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

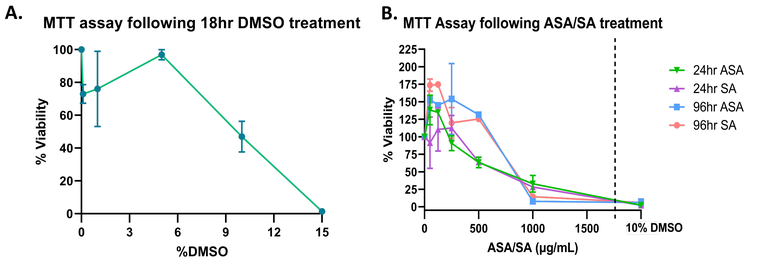

Figure 1. A: Depicts an MTT assay following a DMSO treatment. **B**. Depicts an MTT assay following ASA/SA treatment. This data shows us that MTT is not an appropriate assay to use to test viability of cells following an ASA/SA treatment due to it being a colour based assay and due to the increase in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

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.

Objectives

Objectives:

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

Methods

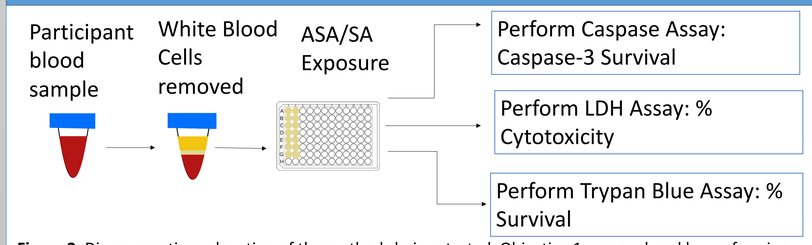

Figure 2. Diagrammatic explanation of the methods beings tested. Objective 1 was analyzed by performing an LDH assay to determine the level of cells undergoing apoptosis. Objective 2 was analyzed by performing a trypan blue assay in order to determine the viability of cells after exposure to ASA/SA.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

Results 1: Aspirin Induced Cytotoxicity

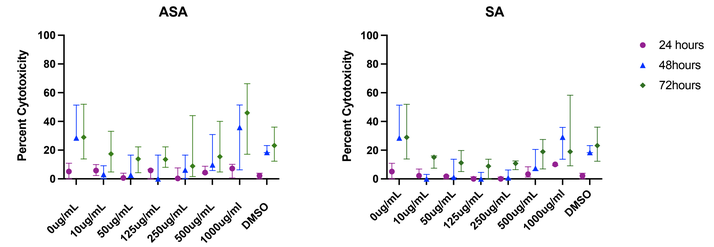

Figure 3. Percentage of cells having undergone apoptosis after being exposed to varying levels of ASA or SA, at three separate exposure time points. Results show for both ASA and SA, there is a larger number of cells undergoing apoptosis as the concentration increases. Each colour represents a different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

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

Results 2: Viability After Aspirin Exposure

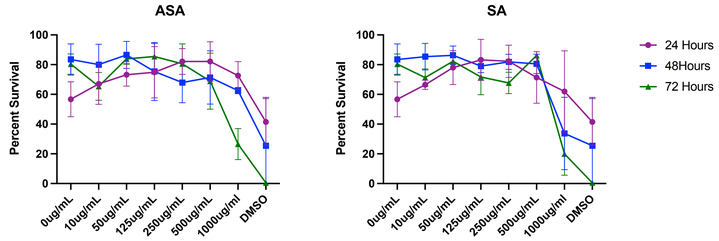

Figure 4. Percentage of cell survival after exposure to varying levels levels of ASA or SA, at three separate exposure time points. Results show a general decreasing trend of decrease in survival as ASA/SA concentrations increase. Each colour represents a separate exposure time point.

