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Dauricine can inhibit the activity of proliferation of urinary tract tumor cells

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ABSTRACT

Objective: To explore the anti–tumor effects of asiatic moonseed rhizome extraction–dauricine on bladder cancer EJ cell strain, prostate cancer PC–3Mcell strain and primary cell culture system. **Methods:** The main effective component–phenolic alkaloids of *Menispermum dauricum* was extracted and separated from asiatic moonseed rhizome by chemical method. MTT method was used to detect dauricine anti–tumor effect. **Results:** Dauricine had an obvious proliferation inhibition effect on the main tumor cells in urinary system. The minimum drug sensitivity concentration was between 3.81–5.15 μ g/mL, and the inhibition ratio increased with the increase of concentration. **Conclusions:** Dauricine, the main effective component extracted from asiatic moonseed rhizome, had a good inhibition effect on tumor cells in urinary system. At the same time, Dauricine has certain inhibition effects on the primary cultured tumor cell.

1. Introduction

Phenolicalkaloids of *Menispermum dauricum* (PAMD) is mixture of dry root extracts from menispermaceae medicinal plants north bean root (*Menispermum dauricum*)^[1], studies have found that the main component are dauricine (Dau) and daurisolone (Ds). From north bean root, a variety of alkaloids component can be separated^[2], pharmacological experiments, Dau can protect the rats nerve when short artery occlusion occurred^[3], can inhibit the effect of potassium ion outflow^[4], it also participate in calcium ion disturbance^[5], induce breast cancer tumor cell apoptosis. Dau also inhibit nuclear factor signal path, inhibit colon cancer cell proliferation^[6], also can produce influence on injury neurons apoptosis and related protein expression rats after ischemia–reperfusion^[7], PAMD has an resisting effect on ischemic arrhythmias, hypertensive, platelet adhesion and accumulation, thrombosis, and also plays a role of immune regulation, inducing tumor cell apoptosis, reversing multi–drug resistant effect. In recent years, to Dau antineoplastic, related research has also been reported, but rare reports about the urinary system tumor, with effects of Dau.

In the study, the chemical method was applied to extract

and assay composition of monomer from north bean root extract, using MTT staining method to observe the in vitro anti–tumor effect of Dau and Ds against tumor cells in urinary system, in order to further mining Dau in cancer development and application.

2. Materials and methods

2.1. Tumor cell lines

Bladder cancer EJ cell line was provided by The Second Affiliated Hospital of Zhengzhou University. People prostate cancer PC–3M cell line was provided by biological laboratory of Zhengzhou University. The original generation cells were taken from resection specimen after urology surgery in Xiangya Hospital, Central South University, 12 cases of bladder cancer, 10 cases of prostate cancer, both by laboratory confirmed.

2.2. Main reagent

MTT was from the Sigma company; Fetal bovine serum, was from Shanghai Yubo biological technology co., LTD.. ZTC1+1 natural clarifying agent was from Tianjin vibration Tiancheng technology co., LTD.. RPMI1640 media, IMDM medium, trypsin were from the United States GIBCO company; SDS was from Biotec company, USA. Thin layer silica gel plates were from Qingdao Marine chemical co., LTD.

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2.3. Cell culture

Bladder cancer EJ cell line and prostate PC-3M cell line were cultured respectively with RPMI1640 nutrient solution containing 12% fetal bovine serum and IMDM nutrient solution, at 37 °C, 5% CO₂ incubator with saturated humidity, change of nutrient solution every other day, until the cells began to grow in single layer, transfer of culture after digestion with 0.25% pancreatic enzyme solution. For log phase, two kinds of cells were collected and adjusted concentration to 1×10⁵/mL for future uses.

