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多重抗藥性抑制劑對小腸排出藥物蛋白質運送抗癌藥物之效應及機轉探討(1/3)

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行政院國家科學委員會專題研究計畫期中報告
多重抗藥性抑制劑對小腸排出藥物蛋白質運送抗癌藥物之效應及機轉探討
Effects and Mechanisms of Multidrug Resistance Reversing Agents on the Anticancer Drug Transport by the Drug Efflux Proteins in the Intestine

計畫編號: NSC 91－2320－B－041－011
執行期限: 91 年 8 月 1 日至 92 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

抗癌藥物於施用後產生多重抗藥性，常是造成癌症化學療法失敗之重要原因。根據文獻報告指出，P 醣蛋白(P-glycoprotein；P-gp)或其它排出藥物蛋白質，例如多重抗藥性相關蛋白質(multidrug resistance-associated protein 1；MRP1) 及小管多重特定有機陰離子運輸蛋白質(canalicular multispecific organic anion transporter；cMOAT；又稱 MRP2)等的過度表現是癌細胞產生多重抗藥性的主要因素之一。在本計劃中，我們以結腸腺癌細胞 Caco-2 模擬小腸細胞，以確定多重抗藥性抑制劑對腸腔排出藥物蛋白質運送藥物之影響。本計劃選用抗癌藥物 epirubicin 爲模式藥物。以反轉錄聚合酵素連鎖反應(RT-PCR)來定量不同之腸腔排出藥物蛋白質及比較加入不同之多重抗藥性抑制劑對其表現強度之影響。使用流式細胞分析儀分析 epirubicin 於加入不同種類之多重抗藥性抑制劑後，其於 Caco-2 細胞之積聚情況之改變。本計劃使用之多重抗藥性抑制劑包括 probenecid, indomethacin, quinidine 及 cyclosporin A。我們發現 P-gp, MRP1 及 cMOAT\MRP2 於 Caco-2 細胞之表現以第三天爲最強。Cyclosporin A 顯著降低 MRP2 之表現。Probenecid, indomethacin 及 quinidine 明顯減少 P-gp 及 MRP1 之表現。這四個多重抗藥性抑制劑均顯著促進 epirubicin 於 Caco-2 細胞之積聚。合併使用 indomethacin 及 cyclosporin A 則能進一步增加 epirubicin 之積聚。本研究使用不同功能之多重抗藥性抑制劑以期能同時拮抗多種排出藥物蛋白質而達到全面反制多重抗藥性之目的。

關鍵詞：排出藥物蛋白質，P 醣蛋白，多重抗藥性，抑制劑，epirubicin，結腸腺癌細胞，流式細胞分析儀，小腸

Abstract

Resistance to structurally and functionally unrelated multiple drugs, known as multidrug resistance (MDR), is a leading obstacle in the treatment of human cancers. MDR is mediated by the increased expression of energy-dependent drug efflux pumps, such as P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1), and canalicular multispecific organic anion transporter (cMOAT; MRP2) in cancer cells. Until now, only scattered information is available regarding the expression and function/activity of these efflux proteins in intestines, a rate-limiting barrier to oral drug absorption. In this study, we aim to evaluate the effects of various MDR modulators on the expression levels of intestinal efflux transporter proteins, such as

P-gp, MRP1, and cMOAT. In addition, by use of inhibitors of these efflux transporters, the correlation among the expression levels of these MDR-related transporters with epirubicin uptake was investigated. The MDR modulators used in this study are probenecid, indomethacin, quinidine, and cyclosporin A. We found that the mRNA expression levels of MDR1, MRP1, and MRP2 in Caco-2 cells were in a time-dependent manner, with the maximal expression was observed in day 3. Cyclosporin A significantly reduced the mRNA expression of MRP2. Probenecid, indomethacin, and quinidine markedly decreased the expression levels of MDR1 and MRP1, but showed marginal effect on MRP2. All the selected MDR modulators markedly enhanced the uptake of epirubicin into Caco-2 cells, as measured by flow cytometry. The modulators with multiple inhibitory function on MDR1, MRP1, and MRP2, e.g., probenecid, indomethacin, and quinidine, showed better enhancement factor on epirubicin uptake. The combined use of indomethacin and cyclosporin A demonstrated further enhancement on the intracellular accumulation of epirubicin, indicating that the pharmacological inhibition of MDR might be intensified by the combination of modulators of MDR1, MRP1, and MRP2. In conclusion, the combined use of epirubicin with multiple-function inhibitors in antagonizing different intestinal transporter proteins may have significant implications to circumvent drug resistance in cancer chemotherapy.

