Do caffeine and more selective adenosine A\textsubscript{2A} receptor antagonists protect against dopaminergic neurodegeneration in Parkinson’s disease?

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Abstract

The adenosine A\textsubscript{2A} receptor is a major target of caffeine, the most widely used psychoactive substance worldwide. Large epidemiological studies have long shown caffeine consumption is a strong inverse predictor of Parkinson’s disease (PD). In this review, we first examine the epidemiology of caffeine use vis-à-vis PD and follow this by looking at the evidence for adenosine A\textsubscript{2A} receptor antagonists as potential neuroprotective agents. There is a wealth of accumulating biological, epidemiological and clinical evidence to support the further investigation of selective adenosine A\textsubscript{2A} antagonists, as well as caffeine, as promising candidate therapeutics to fill the unmet need for disease modification of PD.

Keywords

Adenosine; A\textsubscript{2A} receptors; Caffeine; Neuroprotection; Parkinson’s disease

1. Introduction

The adenosine A\textsubscript{2A} receptor is a major target of caffeine, the most widely used psychoactive substance worldwide. Large epidemiological investigations have identified caffeine consumption as a strong inverse predictor of Parkinson’s disease (PD) (and independent from the similarly inverse association of cigarette smoking) \cite{1,2}. The risk of PD among men routinely consuming a daily intake of 2–3 cups of coffee is nearly half that of men who do not consume caffeine, whereas decaffeinated coffee affords no such reduced risk \cite{3–6}. In
this review, we first examine the epidemiology of caffeine in PD and follow this by examining evidence for caffeine and other, more selective antagonists of the adenosine A<sub>2A</sub> receptor as potential neuroprotective agents. Finally, we suggest how the clinical relevance of this evidence might be tested in future clinical trials pursuing disease-modification in PD.

1.1. Epidemiology of caffeine in PD

1.1.1. Caffeine, a major ‘reduced risk’ factor for PD—Other than cigarette smoking, dietary caffeine is the environmental exposure most robustly and reproducibly linked to a lower risk of developing PD [7]. In addition to numerous case-control studies demonstrating lower caffeine intake in association with PD [1,2], well-designed, rigorously conducted epidemiological studies of prospectively followed cohorts have convincingly shown that the risk of PD is significantly and dose-dependently lower among those who consume coffee, tea and other caffeinated beverages [3,6,8–10]. The lack of effect for decaffeinated drinks [3,4] implicates caffeine as the molecular component of coffee driving its inverse correlation with PD; however, other components remain possibilities given that caffeine is not the only molecule removed during the decaffeination process [11]. Indeed, although caffeine itself is CNS-active and is reproducibly protective in multiple PD models (vide infra) – and possibly in PD if it actually intervenes against its causes – other constituents of coffee or tea have shown neuroprotective properties in animal models of the disease [12–14]. Of note, the design of longitudinal epidemiological studies finding a reduced risk of PD among those consuming less caffeine is based on prospective assessment of caffeine intake years before PD diagnosis, and indeed these predictive associations persisted in lag analyses that exclude PD cases that occurred within 2 [4] or even 6 years [15] from dietary survey. This temporal gap between exposure ascertainment and future PD diagnoses greatly reduces – but does not eliminate – the possibility of reverse causation; i.e., the possibility that early PD features (preceding diagnosis) caused a reduction in caffeine consumption behavior.

