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AN EXPERIMENTAL STUDY ON THE ANTI-EHRLICH ASCITES CARCINOMA EFFECT OF PURIFIED TOAD VENOM EXTRACT

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Abstract

The objective of this paper was to study the anti-Ehrlich ascites carcinoma effect of purified toad venom extract and its mechanism. Mouse model of Ehrlich ascites carcinoma was established with cisplatin as the control to observe the inhibitory effect of purified toad venom extract on malignant peritoneal effusion in mice. The results showed that compared with the control group, ascites volume, number of tumour cells and tumour cell viability decreased and ascites inhibition rate reached over 50% in each treatment group, and with the increase of the dose, incidence of ascites showed a downward trend. The number of tumour cells in ascites and tumour cell viability in the purified toad venom high-dose group were lower than those of the cisplatin group. Compared with the model group, survival time was prolonged in varying degrees in the purified toad venom groups and cisplatin group. The study concluded that purified extract of toad venom has an anti-Ehrlich ascites carcinoma effect.

Keywords: purified toad venom extract; anti-Ehrlich ascites carcinoma; ascites inhibition rate

Introduction

Toad is an animal belonging to the family Bufonidae, order Anura, class Amphibia of the subphylum Vertebrata. Toad venom is the dried secretion of Bufo gargarizans Cantor or Bufo melanostictus Schneider of family Bufonidae, which is an important Chinese medicinal material. It is sweet in nature, pungent in taste, warm and toxic, and has the effects of detoxification, refreshing mind, subsiding swelling, inducing resuscitation, strengthening heart, etc. (Wang et al., 1950; Liu et al., 2002). Clinically, it is widely used in the treatment of periodic facial paralysis, acute pharyngitis, chronic hepatitis B and a variety of diseases such as cancer, and can also be used for anaesthesia and painless pulpotomy of chronic pulpitis (Pastor et al., 2002; Chen et al., 2000). This study investigated whether bufotoxin can inhibit the growth of tumour cells in mice with Ehrlich ascites carcinoma, as well as its toxic and side effects to various internal organs within the effective therapeutic dose range.

Materials and Methods

Test Drugs

Purified extract of toad venom was self-prepared. Other materials included cisplatin injection purchased from Nanjing Pharmaceutical Factory Co., Ltd.

Animals and cells

Kunming mice, half male and half female, weighing 18-22 g, were purchased from the Laboratory Animal Centre of China Medical University. Mouse Ehrlich ascites carcinoma cell lines (201110230) were purchased from Peking Union Medical College.

Model preparation

Mouse Ehrlich ascites carcinoma cells were centrifuged. Supernatant was removed, and cell concentration was adjusted to 2.0×10^7 cells/mL. Under aseptic conditions, each mouse was intraperitoneally administered 0.5 mL of carcinoma cell suspension. Mice bearing tumours

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7-10 d after inoculation with good general state were selected, sacrificed by cervical dislocation, and fixed in the supine position. Ascites were extracted and centrifuged after removal of the supernatant. The remaining was cleaned twice and diluted into a 1.0×10^6 cells/mL carcinoma cell suspension. The above ascites was intraperitoneally administered at 0.2 mL per mouse to establish the animal model of ascites carcinoma.

Grouping and administration

Mice were randomised into 5 groups (n=20), namely the normal saline group; cisplatin group (0.5 mg/kg); purified toad venom low-, medium-, and high-dose groups (0.2, 1.0, and 5.0 mg/kg). After the establishment of ascites carcinoma animal model, 1 ml of different drugs was intraperitoneally injected into mice of respective groups once daily for 10 consecutive days. On the 11th day after inoculation, half of the mice in each group were sacrificed. Ascites were extracted and measured. The total number of tumour cells was counted, and after trypan blue staining, tumour cell viability was calculated by microscopy. Dead mice were dissected, intra-abdominal status was observed, number of peritoneal tumour nodules was counted, and volume of peritoneal tumour nodules was measured. Mice that were not sacrificed were observed for survival time, and life prolongation rate was calculated thus:

Life prolongation rate = (average survival days of the treatment group - average survival days of the model group) / average survival days of the model group.

Statistical methods

The experimental data were statistically analysed using SPSS 11.0 software, and the results were expressed as $\bar{x}\pm s$. The significance of mean differences between treatment groups and model group was compared using t test.

Results

Inhibitory effect of purified toad venom extract on malignant ascites in mice

Compared with the control group, ascites volume, number of tumour cells and tumour cell viability decreased and ascites inhibition rate reached over 50% in each treatment group, and with the increase of the dose, incidence of ascites showed a downward trend. The number of tumour cells in ascites and tumour cell viability in the purified toad venom high-dose group were lower than those of the cisplatin group. The results are shown in Table 1.

