Antiproliferative activity of combination of Thai herbal remedy and chemotherapeutic agents on human cancer cell lines

Tanawan Kummalue M. D.1*, Monthira Suntiparpluacha M. S.2 and Weena Jiratchariyakul3

1Department of Clinical Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand.  
2Medical Molecular Biology Unit, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.  
3Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Accepted 22 November, 2011

Thai herbal remedies have long been used as traditional medicine in Thailand for cancer therapy, however, little is known for their scientific information especially their anticancer effects. In this study, one commonly used Thai herbal remedy for cancer therapy, was demonstrated for its antiproliferative effect on 7 human cancer cell lines; MDA-MB435, SKBR3, MCF-7, T47D, A549, SK-LU1 and Caco-2. Combination of chemotherapeutic drugs and this special herbal remedy was also investigated. The results revealed that the Thai herbal extract alone had no effect on the growth of these 7 human cancer cell lines. The viability of all cancer cells was more than 80% after treated with 30 µg/ml of the extract. Cells treated with doxorubicin plus herbal remedy showed slightly different IC50 value when compared to doxorubicin alone on T47D human breast cancer cell line but no statistical significance. In conclusion, this herbal remedy that was used to treat cancer patients has no significant antiproliferative effect on cancer cells in vitro.

Key words: MDA-MB435, SKBR3, MCF7, T47D, A549, SK-LU1, Caco-2, Thai herbal remedy.

INTRODUCTION

Cancer, one of the most fatal diseases, causes significant morbidity and mortality worldwide. Nowadays, cancer therapy which is usually multimodality strategy with chemotherapeutic agents, surgery, and radiation is in advance, however, it is not fully effective comparing with the survival rate. Therefore, development for novel anticancer drugs to treat cancer patients is needed. This is the reason that natural products from plants sources have frequently been searched as new anticancer agents (Mukherjee et al., 2001).

Medicinal plants have long been used and prescribed by traditional doctors in many countries including Thailand. Lots of cancer patients in Thailand are still using traditional medicine as an alternative medicine for their own diseases (Itharat and Ooraikul, 2007). They use traditional medicines especially herbal remedies together with standard therapy without discussing these usages with their own physicians (Jacobson et al., 2000). Unfortunately, little information about the efficacies of herbal remedies combination with standard chemotherapies has been known. Therefore, this prompted us to investigate the effect of one commonly used Thai herbal remedy for cancer therapy in combination with chemotherapeutic agents on several human cancer cell lines. Three anticancer agents, that is, paclitaxel, doxorubicin, and vinblastine were used in this study to determine the efficacies on human breast cancer (MCF7, MDA-MB435, SKBR3 and T47D), lung cancer (A549 and SK-LU1), and colon (Caco-2) cancer cell lines.

MATERIALS AND METHODS

Preparation of Thai herbal remedy and extract

Components of Thai herbal remedy are stems of Litosanthes biflora Bl., stems of Smilax glabra Roxb. and Smilax china Linn., stems of Derris timoriensis (DC.) Pittier., roots of Phyllanthus emblica Linn.,

*Corresponding author. E-mail: sitkm@mahidol.ac.th. Tel: 662-4181367. Fax: 662-4181367.
stems of Orthosiphon aristatus (Blume) Miq. and rhizomes of Smilax ovalifolia Roxb. All the plant materials were ground and boiled in three litres of water until volume was reduced to one third. It was then filtered through cotton wool. The evaporating and drying processes were done continuously by using desiccators until residues reached a constant weight. The thin layer chromatogram is shown in Figure 1.

Herbal remedy extract was dissolved in sterile water and then filtered through 0.2 μm pore size syringe filter before using. Serial concentrations of sterile herbal extract were prepared by diluting with culture medium.

**Human cancer cell lines and culture**

Human breast cancer cell lines, SKBR3, T47D, MCF7 and MDA-MB435, were kindly provided by Dr. Pornchai O-charoenrat (Mahidol University, Bangkok). Human lung cancer cell lines, A549 and SK-LU1, were purchased from the American Type Culture Collection (Rockville, MD, USA). Human colon cancer cell line, Caco-2, was kindly provided by Ministry of Public Health.

