Impact of IFN-α on the Growth of Human Cervical Cancer Transplanted Subcutaneously in Nude Mice

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Abstract **Purpose** To evaluate the impact of interferon-α on the growth of human cervical cancer transplanted subcutaneously in nude mice. **Methods** twenty-eight nude mice transplanted subcutaneously with human cervical cancer were divided into 4 groups: control group, positive control group(DDP group), IFN-α low dose group(1.5×10^7u/kg) and IFN-α high dose group(3.0×10^7u/kg). Drugs were used from the day 14th day and all mice were sacrificed on the 49th days after transplantation. The weight of the mice and the volume of tumor were measured. FCM was used for measuring the proportion of cell cycle S+G2–M(%) in tumor cells. **Results** The growth inhibiting rates in DDP group, IFN-α low dose group, IFN-α high dose group were 92.8%, 73.8% and 93.1%, respectively. Except for mice in DDP group, there was an obvious increase in the weight of mice in other three groups. As for the propotion of S+G2–M (%), except for the DDP group, there was no difference in three other groups. **Conclusions** IFN-α can obviously inhibit the growth of transplanted cervical cancer, which is correlated with the IFN-α dose. The mechanism of IFN-α inhibiting cervical cancer was not due to the suppression of cancer cell proliferation.

Key Words Cervical neoplasms; interferon-α; Neoplasm transplantation

Carcinoma of the uterine cervix is still the first common malignancies among women with gynecologic tumors, according to the estimation of cancer incidence in the globe in 2000 [1]. It has severely harmed the health of women. So, it is imperative to study the appropriate treatment modalities. Biotherapy, the fourth treatment model following surgery, radiotherapy and chemotherapy, has come to our notice. Among which, IFN-α which is a cytokine has been widely used clinically and has definitive effects on some tumors. The current study is to elucidate the impact of IFN-α on the human cervical cancer transplanted subcutaneously in nude mice and the anti-tumor effect in vivo, and further provide the theoretical basis for the clinical use of IFN-α.

MATERIALS AND METHODS

Drugs

IFN-α1b was provided by the Bioproducts Research Institute of Shanghai and cisplatin (DDP) produced by the Pharmaceutical Factory of Dezhou, Shandong Province. IFN-α and DDP were dissolved in the physiological saline.

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Experimental animals

Female nude mice of BALB/c nu/nu, 4~6 weeks of age and 20g in weight, and the animal model were provided and bred by the Section of Experimental Animal, Cancer Research Institute of Shanghai.

Experimental methods

Inoculation The nude mice bearing tumors were killed and the tumors excised and placed in the physiological saline. Nonnecrotic tumor tissues were selected and cut into small lumps of $1mm^3$. The tumors were inoculated subcutaneously in the right back skin right of nude mice which was prepared with tincture iodine.

Groups After 14 days of inoculation, 28 of nude mice with the tumor being 5 mm in diameter were chosen and randomly divided into 4 groups: Control group, 0.2 ml physiological saline were injected subcutaneosly per day for 5 weeks; DDP group, 0.2 ml DDP (1mg/kg) were administered intraperitoneally per day for 5 days and then rested for 5 days, Sequentially, 20 injections in 5 weeks; low dose IFN-α, 0.2 ml IFN-α (1.5×10^7u/kg) were given subcutaneously per day for 5 weeks; high dose IFN-α, 0.2 ml IFN-α (3.0×10^7u/kg) were given

as above.

Measures of tumor growth After 14 days of inoculation, tumor growth was assessed in serial measurements of long diameter (a) and wide diameter (b) using vernier calipers, tumors were measured at 3~4-day intervals and tumor volumes (V) were calculated ($V=\pi ab^2/6$). Tumor growth curves were depicted at the end of experiment.

Inhibiting rate of tumor After 35 days of drug use, that is, after 49 days of inoculation, mice were killed and tumors were excised and weighed. The inhibiting rate of tumor was defined as the tumor weight in control group minus that in experimental group versus tumor weight in control group.

