行政院國家科學委員會專題研究計畫　期中進度報告

Tamoxifen 預防腹膜纖維化之機轉及其在生體療效之研究

(2/3)

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執行期間：91 年 08 月 01 日至 92 年 08 月 31 日
執行單位：國立臺灣大學醫學院內科

計畫主持人：蔡敦仁

計畫參與人員：洪冠予, 黃政文, 莊惠安

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行政院國家科學委員會專題研究計畫成果報告

Tamoxifen 對於治療腹膜硬化症的分子機制與動物模式研究

計畫編號：NSC 91-2314-B-002-339

執行期限： 91 年 8 月 1 日至 92 年 8 月 31 日

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莊惠安 研究助理

一、中文摘要

腹膜透析是末期腎衰竭病患常用的一種腎臟替代療法，腹膜纖維化會使腹膜功能喪失，使病患無法繼續進行透析。本計畫第一年是研究 Tamoxifen(TAM)對於人類腹膜細胞以及纖維母細胞的影響。結果顯示，TAM 對於第一及第三型膠原蛋白的表現在人類腹膜細胞有抑制效果，可是在纖維母細胞沒有抑制效果。至於細胞生長的影響，TAM 則對於兩種細胞都有抑制效果。證實 TAM 對於腹膜纖維化可能有治療效果。因此在皆下來的研究將針對 TAM 在大鼠誘發腹膜纖維化的動物模式中的治療效果。初步成果顯示，TAM 治療組較對照組活的較久，而且在存活的大鼠中，TAM 治療組有較低的纖維化指數。本計畫將繼續對此動物模式進行更詳細深入的研究。

關鍵詞：腹膜透析，腹膜細胞，腹膜纖維化，纖維母細胞，膠原蛋白，Tamoxifen

Abstract

Peritoneal dialysis (PD) is a common modality of renal replacement therapy for end-staged renal disease (ESRD) patients. Peritoneal fibrosis (PF) will prevent a good performance of PD and lead to technique failure. In the first year of this study, the effect of tamoxifen (TAM) on human peritoneal mesothelial cells (HPMCs) and peritoneal fibroblasts was studied. The TAM inhibit the production collagen I and III in HPMCs but not in fibroblasts. Proliferation of both cells are inhibited by TAM. In the second year, we extend to in vivo study in rat PF model induced by bleach. The results showed that TAM will improve survival and reduce PF. More experiments of *in vivo* studies will be performed in the next two years.

Keywords: Collagen, Fibroblast, Mesothelial Cell, Peritoneal Dialysis, Peritoneal Fibrosis, Tamoxifen

二、緣由與目的

連續可攜式腹膜透析(簡稱 CAPD)是國人常用的透析方式之一，其最嚴重的併發症之一是腹膜纖維化(peritoneal fibrosis，簡稱 PF)。發生 PF 的機轉主要為腹膜表面細胞(human peritoneal mesothelial

cell，簡稱 HPMC)過度增生及細胞外間質堆積[1]。目前對於 PF 還缺乏有效的治療或預防方法。Tamoxifen(TAM)是臨床上廣泛用於癌症輔助治療的藥物，近年來，已有臨床個案報告指出: TAM 對於 CAPD 病人發生 PF 可能也有療效[2]。進一步查尋文獻發現: TAM 對於其他一些人體纖維硬化相關病症，如：後腹膜腔纖維化(retroperitoneal fibrosis) [3, 4]、腸繫膜硬化症(sclerosing mesenteritis) [5]、縱隔纖維化(fibrosing mediastinitis)和子宮頸硬化症[6]等，都曾有治療成功的零星相關報告。既然發生 PF 的機轉與 HPMC 過度增生及細胞外間質堆積有關，而 TAM 對 HPMC 生長是否有抑制之作用，從未有人報告過。本研究計畫第一年已發現 TAM 對於第一及第三型膠原蛋白的表現在人類腹膜細胞有抑制效果。本計畫第二年是利用漂白水誘發大鼠產生腹膜纖維化，並以 TAM 加以治療。

