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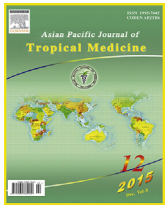

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Anti-tumor effect of matrine combined with cisplatin on rat models of cervical cancer

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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).

Table 1
Comparison of thymus index, spleen index, tumor weight and anti-tumor rate after medication.

Groups	*n*	Thymus index	Spleen index	Tumor weight	Anti-tumor rate
Group I	20	2.02 ± 0.52$^{\#}$	10.40 ± 1.78$^{\#}$	1.77 ± 0.92	–
Group II	20	0.82 ± 0.44	8.14 ± 1.32	0.79 ± 0.45	57.28
Group III	20	4.01 ± 049$^{*\#}$	13.01 ± 2.89$^{*\#}$	1.04 ± 0.64$^{\#}$	38.59$^{\#}$
Group IV	20	3.87 ± 0.25$^{*\#}$	12.87 ± 0.35$^{*\#}$	0.67 ± 0.30$^{\Delta *}$	58.22$^{\Delta *}$

*: $P < 0.05$ compared with Groups I, $^{\#}$: $P < 0.05$ compared with Group II, $^{\Delta}$: $P < 0.05$ compared with Groups III.

Table 2
Comparison of serum creatinine and urea nitrogen levels after medication.

Groups	*n*	Creatinine	Urea nitrogen
Group II	20	72.04 ± 3.95	12.14 ± 2.67
Group III	20	55.11 ± 2.89$^{*\#}$	6.91 ± 2.11$^{*\#}$
Group IV	20	58.99 ± 3.80*	8.59 ± 1.22*

*: $P < 0.05$ compared with Group II, $^{\#}$: $P < 0.05$ compared with Group IV.

3.3. Histological observation

The histological observation showed densely arranged tumor cells, with a crumby structure and appearance of hyperchromatic and large nuclei and abundant cytoplasm in Group I, small number of tumor cells, with extensive degenerative necrosis and sparse arrangement and karyopyknosis as well as karyoclasis in Group II. For Group III, necrosis of tumor cells in different sizes with heterogeneous color in nuclei was observed while for Group IV, less tumor cells than Groups I and III, and an appearance of crumby structure as cancer nests, with more proliferation of connective tissue were observed (Figure 1).

4. Discussion

Cervical cancer is the common malignant disease for women, with high incidence in developing countries, ranking the second in the incidence list of gynecological cancer. According to the statistics, there are 500 000 cervical cancer cases increased every year in the world and 250 000 people died of cervical cancer every year which seriously affecting women's health. The pathogenesis of cervical cancer still remains unclear and it is clinically believed to be related to many factors. There are researches confirming that human papilloma virus plays a significant role in the pathogenesis of cervical cancer.

By far, the clinical treatments for cervical cancer have been surgery combined with radiotherapy and chemotherapy, by which patients can attain satisfactory efficacy. However, the traditional treatments carry unsatisfactory efficacy for patients with relapse, and cervical cancer at late stage. Therefore, it is urgent to find a new treatment option for patients of these kinds. Cisplatin is the cell cycle nonspecific agent and the most common drug that is extensively applied in chemotherapy. Cisplatin inhibits the division of tumor cells by triggering obstacles in DNA replication [3,9–13]. Cisplatin is excreted via kidneys and