Proliferation detection of tumor cells using flow cytometry (FCM) Cells proliferation was analyzed using a flow cytometer (Becton Dickinson Facscalibour) as follows: tumor tissue samples were minced with ophthalmic scissors, and single cells were further isolated throught a 60-um nylon mesh, and were fixed with 95% ethanol, they were washed with PBS and the supernatant was removed after 1500rpm centrifuge. Then, 1 ml Triton-100 was added and incubated for 10 minutes, the supernatant were removed after 1500 rpm centrifuge. 1 ml propidium iodide (PI) was added and the cells were filtered with a 60 um nylon mesh again. Flow cytometry was performed after 20 min incubation at 4℃.

Statistical analysis

The Independent-Samples T test was used.

RESULTS

The impact of IFN-α on the tumor volumes of nude mice

The tumor growth was inhibited in groups of DDP, low and high dose IFN-α, compared with the control group (Fig.1, 2). 7 tumors of control group enlarged obviously at the end of experiment, 5 of which were accompanied by one to three tumor nodules of 2~3 mm in diameters around the tumors; The tumor volumes of low dose IFN-α were smaller than that of control group, 1 of 7 tumors had a 2 mm tumor nodule; The tumors in the high dose IFN-α group and DDP group were much smaller among the four groups and no tumor nodules were formed.

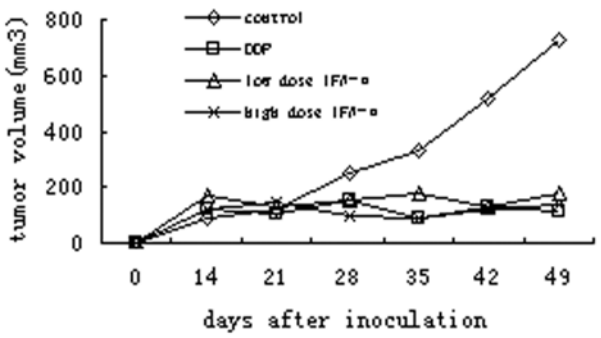

Fig.1 Impact of IFN-α on the Tumor Growth of Nude Mice

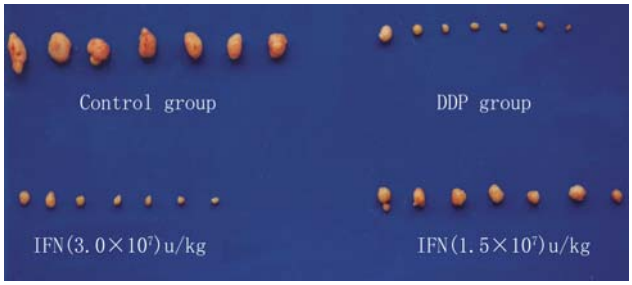

Fig. 2 Tumor size comparison at the end of experiment

Impact of IFN-α on the tumor weight

IFN-α can obviously inhibit the tumor growth. Furthermore, the inhibiting rate of high dose IFN-α was close to that of DDP group (table 1).

Fig.1 Impact of IFN-α on the Tumor Growth of Nude Mice

Group	Tumor weight (mg, $\bar{x}\pm s$)	Inhibiting rate (%)
Control	450±86	
DDP	32±34	92.8***
Low dose IFN-α	118±36	73.8***
High dose IFN-α	31±18	93.1***▲▲▲

Compared to control group, ***P<0.01; Compared to low dose IFN-α, ▲▲▲P<0.01

Impact of IFN-α on Tumor Proliferation

Tumor proliferation was inhibited markedly by DDP, but not by IFN-α. The inhibition mechanisms may be different between IFN-α and DDP (Table 2).

Table 2 The proliferation status of tumor

Group	S+G2-M (%)
Control	37.76 ± 3.86
DDP	31.91 ± 4.14**
Low dose IFN-α	35.43 ± 7.76*
High dose IFN-α	35.79 ± 3.95*

Compared to the control group, $^{*}P>0.05$, $^{**}P<0.05$.

