Study of Neurogenic Inflammation using Acute Pancreatitis as the Model System

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ABSTRACT

Acute pancreatitis is the inflammation of the pancreas and this common clinical condition is frequently associated with edema, pancreatic autodigestion, necrosis and possible hemorrhage. Patients with acute pancreatitis may develop acute lung injury, manifest clinically as the adult respiratory distress syndrome (ARDS). An in vivo model was employed to induce acute pancreatitis by supramaximal stimulation of the pancreas using the secretagogue caerulein. The effect of preprotachykinin-A (PPT-A) gene deletion in balb c mice in neurogenic inflammation was studied using acute pancreatitis as a model system. The severity of acute pancreatitis was determined by the measurements of plasma amylase, the lung and pancreatic water content and pancreatic myeloperoxidase activities. An increase in lung MPO activity suggesting neutrophil sequestration in the lung is employed as criteria for the determination of pancreatitis-associated lung injury. PPT-A gene deletion affords protection against pancreatic damage in acute pancreatitis evidenced by reduced hyperamylasemia, pancreatic water content (edema) and pancreatic MPO activity. Conversely, decreased lung MPO activity suggests that PPT-A gene deletion also provides almost complete protection against pancreatitis-associated lung injury.

INTRODUCTION

Acute pancreatitis, first described by Fitz (1889), is principally viewed as an autodigestion of the pancreas with extravasation of proteolytic enzymes and vasoactive mediators, leading to inflammation of contiguous tissues. It is a potentially fatal clinical condition and the mortality rate in severe episode of the disease is 30% - 50%. There are many similarities between the systemic response in acute pancreatitis and that in other conditions such as trauma, septicaemia, and severe burns, and therefore acute pancreatitis provides an excellent experimental and clinical model for the study of neurogenic inflammation. Although the exact mechanisms by which diverse aetiological factors induce an attack are unclear, common inflammatory and repair pathways are invoked once the disease process is initiated. If this inflammatory reaction is very strong, it leads to a systemic inflammatory response syndrome (SIRS) and multiorgan complications. An excessive SIRS leads to distant organ damage and multiple organ dysfunction syndrome (MODS). MODS associated with acute pancreatitis, is the primary cause of morbidity and mortality. The major component of MODS in acute pancreatitis is impaired lung function due to adult respiratory distress syndrome (ARDS). Patients with acute pancreatitis may develop acute lung injury, manifest clinically as the ARDS. Most patients who die during the early stages of severe acute pancreatitis die either with or because of this lung injury.

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Neurogenic inflammation is triggered by release of neuropeptides such as substance P, a neuropeptide expressed by preprotachykinin-A (PPT-A) gene is characterized by plasma extravasation and neutrophil infiltration. Substance P acts primarily but not exclusively through the neurokinin-1 (NK-1) receptors is involved in inflammation. It has known clinical importance in asthma and growing significance in pancreatic inflammation and pancreatitis-associated lung injury. Knockout mice, which lack NK-1 receptors are protected against acute pancreatitis and the associated lung injury, suggesting that substance P plays a crucial pro-inflammatory role in the regulation of the intensity of acute pancreatitis and pancreatitis-associated lung injury (Bhatia, 1998). Pancreatic edema occurs early in the development of acute pancreatitis, and the overall amount of fluid loss corresponds with disease severity (Grady et al., 2000). The tachykinin substance P is released from sensory nerves, binds to the neurokinin-1 receptor (NK1-R) on endothelial cells and induces plasma extravasation, edema, and neutrophil infiltration, a process termed neurogenic inflammation.

This project was a basic study on the direct effect of PPT-A gene deletion on acute pancreatitis and pancreatitis-associated lung injury based on previously established models for acute pancreatitis. An in vivo model was employed to induce acute pancreatitis by supramaximal stimulation of the pancreas using the secretagogue caerulein. The effect of preprotachykinin-A (PPT-A) gene deletion in balb c mice in neurogenic inflammation was studied using acute pancreatitis as a model system. The severity of acute pancreatitis was determined by the measurements of plasma amylase, the lung and pancreatic water content and pancreatic myeloperoxidase activities.

MATERIALS AND METHODS

Animal facilities in the Animal Holding Unit of the National University of Singapore were utilized for the accommodation of the commercially purchased PPT-A+/+ (wild type) and PPT-A−/− (null) balb c mice produced by targeted gene deletion before they were employed in the experiment. PPT-A+/+ and PPT-A−/− mice (n = 6) were given intraperitoneal injections of saline (control) or saline, which contained a supramaximally stimulating concentration of caerulein (50µg/kg/h each mouse) for 10h. Subsequently, the mice were sacrificed at three intervals, namely one hour after the third, sixth and last injections by carbon dioxide suffocation. The dissection of the mice were performed and mouse blood (plasma), pancreas and lung tissue were isolated simultaneously for each mouse for plasma amylase, water content and myeloperoxidase activities.

Determination of Pancreatic and Lung Water Contents

A portion of the pancreas and lung from each mouse was rapidly removed in plastic bowls and the wet weights of the pancreas and lung from the respective samples were recorded using Precisa XT 220 electronic weighing balance. Subsequently, the samples were dried at 50°C in the Memmert hot air oven over two days. Similarly, on day two and three of the experiment, the dry weights of the samples were measured. The pancreatic and lung water contents were then calculated.

