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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 include medications, surgery and multidisciplinary management can provide relief from the symptoms1. Some of these treatments like drug use need to be cautiously used because of their side effects. This is why scientists are trying to find new forms of treatment by understanding the 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 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.

What exactly are JNK pathways? 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, JNK1 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 families of MAPs. 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.

 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 or another. By using these inhibitors to regulate the JNK pathway scientists would have complete control over process 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 functions10 and it should be noted that it is a subclass of a protein kinase. 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.



**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 regulation cellular functions like cell apoptosis. Obviously the goal of this experiment would be seeing if the results of a new inhibitor do more than the ones already discovered.

 For this experiment, it was decided that instead of using human subjects, mice would be used in vivo instead. The mice that would be for this kind of experiment would be C57BL/6 mice or the common laboratory mouse. It would be made sure that the animal use was in accordance with the European Convention for Animal Care and Use of Laboratory Animals. The experiment would take a lot from Xu Gang Xia et al’s experiment which was basically using lab mice in vivo to transfer MPTP and mice and then perform the experiment to see if the inhibitor used, which in their experiment was JIP-1, had any effect on the JNK pathway. I will probably do what Xu Gang Xia did with her mice almost exactly the same which is8 retrieve Twelve-week-old male C57BL/6 mice and anesthetize them with methohexital . Xu Gang Xia used 50 mg/kg in PBS, and 2.6 × 107 plaque-forming units of the recombinant adenovirus in a volume of 2 μl or 2 μl of PBS (pH = 7.4) but I might play around with the amounts. After anesthizing them the mice were stereotaxically injected into the left striatum with the exact being coordinates: flat skull position, bregma, 2.5 mm laterally, 3 mm below dura8. Another aspect to Xu Gang Xia’s experiment that I would possibly add to mine was basically cardiacally freezing 4 mice, anesthetizing them, and then removing their brains so they could be placed in in 4% PFA overnight at 4°C, then changed to 30% sucrose for 2 days and then stored at −80°C8. The mice would then be killed under deep anesthesia, and their striatum and substantia nigra would be quickly removed and stored at −80°C until Western blot analysis. The blot analysis will be used to see if there were c-Jun phosphorylation peaks in the substanta nigra of the mice.

**III. Discussion**

 Hopefully if all goes well the Tyrosine Kinase will actually yield some result that I can use. I am hoping to see some changes in the blot spot that will allow me to see if the kinase that I chose actually affects the JNK pathway at all or if it should stick to treating cancers. If the results are indeed there then I may be on to something and would probably want to draw some more conclusions and then either do a follow up experiment with humans in vitro. My goal is to see something that will prove to me my kinase of choice actually inhibited the JNK pathway and affect the apoptosis of neuron cells.

**Figure 3.** This is what the blot analysis from the mice should look like after the experiment. Panel a shows a peak in C-jun phosphorylation.

 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 mice with a Tyrosine Kinase 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 a miracle to me if I saw some signs of JNK phosphorylating at a different rate than usual.

 Another problem that comes to mind is if I were to actually get some results from my experiment, how I would go about comparing them to the other ‘successful’ inhibitors like CEP-1347/KT-75157 and SP6001256 whose results were achieved differently than Xu Gang Xia’s experiment for JIP-18. I guess I could get all the results from all different inhibitors and try making a plethora of charts and graphs to compare them in various ways. The whole purpose of this experiment is to find out if there are other inhibitors out there that could do a better job than the ones already mentioned.

 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. I am sure I will accumulate some kind of results. If the experiment does yield the results that I wanted 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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