Impact of IFN-α on Mice Weight

The mice weight was increased with the age growing in the low and high IFN-α groups, the same as in control group. However, the mice of DDP group didn´t gain weight with the mice age increase (table 3). The study elucidated that IFN-α not only has an inhibiting effect on tumor growth, but also has no an impact on mice weight, that is to say, IFN-α has no obvious side effects for nude mice.

Table 3 The weight changes of nude mice

Group	Weight (g)	
	Before experiment	After experiment
Control	20.26 ± 0.95	24.41 ± 0.67▲▲▲
DDP	20.11 ± 0.73	21.31 ± 1.99***▲
Low dose IFN-α	20.29 ± 0.90	23.31 ± 1.33*▲▲▲
High dose IFN-α	20.43 ± 0.74	24.45 ± 2.19*▲▲▲

Before the experiment, the mice weight compared between any two groups, $p>0.05$, After the experiment, Compared to the control group, $^{*}P>0.05$, $^{***}P<0.01$; Compared to the mice weight before experiment, $^{▲}P>0.05$, $^{▲▲▲}P<0.01$。

DISCUSSION

Carcinoma of the uterine cervix is one of the most common gynecologic malignancies. It is estimated that in 2000 there were 470,000 new cases of cervical cancer in the world, and 230,000 cases died of the disease[1]. Either surgery or radiotherapy is an effective approach for early disease. However, the treatment effect is not optimal for advanced cases and has not been improved in the last 30 years. So, it is imperative to search for the appropriate comprehensive modalities.

Biotherapy, the fourth treatment model in recent years, includes cellular therapy, cytokine therapy, gene therapy and antibody therapy. IFN-α, a cytokine, has been used clinically for more than 30 years. It is now verified that IFN-α has definitive effect on some human malignant tumors such as hairy cell leukemia, lymphoma, renal cell carcinoma, malignant melanoma, basal carcinoma, squamous cell carcinoma, and so on. The anti-tumor mechanism of IFN-α may be the direct modulation to tumor cells, modulating the host immunity reaction to tumors and changing the mutual relation between host and tumor[2].

IFN-α has an obvious inhibition on cervical cancer cells in vitro[3,4]. The human cervical cancer transplanted subcutaneously in nude mice was used as the model and the impact of IFN-α on the tumor growth was studied in our current experiment. The results showed that the inhibition rate of IFN-α in low dose IFN-α, high dose IFN-α and DDP group was 73.8%, 93.1% and 92.8%, respectively. Furthermore, the inhibition rate was statistically different between low dose and high dose IFN-α, which indicates that the inhibition effect was associated with the dose of IFN-α. The mice weight in DDP group didn´t increase at the end of experiment, whereas, in both IFN-α and control group it had an increasing tread with the age increase, which reflects the basic difference between biotherapy and chemotherapy. Biotherapy, in which the

biologicals used are its own substances of organism, has no harm to the organism and conversely has an effect of modulation and enforcement on the organism[2]. DDP is an effective drug in the treatment of cervical cancer[5] and that is why it was used as a positive control in our current study. DDP should be given intermittently but not continuously due to its toxic effect. IFN-α has a mild toxic effect and good tolerance and conformability. So, It is different in the use of DDP and IFN-α.

The tumor cell proliferation was detected using FCM. The proportion of S+G2–M was significantly lower in the DDP group than that in the control group. However, It had no obvious difference between low-dose and high dose IFN-α group and the control group. The current study showed that there is a different anti-tumor mechanism for IFN-α and DDP. The anti-tumor effect of IFN-α may be through other mechanism, not through inhibiting tumor cell proliferation. IFN-α is now considered as an angiogenesis inhibitor and one of its important anti-tumor mechanisms may be its ability to inhibit angiogenesis of tumor cell. Some authors, using the corneal micropocket model in nude mice bearing human hepatocellular carcinoma xenografts, found that IFN-α could inhibit the angiogenesis induced by liver cancer tissues [6]. It needs further study whether the anti-tumor effect of IFN-α on the human cervical cancer transplanted subcutaneously in nude mice was correlated with its anti-angiogenic ability.

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