cGMP 調控人類肺臟上皮細胞環氧酵素-2 表現的機制探討

Mechanisms Underlying the cGMP-Mediated Cyclooxygenase-2 Expression in Human Pulmonary Epithelial Cells (A549)

中文摘要

YC-1 [3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole]為一種 soluble guanylate cyclase (sGC)活化劑，其可以刺激細胞內 cGMP 濃度的增加。本論文主要是探討 YC-1 引發人類肺臟上皮細胞(A549)環氧酵素-2 (cyclooxygenase-2, COX-2)表現之訊息傳遞路徑。YC-1 以濃度及時間相關方式刺激 COX 活性增加及 COX-2 表現。我們以 sGC 抑制劑 1H-(1,2,4)oxadiazolo[4,3-a]quinozalin-1-one (ODQ)、protein kinase G (PKG)抑制劑 KT-5823 或 protein kinase C (PKC)抑制劑 Go 6976 及 GF109203X 預先處理細胞，皆可抑制 YC-1 誘發 COX 活性增加及 COX-2 的表現。另外，YC-1 也可刺激 A549 細胞中 PKC 活性的增加，此效應也可被 ODQ、KT-5823 或 Go 6976 所抑制。利用西方墨點法，我們發現在 A549 細胞中存在有 PKC-α, -ι, -λ, -ζ 及 -μ 五種異構?(isoforms)。以 YC-1 或 phorbol 12-myristate 13-acetate (PMA)刺激 A549 細胞後發現只有 PKC-α 會從細胞質轉位到細胞膜上，其它的異構?則不會有此轉位的現象。而以 PMA 長時間(24 小時)處理細胞後會進一步造成 PKC-α 表現下降。MAPK/ERK kinase (MEK)抑制劑 PD 98059 (10~50 μM)可以濃度相關方式抑制 YC-1 所引發之 COX 活性增加及 COX-2 的表現。以 YC-1 刺激 A549 細胞可導致 p44/42 mitogen-activated protein kinase (MAPK)的活化，而 KT-5823、Go 6976、PD 98059 或長時間(24 小時)之 PMA 處理，發現皆會抑制 p44/42 MAPK 的活化，但是 p38 MAPK 抑制劑 SB 203580 則不具有抑制的作用。由以上的結果顯示，在 A549 細胞中，YC-1 先藉由活化 sGC/cGMP/PKG 路徑之後使 PKC-α 轉位活化，接著導致 p44/42 MAPK 的活化，最終引發 COX-2 蛋白表現。

我們接下來繼續探討在 A549 細胞中，Ras、phosphoinositide-3-OH-kinase (PI3K)、Akt 及轉錄因子 nuclear factor-κB (NF-κB)在 YC-1 誘發 COX-2 蛋白表現的訊號傳遞機制中所扮演的角色。我們發現 Ras 抑制劑 manumycin A、PI3K 抑制 wortmannin、Akt 抑制劑 1L-6-Hydroxymethyl-chiro-inositol 2-[(R)-2-O-methyl-3-O-octadecyl carbonate]及 NF-κB 抑制劑 pyrrolidine dithiocarbamate (PDTC)皆可以抑制 YC-1 引發 COX-2 表現。在 YC-1 引發 COX 活性的增加也會被 manumycin A、wortmannin 及 PDTC 等抑制劑或是 Ras、Akt 及 IκBα 的顯性負性突變體(dominant negative mutants) RasN17、Akt DN 及 IκBαM 所抑制。另外 YC-1 引發之 Ras 活性增加也會受到 ODQ、KT-5823 及 manumycin A