Briefly, SKBR3 is a human breast cancer cell line with over-expression of HER2/neu receptor but absence of ER receptor. In contrast, T47D and MCF7 are human breast cancer cell lines in which ER receptor is positive but HER2/neu receptor is absent. Moreover, MDA-MB435 is also a human breast cancer cell line which is absent in both ER receptor and HER2/neu receptor expression (Lacroix and Leclercq, 2004; Pratumvinit et al., 2009; Krumvalue et al., 2007). The human lung cancer cell line, A549, is known to resist to the cytotoxic effects of both tumor necrosis factor alpha (TNF-α) and chemotherapeutic agents, for example, cisplatin, etoposide, and doxorubicin (Sanlioglu et al., 2001; Prewitt et al., 1994 and Sartorius and Krammer, 2002). Moreover, SK-LU1 is also a human lung adenocarcinoma cell line which expresses type 1 insulin growth factor receptors (Price and Stiles, 2000). Caco-2 is a human colon cancer cell line which is relatively insensitive to doxorubicin due to the over-expression of an energy-dependent drug efflux pump (Bellamy, 1997).

All cell lines except SK-LU1 were cultured in advanced Dulbecco’s modified eagle medium (DMEM) supplemented with 5% heat-inactivated fetal bovine serum (FBS), and 1% penicillin-streptomycin (Gibco, Invitrogen, USA) at 37°C in a 5% CO2 atmosphere at 95% humidity. SK-LU1 cell line was cultured in minimum essential medium (MEM) with Earle’s Balanced Salt Solution supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% non essential amino acid, 1 mM sodium pyruvate, and 1% penicillin-streptomycin (Gibco, Invitrogen, USA) in the same environment mentioned previously.

**Chemotherapeutic drugs**

Three anticancer drugs were used in this experiment, that is, doxorubicin (Boryung, Korea), vinblastine (Medline, Thailand), and paclitaxel (Bristol, USA). Briefly, doxorubicin is an anthracycline antibiotic which binds to nucleic acids and interferes cell cycle. This chemotherapeutic agent is used for treatment of various types of cancers such as breast cancer and leukemia (Gieseler et al., 1994). Vinblastine is a plant alkaloid and affects the microtubules and interrupts the process of mitotic spindle formation during the

**Figure 1.** Thin-layer chromatogram of Thai herbal remedy extract. Adsorbent: Silica gel GF<sub>254</sub>, Alufolien, Merck. Solvent system: Ethyl acetate : Methanol : water : Acetic acid (50 : 5 : 3 : 6). Detection: Spray with 10% sulfuric acid and heat in oven. Reference: 1 = G1a (Phytosteryl glucoside) 2000 ppm 10 μl.

Under UV 254 nm

Under UV 366 nm

Spray with 10% sulfuric acid
metaphase stage of mitosis, while paclitaxel (Taxol®) induces microtubules foragathering and stops them from depolymerization which cause the apoptosis of cancer cells (Wilson et al., 1999; Guo et al., 2006). All these anticancer drugs were diluted by medium to the serial final concentrations of 0.025, 0.05, 0.1, 0.5, 1.0 and 1.5 µg/ml.

