行政院國家科學委員會補助專題研究計畫成果報告

※※※※※※※※※※※※※※※※※※※※※※※※※※

※ 藥學賦形劑拮抗多重抗藥性之機轉探討 ※

※ Mechanisms of Multidrug Resistance Reversal ※

※ Mediated by Pharmaceutical Excipients ※

※※※※※※※※※※※※※※※※※※※※※※※※※※

計畫類別：☑個別型計畫　　□整合型計畫

計畫編號：NSC 89-2320-B-041-015

執行期間：89 年 8 月 1 日至 90 年 7 月 31 日

計畫主持人：駱雨利副教授

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：嘉南藥理科技大學藥學系

中　華　民　國 90 年 10 月 23 日

行政院國家科學委員會專題研究計畫成果報告
藥學賦形劑拮抗多重抗藥性之機轉探討
Mechanisms of Multidrug Resistance Reversal Mediated by Pharmaceutical Excipients

計畫編號: NSC 89-2320-B-041-015
執行期限: 89 年 8 月 1 日至 90 年 7 月 31 日
主持人: 駱雨利副教授　　嘉南藥理科技大學藥學系

一、中文摘要

本研究之主要目的是評估低藥理活性之藥學賦形劑 Span 80, Brij 30, Tween 20, Tween 80, Myrl 52, 及 sodium lauryl sulfate 對 epirubicin 於人類結腸腺癌細胞(Caco-2)及老鼠小腸運輸之影響，並探討開發這些賦形劑作為多重抗藥性拮抗劑之可行性。使用流式細胞分析儀，顯示這些賦形劑明顯增進 epirubicin 於 Caco-2 細胞之積聚且其促進效應隨劑量之增加而增加。以 Caco-2 細胞為小腸運輸模型，發現這些賦形劑可顯著促進 epirubicin 於吸收方向之運輸，Tween 20, Tween 80 及 Myrj 52 並可明顯減少 epirubicin 於排出方向之運輸。老鼠小腸實驗證實，不管是在空腸或迴腸，這些賦形劑均能明顯增進 epirubicin 之吸收。本篇研究顯示抑制小腸 P 醣蛋白或其他排出藥物之蛋白質可能跟 epirubicin 之增加吸收及減少排出有關。總結，臨床應用 Brij 30, Tween 20, Tween 80 及 Myrj 52 以當作吸收促進劑及多重抗藥性抑制劑可以促進 epirubicin 之小腸吸收，並可應用於拮抗癌症化學療法上之多重抗藥性。使用這些低毒性之賦形劑於劑型中可增進 P 醣蛋白受質之生體可用率並具有無全身性副作用及因改善藥物口服吸收所帶來之服用方便的優點。

關鍵詞： Epirubicin、賦形劑、界面活性劑、結腸腺癌細胞、老鼠小腸、P 醣蛋白、多重抗藥性

Abstract

The effects of a series of pharmaceutical excipients, including Span 80, Brij 30, Tween 20, Tween 80, Myrj 52, and sodium lauryl sulfate with increasing HLB values on the epirubicin absorption/exsorption and transport kinetics were investigated in both the human colon adenocarcinoma (Caco-2) cell line and the everted gut sacs of the rat jejunum and ileum. The possible use of these excipients as multidrug resistance (MDR) reversing agents also was examined. Epirubicin uptake experiments using a flow cytometer showed that the selected excipients markedly enhanced the intracellular accumulation of epirubicin in Caco-2 cells in a dose-dependent manner. These excipients significantly increase apical to basolateral absorption of epirubicin across Caco-2 monolayers and mucosal to serosal absorption of epirubicin in the rat jejunum and ileum. Moreover, the addition of Tween 20, Tween 80, and Myrj 52 substantially reduced the basolateral to apical efflux of epirubicin across Caco-2 monolayers. The study suggests that inhibition of P-gp or other transporter proteins located in the intestines may be involved, at least partially, in the reduction of epirubicin efflux. In conclusion, the therapeutic efficacy of epirubicin may be improved by the use of such low toxicity

excipients as absorption enhancers and MDR modulators in formulations.

