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| • 系統編號 | RC9008-0066 |
| • 計畫中文名稱 | 革蘭氏陽性菌細胞壁成分 Lipoteichoic Acid 引發上皮細胞環氧酵素-2 表現之訊號傳遞路徑探討 |
| • 計畫英文名稱 | Studies on the Expression of Cyclooxygenase-2 Protein Induced by Lipoteichoic Acid from Gram-Positive Organisms in Human Airway Epithelial Cells |
| • 主管機關 | 行政院國家科學委員會 |
| • 計畫編號 | NSC89-2320-B038-031 |
| • 執行機構 | 台北醫學院醫事系 |
| • 本期期間 | 8808 ~ 8907 |
| • 報告頁數 | 5 頁 |
| • 使用語言 | 中文 |
| • 研究人員 | 林建煌 Lin, Chien Huang |
| • 中文關鍵字 | 脂磷壁質酸；環氧酵素-2；訊息傳遞；人類呼吸道上皮細胞 |
| • 英文關鍵字 | Lipoteichoic acid (LTA)；Cyclooxygenase-2 (COX-2)；Signal transduction；Human airway epithelial cell |
| • 中文摘要 | 本論文主要在探討革蘭氏陽性菌細胞壁成分 lipoteichoic acid (LTA)刺激人類肺臟上皮細胞(A549) cyclooxygenage (COX)活性增加及 COX-2 表現的訊號傳遞路徑。LTA 以濃度相關的方式刺激 prostaglandin E/sub 2/ (PGE/sub 2/)的釋放、COX 活性的增加及 COX-2 的表現。當以 LTA 處理不同的時間，在外加 arachidonic acid (30 μM, 30 min)的情況下，發現 LTA 以時間相關的方式引發 COX 活性增加和 COX-2 的表現。而 dexamethasone、蛋白轉錄抑制劑 actinomycin D 和蛋白轉譯抑制劑 cycloheximide 可抑制 LTA 所引發之 COX 活性增加及 COX-2 的表現，然而內毒素的抑制劑 polymyxin B 則不影響 LTA 所引發之反應。PC-PLC 抑制劑 D-609 和 phosphatidate phosphohydrolase 抑制劑 propranolol 可抑制 LTA 所引發之 COX 活性增加及 COX-2 表現，然而 PI-PLC 抑制劑 U-73122 則不影響 LTA 所引發之反應。Go 6976、Ro 31-8220 和 GF 109203X 三種 PKC 抑制劑顯著地抑制 LTA 所引發之 COX 活性增加及 COX-2 的表現。Ca/sup 2+/ 的螯合劑 BAPTA 也抑制 LTA 所引發之 COX 活性增加及 COX-2 的表現。先前的報告已證實 A549 細胞存在有 PKC-α,-γ,-ι,-λ,-ζ,-μ 六種同功酵素。當以 LTA 刺激 A549 細胞發現在六種 PKC isoforms 中只有 PKC-α 和-γ 會從細胞質轉位到細胞核，這結果暗示 PKC-α 和-γ 可能包含在 LTA 引發 COX-2 表現的訊號傳遞路徑。NF-κB 抑制劑 pyrrolidine dithiocarbamate (PDTC)可抑制 LTA 所引發之 COX 活性增加及 COX-2 的表現。以 LTA 刺激細胞 10 分鐘可使 p65NF-κB 由細胞質轉位至細胞核，亦會造成 1κB-α 在細胞質的分解，兩者反應皆在 60 分鐘後明顯地減少。Electrophoretic mobility shift assay (EMSA)的結果也發現 LTA 可使 NF-κB 的活性隨作用時間而增加，於 10 分鐘時達最大反應，但 60 分鐘後反應明顯地減少，當加入 D-609，U-73122，Go 6976、Ro 31-8220 或 PDTC，發現這些抑制劑，皆會抑制 |

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| | LTA 刺激，NF-κB 活性的增加，表示 LTA 刺激 NF-κB 活性的增加可受到上游 PC-PLC、PC-PLD 及 PKC 的調控。綜合以上的結果得知，在 A549 細胞中，LTA 可經由活化 PC-PLC 及 PC-PLD 的路徑引發 PKC 的活化，並進而引發 NF-κB 的活化，最後導致 COX-2 的表現及 PGE/sub 2/的釋放。 |
| • 英文摘要 | The signal transduction pathway of lipoteichoic acid (LTA)-induced increase of cyclooxygenase (COX) activity and COX-2 expression was studied in human pulmonary epithelial cell line (A549). LTA caused a concentration-dependent increase in COX activity, PGE/sub 2/ accumulation, and COX-2 expression. The LTA-induced increase in COX activity was measured by the formation of PGE/sub 2/ in the presence of arachidonic acid (30 μM; 30 min). LTA also caused a time-dependent increase in the COX activity and COX-2 expression. Dexamethasone, actinomycin D, and cycloheximide inhibited the LTA-induced increase in COX activity and COX-2 expression. Polymyxin B, an agent which binds and inactivates endotoxin, did not affect LTA-induced increase in COX activity and COX-2 expression. The phosphatidylcholine-phospholipase C inhibitor (D-609) and phosphatidate phosphohydrolase inhibitor (propranolol) prevented the LTA-induced increase in COX activity and COX-2 expression, while U-73122 (a phosphatidylinositol-phospholipase C inhibitor) had no effect. The PKC inhibitors (Go 6976, Ro 31-8220 and GF 109203X) and Ca/sup 2+/ chelator (BAPTA) also attenuated the LTA-induced increase in COX activity and COX-2 expression. In our previous studies have demonstrated that A549 cells expressed PKC-α, -γ, -ι, -λ, -ζand -μ. Treatment of A549 cells with LTA caused the translocation of PKC-α and -γ but not other isoforms from cytosol to the membrane fraction, indicating activation of the PKC-α and -γ isoforms. Moreover, the NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC), attenuated the LTA-induced increase in COX activity and COX-2 expression. Treatment of A549 cells with LTA for 10 min resulted in the translocation of p65 NF-κB from cytosol to the nucleus as well as the degradation of Iκ-Bα in the cytosol. Treatment of A549 cells with LTA caused NF-κB activation by detecting the formation of NF-κB-specific DNA-protein complex in the nucleus; this effect was inhibited by dexamethasone, D-609, propranolol, Go 6976, Ro 31-8220, or PDTC. Taken together, these results suggest that LTA might activate PC-PLC and phosphatidylcholine-phospholipase D to induce PKC activation, which in turn initiates NF-κB activation, and finally induces COX-2 expression and PGE/sub 2/ release in human airway epithelial cell line. |