行政院國家科學委員會專題研究計畫成果報告

P 醣蛋白運送抗癌藥物之機轉：磷脂質之重要性探討

Mechanism of anticancer drug transport by P-glycoprotein: the possible role of phospholipids

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一、中文摘要

磷脂質常被用來當作藥物之攜帶體。抗癌藥物若以磷脂質做成之微脂粒包裝，常可降低毒性、增加療效。因此我們研究微脂粒包埋、空的微脂粒或磷脂質對於 Epirubicin 於人類結腸腺癌細胞(Caco-2)及老鼠小腸之積聚及運送之效應。使用流式細胞分析儀分析，發現微脂粒包埋或空的微脂粒明顯增進 Epirubicin 於 Caco-2 細胞之積聚。以 Caco-2 細胞為模型，發現此二種劑型可顯著促進 Epirubicin 於吸收方向之運輸及明顯減少 Epirubicin 於排出方向之運輸。由老鼠小腸之結果發現不管是在空腸或迴腸，此二種劑型均能明顯增進 Epirubicin 之吸收。實驗結果顯示抑制小腸 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，使用磷脂質做成藥物之微脂粒劑型可以經由抑制 P 醣蛋白之機轉以促進藥物之小腸吸收，並增進 P 醣蛋白受質之生體可用率。微脂粒包裝可應用於拮抗癌症化學療法上之多重抗藥性。

關鍵字： Epirubicin、磷脂質、微脂粒、結腸腺癌細胞、老鼠小腸、P 醣蛋白、多重抗藥性

Abstract

Studies using cancer chemotherapeutic agents such as epirubicin encapsulated in liposomes, made of phospholipids and other ingredients, have generally shown reduced toxicity and enhanced therapeutic efficacy. This study investigated the effects of liposomal encapsulation, empty liposome or free phospholipid addition on the uptake and transport of epirubicin in the human colon adenocarcinoma (Caco-2) cell line and everted gut sacs of rats. The flow cytometric experiments showed that liposomal encapsulation or empty liposome addition significantly increased the intracellular accumulation of epirubicin in Caco-2 cells. These two formulations substantially enhanced apical to basolateral absorption and significantly reduced the basolateral to apical efflux of epirubicin across Caco-2 monolayers. The epirubicin concentrations measured in everted gut sacs pretreated with these two formulations were significantly higher than those in epirubicin control in both the jejunum and the ileum. The study suggests that inhibition of P-glycoprotein or other transporter proteins located in the intestines may be, at least partially, involved in the reduction of epirubicin efflux. In conclusion, the therapeutic efficacy of epirubicin may be improved by the use of phospholipids as excipients and MDR modulators in the formulations. Liposomal encapsulation may have significant implications in circumventing drug resistance in cancer

chemotherapy.

Keywords: epirubicin; phospholipids; liposomes; Caco-2; everted gut sacs; P-glycoprotein; multidrug resistance

二、緣由及目的

Phospholipids have been increasingly used as carriers for delivery of a variety of drugs. Studies using anticancer agents such as epirubicin encapsulated in liposomes, made of phospholipid mixtures, have generally shown reduced toxicity and enhanced therapeutic efficacy[1].

Some studies have shown that Class III P-glycoproteins (P-gps) specifically translocated phosphatidylcholine (PC) toward the outer bilayer leaflet of the plasma membrane. It was also suggested that MDR 1 P-gp translocated PC and phosphatidylethanolamine (PE) [2,3]. In our previous study, we provided some evidence to support our hypothesis that bovine serum albumin (BSA) might work as an acceptor protein to extract phospholipids from the cell surface into the medium, resulting in less P-gp substrates and more available P-gps for pumping epirubicin or vinblastine out of Caco-2 cells[4]. These studies explored the physiological role of MDR1 P-gp to act as a flipase in translocating lipids of broad specificity across the plasma membrane, besides its well-known role as a multidrug transporter. We thus propose that modulation of P-gps by phospholipids may antagonize MDR and increase cytotoxicity of epirubicin.

In this study, we demonstrate the effects of various phospholipid formulations on the intracellular accumulation and transport of epirubicin in Caco-2 cells as well as everted gut sacs of rats. These formulations included epirubicin encapsulated in dipalmitoyl phosphatidylcholine (DPPC) or dipalmitoyl phosphatidylethanolamine (DPPE) liposomes, empty DPPC or DPPE liposomes, and free DPPC or DPPE addition.

三、結果及討論

As demonstrated in Figs. 1 and 2, liposomal encapsulation or empty liposome addition significantly increased the intracellular accumulation and mucosal to serosal absorption of epirubicin in Caco-2 cells and everted gut sacs of rats. The effect of free lipid addition on epirubicin uptake was marginal.

