AN UPDATE ON ADENOSINE A_{2A} RECEPTORS AS DRUG TARGET IN PARKINSON'S DISEASE

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Abstract

Adenosine receptors are G protein-coupled receptors (GPCRs) that mediate the physiological functions of adenosine. In the central nervous system adenosine A_{2A} receptors (A_{2A}Rs) are highly enriched in striatopallidal neurons where they form functional oligomeric complexes with other GPCRs such us the dopamine D_2 receptor (D_2R). Furthermore, it is assumed that the formation of balanced A_{2A}R/D_2R receptor oligomers are essential for correct striatal function as the allosteric receptor-receptor interactions established within the oligomer are needed for properly sensing adenosine and dopamine. Interestingly, A_{2A}R activation reduces the affinity of striatal D_2R for dopamine and the blockade of A_{2A}R with specific antagonists facilitates function of the D_2R. Thus, it may be postulated that A_{2A}R antagonists are pro-dopaminergic agents. Therefore, A_{2A}R antagonists will potentially reduce the effects associated with dopamine depletion in Parkinson's disease (PD). Accordingly, this class of compounds have recently attracted considerable attention as potential therapeutic agents for PD pharmacotherapy as they have shown potential effectiveness in counteracting motor dysfunctions and also displayed neuroprotective and anti-inflammatory effects in animal models of PD. Overall, we provide here an update of the current state of the art of these A_{2A}R-based approaches that are under clinical study as agents devoted to alleviate PD symptoms.

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INTRODUCTION

Adenosine is a nucleoside which is mainly regarded as a retaliatory metabolite [1], thus it increases oxygen supply and decreases oxygen consumption. Consequently, adenosine potentially regulates a large amount of physiological processes (i.e. respiratory regulation, hormone action, neural function, platelet aggregation, lymphocyte differentiation and vascular tone). In addition, adenosine exerts a negative chronotropic and dromotropic effect on the heart, as well as it mediates inhibition of neurotransmitter release and lipolysis. In the central nervous system (CNS), adenosine acts principally as a negative neuromodulator for example decreasing striatal dopamine and glutamate release. In the brain, adenosine is produced by metabolism of adenosine triphosphate (ATP) when it is co-released with glutamate from nerve terminals and astrocytes [2]. Also, extracellular adenosine is related to the intracellular concentration of adenosine and nucleotides, such as ATP, adenosine monophosphate (AMP) and cyclic adenosine monophosphate (cAMP) [3]. In fact, in some brain areas, like the hippocampus, most of the extracellular adenosine seems to depend mainly on intracellular adenosine, the concentration of which is related to the rate of breakdown and synthesis of ATP [3]. Thus, adenosine would be released as a neuromodulator [4] by the effector cells in response to an increased metabolic demand [5]. Interestingly, in the striatum, it has been suggested that the main source of extracellular adenosine is intracellular cAMP [6]. Thus, since cAMP can only be generated by the action of the enzyme adenylyl cyclase, striatal extracellular adenosine would mostly reflect an increased activation of metabotropic receptors positively coupled to cAMP as a second messenger, for example the dopamine D_1 receptor. In addition, the dynamics of adenosine release in the cerebellum has been

recently characterized by means of adenosine biosensors. Interestingly, by using this experimental approach it has been possible to demonstrate that adenosine release shares many characteristics of conventional neurotransmitter liberation such as a dependence on action potential width and homosynaptic plasticity [7].

Adenosine mediates its actions through activation of specific G protein-coupled receptors (GPCRs). Four subtypes of adenosine receptors (ARs) have been identified so far, namely A_1R, $A_{2A}R$, $A_{2B}R$ and A_3R, all of them bearing a distinctive pharmacological profile, tissue distribution and effector coupling [6]. Thus, while A_1R are coupled to $G_{i/o}$, $A_{2A}R$ are coupled to $G_{s/olf}$ receptors. Interestingly, in the CNS the effects of adenosine are mainly mediated by A_1Rs and $A_{2A}Rs$ [8]. Hence, while A_1Rs are more widespread in the brain the $A_{2A}Rs$ are highly concentrated in the striatum where they show both presynaptic and postsynaptic localization in γ-aminobutyric acid (GABA)-containing neurons (GABAergic neurons) [9]. Interestingly, presynaptic $A_{2A}R$ form heteromers with the A_1R in the glutamatergic terminals and it is believed that these heteromers act as concentration-dependent processors that exert subtle modulation of glutamate release [10]. Conversely, the postsynaptic $A_{2A}R$ simultaneously heteromerize with the dopamine D_2 receptor (D_2R) and the metabotropic glutamate type 5 ($mGlu_5$) receptor forming higher-order oligomers or receptor mosaics (or RMs) in the dendritic spine of enkephalinergic GABAergic medium spiny neurons (MSN) [11]. Interestingly, the $A_{2A}R/D_2R/mGlu_5$ receptor oligomeric complex, which is located extrasynaptically and adjacent to the glutamatergic synapse of the dendritic spine of MSNs, is activated by volume transmission and the receptor cross-talk within the complex helps to modulate postsynaptic plastic changes at the glutamatergic synapse [10]. Overall, GPCR

oligomerization brings new and exciting possibilities to the therapy of PD, since combined therapies acting at a named oligomer can have very different pharmacological effects from the ones expected through the monomers. Thus, the desired effect, e.g. the antiparkinsonian effect, can be mediated by the integrated activation of multiple effector systems but also by the allosterism or cross-talk mechanisms provided by the activation of the receptor monomers within the oligomer. Consequently, the existence of adenosine receptors containing oligomers in the striatum allows a much more elaborate tuning of the regulation of both presynaptic and postsynaptic neuronal responses in local striatal circuitry (Fig. 1A).

