行政院國家科學委員會專題研究計畫成果報告

以體外及體內試驗研究多重抗藥性抑制劑對抗癌藥物運送之影響

The effects of multidrug resistance reversing agents on the uptake, distribution and efflux of anticancer drugs: *in vitro*, *in situ* and *in vivo* studies

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一、中文摘要

P 醣蛋白可排出許多抗癌藥物，因而降低抗癌藥物例如 Epirubicin 之療效。使用流式細胞分析儀分析發現 Cremophor EL, verapamil 或 trifluoperazine 均明顯增進 Epirubicin 於 Caco-2 癌細胞之積聚。我們並使用老鼠小腸以評估 Cremophor EL, verapamil 或 trifluoperazine 是否可促進 Epirubicin 於小腸之吸收。結果顯示不管是在空腸或迴腸，此三個 P 醣蛋白抑制劑均能明顯增進 Epirubicin 之吸收。以人類結腸腺癌細胞為模型，發現此三個抑制劑可促進 Epirubicin 於吸收方向之運輸及減少 Epirubicin 於排出方向之運輸。總結，實驗結果顯示 Cremophor EL、verapamil 及 trifluoperazine 為有效之多重抗藥性抑制劑，但 Cremophor EL 於臨床使用之可能性較大，因其具有無全身性副作用之優點並能以體內試驗可用之濃度以增加 epirubicin 於小腸之吸收，而具有促進 Epirubicin 生體可用率之潛力。

關鍵詞：P 醣蛋白、Epirubicin、Cremophor EL、verapamil、 trifluoperazine、老鼠小腸、結腸腺癌細胞。

Abstract

P-glycoprotein (P-gp) actively pumps out a number of anticancer drugs, such as epirubicin, from tumor cells. Inhibition of intestinal P-gp function using MDR reversing agents may enhance oral bioavailability of some chemotherapeutic agents. Our previous flow cytometric study showed that Cremophor EL, verapamil or trifluoperazine all increased the intracellular accumulation of epirubicin in Caco-2 cells. In this study, the effect of Cremophor EL, verapamil or trifluoperazine as MDR reversing agents on the enhancement of intestinal absorption of epirubicin was investigated in both everted gut sacs of rats and human intestinal epithelial Caco-2 cell layers. The epirubicin concentrations measured in everted gut sacs pretreated with either of these modulators were significantly higher than those in epirubicin control in both the jejunum and the ileum. The addition of these modulators significantly increased apical-to-basolateral flux and reduced basolateral-to-apical flux of epirubicin across Caco-2 cells. In conclusion, our results demonstrated that Cremophor EL, verapamil or trifluoperazine are potent MDR modifiers of epirubicin. However, Cremophor EL has the advantage of no systemic side effects. Use of Cremophor EL as excipient may increase intestinal absorption of epirubicin and thus improve bioavailability of epirubicin.

Keywords: P-glycoprotein, epirubicin, Cremophor EL, verapamil, trifluoperazine, everted gut sacs, Caco-2

二、緣由及目的

The ability of malignant cells to develop simultaneous resistance to multiple chemotherapeutic agents appears to be a major obstacle to the successful treatment of clinical tumors. Overexpression of P-glycoprotein (P-gp) by resistant malignant cells is considered to be one common underlying mechanism of multidrug resistance (MDR)[1]. Inhibition of P-gp function using MDR reversing agents may increase intestinal absorption and cytotoxicity of anticancer drugs. Some lipophilic agents such as verapamil and trifluoperazine were found to reverse MDR phenotype *in vitro* by directly competing with anticancer drug binding site(s) of P-gp. However, clinical application of these agents has been very disappointing, because of their severe side effects *in vivo* at the doses required to reverse the MDR phenotype. Some pharmacologically inert surfactants such as Cremophor and Solutol HS-15 have been proven to be effective in reversing MDR phenotype in cultured cells at concentrations likely to be achieved clinically[2]. Our previous flow cytometric study showed that Cremophor EL, acacia, Tween 20 and Tween 80 might have MDR reversing effects and increased the intracellular accumulation of epirubicin in Caco-2 cells[3,4]. In this study, Cremophor EL, verapamil and trifluoperazine were selected as model MDR reversing agents to evaluate their effect on the intestinal absorption of epirubicin in everted gut sacs of rats and human intestinal epithelial Caco-2 cell layers.