While reduced PD risk is strongly associated with caffeine consumption among men, the relationship is more complex in women, among whom caffeine’s overall link to PD is generally smaller or absent [3]. Several studies [4,9,15,16] (but not all [6]) have found that the weaker association between PD and women may be explained by estrogen status, with an inverse relationship in women who are not receiving post-menopausal hormone replacement, whereas those taking replacement therapy have shown no consistent PD link. Preclinical experiments support the biological plausibility for an inverse association due to a protective effect of caffeine that can be modulated by estrogen replacement. Using a standard (high-dose MPTP) toxin model of PD, we found a protective effect of caffeine in male, but not female mice unless they were ovariectomized, and while ovariectomy restored the protective effect of caffeine in older female mice, that restoration was prevented by chronic estrogen replacement treatment [17]. Estrogen administered to male mice eliminated caffeine’s neuroprotective actions. Taken together, these convergent epidemiological and laboratory data, with the caveats of epidemiological data consistency and mouse model relevance notwithstanding, raise the possibility of a competitive interaction between estrogen and caffeine’s protective effects in PD.
Epidemiological studies of whether the caffeine-PD link is modified by caffeine metabolism— inferred from genetic (CYP1A2 polymorphism) determinants of slow versus fast caffeine metabolizers— have provided inconsistent evidence. While two cohort studies suggest a stronger protective association in ‘slow metabolizers’ [18,19], several others have found no difference [20–22]. Although, in humans, caffeine has a serum clearance half-life typically less than 6 h, it is demethylated by CYP1A2 to paraxanthine (the primary metabolite of caffeine) and other dimethyl-xanthines (e.g., theophylline), which, like caffeine, are also non-selective adenosine A\textsubscript{2A} antagonists with neuroprotective properties in an MPTP model of PD [23]. Thus, the lack of a clear interaction between CYP1A2 polymorphism and reduced PD risk with caffeine may be consistent with the protective potential of caffeine and its metabolites.

1.1.2. Caffeine, a less certain predictor of PD progression—While greater caffeine use in healthy populations generally predicts a reduced risk of developing PD, the relationship between caffeine consumption by those already diagnosed with PD and their subsequent disease progression is less straightforward. Evaluation of PD patients who were surveyed for daily caffeine intake and then followed prospectively in multiple observational and interventional study cohorts, showed total caffeine ingestion from coffee, tea and other dietary sources was inconsistently correlated with clinical decline. In an Italian study that enrolled 79 early, untreated, ‘de novo’ PD patients [24] higher levels of caffeine intake were associated with significantly slower rates of decline in motor and non-motor symptoms, although the latter was surveyed at the end of the 4-year follow-up period (limiting conclusions on the predictive value of caffeine usage). In contrast, a larger (n = 413) but shorter (1-year) study of de novo participants in NET-PD cohorts (FS1 and FS-TOO) [25] reported no consistent association between caffeine intake and motor progression. The latter was assessed, as in the Italian study, by the time to disability warranting initiation of dopaminergic medication, and by change in Unified Parkinson Disease Rating Scale [UPDRS] score. Similarly, in the even larger LS-1 trial cohort (n = 1549), which was followed for longer (for up to 5 years) [26], caffeine intake was not associated with slower disease progression. In fact, higher caffeine intake among participants randomized to the active study arm (oral creatine treatment) was actually associated with a faster rate of progression by UPDRS score change. However, the LS-1 trial participants were enrolled at a later stage of disease (after initiation of dopaminergic medications) and were not surveyed for their caffeine usage until 1–2 years after baseline – adding to the complexity of data interpretation.

Another intriguing findings is that caffeine use is consistently associated with reduced rates of developing levodopa-induced dyskinesias (LID), a sometimes disabling motor complication of antiparkinsonian dopaminergic drug treatment. Using data from the CALM-PD trial cohort, we reported that early PD participants who reported higher daily intake of caffeine were less likely to develop dyskinesias after nearly six years of treatment with levodopa or a dopaminergic agonist [27]. Similarly in a case-control study of 485 PD patients in Italy, ever-having-been a coffee drinker and greater number of cups ingested per day were associated with a significantly lower likelihood of manifesting dyskinesias [28]. Combined with preclinical evidence that adenosine A\textsubscript{2A} receptors play a critical role in the
development of LID in murine and primate models [29,30], these findings support a hypothesis that coadministration of caffeine or a more selective A$_{2A}$ antagonist may mitigate against the development of LID [27,31,32].