**Cell viability assay**

The cytotoxic effects of Thai herbal remedy and anticancer drugs on cell growth were assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma, USA) assay in triplicate (Rubinstein et al., 1990) which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Briefly, cancer cells at the density of 1×10⁴ cells/well were seeded in 96-well culture plate (Costar corning, NY). After 24 h of incubation, cells were treated with 30 µg/ml of herbal extract (Srisapoomi et al., 2008). Then, doxorubicin, vinblastine, and paclitaxel at different concentrations were further added to the cancer cells to get the final concentrations of 0.025, 0.05, 0.1, 0.5, 1.0 and 1.5 µg/ml. After 48 h of incubation, 50 µl of 1 mg/ml of MTT in phosphate buffer saline (PBS) was added to each well. The plates were then incubated at 37°C for additional 4 h. After the medium and MTT were carefully removed, 100 µl of dimethyl sulfoxide (DMSO) (Sigma, USA) was added to each well. A purple formazan product was immediately measured at 595 nm using an EL808 Ultra Microplate Reader (Biotek laboratories, USA). Control groups (0.5% DMSO, water, 1.0 µg/ml of doxorubicin, vinblastine, and paclitaxel) were also assayed in parallel with each run. The results were determined by three independent experiments. Cell viability (%) was calculated using the formula as follows:

\[
\text{Cell viability (\%)} = (1 - \frac{\text{control OD} - \text{sample OD}}{\text{control OD}}) \times 100.
\]

**Statistical analysis**

The IC₅₀ values were calculated by R². All data were shown as mean ± standard deviation. Statistical significance was analyzed using the independent samples t-test for normally distributed values and by the non-parametric Mann-Whitney U-test for variables that were not normally distributed. Values of p<0.05 were considered significant.

**RESULTS**

**Effect of Thai herbal extract on the proliferation of human cancer cell lines**

Human breast cancer, lung cancer, and colon cancer cell lines were used as a model system to determine the effect of Thai herbal extract on their growth. The antiproliferative effect of extract on various human cancer cell lines was determined by MTT assay. Cells were treated with extract at different concentrations of 5, 10, 15, 20, 25 and 30 µg/ml for 48 h and the percentage of cell viability was analyzed as shown in Figure 2. Our results showed that there was no antiproliferative effect of this herbal extract on any human cancer cell line. Therefore, no IC₅₀ value at 48 h was detected when these cell
lines were treated with Thai herbal remedy extract.

**Effect of chemotherapeutic agents on the proliferation of human cancer cell lines**

MTT assay was used to determine the antiproliferative activity of three chemotherapeutic drugs on 7 cancer cell lines. The highest response to doxorubicin was found in A549 and MDA-MB435 with IC<sub>50</sub> values of 0.576±0.107 and 0.726±0.056 µg/ml, respectively, which is shown in Figure 3A. The IC<sub>50</sub> values of doxorubicin on MCF7, Caco-2 and T47D were 1.138±0.087, 1.050±0.221 and 1.333±0.048 µg/ml, respectively. Resistant were observed in SK-LU1, and SKBR3. All human cancer cell lines were resistant to vinblastine, and paclitaxel as illustrated in Figure 3B and C.

**Effect of Thai herbal extract combination with chemotherapeutic agents on the proliferation of human cancer cell lines**

The Thai herbal remedy might help altering the human cancer cells response to chemotherapeutic agents, although the remedy alone has no direct antiproliferative effect. The herb extract was added to the cells before adding the chemotherapeutic agents in order to determine the synergistic effects of the herbal extract.

The cytotoxic effect between Thai herbal extract plus doxorubicin and doxorubicin alone against T47D was different, but no statistical significance. Moreover, the IC<sub>50</sub> values of herbal extract combination with other chemotherapeutic agents were not different when compared with those from chemotherapeutic agents alone in all cell lines.

