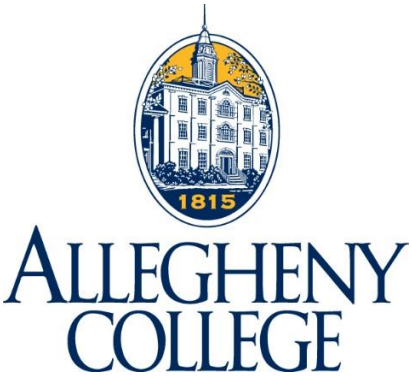
Comparative Effects of Propentofylline and Microglia Inhibiting Gliosis Potentially Limiting Neuronal Damage

Benjamin A. Capella



Submitted to the Neuroscience Department, Allegheny College

In partial fulfillment of the requirements for the degree of

BACHELOR OF SCIENCE

April 3, 2017

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Acknowledgements

Dr. Cross & Dr. Hollerman: Thank you for guiding me through the Comp process and putting up with all my questions.

Mom & Dad: Thank you for giving me the opportunity to attend Allegheny College and for standing by me through all the ups and downs.

Dylan Ukasik, Joe Desanti, and Zach Metkler: Thank you for being there in the computer lab as we struggled together through the Comp process.

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Abstract

Gliosis is a mechanism used by the central nervous system that responds to neuronal damage. Its primary purpose is to limit potential or further damage by targeting the affected area, clearing out necrotic tissue, and facilitating neurological processes that promote regrowth. However, there is a state of secondary neuroinflammtion that leads to further tissue damage associated with the activation of astrocytes and microglia. Both minocycline and propentofylline display neuroprotective as well as anti-inflammatory effects by targeting astrocyte and microglia activity. The present study was designed to look at the effects of minocycline and propentofylline administration following lesion induction on neuronal tissue damage of the motor cortex. It was hypothesized that the group of rats treated with a combination of minocycline and propentofylline would have the least amount structural as well as neurobehavioral deficits. The combination group would display smaller lesion size, increased time spent on vertical hang test, greater variation in which forepaw falls first, and higher frequency of beam test completion compared to rats treated with only minocycline and propentofylline. Lesion surface area was measured to assess severity of lesion damage. Two neurobehavioral assessments were implemented to identify any deficits related to motor cortex function. Statistical analysis revealed no significant differences between drug treatment groups and structural and functional test results. There was a slight decrease in lesion severity for the treatment group administered with a combination of propentofylline and minocycline suggesting a need for further research and refinement of methods used as part of the study.

Introduction

Brain injury causes a wide range of neurodegenerative processes which can cause long-term neurological dysfunction depending on severity of the initial damage (Faden & Loane,

2014). Although the initial neuronal cell death that takes place following brain trauma, may be unavoidable, there is a secondary wave of cellular processes and cascades that occur which may be preventable following clinical or medical intervention. Neuronal degeneration in experimental animal models is evident minutes after external physical trauma leading to unavoidable damage. However, there are observations made of time-dependent increases in the volume of cortical lesions and the presence of degenerating neurons that have led to the idea that delayed neurodegeneration may contribute significantly to the post-traumatic pathology (Raghupathi, 2004). This delayed, potentially avoidable pathology results from a secondary wave of neuro-inflammation carried out by the immune cells of the central nervous system. Processes leading to this inflammation include astrocyte activation, infiltration of peripheral monocytes, as well as activation of resident microglia (Faden & Loane, 2014). This secondary cascade of biochemical changes occurs within minutes of traumatic insult and can last for days, months, or years (Lozano et al., 2015).

Brain injury can occur in various ways. One class of brain injury can be characterized as traumatic brain injury which includes concussions, contusions, or penetrations of external physical objects. There can also be acquired brain injuries such as stroke, toxin exposure, degenerative diseases, etc. (Bach & David, 2006). These causes contribute to high morbidity and mortality in many individuals, specifically ages 15-24 and those above the age of 70 (MacKenzie et al., 2002). The high incidence of brain injury in individuals above the age of 70 may be caused by neurodegenerative diseases exhibiting an increased number of chronically activated microglia leading to greater toxicity following traumatic brain injury (Biber, Owens, & Boddeke, 2014). Although brain injury may differ in its cause, each form shares a similar inflammatory mechanism that follows the initial destruction of brain cells. Traumatic brain injury leads to

mechanical damage of brain tissue disrupting cell membranes, organelles, and blood vessels causing cell death, ischemia, and hemorrhage (Cederberg & Siesjo, 2009).The immediate affected cell region undergoes rapid depolarization which releases glutamate causing a calcium influx through NMDA receptors. This wave of depolarization travels to surrounding tissue, negatively affecting those cells. This mechanism leads to excitotoxic cell death characterized in neurodegenerative pathologies including ischemic stroke-related necrosis (Vedantam & Moller, 2015). Without a proper balance of nutrients, the affected brain region undergoes necrosis, which leads to loss of function, disabilities, or even death in severe cases.

