The effects of Quercetin on seizure like activity in C*aenorhabditis elegans* mutants

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Abstract

Our experiment on *Caenorhabditis elegans* was the first step in providing relief for patients with a clinically recognizable neuronal migration disorder caused by CAMSAP1 mutations. CAMSAP1 is thought to stabilize microtubule minus ends and to be involved in cell signaling. There are currently no treatments for this disorder. Thus, we partnered with the undiagnosed disease network at Washington University School of Medicine for this project. Our goal was to find a drug that will help patients with this disorder. We picked *C. elegans* because they have a homolog for the CAMSAP1 gene called PTRN-1. The worms were grown on NGM (Nematode Growth Medium) plates without calcium. This was how we got them to have convulsion, which presented themselves when we put them in a buffer (100 mM NaCl, 50 mM MgCl2.) We delivered the drugs into the worms by using a "spot dead" method, where we put a drop of the tested drug onto the plate and covered it in dead *E. coli*. The worm then eats the dead *E. coli, ingesting the drug with it.* This method directly bypasses the cuticle of the worm, meaning less of each drug had to be used. The experiment overall was a resounding success, as a handful of the drugs we tested rescued the mutants. This includes Quercetin, which was the drug I chose. It was picked due to its ability to downregulate cell signaling pathways impacted by CAMSAP, specifically DAPK and MAP3K12. The drugs that rescued will be tested on a vertebrate model organism next, and if all goes well, it can be given to the children to help them. This experiment was the first step in the journey of helping these kids with an incredibly debilitating disorder.

Introduction

Our experiment was carried out to try to find a drug that helps children with a Bi-allelic CAMSAP1 Variant that causes a clinically recognizable neuronal migration disorder.[1] One of the symptoms of the disorder is seizures; because of this we conducted the testing on Caenorhabditis elegans who have shown to be a successful model for seizure-like activity.[2] C. elegans have a homolog for the CAMSAP1 protein named PTRN-1, [3] also making them an ideal candidate for the research. The drug I chose to test was Quercetin, selected for the downregulation of MAP3K12 and DAPK. It accomplishes this intervening in the ERK ½ and the JNK signaling cascade involved in apoptosis. This research is an important step to help the children with this disorder, as there are no known treatments for the disorder. Partnering with the undiagnosed disease network at Washington University School of Medicine, we set out to try and remedy that.

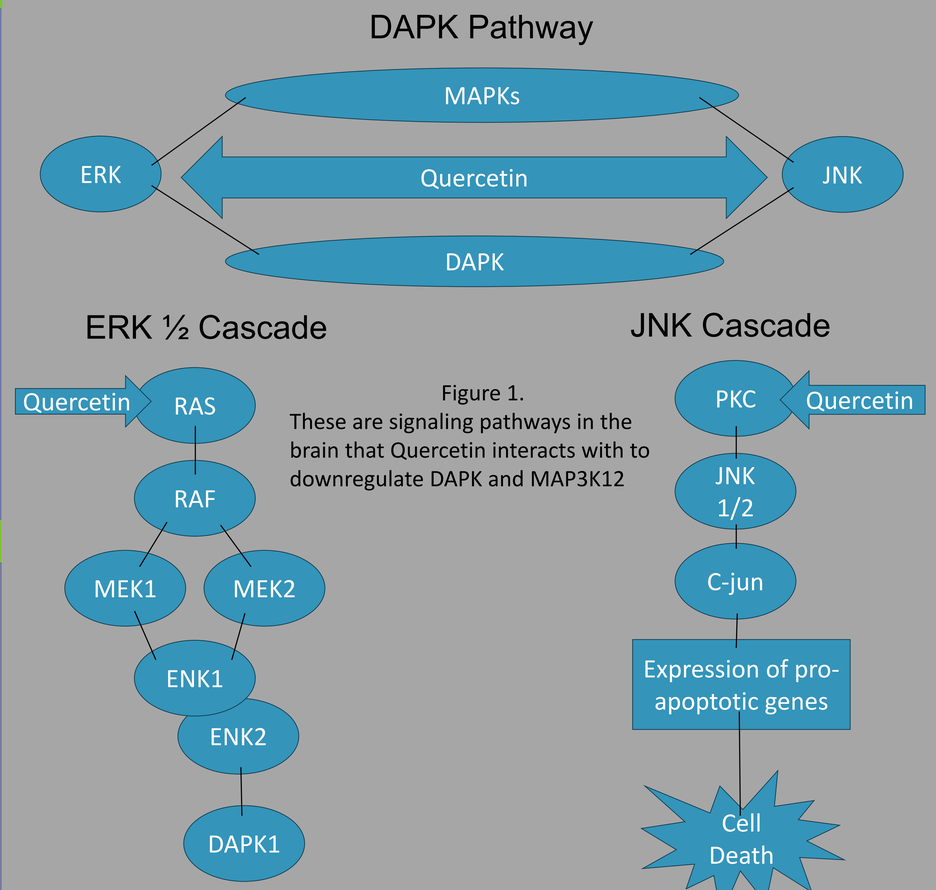


Figure 1.
These are signaling pathways in the brain that Quercetin interacts with to downregulate DAPK and MAP3K12