1.1.3. **Caffeine, as an emerging marker of resistance to genetic PD**—The increasingly routine identification of discrete genetic subtypes of PD (most commonly attributed to mutations in the GBA and LRRK2 genes) provides an opportunity to characterize gene-environment and pharmacogenetic interactions that may influence the development and progression of PD. The relationship between PD associations with LRRK2 and caffeine may be a prototype for such gene-environment interaction based on emerging evidence that links external (dietary [33–35] and internal (plasma [36]) caffeine exposure with a reduced likelihood of developing PD among carriers of a pathogenic LRRK2 mutation. This seems to occur to an even greater extent than among PD patients who do not carry LRRK2 mutation [33,36]. Given caffeine’s association with LRRK2 PD resistance, its neuroprotective properties in PD models (vide infra), and its general safety at doses of at least 400 mg/day [37] (less than the exposure from drinking two Dunkin’ Donut Coffees or two Starbucks Café Americanos per day [38]), caffeine is a reasonable candidate for interventional investigations in PD prevention trials targeting those at high risk for the disease based on the presence of a LRRK2 mutation.

1.2. **Evidence for A$_{2A}$ antagonists as potential neuroprotective agents**

1.2.1. **Neuroprotection by A$_{2A}$ antagonists in PD models**—The first experimental evidence for a neuroprotective effect of A$_{2A}$ receptor antagonists including caffeine arose 20 years ago with the demonstration that genetic inactivation and pharmacological blockade of the A$_{2A}$ receptor attenuate dopaminergic neurotoxicity and neurodegeneration in rodent models of parkinsonism. These models were created using mitochondrial toxins (systemically-administered MPTP and brain infusion of 6-OHDA), which produce relatively selective neuronal cell death [39–41]. A$_{2A}$ receptor antagonist-mediated neuroprotection against dopaminergic neurodegeneration has been confirmed by follow-up studies and has been extended to other neurotoxin models of PD, such as the rotenone rat PD model [42,43]. Protection against dopaminergic neurotoxicity was achieved by selective A$_{2A}$ antagonists (including SCH58261, KW-6002, DMPX, CSC, MSX-3 and IDPU), but not the A1 antagonist DPCPX [39,44–46]. Systemic administration of the A$_{2A}$ antagonist SCH58261 for five days also improved synaptic currents, spine morphology, and dendritic excitability of the striatopallidal neurons after dopamine depletion in mice [47]. Furthermore, acute or chronic treatment with the non-selective adenosine antagonist caffeine attenuates MPTP-induced loss of dopamine content and dopaminergic terminals in the striatum [39,41] as well as loss of dopaminergic neurons in the substantia nigra [40,41,48]. Caffeine conferred protection against the degeneration of dopamine cell bodies in the substantia nigra even when it was administered after the onset of the neurodegenerative process in a chronic MPTP-exposure paradigm [49]. While reproducible in traditional toxin models of PD neurodegeneration, caffeine’s neuroprotective properties are not universal. Indeed caffeine has been shown to exacerbate methamphetamine-induced dopaminergic cell death and neuroinflammation in mouse substantia nigra [50,51]. Overall, these studies demonstrate a potential neurobiological basis for the relationship between increased caffeine consumption...
and reduced risk of developing PD, and support the clinical potential for A2A antagonism as a disease-modifying drug target against PD.

Another line of experimental evidence for A2A receptor mediated neuroprotection against dopaminergic neurodegeneration came from the demonstration of A2A receptor modulation of α-synuclein-mediated neurotoxicity in transgenic PD models. Alpha-synuclein aggregation and misfolding plays a central role in PD pathogenesis and the appearance of α-synuclein inclusions within in neurons is highly correlated with the onset of neuronal loss [52–54]. A2A receptor blockade decreases α-synuclein aggregation in SynT-Synphilin-1 neuroglioma cells [55], and A2A receptor knock out prevents loss of dopaminergic neurons caused by the transgenic overexpression of the human α-synuclein gene containing both A53T and A30P mutations (hmt(2)-α.SYN) [56]. Furthermore, intrastriatal injection of preformed A53T α-synuclein fibrils has been recently developed as a model of PD that recapitulates the robust formation of α-synuclein inclusions characteristic of PD pathology, with the abnormal seeding and spreading of α-synuclein inclusions throughout the brain alongside dopaminergic neuron loss [57,58]. In this α-synuclein fibril model of PD, genetic deletion of A2A receptors [59] or chronic caffeine treatment [60] attenuates neurodegeneration induced by intra-striatal injection of preformed A53T α-synuclein fibril. Collectively, these findings define aberrant A2A receptor signaling as a critical pathogenic mechanism of α-synuclein-triggered neurodegeneration in PD models.

