Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-0034-z
November 2014, Vol. 13, No. 11, P523–P527

Influence of Tamoxifen or the combination of Tamoxifen and Cisplatin on the growth of human lung adenocarcinoma A549 cells*

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Received: 15 July 2014 / Revised: 17 August 2014 / Accepted: 29 September 2014
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Abstract ***Objective:*** The experiment aims to investigate the influence of Tamoxifen and the combination of Tamoxifen and Cisplatin (DDP) on the growth of human lung adenocarcinoma A549 cells. ***Methods:*** We treated human lung adenocarcinoma A549 cells with different concentrations of Tamoxifen, DDP and combination of DDP and Tamoxifen with non-toxicity for 72 h. Then we calculated the inhibition rate through MTT approach and detected the apoptosis rate by flow cytometry. The statistical analysis was performed with SPSS 13.0 software and statistical differences were determined by one-way ANOVA. The data were expressed as the mean ± standard deviation and all experiments were performed in three times. The value of $P < 0.05$ was considered to indicate a statistically significant difference. ***Results:*** 1. The inhibition rates of Tamoxifen with 2.5 μmol/L, 5 μmol/L, 10 μmol/L and 20 μmol/L on the growth of the A549 cells were 18.7%, 25.8%, 54% and 98.8%, respectively ($P = 0.000$). Tamoxifen with concentration of 1 μmol/L has no obvious cytoxicity on the A549 cells ($P > 0.05$). 2. As the increase concentration of Tamoxifen, the S stage and G2/M of the A549 cells decreased while the G0/G1 increased. The apoptosis rate of Tamoxifen with 0 μmol/L, 0.1 μmol/L, 1 μmol/L and 10 μmol/L on the A549 cells were 6.51%, 8.91%, 17.97% and 42.7%, respectively. 3. The inhibition rates of combination of Tamoxifen with 1 μmol/L and DDP with 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL on the A549 cells were 40.4%, 54.4%, 72.9%, 86.1% and 92.4%, respectively ($P < 0.05$). ***Conclusion:*** Tamoxifen can inhibit the proliferation of human lung adenocarcinoma A549 cells and induce the apoptosis of the A549 cells. The combination of Tamoxifen with non-toxicity and DDP can improve the sensitivity of chemotherapy on the A549 cells.

Key words Tamoxifen; Cisplatin (DDP); chemosensitization; A549 cells

Lung cancer is the major cause resulting in cancer death in the United States both in men and women [1]. Almost 65%–75% patients with lung cancer were preliminary diagnosed with stage IIIB or IV, which the surgery was not available. Chemotherapy is still the important treatment for advanced lung cancer and 70%–80% patients took chemotherapy. However, the multidrug resistance (MDR) is not only the mechanism of defense, but also the cause of chemotherapy failure. And the total effective rate of combined therapy is around 20%–40% [2]. Tamoxifen can reverse drug-resistance in some tumors, such as ovarian cancer [3]. Chang *et al* [4] demonstrated that Tamoxifen can increase the drug sensitivity of resistant cells of Adriamycin. Cisplatin (DDP) is the first line of chemotherapy for lung cancer and Docetaxel/cisplatin regimen and gemcitabine/cisplatin regimen for the patients with advanced non-small cell lung cancer (NSCLC) were efficient and well-tolerated chemotherapeutic approaches with low toxicity levels [5]. However, the efficacy of chemotherapy decreased because of the MDR.

The aim of this study was to investigate the influence of Tamoxifen and the combination of Tamoxifen and DDP on the growth of human lung adenocarcinoma A549 cells.

Materials and methods

Materials

Human lung adenocarcinoma A549 cells were provided by Central Laboratory of Second Affiliated Hospital of Dalian Medical University, China. Tamoxifen was purchased from TRC Company in Canada. DDP was purchased from Jiangsu Haosen Pharmaceutical Co., Ltd, China. The PRMI 1640 culture was supplied by Hyclone Co. (USA). The MTT was provided by Ameresco Company in USA. And DMSO and trypsin were purchased from

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* Supported by a grant of Project fund of Science Department of Liaoning Province (No. 2009225009-5).

Table 1 The influence of Tamoxifen on the growth of A549 cells

Concentration of Tamoxifen (μmol/L)	*A* value ($\bar{\chi} \pm s$)	Inhibition rate (%)
0	0.933 ± 0.014	0
1	0.918 ± 0.015	4.9
2.5	0.811 ± 0.012	18.7
5	0.706 ± 0.014	25.8
10	0.653 ± 0.009	54.0
20	0.091 ± 0.017	98.8

Sigma Co. (USA). Fetal bovine serum (FBS) was supplied by TBD biological Co. (Tianjin, China). AnnexinV-FITC apoptosis kit was purchased from Biouniquer Co. (USA).

