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ORIGINAL ARTICLE

rmhTNF- α Combined with Cisplatin Inhibits Proliferation of A549 Cell Line *In Vitro*

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Objective To explore the inhibitory effect of recombinant mutant human tumor necrosis factor- α (rmhTNF- α) in combination with cisplatin on human lung adenocarcinoma cell line A549.

Methods Human lung adenocarcinoma cell line A549 was treated with varying concentrations of rmhTNF- α (0.38, 0.75, 1.50, 6.00 and 12.00 IU/ml) or cisplatin (3.91, 7.81, 15.63, 31.25 and 62.50 μ g/ml) for 24 hours. Viable cell number was analyzed by using crystal violet staining. The inhibitory rates of A549 cells growth by the two drugs were calculated. For analyzing whether there was a synergistic effect of rmhTNF- α with cisplatin, A549 cells were treated with 0.75 IU/ml rmhTNF- α and increased concentrations of cisplatin.

Results rmhTNF- α or cisplatin inhibited the growth of A549 cell lines in a dose-dependent manner. The inhibitory effect of rmhTNF- α combined with cisplatin was significantly greater than cisplatin alone at the same concentration (all $P < 0.01$).

Conclusion rmhTNF- α combined with cisplatin might have synergistic inhibitory effect on human lung adenocarcinoma cell line A549.

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TUMOR necrosis factor (TNF) is one of endogenous immune regulators, which has direct cytotoxicity against multiple tumor cells and is the cytokines having the strongest antitumor activity so far.^{1,2} Recombinant mutant human TNF- α (rmhTNF- α) is one mutant from natural TNF- α . rmhTNF- α having similar biological activities as the natural TNF- α can kill multiple types of tumor cells without cytotoxicity on normal cells (receptor selectivity) and has sensitizing effect on

chemotherapy.^{3,4} Li *et al*⁵ found that rmhTNF- α has higher antitumor activity and lower systemic toxicity in mice models of homogenic tumor transplantation and human tumor gene transplantation when compared with wild-type TNF- α . It was reported that rmhTNF- α has synergistic and enhancing effects with doxorubicin and paclitaxel in treating breast cancer, colorectal carcinoma⁶ and can reverse multidrug resistance when combined with other chemotherapeutic drugs.⁷ The present study preliminarily explored the inhibitory effect of rmhTNF- α in combination with cisplatin on human lung adenocarcinoma cell line A549.

MATERIALS AND METHODS

Cell culture

Human lung adenocarcinoma cell line A549 purchased from Kaiji Biological Co., Ltd. (Nanjing, China) was cultured in high glucose type DMEM medium (Kaiji Biological Co., Ltd.) containing 10% fetal calf serum (Hyclone, UT, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C in 5% CO₂ incubator. The 0.25% trypsin (Kaiji Biological Co., Ltd.)-EDAT was used for generation of cells every 3 days.

Cell grouping and treatment

A549 cells were divided into the vehicle control group, rmhTNF-α group, cisplatin group, and rmhTNF-α+cisplatin group. In the cisplatin group, A549 cells were incubated with 3.91, 7.81, 15.63, 31.25, and 62.50 µg/ml cisplatin (Qilu Pharmaceutical Co., Ltd., Shandong, China), respectively. In the rmhTNF-α group, the concentrations of rmhTNF-α (Shanghai Weike Biopharmaceutical Co., Ltd., China) were 0.38, 0.75, 1.50, 6.00, and 12.00 IU/ml, respectively.

Crystal violet staining

According to previous studies,^{3,4} the cells at exponential stage were digested with trypsin to make cell suspension at a certain concentration and were incubated in 96-well plates at 100 µl/well. The next day, the cells were exposed to RPMI1640/DMEM medium containing 3% fetal calf serum and 0.7-1.0 µg/ml actinomycin D supplemented with different concentrations of chemicals. After incubation at 37°C for 24 hours, cells were stained with 1% crystal violet for 20 minutes. Optical density (OD) was measured with spectrophotometer at the referral wavelength of 570 nm and measuring wavelength of 630 nm. The inhibitory rate was calculated with the following formula: inhibitory rate=(1-experimental group OD/control group OD) ×100%. Each measurement was repeated 3 times and the average was calculated.

Living cells count

After A549 cells at the density of 6.5×10⁴/bottle were incubated for 24 hours, they were treated with 0.75 IU/ml rmhTNF-α, 15.63 µg/ml cisplatin, 0.75 IU/ml rmhTNF-α+15.63 µg/ml cisplatin for 24, 48, and 72 hours, respectively. The living cells were counted under a light microscope and then a scatter diagram was drawn.

Statistical analysis

Statistical analysis was performed using SPSS 17.0. Continuous variables were expressed as means±SD and

compared with analysis of variance (ANOVA). A *P* value less than 0.05 was set as significant level.

RESULTS

Effects of rmhTNF-α or cisplatin alone on proliferation of A549 cells

Both rmhTNF-α and cisplatin showed inhibitory effects on A549 cells. Within the predetermined concentration range, the inhibitory rate of the two chemicals on proliferation of A549 cell lines increased following the increase in rmhTNF-α or cisplatin concentration (Table 1). The concentrations of rmhTNF-α being above 6.00 IU/mL and cisplatin above 31.25 µg/ml did not significantly enhance the inhibitory effects.

