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Effect of Tetrandrine Combined with Epirubicin on the Growth of Human Breast Carcinoma Multidrug Resistance Cell Line

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Multidrug resistance presents a serious problem in cancer chemotherapy. Recent studies have shown that the multidrug resistance of tumor cells can be reversed by tetrandrine by potentiating the cytotoxicity of chemotherapeutic agents. However, whether tetrandrine has such potentiating effect on epirubicin has not been reported. Thus, the combined effect of tetrandrine and epirubicin on the growth of human breast carcinoma multidrug-resistant MCF-7/ADM cells was studied in the present study. It was shown that tetrandrine significantly potentiated the cytotoxicity of epirubicin. To examine the mechanism of the combined effect of tetrandrine and epirubicin on MCF-7/ADM cell growth, cell cycle progression was evaluated by using flow cytometry. The combined use of tetrandrine and epirubicin caused an accumulation of cells at G_2/M phase, accompanied with a concomitant decrement of cell number at G_0/G_1 phase. The present study demonstrated for the first time that tetrandrine potentiated the cytotoxcity of epirubicin on MCF-7/ADM cells. Cell cycle arrest at G_2/M phase may contribute to the combined effect of tetrandrine and epirubicin.

Key words——tetrandrine; epirubicin; multidrug resistance

INTRODUCTION

Multidrug resistance (MDR) presents a serious problem in cancer chemotherapy, because the tumor cells are resistant to antitumor agents which are commonly used in clinical situations. The major mechanism for MDR is attributed to the reduced accumulation of antitumor agents in resistant cells. Although the etiology of MDR is multifactorial, one of the main causes is the overexpression of the membrane-associated 170-kDa glycoprotein P-gp coded by the mdr1 gene.[1–3] P-gp belongs to the superfamily of adenosine triphosphate (ATP)-binding cassette (ABC) transporters and actively pumps out a wide range of structurally and functionally diverse amphipathic anticancer drugs from the inside of tumor cells thereby decreasing their intracellular accumulation.[1] Recent studies have shown that tumor cells expressing MDR-associated protein,[4] lung-resistance protein,[5] and mutation of DNA topoisomerase II[6] also show MDR.

There are several noncytotoxic drugs that sensitize MDR cells to chemotherapeutic drugs *in vitro* and *in vivo*.[7–9] They include calcium channel blockers, calmodulin antagonists, various steroids, quinolines, immunosuppressive drugs, antibiotics, surfactants and yohimbine alkoids, all of which have been shown to reverse MDR *in vitro*.

Tetrandrine, a kind of calcium channel blocker is a benzylisoquinoline alkaloid isolated from the Chinese herb ''Hanfangji'' (Radix Stephania tetrandra) that has been shown to be a potent inhibitor of P-gp drug efflux *in vitro*.[10] Recent studies have shown that the multidrug resistance of tumor cells can be reversed by tetrandrine. For example, tetrandrine potentiated the cytotoxicity of doxorubicin and vincristine on the multidrug resistance cell lines.[11,12] However, whether tetrandrine has such potentiating effect on epirubicin has not been reported. Thus, the combined effect of tetrandrine and epirubicin on the growth of human breast carcinoma multidrug-resistant MCF-7/ADM cells was studied in the present study.

MATERIALS AND METHODS

Chemicals Tetrandrine was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Epirubicin was obtained from Zhejiang Hisun Pharmaceutical Co,.Ltd. RPMI 1640 was from Gibco (Grand Island, USA). Fetal bovine serum (FBS) was purchased from TBD Biotechnology Development (Tianjin, China). Propidium iodide (PI) and 3-(4,5-dime-

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thylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemicals (St. Louis, MO).

Cell Culture Human breast carcinoma multidrug-resistant cell (MCF-7/ADM) was obtained from Institute of Hematology, Chinese Academy of Medical Science (Tianjin, China). The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/l), penicillin (100 U/ml) and streptomycin (100 mg/ml) and maintained at 37°C with 5% CO_2 in a humidified atmosphere.

Cell Viability The effects of tetrandrine and epirubicin on the growth of MCF-7/ADM and MCF-7 cells were measured by MTT method after 72 h culture. The cells were dispensed in 96-well flat bottom microtiter plates at a density of 1×10^4 cells per well. After 24 h incubation, they were treated with tetrandrine at 1 h prior to various concentrations of epirubicin and were cultured for 72 h. After such treatments, the cells were incubated with MTT (0.25 mg/ml) for 4 h at 37°C. The formazan crystals in the cells were dissolved in dimethyl sulfoxide. The level of MTT formazan was determined by measuring its absorbance at the wavelength of 490 nm with a SPECTRA (shell) Reader (TECAN, Austria).

