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Original paper

Aim of the study: To evaluate the effect of combined use of rapamycin and cisplatin against Hela cells *in vitro*.

Material and methods: The inhibitory effects of rapamycin and cisplatin, used alone or combination, on the proliferation of Hela cell were measured with MTT assay and median-effect plot analysis.

Results: Combined use of rapamycin and cisplatin significantly improved the chemotherapeutic effect against Hela cells. The inhibitory rates were dose-dependent. Rapamycin and cisplatin showed synergistic effects in the chemotherapy of Hela cells (q > 1.15, King's formula).

Conclusions: Combined use of rapamycin and cisplatin significantly improves the chemotherapeutic effect against Hela cells.

Key words: rapamycin, cisplatin, cervical carcinoma, Hela cells.

Effect of combination of rapamycin and cisplatin on human cervical carcinoma Hela cells

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Introduction

As the traditional treatment means of cervical carcinoma, operative treatment is limited to early patients. Although radiotherapy can be used for most medium and advanced patients, it is always ineffective for recurrent advanced patients after radiotherapy. The role of chemotherapy in the treatment of cervical carcinoma is increasing, and it is mainly used for patients receiving new adjuvant chemotherapy and advanced or recurrent patients. However, its apparent side effects and drug resistance [1] are also the main reasons why advanced patients cannot adhere to treatment. With increasing understanding of tumor at the cell and molecule level, molecular targeted therapy is of increasing importance. mTOR is the most popular therapeutic target of malignant tumor in current studies. It has been proved that mTOR inhibitor rapamycin (Rapa) and its derivatives have an inhibitory effect on a variety of tumors such as endometrial carcinoma, metastatic renal cell carcinoma, lymphoma, etc. [2–4]. However, the curative effect is poor for gastroenteric tumor [5]. Domestic and foreign researchers have begun to combine the mTOR inhibitor rapamycin with hormones, chemotherapy drugs or other methods to treat tumors and investigate whether combination application of it and chemotherapy drugs has a synergistic effect and whether the reduced side effects of chemotherapy drugs caused by decrease of their dose can reduce or reverse the resistance to chemotherapy drugs [6, 7].

In this study, the combination of the mTOR inhibitor rapamycin with cisplatin was used to affect cervical carcinoma Hela cells cultured *in vitro* to investigate its inhibitory effect on the growth situation of cervical carcinoma Hela cells and analyze the effectiveness of drug combination. In the investigation the combination of the lowest concentration of rapamycin and cisplatin was used to achieve the most apparent inhibitory effect on cervical carcinoma Hela cells cultured *in vitro* in order to provide a new idea and method for the further clinical application of rapamycin and provide a theoretical basis for developing the new combination chemotherapy regimen of cervical carcinoma in clinical practice.

Material and methods

Cell culture

Hela cells were cultured in PRMI 1640 (Gibco) culture medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/l streptomycin in an incubator containing 5% CO_2 (V/V) at 37°C, and cell growth situations were observed. When the cells grew and covered 70%~80% of the culture bottle wall, 0.25% trypsase was used for digestion, and timely passage was conducted. Subsequently, the cells were continuously cultured. Finally, the cells in logarithmic growth phase were selected for trials.

Drug intervention

On the day before administration, the cells were digested with trypsase and counted. The cells (2×10^6 cells /ml) were spread onto a 96-well plate to make the cell density on administration day be 70%~80%. After the cells adhered onto the wall, different concentrations of complete media of 200 μl were added into each well. Next, the cells were incubated in the incubator containing 5% CO_2 at 37°C for 48 h.

Different concentrations of drugs were added into the cells. In the simple drug administration experiment, the whole group was divided into the cell control group, rapamycin group and cisplatin group. For the cell control group, the cells were cultured in the equivalent medium without a drug. For the rapamycin group, 4 concentration gradients were applied: 10 nmol/ml, 20 nmol/ml, 40 nmol/ml and 80 nmol/ml. For the cisplatin group, 4 concentration gradients were applied: 0.125 mg/ml, 0.25 mg/ml, 0.50 mg/ml and 1.00 mg/ml. In addition, in 3 wells only culture liquid was added.

For the drug combination experiment, inhibitory effects of the combinations of 10 nmol/ml and 20 nmol/ml rapamycin with 0.25 mg/ml and 0.50 mg/ml cisplatin on the cells were respectively detected.