Neurobiology of Parkinson's disease

Parkinson's disease (PD) is a progressive systemic neurodegenerative disorder associated with, but not only, the loss of dopaminergic nigrostriatal neurons and is recognized as one of the most common neurological disorders, affecting approximately 1% of individuals over the age of 60. While the initial symptoms within the pre-motor phase may be nonspecific (i.e. fatigue, depression, constipation, decreased sense of smell and sleep problem) the associated features and clinical signs at the motor phase include resting tremor, rigidity, bradykinesia, and postural instability. Unfortunately, as a neurodegenerative disease, the motor phase of this pathology continues to progress late in the disease to affect other brain areas (i.e. cortex), thus patients may then exhibit cognitive dysfunction and dementia [12].

The major neuropathologic findings underlying the clinical symptoms of PD is the loss of pigmented dopaminergic neurons located in the pars compacta of the substantia nigra

(SNpc) [13]. However, some studies indicate that the loss of dopaminergic neurons occurs after there is significant loss and pathology of other brain regions including the locus coeruleus (LC), nucleus basalis of Meynert, dorsal raphe nucleus and the dorsal motor nucleus of the vagus [14, 15], and there is no explanation for this neuronal selection. In addition, a further neuropathological hallmark of PD is the existence of cytoplasmic proteinaceous inclusion bodies, called Lewy bodies, and dystrophic neurites, designated Lewy neurites [13], a feature common to other types of Parkinsonism. Interestingly, both Lewy bodies and Lewy neurites enclose a mixture of proteins, but α-synuclein seems to be the main structural component [16]. Unfortunately, 60-80% of dopaminergic neurons are lost before the motor signs of PD emerge (pre-clinical phase). This fact points out the relevance of finding a biomarker of the pathology, especially for early stages of the disease.

The basal ganglia, which includes the SN, modulates the cortical output necessary for normal movement. Inhibitory output to the thalamocortical motor circuit is directed from the globus pallidus pars interna (GPi) and the substantia nigra pars reticulata (SNr) in the basal ganglia where it acts to suppress movement. Accordingly, within the basal ganglia two major pathways exist and are referred to as the direct and indirect pathways (Fig. 1A), with a third hyperdirect excitatory corticosubthalamic pathway. Dopamine released by neurons of the substantia nigra pars compacta (SNc) acts on the striatum via activation of either dopamine D_1R or D_2R subtypes. These receptors are distributed across two main populations of GABAergic MSNs, one that contains the neuropeptides dynorphin (DYN) and substance P (SP) and the other containing enkephalin (ENK) [17, 18]. It is generally accepted that striatal DYN/SP-expressing neurons, also containing D_1R, constitute the

direct pathway and project to the GPi/SNr (Fig. 1A). On the other hand, striatal ENK-expressing neurons containing D_2R are part of the indirect pathway (Fig. 1A). However, a distinct population of MSNs with a unique phenotype, namely that co-expressing both DYN and ENK and also the D_1R and the D_2R, have been recently described [19]. The role of these D_1R/D_2R-DYN/ENK neurons, which are highly expressed in the nucleus accumbens (NAc) core and shell but with very low expression in the caudate-putamen (CPu), remains to be elucidated.

In normal non-pathological conditions, dopamine released by the SNc activates the direct pathway and inhibits the indirect pathway resulting in net inhibition of the GPi/SNr, thus resulting in a greater activity of the thalamocortical system that facilitates movements, and cognitive and behavioural outputs. However, in PD, because the loss of dopaminergic neurons in the SNc, striatal dopamine is reduced and inhibition of the GPi/SNr decreases. Thus, disinhibition of the GPi/SNr leads to increased inhibitory output to the thalamus which suppresses movement (Fig. 1B). As a result, the central role that the striatum plays in processes such as motor activity control, motor learning and some forms of associative and visual learning [20] is altered. Overall, dopaminergic neurons from the SNc control the direct and indirect pathways within the basal ganglia via activation of dopamine D_1R or D_2R. And, as described above, an interaction of dopamine receptors with glutamate and adenosine receptors in the same striatal neurons has been demonstrated, thus drugs acting on the latter group of receptors have been postulated as therapeutic tools in PD as they can modulate dopaminergic neurotransmission [21]. Furthermore, these receptor-receptor interactions highlight the potential role of the oligomers formed by these GPCRs as new therapeutic targets, since a

multimodal drug-approach would lead restore normal function in pathological states such as PD [21].

The treatment of PD: the rationale for using adenosine A_{2A}R antagonists

The treatment of PD comprises mainly pharmacotherapy, although surgical interventions (i.e. deep brain stimulation) are also in use. However, all surgical procedures have associated risks and surgery is not suitable for everyone, typically only for advanced PD refractory to pharmacotherapy. Research around gene therapy and stem cell therapy is also promising but remains much underdeveloped thus far. So, at the moment the applications of these new therapies are still unknown. Therefore, the best management for most people with PD remains to be pharmacological treatments.

