GDF-15 as a Biomarker for Glaucoma

Caylee Pattison

Western Michigan University, pattca12@gmail.com

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GDF15 as a Biomarker for Glaucoma

Caylee Pattison
Western Michigan University
Abstract

Biomarkers are measurable substances in an organism that are indicative of some phenomenon. Examples of biomarkers in clinical settings are body temperature and blood pressure. Body temperature is indicative of a fever while blood pressure can be used to monitor someone’s risk of a stroke. Previous studies have investigated the possibility of there being a potential biomarker for glaucoma. One possible biomarker identified is growth differentiation factor 15 or GDF15. These studies prompted a study in our lab using a rat glaucoma model. The objective of this study was to show there is a biomarker present for the neurodegenerative disease, glaucoma. To mimic the effects of glaucoma, adult healthy rats were anesthetized using a KAX solution, and NaCl was injected into the episcleral veins of experimental eyes to induce scarring in the trabecular meshwork and induce glaucoma-like conditions. In each animal, only one eye was subjected to this procedure. The other eye was left untreated and acted as an internal control. After a specific period of time, the rats were euthanized, and their retinas and aqueous humor were removed. The retinas and aqueous humor from untreated control eyes and from experimental eyes were quantified for GDF15 protein levels using an enzyme-linked immunosorbent assay (ELISA). After quantifying protein expression in both sets of eyes, a separate experiment was done by adding neuroprotective to the experimental eye to see if it had any effect on the GDF15 expression. Previous studies have found that activation of alpha 7 nicotinic acetylcholine receptors, α7nAChRs, in the eye reduces retinal ganglion cell death associated with glaucoma. The neuroprotective used in this study was PNU-282987. PNU-282987 was added to untreated and experimental eyes to determine if its effect on GDF15 protein expression. It was found that there was an increase in GDF15 expression after injection with hypertonic saline. This increase was most prevalent at 3 days and 7 days post operation.
This increase means that GDF15 is a potential biomarker for glaucoma. The results from the neuroprotective experiment yielded promising results. There was no significant difference between the eyes treated with PNU-282987, the eyes not treated with PNU-282987, and the control eyes. This result suggests that the neuroprotection worked in preventing the expression of GDF15. The results also showed possible cross over between the eyes since the untreated eyes that had been induced with glaucoma-like conditions also showed no significant difference. To know for sure more experiments need to be done. An early glaucoma marker such as GDF15 levels could help lead to a faster diagnosis in those affected and help us gain a better understanding of the disease itself.

**Introduction**

**The Eye**

Understanding the anatomy and function of the eye is crucial to understanding what glaucoma is and the way it is expressed (Figure 1). Human and rat eye anatomy differs very slightly allowing us to use a rat eye as a model. The eye is separated into two chambers known as the anterior chamber and the posterior chamber. The anterior chamber is filled with a liquid known as aqueous humor, while the posterior chamber contains another fluid called vitreous humor. The eyeball is also divided into three separate layers, all with different functions. The sclera makes up the outermost part of the eyeball and is the site where muscles associated with movement of the eye are attached. Inside the sclera is the choroid, which is associated with providing the oxygen and nutrients to the next layer, the retina. The retina is the innermost layer of the eye that contains photoreceptors called cones and rods that convert photons of light into electrical signals. Visual information is conveyed through the retina and to the brain, where visual perception occurs.
There are multiple components of the eye within these chambers crucial for proper function of the eye. At the anterior portion of the eye, the sclera becomes transparent and is known as the cornea. The cornea as well as the lens is used to focus light onto the retina, which is located in the back of the eye. Deeper in the eye structures such as the iris and ciliary body are encountered. The iris is what gives the eye its color and is responsible for pupil contraction or dilation. The ciliary body controls the shape of the lens through use of ciliary muscles. It is also responsible for the production of the aqueous humor that fills the anterior chamber. The most interior layer of the eye, the retina, is possibly the most important component of the eye when it comes to this thesis.