2.4. In vitro tumor inhibition test

Take the log phase of EJ bladder cancer cells and prostate PC-3M cells, for unicellular suspension of 1×10⁵/mL. The tumor cell line suspension was inoculated in 96 hole culture plate, with 0.1 mL in each hole, 10 μL of different concentrations of Dau working liquid was added to each column respectively, with 6 complex holes. At the same time, 10 μL of PBS solution was added to the negative control, with 6 complex holes. And then the liquid was incubated in at 37 °C, in saturated humidity, 5% CO₂ incubator for 24 h, 48 h, 72 h, 10 μL of 4 mg/mL MTT was added 4 h before the end of incubation, moved away the supernant after centrifugation, 100 μL of 15% dimethyl sulfoxide was added to each hole, for staying overnight. Cell proliferation rate was measured repeatedly for 3 times by enzyme league instrument at the 570 nm wavelength (reference wavelength of 630 nm)^[8–10], the inhibition rate (CI)=(OD value of control group–OD value of treatment group)/OD value of control group×100% (Table 1).

Table 1

Drug concentration of *in vitro* inhibitory test (μg/mL).

	1	2	3	4	5	6	7	8	9
A	Control	64	32	16	8	4	2	1	0.5
B	Control	64	32	16	8	4	2	1	0.5
C	Blank	64	32	16	8	4	2	1	0.5
D	–	64	32	16	8	4	2	1	0.5
E	–	64	32	16	8	4	2	1	0.5
F	–	64	32	16	8	4	2	1	0.5

2.5 Drug sensitive test

Standard suppression ratio CI≥50% is sensitive, 30%≤CI<50% is moderate sensitive, CI<30% is regarded as not having sensitive. Negative value indicates that drug stimulates cell growth.

2.6. Data processing and mathematical modeling

All test data are presented by mean±SD. The *t* test was applied to compare difference between groups. SPSS13.0 software was used to analyze single factor variance. IC₅₀

was analyzed by using Microplate Manager TM4.0 data processing software (US BIO–RAD). All test results were repeatedly analyzed for 3 times, by using improved Karber formula to calculation IC₅₀. The processed data was used for mathematical modeling by Matlab 7.0 software, in the inhibition test, drug concentration was set as *X* (independent variables), proliferation inhibition rate was set as *Y* axis (dependent variable), according to the experimental data, regress function was used to get regression curve.

3. Results

3.1. Effect of Dau to bladder cancer EJ cells

Concentration above 8 μg/mL of Dau showed good inhibition effect on EJ cells, with drug concentration rising, inhibition rate was rising, drug concentration and cell absorbance value was negatively correlated, but it had no obvious time dependence relationship, with the extension of time, the inhibition rate presented a rising trend (Figure 1 and Table 2). Along with the drug concentration increases, poison effect is more obvious and have greater effect on killing tumor cells. However, this kind of cell poison effect only positively correlated with drug concentration, rather than with time length (Figure 2).

Table 2

Inhibition rate of EJ cell proliferation.

Drug (μg/mL)	Inhibition rate		
	24h	48h	72h
–	0.0	0.0	0.0
64	83.5*	91.6*	92.4*
32	73.4*	88.2*	89.8*
16	63.6*	75.8*	83.6*
8	57.6*	56.4*	58.1*
4	45.6*	21.8*	37.6*
2	25.4*	12.8	24.6
1	15.6	2.6	14.1
0.5	1.5	1.8	6.5

*: comparing with control group; -: P<0.05.

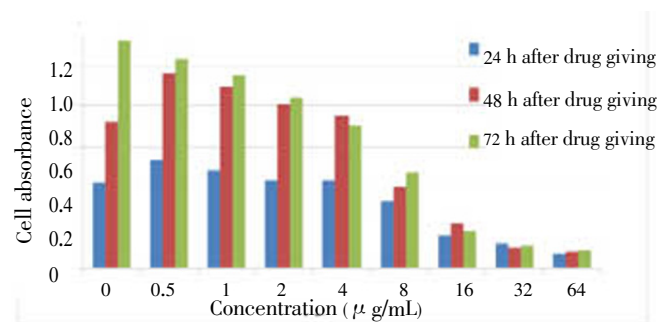


Figure 1. EJ cell absorbance under different drug concentration.

