Developing an Astrocyte Signaling Model to Inform and Improve Stroke Treatment
(Technical Paper)

The Effects of Race and Socioeconomic Status on the Incidence of Stroke
(STS Paper)

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Rebecca Della Croce
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Technical Project Team Members
Annie Ford
Zoe Garman

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Introduction

In 2017, stroke was the fifth leading cause of death in the United States, and in 2015, stroke-related costs in the United States exceeded 46 billion dollars (Murphy et al., 2018; *Stroke Facts | Cdc.Gov*, 2020). In addition to mortality, strokes result in disabilities in most survivors, specifically in the form of neurological deficits (Donnan et al., 2008; Lo et al., 2003). Ischemic stroke is caused by an obstruction of blood flow in the brain, limiting the availability of oxygen and nutrients in the surrounding area (Lo et al., 2003). Currently, the available treatments are limited in both variety and efficacy, with many of them still resulting in significant neurological deficits (Hameed et al., 2017; Hinkle & Guanci, 2007). Additionally, while there are models of specific signaling pathways in stroke, there do not currently exist any models that detail the various connection points between these pathways (Diekman et al., 2013; Dronne et al., 2004). By applying the same principles used to treat ischemia in heart tissue, as well as broadening the understanding of the signaling pathways involved in stroke, new therapeutics can be developed that will reduce the morbidity and mortality of stroke (Masuzawa et al., 2013).

Beyond being an acute medical issue, stroke also appears to be a social issue. There are several notable patterns in the occurrence and outcomes of stroke. Specifically, Black Americans are twice as likely to have a stroke compared to white Americans; there is also a high incidence of stroke among low-income groups (Addo Juliet et al., 2012; *Stroke Facts | Cdc.Gov*, 2020). Beyond having strokes at higher rates, these patient populations are also subject to worse outcomes after stroke than their white, middle- and upper-class counterparts (Bos et al., 2002; Kapral et al., 2002). Thus, a better understanding of the underlying reasons for the increased risk of stroke among racial minorities and low socioeconomic status groups could offer a pathway for addressing this lethal disparity.

Technical Topic: Developing an Astrocyte Signaling Model to Inform and Improve Stroke Treatment[3]

Studies have found that over 50 percent of patients who survive more than two days after their first stroke die within five years due to complications from the stroke, including a subsequent stroke and heart disease, making the effects of stroke both vast and severe (Bae Hee-Joon et al., 2005; Hankey Graeme J. et al., 1998). Treatment of stroke within four and a half hours of its onset is necessary to significantly reduce the risk of long-term disability and mortality; beyond that time, treatment efficacy decreases significantly (Musuka et al., 2015). Thus, given the high rates of stroke occurrence and the severity of outcomes, effective treatments are critical.

Current Therapies and Models

Due to the time-sensitive nature of ischemic stroke, current treatments are limited, and treatments focus primarily on removing the clot that is obstructing blood flow in the brain. Therapies, such as tissue plasminogen activator (tPA), are used to dissolve clots in order to improve blood flow and prevent ischemic damage and appear promising (Baig & Bodle, 2020). However, many patient populations are not eligible due to extensive inclusion criteria, and tPA does little to prevent disability while also increasing the risk of subsequent bleeding in the brain (Donnan et al., 2008; Hinkle & Guanci, 2007). Mechanical devices are designed to retrieve and remove the occlusion, but they are only used in approximately three percent of hospitals (Hameed et al., 2017). These tools are also only effective on the first pass in approximately one

[3]This section was written in collaboration with Annie Ford and Zoe Garman, the other members of the technical project team. The advisors for this project are Dr. Richard Price and PhD candidates Catherine Gorick and Delaney Fisher.

quarter of patients, despite first pass success being necessary for the best outcomes (Zaidat et al., 2018).

There are several existing computational models that relate to stroke, but they typically are not robust enough to be used to identify new therapies. None of the current models paint a full picture of the many signaling pathways involved in normal and stroke conditions, nor do they detail the overlap of these pathways. Models have been developed to simulate recovery of motor function following stroke, but these models cannot be used to identify specific treatment targets in the brain (Casadio et al., 2013; Colombo et al., 2012). Other models have been created to detail intracellular communication following stroke. However, these models depict a limited number of communication pathways, which restricts the extent to which the model can be used to understand interactions within cells (Diekman et al., 2013; Dronne et al., 2004).