**Figure 1.**
The retina is developed from the optic cup and held in position by the sclera and cornea. The neural retina contains six different classes of neurons which are ganglion cells, photoreceptors (rods and cones), bipolar cells, amacrine cells, horizontal cells, and the Muller glia (Figure 2). Photons of light are converted into electrical signals in the photoreceptors in a process known as phototransduction. Those signals are then conveyed through the retina through the bipolar cells and then through the retinal ganglion cells (RGCs), whose axons carry the information to the brain for final visual perception. As information is conveyed through the retina, it is modified by a layer of horizontal cells in the outer retina and then again by a layer of amacrine cells in the inner retina. For purposes of this thesis, the focus will be on RGCs. All the neurons of the retina are arranged in parallel layers. The nuclei of photoreceptors are found in the outer nuclear layer (ONL), the nuclei of bipolar cells, horizontal cells, amacrine cells and Muller glia are found in the inner nuclear layer (INL) and the nuclei of retinal ganglion cells are found in the ganglion cell layer (GCL). Between these nuclear layers lie the inner plexiform layer (IPL) and the outer plexiform layer (OPL). The OPL is where the photoreceptors synapse onto the bipolar and horizontal cells while the IPL contains the site of synapse between bipolar or amacrine cells with RGCs.
Figure 2.

Anatomy of the neural retina. Starting with light entering through the choroid into the retina and encountering the six neurons used to convert it to an electrical impulse. (Image taken from Wiley Online Library http://onlinelibrary.wiley.com/doi/10.1111/j.1442-9071.2010.02363.x/full)

Glaucoma

Glaucoma is most commonly associated with the death of retinal ganglion cells which leads to optic neuropathy. It is a neurodegenerative disease that affects millions of people worldwide and is considered the second leading cause of blindness (Ban et al., 2017). Loss of vision starts in the periphery eventually spreading into the central visual field. RGCs in mammals, like other neurons involved in the central nervous system, cannot regenerate when they die. Once they are damaged and lost, they are gone forever. There are multiple risk factors associated with glaucoma. They include age, genetic background, below average corneal thickness, vascular dysregulation, and the most well-known risk factor is elevated intraocular pressure (IOP) (Almasieh et al., 2012). An increased IOP is caused by a blockage within the eye that prevents outflow of aqueous humor. As mentioned previously the aqueous humor is
continually produced by the ciliary body and fills the anterior chamber of the eye. Blockage of aqueous humor outflow produces a pressure build-up. This elevated pressure in the anterior of the eye results in an increase in total IOP. The higher IOP pushes on the other components of the eye, such as the lens. The lens then applies pressure to the vitreous humor in the posterior chamber of the retina. The jelly-like composition of the vitreous humor then applies pressure to the retina where the RGCs are located. This results in damage and loss of these cells (Figure 3). One or more of these risk factors can contribute to disease progression, but none are conclusively indicative of glaucoma. At present, there is no cure for glaucoma.

![Normal anatomy vs. Glaucoma](image)

**Figure 3.** Glaucoma eye vs. normal eye. (Image taken from Mayo Clinic website https://www.mayoclinic.org/diseases-conditions/glaucoma/symptoms-causes/syc-20372839)

The standard method for measuring IOP is the tonometry (Kaufmann et al., 2003). Most commonly used is the noncontact tonometry which is associated with an air puff. A puff of air is
directed at the cornea, flattening it, allowing the ophthalmologist to measure the IOP. This type of tonometry is not the most reliable, but it is the easiest and most common. A more reliable type is Goldmann tonometry. This type of test is usually done as a secondary method following noncontact tonometry. It is a more invasive test using a small probe to flatten the cornea and using a slit lamp to look at the eye. The amount of force needed to flatten the cornea is used to measure the pressure. The IOP measurement is used in treatment plans.

