

STUDY ON THE INHIBITORY EFFECT OF TOTAL ALKALOIDS OF *SOPHORA ALOPECUROIDES* ON
OSTEOSARCOMA CELL GROWTH

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Abstract

Background: Ku Dou Zi is the dried whole plant, roots and seeds of *Sophora alopecuroides* L. in the genus *Sophora* of family Leguminosae. The entire plant is bitter in taste, cold in nature, and has the heat clearing, detoxifying, pathogenic wind dispelling dampness, analgesic, and insecticidal effects. Modern pharmacological studies have proved that TASA has pharmacological activities of anti-cancer. The objective of this paper was to investigate the inhibitory effect of total alkaloids of *Sophora alopecuroides* (TASA), on osteosarcoma cell growth and its mechanism.

Materials and Methods: MTT assay and flow cytometry were used to study the inhibitory effect of TASA on human osteosarcoma cell line OS732.

Results: The results showed that the inhibition rates of different concentrations of TASA (1.5, 3, and 4.5g/kg), against human osteosarcoma cell line OS732, were: 18.4%, 27.4% and 52.8%, respectively.

Conclusion: TASA has an inhibitory effect on osteosarcoma cell growth.

Key words: total alkaloids of *Sophora alopecuroides*; human osteosarcoma cell line OS732; flow cytometry

Introduction

Ku Dou Zi is the dried whole plant, roots and seeds of *Sophora alopecuroides* L. in the genus *Sophora* of family Leguminosae. The entire plant is bitter in taste, cold in nature, and has the heat clearing, detoxifying, pathogenic wind dispelling, dampness removing, analgesic, and insecticidal effects (Yang et al., 1998). Modern pharmacological studies have proved that TASA not only has pharmacological activities of anti-cancer suppression and killing of various microorganisms, but also possesses a wide range of pharmacological effects on immune system, nervous system, and cardiovascular system (Dong et al., 2005; Liang et al., 2008; Qiu et al., 2007; Qiu et al., 2002). This study investigated the inhibitory effect of TASA on osteosarcoma cell growth and its mechanism mainly by MTT assay and flow cytometry.

Materials and methods**Main reagents and instruments**

MTT reagent (Sigma-Aldrich); automatic microplate reader (BIO 128); flow cytometer (FACSCalibur, BD Biosciences, USA); 5% CO₂ incubator, purchased from Heraeus, USA.

Drugs and experimental tumor strains

Total alkaloids of *sophora alopecuroides* (Wuhan Dinghui Chemical Co., Ltd., calculated by dry base, matrine content was calculated by oxymatrine as 92%, batch number: 200013658); human osteosarcoma cell line OS732, purchased from Institute of Orthopedics of Beijing Jishuitan Hospital.

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Animals

Kunming mice, male, weighing between 18-22g, were purchased from the China Medical University, and adaptively fed for 4, days. All experimental procedures were approved by the Animal Research Ethics Committee of Xinxiang Medical College University.

Cell culturing

OS732, cells were cultured in RPMI 1640, medium containing 15%, mycoplasma-free inactivated FBS, and incubated in a CO₂ incubator set at 37°C, 5% CO₂ with 100% humidity. Cells were maintained in the logarithmic growth phase and set aside.

Inhibition of OS732 osteosarcoma cells by TASA (MTT assay)

The logarithmic-growth-phase cells were digested, collected, and adjusted to a single cell suspension with a concentration of 5×10^4 /ml, then seeded in 96-well plates, 24hrs later, TASA which was prepared in RPMI 1640, medium having final concentrations of 1.5g/kg, 3g/kg, and 4.5g/kg were added, respectively. Five replicate wells were set up for each concentration, on the 3rd day, 30μL of MTT solution ($5\text{g}\cdot\text{L}^{-1}$), was added to each well, and the incubation was continued for another 4hrs, then the supernatant was discarded, DMSO was added, and the plates were shaken for 10min., then the absorbance (A), of each well was measured at 570nm.

Inhibition rate = (A value of negative control group - A value of experimental group) / A value of negative control group $\times 100\%$.

Flow cytometry (FCM) analysis

A total of 1×10^6 OS732, osteosarcoma cells treated by 1.5g/kg, 3g/kg, and 4.5g/kg TASA, were collected, washed with PBS, fixed in ethanol, and allowed to stand overnight at 4°C, detection was performed by conventional PI staining, and apoptotic cell proportion was analyzed by FCM.

Statistical analysis

Experimental data were expressed as $\bar{x} \pm s$, and were processed by analysis of variance using SPSS11 statistical software.

Results

Inhibitory effect of TASA on OS732 cell growth

Compared with the control group, each dose groups of TASA significantly ($P < 0.05$), inhibited OS732, cell growth, and there were clear dose-effect relationships between inhibitory effect and drug concentration (see Table 1).