Inhibitory rate of rmhTNF-α combined with cisplatin on A549 cells' proliferation

According to the above results, in the following test, A549 cells were treated with rmhTNF-α at the fixed concentration (0.75 IU/ml) combined with different doses of cisplatin. Optical density values of A549 cells incubated with 3.91, 7.81, 15.63, 31.25, and 62.50 µg/ml cisplatin for 24 hours were 1.04±0.08, 0.77±0.02, 0.62±0.01, 0.50±0.01, 0.41±0.01, respectively. The inhibitory effect was increased as the concentration of cisplatin augmented. The inhibitory effect of rmhTNF-α combined with cisplatin was significantly greater than cisplatin alone at the same concentration (all *P*<0.01).

Table 1. Inhibitory rate of different concentrations of rmhTNF-α or cisplatin on proliferation of A549 cells exposed to drugs for 24 hours (*n*=3)

Drug	Concentration	Optical density value [§]	Inhibitory rate (%)
rmhTNF-α (IU/ml)	0	1.52±0.08	0
	0.38	1.36±0.05	10.6
	0.75	1.35±0.07	11.2
	1.50	1.16±0.05	23.7
	6.00	1.06±0.08	30.3
	12.00	1.04±0.02	31.6
Cisplatin (µg/ml)	0	1.39 ±0.12	0
	3.91	1.20±0.12	13.7
	7.81	0.90±0.03	35.3
	15.63	0.67±0.02	51.8
	31.25	0.62±0.02	54.4
	62.50	0.54±0.02	61.2

§: Plus-minus values are means±SD.

rmhTNF-α: recombinant mutant human tumor necrosis factor-α.

Growth curve of A549 cells

As shown in Figure 1, the growth speed of A549 cells in the medication groups was slower than that in the control group. The growth speed of A549 cells in the cisplatin+rmhTNF- α group was the slowest among the four groups. Compared with the control group, growth of A549 cells exposed to medication was significantly lower (all $P<0.01$).

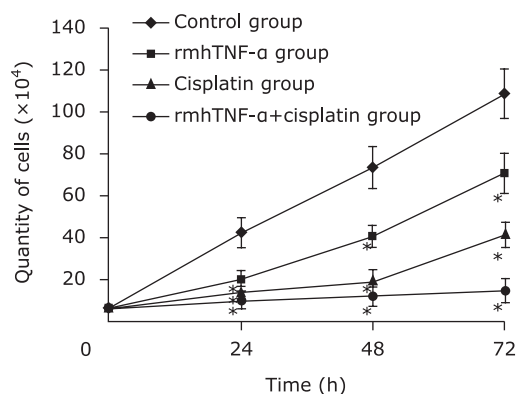


Figure 1. Comparison of A549 cells number between the four groups at the indicated time ($n=3$).

* $P<0.01$ compared with the control group.

DISCUSSION

It has been shown that rmhTNF- α exhibited lower toxicity and higher anti-tumor efficacy compared to wild-type TNF- α .⁸ Preliminary animal studies showed that rmhTNF- α was a promising and safe therapy to use in the clinic.⁵

Using crystal violet staining, we investigated the killing effects of rmhTNF- α on A549 cells. The results indicated that low concentration of rmhTNF- α can obviously decrease the proliferation of A549 cells. Low concentration of rmhTNF- α combined with cisplatin showed better inhibitory effect on lung cancer cells A549 than cisplatin alone, suggesting that rmhTNF- α at low concentration have synergistic effect with cisplatin. Therefore, rmhTNF- α might

have sensitizing effect on chemotherapy in lung cancer. In addition, the effect of rmhTNF- α was not completely correlated with the dosage.

In conclusion, rmhTNF- α might not only inhibit the proliferation of A549, but also enhance the killing effect of cisplatin on A549 cells.

REFERENCES

1. Yang L, Guo J, Wang J, et al. Ad-KDRscFv: sTRAIL displays a synergistic antitumor effect without obvious cytotoxicity to normal tissues. *Int Immunopharmacol* 2012; 13: 37-45.
2. Langdom SP. Cancer cell culture: Methods and protocols. Edinburgh: Humana Press Inc; 2004. p. 267-73.
3. Zhou YY, Zhou NX, Huo JR. Pharmacodynamics and mechanism of rmhTNF in treating human gastric carcinoma. *Gastroenterology* 2010; 15: 261-6.
4. Ren L, Zhou QH, Gou HF, et al. Clinical effect and survival condition of non-small cell lung carcinoma treated by rmhTNF and combined chemotherapy. *West China Med J* 2008; 23: 521-2.
5. Li M, Qin X, Xue X, et al. Safety evaluation and pharmacokinetics of a novel human tumor necrosis factor- α exhibited a higher antitumor activity and a lower systemic toxicity. *Anticancer Drugs* 2010; 21: 243-51.
6. Roberts NJ, Zhou SB, Diaz LA, et al. Systemic use of tumor necrosis factor alpha as an anticancer agent. *Oncotarget* 2011; 2: 739-51.
7. Jinesh GG, Chunduru S, Kamat AM. Smac mimetic enables the anticancer action of BCG-stimulated neutrophils through TNF- α but not through TRAIL and FasL. *J Leukoc Biol* 2012; 92: 233-44.
8. Jiang CL, Niu JZ, Li M, et al. Tumor vasculature-targeted recombinant mutated human TNF- α enhanced the antitumor activity of doxorubicin by increasing tumor vessel permeability in mouse xenograft models. *PLoS One* 2014; 9: e87036.