The current treatments for glaucoma focus primarily on reducing IOP, even though there are significant number of patients with glaucoma that have normal IOPs. These treatments include eye drops, surgery, or pills. Most commonly, glaucoma is partially controlled with eyedrops that act to reduce the IOP. Like all medications, these include side effects such as stinging, red eyes, blurred vision, or changes in eye color (Boyd, 2017). Surgery can be done in two different ways. A laser surgery can be done to help drain the aqueous humor while the other surgery can be done to create a new drainage channel for the aqueous humor. Both surgeries result in a decrease in aqueous humor build-up which in turn should lower IOP. Since all current treatments only reduce IOP and current treatments do not always stop the progression of the disease, it could be suggested that other treatments could be developed to better reduce RGC death.

**Neuronal Cell Death**

Excitotoxicity is the neuronal cell death associated with the over stimulation of receptors. It is associated with several neurodegenerative diseases including glaucoma, retinal ischemia, and diabetic retinopathy (Iwamoto et al., 2013). One specific type of excitotoxicity that plays an important role in glaucoma is glutamate-induced excitotoxicity. Glutamate is an excitatory neurotransmitter released from the bipolar cells onto ganglion cells within the retina. Previous
studies have increased the amounts of glutamate resulting in an excessive activation of glutamate receptors. This activation results in an influx of calcium through NMDA glutamate channels which eventually leads to RGC death through apoptotic mechanisms (Quigley et al., 1995; Kitano et al., 1996; Burnashev, 1998; Lipton, 2001; Li et al., 2002; Aarts and Tymainski, 2004). This idea of excitotoxicity being a factor of glaucoma has led to possible neuroprotective being produced.

Neuroprotection is defined as any technique or procedure that will prevent the loss of neurons due to disease or injury. Previous studies found that acetylcholine (ACh) and nicotine are considered neuroprotective in an isolated RGC culture preparation and prevented glutamate-induced excitotoxicity (Iwamoto et al., 2013). Studies like this one were used to determine that the specific nicotinic acetylcholine receptor (nAChR) involved in ACh-induced neuroprotection was an alpha7 nAChR (Brandt et al., 2011). This identification led to multiple studies whose goals were to determine if the activation of alpha7 nAChR provided neuroprotection of RGCs that were lost as a result of glaucoma-like conditions. To determine if this was so, an ACh agonist was added to eyes of adult Long Evans rats that had the procedure to induce glaucoma-like conditions. The specific α7nAChR agonist added was PNU-282987, which was used to mimic the effects of nicotine and acetylcholine on the alpha7 nAChRs in the eye. The studies concluded that the PNU-282987 was in fact a potent agonist of the alpha7 nAChR and reversed the loss of RGCs normally associated with the procedure to induce glaucoma in adult rats (Iwamoto et al., 2013).

**Biomarker**

Biomarkers are used to measure the presence or progress of a disease. They are beneficial to the identification of many diseases often in early stages. The main objective of this honors
thesis was to identify a possible early biomarker for glaucoma and measure it at different time points, as well as with added neuroprotection. A previous study found that the GDF15, is associated with RGC death within rat and mice models (Ban et al., 2017). The study found that GDF15 expression in the retina increased after RGC axons were injured. The study used an optic nerve crush model to damage the RGC axons. Aqueous humor GDF15 expression was measured in addition to measuring GDF15 expression in the retina. This was done to determine if the GDF15 gene expression led to differences in protein secretion. The results showed that there was an increase in the expression in the aqueous humor as well as the retina. This resulted in the idea that GDF15 may be a biomarker for RGC death and because RGC death is associated with glaucoma it could also be considered a possible biomarker for the disease.

This previous study led to the topic of this honors thesis. Similar to the study, the purpose of this thesis was to determine if GDF15 is a biomarker for glaucoma, but a different model to induce glaucoma was used to show it. An optic nerve crush model was enough to indicate the presence of the marker due to an increase in RGC death, but it is not a very realistic model of glaucoma. As stated earlier, most cases of glaucoma are directly correlated to an increase in IOP. A model where this is the case is a more indicative model of the disease. Hypertonic injection of 2M saline into the episcleral veins of Long Evans rats leads to a significant increase of IOP that corresponds to the typical loss of RGCs associated with glaucoma-inducing model. As a result, we measured GDF15 expression in this more physiologically similar model of glaucoma.
Methods