三、結果及討論

As shown in Fig. 1, our flow cytometric result demonstrated that Cremophor EL, verapamil or trifluoperazine all significantly increased intracellular accumulation of epirubicin with the highest enhancing effect for verapamil and similar effect for Cremophor EL and trifluoperazine. Fig. 2 and Fig. 3 showed that the epirubicin concentrations measured in sacs pretreated with Cremophor EL, verapamil or trifluoperazine were significantly higher than those in epirubicin control for both the jejunum and the ileum ($P < 0.05$, $n = 3$ animals in each group), implying an increase in epirubicin absorption and/or a decrease in epirubicin efflux.

To clarify the involvement of these two factors, the effect of Cremophor EL, verapamil or trifluoperazine on the absorptional or secretory transport of epirubicin was investigated in Caco-2 cells. Fig. 4 showed the transepithelial flux of 100 μg/ml epirubicin across Caco-2 cell monolayers at 37 °C in the absorption (apical to basolateral; a→b) and secretory (basolateral to apical; b→a) directions plotted against time of incubation in the presence and absence of Cremophor EL, verapamil or trifluoperazine. The flux of epirubicin in the basolateral-to-apical direction was 2.3 fold of the flux in the apical-to-basolateral direction. A net flux of epirubicin was therefore observed in the secretory direction for Caco-2 cells. As demonstrated in Fig. 4, the addition of Cremophor EL, verapamil or trifluoperazine all significantly reduced the net efflux of epirubicin across the epithelial cells with the

highest reducing effect for verapamil.

Because epirubicin is one analog of the anthracycline antibiotic doxorubicin and is known to be pumped out by Pgp, our *in vitro* data thus supported the hypothesis that Cremophor EL, verapamil or trifluoperazine may influence the function of Pgp and/or other active transport systems responsible for the efflux of epirubicin in intestine. Inhibition of P-gp function using these MDR reversing agents by substrate competition, ATP-depletion or membrane perturbation[5-8] may antagonize multidrug resistance, thus increase intestinal absorption and cytotoxicity of epirubicin. However, the influence of these modulators in the transcellular and paracellular permeability of epirubicin is also another possible mechanism responsible for the enhancement of epirubicin absorption.

Cremophor EL is a pharmacologically inert surfactant and is usually used as an emulsifying agent and solubilizing agent in pharmaceutical formulations of vitamins, immunosupressants, e.g., cyclosporin A and anticancer drugs, e,g, Taxol. It has been shown to have MDR reversing effect with the advantage of no systemic side effect[9]. Thus, this surfactant has much higher potential to be used safely with epirubicin at concentrations achieved clinically.

Further studies need to be performed to make sure the mechanisms involved in MDR modulating phenomenon mediated by Cremophor EL, verapamil or trifluoperazine and to evaluate the clinical benefit for the combined use of epirubicin with these modulators.

In conclusion, our results demonstrated that Cremophor EL, verapamil or trifluoperazine are potent MDR modifiers of epirubicin in both everted gut sacs of rats and human colon adenocarcinoma cells

四、計畫成果自評

在應用價值方面，本計畫佐以體外及體內試驗來評估多重抗藥性抑制劑對抗癌藥物吸收及排出之影響，此部分實驗將提供臨床合併使用多重抗藥性抑制劑及抗癌藥物治療之直接依據。

在學術價值方面，在這個研究計畫中，藉由二十五個多重抗藥性抑制劑與抗癌藥物 epirubicin 之交互作用之研究，我們已建立適當之老鼠小腸及人體小腸細胞吸收之模型並期望能推而廣之到其它抗癌藥物以發現更多類型之多重抗藥性抑制劑，進而提高癌症化學療法的成功率。