Hypothesis

This lack of an effective treatment and the knowledge gap that limits the development of new treatments have governed the modeling and hypothesis of this research project. The research team hypothesizes that delivering mitochondria to astrocytes following stroke will increase the number of factors that promote recovery in exosomes released from astrocytes.

Astrocytes are specialized glia cells and are the most abundant cell type in the central nervous system (CNS) (Sofroniew & Vinters, 2010). They play an essential role in maintaining normal brain function. Following an ischemic stroke, astrocytes carry out multiple functions that both benefit and damage neurons, making them an excellent therapeutic target to improve functions in the CNS (Liu & Chopp, 2016).

Exosomes are extracellular vesicles that transport proteins, nucleic acids, and lipids between cells over both long and short expanses and are proficient in manipulating target cells.

Exosomes released by neural cells play an important role in communication between these cells and the periphery in both normal and disease conditions (Upadhya et al., 2020). The ability to manipulate the phenotype of these exosomes is important for preventing further brain degradation following stroke and developing new treatments. Recent studies in ischemic heart disease have successfully shown that delivering mitochondria to the affected area promotes recovery (Masuzawa et al., 2013). Ischemic stroke follows a similar mechanism to ischemic injury in the heart, and thus, the impact of mitochondria delivery is also an area of interest in ischemic stroke research. Mitochondria could aid in recovery from ischemic stroke, potentially by altering the biogenesis of exosomes in astrocytes such that they positively impact the surrounding cells in the brain.

Approach

Throughout the 2020-2021 academic year, the research team seeks to construct a model to visualize astrocyte signaling pathways, apply this model to understand the connection between mitochondrial uptake and exosome release, and validate the results with cell culture experiments in both normal and stroke conditions. To construct this model in normal conditions, the team will conduct an extensive literature review of known and common intracellular communications in normal astrocytes to have a complete understanding of astrocyte functions and interactions within the CNS. The research team will use the Netflux program in MATLAB to create the model by inputting variables and downstream targets identified through literature review. NETFLUX is a software developed by the Saucerman Lab at the University of Virginia that is used to "develop models of biological networks" (Kraeutler et al., 2010). To build this model in stroke conditions, the research team will investigate the role of astrocytes in ischemic conditions to determine which parameters will be altered in the Netflux model. The goal of adjusting

parameters is to understand the ways in which changes in the environment exacerbate or prevent damaging effects in the CNS.

To apply the model and test the hypothesis, the team will adjust the model's input parameters to represent mitochondrial uptake and the pathways by which the uptake might impact exosome and neurotrophic factor release following stroke. The research team also aims to model the effects of focused ultrasound, a noninvasive therapy used to open the restrictive blood brain barrier for better treatment of CNS diseases. This will be done by adjusting input parameters to simulate the application of focused ultrasound using data previously collected by the Price Lab as a guide. The model is expected to be complete by the end of the Fall 2020 semester. During the Spring 2021 semester, the team hopes to confirm their findings via experiments using cultured human astrocytes. The cells will be treated with mitochondria that have been isolated from mouse muscle samples, and the exosomes released from the astrocytes will be isolated and analyzed after treatment. It is expected that the model will confirm the hypothesis that delivering mitochondria to astrocytes following stroke will increase the number of neurotrophic factors released in exosomes. The research team also aims to publish these findings in a scientific journal to inform and improve current and future stroke treatments at the end of the Spring 2021 semester.

STS Topic: The Effects of Race and Socioeconomic Status on the Incidence of Ischemic Stroke

Black Americans and low-income populations in the United States have strokes at alarmingly high rates (Addo Juliet et al., 2012; *Stroke Facts | Cdc.Gov*, 2020). However, there is very little research that aims to elucidate the driving forces behind the disparity in incidence. Instead, most studies focus on the different outcomes between racial and socioeconomic groups

following stroke. For example, studies have found that high-income individuals have less risk for mortality after stroke compared to low-income individuals, even after normalizing the data to education levels (Ahacic et al., 2012). The worse outcomes faced by minority and low-income populations further underscore the need for a clear understanding of why race and income are indicators of one's likelihood to have their first stroke.

