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**Using a Tyrosine-Kinase inhibitor to possibly regulate JNK pathways and see its effect as a possible treatment for Parkinson’s disease.**

**I. Introduction**

Parkinson’s disease, abbreviated as PD is an extremely common neurological disorders affecting over approximately one million people in North America1. It is a neurodegenerative disorder that spreads at different rates and can be identified by the loss of neurons in the substantia nigra pars compacta (SNpc) region of the midbrain2. This loss of neurons leads to a decrease in dopamine levels in the striatum. The striatum is where the axons from these neurons are found. As time has gone on many scientists have been trying to find a cure for this disease since there is none. For a while, people who were diagnosed with Parkinson’s disease had a few options to find some relief from the crippling symptoms of PD. These forms of relief included medications and surgery1. Some of these treatments like drug use need to be used cautiously because of their side effects. This is why scientists are trying to find new forms of treatment by understanding the molecular biology involved with PD.

One method that scientists are trying to do is using certain inhibitors to use on specific protein kinases to hopefully find a treatment for PD. According to Wang et al4, the prevalent protein kinase to pay attention to is c-Jun N-terminal kinase (JNK) and that pathway that it is a part of which is the JNK Signaling Pathway. Protein kinases are used in a plethora of intracellular signaling pathways. It is important to note that c-Jun N-terminal kinase is a Mitogen-activated protein (MAP). This kinase is in a family that is composed of three kinases that form a consecutive activation pathway. This pathway is made up of MAP kinases (MAPKs), MAP kinase kinases (MKKs), and MAP kinase kinase kinases (MKKKs) 2. The pathway works like this: MAPKs function as phosphorylation substrates for MKKs which are triggered in turn by phosphorylation via a MKKK2.

JNKs are known as stress activated protein kinases because they respond to extracellular stimuli like heat shock, and ultraviolet irradiation. JNK has ten isoforms which are split into three different categories: JNK1, JNK2 and JNK3. JNK 1 and 2 can be found in cells and tissues and have four isoforms each whereas JNK3 only has 2 isoforms and is found in the brain. Regulation of the JNK signaling pathway is complex and is influenced by multiple MKKKs. The most important function of the JNK pathway is how its reaction to extracellular stimuli affects certain cell functions like apoptosis and proliferation.



**Fig 1:**  A chart to give you an idea of the JNK pathway in regards to PD. Adapted from figure 1 of reference 2

 How does the JNK pathways and Parkinson’s disease correlate to one another? Wang et al states that Parkinson’s disease is characterized by the loss of dopaminergic neurons in the SNpc. The goal in the neurodegenerative therapy is that scientists are trying to find results that will allow them to either see functional restoration and or a decrease/stop of progression of neuronal loss. The role JNK in this disease's pathogenesis is being increasingly recognized. Wang et al also talks about how his evidence suggests that JNK plays an important role in mediating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)/1-methyl-4-phenylpyridnium ion (MPP(+))-induced neurotoxicity. He believes that if the JNK pathway were to be blocked off, then maybe the progression of Parkinson's disease could be slowed down and eventually stopped. Basically in order to stop PD, something must be found that can block the death signaling pathway of the execution for apoptosis in dopaminergic neurons.

 The blocking of JNK pathways can only be done through a kinase inhibitor. A kinase inhibitor is a type of enzyme inhibitor that blocks the action of one or more protein kinases. There are currently a few inhibitors like CEP-1347/KT-75157, SP6001256, and JIP-18, that have been tested and shown to successfully inhibit the JNK pathway in some form. By using these inhibitors to regulate the JNK pathway scientists would have complete control over processes like cell apoptosis and survival signaling. In terms of Parkinson’s disease, if scientists were to inhibit the JNK pathway and have control over cell apoptosis, they would then be in control of neuronal cell death (**Fig 2**)5. It is important to note that none of these inhibitors have reached the goal of blocking off the JNK pathway completely to lead to a cure for PD.

 Since these three inhibitors were already used in an experiment and seemed to get some concrete results, I decided to use an inhibitor that wasn’t mentioned in any research or review article for my experiment. After some searching, it was decided that I would use a tyrosine kinase for my experiment. Tyrosine kinase is an enzyme that has the ability to move a phosphate group from ATP to a protein within the confines of a cell. It can be imagined as an on or off switch in many cellular The specific Tyrosine kinase being used is Gefitinib, which is a drug, used for certain types of cancers like lung, breast, and a few other cancers. I know the idea seems farfetched for using a tyrosine kinase inhibitor that is used for cancer, but I feel like there is some possibility that it could also work as an inhibitor for the JNK pathway.



