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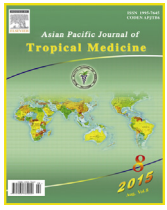

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Antitumor effect of recombinant human endostatin combined with cisplatin on rats with transplanted Lewis lung cancer

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ABSTRACT

Objective: To observe the antitumor effect and mechanism of recombinant human endostatin (Endostar) injection in tumor combined with intraperitoneal injection of cisplatin on subcutaneous transplanted Lewis lung cancer in rats.

Methods: A total of 30 C57 rats were selected, and the monoplast suspension of Lewis lung cancer was injected into the left axilla to prepare the subcutaneous transplanted tumor models in the axilla of right upper limb. The models were randomly divided into Groups A, B, and C. Medication was conducted when the tumor grew to 400 mm^3. Group A was the control group without any interventional treatment. Group B was injected with Endostar 5 mg kg^{-1} d^{-1} for 10 d. Group C was given the injection of Endostar 5 mg kg^{-1} d^{-1} combined with intraperitoneal injection of cisplatin 5 mg kg^{-1} d^{-1} for 10 d. All the rats in three groups were executed the day after the 10 d medication and the tumor was taken off for measurement of volume and mass changes and calculation of antitumor rate, after which the vascular endothelial growth factor (VEGF) concentration in rats' plasma was determined by ELISA. The tumor tissues were cut for the preparation of conventional biopsies. After hematoxylin-eosin staining, the pathologic histology was examined to observe the structures of tumor tissues, VEGF score and microvessel density (MVD) in each group.

Results: The volume and mass of tumor in Groups B and C were significantly lower than Group A ($P < 0.05$) while the tumor volume and mass in Group C were significantly lower than Group B ($P < 0.05$). The antitumor rate in Group C was significantly higher than Group B ($P < 0.05$), but the tumor VEGF score, MVD and plasma VEGF level in Group C were significantly lower than Groups A and B ($P < 0.05$). In Group B, the tumor VEGF score, MVD and plasma VEGF level were significantly lower than Group A ($P < 0.05$). The microscopic image of Group C showed that its number of active tumor cells and the blood capillary around tumor was significantly smaller than that of Groups A and B, and meanwhile atrophy and liquefactive necrosis were seen in local tumor.

Conclusions: Endostar injection combined with intraperitoneal injection of cisplatin is effective in reducing tumor VEGF score and MVD of transplanted tumor tissues in rats with Lewis lung cancer to obstruct the nutrient supply of tumor cells and kill tumor cells, so that the inhibition of tumor cell proliferation and metastasis can be achieved with a remarkable effect.

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1. Introduction

Lung cancer is a common malignancy in respiratory system, ranging the top of incidence of all the malignant cancers [1–3]. According to the pathological characteristics, the lung cancer can be divided into small cell lung cancer and non-small-cell lung cancer; according to statistics, non-small-cell lung cancer in lung cancer patients accounts for about 75%–85% [4]. The conventional therapy for lung cancer is basically surgery, radiotherapy and chemotherapy, with toxic side effects, liability to relapse and metastasize, and low survival rate of 5 years for patients [5]. Therefore, how to improve the efficiency of non-small-cell lung cancer treatment and reduce the toxic side effects have been urgent problems in antitumor research field. It has been widely and clinically recognized that tumor grows depending on angiogenesis and that the tumor invasion and metastasis are closely connected to angiogenesis of tumor tissues; hence, anti-angiogenesis has become the research highlight of oncotherapy [6]. Angiostatin is a new angiogenesis inhibitor and can play a specific inhibitory role in vascular endothelial cells with heparin [5–7]. Researches confirm that vascular endostatin at normal level has the particular inhibitory effect in tumor growth [8,9]. In the present research, the antitumor effect of Endostar injection in tumor combined with intraperitoneal injection of cisplatin on subcutaneous transplanted Lewis lung cancer in rats was observed by taking C57 rats as models of subcutaneous transplanted Lewis lung cancer ready for the medication. The antitumor mechanism and effect were observed, aiming to provide an experimental basis for the clinical treatment.

