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In vivo characterization of a novel dopamine D3 receptor agonist to treat motor symptoms of Parkinson's disease

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ABSTRACT

Synthetic dopaminergic agents have found utility in treating neurological and neuropsychiatric disorders since the beginning of 19th century. The discovery of Levodopa (L-dopa) to effectively treat motor symptoms of Parkinson's disease (PD) revolutionized the therapy and remains a gold standard for treating PD. However, L-dopa therapy has been implicated in worsening of the non-motor symptoms including cognition and long-term therapy leads to plasticity and development of abnormal involuntary movements (AIMs) that are collectively called dyskinesias. In response to these severe side effects, a range of dopaminergic agents from partial agonists to antagonists have been tried either as L-dopa replacement agents or as adjuvants with L-dopa therapy with limited success. Recent studies in rodents, non-human primates and post mortem studies on PD patients have implicated that dopamine D3 receptors may play a role in the etiology of both the motor symptoms and L-dopa induced dyskinesias. We have recently developed SK609, a selective dopamine D3 receptor agonist with atypical signaling properties. In this study, we further characterized this novel small molecule using the unilateral lesioned rodent model of PD. In the forepaw stepping test paradigm, SK609 significantly improved the performance of the impaired paw and also normalized the bilateral asymmetry associated with the hemiparkinson rat. In addition, a chronic treatment of SK609 did not induce any AIMs and when used adjuvantly with L-dopa significantly reduced AIMs induced by L-dopa. Further, an optimal dose combination of SK609 with L-dopa was determined by dose dependent titrations of both SK609 and L-dopa that produced minimal AIMs and maximized the effect on improving motor symptoms. Results from this study suggest that SK609 is a novel dopaminergic agent that has the therapeutic potential to treat PD and LID.

1. Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder after Alzheimer's disease. The incidence of PD has been associated with increased age as well as other genetic and epigenetic factors, such as occupational exposures and environmental triggers (Mandel et al., 2012; de Lau and Breteler, 2006; Wirdefeldt et al., 2011). The prevalence of PD in industrialized countries is estimated to be about 0.3% of the entire population and about 1% of the population older than the age of 60 years (Pringsheim et al., 2014; Willis et al., 2013). In the United States an estimated one million individuals live with PD, while a projected total of seven to ten million people live with PD worldwide (Pringsheim et al., 2014; Willis et al., 2013). Each year approximately 60,000 Americans are diagnosed with PD, not including the potentially thousands of cases that remain undetected. PD is a chronic, progressive movement disorder typically characterized by at least two of its four cardinal features: tremor at rest, rigidity, bradykinesia, and postural instability (Jankovic, 2008). The disease and its progression are attributed to the loss of both dopaminergic and noradrenergic neurons due to the generation of reactive oxygen species (Soldani and Fornai, 1999; Burns et al., 1985; Obata et al., 2001). It has been shown that the neuronal degeneration seems to initiate with the selective loss of

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dopaminergic neurons in the substantia nigra (SN) and furthers into other regions of the striatum (Coyle and Snyder, 1969; Zigmond et al., 1990).

In PD, the dopaminergic cell loss in the SN pars compacta (SNc) leads to a cascade of alteration affecting the entire basal ganglia circuit, consisting of both the direct and the indirect pathways (Gerfen and Surmeier, 2011; Obeso et al., 2008; Calabresi et al., 2014). Consequently, the end result is increased activity in the GABAergic output nuclei involving the medial globus pallidus and the SN pars reticulate (SNr), which increases inhibition of the motor thalamus while potentially reducing thalamo-cortical signaling (Blandini et al., 2000). Thus, the hyperactivity of the subthalamic nucleus (STN) glutamatergic projections to the output nuclei has been hypothesized to be a fundamental player in governing motor movements (Greenamyre, 2001).

Based on this hypothesis, many pharmacological and device based interventions have been designed to restore the normal functional balance of the basal ganglia circuitry to manage the symptoms of PD. Among the device based techniques, deep brain stimulation (DBS) has been performed to improve postural instability and gait disorder symptoms with marked acute improvement in symptoms (Benabid et al., 2009; Potter-Nerger and Volkmann, 2013). However, like any other surgical technique, DBS has its short comings and is not suitable to many patients (Lilleeng et al., 2014).

Currently, treating PD with the administration of dopamine to the patient in the form of levodopa (L-dopa) in combination with a peripheral DOPA decarboxylase inhibitor is considered the gold standard (Mercuri and Bernardi, 2005; Olanow et al., 2004; Poewe et al., 2010). However, L-dopa therapy typically results in numerous side effects, such as nausea, gastrointestinal bleeding, disturbed respiration, disorientation, anxiety, and hallucinations (Olanow et al., 2004; Goodwin, 1971). Unfortunately, long-term L-dopa therapy leads to L-dopa induced dyskinesia (LID), which is the development of motor complications characterized by abnormal involuntary movements (AIMs) and other non-motor symptoms (Davie, 2008; Symptomatic Pharmacologic, 2006; Thanvi et al., 2007; Huot et al., 2013). LID typically presents with advancing PD when patients start to experience "on/off" cycles of motor dysfunction involving periods of dyskinesia alternating with "off" periods (Zesiewicz et al., 2007). LID may occur in nearly 80% of patients who have been on L-dopa treatment for more than five years and also in patients with early onset (Kumar et al., 2005; Sossi et al., 2006).

