Screening the Novel Dopamine D3 Receptor Agonist SK609 in Rodent Model of Parkinson’s Disease

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INTRODUCTION

- Parkinson’s disease (PD) is the second most common age related neurodegenerative disorder after Alzheimer’s disease. The etiology of PD is not well understood but the motor symptoms are associated with loss of dopaminergic neurons in the nigro-striatal regions of the brain.
- However therapy with L-dopa to improve levels of dopamine lead to L-dopa induced dyskinesia (LID), which is characterized by abnormal involuntary movements (AIMs). Analysis of brain tissues from rodent and non-human primate models of PD suggests a key role for Dopamine D3 receptors in PD and LID (1,2).
- We hypothesize that aberrant expression of D3 receptors coupled with desensitization of D3 receptors by agonists such as dopamine could significantly contribute to the development of LID.
- Thus, a selective D3 agonist that does not induce desensitization of D3 receptors in vivo could modulate the motor symptoms of PD and abrogate the symptoms of LID.

DESIGN AND CHARACTERIZATION OF SK609

- Designed using Hybrid structure based method (3) guided by conformational sampling (4)
- SK609 had greater than 10,000 fold selectivity for D3 receptors and minimal effect for D2 receptors (4)

METHODS

Surgery

- Lesioned rats unilaterally microinjected with 6-OHDA (8µg/2µL in 0.9% sterile normal saline with 0.01% ascorbic acid)
- Sham rats unilaterally microinjected with 0.9% sterile normal saline with 0.01% ascorbic acid
- Left intra-striatum injection coordinates: AP: 1.0mm; L: 3.2mm; DV: 4.6mm

Rotation Test

- Two weeks after surgery, the rats were administered apomorphine to be tested in the “apomorphine induced rotation” test to confirm experimental PD model symptoms
- Each rat was administered apomorphine subcutaneously (0.25 mg/kg) and then placed into the rotation testing chamber (30cm diameter Plexiglas cylinder) for 40 minutes (Figure 3)
- Each test was video recorded and analyzed by at least two scorers blinded to the study to count the number of complete contralateral rotations

Stepping Test

- Intended to model motor deficits in the forelimbs that are analogous to limb akinesia and gait deficits seen in PD patients
- Animals were pre-trained for 5 days by handling and practicing the stepping test on the wooden board testing surface (30cm length test with 1.5 cm markings)
- L-Dopa (6mg/kg) or SK609 (4mg/kg) were administered by intraperitoneal injection (i.p.) to the rat 10 minutes before testing
- All rats were tested gently with one hand facing the hind limbs above the test surface (+45° angle) while the other hand fixed the forelimb that is not being monitored (4)
- Recorded initiation time of the first step by each forelimb and total stepping time to travel the full distance (30 cm)

Abnormal Involuntary Movements (AIMs)

- Designed to evaluate the efficacy of SK609 as an adjuvant to L-dopa to treat symptoms of LID
- Performed as described in Morgese, et al. (6)
- AIMs, consisting of axial, limb, and orolingual dyskinesia, was measured daily between 10:00am and 4:00pm over a 2hour period after various doses of SK609 (i.p. administration [20 minutes prior to testing] followed by L-Dopa i.p. administration 15 minutes prior to testing )
- Animals were placed in a Plexiglas cylinder (30 cm diameter) for 120 minutes and video recorded for later analysis (Figure 3)
- Each dyskinesia parameter were scored on a severity scaled ranging from 0 to 4 for every 60 second time interval (modified from Lundblad, et al (7))

RESULTS

Independent Effects of L-dopa and SK609 on Initiation Time in Stepping Tests

- We have demonstrated that PD rats with akinetic forelimb showed significant improvement in forelimb stepping test with L-dopa and SK609. Both initiation time and total stepping time were reduced significantly with SK609 treatment when compared to either the control or L-dopa.
- In PD rats primed for LID with chronic treatment with L-dopa, SK609 significantly reduced total AIMs associated with L-dopa treatment in a dose dependent manner.
- These results suggest that the selective D3 agonist SK609 could be used as an adjuvant with L-dopa to treat symptoms of LID in PD patients without impairing the positive effects of L-dopa.
- Future directions involve delineating the long term effects of SK609 in comparison to L-dopa. Furthermore, optimal combination dosages of L-dopa + SK609 should be researched in order to maximize the positive effects of L-dopa while maintaining and minimizing LID symptoms with SK609.

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REFERENCES