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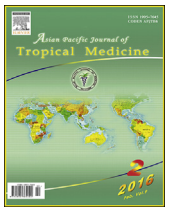

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Therapeutic effect of oridonin on mice with prostate cancer

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ABSTRACT

Objective: To investigate the therapeutic effect and the related mechanism of oridonin on mice with prostate cancer.

Methods: Sixty BALB/C male nude mice were selected. A model of RM-1 cell transplantation tumor of prostate cancer was built by the subcutaneous inoculation of RM-1 cells. After that, those 60 experimental mice were randomly divided into groups A, B and C. Each group had 20 mice. Mice in group A were treated with 0.2 mL of normal saline (0.9%) by intraperitoneal injection once a day; mice in group B received intraperitoneal injection of 1.875 mg/mL of oridonin once a day; and mice in group C received intraperitoneal injection of 7.5 mg/mL of oridonin once a day. Mice in the three groups were treated uninterruptedly for 5 weeks and were all killed. Then, tumors were excised and weighed to calculate their growth inhibitory rate, volume increment and anti-tumor rate. Thymus and spleen of mice in the three groups were collected to calculate the thymus and spleen index. Immunohistochemical staining was applied to observe the expression of caspase-3 in prostate cancer tissue of mice of the three groups.

Results: The qualities and volume increment of tumors in groups B and C were significantly lower than those of group A ($P < 0.05$); the qualities and volume increment of tumors in groups C were evidently lower than those of group B ($P < 0.05$); the tumor volume increment and anti-tumor rate in group C were obviously higher than those of group B ($P < 0.05$); the thymus and spleen indexes of groups B and C were distinctly higher than those of group A ($P < 0.05$); comparison of the thymus and spleen indexes between group B and group C showed no statistical differences ($P > 0.05$). Immumohistochemical staining revealed that the caspase-3 protein in prostate cancer tissue of mice of group A expressed negatively with colorless or light-colored karyon; while the caspase-3 protein in prostate cancer tissue of mice of group B expressed positively with dark-colored karyon, centralized distribution and granular sensation; and the caspase-3 in prostate cancer tissue of mice of group C showed strong positive expression with big and darker colored karyon and dense distribution.

Conclusions: Oridonin can inhibit the growth of RM-1 prostate cancer cells effectively and have great therapeutic effects on RM-1 cell transplantation tumor of prostate cancer.

1. Introduction

Prostate cancer, which is a common malignant disease in male, refers to a kind of epithelial malignant tumors occurring in prostate. Generally, it occurs frequently in males aged over 55 years and its morbidity increases with age [1–3]. According to some statistics, the morbidity of prostate cancer tops the list of male malignant cancers and its mortality ranks only second to lung cancer, which is severely threatening men's health [4]. The pathogenesis of prostate cancer is related to genetic factor, dietary habit and sex activity. Since the pathogenesis of the disease is concealed, the disease has progressed to its middle or advanced stage when it is diagnosed. The tumor cells have already transferred to other organs leading to the missing opportunity of radical operation [5–8]. Therefore, it is significant to find effective drugs to prolong the survival time and promote the life quality for patients. Oridonin is an

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effective active ingredient extracted from natural *Rab-dosia rubescens*. It possesses the abilities to eliminate various cancer cells and fight against cancers and tumors effectively [9]. In this study, in order to investigate the therapeutic effect and the related mechanism of oridonin on mice with prostate cancer, 60 BALB/C male nude mice were selected to build a model of RM-1 cell transplantation tumor of prostate cancer, and treated with oridonin in different concentrations. Now the research results were reported as follows.

2. Materials and methods

2.1. Experimental animals

Sixty 6-month-old BALB/C male nude mice (clean animal) with weights of 18–20 g were chose. They were purchased from Beijing Lihua Experimental Animal Technology Co. Ltd. Those mice took food and drank freely under the room temperature of (23 ± 3) °C. The disposition of the experimental animals was strictly abided by the laboratory animal administration rules.

2.2. Drugs and instruments

RM-1 prostate cancer cell strains were provided by Yanyu Biotechnology Co. Ltd. (Shanghai); oridonin was bought from Xi'an Hao Xuan Biological Technology Co. Ltd.; MTT and Trizol were from Baotaike Company; IMDM nutrient medium was from Gibeo (USA); Olympus invert microscope was from Japan; Bio-rad microplate reader was from USA and Shimadzu ultraviolet spectrophotometer was from Australia. Oridonin was diluted by normal saline into two concentration gradients, 1.875 and 7.5 mg/mL respectively.

