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ANTITUMOR EFFECTS OF POLYETHYLENE GLYCOL-MODIFIED RECOMBINANT HUMAN INTERLEUKIN-2 ON MOUSE UTERINE CERVICAL CARCINOMA *IN VIVO*

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Polyethylene glycol (PEG-8000)-modified recombinant human interleukin-2 (PEG-rIL-2) is a cytokine with prolonged circulatory half-life. In this paper, the antitumor effects of PEG-rIL-2 against mouse uterine cervical carcinoma (U_{14}) transplanted intraperitoneally or subcutaneously is reported. PEG-rIL-2 at different doses was administered intraperitoneally. The results showed that PEG-rIL-2 (4500 IU, i.p., QD×5) prolonged survival time of mice bearing ascites tumor as compared to rIL-2 ($P<0.01$), but PEG-rIL-2 at lower doses was without therapeutic effect. In addition, compared to rIL-2, PEG-rIL-2 at different doses (1500–13500 IU, s.c., QD×5) caused significant dose-dependent growth inhibition of solid tumor ($P<0.01$) when the treatment started at day 4 after subcutaneous inoculation of tumor.

Key words: Cervix neoplasms/therapy, Polyethylene glycol, Interleukin-2, Immunotherapy.

Recombinant human interleukin-2 (rIL-2) is one of the cytokines, which play an important role in antitumor immunotherapy. However, rIL-2 has limited solubility, and rIL-2 is rapidly cleared from circulation of human when it is injected into human, resulting in short-term plasma half-life and limited clinical application. We have modified rIL-2 by polyethylene glycol (PEG), which increased its solubility and prolonged its plasma half-life. In this paper, the antitumor effects of PEG-modified recombinant human interleukin-2 (PEG-rIL-2) against mouse uterine cervical carcinoma (U_{14}) on animal bearing the ascites or solid form of transplanted U_{14} have been investigated.

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MATERIALS AND METHODS

Tumor Cell Lines

U_{14} cells, uterine cervical cancer cells,[1] were kindly provided by Prof. Gan ShengWen (Department of Tumor Immunology Research, Jingxi Medical College). This uterine cervical carcinoma was induced by cervix administration of methylcholanthrene and was carried as an ascitic tumor stock in BALB/C mice.

Animals

Specific pathogen-free, 18–20 g, female BALB/C were obtained from Shanghai Experimental Animal Center, Academia Sinica.

Therapeutics

Highly purified rIL-2 and polyethylene glycol

(8000)-modified rIL-2 were provided by Shanghai Hua Xin Co. The specific bioactivity of the rIL-2 and PEG-rIL-2 were 1×10^7 and 9×10^6 IU/mg, respectively. Before use rIL-2 and PEG-rIL-2 were further diluted in NS.

Preparation of the Single U_{14} Cell Suspensions

The single-cell suspensions were prepared as previously described by Wang et al.[2] Viability was determined by the typan blue dye-exclusion method. Cells viability exceeded 95%.

Antitumor Effects of PEG-rIL-2 and rIL-2 on Host Bearing the Tumor of Ascites Form

BALB/C mice were given an i.p. injection of 5×10^5 U_{14} tumor cells in 0.2 ml, and were randomly segregated into groups of 8 animals. One day after tumor cell inoculation, an i.p. injections with rIL-2 or PEG-rIL-2 (in 0.2 ml) were given daily for 5 days at different dose levels. Control animals received injections with NS in a similar way. The animals were inspected daily and their survival times were recorded. The therapeutic effects of PEG-rIL-2 were expressed by the prolongation of the survival time.

Therapeutic Effects of PEG-rIL-2 and rIL-2 on Host Bearing the Tumor of Solid Form

BALB/C mice were injected subcutaneously in the right anterior flank with 1×10^6 U_{14} tumor cells. After 4 days the animals, bearing established tumors, were randomly divided into treatment groups of 7 to 8 animals, and 0.2 ml injections with rIL-2 or PEG-rIL-2 were started. In the series of experiments intratumor injects were given daily for 5 days at different dose levels. Control animals received injections with NS in a similar way. All groups were killed at day 12, and tumors were dissected out and weight.

Statistical Analysis

Statistical comparisons to controls or among various experimental groups were performed using the Student'*t*-test or *t*'-test. A difference was regarded as significant if P was less than 0.05.

RESULTS

Antitumor Effects of PEG-rIL-2 and rIL-2 on Host Bearing the Tumor of Ascites Form

The effects of 5 daily i.p. injection of rIL-2 and PEG-rIL-2 on the survival time of mice with ascites tumor is shown in Table 1, and Figure 1 depicts survival curves of U_{14} bearing mice treated with PEG-rIL-2 and rIL-2. In animals receiving only NS injections the tumor grew progressively. Administration of PEG-rIL-2 (4500 IU/day) to U_{14} tumor bearing mice prolonged the survival time of the remainder significantly [mean survival days, 23.1±3.6 (SD)] as compared to the control NS or rIL-2 treated mice ($P<0.01$), whereas administration with rIL-2 (4500 IU/day) and low dose levels of PEG-rIL-2 (1500 IU/day) did not prolong the survival time.

