Dipyridamole 對抗腎臟發炎與缺氧作用之研究

The Effects of Dipyridamole on Renal Inflammation and Hypoxia

中文摘要

研究背景. 慢性腎臟病的病理特徵包括腎絲球硬化、腎間質白血球浸潤、腎小管間質纖維化、腎絲球及腎小管周邊微血管喪失。任何形式的腎臟損傷會造成發炎物質的生成，微血管喪失引起組織的缺氧缺血，產生反應性氧族(ROS)，進一步強化發炎反應、組織的修補和纖維化。腎臟慢性的缺氧會活化腎素-血管收縮素系統，局部的血管收縮素 II 增加會引起氧化壓力。同時，腎臟組織的缺氧缺血反應會增加血红素氧合酶 1 的表現，提供腎臟損傷的保護機制。Dipyridamole 是一個核苷運送的抑制劑，也是一個非選擇性磷酸二酯酶(phosphodiesterase)的抑制劑。Dipyridamole 應用在腎臟病治療可改善蛋白尿，然而，作用的機轉並不清楚。本研究將探討 dipyridamole 抗發炎及抗氧化的作用，並研究活化分裂素蛋白激酶磷酸酶 1 (MKP-1)及血红素氧合酶 1 (HO-1)扮演的角色。

研究方法. 白細胞介素 6 (interleukin 6, 簡稱 IL-6)、單核球趨化蛋白-1 (monocyte chemoattractant protein, 簡稱 MCP-1)及骨橋素(osteopontin, 簡稱 OPN) 以 ELISA 方法測量。ROS 的產量以(DCF)螢光強度測量。研究 PI-3K-PKB/Akt，分裂素蛋白激酶(MAPK) 和 NF-kB 訊息傳遞路徑。利用分裂素蛋白激酶磷酸酶 1 和血红素氧合酶 1 的短鏈干擾核糖核酸(siRNA)轉殖的細胞，抑制 MKP-1 及 HO-1 基因表現，來研究 dipyridamole 的分子作用機制。

結果. 在大鼠腎膈細胞(RMCs)，加入 100nM 的血管收縮素 II 會增加 ROS 的產生，PI-3K 的抑制劑 Ly294001 及 wortmannin 會阻斷血管收縮素 II 所誘導 HIF-1α 堆積，顯示血管收縮素 II 是透過 ROS 依賴的 PI-3K-PKB /Akt 路徑，誘導 HIF-1 α 堆積。在大鼠巨噬細胞(RAW 264.7 cells)，dipyridamole 可以活化 MKP-1，以 MKP-1 siRNA 轉殖技術抑制 MKP-1 基因表現或加入 MKP-1 的抑制劑 triptolide，可以抑制脂多醣體(lipopolysaccharide,LPS)在巨噬細胞所誘導環氧化酵素 2 (COX-2)的表現。在大鼠腎膈細胞，我們同樣發現 dipyridamole 可以抑制脂多醣體所誘導 IL-6 和 MCP-1 分泌，ROS 的產量有明顯的減少且和 dipyridamole 劑量相關。另外，dipyridamole 會抑制脂多醣體引起的 NF-kB 和脫氧核醣核酸結合能力及 IkB 的磷酸化。以 MKP-1 siRNA 轉殖的大鼠腎膈細胞則會降低 dipyridamole 對脂多醣體所誘導 IL-6 表現的抑制作用。ERK 1/2 及 p38MAPK 路徑也參與了脂多醣體所引發的 MCP-1 和 COX-2 的表現，可以受到 dipyridamole 及抗氧化劑 l-NAC 所抑制。然而，以 SnPP (HO-1 抑制劑)前處理 RMC 細胞，可逆轉 dipyridamole 對 ROS 及發炎反應的抑制作用。在大鼠腎小管上皮細胞(NRK-52E)，加入化學缺氧劑氯化鈷(CoCl2)，會增加 OPN 蛋白質表現增加。Dipyridamole 可以誘導 HO-1 的表現增加與抑制 CoCl2 所誘導 OPN 的分泌，以 SnPP 或血色素(一氧化碳去除劑)，可逆轉 dipyridamole 對 OPN 的抑制作用，顯

示 HO-1 與 dipyridamole 抑制 CoCl2 誘導 OPN 的表現有關。以 HO-1 siRNA 轉殖的 NRK-52E 細胞會降低 dipyridamole 所誘導 MKP-1 的磷酸化及活化，而以 MKP-1 siRNA 轉殖的 NRK-52E 細胞會則能逆轉 dipyridamole 對 OPN 的抑制作用。

