Testing the Role of Calcitonin Gene-Related Peptide (CGRP) in Scar Formation after Multiple Tissue Injury

Gabrielle Alphonse1; Charlotte Raymond2; Ashlee Williams, B.S.3; Jennifer Simkin, Ph.D.3

Patrick F. Taylor Science & Technology Academy1 St. Mary’s Dominican High School2 Louisiana State University Health Center, Department of Orthopedics3

Introduction

Optimal healing for a traumatic wound or injury is described as completely regenerating the damaged tissue. This study focuses on replacing what the nerve secretes that promotes nerve and tissue regeneration, more specifically in the military population. A study with a sample size of 4,563 soldiers deployed in Iraq and Afghanistan found that 80% of these soldiers suffered musculoskeletal injuries due to explosions or blasts. Out of these injuries, 94% of them resulted in amputations. However, amputations are not the desired outcome of these injuries; and the question becomes: is there a specific protocol that the body should undergo to promote tissue repair? Amputations occur when there is severe and excessive tissue damage and dysfunction, therefore, if more tissue was salvageable, there would be better surgery outcomes. The goal of this study is to develop treatment that promotes regeneration rather than scar tissue formation.

Previous RNA sequencing reveals that several neurotrophic factors are upregulated during scar formation in Mus and downregulated or unchanged during regeneration in Acomys (Figure 3). One notable neuropeptide, the Calcitonin Gene-Related Peptide (CGRP), is not expressed in regeneration in Acomys but expressed in scar formation in Mus. CGRP is a known component of pain and inflammation, but there is still uncertainty around its role in wound healing and scar formation. This study evaluates the direct role CGRP plays in scar formation.

Methods

To determine CGRP’s role in wound healing, Acomys cells were exposed to graded concentrations of CGRP (0.1 ug/mL, 1 ug/mL, 5 ug/mL, and 10 ug/mL) for 24 hours. After the incubation period, the cells are harvested and measured through the ELISA protocol.

Results

Figure 4: Acomys and Mus cells were treated with different concentrations of CGRP (0.1 ug/mL, 1 ug/mL, 5 ug/mL, and 10 ug/mL) for 24 hours. After being exposed to different concentrations of CGRP, the cells were tested for their ability to increase cAMP production.

Discussion

Reactions to CGRP:
- Acomys cells exposed to 1 ug/mL showed the highest expression increase in Col1a1 compared to untreated controls.
- Mus cells showed no change in Col1a1 expression, no matter the concentration of CGRP.
- Acomys cells exposed to 0.1 ug/mL and 5 ug/mL of CGRP show a potential increase in Mmp9 expression.
- Mus cells show a decrease in Mmp9 expression with all concentrations of CGRP.
- More replicates need to be run to determine if these trends are accurate.

Determining profibrotic potential:
- Because the cAMP assay was inaccurate, we cannot fully determine if the cells were responding directly to CGRP. Therefore, our conclusions on the qPCR results are still preliminary.

Conclusions & Further Research

Results suggest:
- If the above trends hold true, it is possible CGRP is increasing Col1a1 expression in Acomys cells which would suggest potential fibrotic activity.

Further Research Tactics:
- Investigate profibrotic potential further to replicating cAMP assay procedure to determine if cells are responsive to CGRP.
- Working with more cells (BCA assay recommends 1000 ug/mL of protein to get consistent cAMP results, more cells = more protein).
- Treat cells with CGRP for longer than 10 minutes.
- Cleaning protein protocol to prevent interfering substances.
- Performing Western blot for different CGRP downstream mediators (ex. pCREB).

References


Acknowledgments: This work is supported by the Office of Assistant Secretary of Defense for Health Affairs through the Orthopaedic Research Program under Award No. W81XWH1110503.

Dr. Jessica Rivera for her help guiding the project directions.