Animals

Adult Long Evans rats three months of age or older were used for this study. Long Evans rats are considered docile animals, have prominent pigmented eyeballs, and do not have as many visual deficits as other rats making them good models for the study. All animals used were a part of Western Michigan University’s animal facility and were used for research purposes. All animals used in the research were treated and euthanized following guidelines set forth by the Institutional Animal Care and Use Committee of Western Michigan University. Collaborative Institutional Training Initiative, CITI, program courses were completed to ensure the correct handling and care of the animals.

Inducing Glaucoma-Like Conditions

The procedure used to induce glaucoma-like conditions in the rat was followed from a previous study published by Western Michigan University (Iwamoto et al., 2014). A KAX solution was prepared as an anesthetic for the animals. KAX is a cocktail containing 5 mL of ketamine, 50 mg of xylazine, 1 mL of acepromazine, and 3 mL of sterile water. Males received a dose and a half for their weight while female rats received a dose. The anesthetic was given via intraperitoneal injections. After administration of the drug, the rat’s reflex activity was monitored. Once the rats had no obvious signs of reflex activity, the procedure to induce glaucoma could begin.

To numb the eye before the surgery and prevent blinking reflex activity, proparacaine ophthalmic drops were added. To induce glaucoma-like conditions, 50 μL of 2 M NaCl was injected into the episcleral vein of the right eyes of all the rats. The injection was done into a
bulging rat eye using a 3 mm long glass micropipette that was attached to polyethylene tubing and a 1 mL syringe. The micropipette was pulled using a vertical electrode puller and beveled. A 23-gauge needle with the tip filed off was used to backflow the hypertonic saline, NaCl, into the beveled micropipette before attaching it to polyurethane tubing (Iwamoto et al., 2014) The NaCl injection creates scar tissue in the trabecular meshwork and increases IOP. The increase in IOP mimics the effects of glaucoma and causes the loss of ~25% of RGCs within a month following the procedure. The right eye was always used as the experimental eye while the left eye was used as the control. Following the injection of NaCl, the eyes were treated with an antibacterial cream. The rats were then returned to their cages and monitored until they were fully awake. Once awake they were placed back in the animal facility.

**Agonist Administration**

For treatment with the neuroprotective nAChR agonist, the same procedure was followed with slight modifications. All rats had their right eyes treated with the PNU-282987 through eye drops. However, half of these animals had surgery on both eyes while the other half only had surgery on their right eyes. The neuroprotective agonist was added to the right eyes three days in advance of the procedure to induce glaucoma-like conditions for all the rats and eye drops of the nAChR agonist was continued for either three or seven days following the surgical procedure. The purpose of performing surgery on both eyes for some rats and only the right for others was to provide three experimental conditions: control untreated, glaucoma-induced, and glaucoma-induced with neuroprotection. This made it possible to gauge the overall effect of the neuroprotective agonist, PNU-282987, on the expression of the GDF15 gene.

**Removal of Retinas and Aqueous Humor**
The retinas and aqueous humor of all the rats’ eyes were removed to measure the expression of GDF15. They were euthanized by carbon dioxide asphyxiation. Aqueous humor was removed using a 31 gauge syringe needle. The needle was inserted laterally adjacent to the episcleral vein of the eye into the anterior chamber and pulled back to withdraw amounts of aqueous humor from the eye. It was then placed in a small centrifuge tube and put on ice until being sent out for ELISA testing at PharmOptima. The aqueous humor of both the control and treated eyes were removed so a comparison of the two levels could be made.

The retinas were also removed after the removal of aqueous humor. First, the eyeball had to be surgically removed from the animal. After removal, the eyes were dipped in a 70% alcohol solution and then placed in sterile phosphate buffered saline. While in the solution, the cornea and lens were carefully removed creating an eye cup. The retina that lines the back of the eye cup was gently scraped out and collected. Once the retina was removed it was weighed and placed in a small centrifuge tube. The retinas were labelled and placed on ice with the aqueous humor samples until they were delivered to PharmOptima for ELISA testing.