The vast majority of PD pharmacological treatments seek to restore dopamine signalling and thus to diminish the intensity of the motor and non-motor symptoms. Since the sixties the most effective therapy for the management of this disease is based on the use of drugs that mimic dopamine, initially the precursor L-DOPA (L-3,4-dihydroxyphenylalanine) [22] and later on other dopaminergic agents, which act to compensate for the loss of this neurotransmitter. For instance, other dopaminergic approaches include inhibition of dopamine turnover using monoamine oxidase type B (MAO-B) inhibitors (i.e. selegiline and rasagiline) [23], catechol *O*-methyl-transferase (COMT) inhibitors (i.e. entacapone and tolcapone) [24, 25] and dopamine receptor agonists (i.e. ergot D_2R agonists: bromocriptine, pergolide, cabergoline; and non-ergot D_2R agonists: pramipexole, ropinirole, rotigitine) (for review see [26]). Interestingly, although L-DOPA has been proved very efficacious in the treatment of PD symptoms, long term treatment can result

in a loss of efficacy as motor functions deteriorate and the disease progresses. In addition, the appearance of motor complications such as dyskinesia and non-motor complications including psychosis and impulse-control disorders become more severe and problematic with continued treatment [27]. Thus, as PD progresses, fewer dopamine neurons are available to store and release L-DOPA-derived dopamine, and the patient's clinical status begins to fluctuate more and more closely in accordance with plasma L-DOPA levels. Exposing striatal dopamine receptors to fluctuating dopamine concentrations may cause a hypersensitivity that is expressed clinically as peak-dose dyskinesia. Finally, fluctuating L-DOPA-derived dopamine concentrations in association with the advancement of the disease may be responsible for the development of motor fluctuations and dyskinesia.

As mentioned above, the dopamine-based agents used in PD therapy have significant limitations, a fact that has forced the researchers to explore new non-dopaminergic PD treatments that circumvent these problems. Consequently, research is nowadays focused on finding better pharmacological therapies that provide efficacious relief of parkinsonian symptoms while evoking less risk of dyskinesia and associated motor-complications. Interestingly, another important area of research associated to these non-dopaminergic-based PD drugs is related to the neuroprotection of the dopaminergic neurons and other neuronal types (i.e. brain stem and LC neurons), in order to slow down the progression of the disease, but unfortunately still no neuroprotective therapy exists. Accordingly, we will delineate here some in vivo studies revolving around neuroprotection (i.e. protection of surviving neurons) and we will leave aside those related to neurorestoration (i.e. return to functioning phenotype of quiescent neurons) as the latter issue may constitute a review into itself [28, 29].

As introduced above, a promising treatment for the disease is based on the modulation of dopaminergic neurotransmission by means of an indirect action towards dopamine receptors. These non-dopaminergic-based PD therapies may offer advantages over dopamine medications, possibly including fewer side effects. Interestingly, one non-dopaminergic approach that has received considerable attention by the scientific community is the modulation exerted by adenosine receptors in general and by the $A_{2A}R$ in particular on the dopaminergic neurotransmission. Adenosine, as a neuromodulator, synchronizes responses to several neurotransmitters including dopamine in brain areas that are important for motor function, mood, and learning and memory [3]. Interestingly, it has been recently described that the D_2R interacts with the $A_{2A}R$ and the $mGlu_5$ receptor in striatopallidal GABAergic neurons [11], in such a manner that $A_{2A}R$ and $mGlu_5$ receptor agonists act synergistically to reduce the affinity of D_2R agonist binding sites [30]. In addition, by using the novel resonance energy transfer technique SRET [31] and through bimolecular fluorescence complementation techniques the existence of $D_2R/A_{2A}R/mGlu_5$ receptor oligomer in living cells has recently been demonstrated [11]. Moreover, coimmunoprecipiation studies in rat striatal tissue have confirmed this interaction [11]. Interestingly, in striatopallidal GABAergic neurons, it has been demonstrated that $D_2R/A_{2A}R$ dimerization results in a reciprocal inhibition and in a final negative outcome in the cAMP- and phospholipase C-mediated signalling [32]. Thus, $A_{2A}R$ blockade facilitates D_2R signalling and thereby might in theory restore D_2R-mediated striatal neurotransmission. Overall, the presence of the $D_2R/A_{2A}R/mGlu_5$ receptor oligomeric complex and the cross-talk within the receptor heteromer might help

to modulate postsynaptic plasticity at the glutamatergic synapse, and thus the output of striatopallidal GABAergic neurons (Fig. 1).

Increased knowledge about oligomerization has helped scientist to the design and development of specific A_{2A}R antagonists for the treatment of PD [33]. Interestingly, the obtained preclinical data suggest that these A_{2A}R antagonist (i.e. istradefylline) might have major applications in the treatment of PD: a) providing motor benefit as monotherapy; b) potentiating the benefit of dopamine agonists and allowing a lowering of L-DOPA dose; c) preventing the development of L-DOPA induced dyskinesias (LID), and; d) allowing the maintenance of the motor response with less dyskinesia using a lower L-DOPA dose [34]. Indeed, A_{2A}R antagonists have been shown to possess antiparkinsonian properties in human PD patients with LID [35]. This antiparkinsonian effect is probably due to the action of the A_{2A}R antagonist at A_{2A}R/D_2R oligomers, thereby reducing the A_{2A}R-mediated inhibition of D_2R signalling. Furthermore, the status of some oligomers might be altered in pathological situations such as PD or LID. In line with this, the appearance of LID has been suggested to be due to the dominance of A_{2A}R/A_{2A}R homodimers versus A_{2A}R/D_2R heterodimers and D_2R/D_2R homodimers, thus with an uncontrolled increment in the A_{2A}R signalling [36]. Indeed, the expression of A_{2A}R has been shown to be increased in the brain of PD patients with dyskinesias and it has been postulated that the increased synthesis of A_{2A}Rs in striatopallidal pathway neurons is associated with the development of dyskinesias following long-term L-DOPA therapy in PD [37]. Interestingly, this hints at another potential therapeutical use of A_{2A}R antagonists in PD, namely their use not only as anti-parkinsonian agents in patients with pre-existing LID but also its use *de novo* in combination with dopaminergic agents to