的抑制。而 YC-1 引發 A549 細胞 Akt 的活化也會受到 ODQ、KT-5823、manumycin A 及 wortmannin 的抑制。進一步我們也證實 YC-1 會刺激細胞內的 NF-κB 和其特異性 DNA 序列結合及刺激 κB-luciferase 活性的增加。而由 YC-1 引發 κB-luciferase 活性的增加會受到 ODQ、KT-5823、manumycin A、wortmannin、Akt 抑制劑及 PDTC 等或是 RasN17、AktDN 及 IκBM 所抑制。同樣地，YC-1 引發 IKKα/β 的活化也受到 ODQ、KT-5823、manumycin A、wortmannin 及 Akt 抑制劑抑制。進一步我們也發現 manumycin A、RasN17、AktDN、PDTC 及 IκBM 皆可抑制 YC-1 引發 COX-2 promoter 活性的增加。綜合這部分實驗結果我們證實在 A549 細胞當中，YC-1 也可經由活化 sGC/cGMP/PKG 路徑之後進而促使 Ras 及 PI3K 活化，接著導致 IKKα/β 及 NF-κB 的活化，最後引發 COX-2 蛋白表現。
除了 PKC-α/p44/42 MAPK 及 Ras/PI3K/Akt/IKKα/β/NF-κB 這兩條路徑外，我們也進一步探討在 A549 細胞中，PKC-α、Ras、Raf-1 及 p44/42 MAPK 在 YC-1 誘發 IKKα/β、NF-κB 活化及 COX-2 蛋白表現的訊號傳遞機制中所扮演的角色。我們發現 YC-1 誘發 COX-2 蛋白的表現會受到 PKC-α 特異性抑制劑 Ro 32-0432、Raf-1 抑制劑 GW 5074 及 p44/42 MAPK 抑制劑 AG 126 所抑制。YC-1 引發之 Raf-1 活化會受到 ODQ、KT-5823、Ro 32-0432、manumycin A 及 RasN17 所抑制。YC-1 引發 Ras 活性的增加會受 manumycin A 抑制，但卻不被 Ro 32-0432 所抑制。接著我們發現 YC-1 引發 p44/42 MAPK 活化會被 Ro 32-0432、manumycin A 及 GW 5074 等抑制劑抑制。而由 YC-1 調控 IKKα/β 活化及 κB-luciferase 活性的增加皆會受到 Ro 32-0432、GW 5074、PD 98059 及 AG 126 所抑制。進一步我們還證實了 YC-1 引發 COX-2 promoter 活性增加也會受到 Ro 32-0432、GW 5074、PD 98059 及 AG 126 所抑制。從這部分實驗結果我們證實在 A549 細胞當中，YC-1 可能經由活化 sGC/cGMP/PKG 路徑之後分別引發 Ras 及 PKC-α 活化，接著這兩條路徑皆會依序引發 Raf-1、p44/42 MAPK、IKKα/β 及 NF-κB 的活化，最後造成 COX-2 蛋白表現。
綜合以上所有研究結果，我們認為在人類肺臟上皮細胞株(A549)中，YC-1 活化 sGC/cGMP/PKG 路徑後進一步調控 IKKα/β 和 NF-κB 活化及引發 COX-2 表現，而其中分別經由 Ras/PI3K/Akt、Ras/Raf-1/p44/42 MAPK 及 PKC-α/Raf-1/p44/42 MAPK 三條不同的訊號傳遞機轉。

英文摘要

YC-1 [3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole], an activator of soluble guanylate cyclase (sGC), has been shown to increase the intracellular cGMP concentration. This study was designed to investigate the signaling pathway involved in the YC-1-induced cyclooxygenase-2 (COX-2) expression in A549 cells. YC-1 caused a concentration- and time-dependent increase in COX activity and COX-2

