行政院國家科學委員會補助專題研究計畫成果報告

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※　Pentoxifylline 預防腹膜纖維化之基礎研究　　　　　※
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計畫類別：■個別型計畫　　□整合型計畫
計畫編號：NSC89－2314－B－002－058－
執行期間：89 年 8 月 1 日至 90 年 8 月 31 日

計畫主持人：洪冠予
共同主持人：蔡敦仁

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：國立臺台灣大學醫學院內科

中　華　民　國　90　年　10　月　31　日

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主持人：洪冠予　執行機構及單位名稱: 國立臺台灣大學醫學院內科
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一. 中文摘要

腹膜硬化症(EPS)**是腹膜透析**(PD)**病人長期嚴重的合併症之一。**EPS **的成因目前認為和人類腹膜表面細胞**(HPMC)**過度製造纖維蛋白有關。吾人曾經報告**pentoxifylline(PTX)**具有抑制** HPMC **纖維蛋白基因表現的作用**(*Kidney Int* 2000)**。本計畫擬進一步探究** PTX **抑制** HPMC **纖維蛋白基因表現的可能作用機轉。**

自手術取得的正常腹膜大網分離出 HPMC **進行培養，再經硬化症相關因子** TGF-β **刺激。採用北方點墨法**(Northern blot)**觀察** HPMC **內纖維蛋白基因**(type I & III collagen)**表現的情形，並利用西方點墨法**(Western blot)**觀察** HPMC **內訊息傳遞路徑**(**包括**: ERK1/2，SMAD family，JNK and $p38^{HOG}$) **的活化情形。**

結果顯示：TGF-β**可以刺激** HPMC **內纖維蛋白基因**(type I collagen)**表現增加，而** PTX **可以抑制此一作用。**TGF-β**刺激** HPMC **內** Smad2, ERK1/2 **和** $p38^{HOG}$ **路徑活化，但是對** JNK **路徑沒有影響。不論是** ERK1/2 **路徑或是** $p38^{HOG}$ **路徑被阻斷時，都會使** TGF-β**刺激** HPMC **纖維蛋白基因表現的效果受到抑制。**PTX **可以抑制** TGF-β**刺激** HPMC **內** ERK1/2 **和** $p38^{HOG}$ **路徑被活化，但是對** Smad2 **沒有影響。**

結論：PTX **會抑制** TGF-β**刺激** HPMC **內纖維蛋白基因表現增加，此種作用的機轉主要透過抑制** HPMC **內** ERK1/2 **和** $p38^{HOG}$ **路徑被** TGF-β**刺激活化有關。本計畫成果可以提供作為** PTX **預防** EPS **的治療基礎。**

(**關鍵詞：腹膜硬化症，**pentoxifylline**，**TGF-β**，纖維蛋白，訊息傳遞**)

二. 英文摘要

Peritoneal matrix accumulation is characteristics of encapsulating peritoneal sclerosis (EPS), which is a serious complication in long-term peritoneal dialysis (PD) patients. We previously had reported that TGF-β stimulates expression of type I and III collagen mRNA in cultured HPMC, and was attenuated by pentoxifylline (PTX). The SMAD family and the mitogen-activated protein kinase (MAPK) (ERK1/2, JNK and $p38^{HOG}$) pathways have been shown to participate in TGF-β signaling. However, the intracellular signaling downstream to TGF-β remains undetermined in HPMC. In this study, we explored these signaling pathways in HPMC, and investigated the molecular mechanisms involved in the inhibitory effects of PTX on TGF-β induced collagen gene expression in HPMC.

HPMC was cultured from human omentum by an enzyme digestion method. Expression of collagen α1(I) mRNA was determined by northern blotting. The SMAD proteins and the MAPK kinase activity were determined by Western blotting.

TGF-β-stimulated collagen α1(I) mRNA expression of HPMC was inhibited by PTX. The Smad2, ERK1/2 and $p38^{HOG}$ pathways were activated in response to TGF-β. However, TGF-β displayed no activation of the JNK pathway in HPMC. Addition of PD98059 and

SB203580, which blocked activation of ERK1/2 and p38^{HOG} MAPK respectively, suppressed TGF-β-induced collagen α1(I) mRNA expression. At concentration that inhibited collagen gene expression, PTX suppressed ERK1/2 and p38^{HOG} MAPK activation by TGF-β. In contrast, PTX had no effect on TGF-β-induced activation of Smad2, under the same concentration.

PTX inhibits TGF-β-induced collagen gene expression in HPMC through modulations of the ERK1/2 and p38^{HOG} MAPK pathways. Our study of PTX may provide therapeutic basis for clinical applications in prevention of EPS.