prevent the development of dyskinesias [38].[39] Recently, the availability of A_{2A}Rs in PD patients with and without LIDs was investigated by using positron emission tomography (PET) scan and the [^{11}C]SCH442416 compound [40]. Interestingly, while the thalamic A_{2A}R availability was similar in PD subjects with and without LIDs (and similar to the control subjects) the A_{2A}R binding in the CPu of PD subjects with LIDs was significantly increased when compared to the PD subjects without LIDs [40]. Overall, as patients with PD with LIDs show increased A_{2A}R expression in the striatum, and thus altered adenosinergic transmission in this brain region, it sounds reasonable to start thinking in clinical trials using A_{2A}R antagonists for the treatment of these motor complications.

As mentioned earlier, another important issue around the research related to PD concerns the direct neuroprotection of the dopaminergic neurons in order to slow down the progression of the disease. Interestingly, A_{2A}R antagonism or gene KO has been found to be neuroprotective in different models of neurodegeneration, such as Alzheimer's disease, Huntington's disease, and cerebral ischemia [41-44]. Indeed, in preclinical studies using an animal model of PD (i.e. the subchronic MPTP mouse model) it was shown that the concomitant treatment with SCH58261, an A_{2A}R antagonist, attenuated dopamine cell loss and gliosis in the SNc and in the striatum [45]. In brief, the SCH58261 injected half an hour before MPTP administration, and keep after MPTP treatment discontinuation, prevented the degeneration of nigrostriatal TH positive neurons [45]. Also, the selective deletion of neuron-specific forebrain A_{2A}R prevented MPTP-induced dopamine neuron degeneration and glial activation [45]. Recently, another A_{2A}R antagonist, the ST1535, showed a neuroprotective and anti-inflammatory effect in the

same animal model [39]. Collectively, these results provide evidence of the key role played by the blockade of A_{2A}R in PD neuroprotection. Overall, with the described neuroprotective effect on dopaminergic neuron toxicity the A_{2A}R antagonists have opened new prospects as symptomatic antiparkinsonian drugs, and thus justifyied the implementation of clinical trials studying the benefit of using these kinds of drugs early in the treatment of PD.

Clinical evaluation of adenosine A_{2A}R antagonists in PD

From the wide-range of non-dopaminergic drugs targeted at the management of PD (for review see [46]), we will focus here particularly on the clinical efficacy and the described outcomes of the A_{2A}R-based approaches that are in at least phase II clinical development for the treatment of PD. Interestingly, several preclinical studies point to the benefits of adenosine A_{2A}R antagonists on the symptomatic management of PD [47-50]. Accordingly, selective adenosine A_{2A}R antagonists, such as istradefylline (KW-6002), preladenant (SCH-420814), vipadenant (BIIB014), SYN115 and ST-1535, are being assessed in clinical studies as potential antiparkinsonian drugs [46, 51, 52]. Thus, we will describe here, as a prototypical A_{2A}R-based antiparkinsonian agent, the development of the selective adenosine A_{2A}R antagonist istradefylline, which is the first selective adenosine A_{2A}R antagonist well studied in preclinical studies and clinical trials.

Istradefylline was originally assessed as monotherapy and adjunctive therapy in rodent and primate models of PD, thus the obtained preclinical data suggested that this A_{2A}R antagonist may have major applications in the management of PD[53-58]. Accordingly, it was initially proposed that istradefylline might provide motor benefits as monotherapy

and also it was postulated that it may potentiate the benefit of dopamine agonists. Hence, the combined use of istradefylline with L-DOPA would allow lowering L-DOPA doses used in PD therapy and thus it would be prevented, in theory, the development of LID (i.e. maintenance of the motor response with less dyskinesia using a lower L-DOPA dose) [34]. Therefore, with these encouraging preclinical data, clinical effects of istradefylline were studied in human beings [59].

Firstly, regarding data from population pharmacokinetic analysis of istradefylline in healthy volunteers and patients with PD it was shown the drug fits to a two-compartment model with first order absorption. Thus, in clinical pharmacokinetics studies, the terminal elimination half life of istradefylline was of 70 to 118 hours, with a peak time of 2 to 5 hours. Istradefylline inhibits and is primarily metabolized by cytochrome P450 3A4 (CYP3A4) and is also an inhibitor of the efflux transporter P-glycoporotein. Smoking and CYP3A4 inhibitors as concomitant drugs alter istradefylline clearance and systemic exposure. As a result, istradefylline area under the concentration-time curve at steady-state increased 35% in the presence of CYP3A4 inhibitors and decreased 38% in smokers [60].

