Microglia Activation in Ageing and Neurodegenerative Diseases

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INTRODUCTION

Microglia are cells located in the **Central Nervous System** (CNS). Also known as the 'scavengers', microglia play an important role in carrying out an inflammatory response and cleaning away dead paths as a result of brain trauma, consequently prohibiting them from becoming toxic to other cells. Because of this, microglia are known to be highly dynamic.

Following a traumatic brain injury (TBI), microglia activate in order to maintain homeostasis in the brain, after this inflammatory phase, most functioning microglia should then transition to the clean up phase of cellular debris through phagocytosis. However, some microglia remain constantly activated, which then results in damage being caused to surrounding tissues. This damage can then lead to Neurodegenerative Diseases with Age.

Some drugs exist that can act as inhibitors against microglia. They are able to halt/ reduce excessive microglia activation, thus reducing inflammation in the brain. The issue with this is that they completely stop the inflammatory process, rather than controlling the cells to remain in the anti-inflammatory phase.

OBJECTIVE

Our aim was to test a new group of drugs to narrow down which of them are effective in modulating excessive microglia activation without unwanted side effects, and also understanding how these processes take place. The drugs that were used were LPS and SAHA.

It is also presumed that, regarding cells contained in a serum, more serum can encourage overgrowth of cells, so we wanted to check if reduced serum = reduced activation.

METHODOLOGY

The method that was used to test these theories were repeated quantitative polymerase chain reaction (qPCR) complementary DNA (cDNA) synthesis. The cells were counted and split into two 6-well plates, each with a drug treatment and a percentage of media.
As well as this, a 24-well plate of cells with different percentages of media was placed under a cell imaging microscope, to produce a time-lapse to track cell movement. During the entirety of the research period, cells had to be split every two days, to avoid them becoming over confluent and eventually dying.

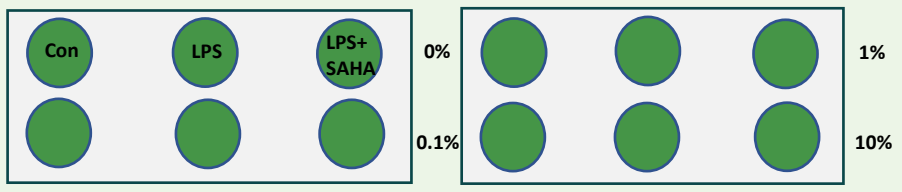

Fig 1. The layout of the cell treatment processes. Each row has a different percentage, and the three columns represent the control, the LPS treated cells and the SAHA treated cells.

RESULTS

The findings were mostly as predicted:

- **The SAHA was able to inhibit microglia activation significantly**
- **More serum used resulted in a higher movement of cells**
- Carrying out Independent two tail t-tests using IBM spss statistics, and setting the significance level to 0.05, we were able to deduce **that there is a significant difference between LPS and SAHA at the 5% significance level**.

The amount of serum used, however, was insignificant to the effect of the SAHA on the cells.

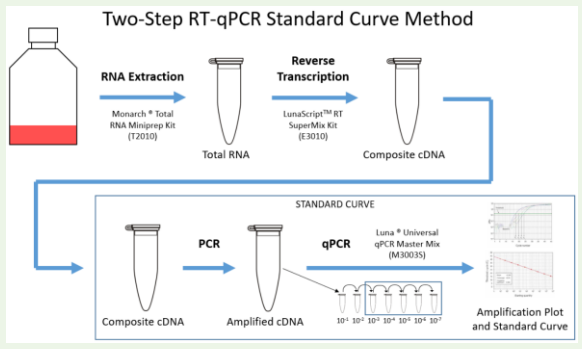

Fig.2 The process of a two-step qPCR used to create a standard curve

ANALYSIS

After imaging the cells under a microscope for 24 hours, because the cells were not incubated under the microscope, they died after some time, resulting in a lot of the images becoming unusable. To overcome this, the time taken under the microscope was reduced to 6 hours, to get images of the cells while they were still healthy. The images were then put together in sequence to form a time-lapse. The cells were tracked using the **Trackmate** plugin on ImageJ. After tracking the cells, the distance moved by each cell was recorded. Then the results were put into a coding software called **jupyter** where the histograms (Fig.4) were created. The graphs show that with the increase in the % serum, the frequency of the total distance moved by the cells shifts to the right, indicating that a larger frequency of cells move more with more serum. The means of the qPCR results were also taken and plotted (Fig.3). The results were generally similar regardless of the serum %.