Central Nervous System (CNS) resident inflammatory cells, including microglia and astrocytes, react to the dysfunction of tissue homeostasis and facilitate secondary tissue damage (Cederberg & Siesjo, 2009). Activated microglia undergo proliferation and morphological alterations limiting the damage to neuronal tissue, as the brain's primary response to trauma-related inflammation. Upon activation, ramified microglia retract their fine processes and morphologically change to a phenotype similar to that of phagocytic macrophages (Yenari et al., 2005). This switch to activation is also sensitive to even minor pathological changes in the CNS. They phagocytose the dead proteins of neurons and produce proinflammatory cytokines and toxic molecules that compromise neuronal survival (Zhang et al., 2010). For example, activated microglia secrete high levels of cytokines, such as tumor necrosis factor α (TNFα), a proinflammatory cytokine involved in demyelinating diseases. The release of these cytokines by microglia dysregulate astrocytes and neurons at the tripartite synapse, but more research is required to further elucidate what to what degree and how they affect neuronal and glial cells (Barres, 2008). In experiments using animal models of stroke, researchers discovered that trauma-related inflammation induces microglia to shift from a transient M2 phenotype to M1-

like phagocytes that express cytotoxic effects on neurons (Prinz & Priller, 2014). Microglia become primed for activation as age increases in an individual. Models examining neurodegeneration show that the aged brain is in a chronic state of increased inflammation, due to a primed activated state microglia are in (Norden & Godbout, 2013).

Activated microglia facilitate astrocyte function, which leads to the subsequent activation of astrocytes through microglia's secretion of cytokines. However, astrocytes release cytokines as well that act on microglia creating a continuous positive feedback loop regulating the activation of both glial cell groups (Zhang et al., 2010). Astrocytes outnumber neurons in the brain and have the capability of redistributing potentially detrimental ions and metabolites over long distances. Also, in cases of extreme excitatory crisis caused by the release of glutamate following traumatic CNS injury, astrocytes' protective function of eliminating glutamate can become reversed and add more free radicals into the environment contributing to further neuronal damage (Pekny & Nilsson, 2005). Many astrocytes at the lesion site become hypertrophic and release extracellular matrix molecules known as chondroitin sulphate proteoglycans that stimulate the formation of dystrophic growth cones on lesioned sensory axons inhibiting the regrowth of new axons (Yiu & He, 2006). Mechanisms such as these led to the idea that reactive astrocytes may cause further damage to neuronal tissue following neuronal damage from trauma.

Loss of neural tissue after brain trauma, as a result of the initial lesion or subsequent neuroinflammtion, leads to cognitive and functional deficits depending on the location of trauma (Schaefer et al., 2007; Scott et al., 2001). Experimental models pertaining to different pathologies of neural tissue damage, including but not limited to stroke, Parkinson's disease, and traumatic brain injury, display these functional deficits (Beal, 2003; Bonita & Beaglehole, 1988;

York Haaland & Delaney, 1981). Understanding the processes surrounding these pathologies of neural tissue damage has become a focal point of CNS inflammatory research.

Administering minocycline, a tetracycline antibiotic that also displays anti-inflammatory as well as neuroprotective effects, reduces infarct size and prevents tissue loss in the damaged brain (Yang et al., 2015). Minocycline affects specific mechanisms associated with the anti-inflammatory response such as inhibiting the inducible form of nitric oxide synthase, microglia activation, and reducing glutamate toxicity (Fagan et al., 2011). It is different from other members of the tetracycline family in the sense that it is lipophilic and an efficient penetrator of the blood-brain barrier (Vedantam & Moller, 2015). Recent research has also showed that early minocycline treatment following stroke facilitated neurovascular remodeling post-stroke and promoted M2-phenotypye microglia activation four weeks after stroke (Yang et al., 2015). Another study revealed that astrogliosis, represented by low levels of GFAP immunoreactivity induced by hypoxia-ischemia, decreased after administering minocycline (Zhang et al., 2010). Minocycline has also been shown to reduce the loss of hippocampal neurons due to cerebral ischemia, by decreasing microglia expression of Interleukin1β-converting enzyme, which creates a powerful cytokine, while also lowering glutamate toxicity in cell cultures (Yrjänheikki et al., 1999).

Propentofylline is a xanthine derivative that has demonstrated anti-inflammatory, anti-proliferative, neuroprotective, glial modulatory effects, as well as inhibiting glutamate. Microglia proliferation has also previously shown to be dose dependently inhibited by propentofylline (Sweitzer & Leo, 2011). Propentofylline has also shown, by measuring GFAP expression seven to twenty-one days following neuronal injury, to reduce and even eliminate glial scar development following injury (Bondan & Dossa, 2014). Minocycline and Propentofylline have

been shown to help prevent neuronal cell death in an ischemic model of stroke. This experiment focused on whether these benefits are specific to stroke related necrosis or if they can be generalizable to a broader range of causes for neuronal cell death. It was hypothesized that rats administered a combination of propentofylline and minocycline would display smaller lesion size as well as lead to the least severe motor deficits compared to rats only treated with propentofylline or minocycline because they act upon two mechanisms of glial inhibition caused by both drugs instead of one.

