Immunoregulation of Cardiac Output *in Vitro*

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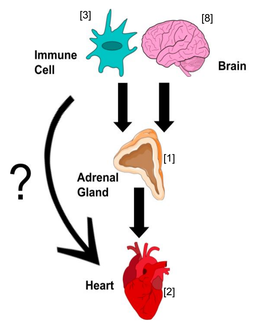
Abstract

Cardiovascular disease (CVD) is the leading cause of death worldwide [4], with around 28.1 million adults in the world diagnosed with heart disease [7], and although there are some existing ways to treat it, targeting inflammatory response has not yet been explored to treat CVD. Therefore, we aim to develop an in vitro model to study cardiac output as a response to chronic inflammation by investigating the role of cytokines in the heart. We hypothesize that the release of cytokines alter cardiac output indirectly by binding to receptors in the adrenal gland and promoting the exocytosis of adrenaline/noradrenaline. To test this hypothesis, our experiments will investigate how cytokines affect the beating of enriched cardiomyocytes both directly and indirectly through the adrenal gland. Here, we will determine the effect of cytokines on cardiac output through the use of video and immunofluorescent microscopy, cardiac cell isolation, and high throughput data analysis. We hope that findings from this study will lead to the development of novel therapeutic strategies to treat CVD. From our results, we can conclude that the cytokines TNFα and IL1β do not directly affect the heart rate of cardiomyocytes.

Introduction

CVD currently remains a clinical challenge; commonly treated with beta blocker pills or pacemakers, there currently remains no effective therapies for cardiac disease that target inflammatory response.

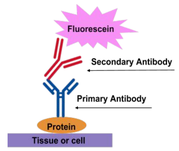


Cytokines are a category of proteins produced by immune cells in response to infection, inflammation, cancer, or many other health factors. Though it is known that they alter cardiac output, their mechanism of action remains unknown. These proteins affect cells through intracellular signaling by binding to specialized cell-surface receptors [5]. While it is unknown if these receptors are present in the heart, the tumor necrosis factor alpha (TNFα) and interleukin-1beta (IL1β) receptors are expressed in the adrenal glands. Through analyzing the effect of these cytokines on cardiomyocyte cells and adrenal gland cells, we can determine if the release of such cytokines directly or indirectly prompt an increased heart rate.

Materials and Methods

- Cardiac cell isolation: dissociated minced heart pieces with collagenase type II to collect cardiomyocytes (CMs).
- Adrenal cell isolation: dissociated whole adrenal glands with collagenase type I to collect adrenal chromaffin cells (ACCs).
- Cell seeding/dosing: seeded CMs at 100 cells/mm^2 into wells and added cytokines (TNFα and IL1β) at concentrations of 1 ng/mL, 10 ng/mL, and 50 ng/mL.
- Video Microscopy: monitored beat rate using the Axio Observer Z1 inverted microscope with live cell imaging at 30 fps.
- Immunofluorescence: using anti-sarcomeric Alpha Actinin (primary mouse antibody) and anti-mouse conjugated Alexa Fluor 555 to stain CMs and anti chromogranin A (primary rabbit antibody) and anti-rabbit conjugated Alexa Fluor 488 to stain ACCs.
- Biostatistics: all data was checked for normality using the lilliefors test in MATLAB and then analyzed and represented by p-values in bar/dot plots.


[6]



Results

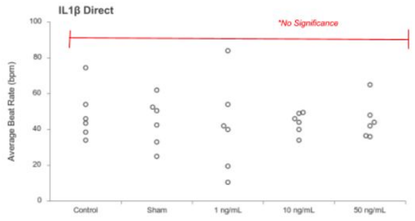


Figure 1: Analysis of IL1β's effect on CM beat rate directly shows no statistically significant difference between controls.

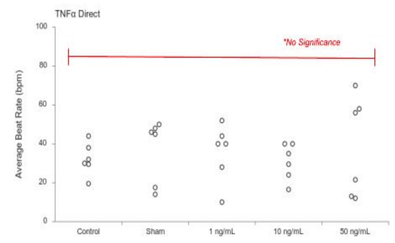


Figure 2: Analysis of TNFα's effect on CM beat rate directly shows no statistically significant difference between controls.

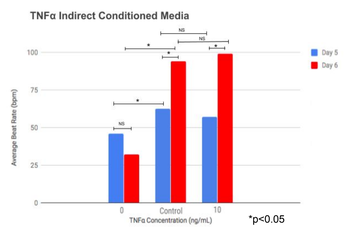


Figure 3: Analysis shows no statistically significant difference between ACC's cultured with/without TNFα, but a significant increase in beat rate compared with CMs treated with conditioned media.

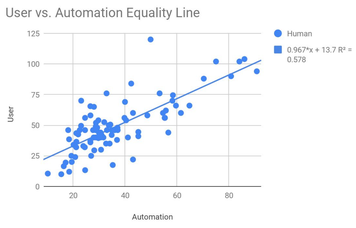


Figure 4: Equality line that shows the accuracy of the MATLAB code counting beats vs. a user.

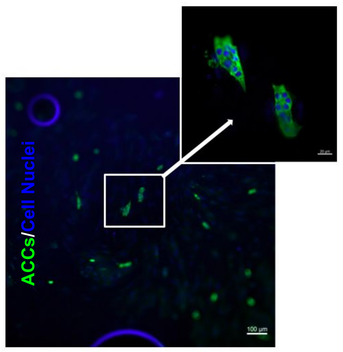


Figure 5: A 10x vs 63x image of ACCs in a transwell insert from the Axio Observer Z1 inverted microscope.

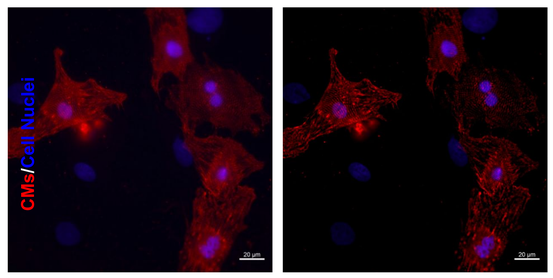


Figure 6: A single z-positioned image vs. a deconvoluted image from the Axio Observer Z1 inverted microscope shows improved clarity and resolution.

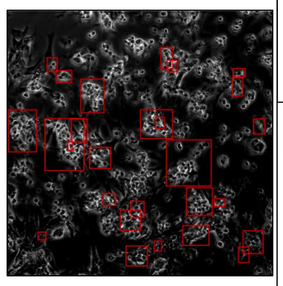


Figure 7: Automated cell identification and quantification of beat rate using a custom MATLAB algorithm developed in the Koppes Lab.

Discussion

- In our experiment for indirect IL1β with transwells, we did not include a control transwell (adrenal cells and cytomyocytes only) for comparison.
- We cannot conclude that IL1β inhibits the exocytosis of catecholamines in adrenal cells because we have no control to compare our data with.
- In future research, it would be beneficial to include a control in this experiment to reach a conclusion on whether or not IL1β affects heart rate through the adrenal gland.

Figure 8: Experimental data testing how IL1β affects heart rate through transwells with adrenal cells.

Conclusion

- TNFα and IL1β do not cause an increase in beat rate in cardiomyocytes directly.
- TNFα does not promote exocytosis of catecholamines in adrenal cells, as there was no significant difference between the CM beat rate in ACC conditioned media with or without the TNFα.
- Findings necessitate further research (increased sample sizes) on how cytokines affect heart rate indirectly to help find cures for heart disease through inflammatory immune response.

References

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