**Figure 2** This figure gives you an idea of the pathway involved in dopaminergic neuron death. Adapted from figure 2 in Reference 2.

**II. Experiment**

The aim of this experiment is to determine if the Tyrosine kinase Geftifinib could serve as an appropriate kinase inhibitor towards the JNK pathway and have an effect on the neural degeneration of neurons by regulating cell apoptosis.

 While doing research for what kind of experiment I should do I came across a plethora of articles (8, 18, and 22) that somewhat followed the same experimental procedure. In these different experiments that I came across there was a common trend of mice being used in vitro and simulating the effects of PD onto them. The experiment that I will do will draw from these three articles and hopefully I can come out with some concrete results.

I will begin my experiment by getting Three months old adult male C57BL/6N mice (the common black lab mouse). I believe using adult mice will yield the best results because their bodies are fully developed. All proper procedures for animal care in experiments were followed and it was made sure that measures were taken to make sure the mice were comfortable.

This experiment will be a week long experiment because it will involve freezing certain body parts for a few days and analyzing said parts. The mice for the experiment will be divided into four groups with seven mice in each group. Groups one through three will be injected with MPTP to simulate PD in their bodies. Group 1 will have the mice injected with the tyrosine kinase Geftifinib. Group 2 will have the mice injected with a known inhibitor of JNK. This inhibitor will be SP600125 and it will serve as a comparison to group 1. Group 4 will just have normal mice to compare all of the group’s results to. It is important to note that all mice that have anything being injected into them are going to be stereotaxically injected into the left striatum.

To get results, a technique called Immunohistochemistry will be administered to the brains of the mice. In order to do this the mice need to be killed and have their brains immediately removed and frozen until further analysis. This will be done to three of the mice in groups one through three. According to Wang et al the brain should be fixed in 4% PFA for about two hours at room temperature and then preserved in 30% sucrose overnight at 4 °C. This is probably done so that the brain is preserved in a way that it can be used to the fullest extent. Once the brain is taken out from its frozen encampment the next day, it will be split into 10 μm coronal sections8. Next the antibodies must be prepared so that they can be detected in the Immunohistochemistry. The anti-bodies that I will use for this experiment are the oens both used in Wang and Xia et al. These antibodies are rabbit anti-phospho-c-Jun (Ser73) antibody, mouse anti-TH antibody, and anti-rabbit IgG (fluorescein isothiocyanate (FITC).

The mice brains should be serially sectioned (35 μm) through the entire midbrain after fixation and stained for TH by immunohistochemistry. Wang et al suggest that the tissue sections should be incubated successively with a mouse monoclonal antibody to mouse TH according to the diaminobenzidine (DAB) staining procedure. Once the experiment is done the TH immunoreactive neurons should be counted to see if there was in an increase or decrease.

**Figure 3** This figure shows what an Immunohistochemistry would yield. The results I would be looking to get would be something similar to the last 3 sub figures (g-i).

**III. Discussion**

 Hopefully if all goes well the Tyrosine Kinase will actually yield some result that I can use. I am hoping to see reduced levels of c-Jun phosphorylation like in figure 3 (g-i). Seeing those results will validate that the kinase that a kinase used to treat something completely different could actually serve as another inhibitor of the JNK pathway. If the results are indeed there then I may be on to something and would probably want to draw some more conclusions by doing a follow up experiment with humans in vivo. Or maybe I could try to fuse multiple inhibitors, if that is possible, and see what that yields.

 The obvious problem is that the kinase I am using has never been used for this kind of situation (to my knowledge). The chance of receiving nothing except dead mice with a Tyrosine Kinase in them and new results is extremely high. I would love to get results that actually show something in regards to the JNK pathway being inhibited, but like I said this is a longshot. It would be extremely interesting if I observed signs of JNK Pathway being inhibited and affecting cell apoptosis. I also have to take into account that Gefitinib is a drug and there are other components in this drug. If I were to yield successful results, I have to remember that it was due to all of the factors inside the Gefitinib inhibitor.

 Despite all these beliefs that I won’t get any results I should look at the bigger picture and realize that I am doing something that to my knowledge has not been done before so there is no need to fear failure. What I am doing is something unconventional, but I believe there is a realm of possibility that using a tyrosine kinase primarily used for cancer can work on inhibiting the JNK pathway. I am sure I will accumulate some kind of results. If the experiment does yield the results that I want, than I may be on the right track for providing a cure, or at least advancement in a cure, for Parkinson’s disease.

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