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Effect of *Stemona curtisii* root extract on P-glycoprotein and MRP-1 function in multidrug-resistant cancer cells

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Abstract

Multidrug resistance (MDR) is the result of overexpression of membrane bound proteins that efflux chemotherapeutic drugs from the cells. Two proteins, P-glycoprotein (P-gp) and multidrug-resistance associated protein-1 (MRP-1) efflux chemotherapeutic agents out of the cancer cell that decrease intracellular drug accumulation, thereby decreasing the effectiveness of many chemotherapeutic agents. In the present study, the ethanolic extract of the roots of *Stemona curtisii* Hook. was tested for the potential ability to modulate the MDR phenotype and function of P-gp and MRP-1. The *S. curtisii* extract reversed the resistance to putative chemotherapeutic agents, including vinblastine, paclitaxel and colchicine of KB-V1 cells (MDR human cervical carcinoma with high P-gp expression) in a dose-dependent manner, but not in KB-3-1 cells (drug sensitive human cervical carcinoma, which lack P-gp expression). The root extract also increased the intracellular uptake and retention of ^{3}[H]-vinblastine in KB-V1 cells dose dependently. The extract did not influence MDR phenotype-mediated MRP-1 in MRP1-HEK293 (human embryonic kidney cells stably transfected with pcDNA3.1-MRP1-H10 which show high MRP-1 expression) and pcDNA3.1-HEK293 (wild type). In summary, the *S. curtisii* root extract modulated P-gp activity but not MRP-1 activity. The result obtained from this study strongly indicated that *S. curtisii* extract may play an important role as a P-gp modulator as used in vitro and may be effective in the treatment of multidrug-resistant cancers. The purified form of the active components of *S. curtisii* extract should be investigated in more details in order to explain the molecular mechanisms involved in P-gp modulation. This is the first report of new biological activity in this plant, which could be a potential source of a new chemosensitizer.

Keywords: *Stemona curtisii*; Multidrug resistance (MDR); P-glycoprotein (P-gp); Multidrug resistance-associated protein-1 (MRP-1); Chemosensitizer

Introduction

Cancer cell resistance is considered to be one of the major reasons for the failure of chemotherapy in most cancer patients. Some tumors are intrinsically resistant to treatment, whereas others acquire resistance with exposure to structurally unrelated drugs. The phenomenon, multidrug resistance (MDR) is the result of overexpression of membrane bound proteins that efflux drugs from the cells, thus decreasing the intracellular concentration of the drugs. Two proteins in particular, P-glycoprotein (P-gp) and MDR associated protein-1(MRP-1) have been linked to MDR associated with a variety of cancers (Ambudkar et al., 1999; Stouch and Gudmundsson, 2002). P-gp and MRP-1 belong to the ATP-binding

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cassette (ABC) superfamily of transporters that utilize the energy of ATP hydrolysis to translocate a wide range of substrates across a variety of cellular membranes. In tumor cells expressing these proteins, this results in reduced intracellular drug concentrations, which decreases the cytotoxicity of a broad spectrum of antitumor drugs including, anthracyclines (e.g. doxorubicin), *Vinca* alkaloids (e.g. vinblastine, vincristine), podophyllotoxins (e.g. etoposide) and taxanes (e.g. paclitaxel) (Teodori et al., 2002; Krishna and Mayer, 2000).

