**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 Brail 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 Brail of family Polygonaceae. 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. 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.

**Materials**

**Cell lines and reagents**

Human hepatoma cell line SMMC-7721 (Catalog 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., Hua-dong Electronics Group); low temperature centrifuge.

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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°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/well after adjusting the cell density to 5×104 cells/mL. After the cells were adherent, different concentrations (20, 40 and 80 μmol/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°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 was added, 48 h later, slides were taken out, fixed, Wright stained, and observed under microscope.

Statistical analysis
The experiment was performed in triplicate. The data were analyzed using SPSS 11.0 software package; comparison among groups was performed by analysis of variance. 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.

Flow cytometric analysis

SmMMC-7721 cells after 48 h action of 20, 40, and 80 μmol/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°C) ethanol, and allowed to stand overnight at 4°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.

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 μmol/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.

Discussion

Studies of domestic and foreign scholars have demonstrated that emodin can inhibit the growth of 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. 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.

References