1.2.2. Up-regulation of the adenosine A2AR signaling in PD brains—Adenosine A2A receptor signaling is important not only in physiological homeostatic regulation, but also in many pathophysiological processes including inflammatory diseases [61,62], ischemia and reperfusion [63] and neurodegenerative disorders [64,65]. The brain response to a variety of insults is characterized by upregulated A2A receptor signaling, which may contribute to development of neurological disorders, including PD. For example, recent studies show that dopamine depleting 6-OHDA/MPTP treatment increased ATP release and upregulated ecto-5'-nucleotidase (CD73, a marker for mesenchymal stem cells) and A2A receptors in striatal synaptosomes [66] and in the striatum of MPTP-treated mice [67]. Furthermore, A2A receptor upregulation in neurons and glial cells of hippocampus is induced by intra-hippocampal injection of preformed A53T α-Syn fibrils [68].

Importantly, A2A receptor upregulation has also been clearly documented in putamen of early stage of PD (Braak stage 1–2) [69]. PET studies have also confirmed up-regulation of striatal A2A receptors in PD with dyskinesia, compared to PD patients without dyskinesia [70,71]. Moreover, a recent study suggests a self-regulating feed-forward adenosine formation (via CD73) can activate A2A receptors and promote neuroinflammation as CD73 inactivation suppressed A2A receptor induction and A2A-mediated pro-inflammatory responses [67]. Thus, increased A2A receptor signaling represents a useful biomarker as well as a therapeutic target of PD. Consequently, the potential to protect against dopaminergic neurodegeneration represents the most exciting and yet-to-be realized aspect of A2A antagonists as a novel therapy for PD.
1.3. Mechanisms of neuroprotection via A<sub>2A</sub> antagonism in PD models

How genetic deletion or pharmacological blockade of the A<sub>2A</sub> receptor affords protection against dopaminergic neuron degeneration in PD remains unclear. Several critical pathological processes at cellular and molecular levels have been identified in PD pathogenesis, including mitochondrial dysfunction, neuroinflammation, and dysregulated proteasome and autophagy pathways [72,73]. In the next part of this review, we examine how A<sub>2A</sub> receptor activity may influence these pathogenic processes to confer neuroprotection against dopaminergic neurotoxicity.

1.3.1. A<sub>2A</sub> receptor modulation of mitochondrial dysfunction—PD pathogenesis is characterized by mitochondrial dysfunction. Studies in MPTP-mice models show reduced (13)C labeling of GluC 4, GABAC2 and GlnC4 from [1,6-(13)C2]glucose in the cerebral cortex, striatum, thalamus and cerebellum, suggesting impaired glutamatergic and GABAergic neuronal activity and neurotransmission [74]. However, pre-treatment with caffeine has been shown to partially preserve the (13)C labeling of amino acids of MPTP-treated mice compared to control values and A<sub>2A</sub> antagonists reduced the mitochondrial toxin rotenone-induced loss of corticostriatal field potential amplitude as well as membrane depolarization in striatal slices [75]. In MitoPark mice—a genetic model of mitochondrial dysfunction with dopamine neurodegeneration, pre-treatment with the A<sub>2A</sub> antagonist MSX-3 prevented the reduction of spontaneous locomotor activity [76], lending further support for A<sub>2A</sub> receptor modulation of mitochondrial dysfunction.

1.3.2. A<sub>2A</sub> receptor interaction with NMDA receptor—Abnormal activation of A<sub>2A</sub> receptors might lead to overactivation of NMDA receptors, which is a prominent synaptic event resulting in excitotoxicity [77]. A<sub>2A</sub> receptors are known to facilitate NMDA receptors function in the hippocampus (possibly by facilitation of presynaptic release of glutamate [78]) and also attenuate traumatic brain injury by control of glutamate-mediated neurotoxicity [79]. In SH-SY5Y cells, A<sub>2A</sub> receptor antagonists also rescue α-synuclein toxicity via an NMDA receptor interaction [80]. However, it is also possible that A<sub>2A</sub> antagonists confer neuroprotective actions at non-neuronal cells (such as glial cells) since A<sub>2A</sub> antagonists and caffeine remain neuroprotective in forebrain neuron-specific A<sub>2A</sub> knockout mice [81,82,90].