Table. 1: Inhibitory effect of purified toad venom extract on malignant ascites formation and tumour cells in mice ($\bar{x}\pm s$)

Group	Dose (mg/kg)	Incidence of ascites (%)	Volume of ascites (ml)	Ascites inhibition rate (%)	Number of tumour cells (10^6/m)	Tumour cell survival rate (%)
Control group		100	12.30		29.38±2.57	91.24±4.59
Cisplatin group	0.5	72	2.92*	76.3	6.75±2.49*	57.49±5.39*
Purified toad venom group	0.2	84	5.81*	52.8	14.68±2.57*	78.65±5.34*
	1.0	73	3.74*	69.6	8.76±2.67*	69.51±2.88*
	5.0	62	1.12*	90.9	3.52±2.31*	48.59±4.12*

Note: Comparison with the control group, * $P<0.05$, the same below.

Effect of purified toad venom extract on survival time of mice

Mice in the model group died generally about 15 d after intraperitoneal inoculation of EAC cells, indicating that the experimental

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conditions were stable, which were in line with the evaluation criteria. Compared with the model group, survival time was prolonged in varying degrees in the purified toad venom groups and cisplatin group, of which the prolongation of survival time was most apparent in the purified toad venom high-dose group, showing a significant difference. Average survival time and life prolongation rate of mice are shown in Tab. 2.

Tab. 2: Effect of purified toad venom extract on survival time of mice ($\bar{x}\pm s$, n=10)

Group	Dose (mg/kg)	Survival time / d	Life prolongation rate (%)
Model group		15.39±2.79	--
Cisplatin group	0.5	18.12±3.51*	17.77
Purified toad	0.2	20.34±4.27*	32.16
venom group	1.0	21.79±4.65*	41.59
	5.0	24.20±3.49*	57.24

Side effects of purified toad venom extract in mice

5 mice showed vomiting, and 2 mice presented mild weight loss in the cisplatin group. In the high-dose purified toad venom group, 3 mice showed vomiting, and 8 mice presented obvious emaciation and weight loss after 5 times of medication. No obvious toxic and side effects were noted in other groups.

Discussion

Toad venom has a complex chemical composition, which mainly includes bufadienolides, cardenolide bufotoxins, indole alkaloids, sterols, as well as adrenaline, proteins and polysaccharides. Toad venom is extremely widely applied in clinical settings, especially in recent years. The anti-tumour effect of toad venom has attracted great attention of scholars from home and abroad (Fan et al., 2006; Hu et al., 2008). In 1969, Sondheimer et al. synthesized bufogenin — bufalin and resibufogenin, which marked the beginning of molecular level research of toad toxins, laying a solid foundation for further revelation of anti-tumour mechanism of bufotoxins, clinical novel drug research and development, as well as batch launch of bufalin and other related products.

The pathogenesis of malignant ascites is very complex, and it mainly includes injury of peritoneal serosa by tumour causing increased capillary permeability, making more protein leakage into the serous cavity; it also includes poor return of blood vessels and lymph vessels due to tumour compression, or the embolization of tumour cells can also lead to ascites, and severe hypoproteinemia can also inhibit the reabsorption process.

Clinical and animal experiments in recent years have found that VEGF can stimulate division and proliferation of tumour vascular endothelial cells and increase vascular permeability, and can play an important role in the formation of ascites. VEGF level is high in more benign ascites, which is of value in the diagnosis of malignant ascites (Mori et al., 2000; Sun et al., 2004a; Sun et al., 2004b; Zhao et al., 2004) We applied purified extract of toad venom to treat the animal model of mouse ascites carcinoma, and got good results, providing an experimental basis for the clinical application of angiogenesis inhibitors in the treatment of malignant ascites.

Our experimental results showed that the purified extract of toad venom could significantly inhibit the growth of Ehrlich ascites carcinoma cells in vivo in tumour-bearing mice and prolong their survival time within the concentration range of 0.5-5.0 mg/kg, which were positively correlated to the dose. The prolongation of survival time of experimental mice was most apparent in the purified toad venom extract high-dose group, which was of significant difference.

Cinobufagin is a water-soluble bufotoxin extracted from toad skin. Researchers often use cinobufagin as a positive control to explore the anti-tumour activity of toad venom in mice in vivo. Our experiment demonstrated that cinobufagin can significantly inhibit the growth of Ehrlich ascites carcinoma cells in vivo in mice and prolong their survival time. But with the increase in the amount of cinobufagin, large dose can cause oedema, degeneration and focal necrosis of myocardial cells, which can limit the clinical application of cinobufagin. This experiment confirmed that the effective dose for anti-Ehrlich ascites carcinoma of further isolated and purified product of toad venom is much lower than cinobufagin, suggesting that the high purity toad venom extract will further broaden the range of clinical medication of toad venom.

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