2. Materials and methods

2.1. Experimental animals

A total of 30 clean C57/6J rats were purchased from Shanghai Lab, Animal Research Center, with age of 5–7 weeks, weight of (21 ± 3) g, humidity for raising of (60 ± 5)%, and temperature at (25 ± 2) °C, and food and water were available *ad libitum*. The whole experimental process was conducted strictly sticking to Regulations for the Administration of Affairs Concerning Experimental Animals.

2.2. Equipments and reagents

Endostar injection was purchased from Shandong Simcere-Medgenn Bio-pharmaceutical Co., Ltd., with batch number 20130404 and standard of 15 mg/piece. Cisplatin injection was provided by Mayne Pharma Pty. Ltd., with batch number U131881AA and standard of 50 mg/piece. The monoclonal antibodies of rabbit-anti-rat endothelial cell CD34 antigen and rabbit-anti-rat VEGF antigen were provided by Beijing Zhongshan Golden Bridge-Biotechnology Co., Ltd. Phosphate buffered saline (buffer, Strept Avidin–Biotin Complex kit, 3,3′-diaminobenzidine color-substrate solution and related reagents were purchased from Shanghai Senxiong Biotechnology Co., Ltd. Olympus BH-2 microscope was from Japan.

2.3. Model preparation and group treatments

Well-grown tumors in tumor-bearing rats were taken for the preparation of monoplast suspension 2×10^6/mL by homogenization. A total of 0.2 mL of the prepared suspension was taken to inoculate in the subcutaneous tissues of axilla beneath rats' right upper limb, after which the rats were divided randomly into Groups A, B, and C, with 10 in each. Medication was performed when the tumor volume reached 400 mm^3. Group A was the control group without any medication. Group B was given Endostar 5 $mg \cdot kg^{-1} \cdot d^{-1}$ injection directly in tumor for continuous 10 d while Group C was given Endostar injection 5 $mg \cdot kg^{-1} \cdot d^{-1}$ combined with intraperitoneal injection of cisplatin 5 $mg \cdot kg^{-1} \cdot d^{-1}$ for continuous 10 d.

2.4. Observation indicators

All the rats were executed the day after the 10 d medication. The volume and mass of tumor were then measured and the antitumor rate was calculated. The blood from rats' eyeballs was taken for the determination of vascular endothelial growth factor (VEGF) level in plasma by double antibody sandwich ELISA assay. After the measurement and weighing of tumor, conventional tissue biopsies were prepared. After hematoxylin-eosin staining was conducted, the necrosis and metastasis of tumor were observed under light microscope. VEGF and microvessel density (MVD) of tumor tissue were determined by immunohistochemical method.

2.5. Result determination

According to method of Rahman *et al.*, the VEGF expression was scored and radio of positive cells were scored based on dyeing range and intensity. Dyeing intensity was ranged 0–3 levels, namely, level 0: negative; level 1: weakly positive; level 2: positive; level 3: strongly positive. Dyeing techniques were ranged from 0 to 4 levels, namely, level 0: negative; level 1: positive cells 1%–25%; level 2: positive cells 26%–50%; level 3: positive cells 51%–75%; level 4: positive 76%–100%. The densest dyeing area of blood vessels of tumor in biopsies at high magnification was for the MVD count and 5 random counts were taken for the average value of microvessel numbers.

2.6. Statistical processing

The data were processed by SPSS 13.0 and measurement data were expressed by mean ± sd. One-way ANOVA was performed by pairwise comparison and Q test was conducted. If $P < 0.05$, statistical significance was considered to exist.

3. Results

3.1. Comparison of tumor volume, mass and antitumor rates in groups

Both tumor volume and mass in Groups B and C were significantly lower than Group A ($P < 0.05$). Tumor volume and mass in Group C were significantly lower than Group B ($P < 0.05$). The antitumor rate in Group C was significantly higher than Group B ($P < 0.05$). Specific results were shown in Table 1.