Inhibitory effect of emodin on human hepatoma cell line SMMC-7721 and its mechanism

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Abstract

Background: Da Huang (Radix et Rhizoma Rhei) is the dried root or rhizome of Rheum palmatum L., Rheum tanguticum Maxim ex Balf. or Rheum officinale Braill of family Polygonaceae. It has heat clearing, damp drying, fire purging and toxin removing effects. Because of its definite curative efficacy, it has been widely applied in clinical settings.

Objective: To study the inhibitory effect of emodin on human hepatoma cell line SMMC-7721 and its mechanism.

Methods: MTT assay, flow cytometry and electron microscopy were used to investigate the inhibitory effect of different concentrations of emodin on human hepatoma cell line SMMC-7721.

Results: 12 h, 24 h and 48 h after the action of 20, 40 and 80 umol/L emodin on SMMC-7721 cells, the proliferation of human hepatoma SMMC-7721 cells was inhibited; the inhibitory effects showed time-and concentration-dependence. 48 h after the action of different concentrations of emodin on SMMC-7721 cells, cells in G2/M phase increased significantly, while the proportion of S phase cells gradually declined.

Conclusion: Emodin can inhibit human hepatoma cell line SMMC-7721.

Keywords: emodin; SMMC-7721; MTT assay

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Introduction

Da Huang (Radix et Rhizoma Rhei) is the dried roots or rhizomes of Rheum palmatum L., Rheum tanguticum Maxim ex Balf. or Rheum officinale Braill of family Polygonaceae[1]. It has heat clearing, damp drying, fire purging and toxin removing effects. As a representative drug of traditional Chinese medicine, Radix et Rhizoma Rhei has a very old history of clinical application, and a very important position. As early as in the "Shen Nong's Herbal Classic", there was the record of Radix et Rhizoma Rhei as "treats blood stasis, blood block, chills and fever ..... can boost metabolism, remove accumulation in stomach and intestines, and pacify the five internal organs". Because of its definite curative efficacy, it has been widely applied in the clinical settings, which has long been reputed as the "great general".

Scholars at home and abroad have done extensive studies on Radix et Rhizoma Rhei, and have confirmed that Radix et Rhizoma Rhei has pharmacological effects such as anti-bacteria, anti-inflammation, invigorating blood circulation, inhibiting enzyme, lowering lipid, protecting liver, nourishing gallbladder, improving circulation and kidney function, etc.[2-6] In this experiment, starting from the inhibitory effect of emodin on growth of hepatoma SMMC-7721 cells, the apoptosis of cells after drug action was studied by different methods, for the purpose of better understanding the antitumor effect of emodin, and providing a new theoretical reference for the pharmacological treatment of liver cancer.

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Materials

Cell lines and reagents

Human hepatoma cell line SMMC-7721(Bath Number: 201302035) was purchased from Shanghai Institute of Cell Biology of Chinese Academy of Sciences; DMEM medium was purchased from Gibco; fetal calf serum (FCS) was purchased from Hangzhou Sijiqing Bioengineering Material Co., Ltd.; MTT, DMSO, DAPI were products of Sigma.

Drugs and instruments

Emodin, self-prepared, purity of 95% (extracted from the dried roots or rhizomes of Rheum palmatum L.); microplate reader (Medical Equipment Co. Ltd., Huadong Electronics Group); low temperature centrifuge.

Inverted microscope (Olympus), CO2 incubator (Heraeus, Germany).

Methods

Cell cultivation

Human hepatoma cell line SMMC-7721 was cultured routinely in 10% FCS-containing RPMI 1640 medium, and incubated in a cell incubator set at 37^{0}C, 5% CO2 with saturated humidity.

Determination of cell proliferation activity by MTT assay

SMMC-7721 cells in the logarithmic growth phase were collected, and added to 96-well culture plates at 100LL per well after adjusting the cell density to 5×104 cells/ml. After the cells were adherent, different concentrations (20, 40 and 80 umol/L) of emodin were added, control group was added with serum-free RPMI 1640 medium. The plates were placed in the incubator set at 37^{0}C, 5% CO2/95% ambient air and cultured for 12, 24 and 48 h; 4 h before the end of the experiment, 180 μl of serum-free RPMI 1640 medium and 20 μl of 5 mg/ml MTT were added, then the cultivation was continued at 37^{0}C. After the cultivation, supernatant was discarded, 150 ul of DMSO was added to each well, and the plates were shaken for 10 min to fully dissolve the crystals. The absorbance was measured at 490 nm.

Cell proliferation inhibition rate = (A value of the control group - A value of the experimental group) / A value of the control group × 100%.

Flow cytometric analysis

SMMC-7721 cells after 48 h action of 20, 40, and 80 umol/L emodin were collected, digested with 0.25% trypsin into single cell suspension, centrifuged at 1000 r/min to remove supernatant, washed in PBS, resuspended, then fixed by addition of 70% precooled (-20^{0}C) ethanol, and allowed to stand overnight at 4^{0}C. Ethanol was discarded, and the remaining was washed twice with phosphate buffer, stained under dark conditions for 30 min by addition of ethidium iodide dye, then the cell cycle changes and apoptosis rate were detected by flow cytometry.