Figs. 3 and 4 show that liposomal encapsulation or empty liposome addition apparently enhanced apical to basolateral (a→b) absorption and significantly reduced basolateral to apical (b→a) efflux of epirubicin across Caco-2 monolayers.

The polarized localization of P-gp has led to the suggestion that its physiological role is as a secretory detoxifying system and is responsible for the efflux of lipophilic molecules[5]. In the gut, P-gp is localized in the apical membrane of enterocytes. It is thus conceivable that P-gp can act as a barrier to the intestinal absorption of drugs, by inducing a net basolateral to apical flux of xenobiotics[6].

Anticancer drugs encapsulated in liposomes made of phospholipid mixtures were found to circumvent P-gp mediated drug resistance[7]. The increased cytotoxicity was suggested to be at least

partly due to the increased intracellular concentration of the cytotoxic agents by the inhibition of the P-gp mediated drug efflux[8].

Phospholipids are the major lipids in the plasma membrane of mammalian cells. They have been proven to be the substrates of Class I and/or Class III P-gps in many cell lines. In addition, human MDR1 P-gp has been demonstrated to be a phospholipid translocase with broad specificity[2,3]. We thus suggest that both epirubicin and phospholipid molecules may be recognized by P-gps within the context of the lipid bilayer and then be translocated to the outer leaflet of the membrane or to the cell exterior, to ultimately reduce the intracellular accumulation. Thus, inhibition of P-gps or other transporter proteins located in the intestines by phospholipids may be, at least partially, involved in the reduction of epirubicin efflux.

In conclusion, modulation of P-gps by exogenous phospholipids through substrate competition, membrane perturbation, or other mechanisms might antagonize MDR and increased cytotoxicity of epirubicin. However, further studies need to be performed to verify the above hypothesis. More importantly, we demonstrate that the combined use of epirubicin with phospholipids may have significant implications to circumventing drug resistance in cancer chemotherapy. Phospholipids may function as MDR reversing agents and have potential therapeutic use.

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磷脂質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磷脂質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，藉由不同磷脂質劑型與 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一個磷脂質 DPPC 與 epirubicin 作用的結果。綜合其它不同磷脂質類多重抗藥性抑制劑及先前共同指導之學生論文[4]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

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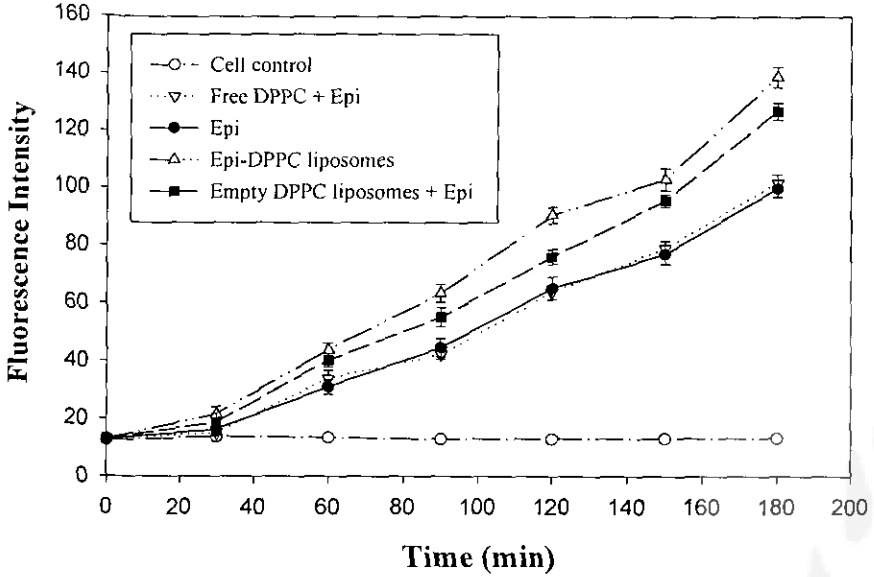

Fig. 1 Time course of the fluorescence intensity of epirubicin in different lipid treatments.