結論. 我們的實驗結果顯示，dipyridamole 會先藉由活化 MKP-1 的方式使得 p38 MAPK 去磷酸化而失去功能。此外，血管收縮素 II 透過 ROS 依賴的路徑來誘導 HIF-1 α 堆積。在大鼠腎膈細胞，dipyridamole 藉由 HO-1 降低 ROS ，抑制脂多醣體所誘導 COX-2 表現及 MCP-1 分泌。在 NRK-52E 細胞，dipyridamole 會經由誘導 HO-1 及 MKP-1 的活化，降低 CoCl2 所誘導 OPN 的分泌。總而言之，dipyridamole 可能透過抗發炎及抗氧化的作用，提供在慢性腎臟病治療的好處。

英文摘要

Background. Chronic kidney disease is characterized by glomerulosclerosis, interstitial leukocyte infiltration, tubulointerstitial fibrosis, and loss of glomerular and peritubular capillaries. Renal injury of any kind generates mediators of inflammation. Hypoxia/ischemia secondary to loss of capillaries also contributes to reactive oxygen species (ROS) generation and may further enhance the inflammatory process, tissue remodeling and fibrosis. Chronic hypoxia can activate the renin-angiotensin system (RAS) and local angiotensin II induces oxidative stress. In the meantime, up-regulation of heme ogygenase-1 (HO-1) provides protection against renal injury that follows hypoxia/ischemia. Dipyridamole is a nucleoside transport inhibitor and a non-selective phosphodiesterase inhibitor, which has been shown to improve proteinuria. However, the mechanisms by which dipyridamole exerts its beneficial effects on renal disease are not completely understood. In the present study, we investigated the roles of mitogen-activated kinase phosphatase-1 (MKP-1) and HO-1 in dipyridamole's anti-inflammatory and anti-oxidative effects.

Methods. IL-6, MCP-1 and OPN secretions were measured by ELISA kits. ROS generation was measured using the fluorescent probe 2',7'- dichlorofluorescein (DCF). PI-3K-PKB/Akt, MAPK and NF-kB signal pathways were studied. HO-1 and MKP-1 siRNA were used for gene knockdown to investigate the molecular mechanisms of dipyridamole.

Results. Treatment of rat mesangial cells (RMCs) with Ang II (100 nM) increased production of ROS. Ang II-stimulated HIF-1alpha accumulation was blocked by the phosphatidylinositol 3-kinase (PI-3K) inhibitors, Ly 294001, and wortmannin, suggesting that Ang II may stimulate a ROS-dependent activation of the PI-3K-PKB/Akt pathway, which leads to HIF-1alpha accumulation. In RAW 264.7 cells, dipyridamole stimulated transient activation of MKP-1, a potent inhibitor of p38 MAPK function. Knockdown of MKP-1 by transfecting MKP-1 siRNA or inhibition

of MKP-1 by the specific inhibitor, triptolide, significantly reduced the inhibitory effects of dipyridamole on COX-2 expression induced by LPS. We also showed that dipyridamole inhibited LPS-induced IL-6 and MCP-1 secretion in RMCs. Pretreated with dipyridamole showed significantly inhibition of ROS generation in a dose-dependent manner. In addition, dipyridamole inhibited the LPS-stimulated NF-kB DNA binding activity and IkB phosphorylation. The dipyridamole inhibitory effect on LPS-induced IL-6 secretion was reduced in MKP-1 siRNA knockdown cells. ERK1/2 and p38 MAPK signaling pathways were demonstrated to be involved in the LPS-induced MCP-1 secretion and COX-2 expression, and were inhibited by dipyridamole and l-NAC treatment. However, pretreatment of RMCs with tin protoporphyrin (Snpp; an HO-1 inhibitor) reversed the inhibitory effect of dipyridamole on ROS and inflammatory responses. Incubation of rat renal tubular NRK52E cells with cobalt chloride (CoCl2) increased OPN production. Dipyridamole could induce HO-1 expression and inhibited CoCl2-induced OPN secretion. Pretreatment of cells with Snpp, or hemoglobin (a CO-scavenging agent), reversed the inhibition of OPN expression by dipyridamole. Transfection of HO-1 siRNA reduced dipyridamole-stimulated MKP-1 phosphorylation. Knockdown of MKP-1 reversed the inhibition of OPN expression by dipyridamole.

Conclusions. Our results demonstrate that dipyridamole may exert its anti-inflammatory effect via activation of MKP-1, which dephosphorylates and inactivates p38 MAPK. In addition, Ang II increases ROS-dependent HIF-1 alpha accumulation. Dipyridamole inhibits the expression of COX-2 and secreted MCP-1 in LPS-treated RMCs via HO-1-mediated ROS reduction. In NRK-52E cells, dipyridamole may suppress CoCl2-induced OPN secretion via induction of HO-1 and activation of MKP-1. Taken together, these data suggest that dipyridamole may contribute to its beneficial effects on chronic kidney disease through its anti-inflammatory and anti-oxidative effects.