The clinical effects of istradefylline were evaluated in multicentre double-blind, placebo-controlled randomised clinical trials involving patients with moderate or advanced PD [35, 61-65][63], while only in one clinical trial the effects of treatment were examined in patients with early PD [66] (Table 1). To briefly summarize, the length of the studies was short (12 weeks) (Table 1). The mean age of patients included in clinical trials ranged from 63 to 65 years old and approximately 60% were men, while the mean time since diagnosis of disease was from 8 to 10 years (Table 2). The clinical trials evaluated a wide

range of istradefylline doses (from 5 to 80 mg), but the most studied doses were 20 and 40 mg (Table 1). On the other hand, patients were usually treated with L-DOPA (mean daily dosage from 400 mg to 857 mg) (Table 2), and often also with other concomitant antiparkinsonian medications (Table 2), while only in one clinical trial patients were treated neither with L-DOPA nor with other dopaminergic drugs (Table 1). The mean time since onset of motor complications of patients was 3 years and the mean time spent by patients in the "off" state was 6 hours (Table 2). Finally, drugs were generally well tolerated in all the clinical trials mentioned above. Thus, on average, 88 % of subjects in each group (i.e. istradefylline and placebo) completed double-blind treatment when all istradefylline clinical trials were analyzed (853 out of 968 istradefylline treated patients and 435 out of 491 placebo treated patients), while about only 11-12% of drug-treated patients discontinued prematurely the treatment (115 out of 968 istradefylline treated patients and 56 out of 491 placebo treated patients). The most common reasons (~50%) for discontinuing treatment were the adverse events (61 out of 115 istradefylline discontinued patients and 27 out of 56 placebo discontinued patients), followed by withdraw consent (16.5% in istradefylline discontinued patients and 25% in placebo discontinued patients), lack of efficacy (7.7% in istradefylline discontinued patients and 19.6% in placebo discontinued patients), non-compliance or protocol violation (13% in istradefylline discontinued patients and 7% in placebo discontinued patients) and other reasons (8.7% in istradefylline discontinued patients and 1.8% in placebo discontinued patients).

The clinical trials evaluating istradefylline efficacy targeted symptoms associated with dopamine replacement and therapy of dyskinesia. The most evaluated primary variable

for assessing clinical efficacy in clinical trials was the change in time spent on "off" state from baseline to end point. The typical primary endpoint was the change from baseline at end point in the clinical rating scale of disease Unified Parkinson's Disease Rating Scale (UPDRS) part III subscale [66] (Table 1). Needless to say, available data on the outcomes are difficult to interpret. However, in clinical trials, istradefylline reduced the time spent in the "off" state in L-DOPA-treated PD patients with motor complications but the magnitude of the drug effect was small and with doubtful clinical significance (Table 3). In addition, most studies did not reported significant benefit of istradefylline on parkinsonian symptoms as measured by the UPDRS, with the exception of a Japanase clinical trial (Table 3). In the Mizuno *et al* study, the UPDRS part III subscale score on state was reduced by 5.7 at endpoint in both istradefylline groups (20 and 40 mg) and 3.7 in the placebo group (Table 3) [65]. Thus, the differences between the two istradefylline groups and placebo were statistically significant, but again the relatively modest improvement with istradefylline (only 2 points on a scale with a total of 68 points) raised the question of clinical relevance. On the other hand, istradefylline as monotherapy in patients with early PD was not effective in improving motor symptoms [66] (Table 3).

With regard to treatment-related adverse events, they were reported in between 59% and 89% (average of 68.4%) of subjects receiving istradefylline and between 56% and 86% (average of 67.6%) of subjects receiving placebo. Globally, the frequency of adverse events was not different between groups of patients treated with doses of 20 mg and those treated with doses of 40 mg. On the other hand, adverse events caused discontinuation of the treatment in 6% of the patients, and were severe in almost 4% of patients treated with istradefylline and 2% of the placebo-treated. Finally, the most frequently reported

treatment-related adverse events in patients receiving istradefylline were nervous system-, gastrointestinal-, psychiatric- and respiratory disorders. In particular, dyskinesia, dizziness, nausea, constipation and hallucinations were the most frequently reported events (Table 4). Overall, a lot of effort has been put in the study of this first adenosine A_{2A}R-based drug designed for the treatment of PD and yet the results are modest.

Interestingly, a new set of A_{2A}R-based antiparkinsonian agents are under study. For instance, a phase II, double-blind, placebo-controlled randomised trial that assessed efficacy and safety of preladenant in 253 patients with PD and motor fluctuations who were receiving L-DOPA and other antiparkinsonian drugs, found significant although slight changes in mean daily "off" time when comparing the effects of the selective adenosine A_{2A}R antagonist with placebo [67]. On the other hand, the most common adverse events in the combined preladenant group versus placebo were similar -i.e. somnolence (10% *vs.* 6%), dyskinesia (9% *vs.* 13%), nausea (9% *vs.* 11%), constipation (8% *vs.* 2%), and insomnia (8% *vs.* 9%). Again, although initial results show that these drugs are safe and well tolerated, its efficacy in PD patients has yet to be shown conclusive.

FUTURE CHALLENGES

A_{2A}R antagonists remain one of the most attractive potential non-dopaminergic classes of drugs for the management of PD as they have shown effectiveness in counteracting parkinsonian motor symptoms and have also displayed potential neuroprotective and anti-inflammatory effects in animal models of PD. However, the translation of these promising pre-clinical observations into benefit in PD patients has yet to be conclusively

demonstrated. Thus, the continued optimisation of new symptomatic A_{2A}R-based treatments for many of the motor and non-motor problems of PD is required. Interestingly, a new generation of A_{2A}R antagonists is now under development. Accordingly, there are several phase II and III ongoing clinical trials evaluating the efficacy and safety of new selective A_{2A}R antagonist drugs in patients with PD (Table 8). Finally, it is important to mention that key to success in this endeavour is the improvement of translation of preclinical findings into phase II and III endpoints through better clinical study designs employing appropriate study populations. Overall, the need for alternative pharmacotherapies in PD is progressively clearer and non-dopaminergic drugs such as A_{2A}R antagonists constitute a realistic opportunity to reach this end.

