鋰鹽誘導 C6 神經膠瘤細胞血紅素氧化酵素-1 之表現

Lithium Induces Heme Oxygenase-1 Expression in C6 Glioma Cells

中文摘要

鋰鹽（Lithium chloride，LiCl）長期用來治療躁鬱症的雙極性情感疾病（bipolar disorder）。但其作用機制尚未完全釐清。

由於血紅素氧化酵素-1（heme oxygenase-1，HO-1）具有抗發炎、抗氧化抗細胞凋亡及免疫調節等功能。本研究利用 C6 神經膠瘤細胞（C6 glioma cells）探討 lithium 是否可以誘導 HO-1 的表現。我們發現鋰鹽的確可以造成 HO-1 蛋白劑量及時間依存性的誘導作用。加入自由基的淨化劑 l-N-acetylcysteine（l-NAC）、phosphatidylinositol 3-kinase（PI3-K）及 p38 抑制劑會抑制 lithium 誘導 HO-1 的表現。Lithium 會藉由活化 reactive oxygen species（ROS）經 PI3-K 及 p38 pathway 表現 HO-1，但不藉由 extracellular responsive kinase（ERK44/42）及 c-Jun NH2-terminal kinase /stress-activated protein Kinase（JNK）訊息傳遞路徑。近年來研究指出，NF-E2 related factor 2（Nrf-2）可經 PI3-K 與 MAPK 訊息傳遞路徑調控 HO-1 基因的表現。我們發現 lithium 可以增加累積細胞核 Nrf-2 蛋白。當加入 lithium 前處理後，以革蘭氏陰性菌細胞壁成份（lipopolysaccharide，LPS）刺激 C6 神經膠瘤細胞，由於可以大幅抑制 LPS 所誘導型一氧化氮合成（inducible NOS，iNOS）表現，利用 tin protophyrin Ⅸ dichloride（SnPP，HO-1 抑制劑）可阻斷鋰鹽抑制 nitrite 產生的抑制作用。由於使用 tricarbonyl dichlororuthenium（II）（CO donor）可以抑制 iNOS 的表現，而以血紅素清除 CO 又可阻斷鋰鹽對 nitrite 產生的抑制作用，進一步顯示鋰鹽可以誘導 HO-1 並藉由血鐵素的降解而產生 CO，而抑制在 C6 神經膠瘤細胞 LPS 刺激的 iNOS 表現。

本篇研究論文證明在 C6 神經膠瘤細胞中，lithium 誘導 HO-1 表現是透過 ROS 活化 PI3-K 與 MAPK 訊息傳遞路徑，使細胞核內 Nrf-2 累積進一步持續表現 HO-1，而 lithium 抗發炎反應是透過產生 HO-1/CO 的訊息傳遞。

英文摘要

Lithium has been used to treat bipolar disorder. However, the underneath mechanisms are not completely elucidated.

In the present study, we investigated whether lithium influences the heme oxygenase-1 (HO-1) protein level in C6 glioma cells. Lithium induced dose- and time- dependent manner increases of HO-1 expression in C6 glioma cells. l-N-acetylcysteine, a free radical scavenger, inhibited lithium-induced HO-1 expression in C6 glioma cells, suggesting reactive oxygen species (ROS) may be involved in the induction of HO-1 expression. Treatment of cells with PI 3-Kinase specific inhibitor, LY294002 or the p38 MAPK specific inhibitor, SB203580, blocked

lithium induced HO-1 expression. However, the specific p42/44 MAPK inhibitor, PD98059 or the specific JNK inhibitor, SP600125, had no effect on lithium induced HO-1 expression. In the present studies, Nrf-2 can regulate HO-1 expression goes through PI3K and MAPK pathway. We find that lithium induced the nuclear accumulation of Nrf-2. Given HO-1 expression has been linked to anti-inflammatory effect, we investigated whether treatment of C6 glioma cells with lithium inhibited LPS-inducible nitric oxide syntheses (iNOS) and nitric oxide (NO) expression. Lithium can inhibit LPS-induced iNOS and NO expression. Pretreatment with tricarbonyl dichorouthenium II (CO donor), inhibited LPS-induced iNOS and NO expression, suggesting CO mediated the inhibitory effect by lithium, and scavenging CO with hemoglobin suppressed lithium inhibition in LPS-induced iNOS expression. In conclusion, these data suggest that lithium can exert an anti-inflammatory effect in C6 glioma cells through a mechanism involving generation of ROS, activation of PI3K signaling pathway, Nrf-2 dependent HO-1 induction. Increase of HO-1 expression catalyzes the degradation of heme, which in turn leads to CO production and suppresses the inhibitory effect of LPS-induced iNOS expression by lithium.