3.2. Effect of Dau to PC-3M cell and primary cells of urinary tract tumor

Comparing with the negative control, concentration above 8 $\mu\text{g/mL}$ of Dau above strong inhibition to PC-3M cell line, and present a good quantity activity relationship, IC_{50} of 24 h, 48 h and 72 h were 31.2 $\mu\text{g/mL}$, 7.2 $\mu\text{g/mL}$ and 6.7 $\mu\text{g/mL}$. In 22 cases of primary culture of carcinoma tissue cell, 1 case did not take part in

drug evaluation due to individual reason, the rest 21 patients with tumor cells presented different degree of sensitive to Dau, half inhibition concentration of 8 cases are between 8 to 16 $\mu\text{g/mL}$, of 4 cases between 16 and 32 $\mu\text{g/mL}$, of 4 cases between 32 to 64 $\mu\text{g/mL}$ and 5 cases with half inhibition concentration more than 64 $\mu\text{g/mL}$, the sensitive degree are lower than the tumor cell line (Figure 3 and Figure 4).

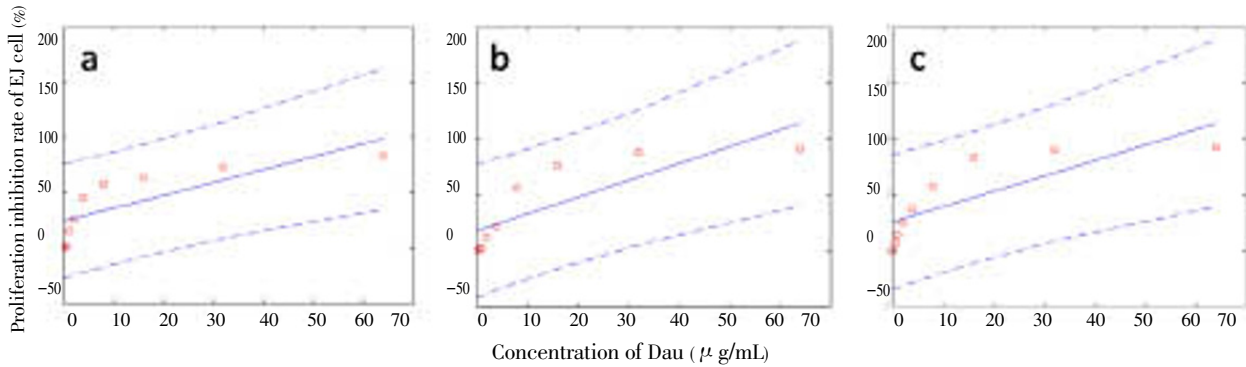


Figure 2. EJ cell proliferation inhibition effect under different concentrations of Dau.
a: inhibition rate of 24 h after drug giving; b: inhibition rate of 48 h after drug giving; c: inhibition rate of 72 h after drug giving.

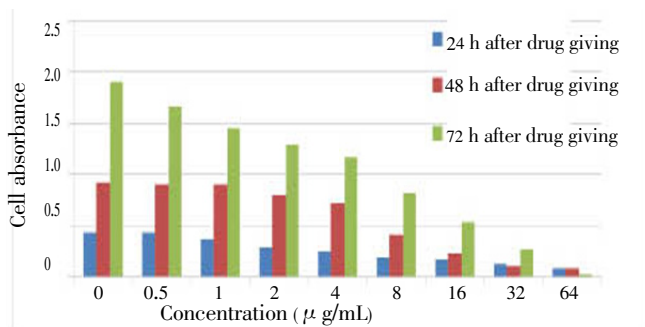


Figure 3. PC-3M cell absorbance under different drug concentration.

