JAK/STAT pathway inhibitors to treat neuroinflammation: a novel treatment for Parkinson Disease patients

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I. BACKGROUND

Parkinson's disease (PD) is a prevalent neurodegenerative movement disorder, affecting approximately 0.5-1% of individuals aged 65 to 69, accounting for more than 10 million people globally (Kouli, Torsney, & Kuan, 2018). Major symptoms of PD include tremors, limb rigidity, bradykinesia (slowness of movement), and impaired balance. The disease is primarily driven by the loss of dopaminergic neurons in the substantia nigra pars compacta, a brain region critical for dopamine production. Additionally, PD is characterized by the accumulation of misfolded alpha-synuclein (α-SYN) proteins, forming Lewy bodies, which further contribute to neurodegeneration (Lee et al., 2014). PD patients also experience a loss of norepinephrine-producing nerve endings, impairing the body's sympathetic nervous functions. Mutations in the α-synuclein gene are linked to familial forms of PD, marked by increased Lewy body aggregation (Singleton, 2003).

Neuroinflammation has emerged as a crucial factor in PD pathogenesis, with research showing its significant role in disease progression (Tansey & Goldberg, 2010). In one study, the immunosuppressant FK506 demonstrated high efficacy in reducing both neuroinflammation and neurodegeneration in PD models (Van der Perren et al., 2015). Despite these advances, there is currently no cure that can reverse PD's neurodegenerative effects. Current treatments are focused on managing symptoms, particularly by increasing dopamine levels or addressing non-motor symptoms. HoIver, more than 98% of novel treatments fail to cross the blood-brain barrier (BBB), limiting their effectiveness.

Existing treatments, such as deep brain stimulation, MRI-guided focused ultrasound, and Levodopa, remain the most effective in managing motor symptoms (Bronstein et al., 2011). Levodopa, the most widely used treatment, alleviates tremors but does not halt disease progression. Furthermore, prolonged Levodopa use often results in Levodopa-induced dyskinesia, causing involuntary movements (Radhakrishnan & Goyal, 2018). To mitigate side effects, patients are also prescribed Carbidopa. Other treatments include dopamine agonists, MAO-B inhibitors, COMT inhibitors, Amantadine, and anticholinergics, all aimed at controlling symptoms but failing to slow disease progression (National Institute on Aging, 2017).

Given the limitations of current treatments, there is an urgent need for novel therapeutic approaches. This research proposal explores the potential of Ruxolitinib, an ATP-competitive inhibitor of the JAK/STAT pathway, as a treatment for PD. Ruxolitinib has demonstrated the ability to cross the BBB in phase 3 trials for HIV-1 infection (Haile et al., 2016) and is FDA-approved for treating myelofibrosis and polycythemia vera (Raedler, 2015). While it has never been tested for PD, its known properties suggest it may offer a promising new avenue for slowing disease progression.

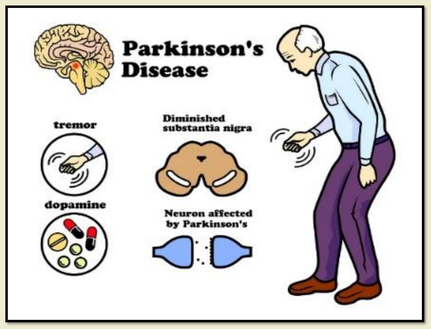

Figure 1: A diagram of the relation between symptoms of PD and the affected brain areas

II. ABSTRACT

Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. A key feature of PD is neuroinflammation, which drives the overexpression of pro-inflammatory cytokines such as IL-6 and IFN-γ. Preclinical rat models have demonstrated that the Janus Kinase/Signal Transducer and Activator of Transcription (JAK/STAT) pathway is excessively activated in PD, particularly involving IL-6, IFN-γ, and MHC Class II. This study proposes a trial using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice to evaluate Ruxolitinib, a JAK/STAT pathway inhibitor, as a potential therapeutic for PD. Mice will be divided into three groups: one receiving 5 mg/kg of Ruxolitinib orally, another receiving 10 mg/kg, and a control group treated with a vehicle. After four weeks, immunoblotting and electrophoresis will assess MHC Class II expression, comparing outcomes between the treated and control groups. A significant reduction in MHC Class II activation is anticipated in the Ruxolitinib-treated groups. This study aims to validate the JAK/STAT pathway as a promising therapeutic target to slow PD progression.

III. RESEARCH HYPOTHESIS

Levels of MHC Class II will decline by inhibiting the JAK/STAT pathway using Ruxolitinib, indicating that Parkinson's disease progression is slowed down.

IV. DISCUSSION

Pre-clinical models have shown elevated levels of cytokines like IFN-γ and IL-6 in PD patients, which are potent activators of the JAK/STAT pathway (Qin et al., 2016; Chen et al., 2007). This establishes a strong link between the JAK/STAT pathway, neuroinflammation, and PD progression, highlighting its importance in disease development.

To explore this connection, I propose testing Ruxolitinib, a JAK inhibitor, as a potential treatment for Parkinson's disease in mouse models. Ruxolitinib is an oral inhibitor of JAK1 and JAK2, known for passing the blood-brain barrier in phase 3 trials for HIV-1 infection (Haile et al., 2016), and is FDA-approved for the treatment of myelofibrosis and polycythemia vera (Raedler, 2015). While its mechanism in treating PD has not been studied, I hypothesize that it may act similarly to its role in treating myelofibrosis by inhibiting JAK1 and JAK2 as an ATP-competitive inhibitor, thereby blocking the phosphorylation of tyrosine residues in cytokine receptors.