**Experiments**

The initial study was done with four rats. The four rats were euthanized at 1 day, 3 days, 7 days, and 14 days post-injection of hypertonic saline. After euthanasia, their retinas and aqueous humors were removed from control untreated and experimental eyes following the procedure stated above. An ELISA was run on both the aqueous humors and retina samples.

ELISA stands for enzyme-linked immunosorbent assay. ELISAs are used to determine how much of a certain protein is in a sample. This is determined using a spectrophotometer that picks up on the amount of antigen present in the sample through light intensity. The light
intensity is produced when an antibody in the ELISA binds to the desired antigen (Figure 4). The ELISAs used in this experiment contained the antibody against GDF15. With the antigen bound to the antibody, a substrate was added to produce a detectable signal for the spectrophotometer.

**ELISA**

![ELISA Diagram](image)

**Figure 4.**
The steps that occur within an ELISA. (Image taken from the website for the Society for Mucosal Immunology http://www.socmucimm.org/elisa-method/)

The aqueous humor was diluted with a RD5P buffer and used with the ELISA to measure GDF15 expression. The retina had to be homogenized with the RD5P buffer before being tested as well. A protease inhibitor also had to be added to prevent the protein from degradation in the process. The aqueous humor was best measured at a dilution factor of 1 to 4 or 12.5 μL AH with 37.5 μL buffer. For the first experiment the results were pooled, but in the following experiments, results were not as it was found beneficial to compare experimentally induced eyes to their specific internal control eyes.
The second and third experiments completed were very similar to the first. They followed the same procedure regarding the surgeries and testing, but the number of animals tested for each experimental condition was increased. However, the final experiment differed quite a bit from the earlier ones. This experiment took into consideration the effect the neuroprotective agonist, PNU-282987, had on the expression of GDF15. The experiment was done for two time points, 3 and 7 days with N’s of 6 for each point. For both time points, the rats had PNU-282987 drops added to their right eyes 3 days prior to surgery. After surgery, 6 rats had drops added for an additional 3 days while the other 6 had drops added for an additional 7 days. After 3 or 7 days the rats were euthanized, and their aqueous humor and retinas were removed following the same procedure as the previous experiments. They were tested for the expression of the GDF15 protein using an ELISA kit just like the prior experiments.

**Statistical Analysis**

The results of the ELISAs were analyzed using ANOVA with Tukey’s multiple comparison test. ANOVA, or the analysis of variance test is used to analyze differences among groups of means. Tukey’s multiple comparison test is often paired with ANOVA to compare those differences in means. The results are presented as a p-value. The data collected was considered significant if p < 0.05.

**Results**

**GDF15 Expression without Neuroprotection**

Injection of 50 μL of 2 M NaCl into the episcleral vein of the experimental eyes of the rats’ results in scar tissue formation in the trabecular meshwork (Morrison et al., 1997). The scar
tissue formation results in the increase of IOP (Morrison et al., 1997; Iwamoto et al., 2014).

After 1 day, 3 day, 7 day, and 14 day intervals following the glaucoma-inducing procedure, the rats’ aqueous humor and retinas were removed. The ELISA pooled results of these rats did not show a significant increase in the expression of GDF15, but the data did have a linear characteristic to it. This suggested that the protein was being expressed, but pooling the data was making it so this expression could not be seen. These results prompted the next experiment. Rather than pooling the data, all rat eyes were kept separate, so their individual experimental eyes could be tested against their internal control eyes (Table 1). The results of this study showed a large increase in expression of aqueous humor GDF15 levels at days 3 and 7.