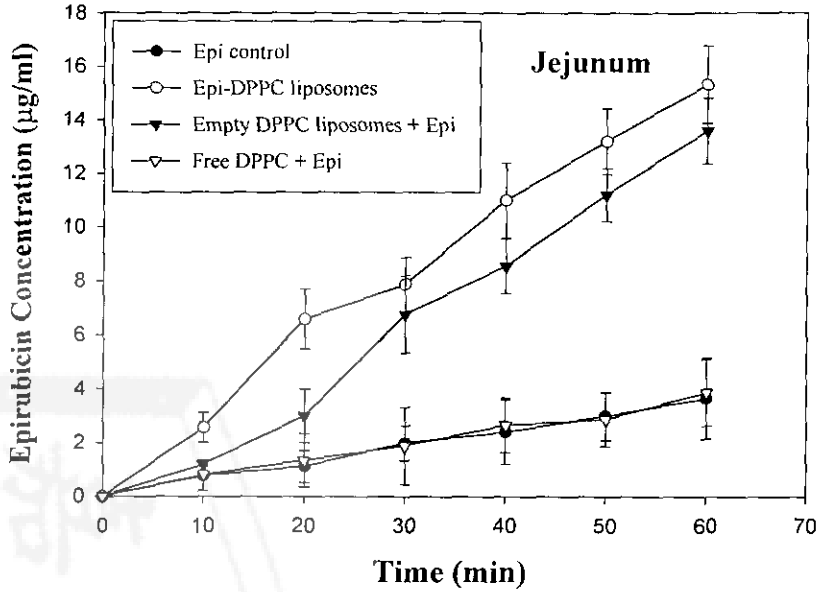

Fig. 2 The time profile of epirubicin concentrations inside the everted jejunum sacs of rats (n = 3) in the presence or absence of various DPPC treatments.

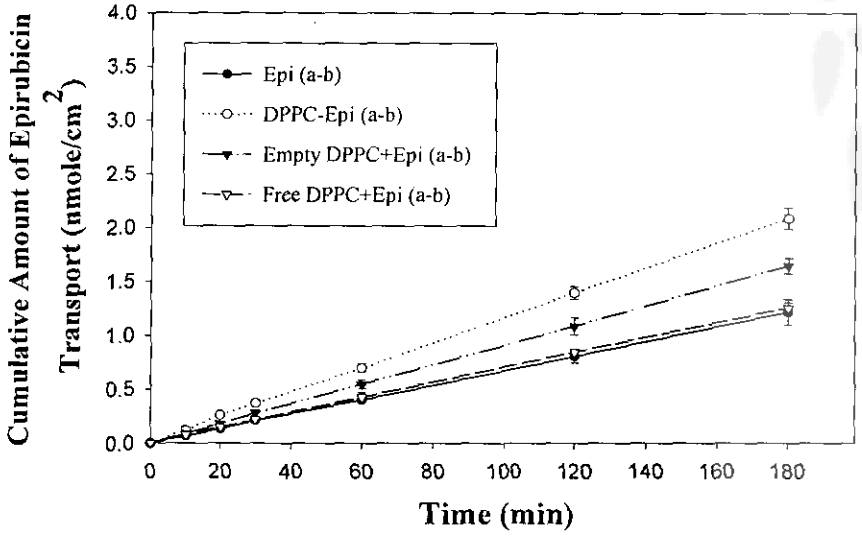

Fig. 3 Transepithelial fluxes of epirubicin in the absorptional (a→b) direction in the presence or absence of various DPPC treatments.

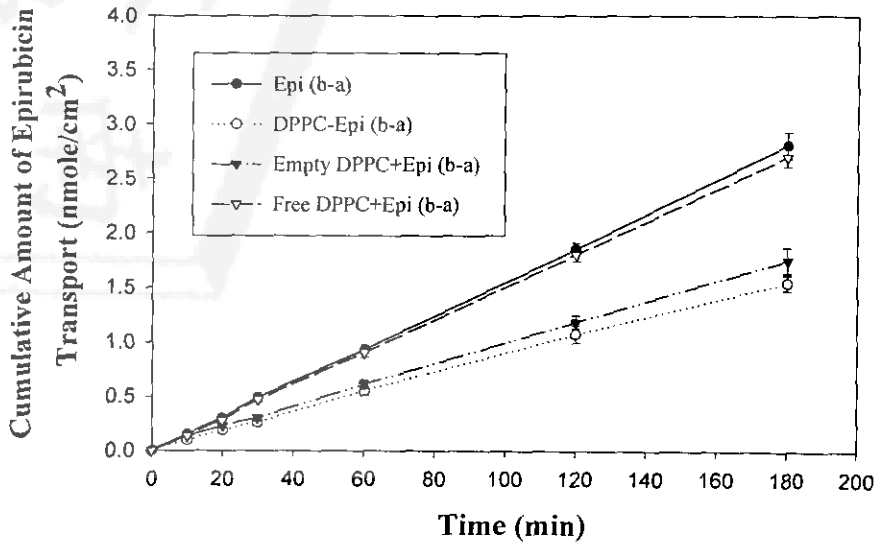

Fig. 4 Transepithelial fluxes of epirubicin in the secretory (b→a) direction in the presence or absence of various DPPC treatments.