1.3.3. A<sub>2A</sub> receptor modulation of microglial activity and neuroinflammation—Extracellular aggregated α-synuclein can trigger massive microglial activation and neuroinflammation by binding to Toll-like receptor 2 (TLR2), CD11b receptors and integrin β1 subunit on microglia, resulting in neuronal death [83–87]. It is thought that A<sub>2A</sub> receptors modulate microglial reactivity and control neuroinflammatory processes to modify α-synuclein-induced neurodegeneration [88,89]. Consistent with this view, α-synuclein-triggered reactive microglial activation, NF-κB p65 activation neuroinflammatory responses were largely reverted in A<sub>2A</sub> receptor knockout mice [59]. Furthermore, genetic inactivation of CD73 reduces LPS-induced microglial release of pro-inflammatory cytokines, but increases microglia process extension, movement and morphological transformation in acute MPTP model of PD [67]. CD73 modulation of microglial activity is associated with microglia-mediated neuroinflammation and protects against dopaminergic...
neurodegeneration and increases motor behaviors in MPTP model of PD. A$_2$A receptor activation can inhibit dopamine-mediated anti-inflammation in glial cells to exacerbate inflammation in the brain. On the other hand, astrocytic A$_2$A receptors may not be involved in neuroprotection as caffeine’s protective properties were undiminished in astrocytic A$_2$A receptor conditional knockout mice [82]. Thus, the homeostatic balance between adenosine and dopamine signaling is critical for the control of microglia immunoresponses.

1.3.4. Direct interactions of A$_2$A receptors with α-synuclein—Studies in a yeast proteotoxicity model with aggregation of recombinant α-synuclein show that caffeine can reduce the toxicity of oligomers and aggregates, and increase cell survival with concomitant reduction in intracellular oxidative stress and decreased oxidative proteome damage [91]. This was achieved presumably by caffeine directly binding to α-synuclein to alter the native conformation of mature α-synuclein aggregates displaying amorphous as well as fibrillar morphology [91]. This concept is supported by a recent study showing that A$_2$A antagonists decrease the percentage of cells displaying α-synuclein inclusions in cultured cells [80]. It is thought that A$_2$A receptor deletion may protect against α-synuclein aggregation and neurodegeneration by decreasing the phosphorylation of α-synuclein on Ser 129, a safeguard procedure for control of α-synuclein toxicity. The notion that A$_2$A receptors may modulate phosphorylation modifications of pathological proteins corroborates the recent finding that A$_2$A antagonist MSX-3 significantly improved memory and reduced Tau hyperphosphorylation in THY-Tau 22 mice [92].

1.3.5. A$_2$A receptor modulation of proteosome and autophagy pathway—Increasing evidence demonstrates that aberrant regulation of autophagy contributes to α-synuclein aggregation and α-synuclein-induced neurodegeneration in PD [93–95]. It is postulated that A$_2$A receptor activity may confer protection by control of ubiquitin-proteasome system (UPS) and autolysosome activity associated with degradation of the accumulated α-synuclein [96,97]. However, this assumption of A$_2$A receptor modulation of UPS is apparently not supported by recent demonstrations that A$_2$A activation enhances proteasome activity and reduces mutant huntingtin aggregations through a PKA-dependent pathway [98,99]. On the other hand, both caffeine and A$_2$A receptor signaling can regulate autophagy activity under different conditions in several cell types [100,101]. Indeed, in the α-synuclein fibril model of PD, chronic caffeine treatment did not affect autophagy processes in the normal striatum, but selectively reversed α-synuclein-induced defects in macroautophagy (by enhancing microtubule-associated protein 1 light chain 3, and reducing the receptor protein sequestosome 1, SQSTM1/p62) and chaperone-mediated autophagy (CMA, by enhancing LAMP2A) [125]. The ability of caffeine to modify autophagy is correlated with that chronic caffeine-mediated attenuation of the pathological cascade leading to α-synucleinopathy, including pSer129α-Syn-rich aggregates, apoptotic neuronal cell death, and microglia and astroglia reactivation [125]. Thus, caffeine may represent a novel pharmacological therapy for PD by targeting the autophagy pathway.
1.4. The challenges in developing A\textsubscript{2A} receptor-based disease-modification therapy for PD