The disparity in stroke incidence is not likely to be a coincidence; research has also found a significantly increased prevalence in several risk factors for stroke among racial minorities, including high blood pressure and diabetes (Bravata et al., 2005). High blood pressure, specifically, has been found to be positively correlated with experiences of discrimination, and racism-related stress is believed to contribute to overall declines in health (Pieterse & Carter, 2007; Ryan et al., 2006). In addition to understanding the patients, it is also important to evaluate the ways in which they interact with the healthcare system as a whole. Minorities have faced years of abuse by medical research, including the Tuskegee Syphilis Study, in which African American men with syphilis were observed without treatment to study the progression of the disease (Brandt, 1978). Likely as a result, there is a significant lack of trust in medicine and healthcare among minority communities (Kennedy et al., 2007; Rhee et al., 2019).

In order to use the wide range of available research and information to explain the disparity in stroke incidence, Actor-Network Theory (ANT) and the Social Construction of Technology (SCOT) will be implemented. ANT looks to identify all of the actors involved in a technology and track their interactions with each other and additional technologies (Cressman, 2009). Notably, this theory treats human and non-human actors equally, which allows technology, policies, and groups of people all to be considered (Rodger et al., 2009). ANT begins its search for actors with those who seem to have built the network of interest and, from there,

expands to include a wide range of actors. Criticisms of ANT include its seemingly infinite nature and the level of subjectivity for which it allows (Cressman, 2009). The key actors that appear to influence the disparity in stroke incidence include minority and low-income groups, white, middle- and upper-class groups, the healthcare system, healthcare providers, technology, insurance companies, and institutions such as education and the government. ANT will be employed to characterize the interactions between these groups, and potentially more actors, in the hope of elucidating the ways in which these interactions might impact the health and risk of stroke among minority and low-income groups.

SCOT evaluates the ways in which social constructions in society shape technologies, specifically in their early stages of development. It is used to follow the design and implementation process of various technologies as relevant social groups interact with them and dictate their development (Klein & Kleinman, 2002). However, SCOT has been criticized for its narrow focus, as it treats social structures as the drivers of technological change without considering the effects technologies can have on those same social structures (Hughes, 1994). Because the constructs of discrimination in society were prevalent during the time of many medical advances, SCOT will be used to analyze the ways in which social constructs relating to income and race influenced the development of the medical field. The goal of this analysis will be to better understand how societal beliefs and norms, especially racism, remain interwoven in healthcare and ultimately impact patient outcomes.

Methodologies

The research paper will answer the question, "Why do race and socioeconomic status influence one's likelihood to have a stroke?" By answering this question, this research will look to connect the experiences of minority and low-income groups in the United States with known

risk factors for stroke in order to detail the reasons behind the well-established disparity in stroke incidence (Addo Juliet et al., 2012; *Stroke Facts | Cdc.Gov*, 2020). To do this, documentary and historical case study research will be the primary methods utilized.

Documentary research will be implemented to perform an in-depth literature review via search engines such as PubMed and Web of Science. Specifically, these searches will gather scientific studies on the causes of ischemic stroke, as well as causes of the risk factors for ischemic stroke. The information gathered from these papers will be synthesized in an effort to connect the unique stressors faced by minority and low-income groups to an increased likelihood of stroke. Sources that will be analyzed include Jood's research on links between psychological stress and ischemic stroke, as well as Ryan's work investigating the correlation between discrimination and high blood pressure (Jood et al., 2009; Ryan et al., 2006). Subsequently, network analysis will be performed to elaborate on the interactions between the established actors and inform the use of ANT. Additionally, literature review, specifically focusing on historical case studies, will assist in analyzing the ways in which racism and classism impacted the development of medicine. This will inform the use of SCOT and potential reasons for distrust in medicine among many communities (Kennedy et al., 2007).

Conclusion

The technical project focuses on designing a model of intracellular communications in astrocytes both in normal physiological conditions and following ischemic stroke. This model will then be used to determine the effects of mitochondria delivery to the cells on the contents of the exosomes they release. In addition to the model, this research will also culminate in a scientific journal-style article that will be submitted to journals for publication in order to provide guidance for researchers looking to improve stroke treatments. The STS research project

aims to understand why minority and low-income groups have ischemic strokes at higher rates than other populations. The outcome of this research will be a research paper that utilizes ANT and SCOT to detail the reasons for the disparity in stroke incidence. This paper will serve as a guide for those looking to understand and correct this disparity.

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