Tumor cell preparation

The human lung adenocarcinoma A549 cells were intensively cultured in the PRMI 1640. The cancer cells were then digested by trypsin until they reached 80% confluence. And when the shape of cells turned round and there was gap between cells, the reaction was terminated by adding complete medium. Single cell suspension was passaged as a ratio of 1:2 or 1:3. After the cryopreservation and resuscitation, the A549 cells were preserved in liquid nitrogen container.

Detection of DDP, Tamoxifen on the inhibition of A549 cells proliferation

The cancer cells were plated in 96-chamber culture plates at 20 000 cells per well, allowed to adhere and incubated with the different concentrations of Tamoxifen, DDP and combination of Tamoxifen with non-toxicity and different concentrations of DDP for 72 h. And then the MTT liquid was added to each plate and cultured for 4 h. Absorbance (*A* value) at 592 nm was measured with Model 550 Type microplate reader after dissolving with DMSO. And the inhibition rate was calculated to determine the inhibition effects of antitumor drugs on tumor cells. Absorbance was detected repeatedly 3 times on different days and expressed as mean ± standard deviation.

Statistical analysis

The statistical analysis was performed with SPSS 13.0 software and statistical differences were determined by one-way ANOVA. The data were expressed as the mean ± standard deviation and all experiments were performed in three times. The value of $P < 0.05$ was considered to indicate a statistically significant difference.

Table 2 The influence of Tamoxifen on the cell circle and cell apoptosis of A549 cells

Concentration of Tamoxifen (μmol/L)	G0/G1	S	G2/M
0	61.77	33.69	4.54
0.1	63.34	30.82	5.84
1.0	68.69	28.26	3.05
10.0	85.21	14.13	0.66

Results

The influence of different concentrations of Tamoxifen on the growth of human lung adenocarcinoma A549 cells

The results of influence of Tamoxifen on the growth of A549 cells by MTT method were shown in Table 1. The results showed that there was significant difference between Tamoxifen groups with the concentrations of 2.5 μmol/L, 5 μmol/L, 10 μmol/L, 20 μmol/L and cell control group ($P = 0.000$). And there was no difference between Tamoxifen group with the concentration of 1 μmol/L and cell control group ($P = 0.112$). Therefore Tamoxifen with concentration of 1 μmol/L had no obvious cytoxicity on the A549 cells.

The results of influence of Tamoxifen on the cell circle and cell apoptosis of A549 cells were shown in Table 2 and Fig. 1–4. The S stage and G2/M stage of A549 cells decreased while G0/G1 stage increased after 72 h. The apoptosis rates were 6.51%, 8.91%, 17.97% and 42.7% with the TAM concentrations of 0 μmol/L, 0.1 μmol/L, 1 μmol/L and 10 μmol/L, respectively.

The inhibition of the combination of Tamoxifen and DDP on the growth of A549 cells (Table 3)

The results showed that the inhibition rates on the A549 cells were 18.2%, 27.1%, 33.3%, 39.8%, 45.5% and 59.1% with the DDP concentrations of 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL after

Table 3 The inhibition rate of different concentrations of DDP and Tamoxifen with non-toxicity on the A549 cells

Concentration of DDP (μg/mL)	Inhibition rate of DDP group (%)	Inhibition rate of combination group of DDP and Tamoxifen (%)	χ^2	*P*
0.625	18.2 ± 0.5	25.6 ± 0.8	1.87	0.172
1.25	27.1 ± 0.7	40.4 ± 1.8	6.31	0.012
2.5	33.3 ± 1.6	54.4 ± 0.7	8.97	0.012
5	39.8 ± 0.6	72.9 ± 1.0	22.15	0.000
10	45.5 ± 1.2	86.1 ± 1.1	34.98	0.000
20	59.1 ± 1.5	92.4 ± 1.6	29.44	0.000

