行政院國家科學委員會專題研究計畫　期中進度報告

Terbinafine 的抗血管增生與抗癌作用之研究(1/2)

計畫類別：個別型計畫
計畫編號：NSC93-2320-B-038-018-
執行期間：93 年 08 月 01 日至 94 年 07 月 31 日
執行單位：臺北醫學大學醫學研究所

計畫主持人：李文森
共同主持人：何元順

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 94 年 5 月 26 日

附件一

行政院國家科學委員會補助專題研究計畫 □ 成 果 報 告 ■期中進度報告

Terbinafine 的抗血管增生與抗癌作用之研究

計畫類別：■ 個別型計畫　□ 整合型計畫
計畫編號：NSC　93－2320　－B　－038　－　018　－
執行期間：　2004　年 08　月　01　日至 2005　年 07　月　31　日

計畫主持人：李文森
共同主持人：何元順
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：□精簡報告　□完整報告

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年■二年後可公開查詢

執行單位：臺北醫學大學醫學研究所

中　華　民　國　94　年　5　月　26　日

中文摘要

關鍵詞：抗血管增生、抗腫瘤生長、TB、內皮細胞

我們的先前研究發現臨床上治療皮膚病的用藥 Terbinafine (TB)具有抑制腫瘤生長及血管增生的作用。在一些腫瘤細胞(如肝癌及直腸癌)培養中，加入 TB 可增加細胞內 p53 蛋白的表現而促進 p21 蛋白的表現，進而抑制 cyclin-dependent kinase 4 (CDK4) 的活性，使細胞週期停滯在 G0/G1 時期，最後達到抑制腫瘤細胞生長的效果 (Lee et al. 2003)。在血管內皮細胞培養中，加入 TB 亦可增加細胞內 p53 及 p21 蛋白的表現而抑制 CDK2 的活性，使細胞週期停滯在 G0/G1 時期，最後達到血管內皮細胞生長的抑制效果。利用 Tube formation 及 chick embryo chorioallantoic membrane (CAM) 兩種方法，我們證實 TB 對血管的增生具有抑制效果(Ho et al. 2003)。本計劃的目的即將利用細胞分子生物學的方法，進一步對 TB 的血管增生抑制作用及抗腫瘤生長進行深入的研究探討。本實驗中我們發現 TB 可以依計量相關模式抑制血管內皮細胞的貼附(adhesion)及遷移(migration)的現象，同時 ERK 及 FAK 的磷酸化都有被 TB 抑制的現象，另外，給予 RAS 抑制劑可以阻止 TB 所產生的內皮細胞增生作用，這些結果顯示 ERK 、FAK 及 RAS 的訊息傳導途徑可能都有參與 TB 對於血管增生的抑制作用。利用 RT-PCR 的方法我們發現在 TB 處理 6 小時後 p21 mRNA 有顯著增加的現象，顯示 TB 對 p21 的調控是藉由增加 transcription。

英文摘要

Keywords: Anti-angiogenesis, anti-tumorigenesis, TB, endothelial cells

Our previous studies have demonstrated that terbinafine (TB), a newly synthesized oral antimycotic drug, exerts anti-tumorigenesis and anti-angiogenesis activities. TB treatment caused cell cycle arrest at the G0/G1 phase through up-regulation of the p53 protein, which in turn caused an increase in p21 expression, and finally inhibited the cyclin-dependent kinase 4 (CDK4) activity in various cancer cells including colon and liver cancer cell lines. Moreover, administration of TB reduced the growth of tumors derived from human colon cancer cells in an *in vivo* setting. (Lee et al. 2003). In the human vascular endothelial cells, treatment with TB also caused growth inhibition through up-regulation of p53 and p21 protein, which in turn inhibited CDK2 kinase activity, and finally arrested cell cycle at the G0/G1 phase. Using tube formation and CAM assays, we further demonstrated that TB exerts anti-angiogenic activity (Ho et al. 2003). Taken together, we results strongly suggest the potential applications of TB in the treatment of human cancer. Although we are very happy with these exciting findings, several important issues still need to be further addressed before it can be applied for the clinical uses. Accordingly, the proposed grant application is aimed to apply the cellular and molecular biology techniques to further study the anti-cancer activity of TB in detail. In the present study, we found that TB dose-dependently inhibited adhesion and migration of HUVEC. The levels of phosphorylated ERK and FAK were downregulated in the TB-treated HUVEC. Pretreatment of HUVEC with TB prevented TB-induced inhibition of [3H]thymidine incorporation. Taken together, our data suggest that RAS, ERK and FAK might be involved in the TB-induced inhibition of angiogenesis. Using RT-PCR technique, we also demonstrated that the p21 mRNA levels were up-regulated in HUVEC after 6 hr treatment with TB, suggesting that TB-induced

increase of p21 protein is at the transcriptional level.

