• 計畫中文名稱	鼠尾草屬植物的丹參酮 IIA 能誘導血紅素氧合腶-1 的表現與在巨噬細胞中抑制細菌脂多糖所誘導環氧腶-2 的表現		
• 計畫英文名稱	Tanshinone IIA from Salvia miltiorrhiza Induces Heme Oxygenase-1 Expression and Inhibits Lipopolysaccharide-Induced Cyclooxgenase-2 Expression in RAW 264.7 Cells		
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• 計畫編號	NSC94-2314-B038-032	• 研究方式	學術補助
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• 研究領域	臨床醫學類		
• 研究人員	陳作孝		
• 中文關鍵字	丹參酮 IIA; 環氧腶-2; 血紅素氧合.-1		
• 英文關鍵字	Tanshinone IIA; Cyclooxygenase-2; Heme oxygenase-1		
• 中文摘要	丹參酮 IIA（tanshinone IIA）是由鼠尾草屬植物（Salvia miltiorrhiza）的乾燥根莖所 純化的雙帖烯（Diterpene），已知能抑制巨噬細胞（macrophage）內誘導型一氧化氮合 成腶（inducible nitric oxide，簡稱 iNOS）的表現，但其分子機制仍不清楚。我們最近還 發現在 RAW264.7 細胞中，丹參酮 IIA 可有效的抑制細菌脂多糖體（lipopolysacchardide， 簡稱 LPS）所誘導環氧腶-2（cyclooxygenase-2，簡稱 COX-2）的表現。因丹參酮 IIA 還 能抑制細胞內脂質過氧化產物對肝細胞 DNA 的傷害，具有抗氧化作用，我們認爲這些 作用都和丹參酮 IIA 可誘導血紅素氧合.-1（Heme oxygenase-1，簡稱 HO-1）的表現有 關。因爲我們的預備實驗顯示丹參酮 IIA 的確可誘導 HO-1 的表現；本研究的目的即爲 探討丹參酮 IIA 抑制巨噬細胞（macrophage）內 iNOS 及 COX-2 表現之分子機制。 我們研究的重點如下： (1) 探討丹參酮 IIA 抑制細菌 LPS 所誘導環氧腶-2（COX-2）和誘導型一氧化氮合成腶 （iNOS）表現的機制：在 RAW 264.7 巨噬細胞，LPS 所誘導 COX-2 和 iNOS 的表 現主要經由 NF- B 和 AP-1 轉錄因子。PKC、ERK、p38 MAPK、和 JNK 參與了 AP-1 上游的訊息傳遞，我們將探討這些訊息傳遞與 NF- B 的活化是否受丹參酮 IIA 調控。 (2) 探討丹參酮 IIA 誘導血紅素氧合.-1（HO-1）表現的訊息傳遞路徑：血紅素氧合. -1 (HO-1) 的基因表現可經由 PI 3-kinase /PDK-1/Akt 活化轉錄因子 Nrf2 由細胞質往 細胞核位移，再經 Nrf2 結合於 HO-1 基因之抗氧化結合元(antioxidant responsive element，簡稱 ARE)而影響 HO-1 的基因轉錄。我們將研究丹參酮 IIA 是否可刺激 Nrf2 轉位並誘導血紅素氧合.-1(HO-1) 的表現。因爲 HO-1 基因的調控可因細胞反 應性含氧物種		

	(reactive oxygen species，簡稱 ROS) 而造成，我們除了將測定丹參酮 IIA 是否增加細胞中 ROS 的產生，也將使用 l-N-acetylcysteine（l-NAC）抑制反應性 含氧物種來探討上述反應是否受影響。並確定丹參酮 IIA 誘導 HO-1 的表現是循 ROS/PI 3-kinase /PDK-1/Akt /Nrf2 的訊息傳遞路徑。 (3) 探討丹參酮 IIA 是否透過誘導血紅素氧合.-1（HO-1）及 HO-1 的反應產物來抑制 LPS 所誘導的 iNOS 及 COX-2 的表現：我們將使用 HO-1 的藥理抑制物或 HO-1 的 siRNA 或顯性負突變基因（dominant negative genes）檢驗 HO-1 在抑制 LPS 所誘導 COX-2 和 iNOS 的表現是否扮演關鍵的角色。我們還將以 hemin 誘導 HO-1 的表現、 或使用 zinc protoporphyrin 抑制血紅素氧合.瞭解 HO-1 增減對 LPS 所誘導 COX-2 和 iNOS 的表現有何影響。並進一步以 tricarbonyl dichloro-ruthenium (II) dimmer 增 加一氧化碳（CO）的釋放或以血紅素來去除一氧化碳，評估丹參酮 IIA 的抗發炎作 用是否經由一氧化碳或其他 HO-1 的反應產物而達成。
• 英文摘要	Tanshinone IIA is a direrpene isolated from Salvia miltiorhiza root and has been shown to inhibit inducible nitric oxide (iNOS) expression and nitric oxide production in RAW264.7 cells. However, the mechanisms by which Tanshinone IIA inhibits iNOS expression have not been elucidated. We previously found that Tanshinone IIA also inhibited cyclooxygenase-2 (COX-2) protein expression in lipopolysaccharide (LPS)-activated murine RAW 264.7 macrophages. Given Tanshinone IIA also exhibits antioxidant activity and prevents DNA damage induced by lipid peroxidation in liver cells, we hypothesized that the inhibitory effect is mediated through the induction of heme oxygenase-1 (HO-1) expression. Indeed, our pilot studies revealed that Tanshinone IIA induced HO-1 expression in time and dose dependent manners. In the present proposal, we propose to investigate the molecular mechanisms by which Tanshinone IIA exerts its inhibitory effects on iNOS and COX-2 induction by LPS. Our specific aims are as follow: Specific aim #1. To delineate the mechanisms by which Tanshinone IIA inhibits LPS-induced COX-2 and iNOS expression in RAW 264.7 cells. NF- B and AP-1 are critical transcription factors in LPS-induced iNOS and COX-2 expression in RAW 264.7 cells. PKC, ERK, p38 MAPK, and JNK are upstream signaling kinases for the induction of AP-1 transcription. We will investigate whether any of these signaling pathways or NF- B activation are regulated by Tanshinone IIA. Specific aim #2. To investigate the signal pathway of Tanshinone IIA-induced HO-1 expression. It is becoming evidenced that induction of HO-1 expression is mediated through translocation of a redox-sensitive transcription factor Nrf2 (NF-E2 related factor 2) from cytosol to nuclei. We will examine the ability of this compound to activate Nrf2 and induce HO-1 expression. Further, HO-1 can be induced by increasing of intracellular ROS level. We will examine whether incubation of cells with Tanshinone IIA increased the intracellular ROS level. We will also examine whether increase of endogenous antioxidant by adding l-N-acetylcysteine (l-NAC) would attenuate the HO-1 expression. Specific aim #3. To investigate the role of HO-1 and its reaction products on LPS-induced iNOS and COX-2 expression in RAW 264.7 cells. We will use pharmacological inhibitor of HO-1, siRNA or dominant negative mutant of HO-1 to elucidate the role of HO-1 in this effect. We will also alter the HO-1 levels by the known HO-1 inducer, hemin, or the known inhibitor, tin protoporphyrin, to investigate the effect of HO-1 on LPS-induced COX-2 and iNOS expression. We will also examine the role of CO by adding CO releasing molecule, tricarbonyldichloro-ruthenium (II) dimmer, or CO scavenger, hemoglobin, to examine the effect of Tanshinone

	IIA.