行政院國家科學委員會補助專題研究計畫成果報告

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計畫主持人：蘇聖芳   國立成功大學臨床藥學研究

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中文摘要

臨床上癌症病患的化學治療對藥物產生之抗藥性(多重抗藥性)為最主要的問題，而許多研究已證明多重抗藥性與P酶蛋白有關。P酶蛋白為胞膜運輸體，能將物質由細胞內運送到細胞外，降低其在細胞內的存積，此種運輸體表現於癌細胞及許多正常組織中。P酶蛋白具有廣泛的受質及抑制劑，但抑制劑的毒性在臨床應用上為一大問題，因此，探討其低毒性的抑制劑為目前治療多重抗藥性的一大目標。先前實驗證實，一合成的tripeptide (ALLN)在小腸腸腔中可明顯地增加etoposide的吸收，同時實驗也發現nonpeptidic物質亦為oligopeptide transporter (hPept1)之受質。因此本計劃主要在探討在抗性細胞中peptide之角色，第一年計畫選擇以ALLN analogs來探討peptide的親脂性及帶鈣性與Pgp抑制作用之關係，再進一步以in vivo study來評估藥動學上之變化。這對於臨床上以peptide治療抗藥性是相當重要的。

關鍵詞：Xenopus oocyte, oligopeptide transporter, ALLM, P酶蛋白

Abstract

Clinical resistance to chemotherapeutic agents is the major obstacle in the treatment of cancer, which P-glycoprotein (Pgp) plays an important role. P-glycoprotein, a membrane transporter protein, can efflux intracellular compounds outward and thus decrease their intracellular accumulation. This transporter is expressed in tumor cells as well as in a variety of tissues including the small intestine. Pgp exhibits broad substrate specificity with structure unrelated compounds as modulators. However, the toxicity of modulator may hinder the clinical application. Investigation into Pgp modulator with low toxicity thus becomes an important goal to overcome MDR. In our previous study, a synthetic linear tripeptide (ALLN) was shown to significantly enhance the transport of etoposide in rat everted gut sacs. And studies have demonstrated the nonpeptidic compound as a substrate of oligopeptide transporter (hPept1). Therefore, the main goal of this project is to characterize the linear peptide in drug resistance therapy. In the first year, ALLN analogs will be chosen to assess the relationship between the hydrophobicity, charge of linear peptides and their Pgp inhibitory effect in Xenopus oocyte expression system. Subsequently, drug interaction in pharmacokinetics will be evaluated using in vivo study in rats. The in vitro/in vivo correlation can be characterized to predict the effect of Pgp modulator on Pgp-mediated drug transport. It is important to understand this issue for clinical application of Pgp inhibitors in drug resistance therapy.

Keywords: P-glycoprotein, Oligopeptide transporter, ALLM, Xenopus oocyte

Introduction

P-glycoprotein, the gene product of mdr1 with 170-kDa plasma protein, functions as an energy-dependent drug efflux pump and thus decreases drug accumulation in a variety of systems (Chen et al., 1986; Gros et al., 1986; Endicott and Ling, 1989). Pgp is expressed in a wide range of tissues, including the small intestine and colon in the physiological conditions. This membrane protein has been considered as an absorption barrier for intestinal drug absorption. And
MDR-reversing agent can overcome the barrier and increase drug absorption. Several chemicals such as verapamil, cyclosporine A, and PSC 833 have been proved to be potent Pgp inhibitors in vitro; however, the toxicities hindered them for clinical application (Bradshaw and Arceci 1998).

In 1992, a study by Sharma and his colleagues showed ALLN, a semi-synthetic peptide as a Pgp substrate and proposed that the secretion of peptide/protein is related to the physiological function of Pgp (Sharma et al 1992). And a study by Burton et al. also observed an increased transport of AcPhe(NMePhe) NH in the apical to basolateral direction in Caco-2 cells and this transport was also increased in the presence of verapamil (Burton et al 1993). Further studies have shown that hydrophobic peptides and peptide ionophores are substrates/modulators of Pgp (Raymond et al 1992; Eytan et al 1994, 1996; Lo et al 1994; Sarkadi et al 1994; Toppmeyer et al 1994; Sharam et al 1995, 1996, 1998, 1999; Borgnia et al 1996; Chen and Pollack 1999). And the interaction mechanism(s) with Pgp remains to be clarified. Most of studies were performed by estimating the ATPase activity or quenching constant (Kq). Therefore, it is interesting to investigate the effect of linear peptide on Pgp-mediated drug transport in vivo and the possibility of linear peptide as Pgp inhibitor for clinical application.

The result from in vitro exerted sac study suggested that the synthetic peptide (ALLN) could be a promising Pgp modulator. Therefore, a series of synthetic tripeptides with various amino acids at the C-terminus will be conducted to investigate the relationship between lipophilicity, charge of peptides and the Pgp inhibitory effect.

**Methods**

**Materials**

N, N-dicyclohexylcarbodiimide (DCC), N, N-dimethylformamide (DMF), palladium, pyridinium chlorochromate (PCC), sulfur trioxide pyridine, dimethyl sulfoxide (DMSO), dimethyl-d_{6} sulfoxide (DMSO-D_{6}), borane-methyl sulfide complex (BH_{3}-SMe_{2}), celeite 545, ethyl acetate, tetrahydrofuran (THF), triethylamine, HOBT, H-Trp-OMe, L-leucine benzyl ester tosylate, L-phenylalaninol, and N-α-acetyl-L-leucine, N-α-CBZ-L-lysine methyl ester hydrochloride were purchased from either Sigma Chemical Co. (St. Louis, MO, USA) or Bachem AG (Switzerland).

**Results and Discussion**

A series of synthetic tripeptides with various amino acids at the C-terminus were synthesized. Figure 1 represents the NMR data.

Four tripeptides were successfully synthesized in the current study. The reaction intermediates were also collected and identified. All the peptide analogues were further used for P-glycoprotein inhibitory study to understand the possible structure-activity relationship.

**Figure 1 Series of synthetic peptides.**

(A) N-Ac-Leu-Leu-Trpohan
(B) N-Ac-Leu-Leu-Trphanal

(C) N-Ac-Leu-Leu-Phenylalaninol

(D) N-Ac-Leu-Leu-benzyloxycarbonyl-Lysinol

References


