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Research report

A_{2A} receptor antagonists do not induce dyskinesias in drug-naive or L-dopa sensitized rats

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ABSTRACT

L-Dopa, the precursor to dopamine, is currently the gold standard treatment for Parkinson's disease (PD). However, chronic exposure is associated with L-dopa-induced dyskinesias (LIDs), a serious side effect characterized by involuntary movements. Adenosine A_{2A} receptor antagonists have been studied as a novel non-dopaminergic PD treatment. Because A_{2A} receptor antagonists do not act on dopamine receptors, it has been hypothesized that they will not induce dyskinesias characteristic of L-dopa. To test this hypothesis in a rodent model, the A_{2A} receptor antagonists SCH 412348 (3 mg/kg), vipadenant (10 mg/kg), caffeine (30 mg/kg), or istradefylline (3 mg/kg) were chronically (19–22 days) administered to Sprague Dawley rats, and dyskinetic behaviors were scored across this chronic dosing paradigm. Unlike L-dopa, there was no evidence of dyskinetic activity resulting from any of the four A_{2A} receptor antagonists tested. When delivered to animals previously sensitized with L-dopa (6 mg/kg), SCH 412348, vipadenant, caffeine or istradefylline treatment produced no dyskinesias. When administered in combination with L-dopa (6 mg/kg), SCH 412348 (3 mg/kg) neither exacerbated nor prevented the induction of LIDs over the course of 19 days of treatment. Collectively, our data indicate that A_{2A} receptor antagonists are likely to have a reduced dyskinetic liability relative to L-dopa but do not block dyskinesias when coadministered with L-dopa. Clinical studies are required to fully understand the dyskinesia profiles of A_{2A} receptor antagonists.

1. Introduction

Current pharmacological treatments for Parkinson's disease (PD) involve compensating for deficits in dopaminergic activity through dopamine replacement therapies such as treatment with the dopamine precursor L-dopa. Although initially effective at delaying the onset of parkinsonian symptoms, efficacy diminishes with continued treatment (Marsden, 1994). In addition, chronic L-dopa administration can produce undesirable side effects that manifest as L-dopa-induced dyskinesias (LIDs), a collection of involuntary movements that compromise a patient's quality of life (Hametner et al., 2010). Alternatives to current treatments that avoid LIDs may lie in non-dopaminergic manipulations of the indirect pathway of the basal ganglia. One possible such approach is through pharmacological antagonism of the A_{2A} receptor (Pinna et al., 2007; Schwarzschild et al., 2006).

The A_{2A} receptor is one of a family of four G protein coupled receptors (A_1, A_{2A}, A_{2B}, and A_3) to which adenosine binds and that plays a modulatory role on various neurotransmitter systems. Within the striatum, A_{2A} receptors are expressed primarily in the enkephalinergic striatopallidal GABAergic neurons where they are colocalized with dopamine D_2 receptors (Ferre et al., 1997). It is hypothesized that the mechanisms by which A_{2A} receptor antagonists exert their effects on motor function result from their inhibitory actions on neurons of the striatopallidal pathway expressing both A_{2A} and D_2 receptors (Martinez-Mir et al., 1991; Morelli et al., 2007; Richardson et al., 1997). Inhibitory D_2 receptor activity is negatively influenced through the A_{2A} receptor's role in second messenger systems or through the formation of receptor heterodimers; conversely activation of A_{2A} receptors disrupts the inhibitory actions of D_2 within the indirect pathway leading to downstream effects not unlike those elicited through administration of D_2 receptor agonists (Morelli et al., 2007). Other evidence shows that A_{2A} receptors play a modulatory role in the excitatory input into the striatum (Hettinger et al., 2001) or that they modulate the activity of cholinergic interneurons within the striatum (Tozzi et al., 2011), although the contribution of these effects on the anti-parkinsonian efficacy of A_{2A} receptor antagonists is less well understood.

Animal studies clearly demonstrate that administration of A_{2A} agonists such as CGS21680 decreases motor activity by stimulating the release of GABA within striatopallidal neurons (Khisti et al., 2000). Conversely, A_{2A} receptor antagonists such as preladenant,

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SCH 412348, and istradefylline increase motor activity (Chase et al., 2003; El Yacoubi et al., 2000; Griebel et al., 1991; Hodgson et al., 2009).

In animal experiments, numerous studies have demonstrated that A_{2A} receptor antagonists have a profile predictive of clinical efficacy for the treatment of PD (Hauser and Schwarzschild, 2005; Morelli et al., 2007; Schwarzschild et al., 2006). Following administration of the A_{2A} receptor antagonist istradefylline, levels of the inhibitory neurotransmitter GABA increased in striatopallidal neurons of 6-OHDA lesioned rats (Ochi et al., 2000), whereas they were decreased in the globus pallidus (Ochi et al., 2004). This finding indicates that A_{2A} receptor antagonists modulate the indirect pathway of the basal ganglia in a way that counteracts the effect of the hypodopaminergic state associated with PD. Acute administration of haloperidol, a D_2 receptor antagonist produces a hypodopaminergic state that produces an akinetic response characteristic of PD that is reliably reversed by A_{2A} receptor antagonists (Kanda et al., 1994; Pinna et al., 2005; Shiozaki et al., 1999). Furthermore, chronic administration of haloperidol, pimozide, or reserpine produces a jaw tremor in rats modeling parkinsonian tremor, which can be reversed by the A_{2A} receptor antagonists istradefylline and MSX-3 (Correa et al., 2004; Salamone et al., 2008). A_{2A} receptor antagonists reverse motor deficits typically seen in a 6-OHDA model of PD (Pinna et al., 2007) and in non-human primates treated with the dopamine neurotoxin MPTP (Hodgson et al., 2010). Clinically, co-administration of istradefylline with L-dopa reduced "off-time" and lowered the dose of L-dopa required to achieve a therapeutic effect in PD patients (Bara-Jimenez et al., 2003; Chase et al., 2003; Hauser et al., 2003). More recently, preladenant produced positive data when co-administered with L-dopa in a clinical trial with PD patients (Hauser et al., 2011).