三、結果與討論

The rat about 450 g were used for this model. They were divided into three groups including vehicle groups (injecting DMSO solution, 2 ul/ml in distilled water), TAM group (injecting TAM 2.5 mg/kg/day), and control group (injecting DMSO, 2 ul/ml in distilled water). The treatments were started two days prior to bleach injection. The bleach was diluted with 2:100 with PBS and injected into the peritoneal cavity, 5ml/100 g, in the vehicle and TAM groups. The treatment solution were injected daily from left lower quadrant of the abdominal wall in all three groups. One week after treatment, we sacrifice the rats and obtained the 1. PF scores. 2. Frozen tissues from parietal peritoneum, omentum, and adhesion bands for mRNA. 3. Tissues from liver of right lobe, intestine, and omentum fixed in formaldehyde for HE and PAS staining.

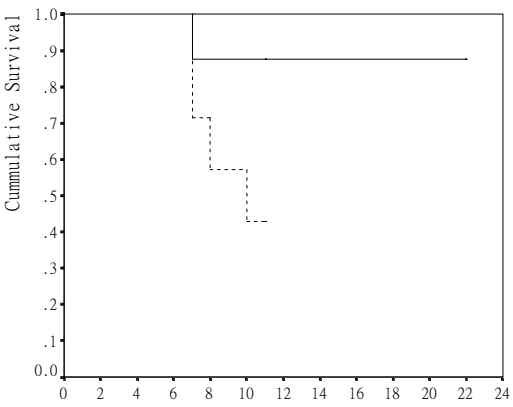


Fig. 1. Kaplan-Meier survival analysis of rats with PF induced by bleach between TAM treated (—, n=8) and vehicle only (---, n=7) (p=0.06).

In the preliminary experiment, the bleach will induce PF 7 days after instillation. The rats will die in the following weeks. TAM could prevent mortality in Kaplan-Meier survival analysis (Fig. 1). The rats were rarely dead in the first week. So the following experiments were conducted one week after instillation of bleach.

Gross evaluation of the fibrosis was according to fibrosis scoring. In brief, we counted the number of adhesion bands between liver-omentum, intestine-abdominal wall, intestine-intestine, intestine-omentum after laparotomy. The score was 0 if there was no adhesion bands, 1 for 1~3 bands, 2 for more than 3 bands, and 3 for sheet like adhesion. The full score was 12. The cacoon formation was 12 points.

Fig. 2 shows the distribution of fibrosis scroe among the three groups. The fibrosis scores in control group (0.3 ± 0.5) was less than the bleach vehicle group (10.4 ± 1.8) and tamoxifen treated group (3.6 ± 2.0). In addition, tamoxifen treated group had lower fibrosis score.

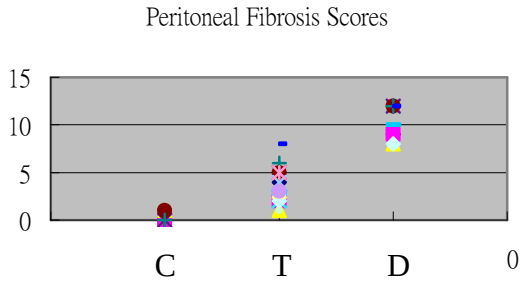


Fig. 2: The PF scores in three groups.(C: control; T: bleach with tamoxifen treatment; D: bleach with vehicle)

Visceral peritoneum around liver was measured under PAS stain. Five measurements were done in each specimen under high power fields. Fig. 3 shows the peritoneum thickness around liver in three groups.

The exact thickeness was presented in the

Fig. 4. The peritoneal thickness was 0.28 ± 0.06 for control group, 1.17 ± 0.45 for tamoxifen group and 3.03 ± 0.11 for bleach with vehicle group. The thickness increased after bleach instillation, and tamoxifen can reduce this effect.

