene expression in striatopallidal neurons. Neuroscience 89, 827–837. doi: 10.1016/S0306-4522(98)00403-5

Tanganelli, S., Sandager Nielsen, K., Ferraro, L., Antonelli, T., and Scheel-Krüger, J. (2004). Striatal plasticity at the network level. Focus on adenosine A2A and D2 on the underlying molecular defect? Front. Aging Neurosci. 9:20. doi: 10.3389/fnagi.2017.00020

Ross, G. W., Abbott, R. D., Petrovitch, H., Morens, D. M., Grandinetti, A., Tung, K. H., et al. (2000). Association of coffee and caffeine intake with the risk of Parkinson disease. JAMA 283, 2627–2629. doi: 10.1001/jama.283.20.2627

Sadikot, A. R., Knight, G. E., and Burnstock, G. (2011). Electrovocuive treatment: a novel hypothesis for the involvement of purinergic signalling. Purinergic Signal. 7, 447–452. doi: 10.1007/s11296-011-9242-y

Frontiers in Neuroscience | www.frontiersin.org 11 November 2017 | Volume 11 | Article 658

Nazario et al. Adenosine Signaling in Dopaminergic Degeneration
interactions in models of Parkinson’s Disease. Parkinsonism Relat. Disord. 10, 273–280. doi: 10.1016/j.parkreldis.2004.02.015
Tomiyama, M., Kimura, T., Maeda, T., Tanaka, H., Kannari, K., and Baba, M. (2004). Upregulation of striatal adenosine A<sub>2A</sub> receptor mRNA in 6-hydroxydopamine-lesioned rats intermittently treated with L-DOPA. Synapse 52, 218–222. doi: 10.1002/syn.20011
Tozzi, A., de Iure, A., Di Filippo, M., Tantucci, M., Costa, C., Borsini, F., et al. (2011). The distinct role of medium spiny neurons and cholinergic interneurons in the D2/A2A receptor interaction in the striatum: implications for Parkinson’s disease. J. Neurosci. 31, 1850–1862. doi: 10.1523/JNEUROSCI.4082-10.2011
Trifilieff, P., Rives, M. L., Urizar, E., Vishwasrao, H. D., Castrillon, J., et al. (2011). Characterization of the antiparkinsonian effects of the new adenosine A<sub>2A</sub> receptor antagonist ST1535: acute and subchronic studies in rats. Eur. J. Pharmacol. 566, 94–102. doi: 10.1016/j.ejphar.2007.03.021
Uchida, S., Kadowaki-Horita, T., and Kanda, T. (2014). Effects of the adenosine A<sub>2A</sub> receptor antagonist on cognitive dysfunction in Parkinson’s disease. Int. Rev. Neurobiol. 119, 169–189. doi: 10.1016/B978-0-12-801022-8.00008-8
Van der Mark, M., Brouwer, M., Kromhout, H., Nijssen, P., Huss, A., and Vermeulen, R. (2012). Is pesticide use related to Parkinson disease? Some clues to heterogeneity in study results. Environ. Health Perspect. 120, 340–347. doi: 10.1289/ehp.1103881
Van der Walt, M. M., Terre’Blanche, G., Petzer, A., and Petzer, J. P. (2015). The adenosine receptor affinities and monoamine oxidase B inhibitory properties of sulfanylphthalimide analogues. Bioorg. Chem. 59, 117–123. doi: 10.1016/j.bioorg.2015.02.005
Villar-Cheda, B., Sousa-Ribeiro, D., Rodriguez-Pallares, J., Rodriguez-Perez, A. I., Guerra, M. J., and Labandeira-Garcia, J. L. (2009). Aging and sedentarism decrease vascularization and VEGF levels in the rat substantia nigra. Implications for Parkinson’s disease. J. Cereb. Blood Flow Metab. 29, 230–234. doi: 10.1038/jcbfm.2008.127
Volkow, N. D., Wang, G. J., Logan, J., Alexoff, D., Fowler, J. S., Thanos, P. K., et al. (2015). Caffeine increases striatal dopamine D2/D3 receptor availability in the human brain. Transl. Psychiatry 5, e549. doi: 10.1038/tp.2015.46
Vortherms, T. A., and Watts, V. J. (2004). Sensitization of neuronal A<sub>2A</sub> adenosine receptors after persistent D<sub>2</sub> dopamine receptor activation. J. Pharmacol. Exp. Ther. 308, 221–227. doi: 10.1124/jpet.103.057083
Watanabe, H., Ikeda, M., and Watanabe, K. (1981). Properties of rotational behaviour produced by methylxanthine derivatives in mice with unilateral striatal 6-hydroxydopamine-induced lesions. J. Pharmacobiodyn. 4, 301–307. doi: 10.1248/bpb1978.4.301
Xu, K., Di Luca, D. G., Orrù, M., Xu, Y., and Chen, J. F. Schwarzchild, M.A. (2016). Neuroprotection by caffeine in the MPTP model of Parkinson’s disease and its dependence on adenosine A<sub>2A</sub> receptors. Neuroscience. 322, 129–137. doi: 10.1016/j.neuroscience.2016.02.035
Yang, M., Soohoo, D., Soelaiman, S., Kalla, R., Zablocki, J., Chu, N., et al. (2007). Characterization of the potency, selectivity, and pharmacokinetic profile for six adenosine A<sub>2A</sub> receptor antagonists. Naunyn Schmiedebergs. Arch. Pharmacol. 375, 133–144. doi: 10.1007/s00210-007-0135-0
Yu, L., Schwarzchild, M. A., and Chen, J. F. (2006). Cross-sensitization between caffeine- and L-dopa-induced behaviors in hemiparkinsonian mice. Neurosci. Lett. 393, 31–35. doi: 10.1016/j.neulet.2005.09.036

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2017 Nazario, da Silva and Bonan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.