LIDs are characterized by jerking and writhing that diminish patients' quality of life (Obeso et al., 2000). With continued therapy, LIDs may increase, leading to a significant and often detrimental reduction in patient compliance (Tarrants et al., 2010). Alteration of neuronal activity, both within the basal ganglia and between circuits crucial to the coordination of movement, are thought to underlie the appearance of dyskinesias (Brotchie et al., 2005). Both preclinical and clinical evidence points to the reduced dyskinesia liability of A_{2A} receptor antagonists. Unilaterally 6-OHDA lesioned rats will rotate contralateral to the lesion site when treated with L-dopa. This phenomenon increases with chronic administration of L-dopa. This behavioral sensitization is thought to be predictive of a drug's dyskinetic liability (Morelli et al., 1994). When low doses of L-dopa are administered to 6-OHDA rats or A_{2A} knockout mice in conjunction with an A_{2A} antagonist, contralateral turning behavior is observed without sensitization (Fredduzzi et al., 2002; Hodgson et al., 2009; Morelli et al., 2007; Pinna et al., 2001).

The most commonly used animal model for assessing dyskinesias of novel drugs involves chronic administration of L-dopa to rats with unilateral 6-OHDA lesions (Cenci et al., 2002; Morin et al., 2013). Typically, when 6-OHDA is unilaterally administered to the medial forebrain bundle (MFB) and following a 2-week period, the animals display a supersensitivity to L-dopa or dopamine agonists (Ungerstedt, 1971). Chronic administration of L-dopa to 6-OHDA lesioned rats results in a characteristic abnormal movement profile that is scored by assessing three body regions: limb movements that are rapid, undirected, and repetitive; trunk twisting; and orolingual movements that involve rapid movements of the jaw and tongue. Treatments that have been shown to have an effect in lowering LIDs in humans also reduce LIDs in 6-OHDA rats (e.g., Bordia et al., 2008). Moreover, these dyskinetic endpoints are dissociable from the anti-akinetic endpoints (Dekundy et al., 2007).

Three A_{2A} receptor antagonists that have been investigated extensively in preclinical models of PD are istradefylline (A_{2A} K_i = 2.2 nM; A_1 K_i = 150 nM) (Shimada et al., 1997), SCH 412348 (A_{2A} K_i = 0.6 nM; A_1 $K_i \geq 960$ nM) (Neustadt et al., 2007), which is structurally and functionally very similar to preladenant and vipadenant (BIIB014) (A_{2A} K_i = 1.3 nM; A_1 K_i = 68 nM) (Armentero et al., 2011). Here we used the rat 6-OHDA model to determine whether the A_{2A} receptor antagonists, SCH 412348, istradefylline, and vipadenant induce dyskinesias both in drug-naive animals and in animals previously treated chronically with L-dopa. In addition, we assessed the non-selective adenosine receptor antagonist caffeine in these models.

2. Methods

2.1. Animals

All studies were conducted using male Sprague Dawley rats (Taconic, Hudson, New York) weighing 350–400 g and aged 11–14 weeks at the beginning of the chronic drug treatment. The group sizes were 6–10 rats per group. Animals underwent unilateral 6-OHDA lesion surgeries at Taconic laboratories (see description of surgical procedure below) and were shipped to Merck Research Laboratories (drug naive) approximately 2 weeks after surgery. Rats were housed individually in Plexiglas hanging cages with food and water available *ad libitum*. Colony rooms were controlled for temperature and humidity and maintained on a 12-h light/dark cycle (lights on 7:00 AM, lights off 7:00 PM). All injections and dyskinesia scoring sessions were performed during the light portion of the light/dark cycle between the hours of 10:00 AM and 2:00 PM. All research procedures and animal care were performed in accordance with the Merck Research Laboratories Animal Care and Use Committee guidelines and complied with the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act.

2.2. Drugs

Istradefylline, vipadenant, and SCH 412348 were synthesized by the Medicinal Chemistry Department of Merck Research Laboratories and administered intraperitoneally (IP) via saline solution containing 0.4% methylcellulose (MC). Caffeine, purchased from Sigma–Aldrich (St. Louis, MO) was also administered IP using a 0.4% MC vehicle. Haloperidol (Sigma–Aldrich) was administered subcutaneously (SC) via distilled water. For the lesion verification studies, turning behavior was induced via SC injection of apomorphine (Sigma–Aldrich) dissolved in 0.4% ascorbic acid saline solution. Benserazide and L-dopa (Sigma–Aldrich) were dissolved together in saline and administered IP. All drugs were administered at a dose volume of 1 ml/kg. All doses are reported as free base.

2.3. Lesion verification

Following 1 week of habituation to the colony room at Merck Research Laboratories (i.e., 3–4 weeks following the surgical procedure), successful 6-OHDA lesioning was validated via automated rotometer quantification of apomorphine-precipitated turning. Specifically, animals were dosed with apomorphine (0.5 mg/kg) and placed in rotometers for 1 h where the number of rotations was automatically counted and recorded. Those animals completing >100 contralateral rotations were considered to have been successfully lesioned and were included in the dyskinesia studies. Approximately 30% of the animals were excluded because they failed to meet this criterion. No histological examination was performed to further confirm the lesion; however, previous studies have indicated that apomorphine-induced turning behavior accurately predicts the quality of the MFB lesion.