巴拉刈誘發人類肺纖維母細胞株 MRC-5 產生膠原蛋白之分子機制

The Molecular Mechanism of Paraquat-induced Collagen Production in Human Lung Fibroblast MRC-5 Cells

中文摘要

巴拉刈（Paraquat, 1,1'-dimethyl-4,4'-bipyridium ; methyl viologen）爲仿間常用之除草劑，根據台灣地區藥物諮詢檢驗中心的統計，巴拉刈中毒的致死率爲所有中毒物質中最高者(Chen et al., 2005)。因此，本論文以人類正常肺纖維母細胞 MRC-5 爲細胞模式，排除巴拉刈之腎毒性造成腎素-血管張力素系統之影響，來探討巴拉刈導致肺臟纖維化之機制。

由結果顯示，巴拉刈刺激 MRC-5 細胞誘導膠原蛋白表現是受到血管張力素 II 的調控，藉由 real-time RT-PCR 發現細胞內血管張力素轉化?第一型、第二型基因表現均下降，但糜蛋白?(chymase)的基因表現卻上升，因而推測 chymase 可能是扮演催化血管張力素 II 生成的角色，之前的報導均認爲 chymase 是由 mast cell 所分泌，這是首次發現纖維母細胞亦能分泌 chymase 並調控血管張力素 II 之產生，而血管張力素 II 的表現會被 chymase 抑制劑(chymostatin)所抑制。

另一方面，細胞在給予巴拉刈刺激後會進一步誘發其血管張力素 II 第一型受體蛋白(angiotensin II type I receptor, AT1R)表現及活化其下游的甲型轉型細胞生長因子與結締組織生長因子之基因與蛋白表現；我們亦發現非選擇性血管張力素 II 受體抑制劑(saralasin)確實會抑制細胞內甲型轉型細胞生長因子、結締組織生長因子與膠原蛋白的表現。此外，實驗結果發現巴拉刈所誘導膠原蛋白增加僅促進細胞內 cathepsin L 的基因表現增加及酵素活性，加入 cathepsin L 抑制劑(Z-Phe-Phe-CH2F)證實 cathepsin L 確實扮演水解膠原蛋白的角色，以維持其細胞內膠原蛋白之恆定性。

綜合以上實驗結果，本論文發現巴拉刈會藉由 chymase 來催化人類正常肺臟纖維母細胞(MRC-5)內血管張力素 II 的產生，同時藉活化其下游路徑之 TGF-β1、CTGF 表現並誘導膠原蛋白的生合成。

英文摘要

Paraquat dichloride (1,1-dimethyl-4,4-bipyridilium dichloride; methyl viologen) is an effective and widely used herbicide. According to epidemiological evidence in the National Poison Center in Taiwan, paraquat poisoning has suggested that was the leading cause of poisoning-induced death in Taiwan. In this study, the normal human fetal lung fibroblast cell line (MRC-5) was used to study the mechanism of paraquat-induced pulmonary fibrosis for eliminating the effect of paraquat nephrotoxicity on renin-angiotensin system (RAS).

The results showed that paraquat-induced collagen expression in MRC-5 was upregulated by angiotensin (ANG) II. However, the responsible enzyme for the conversion of ANG I to ANG II was chymase and the up-secretions of ANG II and collagen were inhibited by chymostatin.
On the other hand, this study also demonstrated the induction of transfoming growth factor (TGF)-β1 and connective tissue growth factor (CTGF) by paraquat was mediated via activation of the angiotensin II type 1 receptor (AT1R) and inactivation of the angiotensin II type 2 receptor (AT2R). The unselective AT1R and AT2R antagonist saralasin also attenuated the increases in TGF-β1, CTGF and collagen synthesis induced by paraquat administration in MRC-5. Furthermore, we also showed that paraquat-induced collagen was hydrolyzed by cathepsin L in MRC-5 cell.
We concluded that ANG II induced-collagen overexpression in paraquat-administrated lung fibroblast was mediated via the activity of chymase rather than angiotensin I converting enzyme I.