In this study, I will use MPTP-induced mice as a model for Parkinson's disease. These mice exhibit abnormal levels of alpha-synuclein phosphorylated at Ser129 and nitrosylated alpha-synuclein, as Ill as 91% destruction of dopaminergic neurons in the substantia nigra pars compacta (Decressac et al., 2012). MPTP is a lipophilic protoxin that crosses the blood-brain barrier and is converted by monoamine oxidase B (MAO-B) into MPP+, which accumulates in dopaminergic neurons, leading to mitochondrial dysfunction, reduced ATP production, and reactive oxygen species generation, ultimately causing neuronal degeneration (Nicklas et al., 1987).

MPTP injection will activate MHC Class II expression in the substantia nigra, simulating Parkinson's disease. The JAK/STAT inhibitor Ruxolitinib is expected to inhibit the activation of these genes, thereby slowing the neurodegeneration associated with PD. To test this, immunoblotting and electrophoresis will be employed to detect MHC Class II levels in the brain tissue of treated mice (Lee and Benveniste, 1996). The results will be compared with vehicle-treated MPTP mice, which will receive dimethylformamide (DMF), to assess the efficacy of Ruxolitinib in mitigating neuroinflammation and slowing disease progression.

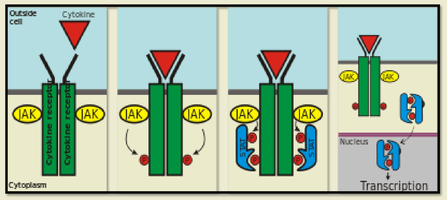

Figure 2: Diagram of the JAK/STAT pathway

V. METHODOLOGY

1. To test the proposed hypothesis, a Parkinson's disease model will be induced in mice using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) according to the protocol outlined by Jackson-Lewis et al. (2007). Mice will be divided into three groups. The first group will receive Ruxolitinib orally at a dose of 5 mg/kg, the second group will receive Ruxolitinib at 10 mg/kg, and the third group will serve as the vehicle control, receiving dimethylformamide (DMF). Oral gavage will be used to administer these treatments daily for two weeks.

2. Following this treatment period, Parkinson's disease symptoms will be evaluated in the MPTP-treated mice, with particular focus on akinesia, rigidity, tremors, gait, and posture disturbances. The over-activation of MHC Class II proteins will be assessed as a marker for Parkinson's disease progression.

3. At the end of the experiment, mice will be deeply anesthetized with isoflurane. Approximately 30 micrograms of the ventral midbrain tissue will be lysed using ice-cold cell lysis buffer and a homogenizer. The brain tissue will be preserved in a tissue collection solution (a mixture of 50% 0.01 M phosphate-buffered saline and 50% glycerol) and stored at -20°C for subsequent immunoblotting analysis. Immunoblotting will be used to measure the concentrations of MHC Class II proteins in the ventral midbrain, as Ill as STAT1 and STAT3 to assess the inhibition of the JAK/STAT pathway.

4. Protein separation will be conducted using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% stacking gel, applying 140 volts to achieve optimal protein separation. The results will be compared to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a loading control. The slowing of Parkinson's disease progression will be assessed by probing for MHC Class II proteins using specific antibodies (Qin et al., 2012). Inhibition of the JAK/STAT pathway will be confirmed by probing STAT1 and STAT3 using their respective antibodies.

5. After electrophoresis, proteins will be transferred to a polyvinylidene fluoride (PVDF) membrane using an electrotransfer setup, which involves creating a transfer sandwich composed of a sponge, filter papers, the gel, and the PVDF membrane. By applying a current, proteins will migrate from the gel to the PVDF membrane. The expression of MHC Class II proteins will be visualized using chemiluminescence in a dark room.

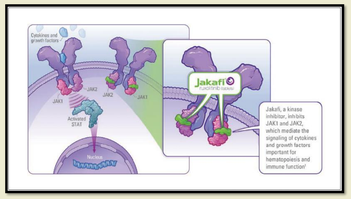

Figure 3: Ruxolitinib, called Jakafi in this figure, binds to the JAK1 and JAK2 and inhibits it, which prevents it from mediating the signaling of cytokines in the cell. This should hypothetically help slow down progression of Parkinson's disease.

VI. EXPECTED RESULTS/CONCLUSION

Based on previous research in Parkinson's disease models, the administration of Ruxolitinib, a JAK/STAT pathway inhibitor, to MPTP-induced mice is expected to result in reduced activation of MHC Class II, which is typically associated with elevated levels of alpha-synuclein in the brain. Over a four-week period, a marked reduction in MHC Class II activation should be observed in the Ruxolitinib-treated group compared to the untreated controls. In immunoblotting assays, MHC Class II protein expression will likely increase following MPTP treatment but should be significantly inhibited by Ruxolitinib administration, indicating a suppression of neuroinflammation in the mice.

Should this study yield promising results, the next step will involve testing Ruxolitinib in MPTP-induced primate models. This will allow for the assessment of drug tolerance and efficacy in a more advanced system, simulating Parkinsonism in primates. Based on the results, I will determine the feasibility of moving forward to Phase I clinical trials. Phase I trials would involve testing the drug and determining the safe dosage range on 20 to 100 healthy volunteers.

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