Observation of cell morphology

Cells in good condition (concentration of 1×105 cells/L) were selected, coverslips were placed in petri dishes, then cell suspension was added to make cells slides, 24 h after seeding, when cells were adherent to the wall, supernatant was discarded and emodin (80 umol/L)-containing medium was added, 48 h later, slides were taken out, fixed, Wright stained, and then observed under microscope.

Statistical analysis

The experimental data obtained were expressed as x±s, and processed using SPSS 11.0 software package; comparison among groups was performed by analysis of variance.

Results

Effect of emodin on SMMC-7721 cell proliferation

The experimental results showed that 12 h, 24 h and 48 h after the action of 20, 40 and 80 umol/L emodin on SMMC-7721 cells, the proliferation of human hepatoma SMMC-7721 cells was inhibited; the inhibitory effects showed time-and concentration-dependence. See Fig. 1.

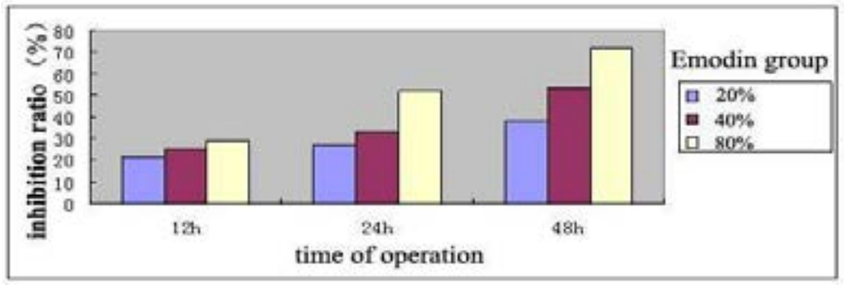


Fig. 1 Effect of different concentrations of emodin on proliferation of SMMC-7721 cells

Flow cytometry results

Cell cycle analysis showed that 48 h after the action of different concentrations of emodin on SMMC-7721 cells, G2/M phase cells increased evidently, its proportions were 18.82%, 50.09% and 53.32%, respectively, while the proportion of S phase cells gradually declined, which was 34.65%, 19.42% and 12.47%, respectively. The results indicated that emodin could induce apoptosis in a dose-dependent manner (see Tab. 1).

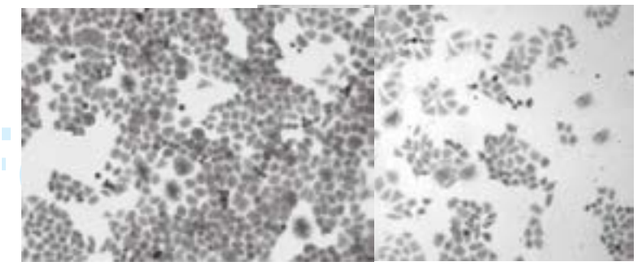
Tab. 1 Results of flow cytometry analysis

Group	Concentration (μ mol/L)	G0/G1	S	G2/M
Control group		56.32±4.16	35.78±4.22	7.90±1.32
Emodin group	20	46.53±3.97	34.65±3.62	18.82±2.74*
	40	30.49±2.41*	19.42±3.52*	50.09±5.41*
	80	34.21±3.49*	12.47±1.74*	53.32±6.32*

Note: Comparison with control group, * P<0.05.

Observation of cell morphology

Morphological observation showed that cells in the control group were well adherent to the wall, grown extensively, and nuclei were large, as shown in Fig. 2 (A). 24 h after treatment and action of emodin, cells were partially detached, volume was gradually reduced, granules in cytoplasm increased, nuclei were pyknotic and became smaller, number of nucleolus decreased, and chromatins were concentrated into pieces and distributed around the nuclear membrane, cytoplasm showed vacuolization and other typical morphological changes of apoptosis, as shown in Fig. 2 (B).



A B

Fig. 2 Effect of emodin on morphology of SMMC-7721 cells (A: control group; B: emodin group)

Discussion

Studies of domestic and foreign scholars have demonstrated that emodin can inhibit the growth of a variety of tumor cells, it not only has an inhibitory effect on the growth of lung squamous carcinoma cell line CH27 and human HER-2/neu overexpressing breast cancer cells, but also has different degrees of inhibitory effect on HER-2 high-expressing T24 bladder cancer cell line, human non-small cell lung cancer cell line H460, etc.[7-8] Although emodin has a high degree of inhibitory activity against various types of tumor cells, the mechanisms of action differ from each other. Some scholars believe that emodin has an apoptosis inducing effect on a variety of tumor cells; its signal transduction pathways for induction of apoptosis mainly include Bax.

Caspase pathway, Fas pathway, PKC pathway and ROS pathway; there are also some scholars who believe that the antitumor mechanism of emodin is the reversal of multidrug resistance of tumor cells, and affection of the cell cycle of tumor cells, thus exerting the tumor treatment effect[9-10].

This experiment investigated the inhibitory effect of emodin on SMMC-7721 cells by MTT assay, the results showed that 12 h, 24 h and 48 h after the action of 20,

40 and 80 umol/L emodin on SMMC-7721 cells, the proliferation of human hepatoma SMMC-7721 cells was inhibited; the inhibitory effects showed time-and concentration-dependence.

Electron microscopy observation showed that 24 h after the action of 80 umol/L emodin on SMMC-7721 cells, nuclei were pyknotic and became smaller, number of nucleolus decreased, and chromatins were concentrated into pieces and distributed around the nuclear membrane, which were all typical characteristic of apoptotic cell transformation into normal cells.

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