Determination of Plasma Amylase Activities

Heparinized blood samples were centrifuged at 12 000rpm for 10min at 4°C using the Labnet microcentrifuge and respective plasma samples were collected. Using the Sigma Diagnostics
Amylase Reagent (Procedure No. 577), the amylase activities in the respective plasma samples were quantitatively determined from the amylase absorbances measured at 405nm between 1 min intervals (from the second to the fourth minute) at 37°C by Tecan SpectroFluro Plus spectrophotometer.

**Extraction of Myeloperoxidase (MPO) from Pancreatic and Lung Tissues**

A portion of the pancreas and lung from each mouse was rapidly extricated, placed into cryovials and deep frozen in liquid nitrogen. The tissues were treated with 1ml of 20 mM sodium phosphate buffer, pH 7.4 and homogenized using the Heidolph homogeniser. The milky suspensions of cell were then centrifuged at 13 000 rpm for 10 min at 4°C and the supernatant was removed. The cell pellet was retained and treated with 1ml of 50mM sodium phosphate buffer, pH 6.0 with detergent, hexadecyltrimethyl ammonium bromide (HDTMAB). The cells were resuspended and subjected to four rounds of freeze-thaw cycles. Subsequently, the cells were sonicated for 10s for four times. Another round of centrifugation was performed and the supernatant were extracted for MPO studies.

**Determination of Pancreatic and Lung MPO Activities**

50µL of neat SureBlue TMB 1-Component Microwell Peroxidase Substrate was added to 50µL of the supernatant in a 96-well microtitre plate. After 2min, 50µL of H₂SO₄ was added into the reaction cells and the MPO activity was read at 405nm using Tecan SpectroFluro Plus spectrophotometer. The absorbance reading was then corrected for the dry weight of the tissue sample used and results were expressed as activity per dry weight (fold increase over control).

**RESULTS AND DISCUSSION**

Statistical data were analyzed and presented as mean ± SEM, ANOVA analysis and t-tests. An interaction plot were generated for the comparison of plasma amylase increase at 3h, 6h & 10h intervals between PPT-A⁺/⁺ & PPT-A⁻/⁻ mice. The comparison of amylase activities between PPT-A⁺/⁺ and PPT-A⁻/⁻ at 10 h did not show a statistical significance. This may be due to the small number of mice (n = 6) per group employed for the experiment. Nevertheless, the ANOVA analysis and the comparison of amylase activities between PPT-A⁺/⁺ and PPT-A⁻/⁻ at 3 h and 6 h respectively did reveal a statistical significance (using t-tests, P < 0.001 and P < 0.01 respectively). Increasing amylase activities were observed in PPT-A⁺/⁺ mice, which signified the occurrence and intensity of pancreatic inflammation.

Increased water contents were observed in the pancreas and lung of PPT-A⁺/⁺ mice but water contents of pancreas and lung of PPT-A⁻/⁻ mice remain within the normal ranges. In fact, during the harvest of the pancreatic and lung tissues, it was noticed that the sizes of pancreas and lung of the caerulein-treated PPT-A null mice were apparently smaller as compared to that of the caerulein-treated PPT-A⁺/⁺ mice and mice treated with saline. Eventually, it was found that the pancreatic and the lung water contents of the caerulein-treated PPT-A null mice fall within the normal range and hence there was no development of edema. On the other hand, an increase in water contents or the presence of edema was determined in the caerulein treated PPT-A⁺/⁺ mice with large, swollen tissues. Hence, the size of the tissue corresponds to the degree to edema in mice. The difference between pancreatic water contents of the PPT-A⁺/⁺ and PPT-A⁻/⁻ mice at 10 h and the difference between lung water contents of PPT-A⁺/⁺ and PPT-A⁻/⁻ mice at 10 h had both
revealed a statistical significance (using t-tests, P < 0.05 respectively) indicating the presence of pancreatic and pulmonary edema in PPT-A^{+/+} mice.

A bar chart comparison of pancreatic MPO increment between PPT-A^{+/+} & PPT-A^{-/-} mice made. A similar comparison of lung MPO levels between PPT-A^{+/+} & PPT-A^{-/-} mice was drawn. Both the pancreas and the lung MPO activities increased gradually over 3, 6 and 10 h. However, the extent of MPO increment for pancreas was lesser than that of the lung. In fact, the MPO increment for PPT-A^{-/-} was found to be statistically insignificant (P < 0.05). The increase of MPO in the pancreas and lung of caerulein-treated PPT-A^{+/+} mice at 3, 6 and 10 h was found to be significant (using t-test, P < 0.001) as compared to the PPT-A^{-/-} mice. Elevated MPO levels with increasing number of caerulein injections in the lung and pancreas of PPT-A^{+/+} mice were determined, signifying the sequestration of neutrophils within the pancreas and lung, which corresponds to the degree of pancreatic inflammation and lung injury. Therefore, PPT-A gene deletion reduces the severity of acute pancreatitis and pancreatitis-associated lung injury.

CONCLUSION

This project provided an excellent opportunity for the familiarization with laboratory procedures and living experimental models. Substance P, the PPT-A gene product, in the pancreas and of NK1 receptors on pancreatic acinar cells in mice plays a vital pro-inflammatory role in acute pancreatitis and associated lung injury. PPT-A gene deletion in mice affords protection against acute pancreatitis-associated lung injury. In the long term, based on the results of these studies, the clinical anti-inflammatory therapy may become available.

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REFERENCES

