# Bradykinin 刺激人類肺臟上皮細胞環氧酵素-2 表現的訊息傳遞路徑探討

# Studies on the signaling pathway of bradykinin-induced cyclooxygenase-2 expression in human pulmonary epithelial cells (A549)


## 中文摘要

Bradykinin (BK)為 kinin 類的成員，是一種具有免疫活性的胜肽，爲 9 個胺基酸所組成。BK 屬於自泌素(autacoid)的一種，在體內會誘導許多發炎的反應。本論文所要探討的是 bradykinin 引發人類肺臟上皮細胞環氧酵素-2(cyclooxygenase-2, COX-2)釋放的訊息傳遞路徑。BK 以劑量與時間相關的反應刺激 COX-2 的表現，此反應可被 actinomycin D (轉錄抑制劑)及 cycloheximide (轉譯抑制劑)所抑制。而給予 B2 receptor antagonist (HOE140)也可以阻斷 BK 誘導的 COX-2 表現。相反地，給予 B1 receptor antagonist (Lys-(leu8)des-Arg9-bradykinin)則不會抑制 BK 所引起的反應。Ras 抑制劑(manumycin A)、Raf 抑制劑(GW 5074)、MEK 抑制劑(PD 98059)、p38 mitogen-activated protein kinase (MAPK) 抑制劑(SB 203580)及 tyrosine kinase 抑制劑(tyrphostin AG126 及 genistein)也會降低 BK 誘導 COX-2 的表現。同樣地，BK 會進一步活化 p44/42 和 p38 MAPK，並且會被 HOE140、tyrphostin AG126 及 genistein 所抑制。此外，bradykinin 所誘導 p44/42 及 p38 MAPK 的活性也分別被 PD 98059 及 SB 203580 特異性地抑制。NF- B 抑制劑 pyrrolidine dithiocarbamate (PDTC)及 I B protease 抑制劑 L-1-tosylamido-2-phenylenylethyl chloromethyl ketone (TPCK)都可以抑制 A549 細胞中，BK 所引發的 COX-2 表現。BK 也可以活化 IKK、使 I B 降解(degradation)及 NF- B p65/p50 轉位到細胞核，而 BK 所引發 IKK 的活性可以被 PD 98059、SB 203580 及 Manumycin A 抑制。我們使用 electrophoretic mobility shift assay (EMSA)與轉染 B-Luciferase 來測量 NF- B 的結合能力與活性，則發現 BK 可以誘發 NF- B 與 DNA 結合能力及活性，此作用也可以被 HOE140、PD 98059 與 SB 203580 所抑制。綜合以上的實驗結果，BK 可以活化 tyrosine kinase、p44/42 MAPK、p38 MAPK 以及 IKK 的作用，促使 NF- B 活化，最終導致 A549 細胞 COX-2 的表現，而這些訊號傳遞是經由 bradykinin B2 receptor 的路徑而來。



## 英文摘要

Bradykinin (BK), a membrane of kinins, is a 9 amino acid peptide which actives the innate immune system. BK, also an autatoid, induces many inflammation response. In this study, we investigated the signaling pathway of bradykinin-induced COX-2 expression in human pulmonary epithelial cells (A549). Bradykinin caused

concentration- and time-dependent increases in COX-2 expression and this effect was inhibited by transcriptional inhibitor (actinomycin D) or translational inhibitor (cycloheximide). The B2 receptor antagonist, HOE140, could block bradykinin-induced COX-2 expression. In constract, B1 receptor antagonist (Lys-(leu8)des-Arg9-bradykinin) did not inhibit the bradykinin response. The Ras inhibitor (manumycin A), Raf inhibitor (GW 5074), MEK inhibitor (PD 98059), p38 MAPK inhibitor (SB 203580) and tyrosine kinase inhibitor (tyrphostin AG126 and genistein) attenuated bradykinin-induced COX-2 expression in A549 cells. In parallel, bradykinin induced p44/42 mitogen-activated protein kinase (p44/42 MAPK) and p38 MAPK activation; these effects were inhibited by HOE140, tyrphostin AG126, genistein. In similar, bradykinin induced activations of p44/42 MAPK and p38 MAPK were inhibited by the MEK inhibitor PD 98059 and the p38 MAPK inhibitor SB 203580, respectively. The NF- B inhibitor (pyrrolidine dithiocarbamate, PDTC) and I B protease inhibitor (L-1-tosylamido-2-phenylenylethyl chloromethyl ketone, TPCK) also decreased bradykinin-induced COX-2 expression in A549 cells. BK also caused I B Kinase activation, I B degradation, NF- B translocation and activation. Furthermore, the BK-induced increase in IKK activity were inhibited by PD 98059, SB 203580 and manumycin A. Using electrophoretic mobility shift assay (EMSA) and transient transfeced with B-luciferase to detect NF- B binding activity and NF- B activity, respectively, we found that bradykinin increased NF- B DNA-binding activity and NF- B activity; These effects were inhibited by HOE140, PD 98059 and SB 203580. In summary, these results indicated that bradykinin might activate tyrosine kinase, p44/42 and p38 MAPK and IKK pathway, which is turn initiates NF- B activation, and finally induced COX-2 expression in A549 cells, and this signaling transduction is through B2 receptor pathway.