

CALIFORNIA STATE SCIENCE FAIR 2013 PROJECT SUMMARY

Name(s)

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Project Number

S1714

Project Title

Preserving the Lung for Transplant: Evaluation of Antioxidant Preservatives at Inhibiting Cell Damage from Free Radicals

Abstract

Objectives/Goals

The time limit for routine cadaveric lung transports in hypothermia is limited to 4 to 6 hours. This project identifies the effectiveness of antioxidant solutions in reducing free radical induced damage to lungs, for transplant.

Methods/Materials

Bovine lungs preserved in ice for 3 hrs since extraction. 3 samples per preservative were taken and immersed in antioxidant preservatives at 4 degree C for additional 3 & 5 hrs. Preservatives evaluated: Ice, Butylated hydroxytoluene (BHT), Vitamin E, Vitamin A, Vitamin C, Lutein, Safflower Oil, Melatonin, Biotin, and Distilled water. Tissue was fixated in 10% Neutral Buffered Formalin (NBF) for 48 hrs. Samples were embedded in paraffin wax, sliced by microtome, and stained with Haemotoxylin & Eosin (H&E). Slides were analyzed for cell damage under a 400x light microscope.

Cell damage assessed: cytoplasm color (lavender is healthy, pink is damaged) and coagulative necrosis (observed by faded chromotin with nuclei going through dissolution). Rating system: Nucleus and cytoplasm health were rated on a scale of 1 # 10 (1 = most cell damage, 10 = least cell damage). The final rating (out of 20) was a sum of these two scores per sample.

Results

The control (lung sample before preservatives) was rated at 18.5/20. Vitamin E was the most effective - 11.5/20 for both 3 hr and 5 hr samples, closely followed by BHT (11/20) and ice (9/20). Right from 3 hour check point onwards, Biotin (2/20) and Melatonin (3.5/20) were the least effective.

Conclusions/Discussion

The goal was to extend transport life of lung being transplanted using existing method of topical icy slush mixture. Significant cell damage (coagulated necrosis) develops due to free radicals during cold ischemia (ice storage).

Antioxidant preservatives improved lung cell health: Even at 3 hrs, oil by itself was a poor preservative (6/20). However, all the antioxidants that used oil as solvent were much better, even after 5 hrs of preservation (Vitamin E # 11.5/20, Vitamin A # 8/20, BHT # 11/20, Lutein # 7.5/20). All the oil soluble preservatives were more effective than water soluble antioxidants (Vitamin C # 5/20, Melatonin # 3.5/20, Biotin # 2/20).

Future research: Longer preservation with combinations of Vitamin E, BHT & Ice, and ViaSpan (default practice) can be evaluated on other organs. Methods to remove preservatives from organs prior to transplant must also be addressed.

Summary Statement

This research proved that readily available antioxidants Vitamin E and BHT are effective in neutralizing free radicals, thereby preventing excessive cell damage in the transport period of the lung transplantation process.

Help Received

I'm grateful to my teacher for guiding me and giving full access to our school lab, Dr. Collinson & Dr. Gardiner for H&E staining, San Jose Valley Veal for providing bovine calf lungs, and my dad for logistical help.