Table 1. Therapeutic effects of PEG-rIL-2 and rIL-2 against U_{14} in BALB/C mice. U_{14} cells (5×10^5) were implanted intraperitoneally in mice

IL-2	Dose* (IU)	Mice	Survival time** (day, $\bar{x}\pm s$)
NS	---	8	15.9±1.2
rIL-2	4500	8	16.5±2.0*
	1500	8	17.4±2.4*
PEG-rIL-2	4500	8	23.1±3.6**
	1500	8	15.5±2.7*

*One day after tumor cell inoculation, an i.p. injections with rIL-2 or PEG-rIL-2 were given daily for 5 days.
**Compared to control: *$P>0.05$ **$P<0.01$

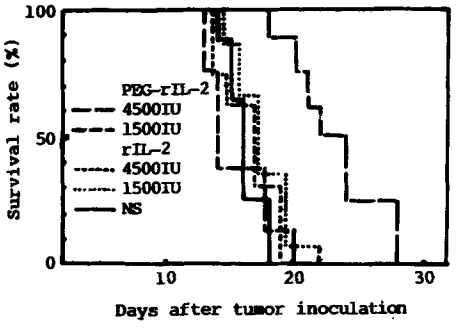

Fig. 1. Survival curves of U_{14} bearing mice treated with PEG-rIL-2 and rIL-2 intraperitoneally. U_{14} cells (5×10^5) were implanted intraperitoneally in BALB/C. One day later the mouse were given 5 daily i.p. injection of the following: PEG-rIL-2; 4500 IU; 1500 IU; rIL-2; 4500 IU; 1500 IU; NS.

Therapeutic Effects of PEG-rIL-2 and rIL-2 on Host Bearing the Tumor of Solid Form

In another experiment, BALB/C mice were injected subcutaneously with 1×10^6 tumor cells. After 4 days the animals had established palpable tumors on the right anterior flank, and treatment were started. Tumor-bearing mice were treated with 5 daily rIL-2 and PEG-rIL-2 intratumorally with dose ranging from 1500 to 13500 IU per injection. In control animals the tumor always showed progressive growth. By day 12 the tumor had grown large in the control mice. Therefore, all animals groups were killed at day 12. During the observation period, no animals were sacrificed, and there was no evidence for disruption of the skin and ulceration. Table 2 shows the results of experiment comparing the effects of 5 daily rIL-2 and PEG-rIL-2 injections, when doses of 1500, 4500 IU of rIL-2 were administered, the significant inhibition of tumor growth were not observed as compared to NS-control animals ($P>0.05$). Treatment with rIL-2 resulted in a significant inhibition of tumor growth at the dose level of 13500 IU ($P<0:01$). With PEG-rIL-2, however, a similar inhibition was achieved at a dose level of 4500 IU ($P<0.01$). Doses of 1500, 4500, 13500 IU of PEG-rIL-2 inhibited significantly tumor growth as compared with the control NS or rIL-2 treated mice ($P<0.01$). However, no complete tumor regression in mice were observed. Furthermore, PEG-rIL-2 inhibited tumor growth in a dose-dependent manner.

Table 2. Growth inhibitory effects of PEG-rIL-2 and rIL-2 on BALB/C mice subcutaneously inoculated with 1×10^6 U_{14} cells. Treatment was initiated 4 days after tumor implanted, s.c. daily for 5 days. U_{14} tumors were excised at day 12 after tumor cell inoculation

IL-2	Dose (IU/day)	Mice	Tumor weight* (mg, $\bar{x}\pm s$)
NS	---	8	342±57
rIL-2	13500	7	143±55**
	4500	8	294± 50*
	1500	7	358±47*
PEG-rIL-2	13500	8	45±10***
	4500	8	102±26***
	1500	8	195±79***

*Compared to control: *$P>0.05$ **$P<0.01$
Compared to rIL-2: ***$P<0.01$

DISCUSSION

Interleukin-2 (IL-2), a cytokine initially described by Morgan et al.[3] is an essential growth factor for all subsets of T cells but it also effects macrophages and natural killer cells. Since rIL-2 has become available, its potential as an immune stimulant in anticancer immunotherapy has been studied on a large scale. Recombinant human interleukin-2 can cause partial and complete responses in certain advanced neoplasms including lymphoma, renal cell cancer and melanoma when used alone or in combination with other agents such as LAK or TILs.[4,5] Systemic immunotherapy with high doses of rIL-2 can induce tumor regression in man. However, the number of responding patients is limited and the treatment is accompanied by serious toxicity.

Recently, chemical modification of rIL-2 with the water-soluble polymer polyethylene glycol increased the solubility of interleukin-2, decreased its plasma clearance, and increased its antitumor potency in Metha A sarcoma and B_{16} melanoma model.[6,7]

In the present report, the antitumor effects of PEG-rIL-2 against mouse uterine cervical carcinoma on animal bearing the ascites or solid form of transplanted U_{14} have been evaluated. The results showed that PEG-rIL-2 significantly prolonged survival time of mice bearing the tumor of ascites form as compared to rIL-2. In addition, compared to rIL-2, locoregionally administered PEG-rIL-2 at different doses caused significant growth inhibition of s.c. solid tumor, the antitumor effects depended on the doses of PEG-rIL-2, and a lower dose of PEG-rIL-2 had effects comparable to a higher dose of rIL-2. The similar results have been reported by Mattijssen and Baleman et al.[8,9] The increased antitumor activity *in vivo* of locally administered PEG-rIL-2 as compared to rIL-2 can probable be explained by a prolonged half-life and prolonged exposure time. Attachment of PEG to rIL-2 markedly increased its circulatory half-life. The larger the protein, the slower the clearance by the kidney. Furthermore, when high doses of PEG-rIL-2 were administered locoregionally, no sever toxicity were observed in mice. It suggested that the best results would be obtained when higher doses of PEG-rIL-2 were administered. The tumor model used in this study is a suitable experimental model for locoregional immunotherapy. Because human uterine cervix belongs to luminal organ and locates in surface parts

of the body, local immunotherapy against uterine cervical carcinoma is convinant.

In conclusion, PEG-rIL-2 has a higher antitumor potency than nonmodified rIL-2, and appears to be a valuable substance for intratumoral immunotherapy of human uterine cervical cancer.

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