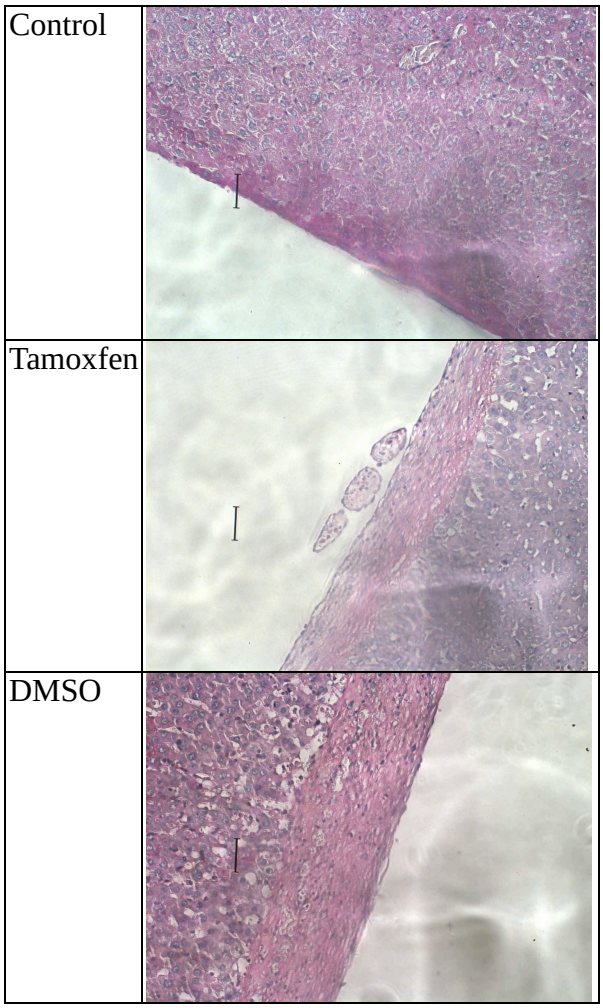


Fig. 3: The thickness of visceral peritoneum of liver. The thickness of tamoxifen treated group was less than bleach with vehicle group (DMSO)

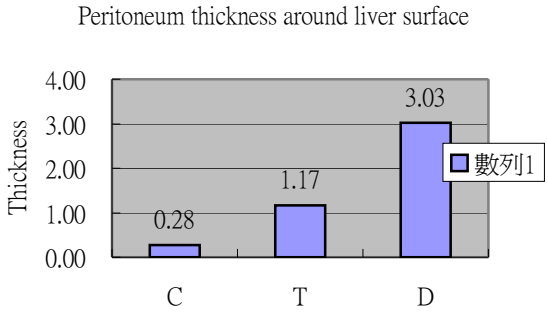


Fig. 4: The visceral peritoneum thickness around the liver surface. The thickness of control group was significantly less than the tamoxifen (T) and bleach vehicle (D) groups (P was less than 0.0001 in both groups.) The thickness was also significantly reduced in tamoxifen group (P< 0.0001).

