• 系統編號	RN9607-4346
• 計畫中文名稱	子計畫二---ASK1 在氧化態低密度脂蛋白誘導腦內皮細胞功能喪失及死亡所扮演的角色(II)
• 計畫英文名稱	The Role of ASK1 in ox-LDL Induces Cerebral Endothelail Cell Dysfunction and Death (II)
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC94-2745-B038-002-URD
• 執行機構	台北醫學大學呼吸治療系
• 本期期間	9408 ~ 9507
• 報告頁數	12 頁
• 使用語言	中文
• 研究人員	陳炳常; 許重義; 林建煌 Chen, Bing-Chang; Lin, Chien- Huang; Lin, Chien-Huang
• 中文關鍵字	中風; 氧化低密度脂蛋白; 腦部內皮細胞; 細胞凋亡; 訊息傳遞
• 英文關鍵字	Stroke; Oxidized low density lipoprotein (ox-LDL); Cerebral endothelial cell (CEC); Apoptosis; ; Signal transduction; Signaling-regulation kinase 1 (ASK1); JNK; AP-1; Bim
• 中文摘要	在許多的研究顯示，中風病人其血中的低密度脂蛋白(LDL)含量過高的現象。腦部血管內皮細胞(cerebral endothelial cells,CECs)爲構成血腦障蔽的主要組成，防止一些傷害性物質進入腦部。一旦氧化態 LDL (ox-LDL)誘導腦部內皮細胞凋亡將促使內皮細胞通透性增加及血腦障蔽破壞，而造成腦部的傷害如中風。然而，ox-LDL 誘導 CECs 的凋亡機制目前還不清楚。於是在本計劃中，我們將探討 apoptosissignaling-regulation kinase 1 (ASK1)及 JNK 在 ox-LDL 誘導腦部內皮細胞凋亡所扮演的角色及詳細的作用機轉。CECs 細胞給予 ox-LDL 可依濃度依賴誘導腦部內皮細胞的凋亡。細胞給予 κ-carrageenan (Lox-1 受體抑制劑)及 Lox-1 抗體二者皆可抑制 ox-LDL 誘導腦部內皮細胞的凋亡。細胞短暫轉染 dominant negative mutant ofASK1 (ASK1 DN)可抑制 ox-LDL 誘導腦部內皮細胞的凋亡。我們也發現 CECs 細胞給予 ox-LDL 可誘導 ASK1 蛋白激?的活性及 ASK1 在 Ser967 去磷酸化的作用。再者，CECs 細胞給予 ox-LDL 明顯刺激 14-3-3 與 ASK1 分離。SP 600125 (JNK 選擇性抑制劑)、JNK1 DN 及 JNK2 DN 皆可抑制 ox-LDL 誘導腦部內皮細胞的凋亡。CECs 細胞給予 ox-LDL 可依時間依賴誘導 JNK 的活性及其磷酸化。細胞短暫轉染 ASK1 DN 可抑制 ox-LDL 誘導 JNK 的磷酸化。細胞給予 curcumin (AP-1 抑制劑)可抑制 ox-LDL 誘導腦部內皮細胞的凋亡。CECs 細胞給予 ox-LDL 可誘導 AP-1 特異性蛋白-DNA 結合的作用。更進一步研究 Bim 在 ox-LDL 誘導腦部內皮細胞的凋亡所扮演的角色。CECs 細胞給予 ox-LDL 可依時間依賴誘導腦部內皮細胞 BimS 的表現及 Bim-luciferase 的活性。經由以上的結果顯示，在 CECs 細胞中，ox-LDL 可經由活化 ASK1/JNK/AP-1 的訊息傳遞路徑來誘導 Bim 表現及腦部內皮細胞的凋亡。
• 英文摘要	Several clinical studies demonstrated that plasma level of low density lipoprotein (LDL) are significantly higher in patients with stoke. Dysfunction of the endothelium is a hallmark of the early atherosclerotic lesion and ox-LDL activates cerebral endothelial cells (CEC), leading to an alteration of the functional and structural integrity of the endothelial barrier. CECs have distinct morphological and functional properties that are essential for strict exchange of water and nutrients between blood and brain. Since oxidant LDL (ox-LDL) might play an important role in the pathology of stroke due to cerebral vasculature dysfunction. However, the mechanism of ox-LDL-induced CEC apoptosis is still unclear. In this project, we interested to explore the role of apoptosis signaling-regulation kinase 1 (ASK1) and JNK on ox-LDL-induced CEC apoptosis. Treatment of CECs with Ox-LDL induced CEC apoptosis in a concentration-dependent manner. Treatment of CECs with kappa-carrageenan

(Lox-1 receptor inhibitor) and Lox-1 antibody both inhibited ox-LDL-induced CEC apoptosis. Cells transient transfection with dominant negative mutant of ASK1 (ASK1 DN) inhibited ox-LDL-induced CEC apoptosis. We also found that ox-LDL induced ASK1 kinase activity and dephosphorylation of ASK at Ser967. Moreover, CECs treatment with ox-LDL induced 14-3-3 disassociation with ASK1. SP 600125 (a selective JNK inhibitor) and JNK1 DN and JNK2 DN all inhibited ox-LDL-induced CEC apoptosis. Treatment of CEC with ox-LDL caused JNK kinase activity and phosphorylation in a time-dependent manner. Cells transient transfection with ASK1 DN inhibited ox-LDL-induced JNK phosphorylation. Treatment of cells with curcumin (AP-1 3 inhibitor) inhibited ox-LDL-induced CEC apoptosis. Treatment of CECs with Ox-LDL induced AP-1 specific protein-DNA binding activity. To further investigate the role of Bim in ox-LDL-mediated CECs apoptosis. Treatment of CECs with Ox-LDL induced BimS expression in a dose dependent manner. These results indicate that ox-LDL induced ASK1/JNK/AP-1 signal pathway to mediate Bim expression and finally induced CEC apoptosis. '