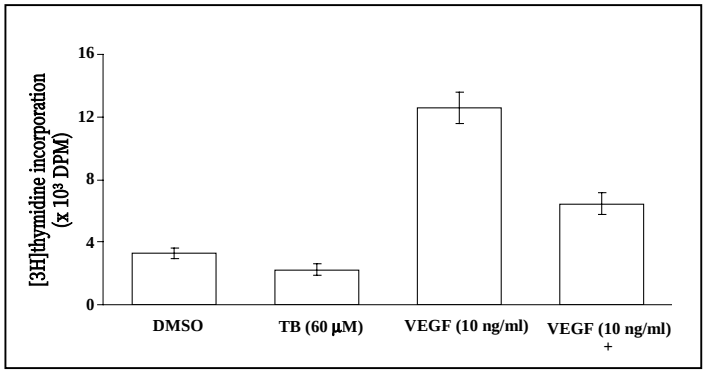
Introduction

TB is a newly synthesized oral antimycotic drug in the allylamines class: a fungicidal agent that inhibits ergosterol synthesis at the stage of squalene epoxidation. It shows a good safety profile and relatively few drug interactions. Recently, we have demonstrated that TB at a range of concentrations (0-120 μM) dose-dependently decreased cell number in various cultured human malignant cells. Flow cytometry analysis revealed that TB interrupts the cell cycle at the G0/G1 transition (Lee et al. 2004). The TB-induced cell cycle arrest in colon cancer cell line (COLO 205) occurred when the cyclin-dependent kinase (CDK) system was inhibited just as the levels of p53, p21 and p27 protein were augmented. In the TB-treated COLO 205, the binding between p53 protein and p53 consensus binding site in p21/Cip1 promoter DNA probe was increased. Pre-treatment of COLO 205 with p53-specific antisense oligodeoxynucleotide decreased the TB-induced elevations of p53 and p21/Cip1 protein, which in turn led to arrest the cell cycle at the G0/G1 phase. Moreover, in the p53 null cells, HL60, TB treatment did not induce cell cycle arrest. Taken together, these results suggest an involvement of the p53-associated signaling pathway in the TB-induced antiproliferation in COLO 205. We further examined whether administration of TB could affect the growth of tumors derived from human colon cancer cells in an *in vivo* setting. COLO 205 cells implanted subcutaneously in nude mice formed solid tumor; subsequent intraperitoneal injections of TB (50 mg/kg) led to obvious decline in tumor size of these tumors of up to 50-60%. In these tumors, increases in the p21, p27, and p53 protein and the occurrence of apoptosis were observed.

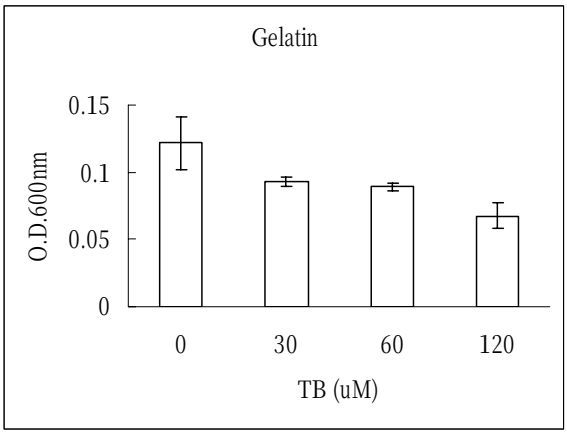
We also demonstrated that TB (0-120 μM) inhibited DNA synthesis and decreased cell number in cultured human vascular endothelial cells in a dose-dependent manner (Ho et al. 2004). TB was not cytotoxic at the concentrations used in the studies of cell growth and [3H]thymidine incorporation and this indicates that they may have an inhibitory effect on cell proliferation in the subcultured human vascular endothelial cells. Moreover, studies of [3H]thymidine incorporation revealed that treatment of the human vascular endothelial cells with TB decreased DNA synthesis and arrested the cells at the G0/G1 phase of the cell cycle. Western blot analysis demonstrated that the protein levels of cyclin A, but not cyclins B, D1, D3, and E, CDK2 and CDK4, decreased after TB treatment. The TB-induced cell cycle arrest in HUVEC occurred when the cyclin-dependent kinase 2 (CDK2) activity was inhibited just as the protein level of p21 was increased and cyclin A was decreased. Pretreatment of HUVEC with a p21 specific antisense oligonucleotide reversed the TB-induced inhibition of [3H]thymidine incorporation. Taken together, these results suggest an involvement of the p21-associated signaling pathway in the TB-induced antiproliferation in HUVEC. Capillary-like tube formation and chick embryo chorioallantoic membrane (CAM) assays further demonstrated the anti-angiogenic effect of TB. These findings demonstrate for the first time that TB can inhibit the angiogenesis.