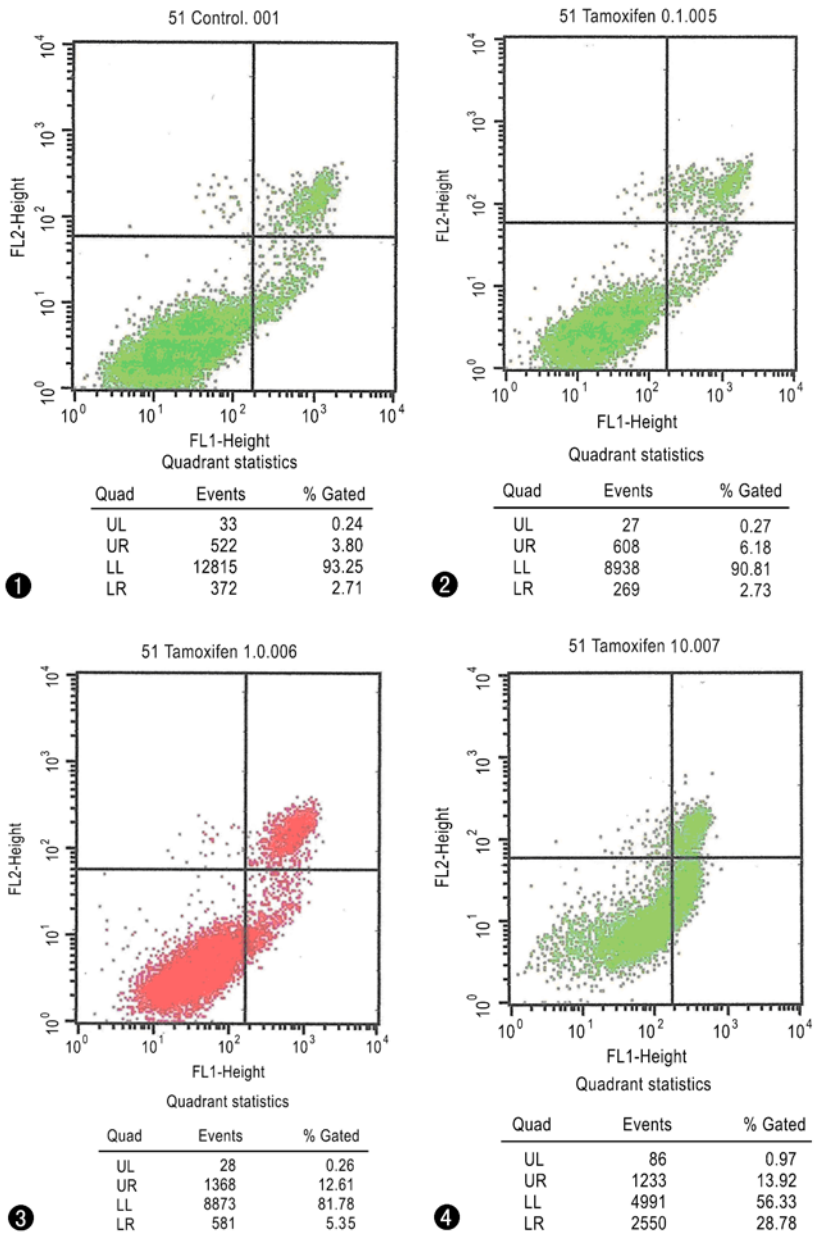

Fig. 1 Control Group. The apoptotic rate was 6.61%
Fig. 2 Tamoxifen with concentration of 0.1 μmol/L group. The apoptotic rate was 8.91%
Fig. 3 Tamoxifen with concentration of 1 μmol/L. The apoptotic rate was 17.96%
Fig. 4 Tamoxifen with concentration of 10 μmol/L. The apoptotic rate was 42.7%

72 h, respectively. The IC50 of DDP single was 11.2 μg/mL. According to the Table 1, Tamoxifen with the concentration of 1 μmol/L had no obvious cytotoxicity. The inhibition rates on the A549 cells in the combination of Tamoxifen with the concentration of 1 μmol/L and DDP with the concentrations of 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL, 5 μg/mL, 10 μg/mL and 20 μg/mL were 25.6%, 40.4%, 54.4%, 72.9%, 86.1% and 92.4% respectively. The IC50 of the combination was 2.9 μg/mL. The inhibition rate in the combination group improved more than that of the DDP group while the IC50 decreased.

Discussion

Lung cancer is one of the most common malignant tumors. There are some therapies such as surgery, chemical therapy, radiotherapy, target therapy, Chinese medicine therapy and so on and chemotherapy still takes important position. However, the curative efficacy of chemotherapy produces unsatisfactory results because of the acquired resistance. The drug which has the function of chemosensitization or reversal of multi-drug resistance can improve the efficacy of chemotherapy [6].

