Methods For Optimizing Aspirin Dosage and Treatment Duration

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Introduction

• HIV risk is linked with increased immune activation
• Acetyl Salicylic Acid (Aspirin) and Salicylic Acid (SA) have been shown to reduce immune activation in Kenyan women
• The Fowke lab speculates that Aspirin is an effective prevention tool against HIV infection
• MTT assays are not an accurate method to study aspirin due to the fact that MTT is a colour based assay and increased cytotoxicity showed increased metabolism

Objectives

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1. Determine the level of cellular apoptosis in exposure to different levels of ASA/SA
2. Determine the viability of the cells after the exposure of different levels of ASA/SA
3. Determine the number of cells that are pre-apoptotic after exposure to different levels of ASA/SA
4. Determine that all three methods of analysis correlate with each other in their results

Hypothesis

The three chosen assays tested (LDH, Trypan Blue, and Caspase-3) will produce similar results and therefore only one method will be needed in order to determine concentration and exposure time of aspirin.

Methods

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Figure 1. A. Depicts an MTT assay following a DMSO treatment. B. Depicts an MTT assay following ASA/SA treatment. This data shows us that MTT is not an appropriate assay to use to test viability of cells following an ASA/SA treatment due to it being a colour based assay and due to the increase in metabolism in cells exposed to aspirin that are pre-apoptotic.

Figure 2. Diagrammatic explanation of the methods being tested. Objective 1 was analyzed by performing an LDH assay to determine the level of cells undergoing apoptosis. Objective 2 was analyzed by performing a trypan blue assay in order to determine the viability of cells after exposure to ASA/SA. Objective 3 was analyzed by performing a Caspase-3 assay to determine the level of cells that were pre-apoptotic.

Figure 3. Percentage of cells having undergone apoptosis after being exposed to varying levels of ASA or SA, at three separate exposure time points. Results show for both ASA and SA, there is a larger number of cells undergoing apoptosis as the concentration increases. Each colour represents a different exposure time point.

Results 1: Aspirin Induced Cytotoxicity

The LDH assay results demonstrated increasing cytotoxicity with both time and concentration.

Results 2: Viability After Aspirin Exposure

The Trypan Blue assay results of ASA and SA are apoptotic.

Results 3: Induction of Apoptosis In Surviving Cells

The caspase-3 assay results of ASA and SA are similar at different concentrations and time points.

Results 4: Correlations Of Methods

Correlation of Cell Survival and Proportion of Caspase-3+ T cells is significant. All other correlations of assays are insignificant.

Discussion

By being able to accurately characterize different parameters of cell viability, it allows us to accurately determine the most optimal drug concentration and treatment duration to study aspirin in vitro.

Conclusion

All three methods of analysis are needed in order to accurately determine concentration and time in vitro.

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References