Keywords: drug efflux transporter proteins, P-glycoprotein, multidrug resistance, modulators, epirubicin, Caco-2, flow cytometer, intestines

二、緣由及目的

Resistance to structurally and functionally unrelated multiple drugs, known as multidrug resistance (MDR), is a leading obstacle in the treatment of human cancers. MDR is mediated by the increased expression of energy-dependent drug efflux pumps, such as P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1), and canalicular multispecific organic anion transporter (cMOAT; MRP2) in cancer cells. These transporter proteins actively pump out a number of drugs, including epirubicin, from tumor cells. These efflux proteins are expressed in various organs and cancer cells, including intestines and human colon adenocarcinoma (Caco-2) cells. These intestinal drug efflux proteins confer resistance to a similar but not identical spectrum of MDR.

Inhibition of function of intestinal pump proteins by MDR reversing agents, through the mechanism of substrate competition, ATP-depletion, or membrane perturbation, may antagonize MDR, and thus increases the intestinal absorption and cytotoxicity of anticancer drugs. Until now, only scattered information is available regarding the expression and function/activity of these efflux proteins in intestines, a rate-limiting barrier to oral drug absorption. In this study, we aim to evaluate the effects of various MDR modulators on the expression levels of intestinal efflux transporter proteins, such as

P-gp, MRP1, and cMOAT. In addition, by use of inhibitors of these efflux transporters, the correlation among the expression levels of these MDR-related transporters with the intracellular accumulation of epirubicin was investigated.

The MDR modulators used in this study are probenecid, indomethacin, quinidine, and cyclosporin A.

三、結果及討論

As illustrated in Figure 1, the results show that the mRNA expression levels of cMOAT/MRP2 in Caco-2 cells was in a time-dependent manner, as measured by RT-PCR measurement. The maximal expression was observed in day 3, and this incubation period was used for the following study. The similar phenomenon was also observed in the mRNA expression levels of MDR1 and MRP1 (data not shown). We found that cyclosporin A showed mild effect on the mRNA expression level of MDR1, but significantly reduced the expression of MRP2 ($P < 0.001$). Probenecid, indomethacin, and quinidine markedly decreased the expression levels of MDR1 and MRP1 ($P < 0.01$), but showed marginal effect on MRP2. Fig. 2 depicts that all the selected MDR modulators markedly enhanced the uptake of epirubicin into Caco-2 cells, as measured by flow cytometry. In combination of Table 1 and Fig. 2, the modulators with multiple inhibitory function on MDR1, MRP1, and MRP2, e.g., probenecid, indomethacin, and quinidine, showed better enhancement factor on intracellular accumulation of epirubicin than that with limited function on MRP2, e.g., cyclosporine A ($P < 0.05$). Especially, indomethacin, which showed superior inhibitory effect on MDR1 and MRP1, and marginal effect on MRP2, demonstrated the best enhancement on epirubicin uptake among the modulators used in the current study. The combined use of indomethacin and cyclosporin A demonstrated further enhancement on the intracellular accumulation of epirubicin, indicating that the pharmacological inhibition of MDR might be intensified by the combination of modulators of MDR1, MRP1, and MRP2.

Organic anion transport inhibitors, such as probenecid and indomethacin, have been shown to modulate the MRP-mediated drug transport. Although Regina et al (1998) showed that probenecid and indomethacin did not affect Pgp-mediated transport, our result demonstrated the excellent inhibitory effect of these two modulators on MDR1/Pgp in Caco-2 cells.

Indomethacin, one glutathione S-transferase inhibitor and a modulator of anion transport, has been shown to be a specific inhibitor of MRP, possibly functioning by inhibition of glutathione S-transferase or by direct competition with the drug at the transport site (Perloff et al., 2001). Indomethacin increased the accumulation of vincristine, one specific MRP substrate, in MRP-overexpressing cells (Perloff et al., 2001). Our study found that the pronounced increase of indomethacin on the epirubicin uptake into Caco-2 cells might be correlated to the excellent multiple inhibitory effect of indomethacin on the expression of MDR1,

MRP1, and MRP2.

Quinidine, one organic cationic antiarrhythmic agent, is lipophilic in nature and includes a heterocyclic ring nucleus separated at a distance from an amino group. It shares a broad structural similarity with some anticancer drugs, such as epirubicin. Quinidine was shown to be a potent P-gp inhibitor. In the current study, it was demonstrated to show significant inhibition on P-gp and MRP1 expression ($P < 0.05$). This indicates the possible further application of this compound for the modulation of MRP- family proteins.