Three days following the procedure to induce glaucoma-like conditions, the mean GDF15 value in the aqueous humor after injection was 373.6 pg/ml + 58.4. The control eye at 3 days had a value of 147.1 pg/ml + 17.28. At 7 days post injection the mean value of GDF15 in aqueous humor was 1,122 pg/ml + 338.8. This was significantly different than the control eye level at 7 days of 188 pg/ml + 42.08. This showed that 7 day post injections was the best time to measure GDF15 values (Figure 5). While days 3 and 7 showed the greatest difference in control and injected eyes, there were still slight increases at day 1 and day 14 as well. Although there were slight changes, they were not enough to be considered statistically significant.
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Table 1.
Results of an ELISA run showing the different dilution factors of the AH and retinas in the wells. Wells in rows 3 and 4 were AH and rows 5 and 6 were for the retinas. A-D were injected eyes.
while E-H were control eyes. N=4 for each type of sample. The R column represents values in or out of the range of the standard. All values that have an R are out of range and all that do not are in range. SD represents the standard deviation. CV represents the coefficient variation or the ratio of the standard deviation to the mean.

Figure 5.
This graph shows the difference in GDF15 expression in control eyes (orange/bottom line) and injected eyes (blue curve). The x-axis represents the days after injection while the y-axis is the mean value of GDF15 on those days. Clearly, 7 days post injection shows the peak level of expression of GDF15.

GDF15 Expression with Neuroprotection

To determine the effects of the neuroprotective PNU-282987 on the expression of GDF15, it was added as eye drops to glaucoma induced rats, before and after the procedure. It
had to be added prior to injection of the hypertonic solution so the neuroprotective cell survival pathway mechanism of the retinal ganglion cells could be initiated (Asomugha et al., 2010). Rat eyes were treated for 3 or 7 days post injection as well, with drops being administered once every day (1 mM PNU-282987, 20 µL drops). After the rats were euthanized, the retinas and aqueous humors were removed, and an ELISA was performed. In the presence of PNU-282987, there was no significant difference in the GDF15 levels in either the aqueous humor or the retina homogenates (Figures 6, 7, 8, 9) compared to internal control retinas. Previous studies outside of this thesis have shown that PNU-282987 is sufficient in blocking RGC death. This study seemed to correlate with those results. The results pertaining to the glaucoma-induced eyes only need to be investigated further as they could indicate some crossover event of the PNU-282987. In the end, it was concluded that GDF15 expression is increased in the glaucoma model used in this experiment and that expression was affected by the neuroprotective nAChR agonist, PNU-282987 by reversing the increase seen without the neuroprotection.

![Figure 6.](image)
Day 3 aqueous humor results. The figure shows the amount of GDF15 in pg/mL for each rat in each eye. The results were collected from the ELISA and because they were diluted the amounts had to be corrected by multiplying each amount by the dilution factor of 4. The results show that there is little difference between the amount of GDF15 in the different treated eyes. The overall amounts of GDF15 do not differ significantly from the control values in the earlier studies. The lines represent the range from the lowest level of GDF15 to the highest. Each symbol represents a different rats’ levels with a total of six different rats.

![Day 7 AH](image)

**Figure 7.**

The figure shows the aqueous humor results in the 7 day rats. The results shown were fixed just like in the 3 day results by multiplying the results from the ELISA by the dilution factor. Just like in the 3 day rats there is little difference in the amount of GDF15 in the eyes even though they did not have the same treatments. The figure shows that the amount of GDF15 is not significantly different in the eyes treated with the PNU-282987 and the eyes not treated. The lines represent the range from the lowest level of GDF15 to the highest. Each symbol represents a different rats’ levels with a total of six different rats.
Figure 8.

The figure shows the GDF 15 levels within the retina of the 3 day rats. All results were corrected by multiplying by the dilution factor of 5. The samples taken were diluted with a 1:5 ratio of the retina sample to the buffer used. The results, like the AH results, show no significant difference between the different eyes. The figure shows the results for all six rats used containing three that had surgery on both eyes and three that only had surgery on the right. All rats had their right eyes treated with PNU-282987 and none of the left eyes were. The lines represent the range from the lowest level of GDF15 to the highest. Each symbol represents a different rats’ levels with a total of six different rats.
Figure 9.
The figure above shows the GDF 15 levels in the day 7 rats retinas. Similarly, to the day 3 retinas and the AH results there was no significant difference in the levels. The results were corrected just like the results for the day 3 retinas by being multiplied by the dilution factor of 5. The lines represent the range from the lowest level of GDF15 to the highest. Each symbol represents a different rats’ levels with a total of six different rats.

