• 計畫中文名稱	Dipyridamole 的抗發炎作用		
• 計畫英文名稱	Antiinflammatory Effects of Dipyridamole		
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• 英文關鍵字	Antiinflammatory effects; iNOS; Cox-2; dipyridamole		
• 中文摘要	Dipyridamole (persantin) 是一種核甘酸運送抑制物(nucleoside transport inhibitor)也是磷酸 雙酯脢的抑制物 (phosphodiesterase inhibitor) 可以增加細胞內的 cAMP and cGMP 濃度。臨床 上 dipyridamole 能有效的改善蛋白尿是腎臟科常用的藥物之一，可是確實的機轉仍有待進一步釐 清。磷酸雙酯脢的抑制物(尤其第四型)常具有抗發炎作用，本研究擬探討 dipyridamole 是否具有 抗發炎作用。我們最近發現在 RAW 264.7 細胞中，dipyridamole 可以抑制細菌之脂多醣體 (lipopolysaccharide，簡稱 LPS)-所誘導的誘導型一氧化氮合成脢(inducible nitric oxide，簡稱 iNOS) 及環氧脢-2 (cyclooxygenase，簡稱 COX-2) 的表現。我們進一步發現 dipyridamole 可以 抑制 LPS 所刺激的 IkB 磷酸化、IkB 的降解、p65 NFkB 的轉位和 NFkB-luciferase 報告基因(reporter gene)的表現，顯示 dipyridamole 可以抑制 NFkB 的活化。由於 NFkB 是發炎反應中很重要的訊息媒 介物，很可能 dipyridamole 有更多抗發炎作用。本計劃擬深入研究 dipyridamole 的抗發炎作用。 我們的研究重點如下： (1) Dipyridamole 抑制 iNOS 及 COX-2 表現的機制：雖然我們有相當 promising 的數據顯示 dipyridamole 可抑制 NFkB 的活化，我們仍需瞭解 IkB 的上游 IKK、NIK、Vav-1 及 SHP-1 是否受 dipyridamole 所 調控。另外，在 RAW 264.7 巨噬中細胞，LPS 所誘導的 iNOS 及 COX-2 表現還可經由 p38 MAPK、 Erk-1/-2、AP-1 等訊息傳遞路徑，我們將利用藥理抑制物和分子生物學的工具(如 dominant negative mutants 和 constitutive active mutants)來瞭解這些訊息傳遞路徑是否受 dipyridamole 調 控。 (2) Dipyridamole 是否影響人類巨噬細胞 cytokines 的分泌：我們將以 dipyridamole 前處理人類巨噬細 胞，再以 LPS 刺激巨噬細胞後，收集培養液，以 cytokine beads array 瞭解 LPS 所刺激的 cytokines 分泌升高是		

	否受 dipyridamole 前處理所影響。可受 dipyridamole 調控的 cytokines，將再以北方墨點 法或 real time PCR 定量 cytokine 基因轉錄的差異。 (3) 在 thy-1 誘導的腎絲球體腎炎動物模式中，dipyridamole 是否具抗發炎活性。我們已順利的用抗 thy-1 抗體誘導腎絲球體腎炎的動物模式，我們將進一步以免疫組織染色評估 dipyridamole 在大白 鼠的腎絲球體腎炎中是否同樣可抑制 iNOS 及 COX-2 的表現。我們也將萃取組織中之 mRNA 並以 北方墨點法檢驗各種 cytokines 以及 PAI-1、chymase、MCP-1、HO-1 的表現是否有所不同。
• 英文摘要	Dipyridamole is a nucleoside transport inhibitor and a non-selective phosphodiesterase inhibitor that increases intracellular level of cAMP and cGMP through phosphodiesterase inhibition. Phosphodiesterase has been demonstrated to have anti-inflammatory effects in many experimental systems. We previously found that dipyridamole inhibits lipopolysaccharide (LPS)-induced inducible nitric oxide (iNOS) and cyclooxygenase (COX-2) expression in RAW 264.7 macrophages. The LPS-induced iNOS and COX-2 expressions were inhibited by pretreatment of cells with the nuclear factor-kappa B (NF-kB) inhibitor, pyrrolidone dithiocarbamate (PDTC) suggesting NFkB is involved in the induction of both iNOS and COX-2. Dipyridamole inhibited the LPS-stimulated IkB phosphorylation, IkB degradation, transloction and the transcription of reporter gene, suggesting dipyridamole inhibits NFkB activation. Taken together, these results indicate a novel effect of dipyridamole on LPS-induced iNOS and COX-2 expression and suggest that dipyridamole may have other beneficial effects through inhibiting NFkB signaling pathway. Our specific aims are as follow: (1) Specific aim #1. To delineate the mechanisms by which dipyridamole inhibit LPS-stimulated responses. We will address further whether the upstream regulating molecules including IKK, NIK, Vav-1 and SHP-1 are regulated by dipyridamole. Additionally, LPS is known to activate p38 MAPK, Erk-1/-2, and AP-1 signaling pathways. We will also investigate whether any of these signaling pathways are regulated by dipyridamole. We will use pharmacological inhibitors, dominant negative and/or constitutive active mutants to adress this question. (2) Specific aim #2: To investigate whether dipyridamole inhibits LPS-stimulated cytokine secretion in human macrophages. Inhibition of cytokine release is an important aspect of antiinflammatory effect. LPS may stimulate a variety of cytokine release in human macrophages. We will examine whether these responses are affected pretreatment of macrophages with dipyridamole using cytokine beads array. The results will be further confirmed by Northern blot analysis and/or real time PCR.. (3) Specific aim #3: To evaluate the antiinflammatory effects of dipyridamole in Thy-1 induced glomerulo- nephritis. Recently, we have developed an animal model of Thy-1 induced glomerulonephritis. We will use this model to evaluate the antiinflammatory effect of dipyridamole in vivo. Control or Thy-1 induced rats will be treated without or with dipyridamole for different time periods. The protein urea will be monitored. Rats will be sacrificed and the iNOS and COX-2 expression in the kidney will be examined by immunohistochemical staining. The mRNA will be extracted from the tissues and the expression of different cytokines, PAI-1, chymase, MCP-1, and HO-1 will be examined using Nothern blot analysis.