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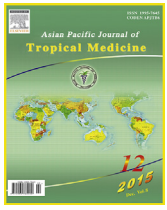

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Anti-tumor effect of matrine combined with cisplatin on rat models of cervical cancer

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ABSTRACT

Objective: To observe the anti-tumor effect of matrine combined with cisplatin on U14 rat models of cervical cancer.

Methods: A total of 80 female Kunming rats were used to establish U14 rat models of cervical cancer and then divided into groups I, II, III, IV, with 20 rats in each. For Group I, the control group, injection of normal saline was given around the tumors. For Group II, injection of 2 mg/kg cisplatin was given around the tumors. For Group III, injection of 75 mg/kg matrine was given around the tumors while the combined injection of matrine and cisplatin was given for Group IV with the same doses as Groups II and III. The animals were sacrificed 10 d after the injection and tumors were taken out for the comparisons of tumor weights after injection and calculation of anti-tumor rates, while thymus and spleen were taken for thymus index and spleen index. Blood in eyeball was collected for determination of changes in serum creatinine and urea nitrogen levels. Sections of tumor issue were prepared and morphological changes in tumor tissue cells were observed by using immunohistochemistry technique.

Results: After injection, the thymus index and spleen index in Groups III and IV were significantly higher than those in Groups I and II ($P < 0.05$) while the two indexes in Group II were significantly lower than Group I ($P < 0.05$). The tumor weights in Groups II and IV were significantly smaller than those in Groups I and III ($P < 0.05$) with significantly higher anti-tumor rates than Groups I and III ($P < 0.05$). The serum creatinine and urea nitrogen levels in Groups III and IV were significantly lower than Group II ($P < 0.05$) and the two indicators in Group III were significantly lower than those in Group IV ($P < 0.05$). The observation under the histological microscope showed densely arranged tumor cells in Group I, growing as a crumby structure and diffuse appearance, with hyperchromatic and large nuclei, and abundant cytoplasm. In the case of Group II, it showed less tumor cells, with extensive degenerative necrosis, sparse arrangement and karyopyknosis as well as karyoclasis. For Group III, necrosis of tumor cells in different sizes and heterogeneous color in nuclei were observed. For Group IV, the number of tumor cells was significantly smaller than Groups I and III and the tumor cells presented an appearance of crumby structure as cancer nests, with more proliferation of connective tissue.

Conclusions: The treatment of matrine combined with cisplatin can significantly improve the anti-tumor effect on U14 rats with cervical cancer, which can be a new option for the treatment for cervical cancer.

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1. Introduction

Cervical cancer is the malignant tumor in female genital organs with high incidence worldwide, seriously affecting women's life and health [1]. According to the statistics [2], there are about 500 000 cervical cancer patients increased every year all around the world and around 250 000 people died of cervical cancer every year, with cervical cancer ranking the top of lethality list of gynecological malignant diseases. The pathogenesis of cervical cancer still remains unclear and may be related to many factors. There have been researches confirming that human papilloma virus is the key factor in pathogenesis of cervical cancer [3–5]. At the moment, surgery, radiotherapy and chemotherapy are the major means in the treatment of cervical cancer at early stage, by which the survival time and life quality of majority of patients can be notably prolonged and improved [6]. However, for the patients with relapse, at late stage or without tolerance to surgery, all the aforementioned treatments show no good effects. Therefore, it is urgent to find a better treatment option. Cisplatin is the cell cycle nonspecific agent and the most common drug extensively applied in chemotherapy, with notable effect and serious adverse effect which creates the least tolerance for patients [7]. Traditional Chinese medicine is indispensible in treatment of cancer, notably regulating the patients' immunologic function and significantly decreasing the toxic reaction in chemotherapy [8]. In the present study, the anti-tumor effect of matrine combined with cisplatin in U14 rat models of cervical cancer is studied.

2. Materials and methods

2.1. Animal origin

A total of 80 female clean grade Kunming rats, aged 6–8 wk and weighed (20 ± 2) g were provided by Experimental Animal Center of Wuhan University School of Medicine and raised at room temperature, with a free access to feed and water. All the animal handlings in the experimental process stuck strictly to Regulations for the Administration of Affairs concerning Experimental Animals.

2.2. Reagents and equipments

The cervical carcinoma cell line U14 was provided by Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences. Matrine (0.15 g/each) was purchased from Jiangsu Kanion Pharmaceutical Co. Ltd., with national medicine permission number of H20041496. Cisplatin (10 mg/each) was purchased from Qilu Pharmaceutical Co. Ltd. with national medicine permission number of H20023460. Inverted microscope was purchased from Olympus company, Japan. Plastic petri dish was purchased from Costar company, USA. Low temperature super-centrifuge, SMART CELL 5% CO_2 constant temperature incubator and constant temperature water bath were purchased from Heraeus company, Germany. The electrothermal constant-temperature dry box (DHG-9245A) was purchased from Shanghai Yiheng Instruments Co., Ltd. The fully automatic biochemical analyser (LX-20) was purchased from BECKMAN-COULTER company, USA.

2.3. Modeling

U14 cell lines at logarithmic phase were injected into rats' abdominal cavity and gone through the passage for three times until the cell concentration reached 5×10^6/mL with addition of sterile normal saline. The subcutaneous injection of 0.2 mL U14 cell lines was conducted into the right axilla to establish U14 rat models of cervical cancer.

2.4. Grouping and treatments

After modeling, the animals were randomly divided into Groups I,II, III and IV, with 20 rats in each. Group I, as the control group, received the injection of 0.2 mL normal saline around the tumors. Group II received the injection of 2 mg/kg cisplatin around the tumors. For Group III, the injection of 75 mg/kg matrine was performed around the tumors. In the case of Group IV, injection of matrine combined with cisplatin was given with the same dose as Groups II and III. All the injection was performed continuously for 10 d.

2.5. Indexes observation

After injection of medicine, the animals were sacrificed and tumors were taken out for weights of tumor, calculation of anti-tumor rate, while thymus and spleen were taken for thymus index and spleen index. The blood in eyeball was collected for determination of changes in serum creatinine and urea nitrogen levels. Sections of tumor tissue were also prepared and the morphologic changes in tumor tissue cells were observed by using immunohistochemistry technique.

2.6. Statistical analysis

Experimental data were expressed as mean ± SD. Comparison between groups was performed by using the least significant difference method. One-way ANOVA method was for measurement data. Differences with $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Comparison of thymus index, spleen index, tumor weight and anti-tumor rate after medication

After the injection of medication, the thymus index and spleen index in Groups III and IV were significantly higher than those in Groups I and II ($P < 0.05$) while the two indexes in Group II were significantly lower than Group I ($P < 0.05$). In Groups II and IV, the tumor weights were significantly lower than those in Groups I and III ($P < 0.05$), and the anti-tumor rates were significantly higher than those in Groups I and III ($P < 0.05$) (Table 1).

3.2. Comparison of serum creatinine and urea nitrogen levels after medication

The serum creatinine and urea nitrogen levels in Groups III and IV were significantly lower than Group II ($P < 0.05$) while those two indicators in Group III were significantly lower than those in Group IV ($P < 0.05$) (Table 2).