Despite considerable strengths of epidemiological evidence and consistent demonstration that A\textsubscript{2A} antagonists afford neuroprotection against MPTP- or 6-OHDA- and alpha-synuclein induced dopaminergic neurotoxicity, the therapeutic potential of A\textsubscript{2A} antagonists (including caffeine) remains unclear. Significant knowledge gaps remain to be closed before the therapeutic potential of A\textsubscript{2A} antagonists in disease-modifying effect in PD can be realized. First, it would be important to validate and cement the neuroprotective effect of A\textsubscript{2A} antagonists in a non-human primate model of PD which has much evolutional similarity to humans. Second, the mechanism by which A\textsubscript{2A} inactivation protects against the loss of dopaminergic neurons remains unknown with the particular challenge lying in explaining the apparent dichotomy between restricted expression of the A\textsubscript{2A} receptor in striatopallidal neurons and neuroprotection against degeneration of dopaminergic neurons in the substantia nigra where only scattered (if any) expression of A\textsubscript{2A} receptors is detected. Third, we need to develop better biomarkers to closely monitor any neuroprotective effect of A\textsubscript{2A} antagonism given the relatively short duration of clinical trials with respect to the slow pace of neurodegeneration in PD pathogenesis. Sensitive and reliable biomarkers may directly detect effects of A\textsubscript{2A} antagonists on dopaminergic neuron degeneration (rather than indirectly assess them based on changes in worsening motor symptoms). Tackling these challenges would greatly facilitate development of novel and promising strategies in PD by targeting adenosine production and A\textsubscript{2A} receptor signaling.

1.4.1. Disease modification trials of A\textsubscript{2A} receptor antagonism in PD—Although caffeine [37,102] and more selective adenosine A\textsubscript{2A} receptor antagonists (such as istradefylline [KW-6002] [103], preladenant [SCH420814/MK 3814] [104], tozadenant [SYN115] [105], and vipadenant [BIIB014] [106,107]) have been extensively tested in Phase 2 and 3 randomized, placebo-controlled trials (RCTs) as candidate therapeutics for symptomatic indications in PD, few trials have encompassed designs capable of assessing long-term disease modification with these agents and early trial terminations have precluded these assessments.

Preladenant was tested as monotherapy in 1007 early stage PD participants in a large, 52-week long phase 3 double-blind RCT initially under a delayed-start, two-period trial design [108], which was structured to support regulatory approval for a ‘disease modification’ indication [109,110]. In keeping with such a goal, the trial included an active comparator treatment arm with rasagiline, a drug that had been employed in a delayed-start, two-period trial [111] also in pursuit of a disease-modification indication. However, nine months after starting enrollment, the study design was modified to eliminate the delayed start treatment arm (see [108] and ClinicalTrial.gov registration NCT01155479 [112] entries under “History of Changes” from June 30, 2010 until April 6, 2011), in which placebo was used during the first 26-week period before replacement by preladenant during the second 26-week period.

Similarly, caffeine was investigated for disease modification in PD as a secondary outcome of a smaller (n = 121), but longer (planned for 5 years), Phase 3, two-arm, double-blind RCT, with a primary outcome focused on assessing improvement in motor symptoms. Its
ambitious two-period, delayed-start study design entailed comparison of PD patients who
were enrolled on stable symptomatic therapy and then randomized to receive either caffeine
at 200 mg twice daily for 5 years, or placebo twice daily for 4.5 years before being switched
to caffeine treatment for the final 6 months [113]. Unfortunately, with the primary outcome
analysis demonstrating no significant symptomatic benefit after 6 months [37], the study was
terminated early, precluding the opportunity to test for disease modification by caffeine
treatment.

