• 系統編號	RN9408-0725
• 計畫中文名稱	肝細胞藉由 HO-1 排出鐵離子之機轉研究，以及此機轉對於貧血、繼發性鐵離過載治療的可能意義(II)
• 計畫英文名稱	Studies on the Mechanism of the Release of Iron from Liver by Heme Oxygenase-1 and Its Possible Implication for the Treatment of Anemia and/or Secondary Iron Overload (II)
• 主管機關	行政院國家科學委員會
• 計畫編號	NSC92-2320-B038-015
• 執行機構	臺北醫學大學生理學科
• 本期期間	9208 ~ 9307
• 報告頁數	11 頁
• 使用語言	英文
• 研究人員	阮淑慧 Juan, Shu-Hui
• 中文關鍵字	原血紅素氧化酵素; 血晶素
• 英文關鍵字	Heme oxygenase; Hemin
• 中文摘要	研究的目的是探討原血紅素氧化酵素 (HO-1) 對肝細胞及網狀內皮系統中，如何重新利用鐵的可能機轉。老化的紅血球經由網狀內皮系統清除分解，將鐵離子交予肝細胞循環利用，這是體內細胞中最大的鐵離子運送過程。目前對於這個機轉仍不了解，對於非小腸上皮細胞 (如肝臟、腎臟)的鐵離子運輸，也是所知有限。於是，我們將闡明 HO-1 對鐵的重新利用在生理上所伴演的角色及其分子機轉。在計畫第一年中，實驗室已能穩定產生相當高濃度的腺病毒(1 x 109pfu)攜帶 HO-1 基因。此外，實驗室也能從老鼠活體中取出肝臟細胞在體外培養。利用免疫組織染色，我們證實，腺病毒攜帶的 HO-1 基因及控制組 GFP 可 homing 到肝臟並表達蛋白質。動物實驗結果顯示，大量表達 HO-1 蛋白質可降低血晶素在肝細胞上的沉積，建議 HO-1 蛋白質可協助鐵離子的釋出細胞外。計畫第二年的重點，強調蛋白質之間的互動，尋找參與並輔助 HO-1 排出鐵離子的輔助蛋白質。利用酵母菌雜交系統，我們發現五個能與 HO-1 蛋白質反應的蛋白質克隆(clones)，其中四株是尚未被發表的序列，其中一株是高度 homologous 金 屬傳送蛋白質 (metal transporter protein(MTP1))。利用基因重組的技術，我們已能大量表達 MTP 及 HO-1 蛋白質在肝臟細胞。免疫沉澱法，確實了 MTP 及 HO-1 蛋白質有結合的證據。
• 英文摘要	The aim of this study was to investigate the role of HO-1 in hepatocytes for iron reutilization. This pathway represents the largest efflux of iron from cells, as we know that the regeneration of iron in reticuloendothelial cells after the catabolism of senescent erythrocytes is passed to hepatocytes for erythropoiesis and returned blood. We tend to unravel the physiological role and molecular mechanism of HO-1 for iron

reutilization in hepatocytes. The strategy in this investigation is to generate adenovirus carrying HO-1 gene for in vivo and in vitro studies. We already develop the technique to stably produce advenovirus carrying HO-1 gene to the concentration of 1 x 109pfu. Additionally, we are able to outgrow hepatocyte in culture. Both in vivo and in vitro studies, we showed that HO-1 overexprssion decreased the extent of iron accumulation in hypatocytes challenged by both hemn in vitro and iron dextran in vivo. For iron expulsion from plasma membrane by HO-1, this process must involve some not yet identified proteins since HO-1 is resided in microsomes. Thus, we employed yeast 2-hybrid system to pull down those candidate proteins using HO-1 as bait. Our lab already set up yeast 2-hybrid system for screening HO-1 associated protein in human liver library. We obtained five candidate clones and then have them sequenced. One of them are higher homologous to the sequence of metal transporter protein (MTP-1) and the others are novel sequences not being identified. Furthermore, We attempted to overexpression MTP-1 in hepatocytes cells to investigate the extent of iron accumulation. We also intended to co-overexpression of HO-1 and MTP-1 in hepatocyte cell to prove that MTP-1 could facilitate HO-1 for iron efflux to occur in vitro and in vivo.