Cisplatin (DDP) is the common chemotherapeutic drugs for lung cancer treatment, which acts on the DNA interstrand and DNA intrastrand resulting in the apoptosis of cancer cells. Tamoxifen (TAM) is the most common estrogen receptor (ER) antagonist, which outcompetes with estradiol about the ER of cancer cells. Some researchers demonstrated that estradiol combined with ER can promote the synthesis of nucleic acid and the synthesis of c-myc mRNA, erbB-2 mRNA, EGFR mRNA in order to stimulate the proliferation of cancer cells [7]. Tamoxifen can block the increase of c-myc mRNA, erbB-2 mRNA, EGFR mRNA induced by estradiol as the ER antagonist, in order to inhibit the growth of cancer cells [8]. Tamoxifen below the concentration of 10^{-6} mol/L or lower can inhibit the cancer cells through the combination with estrogen receptor. However, when the concentration of Tamoxifen was higher than 10^{-6} mol/L, the anti-tumor effect was mediated by inhibiting the calmodulin and protein kinase C and other mechanism instead of estrogen receptor. And calmodulin and protein kinase C are important growth promoting factors of cancer cells, which partially rely on the downregulation of Ca^{2+}. But Tamoxifen with high concentration can upregulate the concentration of Ca^{2+} and then promote to conduct the cytotoxicity by Ca^{2+} in order to inhibit the cell growth. Our experiment demonstrated that the concentration of Tamoxifen above 10^{-6} mol/L can obviously inhibit the A549 cells and the inhibition rate improved with the increase of the concentration after 72 h.

It has been reported that Tamoxifen can prolong the cell transition phase or block the cell cycle at G1 stage resulting in the decrease of S, G2 and M cells in order to inhibit the cell proliferation. Our experiment detected that the S stage and G2/M of the A549 cells decreased while the G0/G1 increased. The resting stage cells increased while proliferative stage cells decreased.

Kang *et al* [9] discovered that 1 μmol/L of Tamoxifen can induce the apoptosis of breast cancer cells with positive ER or negative ER. And the former can be blocked by estradiol, and the latter can not be influenced, which indicated that the apoptosis induced by Tamoxifen may be related to the downregulation of anti-apoptosis gene [10]. Our experiment showed that the apoptosis rate of Tamoxifen with 0 μmol/L, 0.1 μmol/L, 1 μmol/L and 10 μmol/L on the A549 cells was 6.51%, 8.91%, 17.97% and 42.7%, respectively. Tamoxifen can induce the apoptosis of A549 cells, and the apoptosis rate increases with the concentration of Tamoxifen, which indicates that the antitumor mechanism may relate to the induction of cell apoptosis.

Yu *et al* [11] demonstrated that Tamoxifen may reverse the multidrug resistance. MDR1 gene locates on the chromosome 7q21.1, which encodes the transmembrane resistance protein p-gp. The multidrug resistance which induced by MDR1/p-gp can be reversed, and MDR1/p-gp is the main target. There are a lot of reversal agents such as calcium ion antagonist, cyclosporin, Tamoxifen, IFN-2, IL-2 and so on [12]. The reversal agents can reverse the resistance of chemotherapeutic, through overcompeting with the anti-tumor drug about the binding site on the p-gp resulting in that chemotherapeutic can not pump outside the cell, thus increase the concentration of the drug [13]. Some reporters demonstrated that p-gp can lower the intracelluar concentration of chemotherapeutic rely on the adenosine triphosphate [14]. And other reporters investigated that multidrug resistance mediated by p-gp can be reversed, especially the resistance for antharcycline in order to improve the curative efficacy [15].

Kadzhoian *et al* [16] reported that one-year and median survival at high-dose Tamoxifen therapy use in combination with chemotherapy were established to be significantly higher than in patients which received only chemotherapy. Our experiment showed that 1 μmol/L of Tamoxifen combined with DDP enhances the inhibition on the growth of A549 cells rather than DDP single. And the IC50 of DDP decreases from 11.2 μg/mL to 2.9 μg/mL, which indicates that Tamoxifen can improve the sensitivity of chemotherapy. Further research on the mechanism that how Tamoxifen reverse the resistance of chemotherapeutic is still to be needed.

Conclusion

Tamoxifen can inhibit the proliferation of human lung adenocarcinoma A549 cells and induce the apoptosis of the A549 cells. The combination of Tamoxifen with nontoxicity and DDP can improve the sensitivity of chemotherapy on the A549 cells.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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DOI 10.1007/s10330-014-0034-z
Cite this article as: Che YX, Sun XH, Huang CM, *et al*. Influence of Tamoxifen or the combination of Tamoxifen and Cisplatin on the growth of human lung adenocarcinoma A549 cells. Chinese-German J Clin Oncol, 2014, 13: 523–527.

Oncology and Translational Medicine
2015年征稿启事

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《肿瘤学与转化医学（英文）》编辑部
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