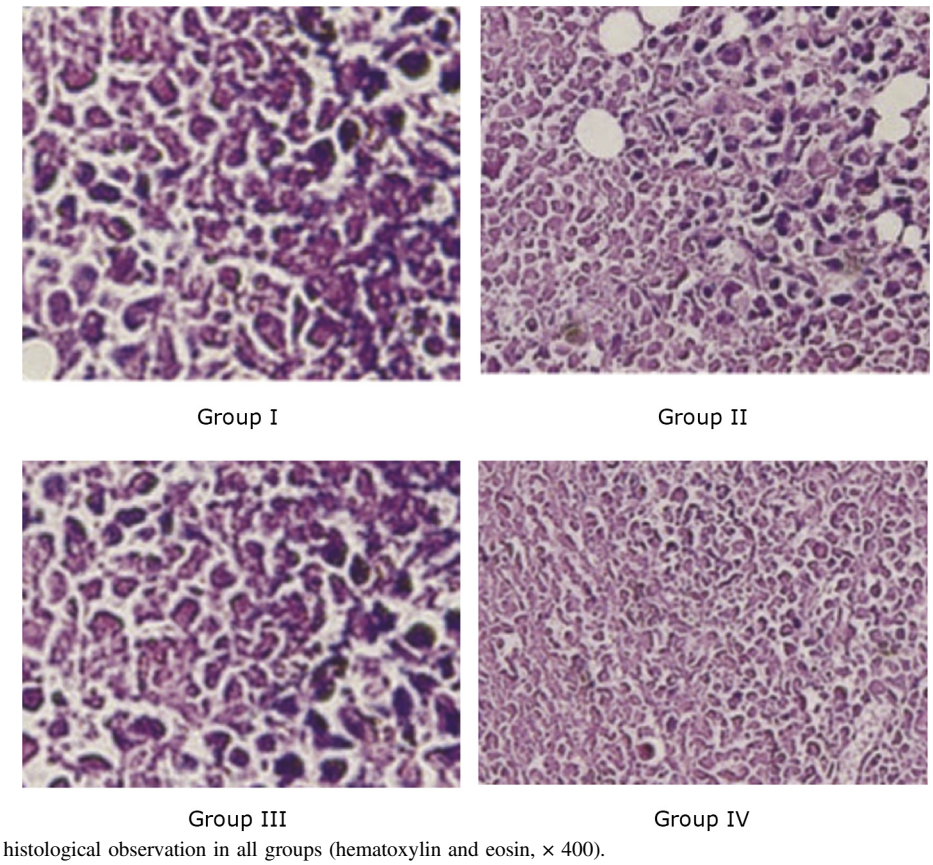

Figure 1. Results of histological observation in all groups (hematoxylin and eosin, × 400).

renal toxicity is the most common toxic reaction in the process of treatment, like large dose or high frequency of usage, which can cause damage in renal proximal tubules and even kidney failure or patients' death in the end [14–16]. Therefore, how to reduce the toxic effect of cisplatin in the application during the process of treatment has been the hot issue for clinical research. Matrine is the extraction of traditional Chinese herb *Sophora flavescens* with functions of clear heat and dry dampness, and belongs to tetracyclic thiazides with molecular weight of 266; besides the traditional medicinal functions, it also has the functions of protecting cardiovascular system, improving patients' immunologic function, protecting liver along with antiviral and anti-tumor roles [4,17,18]. Experiments confirm that matrine carries inhibition effect on many kinds of cancer cells, with wide-spectrum application and low toxicity, and can effectively improve the patients' immunologic function [19]. The main anti-tumor mechanisms of matrine are (1) inhibition of proliferation of tumor cells, and induction of cell differentiation and apoptosis; (2) inhibition of expression and activity of telomerase; (3) induction of changes in DNA methylation and cell signals; (4) decrease in activity of adherence factors and migration of tumor cell infiltration [20]. In the present study, combined treatment of matrine and cisplatin was used for U14 rat models of cervical cancer and 10 d after treatment, it is found that Groups II and IV showed significantly lower tumor weights and significantly higher anti-tumor rates than Groups I and III ($P < 0.05$), suggesting that treatment of matrine combined with cisplatin displays notable inhibition effect on tumor growth in the U14 rat models of cervical cancer. Thymus and spleen are the most important immune organs in human body, which can inhibit the immunologic function of organisms via sorts of ways in the tumorigenesis to make the host present the state of immunosuppression, hence, the indexes of immune organs are important indicators for immunologic function [21–23]. In the present study, the thymus index and spleen index in Groups III and IV are significantly higher than Groups I and II ($P < 0.05$) and the two indexes in Group II are significantly lower than Group I ($P < 0.05$), suggesting that treatment of matrine combined with cisplatin can apparently reduce the immunosuppressive reaction of U14 rats with cervical cancer, accelerate the growth of immune organs and strengthen the immunocompetence of the host. It also shows in the present study that the serum creatinine and urea nitrogen levels in Groups III and IV are significantly lower than Group II ($P < 0.05$) and the two indicators in Group III are significantly lower than Group IV ($P < 0.05$), suggesting that treatment of matrine combined with cisplatin can significantly reduce the toxic damage in the process of treatment and improve the patients' life quality. In addition, histological observation reveals that Group IV presents significantly less tumor cells than Groups I and III, crumby structure as cancer nests, more proliferation of connective tissue, suggesting that the combined treatment of matrine and cisplatin has synergistic effect and can improve the therapeutic effect [19].

The results of the present study indicate that the combined treatment of matrine and cisplatin, with synergistic effect, can notably inhibit the growth of tumor in U14 rats with cervical cancer, significantly improve the immunologic function of rats and decrease the toxic reaction in process of treatment. The combined treatment of matrine and cisplatin can be a new option for treatment of cervical cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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