Despite significant advances in research, the molecular origins and pathophysiology of LID still remain incompletely understood (Thanvi et al., 2007). A possible mechanism for LID involves pulsatile stimulation of dopaminergic receptors leading to consequent alterations in molecular and neurophysiologic function of downstream basal ganglia neurons. Thus, the postsynaptic striatal neurons experience dysregulation of genes and proteins with changes to the firing pattern of the basal ganglia output neurons, ultimately leading to the clinical expression of dyskinesia (Obeso et al., 2000; Nutt and Holford, 1996).

In managing the symptoms of PD and LID, other therapeutics have shown varying degrees of efficacy, including dopamine agonists (Hobson et al., 1999; Connolly and Lang, 2014; Pechevis et al., 2005), MAO-B inhibitors (Connolly and Lang, 2014; Bortolato et al., 2008), and NMDA inhibitors (Kieburtz et al., 1991; Greenamyre and O'Brien, 1991). MAO-B inhibitors act to increase the level of dopamine in the basal ganglia by blocking dopamine metabolism (Youdim et al., 2006). Similar to dopamine agonists, MAO-B inhibitors, when used as a monotherapy, improve motor symptoms and delay the need for L-dopa therapy early in the disease course. However, not only do MAO-B inhibitors hold a lower degree of efficacy than L-dopa, but they also lead to significant side effects, drug interactions, and potentially lethal dietary interactions (Symptomatic Pharmacologic, 2006; Youdim et al., 2006). NMDA inhibitors work to reduce the over-activity of the glutamatergic systems in the basal ganglia of LID patients with the intention of reducing dyskinesia (Papa and Chase, 1996). Amantadine, an antiviral drug with weak NMDA antagonist activity, may be used for managing PD; however, studies show mixed results for efficacy for PD treatment (Crosby et al., 2003; da Silva-Junior et al., 2005; Wolf et al., 2010). Moreover, amantadine may be limited by tachyphylaxis and has been associated with central nervous system side effects (Thanvi et al., 2007; American Hospital Formulary Service – Drug Information, 2005).

Dopamine receptor agonists such as Pramipexole, have been shown to be effective in controlling the symptoms of PD during the early stages of the disease (Connolly and Lang, 2014), but eventually need to be combined with L-dopa to optimize the management of PD (Noyes et al., 2005). Thus, dopamine agonists are typically implemented in the treatment program to either delay the use of L-dopa (L-dopa sparing) or to reduce the dosage of L-dopa (Thanvi et al., 2007). Furthermore, dopamine agonists produce significant side effects, including somnolence, nausea, insomnia, constipation, and hallucinations (Symptomatic Pharmacologic, 2006). Apomorphine, a non-selective parenteral dopamine agonist, may be used to off-set the fluctuations of L-dopa doses, namely the "on" and "off" effects. However, Apomorphine is emetic, is ineffective in some patients, and is associated with adverse neuropsychiatric effects (Deleu et al., 2004).

A recently discovered potential mechanism for the etiology of motor symptoms of PD and LID involves dopamine D3 receptors, whose expression is decreased in PD while increased in brain regions involved in motor control in patients exhibiting LID (Bezard et al., 2003; Guigoni et al., 2005; Berthet and Bezard, 2009; Aviles-Olmos et al., 2012; Mahmoudi et al., 2014). More specifically, in rodent and non-human primate models of LID, the D3 receptor has been shown to be abnormally over-expressed in the direct striatonigral output pathway; as a result, it plays a significant role in the improper balance between the indirect and direct pathways of the basal ganglia (Guigoni et al., 2005; Mahmoudi et al., 2014). Previous studies using selective D2/D3 receptor agonists, partial agonists, and several selective D3 receptor antagonists indicated the role of D3 receptor in the development of LID (Bezard et al., 2003; Blanchet et al., 1997; Visanji et al., 2009). Furthermore, the D3 receptor has been shown to exhibit biased signaling and desensitization pattern in response to certain agonists, including dopamine; however, the closely related D2 receptor does not demonstrate these D3 characteristics (Gil-Mast et al., 2013; Kuzhikandathil and Kortagere, 2012; Westrich et al., 2010). Therefore, aberrant expression of D3 receptors coupled with selective desensitization of D3 receptors by agonists such as dopamine could significantly contribute to the development of motor and hyperkinetic symptoms in PD and LID respectively (Guigoni et al., 2005; Kortagere and Kuzhikandahil, 2012; Cote and Kuzhikandathil, 2014). Thus, we hypothesize that a novel selective D3 agonist that does not induce desensitization of D3 receptors in vivo could modulate the motor symptoms of PD and LID (Westrich et al., 2010; Kortagere and Kuzhikandahil, 2012). To test this hypothesis, we have designed and characterized SK609 (previously called ES609) – a novel D3 selective agonist that does not induce desensitization of D3 receptors in vitro (Kuzhikandathil and Kortagere, 2012; Kortagere and Kuzhikandahil, 2012). Our previous studies on this molecule describes the in vitro effect of ES609 on AtT-20 cells over expressing either the D2 or D3 receptors. We demonstrated that ES609 was a selective D3 agonist and did not induce tolerance or slow receptor termination properties characterized using whole cell patch clamp techniques measuring the GIRK channel currents

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