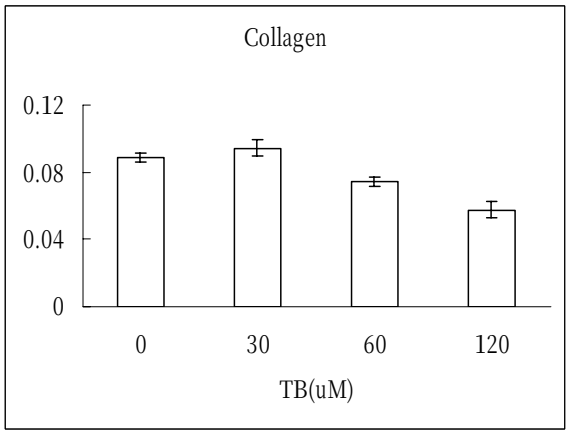
Results

TB inhibits VEGF-induced [3H]thymidine incorporation in HUVEC

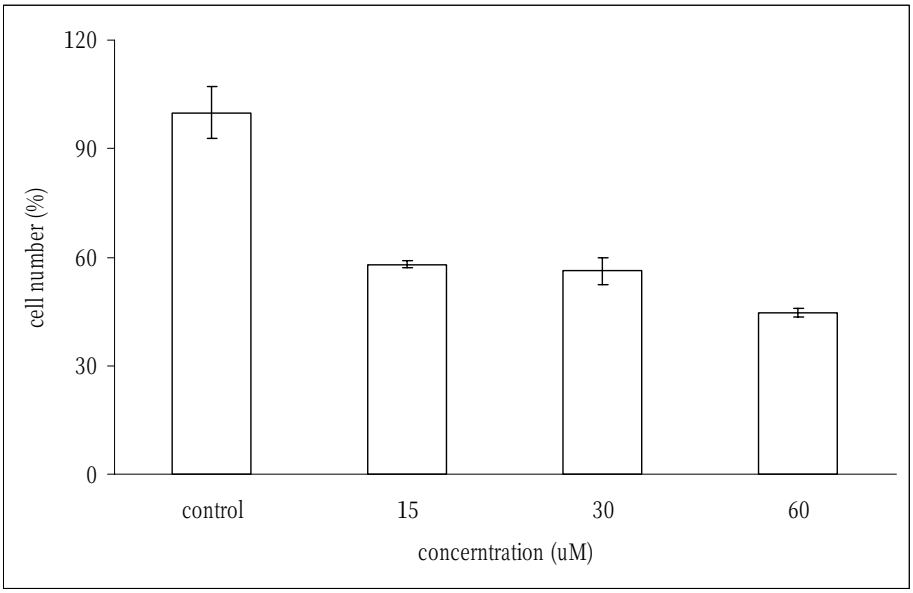


TB dose-dependently inhibits HUVEC adhesion in gelatin and collagen-coating plates