Materials & Methods

This study used fifteen rats issued by the neuroscience department at Allegheny College. The procedures for this study were reviewed and approved by Allegheny College's Animal Research Committee. An animal model for brain lesion was implemented because animals and humans have similar neural and vascular substrates while also displaying similar molecular mechanisms for brain injury. In vivo studies were preferable to in vitro ones because the brain trauma pathophysiology is dependent on the dynamic mechanisms and environmental conditions within the brain that are nearly impossible to simulate in cell cultures (Wang-Fischer, 2009). Accounting for gender differences among rodent subjects was important to study because hormonal and chemical differences among sex's leads to variations in infarct size and volume because of estrogen's neuroprotective effects (Lisabeth & Bushnell, 2012). Therefore, the fifteen subjects were all male rats in order to assess the effects of these drugs, minocycline and propentofylline, have on one specified gender.

A permanent localized lesion of the motor cortex was induced using a direct current (DC) or electrolytic lesion using a modified micro-syringe electrode also capable of administering minocycline and propentofylline. The needle of the syringe was insulated 3mm up from the

bevel to properly direct the current into the specified region of the motor cortex. This allowed the glial inhibitors to be administered directly to the lesion site immediately after its formation, allowing minimal time to pass between lesion induction and drug treatment. In order to induce a permanent lesion, the brain bregma was exposed following anesthesia with an intraperitoneal (ip) injection of a mixture of 70mg ketamine and 6mg xylazine at a dose of 0.1cc/100 grams of body weight. In order to reach the site of the motor cortex, 2.5 mm anterior and 3.0 mm lateral were measured from the bregma. A 1mm diameter hole was drilled through the skull of the rat exposing the brain at the site of the motor cortex responsible for forelimb movement. The micro-syringe was then lowered to the site of the motor cortex responsible for forelimb movement and a 5 mA anodal direct current was administered to the site for 10 seconds. The Ugo Basile Lesion Maker was reset after subject seven in order to ensure that the charge remained at a constant 5 mA for each subject. Directly following the creation of the lesion, the glial inhibitors were administered to the lesion site via micro-syringe. The syringe was then removed and the incision sutured.

Drugs were administered once via a single 5µl intra-lesion intracerebral injection of minocycline at 2mg/µl and propentofylline 0.5mg/µl respectively following induction of the electrolytic lesion. Within the fifteen rats, five were given a dose of minocycline, another five were given propentofylline, and the last five were given a combination of minocycline and propentofylline at amounts equal to the totals of the previous groups.

Experimental trials of lesion related neuronal damage also looked at functional behavioral deficits following motor cortex lesions in order to gain a more complete understanding of the mechanisms affected by motor cortex cell necrosis (Jones et al., 1999). Neuro-behavioral deficits were assessed at two separate times using both quantitative and

qualitative observations following lesion induction. The first assessments occurred 5 days following a lesion to the motor cortex. The first neurobehavioral test was a vertical hang test where the rat was suspended on a metal bar by its forepaws. The time it took for the first forepaw, right or left, to fall off the bar was recorded. The second test was a traction beam test. The rat was placed on a wooden beam at one end and then observed. Notes were recorded pertaining to how the rat walked and oriented itself on the beam. Those notes were converted to scores representing the degree of success each rat had on the traction beam test using a scale created for this study (Table 1). The second session of neurobehavioral assessments took place 10 days following lesion induction in order to observe any changes from the first session. The rats were euthanized on the eleventh day following surgery with a single dose of Euthasol at 0.1 cc/100g.

Level of Success	Behavioral Characteristics
0	Immediate falling off of beam or turning around
1	Half of bar covered before falling or turning to right
2	Half of bar covered before falling or turning left
3	Complete coverage of bar with minor falters right
4	Complete coverage of bar with minor falters left
5	Complete coverage of bar with no falters

Table 1. Scale created to score similar rat performances on traction beam test.

After respiratory arrest, the rats were then perfused with 10% formolsaline and the brains extracted. The brains were sectioned and prepared for staining in order to observe anatomical differences in cell body populations at and surrounding the lesion site. All brains were sectioned at 50µm via a cryostat and slices were mounted on subbed slides. A cresyl violet stain was used to assess damage to the motor cortex and surrounding region. Lesion surface area was measured using the area line tool on the Leica Application Suite microscope imaging software. Each lesion was outlined and surface area was measured and recorded for seven brain slices as depicted in

Appendix A. The average surface area for each individual was then calculated using the surface area gathered from seven brain slices. Cresyl violet stains the nissl substance in all neurons and the DNA of all nuclei including glia (Pearce et al., 2016).

