Argon-Mediated Survival in Stroke Models

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Introduction

An ischemic stroke is the result of an artery to the brain being blocked. This prevents the delivery of vital oxygen and nutrients to neurons, thus leading to cell death. Studies indicate that the noble gas, Xenon has neuroprotective effects following stroke like injuries. Argon has also shown potential neuroprotective effects, however the exact signaling mechanisms are unknown. Argon is also abundant and economical, and thus it is a prospective medical treatment for stroke victims. Preliminary results in our lab confirm the ability of argon to promote survival in neuron-like cell cultures.

The purpose of this research project is to determine the neuroprotective effects of argon gas on cell survival following an in vitro injury.

Methods

HeLa cells were grown and replicated in the cell culture lab at High Point University. Four, 12-well plates were used for the experiment. Quadruplets were plated in each well-plate with a total of 16 wells plated containing about 20K cells/well.

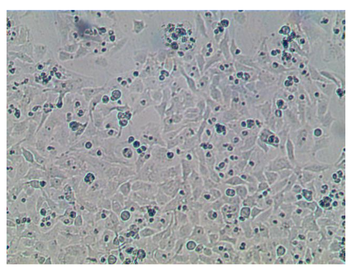
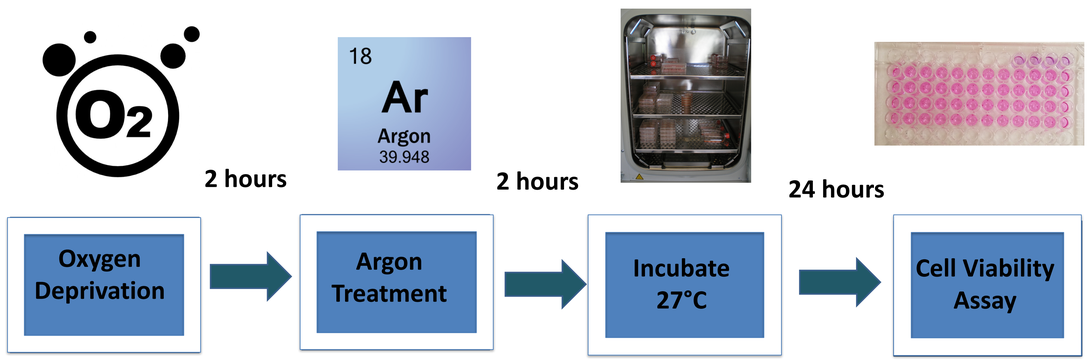


Figure 1: Image of HeLa cells under microscope.

Oxygen Deprivation: Cells were incubated in an environment of 0% Oxygen, 95% N_2, and 5% CO_2 at 37°C for 2 hours.

Argon Treatment: Cells were incubated in an environment of 21% Oxygen, 74% Argon gas, and 5% CO_2 at 37°C for 2 hours.



Results

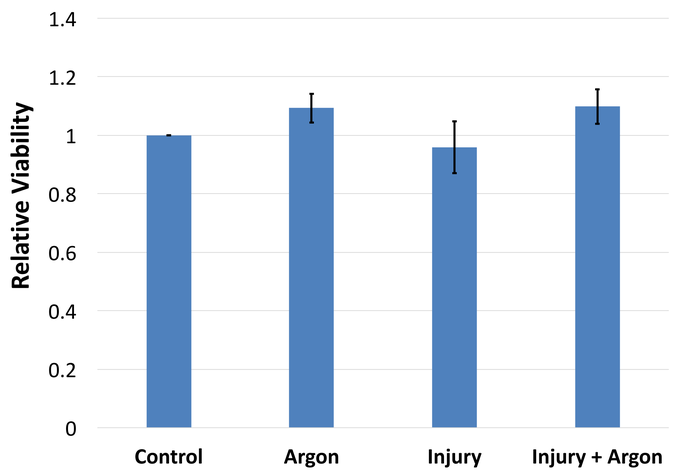


Figure 2: alamarBlue® cell viability assay. alamarBlue® reagent was added to each well and the well-plates were incubated at 37°C for 1-4 hours. The fluorescence signal was then measured using a spectrophotometer. The results were evaluated by plotting the relevant alamar blue signal versus our treatments. Error bars represent the SEM.

Discussion/Future Aims

Previous research indicated neuroprotective effects of Argon on retinal ganglion cells. (Ulbrich, Kaufmann et al. 2015) Their findings support their hypothesis that Argon exerts neuroprotection via an ERK-1/2 dependent pathway following ischemia/reperfusion injury (R=IRI).

However, our results indicate that after exposing HeLa cells to 0% oxygen for 2 hours there is no significant difference in cell death compared to our control. Our results also signify that argon treatment following injury does not have an effect on the amount of cell death compared to injury alone.

In the future, we plan to utilize PC12 cells. This cell line can be differentiated into a neuron-like state making them a more realistic model for our experiment. Our future goals also include the use of flow cytometry to confirm relationships between molecular signaling molecules and argon-mediated cell survival.

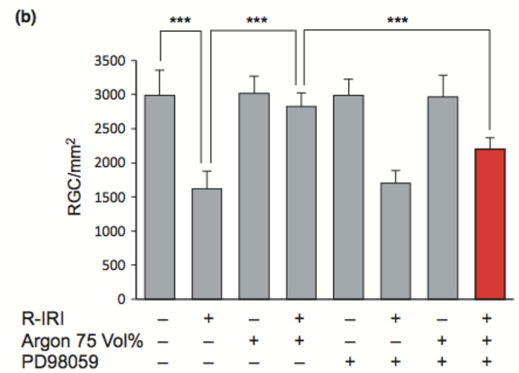


Figure 3: Inhibition of ERK-1/2 reduces the effect of Argon inhalation on retinal ganglion cells count following R-IRI. (Ulbrich, Kaufmann et al. 2015)

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- Ulbrich, F., et al. (2015). "Neuroprotective effects of Argon are mediated via an ERK-1/2 dependent regulation of heme-oxygenase-1 in retinal ganglion cells." Journal of neurochemistry **134**(4): 717-727.