MTT

After the drugs affected the cells for 48 h, 15 μl of MTT (5 mg/ml) was added into each well. After incubation for 4 h at room temperature, 100 μl of DMSO was added into each well. After gently shaking for 10 min, the absorbance value of each well was immediately detected with the microplate reader at 589 nm. Trials were repeated three times, and absorbance values of various repeated wells were averaged. Finally, the inhibition ratio of each group of cells (%) was calculated: inhibition ratio of tumor cell proliferation = (1 – absorbance value of drug administration group/absorbance value of the treatment group) × 100%.

Jin's formula q = Ea + b/(Ea + Eb – Ea × Eb) was used to evaluate whether two drugs had a synergistic effect. Ea + b represented the inhibition ratio of the combination group shozhe, and Ea and Eb represented respectively the inhibition ratios of simple drug a and simple group b. If the calculated q value was between 0.85 and 1.15, the effect of combination of two drugs was the simple summation of respective effects; if $q > 1.15$, the two drugs had a synergistic effect; if $q < 0.85$, the two drugs had an antagonistic effect.

Results

Inhibitory effects of simple rapamycin and cisplatin and their combination on growth of cervical carcinoma Hela cells

After rapamycin affected cervical carcinoma Hela cells for 48 h, it had an apparent inhibitory effect on their growth. This inhibitory effect was dose-dependent, and it was more apparent with increase of drug concentration. Between various drug groups and the control group, there were significant differences ($p < 0.05$). Comparison of the result between two adjacent concentration groups showed that there was a significant difference between the 10 nmol/ml group and the 20 nmol/ml group ($p < 0.05$), and there was no significant difference between two other adjacent concentration groups.

After cisplatin affected the cells for 48 h, compared with the control group, the inhibition ratio increased. It was suggested that cisplatin had an apparent inhibitory effect on growth of cervical carcinoma Hela cells. This inhibitory effect was dose-dependent, and between various drug groups and the control group, there were significant differences ($p < 0.05$). Comparison of the result between two adjacent concentration groups showed that there were significant differences between two groups among the 0.25 mg/ml group, 0.50 mg/ml group and 1.00 mg/ml group ($p < 0.05$), and there was no significant difference between two other adjacent concentration groups.

According to the ratios of inhibition of two simple drugs on Hela cells, drug concentrations in the case of drug combination were selected. As drug combinations, 10 nmol/ml and 20 nmol/ml rapamycin were respectively combined with 0.25 mg/ml and 0.50 mg/ml cisplatin to affect cervical carcinoma Hela cells. Comparisons of various drug groups with the control group and comparisons between two adjacent concentration groups showed that there were significant differences ($p < 0.05$) (Table 1).

Calculation of the synergistic effect of the two drugs according to Jin's formula

In terms of the ratios of inhibition of two simple drugs and their combination on Hela cells, it was calculated according to Jin's formula q = Ea + b/(Ea + Eb – Ea × Eb) whether the two drugs had a synergistic or antagonistic effect. Ea + b represented the inhibitory ratio of the combination group, and Ea and Eb respectively the inhibition ratios of simple drug a and simple group b. If the calculated q value was between 0.85 and 1.15, the effect of combination of two drugs was the simple summation of respective effects; if $q > 1.15$, the two drugs had a synergistic effect; if $q < 0.85$, the two drugs had an antagonistic effect. As a result, in the case of the combination of rapamycin and cisplatin, q values all were more

Table 1. The influence of rapamycin and cisplatin individually or combined on the growth of Hela cells *in vitro*

Group	Absorbance A (X ± S)	Inhibition rate (%)
control	0.7012 ±0.0201	0
rapamycin (nmol/ml)		
10	0.6160 ±0.0325	12.15*Δ
20	0.5459 ±0.0318	22.14*Δ
40	0.5173 ±0.0126	26.23*
80	0.4759 ±0.0218	32.23*
cisplatin (mg/ml)		
0.125	0.6146 ±0.0184	12.34*
0.25	0.5740 ±0.0214	18.13*Δ
0.50	0.5014 ±0.0179	28.33*Δ
1.00	0.4324 ±0.0218	38.33*Δ
rapamycin (nmol/ml) + cisplatin (mg/ml)		
10 + 0.25	0.4464 ±0.0263	36.34*Δ
20 + 0.25	0.3201 ±0.0198	54.36*Δ
10 + 0.5	0.2153 ±0.0315	69.29*Δ
20 + 0.5	0.1497 ±0.0326	78.65*Δ