Results

There were no significant differences between group means as determined by one-way ANOVA ($F(2,12) = 0.88$, $p = 0.44$) for lesion size (Appendix A). This means that propentofylline, minocycline, and a combination of these two drugs led to no significant differences of infarct size between groups.

However, there was a trend for a slight decrease of average lesion surface area in the combination drug treatment group compared to minocycline and propentofylline treatment groups (Figure 1).

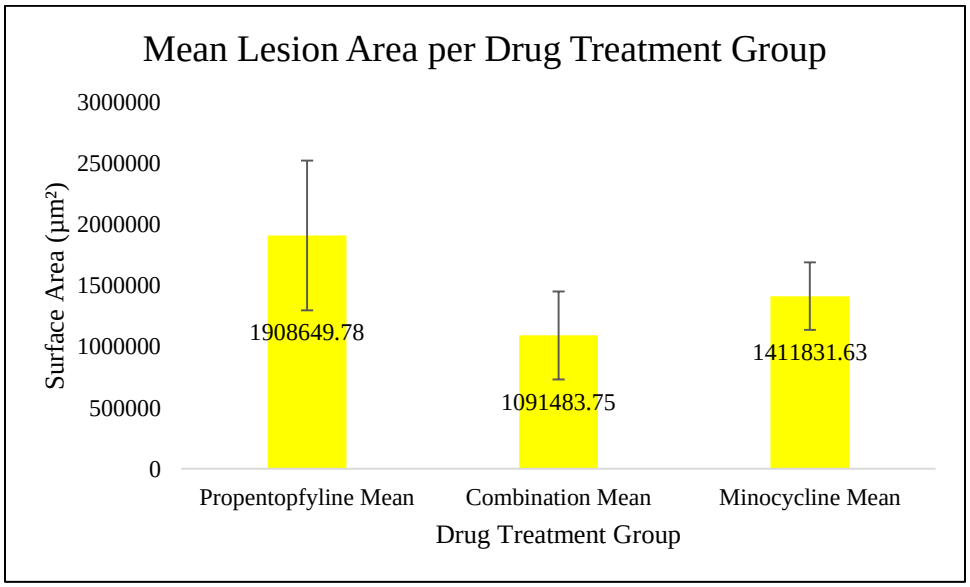

Figure 1. Mean lesion surface area recorded between drug treatment groups. Each vertical yellow bar represents the average lesion surface area of rats treated with propentofylline (n=5), a combination of propentofylline and minocycline (n=5), and with minocycline (n=5). Averages are displayed near the top of each part in micrometers. Error bars represent the standard error of the mean for each group.

There was no significance between group means for time spent hanging from the bar or which forepaw dropped from the bar first. Using a repeated measures design, statistics relating to time spent hanging from the bar showed no significance ($F(2,12) = 0.14$, $p = 0.87$) (Appendix C). Statistics relating to which forepaw fell from the bar first, displayed no significance after running a repeated measures design ($F(2,12) = 0.19$, $p = 0.83$) (Appendix C). The group administered a combination of propentofylline and minocycline reported increased time spent hanging from the bar compared to groups administered propentofylline or minocycline (Figure 2).

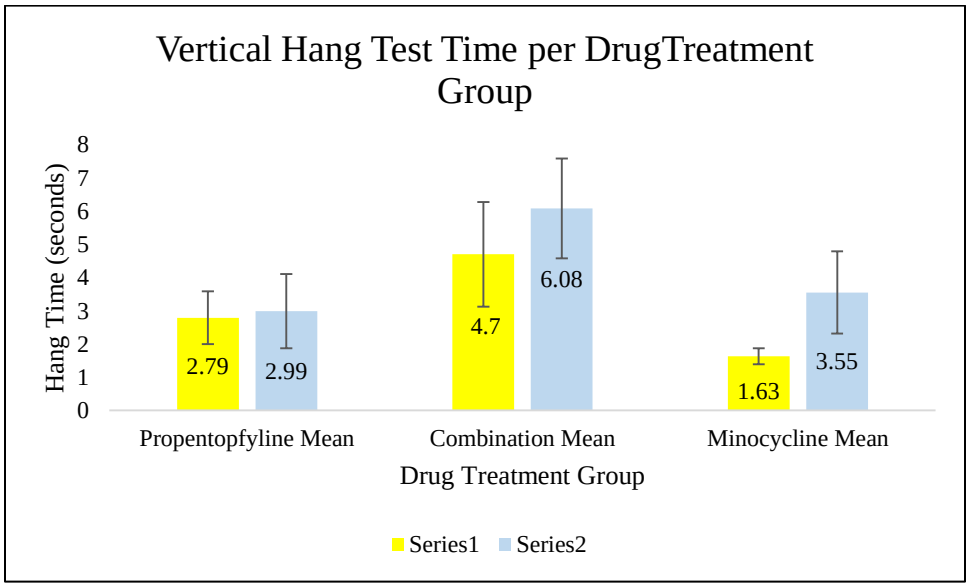

Figure 2. Mean time spent hanging from vertical bar recorded between drug treatment groups. Each yellow vertical bar represents the mean time spent hanging from the bar during the test that took place five days post-surgery. Blue bars represent the average time spent hanging from bars 10 days post-surgery. Error bars represent standard error of the mean. Each pairing of blue and orange bars represent rats treated with propentofylline (n=5), a combination of propentofylline and minocycline (n=5), and with minocycline (n=5). Averages are displayed near the top of each part in seconds.