本篇成果報告僅節錄其中三個多重抗藥性抑制劑與 epirubicin 作用的結果。綜合其它二十二個多重抗藥性抑制劑之結果，將可得到全面性之結論。這些結果目前已在整理階段，將發表於學術期刊上，並亟具有臨床應用之遠景。

五、參考文獻

1 Gottesman, MM and Pastan I. Annu Rev Biochem *62:* 386-427, 1993.

2 Buckingham et al., Int J Cancer *62:* 436-42, 1995.

3 Lo YL, Hsu CY and Huang JD. Anticancer Res *18*: 3005-3009, 1998.

4 Lo YL, Hsu CY and Huang JD. Chia Nan Annual Bulletin *23*: 18-24, 1997.

5 Ho NF, Burton, PS and Barsuhn CL. J Pharm Sci *84*: 21-27, 1995.

6 Leu BL and Huang JD. Cancer Chemother Pharmacol *35*: 432-436, 1995.

7 Hosoya KI, Kim KJ and Lee VHL. Pharm Res *13*: 885-890, 1996.

8 Woodcock et al., Cancer Res *50:* 4199-4203, 1990.

9 Schuurhuis et al., Br J Cancer *62:* 591-594, 1990.

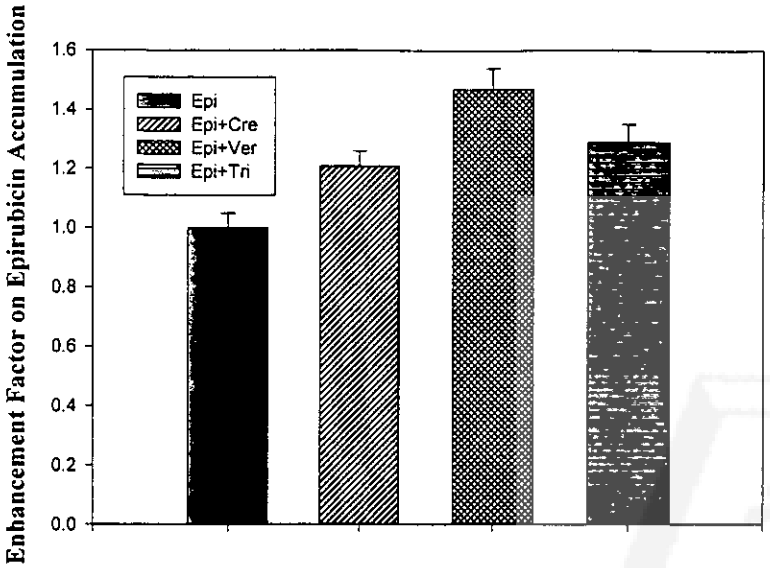

Fig.1. Enhancement factor for 3 hr intracellular accumulation of 1μg/ml epirubicin after pretreatment with various MDR reversing agents for 30 min

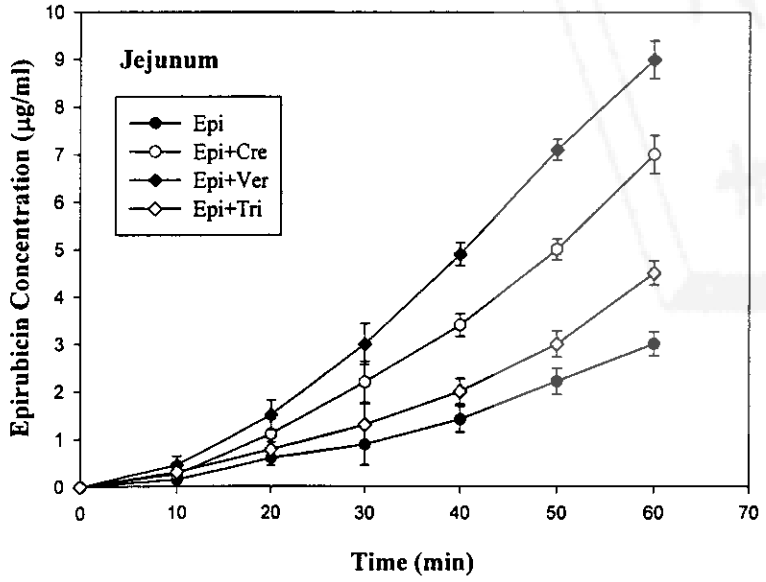

Fig. 2. The time profile of epirubicin concentration (Epi) inside the everted jejunum sacs of rats (n = 3 animals in each group) in the presence and absence of Cremophor EL (Cre), verapamil (ver) or trifluoperazine (Tri).