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ABBREVIATIONS

A_1R = adenosine A_1 receptor

A_{2A}R = adenosine A_{2A} receptor

ATP = adenosine triphosphate

cAMP = cyclic adenosine monophosphate

CNS = central nervous system

CPu = caudate-putamen

CYP3A4 = cytochrome P450 3A4

D_1R = dopamine D_1 receptor

D_2R = dopamine D_2 receptor

DYN = dynorphin

ENK = enkephalin

GABA = γ-aminobutyric acid

GPCR = G protein-coupled receptor

GPi = globus pallidus pars interna

LC = locus coeruleus

L-DOPA = L-3,4-dihydroxyphenylalanine

LID = L-DOPA induced dyskinesias

$mGlu_5$ = metabotropic glutamate type 5 receptor

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

MSN = GABAergic medium spiny neurons

NAc = nucleus accumbens

PD = Parkinson's disease

SN = substantia nigra

SNc = substantia nigra pars compacta

SNr = substantia nigra pars reticulata

SP = substance P

UPDRS = Unified Parkinson's Disease Rating Scale

FIGURE LEGENDS

Fig. 1. Scheme of the basal ganglia circuitry and its connection to the thalamocortical circuit in both normal (A) and PD (B) conditions. Dopaminergic neurons from the substantia nigra pars compacta (SNc) control the direct and indirect pathways within the basal ganglia via activation of D_1R or D_2R. Thus, the striatum is linked to substantia nigra pars reticulata and globus pallidus pars interna complex (SNr–GPi) via direct (striatonigral) and indirect (striatal–pallidal–subthalamic–nigral) pathways. The co-localisation of receptors for dopamine, glutamate and adenosine in the same striatal neurons puts into relevance the potential role of oligomers formed by these GPCRs and postulated the use of striatal glutamate and adenosine receptors as therapeutic targets in PD. GPe: globus pallidus pars externa; STN: subthalamic nucleus; ENK: enkephalinergic neuron; DYN: dynorphinergic neuron.

Table 1. Randomised clinical trials with istradefylline in Parkinson's disease (PD)

Authors (year) Study Country/ies	Phase and Design clinical trial	Patients	Treatment (drugs, doses and length)	Primary outcome	Other outcomes
Bara-Jimenez et al (2003) USA [61]	●Proof-of principle ●Double-blind, placebo-controlled randomised trial (randomisation ratio 3:1)	15 subjects with moderate to advanced PD	●Istradefylline 40 mg/day oral vs. placebo ●Istradefylline 80 mg/day oral vs. placebo →6 weeks	●Exploratory study ●No primary efficacy variable was defined.	●UPDRS III motor scale scores ●Dyskinesia scales ●Duration of LD action ●Adverse events
Hauser et al (2003) US-001 Study USA [62]	●Phase II ●Multicentric double-blind, placebo-controlled randomised trial (randomisation ratio 1:1:1)	83 LD treated patients with motor fluctuations and dyskinesias	●Istradefylline 5/10/20 mg/day oral vs. placebo ●Istradefylline 10/20/40 mg/day oral vs. placebo →12 weeks	●Exploratory study. ●No primary efficacy variable was defined.	●Off time ●On time with dyskinesia ●UPDRS scores ●Dyskinesia scales ●Adverse events
LeWitt et al (2008) 6002-US-005 Study USA, Canada [35]	●Phase III ●Multicentric double-blind, placebo-controlled randomised trial (randomisation ratio 2:1)	196 LD subjects with PD experiencing prominent wearing-off motor fluctuations	●Istradefylline 40 mg per day oral vs. placebo →12 weeks	●Percentage of "off" time from baseline to end point	●On time with dyskinesia ●On time without dyskinesia ●UPDRS scores ●CGI-I scores ●Adverse events
Hauser et al (2008) 6002-US-013 Study USA [63]	●Phase III ●Multicentric double-blind, placebo-controlled randomised trial (randomisation ratio 1:1)	231 subjects with LD treated PD who have motor fluctuations	●Istradefylline 20 mg per day oral vs. placebo →12 weeks	●Percentage of "off" time from baseline to end point.	●On time with dyskinesia ●On time without dyskinesia ●UPDRS scores ●CGI-S scores ●PGI-I scores ●Adverse events
Stacy et al. (2008) 6002-US-006 Study USA [64]	●Phase III ●Multicentric double-blind, placebo-controlled, randomised trial (randomisation ratio 1:2:2)	395 subjects with LD treated PD who have motor fluctuations	●Istradefylline 20 mg per day oral vs. placebo ●Istradefylline 40 mg per day oral vs. placebo →12 week	●Percentage of "off" time from baseline to end point.	●On time with dyskinesia ●On time without dyskinesia ●UPDRS scores ●CGI-S scores ●Adverse events