There was no significance reported between group means for success scores on traction beam test and drug treatment group. A repeated measures design was implemented and displayed

no significance ($F(2,12) = 0.59$, $p = 0.57$) (Appendix C). Out of fifteen rats, there were only two rats within the propentofylline group, subjects four and five that completely made it across the beam. They achieved this 5 days following surgery as well as 10 days (Appendix D).

Discussion

It has been previously researched that targeting and suppressing the activated state of glial cells limits their ability to facilitate further structural damage leading to fewer functional deficits (Fitch & Silver, 2008; York Haaland, 1981). Glial cells such as astrocytes and microglia, carryout specific mechanisms contributing to secondary tissue damage following the initial loss of neuronal tissue (Cederberg & Siesjo, 2009). Targeting the secondary stage of tissue loss characterized in neurodegenerative pathologies, represents a therapeutic opportunity to minimize the potential overall damage. However, finding a consistently effective agent that limits neuronal damage remains a problem.

The current study resulted in no statistically significant outcomes. These results were supported by previous studies discussing inconsistencies of propentofylline and minocycline's effectiveness on limiting neural tissue loss. One study looked at the level of basal fibroblast growth factor (bFGF), as a measure of decreased tissue loss as well as greater capability of recovery, after repeated propentofylline administration over a seven day period. They found that propentofylline intervention only led to a significant increase in bFGF expression for the peri-lesional cortex but not in the ipsilateral dorsal striatum compared to controls (Buytaert-Hoefen et al., 2002). Minocycline has been tested in several pathological neurodegenerative models and has shown inconsistent results addressing benefits and disadvantages. One study displayed minocycline's ability to decrease microglia activity, but exacerbated effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin that attacks nigrostriatal dopaminergic

neurons, increasing overall cell loss (Yang et al, 2003). Another study addressed the inability of minocycline to inhibit TNF-α and receptor induction. This caused the transient activation of microglia despite minocycline inhibiting the activation of microglia in the first place (Sriram et al., 2006).

Looking at research addressing minocycline and propentofylline, it is more than likely that mechanisms outside of glial inhibition influence tissue damage following trauma or inflammation. On the other hand, how these two drugs target glial inhibition may be a reason why the combination group in this study resulted in a slight decrease of lesion surface area and a slight increase of hang time on the vertical hang test. Both minocycline and propentofylline have demonstrated to inhibit microglial activation in turn suppressing proliferation. However, minocycline has also neuroprotective effects such as acting as a reactive oxygen species scavenger and inhibitor of ischemic induced astrogliosis (Vedantam & Moller, 2015; Zhang et al., 2010). Propentofylline has shown to affect glial scar development, enhance astrocyte mediated glutamate clearance, and decrease the reactive phenotype of astrocytes (Sweitzer & Leo, 2011). These drugs have shown differing dose dependent effects as well. Minocycline exacerbated ischemic injury and became toxic at high doses to oxygen glucose deprived cell cultures and propentofylline did not effectively reduce glutamate activity and microglia proliferation at small doses (Matsukawa et al., 2009; Sweitzer & Leo, 2011).

The current study had a few limitations that hindered the validity of the findings as well as influenced the results. The first limitation, absence of a control group, decreased the degree of internal validity the experiment was capable of achieving. By not having a control group, if there was a significant difference of lesion size and functional impairment of drug administration compared to no drug administration, it was not seen. Therefore, this study led to no significant

differences between groups' effectively limiting lesion size and functional deficits. Another limitation came from only assessing lesion size by measuring the surface area of lesions. Studies also looked at lesion volume and density with the use magnetic resonance imaging to gather a more complete evaluation of lesion size (Baird et al., 1997). The last limitation for this study was not recording a baseline measurement for individuals before lesion induction. There may have been functional deficits between rats prior to lesion induction that may have affected scores following surgery.

Considering the lack of significant findings from this study and the body of literature addressing similar inconsistencies that minocycline and propentofylline have on effectively limiting neuronal tissue damage and associated functional impairments, there are still directions to go for future research. Addressing the limitations of this study is a place to start for future research by implementing a control group, using other measurements to determine lesion size, and recording baseline scores for neurobehavioral tests. Assessing differences between genders would also lead to differences in results because the hormone estrogen has already demonstrated neuroprotective effects in the brain, supporting the idea as to why women are at a lower risk of having a stroke during the premenopausal phase of development (Lisabeth & Bushnell, 2012). Future research could also look at the effects of propentofylline and minocycline have on rats during different stages of development. Research points out that microglia become primed for activation and can become chronically activated in later stages of development. This causes an increase in the amount of pro-inflammatory cytokines in the neural environment leading to greater neuronal damage following an insult (Norden & Godbout, 2013).

