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• 計畫英文名稱	Analysis of the Signal Transduction in the Induction of Cyclo- Oxygenase-2 by Lipopolysaccharide in Human Airway Epithelial Cells
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• 研究人員	林建煌 Lin, Chien-Huang
• 中文關鍵字	內毒素；環氧酵素；上皮細胞；訊息傳遞；脂多醣；呼吸道
• 英文關鍵字	Endotoxin；Cyclooxygenase；Epithelial cell；Signal transduction；Lipopolysaccharide (LPS)；Respiratory tract
• 中文摘要	Lipopolysaccharide 可引發人類肺臟上皮細胞(A549)釋放 IL-1.beta.或 TNF-.alpha.等細胞激素。本研究主要探討 IL-1.beta.引發人類肺臟上皮細胞 COX-2 表現及 PGE/sub 2/釋放的訊息傳遞路徑。IL-1.beta.以劑量及時間相關的方式刺激 COX-2 表現及 PGE/sub 2/釋放。Tyrosine kinase 抑制(Genistein 及 Tyrphostin AG126)和 PC-PLC 抑制劑(D-609)可抑制 IL-1.beta.引發 COX-2 表現及 PGE/sub 2/釋放;而 PI-PLC 抑制劑 U73122 和 Phosphatidate phosphoydrolase 抑制劑 Propranolol 則不影響 IL-1.beta.所引發之反應。兩種 PKC 抑制劑(Ro 31-8220 和 Go 6976)也抑制 IL-1.beta.引發之 COX-2 表現及 PGE/sub 2/釋放。由 Western bloting 之分析發現 A549 細胞存在有 PKC-.alpha.,-.gamma.,-.iota.,-.lambda.,-.mu.,和-.zeta.六種 isoforms。以 IL-1.beta.刺激 A549 細胞發現在六種 isoforms 中只有 PKC-.gamma.會從細胞質轉位到細胞核。而 Genistein 及 D-609 可抑制 IL-1.beta.所引發之 PKC-.gamma.轉位,但 U-73122 則不影響 IL-1.beta.所引發之反應。 Ras 活化的抑制劑(FPT inhibitor II)、MEK 抑制劑(PD 98059)和 p38 MAPK 抑制劑(SB 203580)可抑制 IL-1.beta.所引發之 COX-2 表現及 PGE/sub 2/釋放;但 PI-3 kinase 抑制劑 Wortmannin 則不影響 IL-1.beta.所引發之反應。以 IL-1.beta.刺激 A549 細胞可引發 p44/42 及 p38 MAPK 的活化,加入 genistein,Go 6976,Ro 31-8220,FPT inhibitor II,PD 98059 及 SB 203580,發現除 FPT inhibitor II 及 PD 98059 可抑制 p44/42 MAPK 的活化外,其餘的抑制劑均不會抑制 p44/42 MAPK 的活化。另外,Go 6976 及 FPT inhibitor II 亦不會抑制 p38 MAPK 的活化。 NF-kB 抑制劑 PDTC 可抑制 IL-1.beta.所引發之 COX-2 表現及 PGE/sub 2/釋放。而 IL-1.beta.可刺激 p65 NF-kB 從細胞質轉位到細胞核,及造成 IkB-.alpha.的分解。加入 Genistein,Go 6976,FPT inhibitor II,PD 98059,SB 203580 和 PDTC 發現其均可抑制 p65 NF-kB 的轉位。因此在 A549 細胞中,IL-1.beta.刺激 COX-2 表現及 PGE/sub 2/生成的訊號傳遞路徑至少有三條路徑,可分爲:(一) IL-1.beta.作用

	於細胞膜上的受體,而活化 Tyrosine kinase,使得 PC-PLC 活化而產生 DAG,DAG 再促使 PKC-.gamma.活化,再進一步活化 NF-kB,最後引發 COX-2 表現及 PGE/sub 2/的生成。(二)IL-1.beta.作用於細胞膜上的受體,而活化 Ras,使得 p44/42 MAPK 活化,再進一步活化 NF-kB,最後引發 COX-2 表現及 PGE/sub 2/的生成。(三)IL-1.beta.作用於細胞膜上的受體,經由未知的機制活化 p38 MAPK,再進一步活化 NF-kB,最後引發 COX-2 表現及 PGE/sub 2/的生成。
• 英文摘要	The signal transduction pathway of IL-1.beta.-induced cyclooxygenase-2 (COX-2) expression and prostaglandin E/sub 2/(PGE/sub 2/) release was studied in human pulmonary epithelial cell line (A549). IL-1.beta. caused a concentration- and time-dependent increase in PGE/sub 2/ formation and COX-2 expression. The tyrosine kinase inhibitors (genistein and tyrphostin AG126) and phosphatidylcholine-phospholipase C (PC-PLC) inhibitor (D-609) prevented IL-1.beta.-induced PGE/sub 2/ release and COX-2 expression, while U-73122 (a phosphatidylinositol-phospholipase C inhibitor) and propranolol (a phosphatidate phosphohydrolase inhibitor) had no effect. The PKC inhibitors (Go 6976 and Ro 31-8220) also attenuated IL-1.beta.-induced PGE/sub 2/ release and COX-2 expression. Western blot analysis using PKC isoenzyme-specific antibodies indicated that A549 cells expressed PKC-.alpha., -.gamma., -.iota., -.lambda., -.zeta., and -.mu.. Treatment of A549 cells with IL-1.beta. caused the translocation of PKC-.gamma. but not other isoforms from cytosol to the membrane fraction, indicating activation of the PKC-.gamma. isoform. Moreover, the IL-1.beta.-induced PKC-.gamma. translocation was inhibited by genistein or D-609, but not by U-73122. The IL-1.beta.-mediated PGE/sub 2/ release and COX-2 expression were also inhibited by FPT inhibitor II, MEK inhibitor (PD 98059) and p38 MAPK inhibitor (SB 203580) but not by wortmanin (a PI-3 kinase inhibitor). Treatment of A549 cells with IL-1.beta. caused the activation of both p44/42 and p38 MAPK. Moreover, the IL-1.beta.-induced p44/42 MAPK activation was inhibited by FPT inhibitor II or PD 98059, but not by genistein, Go 6976, or SB 203580. The IL-1.beta.-induced p38 MAPK activation was not affected by FPT inhibitor or Go 6976. The NF-kB inhibitor, pyrolidine dithiocarbamate (PDTC), attenuated IL-1.beta.-induced PGE/sub 2/ release and COX-2 expression. IL-1.beta. caused the translocation of p65 NF-kB from cytosol to the nucleus as well as the degradation of IkB-.alpha. in cytosol. Furthermore, the translocation of p65 NF-kB was inhibited by genistein, Go 6976, FPT inhibitor II, PD 98059, SB 203580 or PDTC. These data indicate that in pulmonary epithelial cells, IL-1.beta. has at least four distinct signaling pathways in regulating COX-2 expression and PGE/sub 2/ release. First, IL-1.beta. may activate PC-PLC through an upstream tyrosine phosphorylation to elicite PKC-.gamma. activation, which in turn initiates NF-kB activation, and finally induces COX-2 expression and PGE/sub 2/ release. Second, IL-1.beta. may activate Ras to elicite p44/42 MAPK activation, which in turn initiates NF-kB activation, and finally induces COX-2 expression and PGE/sub 2/release. Third, IL-1.beta. may activate p38 MAPK through an unknown pathway to initiate NF-kB activation, and finally induces COX-2 expression and PGE/sub 2/ release.