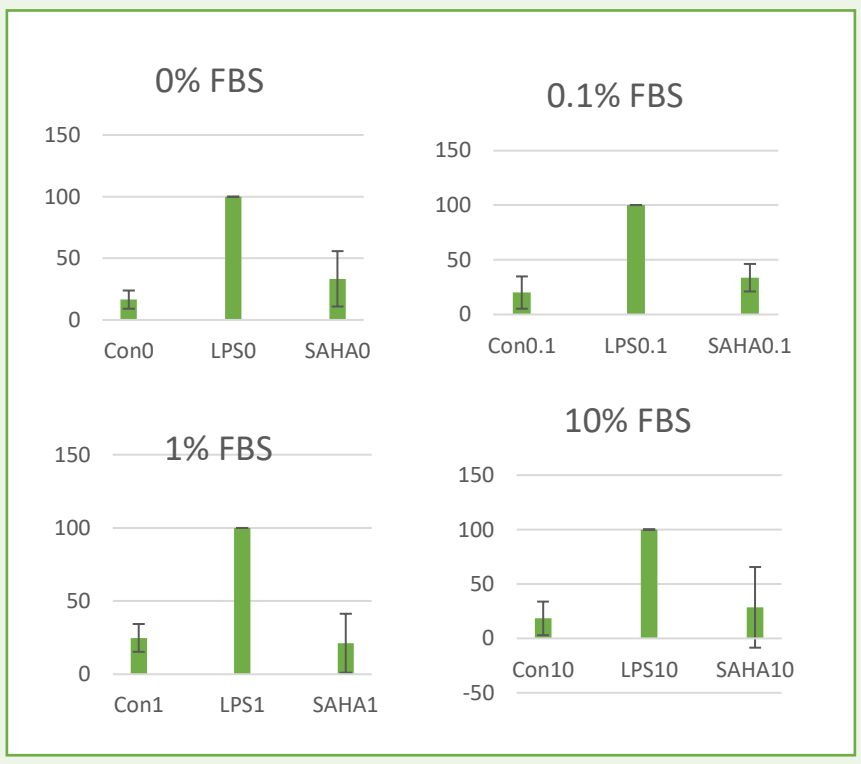

Fig.3 The concentrations of each group of treated cells after qPCR

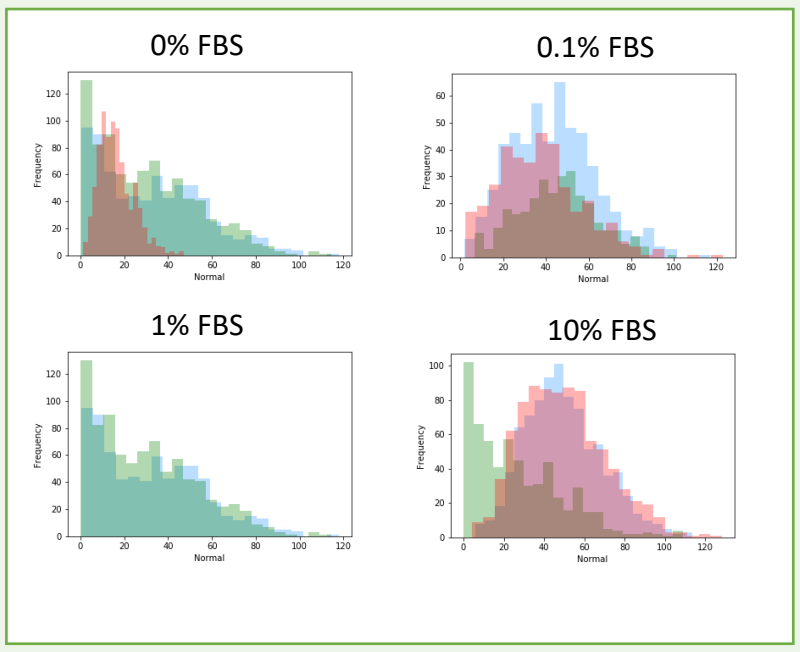

Fig.4 The total distance moved by BV2 cells under the microscope at different percentages of media, taken on 12/07/22.

CONCLUSION

In conclusion we were able to successfully prove that SAHA is an effective drug in inhibiting microglial activation. Also, the percentage of serum used does affect the movement of cells but does not have an effect on how well the drugs work on the cells.

BV-2 cells were used to mimic microglia since they are cheaper, easier to use and save the unnecessary excessive use of animals. Also, 90% of genes regulated in BV-2 are also found in primary microglia.

One thing that could be improved is the fact that whilst the cells were in the dishes, they were activated but had no specific area to go to since there was 'damage' all around, so the cells moved aimlessly. Next time we could somehow have a specific point of 'damage' for the cells to flock around and act upon.

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