1.4.2. Future designs with caffeine and more selective A₂A receptor antagonists—Despite these missed clinical trial opportunities to test the hypothesis of
neuroprotection by adenosine A₂A receptor antagonism, new prospects are appearing on the
horizon. With advances in identifying large numbers of individuals who are at risk
(genetically and/or prodromally) of developing PD, but otherwise healthy, it is now plausible
to begin planning trials for prevention of the disease. Carriers of pathogenic LRRK2
mutations with a lifetime penetrance of PD generally exceeding 30%, who have family
members suffering from the disease as well as children or grandchildren who are also at-risk
carriers, may be motivated to enroll in prevention trials testing compelling and low-risk
candidate protectants like caffeine. The preclinical and biomarker data mentioned above,
which suggest a greater potential benefit in LRRK2 mutation-driven as compared to
idiopathic PD, further encourage consideration of such trials. At present the greatest
limitation to embarking on prevention trials may be the inadequacy of currently envisioned
outcome measures. While phenoconversion to manifest disease is intuitive and a seemingly
definitive endpoint, the wait for it might be prohibitive long and would require a large
sample size. Enriching a target population for older participants (e.g., >60 years among
genetically at-risk subjects) and/or for additional or prodromal risk factors (e.g., presence of
hyposmia or REM sleep behavior disorder) may lessen this challenge. Alternatively,
sensitive tracking of prodromal motor features (e.g., subtle motor slowing assessed by
wearable or smartphone sensors) or neuroimaging (e.g., dopamine transporter brain scan
changes) markers of pre-diagnostic progression may become sufficiently validated to
employ in a proof-of-concept prevention trial designs.

Finally, there may be an opportunity to build on the established safety and efficacy of
istradefylline, now that it has secured regulatory approval in Japan and the United States, for
use as adjunctive treatment of PD symptoms among those experiencing wearing off of
levodopa benefit. As reviewed above, there is considerable evidence for the disease-
modifying potential of istradefylline and other adenosine A₂A receptor antagonists in PD.
Collectively, the data suggest that this strategy might slow not only the neurodegeneration
underlying the inexorably progressive loss of motor function, but also help prevent the
maladaptive neuroplasticity that leads to dyskinetic motor complications in PD. Among
many trial strategies, one powerful Phase 2 design that could explore and support several
indications by employing staggered co-primary outcomes would entail enrolling early,
untreated PD patients – who are not heavy caffeine consumers and who are expected to
presently warrant treatment with dopaminergic therapy. In this scenario (Fig. 1), participants
would be randomized to receive an A₂A receptor antagonist (or one of two doses) or placebo
in equal proportions. After an initial treatment period (e.g., of 12 weeks) to assess for effects
on motor (and other) features, then all participants would initiate standard carbidopa/levodopa 25/100 dosing three times daily while continuing on and blinded to treatment with an A_{2A} receptor antagonist or placebo for another two to four years as levodopa and other antiparkinsonian medications are adjusted to optimize management, before a final 12–24 week period when the placebo is replaced with the A_{2A} receptor antagonist. This delay-start design would sequentially elucidate the potential for valuable (extended) indications for symptomatic benefit as monotherapy or as early adjunctive therapy (Fig. 1C; panel C/outcomes 1a and 1b), and then for preventing dyskinesia (Fig. 1A; panel A/outcome 2), and finally for reducing long-term parkinsonian deficits and/or of dopamine transporter (DAT) binding site loss, a marker for dopaminergic neuron degeneration (Fig. 1B; panels B/outcome 3a and panel C/outcome 3b).

Overall, a remarkable convergence of biological, epidemiological and clinical data support the further investment in selective adenosine A_{2A} antagonists (as well as the possibility of caffeine fulfilling this role) as promising candidate therapeutics to fill the unmet need for disease-modifying treatment of people with PD.

