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How Important Is Gastroduodenal Reflux In Lung Transplant Rejection?

Background Information

Lung transplantation has become a valuable treatment for end-stage pulmonary disorders in an attempt to improve survival and quality of life for these patients. Chronic allograft rejection, also known as bronchiolitis obliterans syndrome (BOS) contributes to the poor long term survival. Reflux of gastric and duodenal contents into the oesophagus and extra-oesophageal areas has been implicated in the development of BOS in patients post lung transplantation. Statistics indicated up to 50% of transplant patients develop BOS in the first 5 years. In addition trypsin, a duodenal protease and component of the refluxate, has been shown to stimulate the production of inflammatory mediators. It also plays an important role in mucosal damage by gastroduodenal reflux and can therefore be a therapeutic target for patients with BOS. Recently reflux components such as bile salts and trypsin have been shown to have the potential to disrupt oesophageal barrier function by modulating tight junction proteins.

Aims

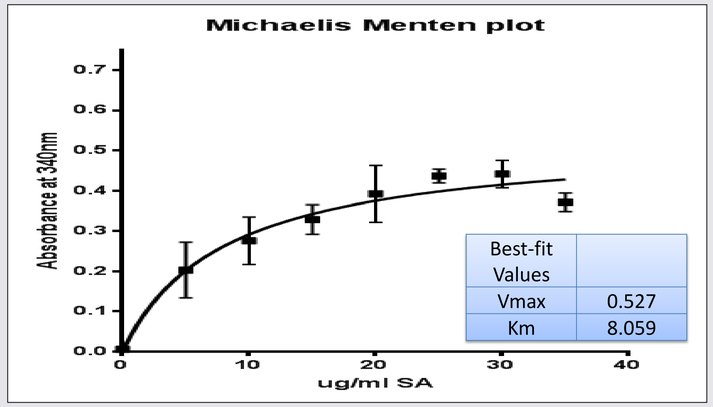
- To assess the effect of duodenal aspiration/contents on airway epithelia and its importance in airway diseases and lung transplant rejection
- To optimise experimental assays used to detect trypsin in refluxate samples
- To grow a cancer cell line (HT29-MTX goblet cells) to apply various concentrations of trypsin and measure cell viability using metabolic assays

Methods

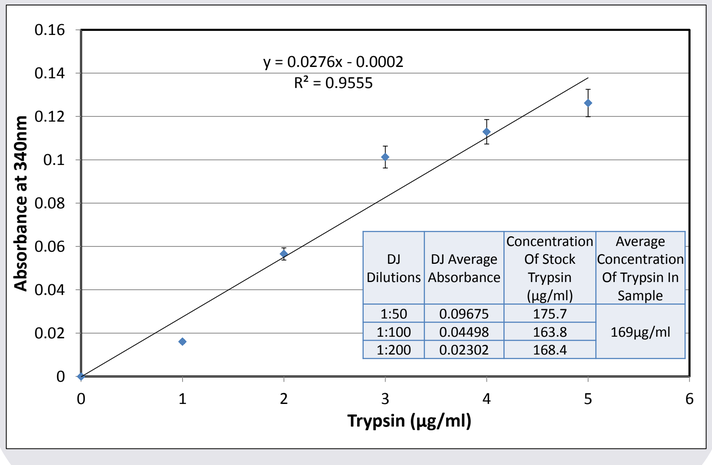
- A proteolytic activity assay was used to assess the total amount of active trypsin present within the duodenal sample. The assay was first modified to find the correct concentration of substrate to produce Vmax.
- A DGOR event was stimulated by applying a sample of duodenal juice to lung epithelial cells. The cells within the wells of the plate were then tested with varying concentrations of the duodenal sample, active trypsin and heat killed trypsin. The cell viability was then measured using the titer-blue cell viability assay (Promega) which determines the metabolically active cells as viable cells by measuring the absorbance shift of each well after exposure to a substrate. The absorbance shift from the stimulated cells was compared to that of the control well (=100% viable cells) and the dead cell control. As well as this the cell supernatant was collected from each well exposed to the different conditions and the immune response tested by IL-8 analysis of the supernatants with an ELISA kit (R&D Systems).

Results

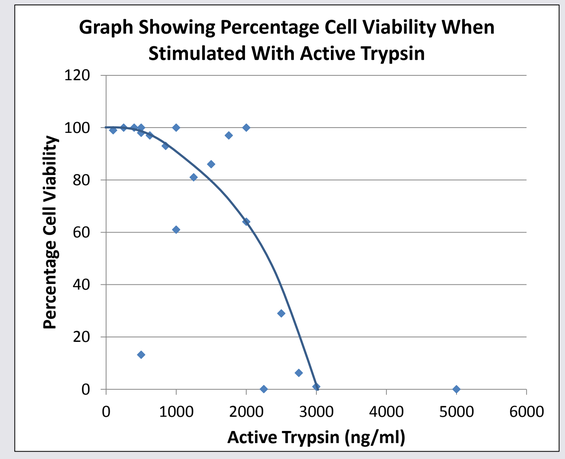
Activity Assay Optimisation. Using data from the Michaelis Menten plot the concentration of substrate succinyl albumin with which Km occurs is 8mg/ml on the basis of this the Vmax concentration used was 16mg/ml.



Activity Assay. An accurate standard graph was produced showing a linear relationship between increasing trypsin concentration and active trypsin present identified by in the increase in absorbance due more trypsin reacting with the albumin. Duodenal samples were then tested at various dilutions to calculate the amount of active trypsin present in the sample. Literature states that the trypsin concentration in duodenal juice can vary between 150 and 290µg/ml, with the results indicating that there was an average of 169µg/ml active trypsin present in the sample used.



Cell Viability Assay. Increasing the concentration of heat-killed trypsin was shown to have no effect on cell viability as the enzyme is degraded. Duodenal samples tested were shown to cause damage at dilutions of 1:300 and above. The effect of active trypsin on epithelial cells was looked at extensively and as shown on the graph, cell viability decreases slowly before an increasing rate of decrease, with no cells surviving at concentrations of 3000ng/ml and higher.



Conclusion

- The amount of active trypsin from a duodenal sample can be calculated to ascertain how much damage will occur in a reflux event
- Reflux events where the concentrations of trypsin is above 1000ng/ml will result in damage and above 3000ng/ml will cause cell death
- As a result patients who experience gastroduodenal reflux after lung transplantion are at risk of rejection due to the potential cell damage caused by trypsin. Patients may receive proton pump inhibitor therapy if acid reflux is though to cause BOS, but further studies are looking at other methods of treatment.

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