$p < 0.05$ vs. control group; Δ$p < 0.05$, comparison between two adjacent concentrations

Table 2. Inhibitory effect of rapamycin combined with cisplatin on the proliferation of Hela cells

Rapamycin	Cisplatin	
	0.25 mg/ml	0.5 mg/ml
10 nmol/ml	1.18	1.67
20 nmol/ml	1.31	1.49

all q values > 1.15

than 1.15, as shown in Table 2. The result showed that the combination of rapamycin and cisplatin had an apparent effect on Hela cell proliferation, and they had a synergistic effect.

Discussion

Akt/mTOR is one of the cell proliferation-related signal pathways, and it is closely related to occurrence and development of multiple malignant tumors [8–10]. In recent years, mTOR has become a new target of molecular targeted therapy of tumors. Recent studies show that the mTOR specific inhibitor rapamycin has a treatment effect on a variety of tumors such as breast carcinoma, leukocythemia, liver cancer, gallbladder carcinoma, etc. [11–15]. However, at present there are fewer reports on the effect of this drug on cervical carcinoma at home and abroad.

The anti-tumor inhibition mechanism of the mTOR specific inhibitor rapamycin is mainly to inhibit conversion of the cell cycle from G1 phase to S phase, induce apoptosis of mutational tumor cells with P53 function deficiency, shut off the energy cycle route and block energy synthesis and utilization of the tumor cell by inhibiting mTOR and block tumor cell growth by inhibiting angiogenesis [16–18], which indicates that rapamycin achieves tumor inhibition by inhibiting tumor cell growth and proliferation and has a targeted antitumor effect on a number of pathways. Some studies have suggested that although rapamycin can kill tumor cells, it could attack normal cells, which was also confirmed in nude mice [19].

At present, chemotherapy of cervical carcinoma mainly uses cisplatin. However, its administration is usually stopped due to non-specific toxic side effects of cisplatin after multiple treatment courses of chemotherapy. Also, clinical effectiveness of drug resistance is not especially ideal. Yuan studied the effects of cisplatin on oophoroma and breast carcinoma tissues and found that increase of Akt activity was possibly one of the main factors causing drug resistance and thus causing failure of cisplatin chemotherapy [20]. Fraser *et al.* drew the same conclusion in a study of oophoroma [21]. Therefore, it is indicated that we can inhibit tumor cell growth by inhibiting Akt/mTOR to reverse drug resistance of cisplatin.

This experiment used simple rapamycin and cisplatin and their combination to carry out *in vitro* intervention of cervical carcinoma Hela cells, and the research results showed that simple application of rapamycin or cisplatin could inhibit human cervical carcinoma Hela cell strains and the inhibitory effect was dose-dependent. The combination application had a more apparent inhibitory effect on Hela cell proliferation than application of simple drugs. Also, q values of various drug combination groups were more than 1.15, suggesting that combination of the two had a synergistic effect. In a study on lung cancer, Wu *et al.* [15] proved that mTOR inhibitor could reverse cisplatin resistance and restore sensitivity of lung cancer resistance strains to cisplatin to cause apoptosis of resistant cells, and combination application had an apparent synergistic effect, which was in line with the study result about cervical carcinoma Hela cells in this study. Similarly, Bae-Jump *et al.* confirmed that combination of rapamycin and cisplatin had a synergistic inhibitory effect on endometrial carcinoma cells [22].

It can be found that rapamycin can really inhibit the growth of human cervical carcinoma Hela cells, and combination application of rapamycin and cisplatin can generate an apparent synergistic effect to increase the inhibition ratio of tumor cells. Therefore, rapamycin can be considered as a complementary drug. Under the premise of equivalent efficacy, concerted application of it and a chemotherapy drug can decrease the application amount of cisplatin, reduce toxic side effects of cisplatin and achieve a more satisfactory drug treatment effect of cervical carcinoma. Rapamycin has a broad-spectrum antitumor effect, and it has been safely and effectively used for tumor patients after organ transplantation. It is still necessary to investigate and explore the discussed application of rapamycin in tumor patients after non-organ transplantation.

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The authors declare no conflict of interest.

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