肋膜積液中纖維蛋白分解活性的研究

Studies on fibrinolytic activity in pleural effusions

中文摘要

肋膜積液是臨床上常見的問題，也是許多疾病常有的併發症。目前研究觀察到不同病因的肋膜積液中，其纖維蛋白分解系統都有調控異常的現象。肋膜積液中的纖維蛋白分解活性受抑制，將導致肋膜腔產生纖維蛋白沉積及肋膜沾黏，形成腔室化肋膜積液(loculated effusion)及肋膜纖維化(pleural fibrosis)，進而損害病患的肺功能；這些現象與肋膜發炎、細胞激素及肋膜細胞的交互作用有關，但其調控機轉目前尚未完全釐清。

本研究分四個子題進行，第一，腔室化及自由流動之漏出性肋膜積水中細胞激素與纖維蛋白分解活性的比較分析；第二，多次胸腔穿刺引流對滲出性肋膜積水中細胞激素與纖維蛋白分解活性的影響；第三，肋膜腔注射纖維蛋白分解酵素對腔室化結核性肋膜炎產生肋膜纖維化及臨床預候的影響；第四，Dynasore，一種可滲透入細胞之 dynamin 抑制劑，對於 TGF-β1 誘發 MeT-5A 人類肋膜間皮細胞株產生 PAI-1 之影響。希望研究結果有助於釐清纖維蛋白分解活性失調的病理機轉並裨益病患的照護。

第一個子題的研究結果顯示，在包括肺炎、結核或惡性腫瘤所造成的漏出性肋膜積液中，相較於自由流動之肋膜積液，腔室化積液的纖維蛋白分解活性被更明顯抑制。腔室化積液中的 TNF-α, IL-1β, TGF-β1 及 PAI-1 的濃度皆明顯高於自由流動之肋膜積液；而不論是腔室化或自由流動積液，其 PAI-1 的濃度及 PAI-1/tPA 比值皆與 TNF-α, IL-1β及 TGF-β1 的濃度呈明顯正相關。肋膜腔中 PAI-1 與 tPA 的不平衡，將導致纖維蛋白沉積及肋膜沾黏，形成肋膜積液腔室化。此外，結核性肋膜積液最後產生殘餘性肋膜纖維化者，其肋膜積液中的 TGF-β1 的濃度明顯高於未產生殘餘性肋膜纖維化者，此結果顯示 TGF-β1 在結核性肋膜炎產生肋膜纖維化扮演重要角色。

第二個子題的研究結果顯示,對於心臟衰竭合併滲出性肋膜積水患者，多次胸腔穿刺引流(連續三天)會導致肋膜積液中的 LDH 值、 紅血球數、白血球數、嗜中性白血球、TNF-α、 IL-1β、 IL-8、 VEGF 及 PAI-1 濃度顯著地增加。且第二天及第三天穿刺引流所得的肋膜積液中，PAI-1 及 PAI-1/tPA 比值與 TNF-α、 IL-1β、 IL-8 及 VEGF 的濃度呈現明顯正相關。多次胸腔穿刺引流可能會誘發滲出性肋膜積水產生肋膜發炎、抑制其纖維蛋白分解活性，導致纖維蛋白沉積。此外，多次胸腔穿刺引流後形成肋膜纖維蛋白沉積之患者，其七日內肋膜積液未吸收的機率明顯偏高，因此推論纖維蛋白分解活性被抑制，可能阻礙肋膜積液的吸收。

第三個子題的研究結果顯示, 肋膜腔注射纖維蛋白分解酵素 streptokinase，提高肋膜腔內纖維蛋白分解活性，可分解清除纖維蛋白，安全且有效地引流出腔室化

結核性肋膜積液，配合抗結核藥物治療，可加速肋膜積液的吸收、減少產生肋膜纖維化的機率、並長期改善病患的肺功能。
第四個子題的研究結果顯示，dynasore 可藉著活化 non-Smad TGF-β訊息路徑，來加強 TGF-β1 誘發 PAI-1 在 MeT-5A 人類肋膜間皮細胞的表現。而且，dynasore 本身可活化 JNK MAPK，導致 Smad4 的核轉移(nuclear translocation)，促進 PAI-1 的基因表現及蛋白合成。本研究的初步報告認爲，dynasore 雖然可以抑制與 dynamin 有關的 endocytosis，卻反而會促進 TGF-β/JNK 的訊息傳遞，進而增加 PAI-1 的表現。
簡要言之，本研究顯示，肋膜發炎反應可抑制肋膜積液中纖維蛋白分解活性，導致肋膜腔中 PAI-1 與 tPA 的不平衡，形成纖維蛋白沉積、肋膜積液腔室化及肋膜纖維化。纖維蛋白分解活性被抑制，可能阻礙肋膜積液的吸收；而提高纖維蛋白分解活性，可以促進肋膜積液的吸收，並減少肋膜纖維化。Dynaosre 可促進肋膜間皮細胞內 PAI-1 的基因表現及蛋白合成，臨床上或許可作爲肋膜沾粘(pleurodesis)的藥劑。仍需要進行更多細胞、動物及人體研究，探討肋膜腔中細胞激素、發炎細胞及肋膜間皮細胞之間的交互作用，才能更深入了解肋膜積液中纖維蛋白分解活性的調控與臨床上的意義。