expression in A549 cells. Pretreatment of the cells with the sGC inhibitor, 1H-(1,2,4)oxadiazolo[4,3-a]quinozalin-1-one, ODQ, the protein kinase G (PKG) inhibitor (KT-5823), or the PKC inhibitors (Go 6976 and GF109203X), attenuated the YC-1-induced increase in COX activity and COX-2 expression. Exposure of A549 cells to YC-1 caused an increase in PKC activity; this effect was inhibited by ODQ, KT-5823 or Go 6976. Western blot analyses showed that PKC-α, -ι, -λ, -ζ and –μ isoforms were detected in A549 cells. Treatment of A549 cells with YC-1 or PMA caused a translocation of PKC-α, but not other isoforms, from the cytosol to the membrane fraction. Long-term (24 h) treatment of A549 cells with PMA down-regulated the PKC-α. The MAPK/ERK kinase (MEK) inhibitor, PD 98059 (10~50 μM), concentration-dependently attenuated the YC-1-induced increases in COX activity and COX-2 expression. Treatment of A549 cells with YC-1 caused an activation of p44/42 mitogen-activated protein kinase (MAPK); this effect was inhibited by KT-5823, Go 6976, long-term (24 h) PMA treatment, or PD98059, but not the p38 MAPK inhibitor, SB 203580. These results indicate that in human pulmonary epithelial cells, YC-1 might activate PKG through an upstream sGC/cGMP pathway to elicit PKC-α activation, which in turn, initiates p44/42 MAPK activation, and finally induces COX-2 protein expression.
We continue to explore the role of Ras, phosphoinositide-3-OH-kinase (PI3K), Akt, and transcription factor nuclear factor-κB (NF-κB) in YC-1-induced COX-2 expression in A549 cells. A Ras inhibitor (manumycin A), a PI3K inhibitor (wortmannin), an Akt inhibitor (1L-6-Hydroxymethyl-chiro-inositol2-[(R)-2-O-methyl-3O-octadecylcarbonate]), and an NF-κB inhibitor (pyrrolidine dithiocarbamate, PDTC) all reduced YC-1-induced COX-2 expression. The YC-1-induced increase in COX activity was also blocked by manumycin A, wortmannin, PDTC, and the dominant negative mutants for Ras (RasN17), Akt (Akt DN), and IκBα (IκBαM). The YC-1-induced increase in Ras activity was inhibited by ODQ, KT-5823, and manumycin A. YC-1-induced Akt activation was also inhibited by ODQ, KT-5823, manumycin A, and wortmannin. YC-1 caused the formation of an NF-κB-specific DNA-protein complex and an increase in κB-luciferase activity. YC-1-induced κB-luciferase activity was inhibited by ODQ, KT-5823, manumycin A, wortmannin, an Akt inhibitor, PDTC, RasN17, Akt DN, and IκBαM. Similarly, YC-1 caused IKKα/β activation was inhibited by ODQ, KT-5823, manumycin A, wortmannin, and an Akt inhibitor. Furthermore, YC-1-induced COX-2 promoter activity was inhibited by manumycin A, RasN17, Akt DN, PDTC, and IκBαM. These results indicate that YC-1 might also activate the sGC/cGMP/PKG pathway to induce Ras and PI3K/Akt activation, which in turn initiates IKKα/β and NF-κB activation, and finally induces COX-2 expression in

A549 cells.

Except the PKC-α/p44/42 MAPK cascade and the Ras/PI3K/Akt/IKKα/β/NF-κB cascade which involved in YC-1-induced COX-2 expression in A549 cells. We further investigated the role of PKC-α, Ras, Raf-1, and p44/42 MAPK in YC-1-induced IKKα/β and NF-κB activation, and COX-2 expression in A549 cells. YC-1-induced COX-2 expression was attenuated by a specific PKC-α inhibitor (Ro 32-0432), a Raf-1 inhibitor (GW 5074), and a p44/42 MAPK inhibitor (AG 126). YC-1-mediated Raf-1 activation was inhibited by ODQ, KT-5823, Ro 32-0432, manumycin A, and RasN17. The YC-1-induced increase in Ras activity was inhibited by manumycin A, but not by Ro 32-0432. YC-1-induced p44/42 MAPK activation was inhibited by Ro 32-0432, manumycin A, and GW 5074. The YC-1-mediated increases in IKKα/β activation and κB-luciferase activity were attenuated by Ro 32-0432, GW 5074, PD 98059, and AG 126. Furthermore, YC-1-induced COX-2 promoter activity was also inhibited by Ro 32-0432, GW 5074, PD 98059, and AG 126. These results indicate that in A549 cells, YC-1 might activate the sGC/cGMP/PKG pathway to independently elicit Ras and PKC-α activation, which both in turn induce sequential Raf-1, p44/42 MAPK, IKKα/β, and NF-κB activations and ultimately cause COX-2 expression.

Taken together, all of our results show that treatment of A549 cells with YC-1 caused sGC activation, cGMP accumulation, and PKG activation, which in turn caused IKKα/β and NF-κB activation, and finally induced COX-2 expression through three separate pathways: the Ras/PI3K/Akt cascade, the Ras/Raf-1/p44/42 MAPK cascade, and the PKC-α/Raf-1/p44/42 MAPK cascade.