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Fig. 1. Envisioned design for a phase 2, randomized, double-blind clinical trial of an adenosine $A_{2A}$ receptor antagonist to investigate its multiple potential indications spanning short-term symptomatic and long-term, disease course benefits in PD. The study proposed here would evaluate three co-primary outcomes based on three measures: development of dyskinesia, a motor complication induced by repeated levodopa treatment (A), striatal dopamine transporter (DAT) binding, a marker nigrostriatal neuron integrity, whose progressive loss can reflect dopaminergic neuron degeneration (B), and levodopa equivalent daily dose (LEDD)-adjusted Movement Disorder Society Unified Parkinson’s Disease Rating Scale (MDS-UPDRS), which can show progressive clinical
worsening (increasing score) over years in early PD [116] as well as short-term placebo or medication effects (C).

By enrolling only participants who have been recently diagnosed, who have a DAT deficit on neuroimaging [117] and who are not expected to require anti-parkinsonian medication until at least a few months after enrollment, the trial would likely be more sensitive to interventions targeting A2A-dependent pathophysiology in the early stages of PD, which has been implicated by epidemiological and preclinical data (see text). And by enrolling only participants with modest caffeine intake (< 100 mg/day) the study would enrich for those whose targeted A2A receptors are available (i.e., given that normal caffeine use in humans may substantially block striatal A2A receptors) [118]. Alternatively, simply monitoring caffeine consumption would allow stratification or adjustment of results by caffeine levels, and may improve interpretation of the findings [31,119]. Of note, negative results of prior trials of selective A2A antagonists as symptomatic therapy, particularly as monotherapy targeting de novo PD subjects [e.g., [114]], may be attributed at least in part to enrollment of a small but significant proportion of non-PD patients who do not have a striatal dopaminergic deficit, and of patients whose caffeine use greatly reduced the availability of striatal A2A receptors. The proposed trial’s exclusion of people who have DAT scans without evidence of dopaminergic deficit (SWEDDS), and of people regularly consuming ≥100mg or more mg of caffeine daily, increases the likelihood of identifying benefits of selective A2A antagonist therapy in early PD.

Sequential outcomes would be analyzed to test 3 hypotheses, that beginning treatment with an A2A antagonist prior to levodopa initiation in this enriched early PD population 1) improves parkinsonian symptoms and deficits (assessed on the MDS-UPDRS) in the short-term, both as monotherapy (1a) and perhaps more substantially as an adjunct (1b) to initial levodopa treatment with 25/100 carbidopa/levodopa t.i.d. for 3 months (panel C); 2) delays or prevents the development dyskinesia (LID), which can be experienced in a quarter of PD within 18 months of starting standard levodopa treatment [120] (panel A); and 3) slows long-term progression of dopaminergic parkinsonian deficits measured radiographically by serial DAT imaging (3a, Panel B; with DAT scans conducted at screening/baseline and thereafter including 1–2 months into Period 2 in order to avoid any acute confounding effect A2A antagonist treatment may have on DAT signal) or clinically by adjusted MDS-UPDRS (panel C). The latter scale can be used to gauge differences in progression of clinical disability after years of A2A antiagonist versus placebo treatment, based not only on the residual group difference after delayed-start of the drug in the 2nd period (3b) but also on a slope difference in Period 1, and on lack of convergence of slopes in Period 2 – as expected in a classic 2-period design [110], which is particularly well suited to candidate neuroprotectants that possess symptomatic antiparkinsonian properties like A2A antagonists. Note also that A2A antagonist are well known to modestly exacerbate or unmask LID after chronic treated with levodopa [e.g., [115], their potential for LID prophylaxis notwithstanding, as depicted for in panel A upon addition of A2A antagonist at the start of Period 2 at 24 months in the (early) placebo arm.

Although the MDS-UPDRS score is a well characterized composite patient- and clinician-reported outcome designed for in-clinic assessment, it may be modified for remote evaluation via televisits [121]. This variant and other remote assessments of parkinsonism currently being developed or validated (e.g., [122]) may offer attractive alternative measures
of clinical progression given the convergence of impediments to long-term serial in-person clinic visits (e.g., the 2020 pandemic [123]) with advances in digital and wearable technologies [124].