Cell Cycle Analysis MCF-7/ADM cells (1×10^6 cells) were harvested and washed once in cold PBS. Cell pellets were fixed in 70% ethanol and washed in cold PBS. Then the pellets were suspended in propidium iodide (PI) solution (1 ml) containing 50 μg/ml of PI, 0.1% (w/v) sodium citrate, 0.1% (v/v) Triton X. Cell samples were incubated at 4°C in the dark for at least 15 m, and analyzed by a FACScan flow cytometer (Becton Dickinson).[13]

Data Analysis Results were expressed as mean ±SD. Statistical significance ($p<0.05$) was assessed by one-way ANOVA followed by least significant difference method (SPSS12.0 software, SPSS, USA).

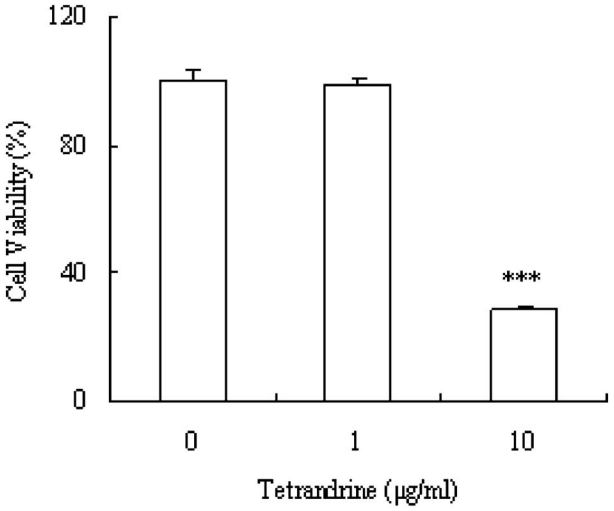

Fig. 1. Effect of Tetrandrine on MCF-7/ADM Cell Viability

MCF-7/ADM cells were treated with tetrandrine for 72 h. Cell viability was examined by MTT reduction assays and the results were expressed as percentage of surviving cells over control cells. Data are presented as mean ± SD ($n=3$). ***$p<0.001$ compared with the control group.

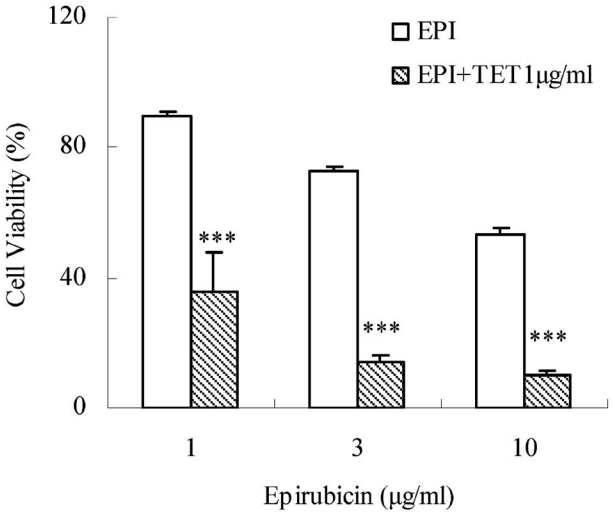

Fig. 2. Effect of Tetrandrine and Epirubicin on MCF-7/ADM Cell Viability

MCF-7/ADM cells were treated with tetrandrine at 1 h prior to various concentrations of epirubicin and were cultured for 72 h. Cell viability was examined by MTT reduction assays and the results were expressed as percentage of surviving cells over control cells. Data are presented as mean ± SD ($n=3$). ***$p<0.001$ compared with the corresponding epirubicin group.

RESULTS AND DISCUSSION

The effect of tetrandrine on cell growth in the MCF-7/ADM cells was first investigated. The cytotoxic effect of tetrandrine after a 72-h treatment is shown in Fig. 1. Tetrandrine, at the concentration of 1 μg/ml, did not show significant effect on the cell growth, compared with the control group. When the concentration increased to 10 μg/ml, tetrandrine significantly inhibited the cell growth of MCF-7/ADM cells. So tetrandrine of 1 μg/ml was used in the following study.

Figure 2 presents the combined effect of tetrandrine and epirubicin on cell growth in the MCF-7/ADM cells. The cells were incubated with 1 μg/ml tetrandrine and various concentrations of the chemotherapeutic agent, epirubicin. The aim of the experiments was to see if tetrandrine could modulate the sensitivi-

ty of MDR cells to epirubicin. It was clear that tetrandrine significantly potentiated the cytotoxicity of epirubicin. The effect of combined treatment of tetrandrine and epirubicin on parent MCF-7 cells was also investigated (Fig. 3). Tetrandrine did not show significant effect on the cytotoxicity of epirubicin in the MCF-7 cells.