The Trypan Blue assay results of ASA and SA are similar at different concentrations and time points

Results 3: Induction of Apoptosis In Surviving Cells

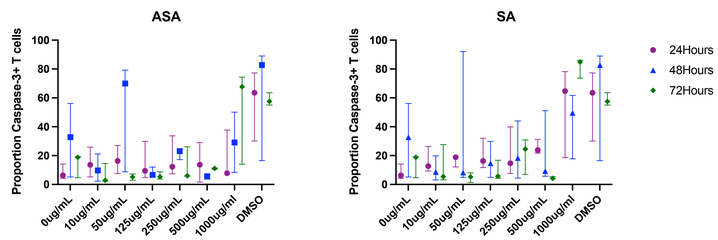

Figure 5. Proportion of cells that were in a pre-apoptotic state after being exposed to varying levels of ASA or SA at three separate exposure time points. Results show a general increase in pre-apoptotic cells as concentration of ASA/SA increases. Each colour represents a separate exposure time point.

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

References

Card, C.M., Ball, T.B. & Fowke, K.R. Immune quiescence: a model of protection against HIV infection. *Retrovirology* **10,** 141 (2013). https://doi.org/10.1186/1742-4690-10-141
Hussain, M., Javeed, A., Ashraf, M., Zhao, Y., Mukhtar, M. M., & Rehman, M. U. (2012). Aspirin and immune system. *International Immunopharmacology*, *12*(1), 10–20. https://doi.org/10.1016/j.intimp.2011.11.021
McLaren, P. J., Ball, T. B., Wachihi, C., Jaoko, W., Kelvin, D. J., Danesh, A., Kimani, J., Plummer, F. A., & Fowke, K. R. (2010). HIV-exposed seronegative commercial sex workers show a quiescent phenotype in the CD4+ T cell compartment and reduced expression of HIV-dependent host factors. *The Journal of infectious diseases*, *202 Suppl 3*, S339–S344. https://doi.org/10.1086/655968

Results 4: Correlations Of Methods

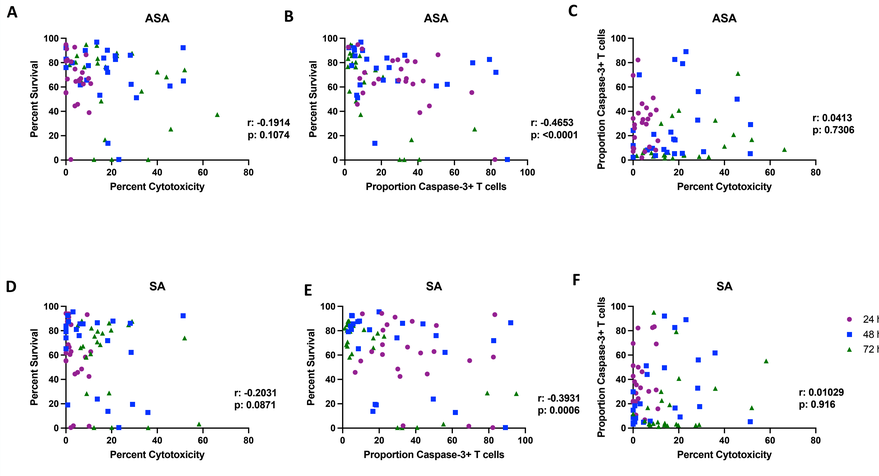

Figure 6. A and D: Represent the correlations between the percentage of cells surviving and the percentage of cells having undergone apoptosis after being exposed to varying levels of ASA/SA. Results were non-significant. **B and E:** represents the correlations between the percentage of cells surviving and the percentage of cells in a pre-apoptotic state after being exposed to varying levels of ASA/SA. Results were significant. **C and F:** Represents the correlations between the percentage of cells in a pre-apoptotic state and cells that underwent apoptosis after exposure to varying levels of ASA/SA. Results were non-significant.
* All results were analyzed using a nonparametric Spearman correlation. For all results p value <0.005 was considered significant.

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

Significance

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*.

Acknowledgments

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- Monika Kowatsch
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- Participants