Determining Time Dependent Effect of Hypoxia on the Expression of von Willebrand Factor in Lung Endothelial Cells

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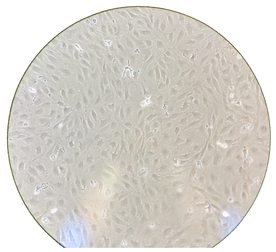
INTRODUCTION

- Von Willebrand Factor (VWF) is a protein that plays an important role in the formation of blood clots
- This protein is crucial for maintaining the health of blood vessels because it helps to repair damages
- It is synthesized and stored in storage organelles in endothelial cells
- VWF is triggered to be released when a vasculature is damaged but it can also be released in response to hypoxia or other stimuli
- A previous study showed that in some organs VWF production increases when exposed to hypoxia
- This can be problematic for transplanted organs as their time outside of the body can trigger an increase in VWF release
- The purpose of this study is to explore how long cells need to be in hypoxia before the amount of VWF being released increases

METHODS

- Lung endothelial cells were cultured for several days and separated into 48 dishes
- 24 dishes of cells were labeled Hypoxia and the remaining 24 were labeled Control
- The Hypoxia cells were put into a hypoxia chamber for different amounts of time. Two dishes were kept in the chamber for each amount: 1 hour, 4 hours, 8 hours, 24 hours, 48 hours, and 72 hours
- The Control cells were kept in normal conditions for the same amounts of time



- The cells were then collected and the RNA was extracted
- cDNA was synthesized from the RNA by using reverse transcriptase

RT-PCR Analysis
- This method of analyzing data works by first denaturing the double stranded cDNA into single strands
- After this, it uses a primer to begin new strands of cDNA
- Finally, a dye is used to identify the amplified product

Western Blot Analysis
- This is a method of detecting and analyzing specific proteins
- First, samples are loaded into a gel mold and the proteins separate based on size
- Later, the gel is transferred onto a membrane
- Specific antibodies are used to detect different proteins

RESULTS

RT-PCR

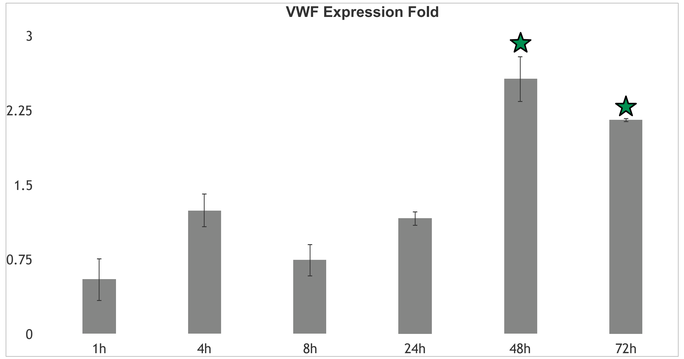


***Figure 1**. Levels of VWF expression after being exposed to hypoxia. Cells were fed the day before exposure. Stars indicate a significant increase.*

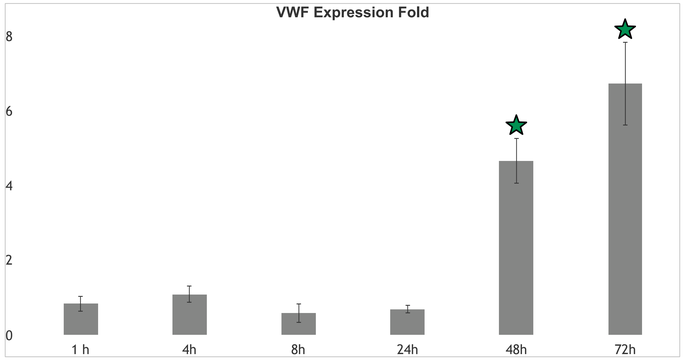


***Figure 2**. Levels of VWF expression after being exposed to hypoxia. Cells were fed the day of exposure. Stars indicate a significant increase.*

Western Blot Analysis

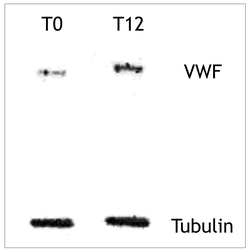


***Figure 3**. This western blot analysis shows VWF levels for T0 and T12. A pig organ was taken out of the body for 12 hours. T0 is a sample taken from that organ at 0 hours outside of the body and T12 is a sample taken from that organ after 12 hours.*

CONCLUSION

RT-PCR
- These results show little difference in VWF expression up to 24 hours
- After 48 hours, there is a significant increase. This aligns with a previous study that showed increases in VWF expression in the lungs of mice after 48 hours in hypoxia

- This experiment proves that hypoxia does cause an increase in VWF expression
- The results of this experiment can help us learn how to improve transplanting techniques and minimize health complications
- These results show the sensitivity of cells. The difference in figures 1 and 2 is the time that the cells were fed before hypoxia exposure

Western Blot Analysis
- The blot showed an increase in levels of VWF in pig lungs after 12 hours outside of the body
- This means that organs that are exposed to hypoxia will have increased levels of VWF
- This can lead to complications such as unwanted blood clotting which can cause stroke or, in some cases, rejection of a transplanted organ

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Literature Cited

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