Keywords: epirubicin; excipients; surfactants; Caco-2; everted gut sacs; P-glycoprotein; multidrug resistance

二、緣由及目的

Overexpression of the P-glycoprotein (P-gp), product of the MDR-1 gene, is known to be one of the well-characterized mechanisms of multidrug resistance (MDR)[1]. In our lab, a series of MDR-reversing agents, including non-toxic surfactants, have been proved for their enhancing effects on the intracellular accumulation of epirubicin in Caco-2 cell line[2]. In the current project, the effects of a series of pharmaceutical excipients, including Span 80, Brij 30, Tween 20, Tween 80, Myrj 52, and sodium lauryl sulfate with increasing HLB values on the epirubicin absorption/exsorption and transport kinetics were evaluated in both the human colon adenocarcinoma (Caco-2) cell line and the everted gut sacs of the rat jejunum and ileum. These excipients are widely added to pharmaceutical formulation to facilitate the preparation, patient acceptability and functioning of the dosage form. They can be used as disintegrating agents, diluents, suspending agents, emulsifying agents, and solubilizing agents. The relationship between HLB values of these excipeints and the degree of their MDR reversing effects was also investigated.

三、結果及討論

As illustrated in Figure 1, the results show that all the selected excipients markedly enhanced the uptake of epirubicin into Caco-2 cells in a dose-dependent manner, as measured by flow cytometry. With Caco-2 monolayers grown in Transwell filters as a model, the measurement of transepithelial electrical resistance (TEER) and the transport studies across Caco-2 monolayers were performed. As demonstrated in Figure 2, the reduction in the TEER after the addition of Myrj 52 implies that Myrj 52 may affect the paracellular route through the opening of tight junctions, and they may also reduce the cell integrity of Caco-2 cells. However, the recovery of TEER after removal of Myrj 52 was consistent with its reversible effect on tight junction widening. The similar trend was observed for other excipients used in this study (data not shown). Figure 3 shows that Tween 20, Tween 80, and Myrj 52 significantly increased the apical to basolateral absorption of epirubicin across Caco-2 monolayers. The substantial decrease in the basolateral to apical efflux of epirubicin was also observed. Some other excipients used in this study displayed the similar trend (data not shown).

Surfactants are though to enhance drug absorption via both the lipophilic and the hydrophilic pathways. They may disrupt the lipid arrangements in cell membranes and increase the water content of the membrane proteins.

Our study suggests that such membrane perturbation caused by these excipients may result in a change in the fluidity of Caco-2 cell membranes, and thus inhibit the activity of membrane-spanning proteins, such as P-gp[3].

These excipients may also inhibit protein kinase C (PKC) activity, reduce phosphorylation of P-gp, and modulate P-gp mediated drug efflux[4]. Our study implies that inhibition of P-gp or other transporter proteins located in the intestines may have at least a partial role in the reduction of epirubicin efflux in the secretory direction.

The dependency of the epirubicin enhancement factor on the HLB values of excipients is graphically presented in Figure 4. It is shown that the cytotoxicity of epirubicin formulated in different excipients increased to reach a maximal value and then decreased as the HLB values of excipients increased. These data suggest that maximal effects on the epirubicin uptake were characteristic of excipients with intermediate HLB values ranging from 14 to 18. As a result, the optimal net efficacy was observed for excipients with polyoxyethylene chains and intermediate fatty acid length. Tween 20 and 80 contain a sorbitan segment between polyoxyethylene and fatty acid groups. However, Myrj 52, which includes no sorbitan segment in their structure, still kept the optimal enhancement efficacy. Excipients with medium fatty acid chain length may penetrate the lipid bilayer easily, because of its proper aqueous solubility [5].

In conclusion, our study suggests that Tween 20, Tween 80, Myrj 52, and Brij 30 may have MDR reversing effects. Therapeutic use of these excipients for the inhibition of intestinal P-gp may improve the oral bioavailability of epirubicin. As MDR-reversing agents in drug formulations, they may reduce systemic side-effects and improve oral absorption of drugs in MDR spectrum. The combined use of anticancer drugs with these excipients may have significant implications in circumventing multidrug resistance in cancer chemotherapy.

四、計畫成果自評

在應用價值方面，本計畫佐以細胞及動物試驗來評估賦形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抗癌藥物及賦形劑為多重抗藥性抑制劑作治療之依據。

在學術價值方面，在這個研究計畫中，藉由不同賦形劑與 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廣到其它抗癌藥物以發現更多低毒性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數個賦形劑與 epirubicin 作用的結果。綜合其它賦形劑類多重抗藥性抑制劑之結果，將可得到全面性之結論。這些結果目前已發表三篇文獻於學術期刊上，並亟具有臨床應用之遠景。