Cyclosporin A, which are very potent reversing agents of P-gp (Lo et al., 2001), usually show no or only small effects on the drug sensitivity of MRP-overexpressing MDR cells. However, Chen et al. (1999) found that cyclosporin A increased the sensitivity of LLC/cMOAT cells to vincristine and cisplatin. This suggests that cyclosporin A can be used as a combined inhibitor of P-gp and cMOAT. Our result agrees with their study by showing the inhibitory effect of cyclosporin A on the mRNA expression levels of MDR1 and cMOAT/MRP2, but not on MRP1.

In conclusion, the combined use of epirubicin with multiple-function inhibitors in antagonizing different intestinal transporter proteins may have significant implications to circumvent drug resistance in cancer chemotherapy.

四、計畫成果自評

在**應用價值**方面，本計畫佐以分子生物學及細胞試驗來評估多重抗藥性抑制劑對抗癌藥物於細胞吸收之影響，此部分實驗將提供臨床合併使用抗癌藥物及佐劑以作為化學療法之治療依據。

在**學術價值**方面，在這個研究計畫中，藉由不同多重抗藥性抑制劑與 epirubicin 之合用之研究，我們已建立適當之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具 multiple function 之多重抗藥性抑制劑以與藥物合用，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多重抗藥性抑制劑與 epirubicin 作用的結果。綜合其它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一篇文獻於學術期刊上，其它結果則在整理及投稿階段，亟具有臨床應用之遠景。

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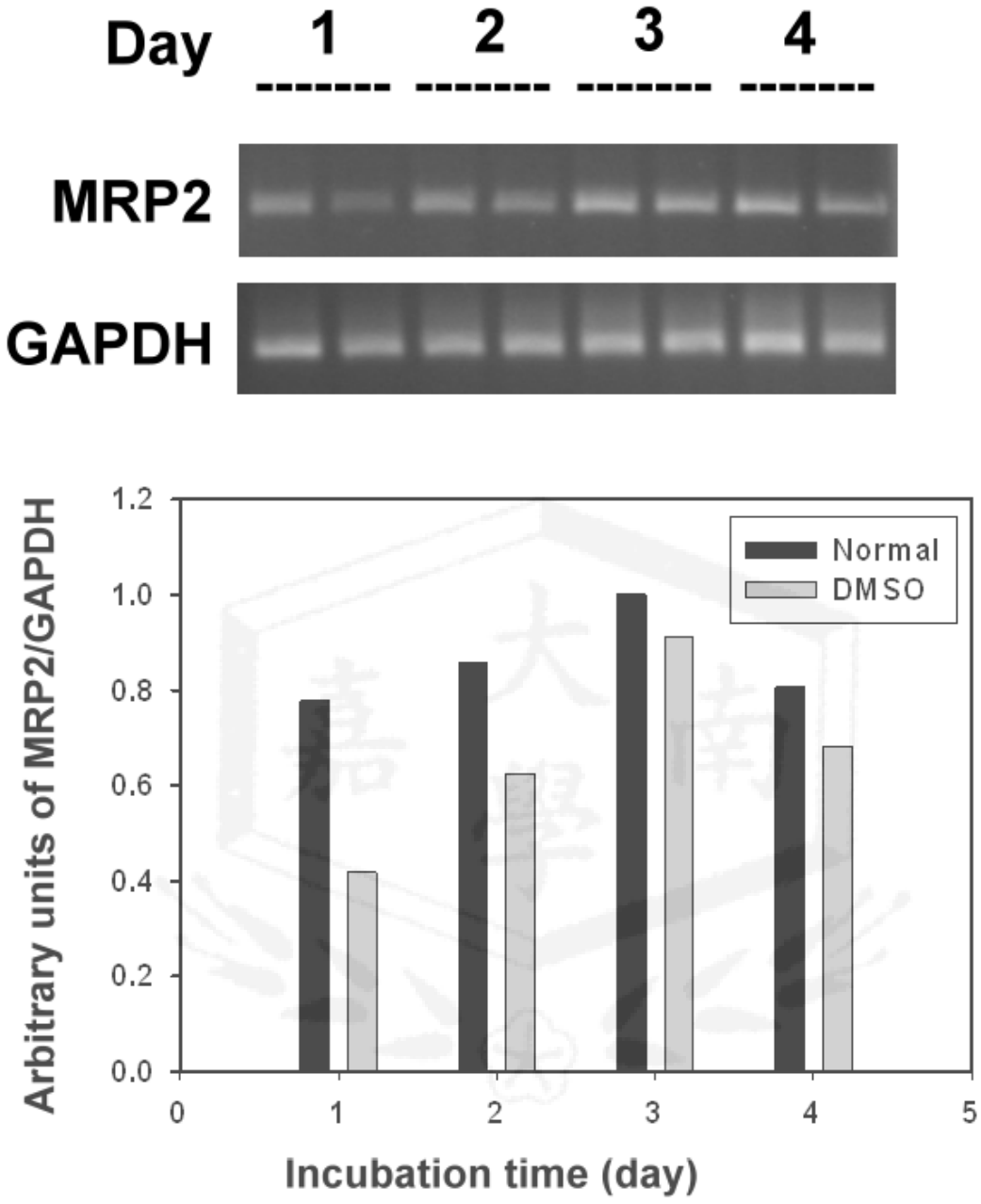

Fig. 1 RT-PCR screen for mRNA expression of cMOAT/MRP2 in Caco-2 cells in incubation day from 1 to 5.

