革蘭氏陽性菌細胞壁成分 lipoteichoic acid 引發上皮細胞環氧酵素-2 表現的訊號傳遞路徑之探討

The Signaling Pathway Involved in Lipoteichoic Acid-Induced Cyclooxygenase-2 Expression in Human Pulmonary Epithelial Cells.

中文摘要

本論文主要在探討 lipoteichoic acid (LTA) 刺激人類肺臟上皮細胞 (A549) cyclooxygenase (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 E2 (PGE2)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 acd (30 mM, 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。Ca2+ 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-a, -g, -i, -l, -z, -m 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-a 和-g 會從細胞質轉位到細胞核，這結果暗示 PKC-a 和 -g 可能包含在 LTA 引發 COX-2 表現的訊號傳遞路徑。Adenylate cyclase 抑制劑 2,5-dideoxyadenosine (DDA) 及 PKA 抑制劑 KT-5720 和 H-8 可抑制 LTA 所引發之 COX 活性增加及 COX-2 表現。Tyrosine kinase 抑制劑 genistein 及 tyrphostin AG126 可抑制 LTA 所引發之 COX 活性增加及 COX-2 的表現。MEK 抑制劑 PD 98059 和 p38 MAPK 抑制劑 SB 203580 亦可抑制 LTA 刺激 COX-2 活性增加及 COX-2 的表現。LTA 以劑量及時間相關的方式引發 p44/42 MAPK 之活化，當加入 genistein、Ro 31-8220、SB 203580、PD 98059 或 KT-5720，發現 genistein 可部分抑制 LTA 所引發之 p44/42 MAPK 的活化，PD 98059 幾乎可完全的抑制 LTA 的作用，但 Ro 31-8220、SB 203580 及 KT-5720 這些抑制劑則皆不會影響 LTA 的作用，表示 LTA 所引發之 p44/42 MAPK 的活化可受到上游 tyrosine kinase 之調控，但並不會受到 PKC 、 PKA 及 p38 MAPK 的調控。LTA 也以劑量及時間相關的方式引發 p38 MAPK 活性的增加，當加入 Ro 31-8220 、 genistein 、 PD 98059、 SB 203580 或 KT-5720，發現這些抑制劑，除了 PD 98059 不影響 LTA 所引發之 p38 MAPK 活性的增加，其他抑制劑則皆有抑制作用，表示 LTA 刺激 p38 MAPK 的活性增加可受到上游 PKC、tyrosine kinase 及

PKA 之調控，但並不會受到 MEK 的調控。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 p65 NF-κB 由細胞質轉位至細胞核，亦會造成 IκB-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 Go 6976、Ro 31-8220、PDTC、KT-5720、genistein、PD 98059 或 SB 203580，發現這些抑制劑，除了 KT-5720 不影響 LTA 所刺激 NF-κB 活性的增加，其他抑制劑則皆有抑制作用，表示 LTA 刺激 NF-κB 活性的增加可受到上游 PKC、tyrosine kinase、MEK 及 p38 MAPK 之調控，但並不會受到 PKA 的調控。綜合以上的結果得知，在 A549 細胞中，LTA 至少經由三條訊號傳遞路徑調控 COX-2 的表現。

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 the accumulation of PGE2, increase of COX activity, and increase of COX-2 expression. The increase of COX activity in LTA-activated A549 cells was measured by the formation of PGE2 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 LTA-induced accumulation of COX activity and COX-2 expression. Polymyxin B, an agent which binds and inactivates endotoxin, did not affect LTA-induced increase of COX activity and COX-2 expression. The phosphatidylcholine-phospholipase C inhibitor (D-609) and phosphatidate phosphohydrolase inhibitor (propranolol) prevented LTA-induced increase of 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 Ca2+ chelator (BAPTA) also attenuated LTA-induced increase of COX activity and COX-2 expression. In our previous studies have demonstrated that A549 cells expressed PKC-α, -γ, -ι, -λ, -z and -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. In addition, the adenylate cyclase inhibitor, 2,5-dideoxyadenosine (DDA), and protein kinase A inhibitors, KT-5720 and H-8, prevented LTA-induced increase of COX activity and COX-2 expression. The LTA-induced the increase of COX activity and COX-2 expression were also inhibited by tyrosine kinase inhibitors (genistein and tyrphostin AG126). The MEK inhibitor (PD 98059) and p38 MAPK inhibitor (SB 203580) also prevented LTA-induced increase of COX activity and COX-2 expression.

LTA caused a concentration- and time-dependent activation of p44/42 MAPK. Moreover, the LTA-induced p44/42 MAPK activation was inhibited by genistein or PD 98059, but not by Ro 31-8220, SB 203580 and KT-5720. LTA caused a concentration- and time-dependent increase in p38 MAPK activity. The LTA-induced p38 MAPK activation was inhibited by Ro 31-8220, genistein, SB 203580 and KT-5720, but not by PD 98059. Moreover, the NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC), attenuated LTA-induced increase of 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. NF-κB binding to DNA-protein was also enhanced by LTA. Go 6976, Ro 31-8220, PDTC, genistein, PD 98059 and SB 203580 all inhibited the DNA-protein binding activity stimulated by LTA, while KT-5720 had no effect. Taken together, these data indicate that in pulmonary epithelial cells, LTA regulates COX-2 expression by at least three distinct signaling pathways.