INTRODUCTION

Approximately 360,000 people in the United States suffer from upper extremity paralytic syndromes annually and by 2012 an estimated 1.5 million people will suffer from peripheral nerve injury (PNI)\(^1\). PNI is a critical issue for many patients and there are no suitable clinical options for restoration of function when such injuries present a critically large cut to the nerve greater than 1.5 cm. Acute sensory problems, pain and loss of sensation, and chronic problems such as desensitization of the injured area are common clinical outcomes, which lead to a reduced quality of life, large health care costs and complications over time\(^2\). Nerve injuries are most commonly caused by trauma, bone fractures or joint dislocations. A major challenge in nerve repair is the regeneration of peripheral nerves with a gap greater than 1.5 cm in humans\(^3\). There is a lack of off-the-shelf, therapeutic modalities for long gap nerve repair. As the axons regrow toward the distal end after injury, they lose both direction and guidance as the distance increases from the proximal end to the distal end\(^2\).

To fix this problem, many researchers have investigated the use of biodegradable nerve conduits. Conduits are an artificial way of guiding nerves as they regrow. Different chemicals and proteins can be placed inside the conduits. It was already determined that nerve conduits with glial cell-line derived neurotropic factor (GDNF) loaded in them result in activation of Schwann cells. Now, a drug, Lithium (Li), which causes nerve elongation, was tested. Li has been shown to inhibit enzymes that adversely affect neuronal regeneration and has been shown to have neuroprotective properties\(^3\).

Neurotropic factors, such as GDNF, are proteins that are essential for \textit{in vivo} studies to guide the regenerating axons. They also aid in the growth and survival of neurons. They are naturally apart of the human body and cause natural regeneration, which occurs from the proximal to distal end of a nerve\(^3\). Also helping in the regeneration process are Schwann cells, which release chemotactic factors that assist the regenerating axon to continue regenerating. It is unknown what effect Li, in combination with GDNF, will have on functional recovery and axonal regeneration of a damaged nerve.

OBJECTIVE

The objective of the summer research project was to examine the relationship of the synergistic combination of GDNF and Li and its effect on nerve regeneration. It was hypothesized that a drug delivery system that targets both neuronal regeneration (Li) and Schwann cell migration and proliferation (GDNF) will result in accelerated functional recovery and axonal regeneration following peripheral nerve injury compared to one that only targets Schwann cell migration and proliferation (GDNF). It was expected that the highest dosage group of Li, 5.0 mEq/Kg LiCl, would yield the most significant results and cause the greatest regeneration due to its neuroprotective properties.

METHODS

Six weeks following the operation that placed the nerve conduits with dosage groups of 0.0, 1.0, 2.5 and 5.0 mEq/Kg LiCl, in the rats, nerve samples were harvested. All tissue was fixed in 4% paraformaldehyde and cross-sectioned at the level of the proximal nerve conduit (PN), proximal third area of the conduit (PC), mid-area of the conduit (MC), distal third area of the conduit (DC), and the distal nerve conduit (DN). After PN, PC, MC, DC and DN regions were sectioned, S-100 and PGP-9.5 staining of the samples was done to detect the presence of Schwann cells and axons, respectively.

Images of the different stained cross sections were captured using a microscope. The images were then analyzed using ImageJ software, which reported the amount of Schwann cells and axons per a given area. The data collected was statistically analyzed across the different dosage groups for each corresponding region using a one sample z-test. The alpha value for all conditions was set at 0.05. Upon calculation of p values for each variable of interest, the data was considered statistically significant if the p value was less than 0.05.

RESULTS

The results of the S-100 staining are depicted in Figure 1. The different rows correspond to the different segments of the nerve guide that were sectioned, and the different columns correspond to the different dosage groups. The data in the figure represents the amount of Schwann cells per a given area each dosage group for a specific region. The boxes shaded in a darker color represent significantly different results compared to other results within the same region (p < 0.05). The dosage group with a significantly higher density of Schwann cells was the 2.5 mEq/Kg group. The significantly higher densities were observed in the PC and MC regions. Also, one 5.0 mEq/Kg showed a significant result in the PC region.

PGP-9.5 staining yielded similar results. Significantly higher densities of axons were detected in only the 2.5 mEq/Kg group. Once again, these significant results occurred only in the proximal and middle conduit regions. Results are shown in Figure 2.
The objective of the project was met, because differences in axonal regeneration, in terms of Schwann cell and axonal presence, were noted amongst the various experimental groups and the control group. The hypothesis was supported because the LiCl dosage group of 2.5 mEq/Kg was observed to accelerate axonal regeneration significantly more than the control group of 0.0 mEq/Kg LiCl. This, however, differed from what was expected. It was expected that the 5.0 mEq/Kg group would yield the most significant results being the most concentrated dosage group. A possible explanation for this observation is that the 5.0 mEq/Kg group was too toxic for the nerve to regenerate. Although Li can inhibit enzymes that adversely affect regeneration and it has neuroprotective properties, too much of it can create a toxic environment. If the environment is too toxic, then the chemotactic factors released by the Schwann cells are destroyed; thus, inhibiting regeneration and it has neuroprotective properties, too much of it can create a toxic environment. If the environment is too toxic, then the chemotactic factors released by the Schwann cells are destroyed; thus, inhibiting regeneration.

Li, in combination with GDNF, was tested and found to be effective in the proximal region, PN. This is due to the fact that natural regeneration occurs without the aid of any external drug. Because regeneration begins at the proximal region, similar amounts of Schwann cells and axons should be expected to be detected there. The Li dosages have essentially no effect at the most proximal region. It is as the nerve continues to regenerate further away from the proximal region that the Li dosage begins to factor in by assisting the nerve to regenerate towards the more distal regions of the conduit.

This project was mainly limited by time. The histological analysis was done six weeks post operation. If more recovery time was given for the nerves to regenerate, then it is possible that more significant results may have been observed in more distal regions. Also, only rat models were used in this project. The utilization of different animal models, such as rabbits, would have yielded more comprehensive results.

Future ideas for this work include myelin staining. Myelin staining detects the presence of myelin on the regenerating axons. The presence of myelin on the axon indicates healthy axonal regeneration. Another idea is to begin using small primate models. If successful results are obtained for this study, it is a step closer to finding a more effective and rapid treatment for peripheral nerve damage; thus, improving the lives of millions around the world.

**CONCLUSION**

PNI is a major clinical challenge, affecting millions around the world. Finding a more rapid treatment that also ensures effective regeneration will help alleviate the PNI suffered by millions. Li, in combination with GDNF, was tested and yielded positive results. A dosage group of 2.5 mEq/Kg was observed to have significant effects of axonal regeneration. Further studies will continue to provide translational groundwork for eventual human implementation.

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**REFERENCES**