Conclusion

The purpose of this study was to examine the effects of propentofylline and minocycline on limiting lesion size and neurobehavioral deficits. Despite no statistically significant results, there was still potential shown for these drugs to be administered in a combined dose, that caused smaller lesion size and a slightly longer hang time than did the other drug treatment groups. This study restated the need for further research to look at the possible benefits these drugs have on limiting secondary neuronal damage as a result of the CNS inflammatory response.

References

Abbott, N. J. (2013). Blood–brain barrier structure and function and the challenges for CNS drug delivery. *Journal of Inherited Metabolic Disease, 36*(3), 437-449.

Bach, L. J., & David, A. S. (2006). Self-awareness after acquired and traumatic brain injury. *Neuropsychological Rehabilitation, 16*(4), 397-414.

Barres, B. A. (2008). The mystery and magic of glia: A perspective on their roles in health and disease. *Neuron, 60*(3), 430-440.

Baird, A. E., Benfield, A., Schlaug, G., Siewert, B., Lövblad, K. O., Edelman, R. R., & Warach, S. (1997). Enlargement of human cerebral ischemic lesion volumes measured by diffusion-weighted magnetic resonance imaging. *Annals of Neurology, 41*(5), 581.

Beal, M. F. (2003). Mitochondria, oxidative damage, and inflammation in parkinson's disease. *Annals of the New York Academy of Sciences, 991*(1), 120-131.

Biber, K., Owens, T., & Boddeke, E. (2014). What is microglia neurotoxicity (not)? *Glia, 62*(6), 841-854.

Bondan, E. F., & Dossa, P. D. (2014). Propentofylline decreases GFAP immunoreactivity in astrocytes after gliotoxic injury in the rat brainstem. *Journal of Neuroimmunology, 275*(1), 139.

Bonita, R., & Beaglehole. (1988). Recovery of motor function after stroke *Stroke, 19*(12), 1497-1500.

Buytaert-Hoefen, K. A., Kreber, L. A., Millar, C. J., Walsh, U. T., Brannigan, C., & Hernandez, T. D. (2002). Propentofylline after focal cortical lesion in the rat: Impact on functional

recovery and basic fibroblast growth factor expression. *Neuroscience Letters, 331*(3), 188-192.

Bye, N., Habgood, M. D., Callaway, J. K., Malakooti, N., Potter, A., Kossmann, T., & Morganti-Kossmann, M. C. (2007). Transient neuroprotection by minocycline following traumatic bra in injury is associated with attenuated microglial activation but no changes in cell apoptosis or neutrophil infiltration. *Experimental Neurology, 204*(1), 220-233.

Cederberg, D., & Siesjö, P. (2010). What has inflammation to do with traumatic brain injury? *Child's Nervous System, 26*(2), 221.

Faden, A. I., & Loane, D. J. (2015). Chronic neurodegeneration after traumatic brain injury: Alzheimer disease, chronic traumatic encephalopathy, or persistent neuroinflammation? *Neurotherapeutics, 12*(1), 143-150.

Fagan, S. C., Cronic, L. E., & Hess, D. C. (2011). Minocycline development for acute ischemic stroke. *Translational Stroke Research, 2*(2), 202-208.

Fitch, M. T., & Silver, J. (2008). CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. *Experimental Neurology, 209*(2), 294-301.

Jones, T. A., Chu, C. J., Grande, L. A., & Gregory, A. D. (1999). Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats. *Journal of Neuroscience, 19*(22), 10153-10163.

Lisabeth, L., & Bushnell, C. (2012). Menopause and stroke: An epidemiologic review. *Lancet Neurology, 11*(1), 82-91.

Lozano, D., Gonzales-Portillo, G. S., Acosta, S., de la Pena, I., Tajiri, N., Kaneko, Y., & Borlongan, C. V. (2015). Neuroinflammatory responses to traumatic brain injury: Etiology, clinical consequences, and therapeutic opportunities. *Neuropsychiatric Disease and Treatment, 11*, 97-106.

MacKenzie, J. D., Siddiqi, F., Babb, J. S., Bagley, L. J., Mannon, L. J., Sinson, G. P., & Grossman, R. I. (2002). Brain atrophy in mild or moderate traumatic brain injury: A longitudinal quantitative analysis. *American Journal of Neuroradiology, 23*(9), 1509-1515.

Matsukawa, N., Yasuhara, T., Hara, K., Xu, L., Maki, M., Yu, G., . . . Borlongan, C. V. (2009). Therapeutic targets and limits of minocycline neuroprotection in experimental ischemic stroke. *BMC Neuroscience, 10*(1), 126.