Many investigations have explored the compounds that can modulate MDR chemo-resistant cells to become sensitive for chemotherapeutic agents. These compounds are called chemosensitizers, modulators or MDR-reversing agents. These compounds have a broad spectrum of chemical structures, which cause great difficulty in identifying the chemosensitizing properties of their structures. Several reviews have illustrated the required chemical structures of P-gp modulators, in which molecules should contain relatively hydrophobic and/or cationic functional groups at physiological pH (Stouch and Gudmundsson, 2002). Many alkaloids whose structures consist of these required structures had been reported for their modulating activities of P-gp and MRP (Teodori et al., 2002). In this study, we focused on chemosensitizing properties of an extract derived from *Stemona curtisii* in the Family Stemonaceae. Plants in this family contain an interesting group of alkaloids, the so-called *Stemona* alkaloids, which constitute a unique chemical character (pyrrolol[1,2-a]azepine core) and are not detected in any other plant family (Greger, 2006). The roots of various Stemonaceae species have been used in traditional Chinese, Japanese, and Thai medicine to treat respiratory diseases, antifungal, insecticides and anti-cancer (for a recent review, see Pilli and Ferreira de Oliveira, 2000). The roots of *S. japonica* and *S. sessilifolia* have been used as insecticides and anthelmintics in the East, and are known to possess antitubercular and antitussive activities (Ye et al., 1994; Cong et al., 1995). More recently, extracts and pure alkaloids derived from extracts of the leaves and roots of *S. collinsae* and *S. tuberosa* were shown to have antifungal, insect toxicity, antifeedant, and repellent activities (Pacher et al., 2002; Brem et al., 2002; Kaltenegger et al., 2003). There are recent reports of free radical scavengers of four new dehydrotocopherols (chromenols) isolated from the roots of various Stemonaceae species including *S. curtisii*, *S. tuberosa* and *S. collinsae* (Brem et al., 2004). In addition, the root extracts of *S. tuberosa* and *S. collinsae* were compared for their anti-tumor effect on medullary and thyroid carcinoma cells. This type of cancer cell is known to be relative resistant to chemotherapy; therefore, anti-tumor activity of these extracts could offer a new approach to successful chemotherapy (Rinner et al., 2004).

S. curtisii Hook. is a herbaceous plant found in the South and North-East region of Thailand. This plant is known in the Thai vernacular as "Non Tai Yak" and has long been used by rural people of Thailand as a useful herbal medicine for the treatment of respiratory disorders and cancer, but no scientific studies have yet been undertaken to verify these claims. Since *Stemona* alkaloids seem to be involved in apoptotic effects of chemo-resistant cancer cells (Rinner et al., 2004), we set out to ascertain how *Stemona* extract affects MDR and MRP-1 cancer cells.

The present study is an attempt to screen the roots of *S. curtisii* extract for its modulating effect on P-gp and MRP-1-mediated efflux. In this report, we demonstrate, for the first time, that the roots extract from *S. curtisii* plays an important role in inhibition of P-gp but not MRP-1-mediated drug efflux, resulting in an increase in the intracellular uptake and cytotoxicity of chemotherapeutic drugs in drug-resistant human cervical carcinoma cells in vitro.

Materials and methods

Chemicals

Vinblastine, paclitaxel, colchicine, etoposide (VP-16), verapamil, indomethacin and 3-(4,5 dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), HRP-conjugated goat anti-mouse IgG and mouse monoclonal anti-Pgp (MDR) clone F4 were obtained from Sigma Chemical Company (St. Louis, MO, USA). MRPm6 monoclonal antibody was obtained from Chemicon, USA. Dulbecco's Modified Eagle's Medium (DMEM), geneticin (G418), L-glutamine and penicillin–strepomycin were purchased from Gibco BRL (Grand Island, NY, USA). ^{3}[H]-vinblastine (10.8 Ci/mmol) was obtained from Amersham Bioscience (Cardiff, UK). A supersignal detection kit was purchased from Pierce (Rockford, IL, USA).

Plant material

Fresh roots of *S. curtisii* were collected from Udornthani, Thailand in May, 2003 by Mr. Supachai Yodkeeree. The botanical identity of the sample was confirmed by Mr. James F. Maxwell, a botanist at the CMU Herbarium, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand. A voucher specimen (No. 5) has been deposited in the CMU Herbarium, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Preparation of *S. curtisii* extract

Fresh roots of *S. curtisii* were dried at 30–45 °C and ground. Dried powdery plant samples were extracted