Table 1 Effect of MDR modulators on the expression levels of different efflux proteins

Drug	Concentration	Types of efflux proteins		
		MDR1	MRP1	MRP2
Cyclosporin A	10 μM	↓ 10 %	↔	↓ 40 %
Quinidine	100 μM	↓ 20 %	↓ 40 %	↓ 10 %
Indomethacin	200 μM	↓ 40 %	↓ 40 %	↓ 10 %
Probenecid	1 mM	↓ 40 %	↓ 20 %	↓ 10 %

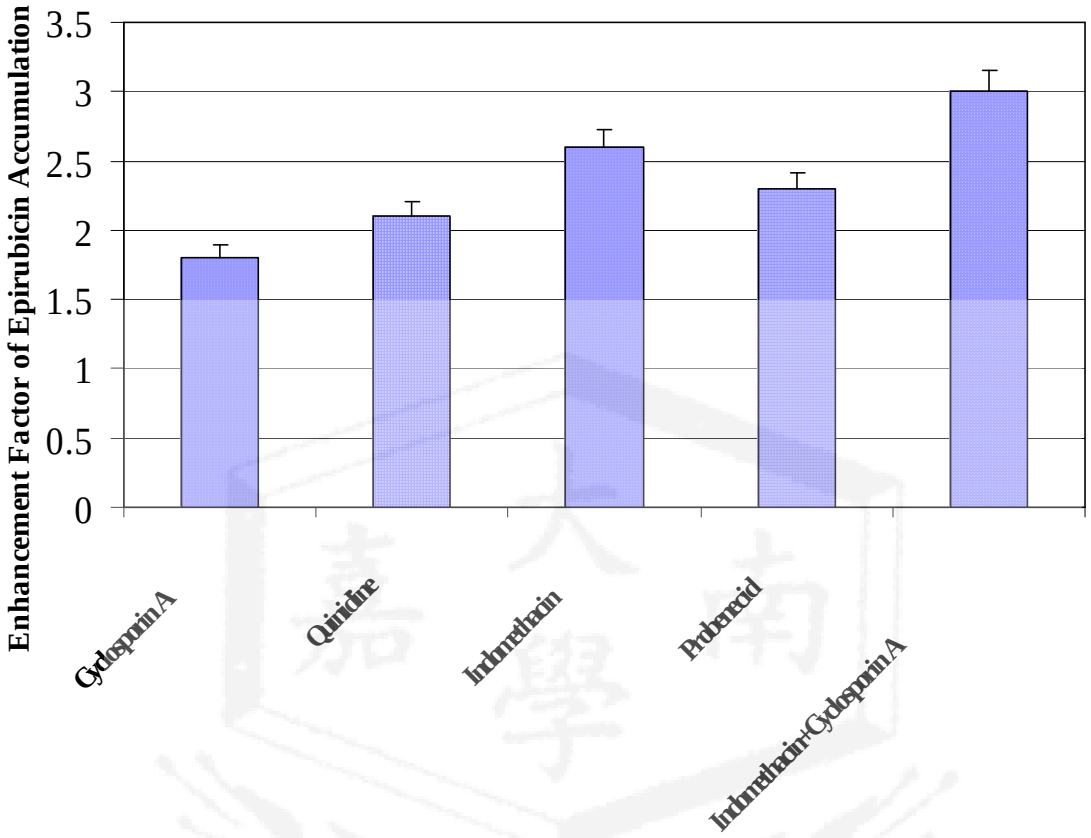

Fig. 2 Enhancement factor of intracellular accumulation of epirubicin in Caco-2 cells. Cells were pretreated with various MDR modulators of 10 μM of cyclosporine A, 100 μM of quinidine, 200 μM of indomethacin, or 1 mM of probenecid for 30 min, and incubated with 1 μg/mL of epirubicin for 180 min. Enhancement factor is the ratio of fluorescence intensity of epirubicin with modulators divided by fluorescence intensity of epirubicin control. Each bar represents the mean and each vertical bar the SD. Data is means ± SD of three independent experiments. Statistics were performed using student's t test. In all cases, we found that $P < 0.001$ when compared with the epirubicin control (enhancement factor = 1).