五、參考文獻

1 Germann UA. Eur J Cancer 32A: 927-944, 1996.

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

3 Lindmark T, Kimura Y and Artursson P. J Pharmacol Exp Ther 284: 362-369, 1998.

4 Komarov PG, Shtil AA, and Coon JS. Int J Cancer 68: 245-50, 1996.

5 Park YS, Chang EY, and Chi YC. Int J Pharm 210:109-19, 2001.

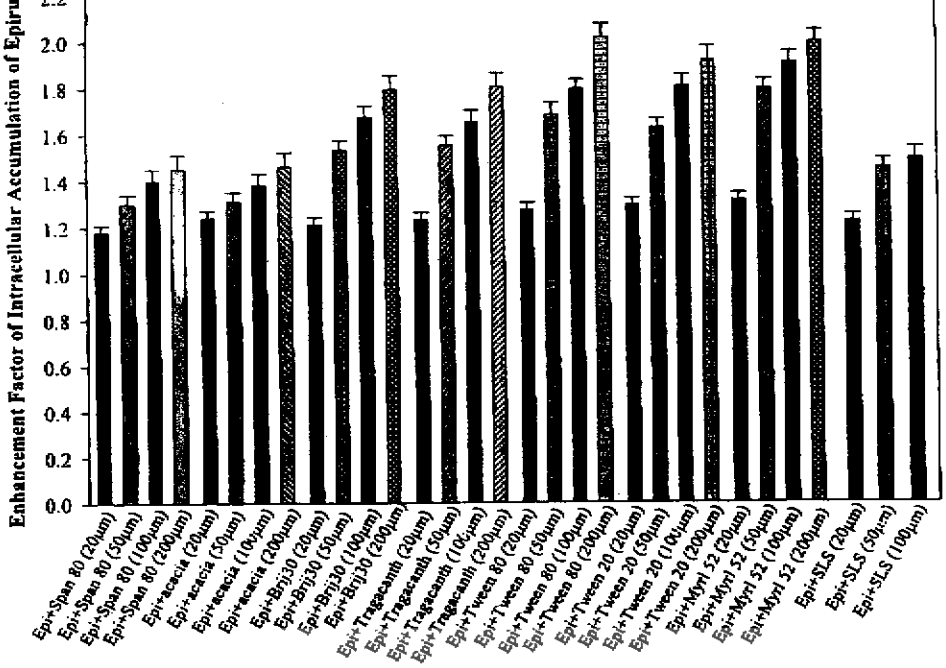

Figure 1. Enhancement factor of intracellular accumulation of epirubicin measured by flow cytometry in Caco-2 cells. Cells were pretreated with various concentration of surfactants for 30 min and incubated with 1 μg/ml epirubicin for 180 min. Enhancement factor is calculated as fluorescence intensity of epirubicin after appli of surfactants divided by fluorescence intensity of epirubicin control (n =4). Epi: epirubicin; SLS: sodium lauryl sulfate

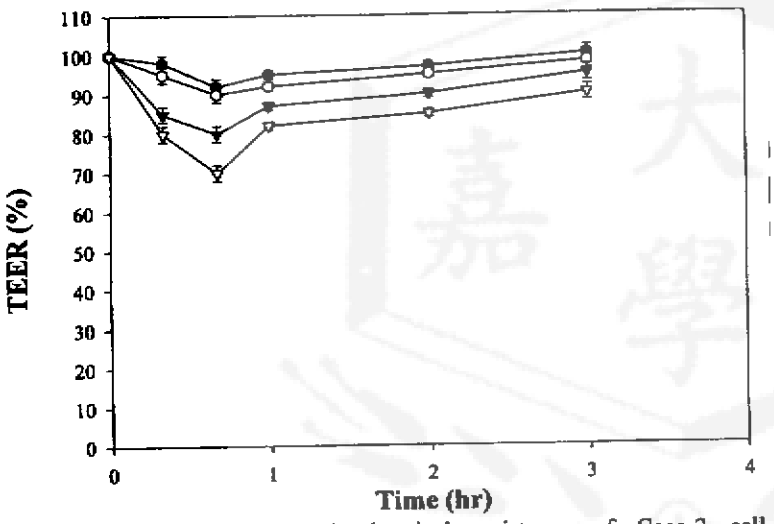

Figure 2. Transepithelial electrical resistance of Caco-2 cell monolayers at 37° plotted against time of incubation after treatment of various concentrations of Myrj 52 for 30 min. Each point represents the means ± SD of four determinations. ●: 20 μM; ○: 50 μM; ▼: 100 μM; ▽: 200 μM.