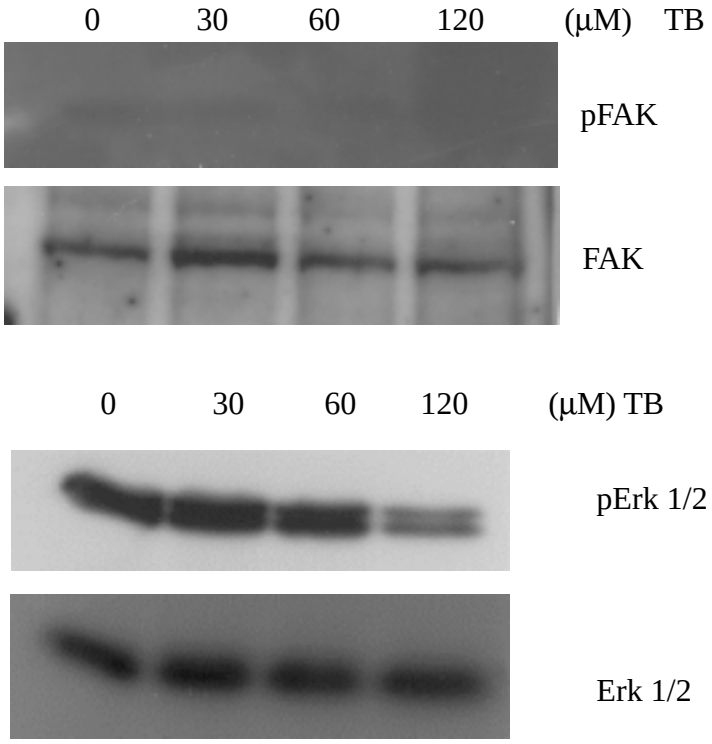




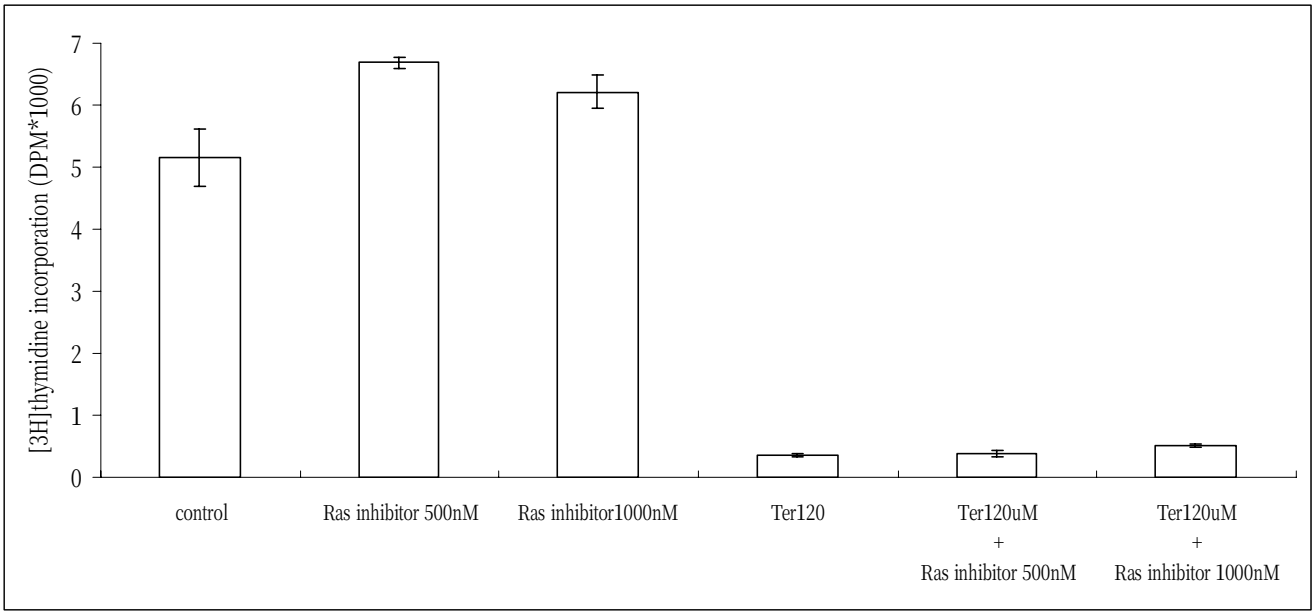
TB dose-dependently inhibits HUVEC migration



TB dose-dependently inhibits phosporylations of FAK and ERK



RAS inhibitor reverses TB-induced inhibition of [3H]thymidine incorporation



The levels of p21 mRNA are increased at 6 hr after TB treatment