Table 1: Inhibitory effect of TASA on OS732 cell growth ($\bar{x} \pm s$)

Group	Dose (g/kg)	A value	Inhibition rate (%)
Control group		86.5 ± 5.2	
TASA	1.5	$70.6 \pm 2.3^*$	18.4
	3	$62.8 \pm 3.5^*$	27.4
	4.5	$40.8 \pm 2.2^*$	52.8

Note: Comparison with the control group * $P < 0.05$

Effect of TASA on OS732 cell cycle

72hrs, after the action of each TASA dose group on OS732, cells, cell cycle changed apparently. It can be seen from the experimental

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results that with the increase of drug concentration, S phase cells decreased, and proportion of G₀/G₁ phase cells increased, indicating that TASA could arrest the cell cycle in G₀/G₁ phase (see Table 2).

Table 2: Effect of TASA on OS732 cell cycle

Group	Dose (g/kg)	Proportion of each cell cycle (%)			Apoptotic cells (%)
		G ₀ /G ₁	S	G ₂ /M	
Control group		45.52	32.92	21.56	1.5
TASA	1.5	70.35	20.61	9.04	6.9
	3	72.68	19.34	7.98	30.5
	4.5	75.36	18.67	5.97	48.9

Observation of cell morphological changes under optical microscope

Each TASA dose group could induce apoptosis; manifestations were: integrated cell membrane structure; cell shrinkage; nuclear fragmentation; after chromatin condensation of apoptotic cells, density increased, and volume diminished. Meanwhile, organelles maintained intact shape, showing the typical apoptotic features (Figures 1, 2).

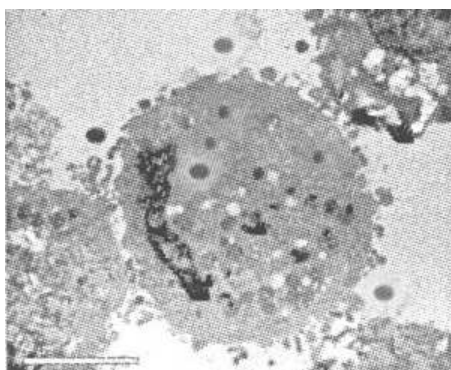


Figure 1: Experimental group (electron microscopy × 3700)

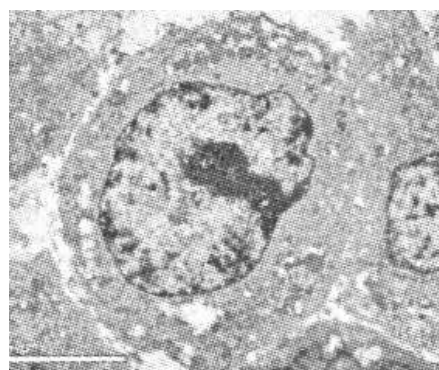


Figure 2: TASA dose group (electron microscopy × 3700)

Side effects of TASA in mice

3, mice showed slight weight loss and 5, mice presented vomiting in the control group. In the high-dose TASA group, 3, mice showed vomiting, and 8, mice presented apparent weight loss after medication. No toxic and side effects were noted in other groups.

Discussion

Osteosarcoma is a malignant osteogenic tumor, and the overall incidence of osteosarcoma accounts for about 0.2%, of all human malignant solid tumors. Osteosarcoma is commonly treated using alkylating agents, their efficiency is about 15%, and the overall curative efficacy is unstable (Niu et al., 2010; Li et al., 2004). At present, surgery-based comprehensive treatments are generally adopted for the treatment of osteosarcoma, including chemotherapy, and radiotherapy. The exploration of anti-osteosarcoma effects of Chinese medicines, and their mechanisms are of great significance for enriching clinical medication, as well as reducing the burden and pain of patients.

This study found by MTT assay that TASA had an apparent inhibitory effect on proliferation rate of human osteosarcoma OS732, cells within a range of 1.5-4.5g/kg, and with the increase of concentration and the extension of action time, tumor cell growth inhibition rate also increased, showing a clear-cut dose-effect relationship, that suggests that, the increase of dosage could enhance the in-vitro inhibitory effect on osteosarcoma cells.

Flow cytometric analysis of cell cycle showed that (Si et al., 2001a): 3, days after action of 1.5, 3, and 4.5g/kg TASA on human osteosarcoma OS732, cells, the number of G₀/G₁ phase cells increased significantly, while the proportion of S, and G₂/M phase cells decreased, suggesting that TASA may arrest cell cycle in G₀/G₁ phase by preventing entry into S phase, thereby achieving the inhibition of OS732, cell proliferation (Si et al., 2001b).

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Therefore, with regards to TASA inhibitory mechanism of action on osteosarcoma cell growth, the authors believe that cell cycle regulatory protein or, abnormal oncogene expression mediated apoptosis is its important molecular mechanism.

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