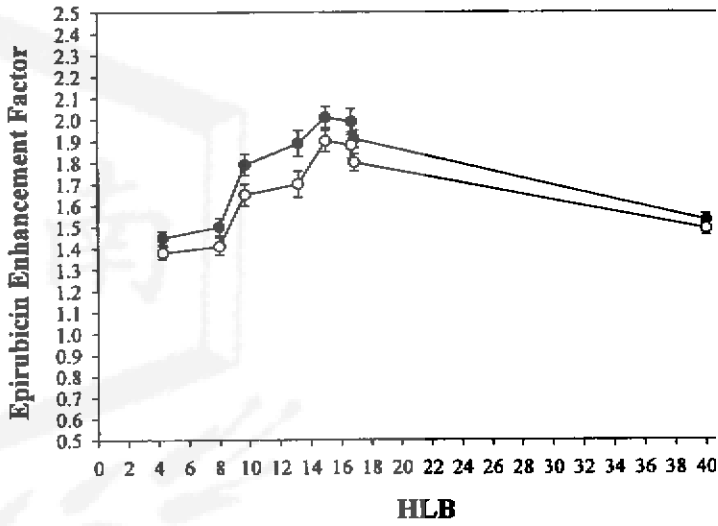

Figure 4. Effects of HLB values of excipients on the enhancement fac of intracellular epirubicin accumulation in Caco-2 cells. Each po represents the means ± SD of four determinations.
●: epirubicin pretreated with 200 μM of excipients for 30 min;
○: epirubicin pretreated with 100 μM of excipients for 30 min.

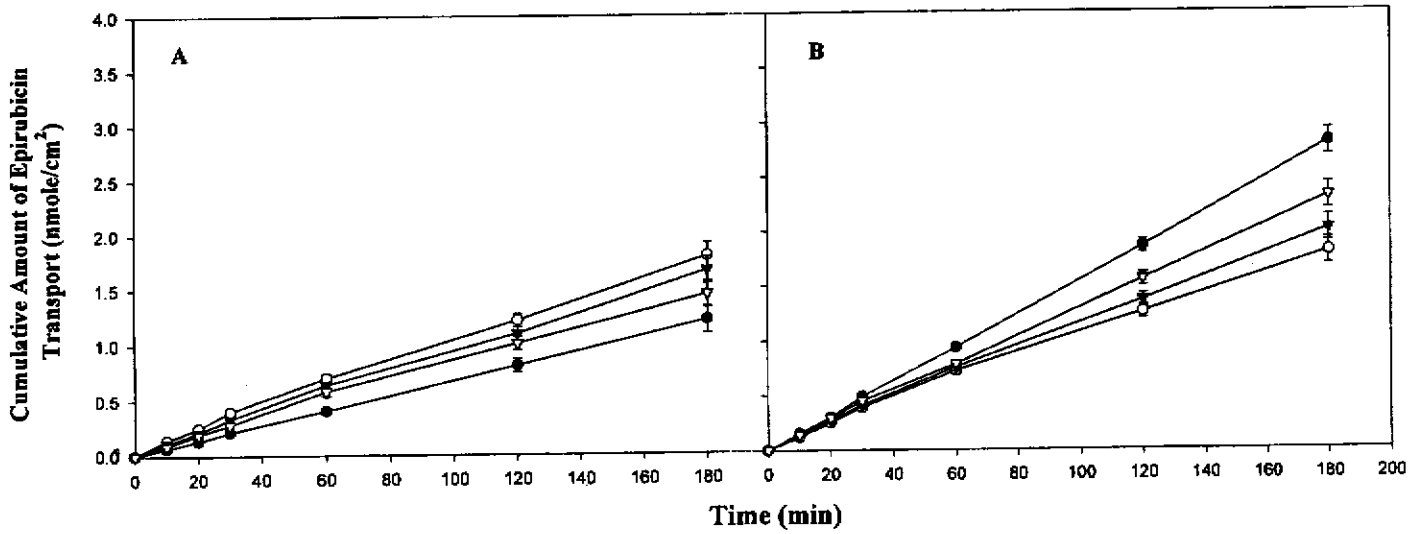

Figure 3. Transepithelial fluxes of epirubicin across Caco-2 cell monolayers at 37° in the (A) absorptive (apical to basolateral; a→b) and (B)secretory (basolateral to apical; b→a) directions plotted against time of incubation in the presence or absence of various excipients. Each point represents the means ± SD of four determinations.
●: epirubicin control; ○: epirubicin pretreated with Tween 80; ▼: epirubicin pretreated with Tween 20; ▽: epirubicin pretreated with Myrj 52