Fernández et al (2010) 6002-US-051 trial USA [66]	●Phase II ●Multicentric double-blind, placebo-controlled randomised trial (randomisation ratio 1:1)	197 patients without treatment with dopaminergic agents or LD	●Istradefylline 40 mg per day oral vs. placebo →12 week	●Change from baseline at endpoint in the UPDRS part III subscale score	●UPDRS subscale I, II, IVA scores ●UPDRS total score ●WPI score ●PGI-I score ●CVLT score ●VFT score ●Adverse events
Mizuno et al (2010) The Japanase Istradefylline Sudy Japan [65]	●Phase III ●Mulicentric double-blind, placebo-controlled randomised trial (assigned by 1:1:1 randomisation ratio)	363 patients with LD treated PD who have motor fluctuations	●Istradefylline 20 mg per day oral vs. placebo ●Istradefylline 40 mg per day oral vs. placebo →12 week	●Change in daily off time from baseline at endpoint	●On time with dyskinesia ●UPDRS part III subscale score ●CGI-I score ●Adverse events

UPDRS: Unified Parkinson's Disease Rating Scale; CGI-I: Clinical Global Impression-Improvement scale; CGI-S: Clinical Global Impression-Severity of Illness scale; PGI-I: Patient Global Impression-Improvement scale; WPI: Webster Performance Index; CVLT: California Verbal Learning Test; VFT: Verbal FluencyTest.

Table 2. Baseline demographic and disease characteristics of the patients.

	Bara-Jimenez 2003	Hauser 2003	LeWitt 2008	Hauser 2008	Stacy 2008	Fernández 2010	Mizuno 2010
Age	63	64	63	63	63-65	63	65
Gender male%	53%	57%	60%	66%	64-68%	60%	43,5-44%
Duration of illness and symptoms							
Mean time since diagnosis of PD	8.1 years	NR	9.3 years	10 years	3 years	1.4 years	8 years
Mean time since onset of motor complications	NR	NR	3.3 years	4 years	NR	NR	3 years
Mean time spent in the OFF state	NR	6 hours	6 hours	6.7 hours	5.7 hours	-	6 hours
Mean time ON state without dyskinesia	NR	NR	7.3 hours	7.3 hours	7-8 hours	-	NR
Mean time ON state with dyskinesia, mean	NR	5.1 hours	2.9 hours	2.8 hours	3-4 hours	-	NR
Mean time ON state with troublesome dyskinesia	NR	NR	0.6 hours	0.7 hours	0.6-1 hours	-	NR
Mean time ON state without troublesome dyskinesia	NR	NR	9.5 hours	9.4 hours	10 hours	-	NR
Clinical Rating scales of the disease							
UPDRS Part II subscale score	NR	NR	6.4	6.8	6-7	8.2	NR
UPDRS Part III subscale score (on-state), mean	NR	18.2	17.7	23.9	17-18	20.6	21
Concomitant medications							
L-DOPA %	100%	100%	100%	100%	100%	0	100%
Daily dosage of L-DOPA	857 mg	818 mg	560 mg	652 mg	NR	-	400 mg
Dopamine agonists	NR	52%	89,9%	4%-42%	91%	-	92-96%
Selegiline	NR	22.2%	18.6%	11.3%	15-16%	-	50-54%
Entacapone	NR	29.6%	41.1%	47.8%	41-44%	-	13-19%
Amantadine	NR	NR	27.1%	32.2%	26-28%	-	31.5-37%
Other	NR	NR	NR	9%-42%	20-40%	-	16-18%

UPDRS: Unified Parkinson Disease Rating Scale; NR: Not reported.

Table 3. Efficacy results. Change in outcomes from baseline to end point

Study	Time spent in the OFF stat Mean Change		UPDRS subscale III On state	
	%	Hours	LS mean change	LS mean difference from placebo
Hauser 2003				
stradefylline 5/10/20 mg/da	-8.2*	-1.4*	NR	NR
stradefylline 5/10/20 mg/da	-6.1*	-1.1*	NR	NR
Placebo	2.2	0.5		
LeWitt 2008				
Istradefylline 40 mg/day	-10.81*	-1.79*	-0.4	NR
Placebo	-4.04	-0.64	-0.2	
Hauser 2008				
Istradefylline 20 mg/day	-9.3*	-1.6*	NR	NR
Placebo	-5.0	-0.9	NR	
Stacy 2008				
Istradefylline 20 mg/day	-7.83 *	-1.24	NR	-0.30
Istradefylline 60 mg/day	-7.96*	-1.37	NR	-0.87
Placebo	-3.47	-0.60	NR	
Fernández 2010				
Istradefylline 40 mg/day	NR	NR	-0.74	-1.11
Placebo	NR	NR	0.36	
Mizuno 2010				
Istradefylline 20 mg/day	NR	-1.31*	-1.31*	-2.0*
Istradefylline 40 mg/day	NR	-1.58*	-1.58*	-2.0*
Placebo	NR	-0.66	-0.66	

*P-value<0.05 when compared with placebo; LS Least Square; NR Not reported.

Table 4. Most frequent adverse events possible or probably related to study reported by 5% or more subjects in either treatment group, by system organ class and preferred term (safety population)

MedDRA System Organ Class/ Preferred term	Istradefylline (N = 953) n (%)	Placebo (N =488) n (%)
Nervous system disorders		
Dyskinesia	166 (17.4)	42 (8.6)
Dizziness	60 (6.3)	13 (2.7)
Lightheadedness	17 (1.8)	8 (1.6)
Dizziness postural	11 (1.1)	1 (0.2)
Headache	11 (1.1)	4 (0.8)
Gastrointestinal disorders		
Nausea	101 (10.6)	28 (5.7)
Constipation	47 (4.9)	18 (3.7)
Diarrhoea	11 (1.1)	10 (2.0)
Psychiatric disorders		
Hallucinations	27 (2.8)	5 (1.0)
Insomnia	27 (2.8)	17 (3.5)
Respiratory, thoracic and mediastinal disorders		
Nasopharyngitis	22 (2.3)	6 (1.2)
Upper respiratory tract inflammation	11 (1.1)	1 (0.2)
Upper respiratory tract infection	10 (1.0)	3 (0.6)

Table 5. Ongoing clinical trials testing drugs acting on A_2R registered with www.ClinicalTrials.gov (on March 30, 2011).