al had confirmed that Dau showed strong cell toxicity on B cell lymphoma daudi cell, can restrain the growth of daudi cell proliferation^[16]. Researchers used MTT experiments of different concentrations of Dau for prostate cancer PC-3M cell line and bladder cancer cell lines BT5637, Dau had an inhibition effect with concentration dependency^[17]. the researchers also find that Ds modulate myocardial's active transport process of $\text{Na}^+ \text{K}^+ \text{Ca}^{2+}$ with nonspecific inhibition, Dau can inhibit platelet aggregation and thrombosis^[18], and protect nerve of short focal cerebral ischemia rats by mitochondrial pathway^[19], can promote apoptosis of colon cancer through the NF- κ B signal transduction channel, and have a variety of inhibition of proliferation and apoptosis induction effect against tumor certain cells^[20-22]. Dau have a significant value-added inhibition to different main tumor in urinary system, and the lowest sensitive drug concentration are between 3.81 and 5.15 $\mu\text{g/mL}$, the inhibition rate increased with increasing concentration time,

4. Discussion

Many researches have confirmed that Dau had a stronger inhibitory proliferation effect on tumor cells^[11-15]. Chen *et*

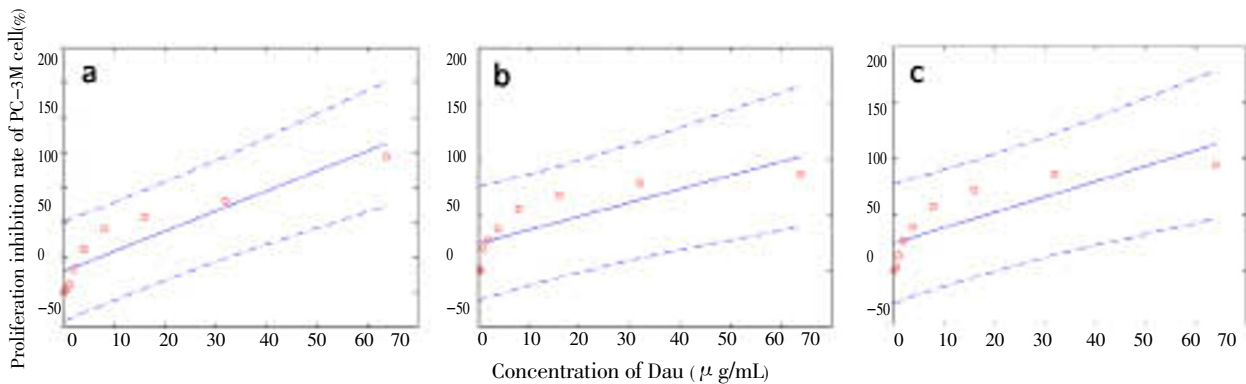


Figure 4. PC-3M cell proliferation inhibition effect under different concentrations of Dau.
a: inhibition rate of 24 h after drug giving; b: inhibition rate of 48 h after drug giving; c: inhibition rate of 72 h after drug giving.

showed that Dau have a broad spectrum of anti-tumor effect, the mechanism is Dau and Ds combine, inducing morphology change of cell apoptosis, volume shrinks, cytoplasmic enriches, followed by karyopyknosis and karyorrhexis. Dau and Ds have significant inhibition effect against proliferation of K562 (human erythroleukemia cell line), BGC823 (human gastric cancer cell line) and TE13 tumor cell. also has inhibition effect on neuroepithelium in primary culture.

This study found that whether cell function is normal has a direct relationship with cancerous tissues[24]. At present, there are not many the researches about Dau application on resistance to urinary tract tumor cell, there are only some reports about the reverse multi-drug resistance. We have preliminarily studied the Dau's cytotoxic effects against bladder tumor cell line and prostate cancer EJ and PC-3M tumor cell line, but the antitumor mechanism of Dau remains to be further studied. In addition, antitumor function of other monomer should still be further studied, such as proliferation inhibition against Hela cell by polysaccharides from Dau, provide new drugs and approaches for clinical treatment of tumor diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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