Rosiglitazone 在大鼠神經膠質瘤細胞中誘導

Mitogen-Activated Protein Kinase Phosphatase-1

表現的作用機制探討

The mechanism of rosiglitazone-induced mitogen-activated protein kinase phosphatase-1 expression in rat C6 glioma cells

中文摘要

Rosiglitazone（RSG）是一種化學合成的 PPAR-γ (peroxisome proliferators-activated receptor- .agonist，可用於第二型糖尿病的治療， 它與 PPAR-γ 具有很強的親和力，爲 TZDs (thiazolidinediones)類的藥物。RSG 還有許多其他的作用，例如: 抗發炎，但其調控的機制目前還不是很清楚。在過去的研究中指出，MKP-1(Mitogen- Activated Protein Kinase Phosphatase-1)能夠使 MAPK(Mitogen- Activated Protein Kinase)去活化。爲了探討 MKP-1 在 RSG 抗發炎反應中扮演的角色，利用 RSG 處理大鼠神經膠質瘤細胞(C6 glioma)，發現 RSG 會誘導 MKP-1 表現，在兩小時達到最大量，八小時就回復到原背景値。而以 MG-132(蛋白分解?抑制劑)處理，能夠使 MKP-1 蛋白表現延長至八小時。爲了探討 RSG 調控 MKP-1 是經由轉錄或轉譯作用，以 actinomycin D 及 cyclohexamide 處理皆能抑制由 RSG 引起的 MKP-1 表現，表示 MKP-1 是經由 de novo 合成而來。爲了探討其中的訊息傳遞路徑，利用各種藥理性的抑制劑去抑制 ROS、PI3-K、ERK 、NF B,、p38 MAPK、JNK 路徑，發現 ERK 1/2 抑制劑(PD098059)，NF- B 抑制劑(PDTC)、 p38 MAPK 抑制劑(SB203580) 和 JNK 抑制劑(SP600125)能夠抑制 MKP-1 表現，但 PI3-K 抑制劑(LY294002)和 ROS 抑制劑(l-NAC)不能抑制由 RSG 誘導的 MKP-1 表現，而 RSG 作用後，磷酸化 ERK 也明顯上升，表示 RSG 經由 ERK 的訊息傳遞路徑而活化 MKP-1。在其他研究報告發現，LPS(Lipopolysacchride)可以引起發炎反應，而 iNOS 可以作爲其指標，本篇研究發現 RSG 可以抑制由 LPS 所誘導的 iNOS 表現，而以 triptolide (MKP-1 inhibitor) 前處理之後，原先抑制的情況即回復，推斷 MKP-1 在抗發炎的反應中扮演一個很重要的角色。此外，基質金屬蛋白?(Metalloproteinase ,MMPs)爲惡性腫瘤細胞的轉移的一個要素，本研究發現 RSG 能夠抑制 C6 glioma cell 所產生的 MMP-2。綜觀以上結果可知 MKP-1 爲細胞內訊息傳遞的調控樞紐，而 RSG 能夠活化 MKP-1 而抑制 iNOS 及 MMP-2 的表現。

英文摘要

Rosiglitazone(RSG) or thiazolidinediones is a ligand of the peroxisome proliferators-activated receptor- . PPAR- ..which has been used in the treatment of type II diabetes. Additionally, RSG has been shown to exert a variety of beneficial effects, such as anti-inflammatory. However, the underneath mechanisms are not clear. MAPK phosphatase-1 (MKP-1) has been shown to dephosphorylate and inactivate MAP kinase. To investigate whether MKP-1 plays a role in mediating RSG-induced anti-inflammatory effects. Treatment of C6 glioma cells with RSG, induced a dose-dependent induction of MKP-1, the maximum effect was seen at 2 hours and decreased at 8 hours. Pretreatment with MG-132 prolonged the increasing of MKP-1 protein levels to 8 hours, suggesting RSG may increase MKP-1 stability. To investigate whether RSG regulate the transcription and translation levels of MKP-1, we find that Act. D (actinomycin D) and CHX (cyclohexamide) can inhibit RSG-stimulated MKP-1 expression demonstrated that MKP-1 goes through de novo synthesis. In order to investigate the signaling mechanism, several pharmacological inhibitors were used to block ROS, PI3-K, ERK1/2, NF- B, p38 MAPK and JNK pathways. Pretreatment cells with ERK inhibitor (PD098059), NF- B inhibitor (PDTC), p38 MAPK inhibitor (SB203580) and JNK inhibitor (SP600125) but not PI3-K inhibitor (LY294002) nor ROS blocker(l-NAC) inhibit RSG-stimulated MKP-1 expression. After RSG treatment, phosphorylated- ERK rise was detected. To examine the anti- inflammation role of MKP-1, we demonstrated that RSG can inhibit LPS-induced iNOS expression. Blocking MKP-1 with triptolide reversed iNOS expression. In addition, tumor invasion is regulated by metalloproteinase (MMPs), in our experiments, RSG inhibits MMP-2 mRNA expression. Blocking MKP-1 with triptolide, MMP-2 mRNA expression was reversed. We conclude that RSG exerts anti-inflammatory and MMP-2 inhibition effects through MKP-1 modulation.