Norden, D. M., & Godbout, J. P. (2013). Review: Microglia of the aged brain: Primed to be activated and resistant to regulation. *Neuropathology and Applied Neurobiology, 39*(1), 19-34.

Pearce, P. S., Wu, Y., Rapuano, A., Kelly, K. M., de Lanerolle, N., & Pan, J. W. (2016). Metabolic injury in a variable rat model of post–status epilepticus. *Epilepsia, 57*(12), 1978-1986.

Pekny, M., & Nilsson, M. (2005). Astrocyte activation and reactive gliosis. *Glia, 50*(4), 427-434.

Prinz, M., & Priller, J. (2014). Microglia and brain macrophages in the molecular age: From origin to neuropsychiatric disease. *Nature Reviews. Neuroscience, 15*(5), 300-312.

Scott, R. B., Stoodley, C. J., Anslow, P., Paul, C., Stein, J. F., Sugden, E. M., & Mitchell, C. D. (2001). Lateralized cognitive deficits in children following cerebellar lesions. *Developmental Medicine and Child Neurology, 43*(10), 685-691.

Schaefer, S. Y., Haaland, K. Y., & Sainburg, R. L. (2007). Ipsilesional motor deficits following stroke reflect hemispheric specializations for movement control. *Brain, 130*(8), 2146-2158.

Sriram, K., Miller, D. B., & O'Callaghan, J. P. (2006). Minocycline attenuates microglial activati on but fails to mitigate striatal dopaminergic neurotoxicity: Role of tumor necrosis factor-α. *Journal of Neurochemistry, 96*(3), 706-718.

Sweitzer, S., & De Leo, J. (2011). Propentofylline: Glial modulation, neuroprotection, and alleviation of chronic pain. *Methylxanthines* (pp. 235-250). Berlin, Heidelberg: Springer Berlin Heidelberg.

Vedantam, S., & Moller, A. R. (2015). Minocycline: A novel stroke therapy. *Journal of Neurology & Stroke, 2*(6), 1-10.

Wang-Fischer, Y. (2009). *Manual of Stroke Models in Rats*. Boca Raton: CRC Press

Yang, Y., Salayandia, V. M., Thompson, J. F., Yang, L. Y., Estrada, E. Y., & Yang, Y. (2015). Attenuation of acute stroke injury in rat brain by minocycline promotes blood-brain barrier remodeling and alternative microglia/macrophage activation during recovery. *Journal of Neuroinflammation, 12*(1), 245.

Yang, L., Sugama, S., Chirichigno, J. W., Gregorio, J., Lorenzl, S., Shin, D. H., . . . Albers, D. S. (2003). Minocycline enhances MPTP toxicity to dopaminergic neurons. *Journal of Neurosci ence Research, 74*(2), 278-285.

Yenari, M. A., Xu, L., Tang, X. N., Qiao, Y., & Giffard, R. G. (2006). Microglia potentiate damage to Blood–Brain barrier constituents: improvement by minocycline in vivo and in vitro. *Stroke, 37*(4), 1087-1093.

Yiu, G., & He, Z. (2006). Glial inhibition of CNS axon regeneration. *Nature Reviews Neuroscience, 7*(8), 617-627.

York Haaland, K., & Delaney, H. D. (1981). Motor deficits after left or right hemisphere damage due to stroke or tumor. *Neuropsychologia, 19*(1), 17-27.

Yrjänheikki, J., Tikka, T., Keinänen, R., Goldsteins, G., Chan, P. H., & Koistinaho, J. (1999). A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proceedings of the National Academy of Sciences*, *96*(23), 13496-13500.

Zhang, D., Hu, X., Qian, L., O'Callaghan, J. P., & Hong, J. (2010). Astrogliosis in CNS pathologies: Is there a role for microglia? *Molecular Neurobiology, 41*(2-3), 232-241.

6.1 Appendix A

ANOVA Test Results

Table 2. One-way ANOVA table displaying significance value between groups. Statistics reported no significant association between drug treatment group and lesion size.

	Sum of Squares	*df*	Mean Square	*F*	*p*
Between Groups	1.70E12	2.00	8.48E11	.88	.44
Within Groups	1.16E13	12.00	9.70E11		
Total	1.33E13	14.00			

6.2 Appendix B

Lesion surface area images

Figure 3. Using the area line tool on the Leica Application Suite microscope imaging software, lesions were outlined for each rat. The top row depicts the lesions for propentofylline treated rats

Table 4. Repeated Measures test displaying within-subject effects between treatment groups. Significance was calculated and was determined that there was no significance between drug treatment groups and which paw fell from the bar first.