英文摘要

Pleural effusion is a common clinical problem and can occur as complications of many different diseases. It is well recognized that the fibrinolytic system does not function properly in the pleural space in patients with pleural effusions of various etiologies. The impaired fibrinolytic activity contributes to the development of pleural fibrin deposition, loculated effusions and pleural fibrosis, of which the pathogenesis involves interactions among pleural cytokines, inflammatory cells and pleural mesothelial cells. However, the regulatory mechanism of fibrinolytic activity in pleural effusions remains incompletely understood.
In this study, we would like to explore (1) the difference in levels of cytokines and fibrinolytic activity between loculated and free-flowing pleural exudates; (2) the effect of repeated thoracenteses on cytokines and fibrinolytic activity in pleural transudates; (3) the effect of intrapleural streptokinase on pleural fibrosis and clinical outcome in patients with loculated TB pleurisy; and (4) the effect of dynasore, a cell permeable dynamin inhibitor, on TGF-β1-induced PAI-1 expression in MeT-5A human pleural mesothelial cells.
We hope the results obtained can be of value in elucidating the pathogenesis of impaired fibrinolytic activity in pleural diseases and in the management of the patients with large transudative effusions, loculated pleural exudates and pleural fibrosis.
The results of the first study on pleural exudates caused by malignancy, TB or pneumonia indicated that fibrinolytic activity was depressed in loculated effusions,

compared to free-flowing effusions. The levels of TNF-α, IL-1β, TGF-β1 and PAI-1 were significantly higher in loculated than in free-flowing effusions. In both loculated and free-flowing effusions, the levels of PAI-1 and the PAI-1/tPA ratio correlated positively with those of TNF-α, IL-1β and TGF-β1. The imbalance of PAI-1 and tPA in pleural spaces may lead to fibrin deposition and loculation of pleural effusions. In addition, the pleural levels of TGF-β1 were significantly higher in those with residual pleural thickening, suggesting that TGF-β may play a role in the development of pleural fibrosis in TB pleurisy.

The result of the second study demonstrated that significant increase in amount of LDH, erythrocytes, total leukocytes, neutrophils, TNF-α, IL-1β, IL-8, VEGF and PAI-1 in the pleural fluid after repeated thoracenteses in patients with transudative pleural effusions caused by congestive heart failure. The amount of PAI-1 and PAI-1/tPA ratio on day 2 and day 3 in the pleural fluid were highly correlated with those of TNF-α, IL1-β, IL-8 and VEGF. Repeated thoracenteses may cause pleural inflammation, impair fibrinolytic activity and lead to fibrin deposition in transudative effusions. Moreover, the patients who developed pleural fibrins had significantly lower rate of complete resolution of pleural effusions by 7 days after repeated thoracenteses, which suggests that impaired fibrinolytic activity may impede resolution of pleural effusion.

The result of the third study on patients with loculated TB effusions revealed that tube drainage with streptokinase irrigation may increase the fibrinolytic activity in the pleural space, increase clearance of pleural fibrins and is safe and effective for evacuation of loculated TB effusions. Effective drainage adjuvant to anti-TB treatments may hasten resolution of pleural effusion, reduce the incidence of residual pleural fibrosis and accelerate recovery of pulmonary function in patients with loculated TB pleurisy.

The result of the fourth study revealed that dynasore may enhance PAI-1 expression induced by TGF-β1 in MeT-5A human pleural mesothelial cells through the activation of non-Smad TGF-β signaling pathway. Dynasore may activate JNK MAPK and/or lead to nuclear translocation of Smad4, and increase PAI-1 gene expression and protein synthesis in MeT-5A cells. The preliminary data of the study indicate that dynasore, a potent inhibitor of endocytic pathways known to depend on dynamin, does not inhibit but, on the contrary, augment the activation of TGF-β/JNK signaling and amplify PAI-1 expression. Further studies are needed to verify the role of receptor trafficking in the regulation of TGF-β signaling in human pleural mesothelial cells.

In summary, the impaired fibrinolytic activity in pleural effusions, as reflected by higher PAI-1 to tPA ratio, depends on pleural inflammation and is very important in the development of fibrin deposition, fluid loculation and residual fibrosis in pleural

spaces. Impaired fibrinolytic activity may impede resolution of pleural effusion, whereas enhanced fibrinolytic activity may facilitate clearance of pleural effusion and prevent pleural fibrosis. Dynasore enhances PAI-1 expression in human pleural mesothelial cells and may be used as a pleurodesing agent. More cellular, animal and human studies on interactions among cytokines, inflammatory cells, and mesothelial cells in the pleural space are needed for elucidation of fibrinolytic activity in pleural effusions and the clinical significance.