Materials/Methods

We had five strains of C. elegans for this experiment, all but one with glowing neurons, wild-type (only glowing neurons, jsls973, jsls 609), unc-49, unc-25, N2 (truly wild-type) and finally the ptrn-1 (Camsap1 homolog) worm, which was the focus of this experiment. The other worms served as controls; however, all the worms were labeled with numbers to ensure we would not know which worm was which during the experiment. This kept us objective while observing the data. The way we ended up doing the experiment was due to several factors. Some of which include what we had to do to get the worms to convulse, the amount of drug we ordered for testing, as well as the time frame in which we worked. To cause the convulsions, we went to an experiment that Dr. Jana Marcette had done previously with the PTRN-1 mutants.[3] Which was detailed in a different paper. [4] We decided on the "dead spot" method for delivery of the drugs, which we found in a paper about drug efficiency in C. elegans.[5] The C. elegans were grown on NGM plates (nematode growth medium) without calcium. The lack of calcium on the plates helped induce convulsion when the worms were placed into the buffer solution.(100 mM NaCl, 50 mM MgCl2) We placed the worms on a plate with this buffer, and the solvent we would use for each drug. Then we recorded their seizure activity, to establish a baseline. Then after synchronizing new worms, they were raised on plates with the drugs under the dead E. coli. Then we put them into the same buffer solution and record the seizure activity and compared the two data sets.

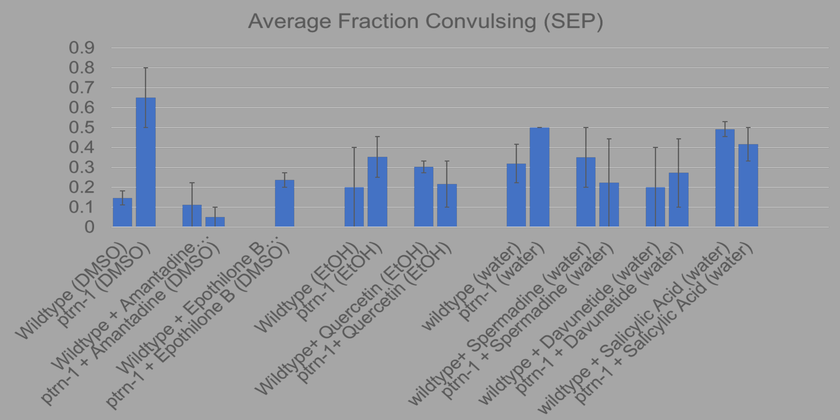
Results

Figure 1 shows the drugs that showed promise, including Quercetin. We had three drugs that we are confident rescued the mutants: Amantadine, Epothilone B, and Quercetin. Some of the data was unclear, but after a third test it is clear these three worked. During the third test none of the worms in water seized at all, which is inconsistent with all other data we had. Thus, while Spermadine, Davunetide, and Salicylic Acid all show promise, I would not be confident in saying they rescued without another test.

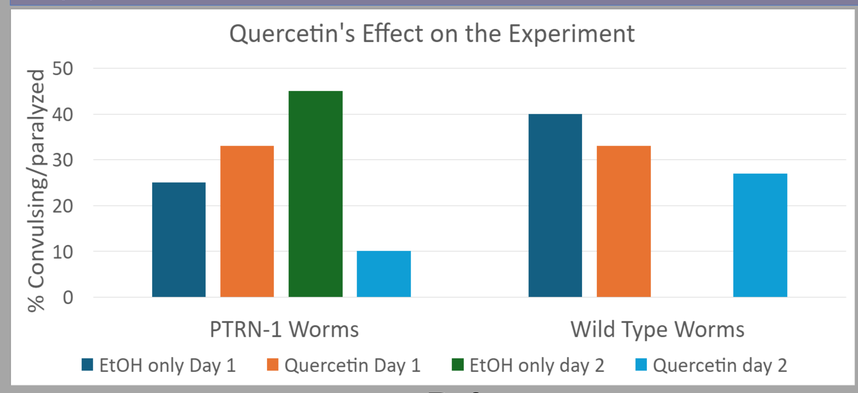
Conclusion

The experiment was a resounding success. We found a couple of drugs that rescued the PTRN-1 (CAMSAP-1 homolog) worms; Amantadine and Epothilone B. The other ones on the figure below we are not confident in saying that with just the data we have. Of course, when we do our third run we will test Amantadine and Epothilone B again as well. There are more steps before doctors start giving these drugs to the kids of course. There will be trials of these drugs on different model organisms, inching closer and closer to humans. However, this was an immensely important, and necessary, first step. There is still a lot that needs to be learned. I also believe if we investigated the mechanisms of the drugs that helped, and the similarities between them, it will provide more insight into what this CAMSAP1 mutation does to cause the symptoms in these children.

Figure 1



Caenorhabditis elegans (Microscopic Roundworms)



Acknowledgments

The CGC: " *C. elegans* worm strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440)."
Joe Walsh and Landon's League Foundation
Aleksandra Foksinska | Program Manager UAB | The University of Alabama at Birmingham
The Undiagnosed Disease Network

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