Source		Type III Sum of Squares	*df*	Mean Square	*F*	*p*
Test	Sphericity Assumed	0.13	1.00	0.13	0.57	0.46
	Greenhouse-Geisser	0.13	1.00	0.13	0.57	0.46
	Huynh-Feldt	0.13	1.00	0.13	0.57	0.46
	Lower-bound	0.13	1.00	0.13	0.57	0.46
Test * Condition	Sphericity Assumed	0.07	2.00	0.03	0.14	0.87
	Greenhouse-Geisser	0.07	2.00	0.03	0.14	0.87
	Huynh-Feldt	0.07	2.00	0.03	0.14	0.87
	Lower-bound	0.07	2.00	0.03	0.14	0.87
Error (Test)	Sphericity Assumed	2.80	12.00	0.23		
	Greenhouse-Geisser	2.80	12.00	0.23		
	Huynh-Feldt	2.80	12.00	0.23		
	Lower-bound	2.80	12.00	0.23		

Table 5. Repeated measures design displaying within-subject effects between treatment groups. Significance scores reveled that there was no significance between drug treatment groups and observed success on traction beam test.

Source		Type III Sum of Squares	*df*	Mean Square	*F*	*p*
Test	Sphericity Assumed	0.83	1.00	0.83	1.14	0.31
	Greenhouse-Geisser	0.83	1.00	0.83	1.14	0.31
	Huynh-Feldt	0.83	1.00	0.83	1.14	0.31
	Lower-bound	0.83	1.00	0.83	1.14	0.31
Test * Condition	Sphericity Assumed	0.87	2.00	0.43	0.59	0.57
	Greenhouse-Geisser	0.87	2.00	0.43	0.59	0.57
	Huynh-Feldt	0.87	2.00	0.43	0.59	0.57
	Lower-bound	0.87	2.00	0.43	0.59	0.57
Error (Test)	Sphericity Assumed	8.80	12.00	0.73		
	Greenhouse-Geisser	8.80	12.00	0.73		
	Huynh-Feldt	8.80	12.00	0.73		
	Lower-bound	8.80	12.00	0.73		

6.4 Appendix D

Recorded Traction Beam Test scores

Table 6. Recorded scores assessing degree of success on traction beam test. Scores were recorded using scale mentioned on Table 1 in methods section.

Drug Treatment Group		Time Spent Since Surgery	
		5 days	10 days
Propentofylline	PPF1	0	0
	PPF2	2	1
	PPF3	4	5
	PPF4	4	5
	PPF5	0	0
Combination Group	PPF/Min1	0	0
	PPF/Min2	0	2
	PPF/Min3	0	0
	PPF/Min4	1	1
	PPF/Min5	3	1
Minocycline	Min1	0	2
	Min2	0	2

	Min3	1	1
	Min4	1	0
	Min5	0	1

6.5 Appendix E

Neuroimaging examples

Figure 4. Activated astrocyte surrounding the lesion site in a rat treated with propentofylline. The activated astrocyte was carrying out part of the central nervous system's inflammatory response.

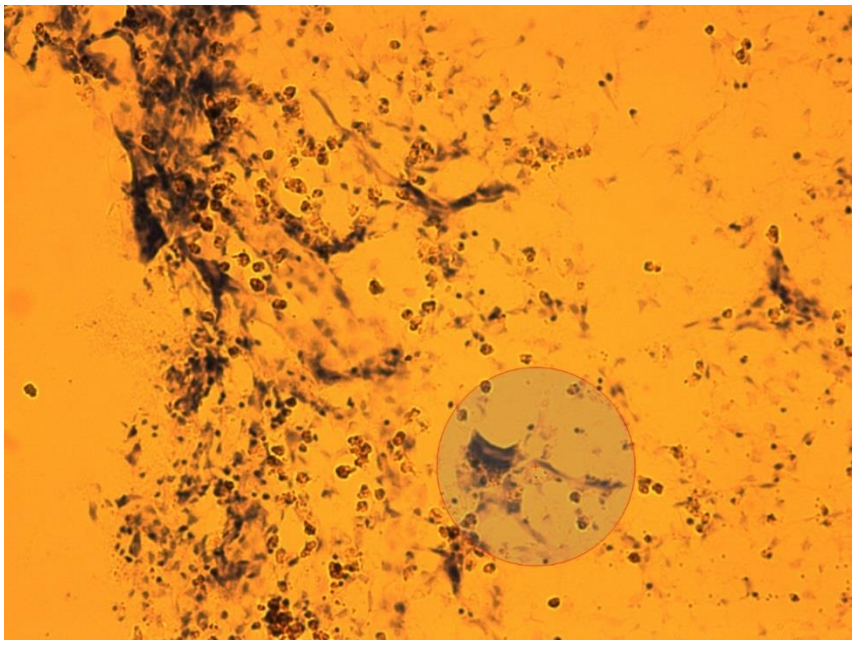

Figure 5. Image of the needle track left after the syringe passed through the neural tissue of a minocycline treated rat Necrotic tissue surrounding needle track was measured as part of lesion size.