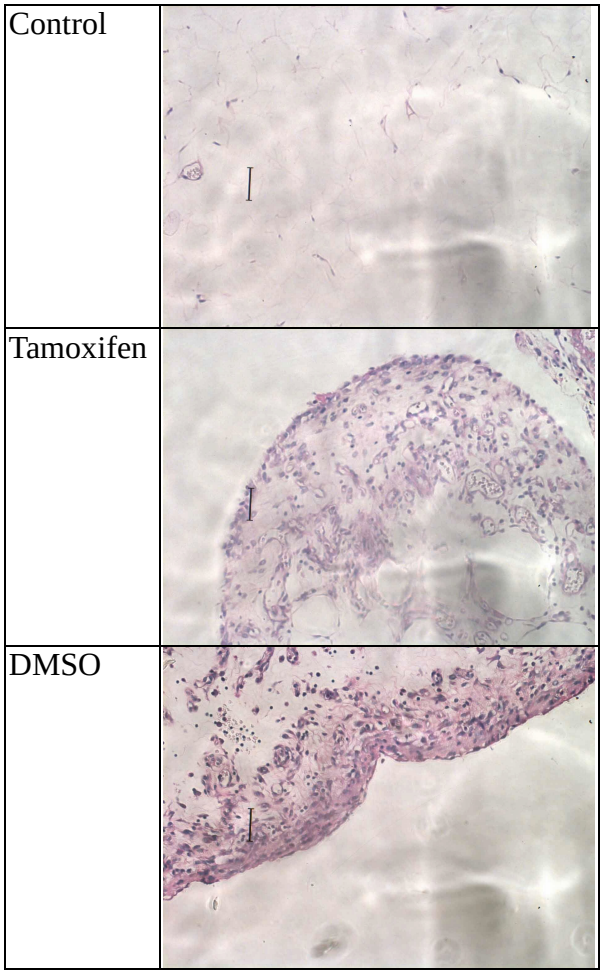
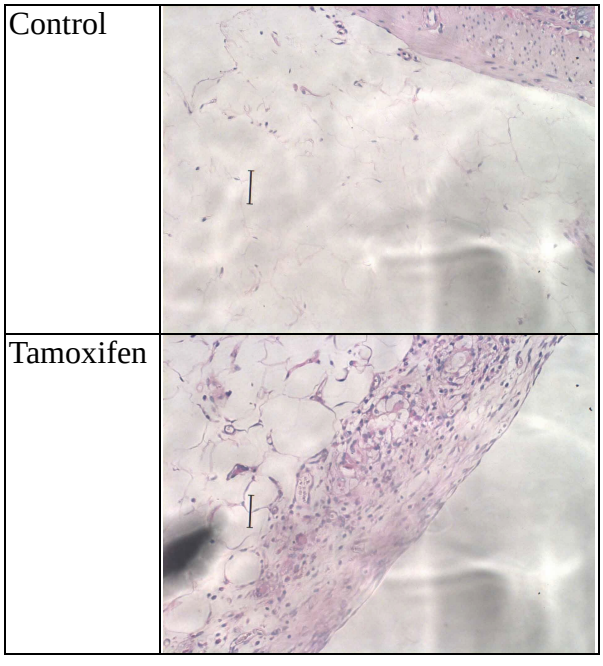


Fig. 5: Border of omentum was thickened after bleach instillation.



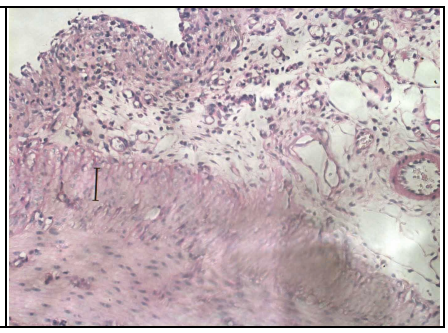
DMSO	

Fig. 6: The border of mesentery thickened with neovasclarization after bleach instillation.

Although it was difficult to exactly measure the peritoneal thickness around intestine and omentum, the borders of the omentum and intestine mesentery were also thickened (Fig. 5, 6). In addition, the neovascularization was also noted in the subperitoneal area.

四計畫成果自評

In the past two years, we found that tamoxifen was effective in reduce collagen production in vitro and reduce peritoneal fibrosis in vivo. The subperitoneal area neovascularization was also noted in our animal model. The tamoxifen had been noted to reduce angiogenesis in cancer. The relationship between tamoxifen and bleach induce angiogenesis is worth our further investigation.

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附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

Tamoxifen 對於治療腹膜硬化症的分子機制與動物模式研究

計畫類別：■個別型計畫　　□整合型計畫

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共同主持人：　洪冠予

計畫參與人員：　黃政文

莊惠安

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