To examine the mechanism of the combined effect of tetrandrine and epirubicin on MCF-7/ADM cell growth, cell cycle progression was evaluated by using flow cytometry. Figure 4 presents the results of the DNA flow cytometric analyses of MCF-7/ADM cells cocultured with tetrandrine and epirubicin for 48 h. The percentage of cells in G_0/G_1 phase, S phase and G_2/M phase was not significantly altered by epirubicin treatment. However, the combined use of tetrandrine and epirubicin caused an accumulation of cells at G_2/M phase. It was found that the increment in G_2/M cell population was accompanied with a concomitant decrement of cell number at G_0/G_1 phase.

Tetrandrine has been reported to potentiate the cytotoxicity of doxorubicin and vincristine on the multidrug resistance cell lines.[11,12] Tetrandrine potentiated the cytotoxicity of doxorubicin *via* inhibiting the P-gp-mediated drug efflux and lowering cell membrane fluidity.[12] Tetrandrine reversed resistance to vincristine in KBv200 cells *via* directly binding to P-gp and increasing intracellular vincristine accumulation.[11] Whether such mechanisms were involved in the action of tetrandrine in potentiating the cytotoxcity of epirubicin merits further investigation.

In conclusion, the present study demonstrated for the first time that tetrandrine potentiated the cytotoxcity of epirubicin on MCF-7/ADM cells. Cell cycle arrest at G_2/M phase may contribute to the combined effect of tetrandrine and epirubicin.

Acknowledgement This research was support-

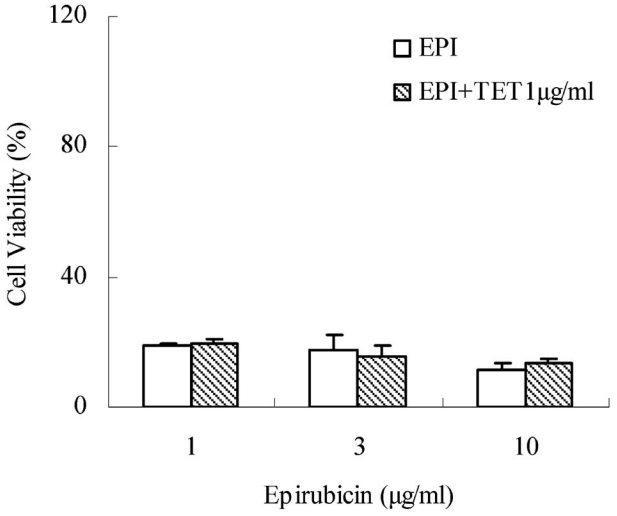

Fig. 3. Effect of Tetrandrine and Epirubicin on MCF-7 Cell Viability

MCF-7 cells were treated with tetrandrine at 1 h prior to various concentrations of epirubicin and were cultured for 72 h. Cell viability was examined by MTT reduction assays and the results were expressed as percentage of surviving cells over control cells. Data are presented as mean±SD ($n=3$).

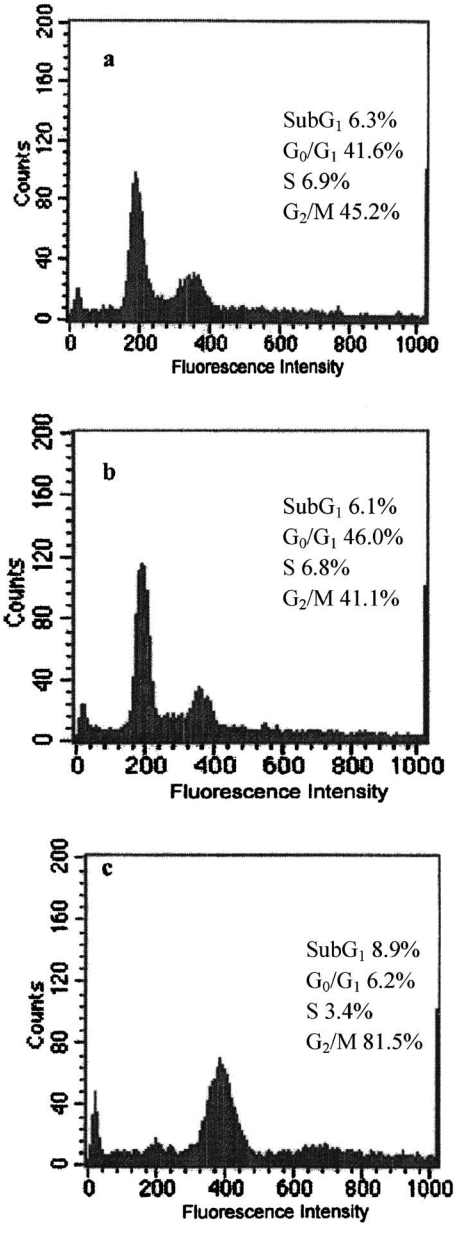

Fig. 4. Cell Cycle Analysis of MCF-7/ADM Cells after Treatment with

(a) medium, (b) 1 mg/ml epirubicin and (c) 1 mg/ml tetrandrine combined with 1 mg/ml epirubicin.

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