ID	Sponsor	Status	Design/ Clinical trial Phase	Experimental Drug / Duration	Control	Size (n)	Condition PD
NCT00955526	Kyowa Hakko Kirin	A (ESCD May 2011)	DB RCT Phase III	●Istradefylline 20 and 40 mg/day / 12-weeks	-Placebo	360	UKPDS step 1-2 MHYS stages 2-4 off state On LD/DC 1year
NCT00957203	Kyowa Hakko Kirin	A (ESCD June 2012)	OL NRCT SG Phase III	●Istradefylline 20 and 40 mg/day / long-term	-	150	Advanced PD treated with LD
NCT01155479	Schering-Plough (P05664AM2)	R (June 2013)	DB RCT Phase III	●Preladenant 4, 10, 20 mg daily / 52 weeks	-Placebo (26 weeks) and after Preladenant (26 weeks) -Rasagiline 1mg daily	1,000	-PD < 5 years -UKPDS Part III ≥ 10 -HYS stage ≤ 3
NCT01227265	Schering-Plough (P07037AM1)	R (September 2012)	DB RCT Phase III	●Preladenant 4, 10, 20 mg daily/ 12 weeks	-Placebo	450	-On LD≥1 year -HYS 2.5-4 "on" state - ≥ 2 hours day "off" time
NCT01155466	Schering-Plough (P04938AM3)	R (February 2013)	DB RCT Phase III	●Preladenant 4, 10, 20 mg daily/ 12 weeks	- Placebo - Rasigiline 1mg daily	750	-On LD≥1 year -HYS 2.5-4 "on" state - ≥ 2 hours day "off" time
NCT01294800	Schering-Plough (P06402)	R (June 2013)	DB RCT Phase III	●Preladenant 4, 10, 20 mg daily/ 12 weeks	- Placebo	440	-On LD≥1 year -HYS 2.5-4 "on" state - ≥ 2 hours day "off" time
NCT01215227	Schering-Plough (P06153AM1)	R (January 2013)	DB RCT Phase III	●Preladenant 4, 10, 20 mg daily/ 40 weeks	- Placebo - Rasigiline 1mg daily	750	-On LD≥1 year -HYS 2.5-4 "on" state - ≥ 2 hours day "off" time
NCT00406029	Schering-Plough (P04501AM3)	C	DB RCT Phase II	●Preladenant 2, 4, 10 mg daily/ 12 weeks	- Placebo	200	PD moderate to severe ≥ 5 years
NCT00537017	Schering-Plough (P05175AM1)	C	OL, SG NRCT Phase II	●Preladenant 10 mg daily/ 12 weeks	-	200	PD moderate to severe ≥ 5 years

NCT01323855	Schering-Plough (P06512AM1)	NYR (September 2011)	OL, SG NRCT Phase I	●Preladenant 5 mg daily/ 72 hours	-	54	Mild to severe chronic renal impairment
NCT00845000	Schering-Plough (P05550AM2)	C	SB RCT Phase II	●Preladenant 10 or 100 mg daily and LD intravenous and oral carbidopa/ 5 hours	- Placebo	18	-On LD≥1 year - Motor fluctuations and UPDRS 20%improvement when "on" - Dyskinesia when "on" ≥ 2 on scale (0-4)
NCT00442780	Biogen IDEC	C	DB RCT Phase II	●BIIB014 dose escalation /NR	Placebo	36	PD ≤ 5 years MHYS stages 1-2.5
NCT00438607	Biogen IDEC	C	DB RCT Phase II	●BIIB014 escalation /NR	Placebo	83	MHYS stages 2-4 off state
NCT00451815	Biogen IDEC	W	DB RCT Phase II	●BIIB014 10,30, 100, 300 mg once daily	Placebo	NR	MHYS stages 1-3
NCT01283594	Synosia Therapeutics	NYR (August 2012)	DB RCT Phase II/III	●SYN115 60, 120, 180, 140 mg / NR	Placebo	400	PD with good response LD
NCT00605553	Synosia Therapeutics	C	DB Crossover RCT Phase II	●SYN115 20, 60 mg daily / 1 week	Placebo	30	MHYS stages 1-3

ID: ClinicalTrials.gov Identifier; Size (n): estimated enrolment of the number of patients; PD: Parkinson Disease; Status: C (Completed) the study has been completed, A (Active) the study is ongoing, but not recruiting participants, R (Recruiting) the study is currently recruiting participants, W (Withdrawn) the study has been withdrawn prior enrollment of patients, NYT The study is not yet open for participant recruitment.; ESCD: Estimated Study Completion Data; DB: Double blind masking; OL: Open label masking; SB: Single blind masking; SG: Single group; RCT: randomised clinical trial; NRCT: nonrandomised clinical trial; UKPDS: UK Parkinson's Disease Society (UKPDS) criteria for PD; HYS: Hoehn and Yahr Scale; MHYS: Modified Hoehn and Yahr Scale: LD: L-DOPA; NR: Non reported.

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