**DISCUSSION**

Thai herbal remedies have long been widely used for many decades as anticancer agents. However, scientific data on these effects of Thai herbal remedies have rarely been demonstrated. As we mentioned previously, the Thai herbal remedy in this study contains seven medicinal plants and some of these 7 plants have been studied and demonstrated to have biological effects. The previous findings showed that β-Sitosterols and polyphenols in *S. glabra* and *S. china* have anticancer effects in colon cancer and breast cancer cell lines, respectively (Baskar et al., 2010; Wu et al., 2010). In addition, Hsu et al (2010) found an anti-inflammatory and antioxidant effects of *O. aristatus* alcohol extracts to mouse leukemic cell (RAW 264.7). Some study reported that *S. glabra* has anti-inflammatory activities and prevents damage of hepatocytes by immune reaction (Itharat and Ooraikul, 2007). In this study, the antiproliferative effects of Thai herbal extract combination with chemotherapeutic agents on the growth of human cancer cell lines were investigated by MTT assay. By using this approach, we were able to demonstrate that Thai herbal extract had no direct cytotoxic effects on the proliferation of these human cancer cell lines. The possible explanations for this finding might be due to the fact that the activities of chemical components from the herbal remedy alone were not in sufficient doses to produce anticancer effect on cancer cells. In a study by Takara et al. (2005) several herbal extracts were tested on HeLa cells, and IC<sub>50</sub> values obtained were over 60 µg/ml, which were higher than the concentrations we used in this study. One important possibility is this special Thai herbal remedy does not really have direct antiproliferative effect on human cancer cell lines.

In this present study, chemotherapeutic agents showed different antiproliferative effects on the growth of various human cancer cell lines. The phenomenon of resistance was observed in all 7 human cancer cell lines treated with vinblastine and paclitaxel. As previously reported, the possible mechanism of resistance to vinblastine and paclitaxel resulted from the alterations in their cellular target, tubulin (Hadfield et al., 2003). Interestingly, p-glycoprotein, an ATP-binding cassette (ABC) transporter superfamily, has been known to play the major role in mediating resistance to several anticancer drugs such as vinblastine, paclitaxel, and daunorubicin (Nabekura, 2010). Based on some report, over expression of glucosylceramide synthase, especially in MCF7 cells, caused the resistance to the vinca alkaloid and vinblastine (Gouaze et al., 2004). In this study, doxorubicin had the highest cytotoxic effect against cancer cell lines which was similar to previous report (Srisapoomi et al., 2008).

In addition, in T47D cell line, antiproliferative activity of doxorubicin at 1.5 µg/ml combination with 30 µg/ml of herbal extract was slightly higher than doxorubicin alone which indicated a possible synergistic effect of this herbal remedy to doxorubicin on these cells. The synergistic effect of the extract might be from phytosterol components in the herbal extract, such as β-Sitosterols and polyphenols in *Smilax sp.*, which slow down cell growth by interfering cell mitosis and thus, doxorubicin can kill the cancer cells more effectively. However, this difference was not statistically significant. Moreover, there was no difference between IC<sub>50</sub> value of doxorubicin plus herbal extract and doxorubicin alone in all the rest of the cell lines. Based on previous results, the IC<sub>50</sub> values of all 3 chemotherapeutic agents on human cancer cell lines were different when compared to those reported by Srisapoomi et al. (2008) which might be the effects from the MTT assay itself. For example, incomplete solubilization of formazan crystal will certainly affect the accuracy and precision of the MTT testing. Furthermore, high manipulation of this assay might cause more difficulties in interpretation (Martin et al., 2005).
Figure 3. Demonstration of the effects of doxorubicin (A), vinblastine (B), and paclitaxel (C) on the proliferation of breast cancer cell lines (SKBR3, MCF7, MDA-MB435, and T47D), lung cancer cell lines (A549 and SK-LU1) and colon cancer cell line (Caco-2). Cells at density of $1 \times 10^4$ cells/well were seeded in 96-well plates. The next day, cells were treated with chemotherapeutic agents at different concentrations of 0.025, 0.05, 0.10, 0.50, 1.0 and 1.5 $\mu$g/ml. Cell proliferation was determined by MTT assay. The data shown are the mean±SD from three independent experiments, each with triplicate wells.
In conclusion, traditional doctors have prescribed several herbal remedies to treat cancer patients, however, some of these herbal remedies have never been investigated for their efficacies especially antiproliferative activities on cancer cells. This Thai herbal remedy which has long been used by Thai cancer patients showed no obvious synergistic effect with chemotherapeutic drugs in vitro.

ACKNOWLEDGEMENTS

The authors would like to thank Assistant Professor Dr. Sathien Sukpanichnant for his kind support. This work was granted by "Chalermphrakiat Grant", Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

REFERENCES