2.3. Model establishment

The revived RM-1 prostate cancer cells were inoculated in the IMDM nutrient medium and then it was cultured and generated in a 5% CO_2 humidity incubator under the temperature of 37 °C. The logarithmic growth phase cells were collected and prepared into cell suspension with the concentration of 10^7 cells/mL.

2.4. Animal grouping

In order to establish the model of RM-1 cell transplantation tumor of prostate cancer, 0.2 mL of the prepared cell suspension of RM-1 prostate cancer cells was inoculated subcutaneously in the axilla of those experimental mice. The tumors formed successfully in all the experimental mice. After inoculation, those mice were randomly divided into groups A, B and C. Each group had 20 mice. Mice in group A were treated with 0.2 mL of normal saline (0.9%) by intraperitoneal injection once a day; mice in group B received intraperitoneal injection of 1.875 mg/mL of oridonin once a day; and mice in group C received intraperitoneal injection of 7.5 mg/mL of oridonin once a day. Mice in the three groups were treated uninterruptedly for 5 weeks.

2.5. Observation

During the first, third and fifth weeks after inoculation, vernier caliper was used to measure the tumor volume and draw the tumor growth curves. Then, those mice were all killed after finishing the treatment. And the tumors were excised and weighed to calculate their growth inhibitory rate, volume increment and anti-tumor rate. Thymus and spleen of mice were collected to calculate the thymus and spleen index. Immumohistochemical staining was applied to observe the expression of caspase-3 in prostate cancer tissue of mice of the three groups.

2.6. Statistical arrangement

SPSS 10.0 was used to analyze research data and mean ± SD was applied to express the tumor volume, volume increment and anti-tumor rate, *etc*. One-way ANOVA was used for intergroup comparison. Differences indicated statistical significances when $P < 0.05$.

3. Results

3.1. Comparison of tumor growth inhibition rates in different treatment periods of three groups

Since tumors in mice of group A grew continuously in all treatment periods, the tumor growth inhibition rate of group A was ignored. The tumor growth inhibition rates of group C for 1, 3 and 5 weeks were all higher than those of group B. Comparative differences between groups had statistical significances ($P < 0.05$) (Table 1).

3.2. Comparison of qualities, volumes, volume increments of tumors and anti-tumor rates of three groups

The quality and volume increment of tumors in groups B and C were significantly lower than those of group A, and the comparative differences between groups had statistical significances ($P < 0.05$); the qualities and volume increment of tumors in groups C were evidently lower than those of group B and the tumor volume increment and anti-tumor rate in group C were obviously higher than those of group B, and the comparative differences between groups also showed statistical significances ($P < 0.05$) (Table 2).

3.3. Comparison of thymus and spleen index of mice in three groups

The thymus and spleen indexes of groups B and C were distinctly higher than those of group A, and comparative differences between groups had statistical significances ($P < 0.05$); while comparison of the thymus and spleen indexes between group B and group C showed no statistical differences ($P > 0.05$) (Table 3).

Table 1
Comparison of tumor growth inhibition rates in different treatment periods of three groups.

Group	*n*	One week	Three weeks	Five weeks
A	20	–	–	–
B	20	19.10 ± 9.48	30.09 ± 1.19	36.47 ± 3.30
C	20	52.16 ± 2.05$^{\#}$	63.07 ± 1.71$^{\#}$	69.60 ± 3.40$^{\#}$

Compared with group B, $^{\#}P < 0.05$.

Table 2
Comparison of qualities, volumes, volume increments of tumors and anti-tumor rates of three groups.

Group	*n*	Tumor quantity (g)	Tumor volume (mm^2)	Tumor volume increment	Anti-tumor rate (%)
A	20	2.75 ± 1.61	163.58 ± 58.84	0.10 ± 0.04	–
B	20	1.80 ± 0.95*	86.93 ± 33.24	0.07 ± 0.04*	34.1
C	20	0.90 ± 0.22*#	68.44 ± 39.48	0.04 ± 0.01*#	66.8#

Compared with group A, *$P < 0.05$; Compared with group B, #$P < 0.05$.

Table 3
Comparison of thymus and spleen index of mice in three groups.