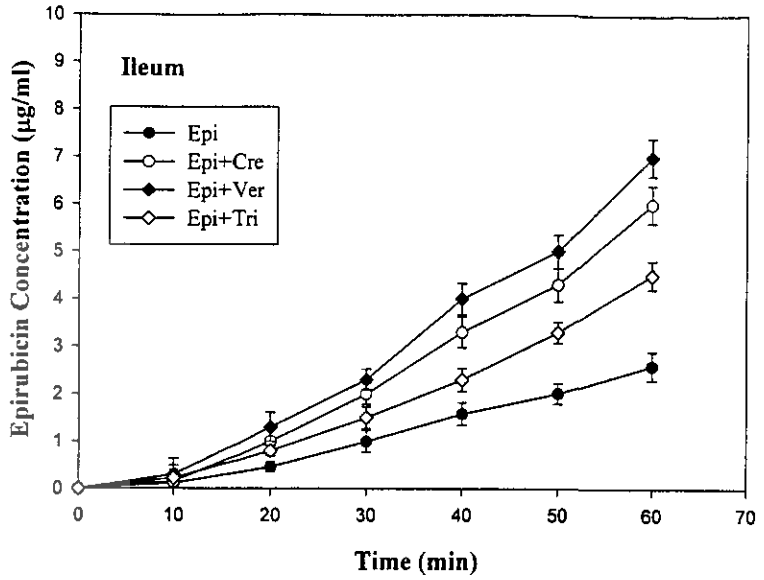

Fig. 3. The time profile of epirubicin concentration (Epi) inside the everted ileum sacs of rats (n = 3 animals in each group) in the presence and absence of Cremophor EL (Cre), verapamil (ver) or trifluoperazine (Tri).

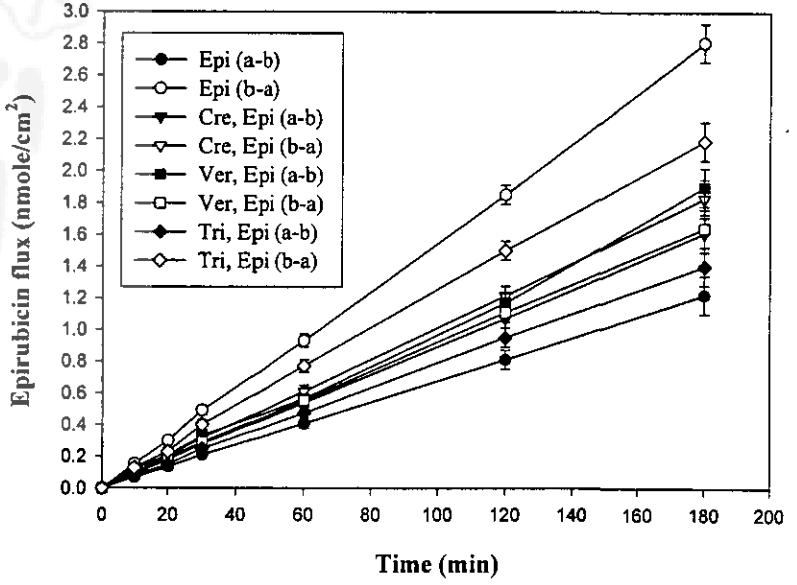

Fig. 4. Transepithelial flux of epirubicin (Epi) across Caco-2 cell monolayers in the absorptional (apical to basolateral) and secretory (basolateral to apical) directions plotted against time of incubation in the presence and absence of Cremophor EL (Cre), verapamil (ver) or trifluoperazine (Tri).