(Keywords: encapsulating peritoneal sclerosis, pentoxifylline, mesothelial cell, TGF-β, signal transduction)

三. 緣由與目的

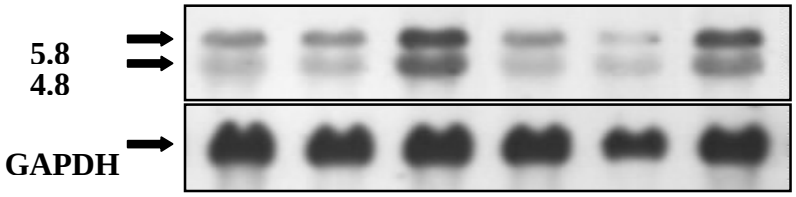
Encapsulating peritoneal sclerosis (EPS) is a serious complication developed in long-term peritoneal dialysis (PD) patients [1]. Over-proliferation of human peritoneal mesothelial cells (HPMC) accompanied by matrix accumulation is important in the pathogenesis EPS [2]. Transforming growth factor-β (TGF-β) has been regarded as the central mediator of fibrosing process in clinical diseases. PD patients who have persistent TGF-β in their drained effluent were found to associate with an increased risk of EPS [3]. We previously have reported that TGF-β stimulates expression of type I and III collagen mRNA in cultured HPMC [4]. As TGF-β may mediate the development of EPS, pharmacological agents which can attenuate TGF-β–induced matrix accumulation in HPMC may have clinical implications for the prevention or retardation of EPS.

Despite the well-recognized association between TGF-β and matrix accumulation, limited information is available regarding the mechanisms of TGF-β to induce this process [5]. The SMAD family members have been identified as major intracellular mediators of TGF-β signaling [6]. TGF-β, first binds to the type II receptor on the cell membrane, then recruits the type I receptor into a complex. The phosphorylated type I receptor activates Smad2 and allows it to form a heteromultimer with Smad4. This complex then is translocated to the nucleus to regulate transcription of target genes. In addition to the SMAD proteins, the mitogen-activated protein kinase (MAPK) pathways have been recently proposed to transmit parts of downstream signalings of TGFβ [5, 7]. The MAPK pathways contain three phosphorylation cascades: the extracellular signal-regulated protein kinase (ERK), the c-Jun N-terminal kinase (JNK), and the p38^{HOG} MAPK. In our previous report [8], we had shown that ERK1/2 and Smad2 were activated by TGF-β, and the blockade of ERK1/2 activity resulted in decrease of TGF-β-induced α1(I) collagen gene expression. In other cell systems, the p38^{HOG} MAPK [9] and/or JNK [10] pathways have been demonstrated to be one of the downstream targets required for TGF-β-mediated matrix expansion. However, in HPMC the role of the JNK and the p38^{HOG} pathways in response to TGF-β has never been investigated.

Pentoxifylline (PTX) is a widely-used anti-platelet agent. In addition to its anti-platelet effect, we previously have demonstrated in human vascular smooth muscle cells [11] and in HPMC [4] that PTX may attenuate TGF-β-induced collagen synthesis. Nevertheless, the molecular mechanism of this inhibitory effect of PTX on TGF-β-induced collagen gene expression in HPMC remains undetermined. In this work we aimed to explore the inhibitory mechanism of PTX on TGF-β-treated HPMC. Our results may provide a pharmacological basis of PTX for the treatment of EPS.

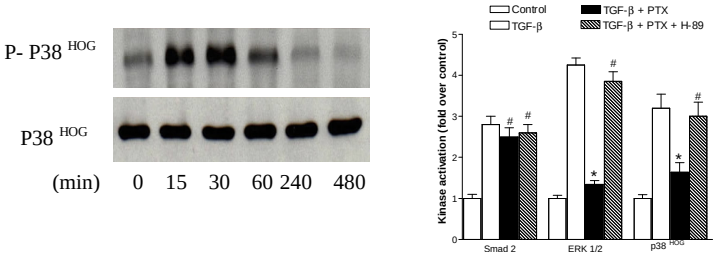
四.結果:

1. PTX inhibits TGF-β induced α1(I) mRNA expression in HPMCs

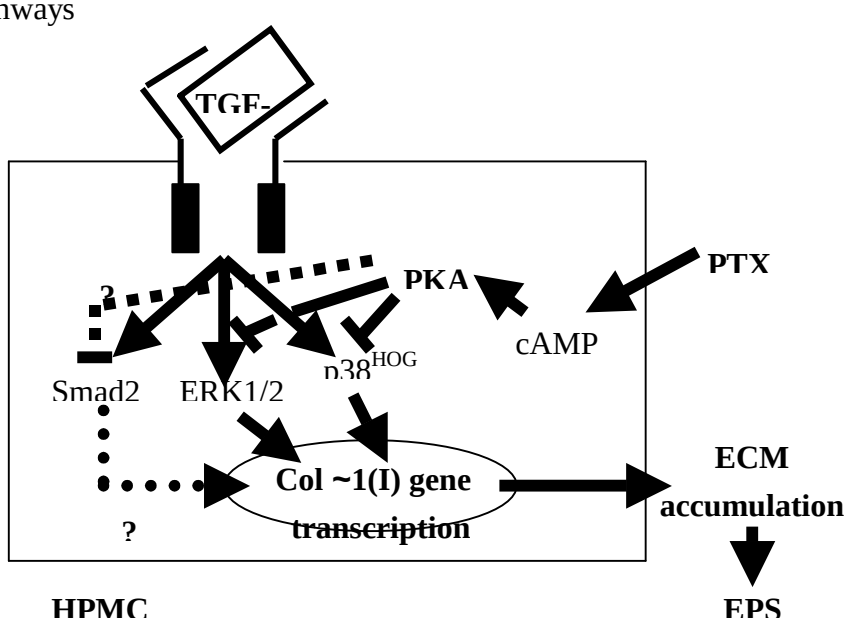


Collagen/GAPDH ratio	0.64	0.62	1.63	0.59	0.52	1.38
TGF-β	-	-	+	+	+	+
PTX	-	+	-	+	-	+
DBcAMP	-	-	-	-	+	-
H-89	-	-	-	-	-	+

2. TGF-β activates ERK1/2, Smad2, and the p38^{HOG} pathways in HPMC



3. PTX suppressed downstream signaling of TGF-β through modulations of ERK1/2 and p38^{HOG} pathways



五.計畫成果自評

Our work implicated that PTX may serve as a therapeutic agent for prevention or retardation of EPs. The molecular mechanism of PTX was also much clarified after this study. An animal *in vivo* study and possibly a human clinical trail are mandatory in the future.

六. 參考文獻

1. Kawaguchi Y, Kawanishi H, Mujais S, Topley N, Oreopoulos DG : Encapsulating peritoneal sclerosis: definition, etiology, diagnosis, and treatment. *Perit Dial Int* 20 (Supl 4): S43 - S55, 2000
2. Dobbie JW : Pathogenesis of peritoneal fibrosing syndrome (scelerosing peritonitis) in peritoneal dialysis. *Perit Dial Int* 12: 14-27, 1992
3. Lin CY Chen WP, Yang LY, Chen A, Huang TP. Persistent transforming growth factor-beta 1 expression may predict peritoneal fibrosis in CAPD patients with frequent peritonitis. *Am J Nephrol* 18: 513-519, 1998
4. Fang CC, Yen CJ, Chen YM, Shyu RS, Tsai TJ, Lee PH, Hsieh BS. Pentoxifylline inhibits human peritoneal mesothelial cell growth and collagen synthesis - Effects on TGFβ. *Kidney Int* 57: 2626-2633, 2000
5. Zimmerman CM. Padgett RW : Transforming growth factor beta signaling mediators and modulators. *Gene* 249:17-30, 2000
6. Miyazono K : TGF-beta signaling by Smad proteins. *Cytokine & Growth Factor Reviews* 11:15-22, 2000
7. Hayashida T, Poncelet AC, Hubchak SC, Schnaper HW. TGF-β1 activates MAP kinase in human mesangial cells: a possible role in collagen expression. *Kidney Int* 56: 1710-1720, 1999
8. Hung KY, Chen CT, Huang JW, *et al.* : Dipyridamole Inhibits TGF-β-Induced Collagen Gene Expression in Human Peritoneal Mesothelial Cells Through Modulations of the Extracellular Signal-Regulated Kinase (ERK) Pathway. *Kidney Int* (accepted)
9. Kucich U, Rosenbloom JC, Shen G, Abrams WR, Hamilton AD, Sebti SM, Rosenbloom J: TGF-beta1 stimulation of fibronectin transcription in cultured human lung fibroblasts requires active geranylgeranyl transferase I, phosphatidylcholine-specific phospholipase C, proteinase kinase C-delta, and p38, but not erk1/erk2. *Arch Biochem Biophy* 374: 313-324, 2000
10. Hocevar BA. Brown TL. Howe PH : TGF-beta induces fibronectin synthesis through a c-Jun N-terminal kinase-dependent, Smad4-independent pathway. *EMBO J* 18: 1345-1356, 1999
11. Chen YM, Chien CT, Hu-Tsai MI, Wu KD, Tsai CC, Wu MS, Tsai TJ. Pentoxifylline attenuates experimental mesangial proliferative glomerulonephritis. *Kidney Int* 1999; 56: 932-943