Group	*n*	Thymus index	Spleen index
A	20	2.01 ± 0.53	10.22 ± 1.96
B	20	3.93 ± 0.33*	12.92 ± 0.43*
C	20	4.02 ± 0.41*	13.03 ± 0.89*

Compared with group A, *$P < 0.05$.

3.4. Expression of caspase-3 gene protein in prostate cancer tissue of mice of three groups

The caspase-3 protein in prostate cancer tissue of mice of group A expressed negatively with colorless or light-colored karyon; while the caspase-3 protein in prostate cancer tissue of mice of group B expressed positively with dark-colored karyon, centralized distribution and granular sensation; and the caspase-3 in prostate cancer tissue of mice of group C showed strong positive expression with big and darker colored karyon and dense distribution.

4. Discussion

Prostatic cancer is a common malignant disease in male urology department and its mortality rate ranks only second to lung cancer, which can bring great harm to patients' health. The morbidity rate of prostatic cancer is lower in people under 55 years old and it increases with age. The peak stage of prostatic cancer prevalence is from 70 to 80 years old. In recent year, the incidence of prostatic cancer increased year by year due to the aging population trend [10–15]. The main pathological types of prostatic cancer include adenocarcinoma, urothelium carcinoma, ductal adenocarcinoma, adenosquamous carcinoma and squamous-cell carcinoma. Among them, the adenocarcinoma accounts for more than 95% of pathogenesis of prostatic cancer [2,16–21]. Since the pathogenesis of the disease is concealed, the disease has progressed to its middle or advanced stage when it is diagnosed. The tumor cells have already transferred leading to the missing opportunity of radical operation. Hence, it is meaningful to search the effective drug for patients with prostatic cancer.

Oridonin is an effective active ingredient extracted from natural *Rabdosia rubescens* and it can exterminate various cancer cells effectively. Oridonin are mainly clinically applied in anti-cancer, anti-bacterial, anti-tumor, insecticide, heat-clearing and detoxifying, anti-inflammatory and analgesics, invigorating the stomach and activating blood treatment, *etc*. There were researches showing that oridonin has a significant inhibitory effect on prostatic cancer and breast cancer by inhibiting the *in vitro* proliferation and invasion of lung cancer A549 and PC9 cells to restrain the tumor [22,23]. In this study, oridonin with different doses were used to treat RM-1 cell transplantation tumor in mice with prostatic cancer by intraperitoneal injection and found that the tumor growth inhibiting rate at different treatment periods of mice in group C was significantly higher than those of group B ($P < 0.05$), which indicated that the megadose of oridonin has a more positive effect on RM-1 cell transplantation tumor of prostatic cancer treatment. The quality and volume increment of tumors in groups B and C were significantly lower than those of group A ($P < 0.05$) after the termination of treatment in this study, and the quality and volume increment of tumors in group C were significantly lower than those of group B ($P < 0.05$) and the volume increment of tumor and anti-tumor rate of group C were significantly higher than those of group B ($P < 0.05$), which also confirmed that oridonin has a significantly inhibitory effect on the growth and proliferation of RM-1 cell transplantation tumor in prostatic cancer. The thymus and spleen are the most important immune organs in human body and they can inhibit body's immune function by various approaches to provide an immunosuppressive state for hosts when tumors occur. Thus, the indexes of immune organs are used as indicators to measure the of body's immune function [24]. In this study, the indexes of thymus and spleen in groups B and C were significantly higher than those of group A ($P < 0.05$), which indicated that oridonin can improve the immune function in mice with prostatic cancer.

Some studies have shown that apoptosis is a programmed death regulated by gene [24–27], and there are three signal channels participating in the progress of apoptosis. Besides, some other studies have confirmed that *caspase* is the core of apoptosis and *caspase-3*, *caspase-6* and *caspase-7* participate in the effective stage of apoptosis, while *caspase-3* is the major executive in the apoptosis [28,29]. In this research, immumohistochemical staining revealed that the caspase-3 protein in prostate cancer tissue of mice of group C showed strong positive expression with big and darker colored karyon and dense distribution, which showed that oridonin can cause the cascade reaction of caspase substrate and induce apoptosis of transplantation tumor to inhibit the tumor by activating caspase-3.

The results of this study showed that oridonin can effectively inhibit the growth of RM-1 cells of prostatic cancer and has a significantly therapeutic effect on prostatic cancer.

Conflict of interest statement

We declare that we have no conflict of interest.

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