Discussion

Previous experiments showed that the injection of a hypertonic solution in the episcleral vein increases IOP (Morrison et al., 1997; Iwamoto et al., 2014) as well as GDF15 expression. This increase of GDF15 protein expression is seen at a time significantly earlier than the loss of RGCs. This shows that GDF15 could potentially serve as an early biomarker for glaucoma. The procedure to induce glaucoma in adult Long Evans rats is an excellent model of glaucoma. The glaucoma-like conditions used in these studies increased IOP which in turn resulted in the death of retinal ganglion cells gradually. Although increased IOP is one of the main risk factors of glaucoma it is not the only one. It has also been shown that not all cases of glaucoma involving
increased IOP get better when the IOP is decreased. As a result, the model is not perfect for demonstrating all types of glaucoma, but it was still better than many models out there such as the optic nerve crush model used in previous studies (Ban et al., 2017). Very rarely are glaucoma cases a result of direct injury to the optic nerve like the optic nerve crush model. It is more likely than not that the glaucoma is a result of increased IOP putting pressure on RGCs resulting in their death.

The experiments above also showed that day 7 post injection showed peak GDF15 expression. The fact that the protein was expressed so early after injection suggests that it could possibly identify glaucoma at an earlier stage than relying on vision loss that accompanies loss of RGCs. At 7 days post injection, the eye has not significantly increased IOP yet (Iwamoto et al., 2014; Cooley-Themm et al., 2017) and significant RGC loss is not reported. The fact that there is a possible biomarker that can detect glaucoma before it kills off RGCs could save someone from going blind. The data collected in this lab showed a trend that the GFP15 increased until a certain time and then decreased. This increase makes sense, but the decrease seems a little out of place. It could be hypothesized that GDF15 is involved in early signaling pathways before RGC death is reported. It could act as a warning sign to later events that lead to RGC death. Levels of GDF15 decrease as other signaling molecules are activated that eventually lead to the death of RGCs. Identification of targets downstream from GDF15 that lead to loss of RGCs still need to be investigated. This is a hypothesis that would need future testing.
Future Studies

This thesis was just the start of possible research that can be done to better understand GDF15. The studies should be repeated to show that they are in fact replicable. The sample size should also be increased. Most of the data collected for this lab came from sample sizes less than 5. This is acceptable for initial findings but should definitely be expanded in future studies. Other neuroprotective treatments should be added to the study to see if one works better than another. From completing more studies similar to this one, the pathway of the expression of GDF15 could be mapped out. It could be determined what exactly causes the protein to be expressed and what could possibly repress the expression of the protein.

Not only should studies regarding GDF15 be continued, but studies regarding glaucoma as a neurodegenerative disease should be more prevalent. There is no cure for neurodegenerative diseases, and many people worldwide are affected by them. As stated earlier, glaucoma affects millions of people across the world, and that is just one neurodegenerative disease. Other common neurodegenerative diseases such as Parkinson’s, Alzheimer’s, and Huntington’s affect even more people. All neurodegenerative diseases are a result of loss of neuron function. Connections can be made between all these diseases that help better explain just how these diseases operate. Studies could be done to help find a possible cure or better treatment options.

Future Applications

One day it could be possible that testing for expression of GDF15 could be used as a risk factor for glaucoma. It could be used as a biomarker like temperature is for a fever. In a clinical setting, it is possible to remove a small amount of the aqueous humor from a human’s eye ball well in vivo. This could benefit those with genetic predispositions for the disease, if they have the
biomarker. The idea of implementing into a clinical setting is a far-off idea that would not happen for many years, but it is still a possibility. One should always hope for better applications when it comes to diagnosing diseases such as glaucoma, and this could be one of them.
Works Cited


