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Differential apoptotic induction of gambogic acid, a novel anticancer natural product, on hepatoma cells and normal hepatocytes

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Abstract

Gambogic acid (GA) is the major active ingredient of gamboge, a brownish resin exuded from *Garcinia hanburryi* tree in Southeast Asia. In this study, we compared the different apoptotic induction of GA on human normal embryon hepatic L02 cells and human hepatoma SMMC-7721 cells by detecting growth inhibition, observing morphological changes, and the expressions of the relative apoptotic proteins (Bax, Bcl-2 and caspase-3). The results indicated that GA could selectively induce apoptosis of SMMC-7721 cells, while had relatively less effect on L02 cells. To illustrate the distinct selective antitumor mechanism of GA, we further study its distribution in cultured cells and in tumor-bearing mice. The results indicated that SMMC-7721 cells have higher GA binding activity than L02 cells. The retention time of GA in grafted tumor was longer than in liver, renal and other organs. Collectively, the selective anticancer activity of GA could be due to its significant apoptotic inducing effects as well as its higher distribution and longer retention time in tumor cells compared to the normal cells. So GA might be a kind of highly effective anticancer drug candidate with low toxicity to normal tissue.

Keywords: Gambogic acid; Apoptosis; Bcl-2; Bax; Caspase-3

1. Introduction

Hepatocellular carcinoma (HCC) is an aggressive malignant tumor with high prevalence, especially in China. Currently although many chemotherapy drugs are used in clinical, the overall survival of patients with HCC is still unsatisfactory [1]. They killed the tumor cells as well as the normal cells, which induce significant adverse effects and cause patients hard to tolerance, resulting in a failure of chemotherapy. So many efforts have been focused on developing novel tumor targeted drugs.

Gambogic acid (GA, $C_{38}H_{44}O_8$, Fig. 1) is the major active ingredient of gamboge, a brownish resin exuded from *Garcinia hanburryi* tree in South-

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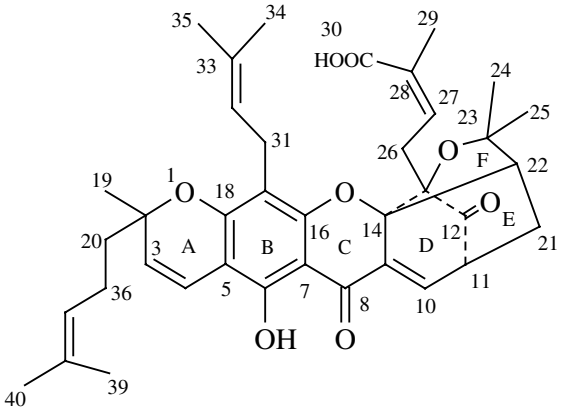

Fig. 1. Chemical structure of GA.

east Asia [2,3]. Previous studies have revealed its significant anticancer activity *in vitro* and *in vivo* [4–8]. In preclinical research, we found that GA at therapeutical dose did not inhibit the proliferation of bone marrow, peripheral leucocyte count and phagocytotic function of macrophage in tumor-bearing mice. It also had no dramatic effect on the count of peripheral leucocyte, marrow karyote and the coefficient of thymus gland and spleen in rat. Moreover, GA treated animal did not show any behavior abnormalities and gross changes compared with animals in the positive group [9]. To further explore the mechanism for this property of GA, in this study we examined the effects of GA on primary rat hepatocytes and compared the different growth inhibition and apoptotic induction of GA on human normal embryon hepatic L02 cells and human hepatoma SMMC-7721 cells by detecting morphological changes, Annexin-V/PI double-staining assay and the expressions of the apoptotic relative proteins (Bax, Bcl-2 and caspase-3). Then we further compared the binding activities of these two kind cells with GA and investigated its distribution as well as retention time in tumor-bearing mice.

2. Materials and methods

2.1. Reagents

^{125}I-labeled GA was offered by Wuxi Nuclear Medical Institute (Jiangsu, China). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was purchased from Fluka, U.S.A. and was dissolved in 0.01 M PBS. Primary antibodies were obtained from Santa Cruz Biotechnology Inc. (U.S.A.), and IRDye™ 800 conjugated anti-goat and anti-rabbit second antibodies were obtained from Rockland Inc. (U.S.A.). Annexin V-FITC Apoptosis Detection kit was purchased from BioVision (U.S.A.).

2.2. Cells culture

Primary rat hepatocytes were isolated from male Sprague–Dawley rats and cultured according to the protocols described previously [10]. Before assay, the cells were resuspended at 2×10^6 cells/mL in Krebs Improved Ringer II buffer with 2% BSA and incubated for 30 min at 37 °C with constant flow of O_2/CO_2 (95/5).

Human hepatoma cell line SMMC-7721 was purchased from Cell Bank of Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences (Shanghai, China). Human normal embryon hepatic cell line L02 was purchased from KeyGen Biology Technology Company (Nanjing, China). Cells were cultured in RPMI-1640 medium (lot No. 20050205, GIBCO, U.S.A) at 37 °C in a Water Jacketed CO_2 incubator (Thermo Forma, U.S.A.) in a humidified atmosphere with 5% CO_2. The culture medium was supplemented with 10% heat-inactivated calf serum (Sijiqing, Hangzhou, China), 100 U/ml penicillin G, and 100 U/ml streptomycin.

2.3. Cell morphological assessment

SMMC-7721 and human normal liver cell line L02 were cultured in RPMI-1640 till mid-log phase. 1.5, 3.0 and 6.0 μM of GA were then added to the culture media and the cells then were incubated for 2 h. At the end of incubation, the morphology of cells was monitored under an inverted light microscope. All floating and attached cells were harvested with 0.02% (w/v) EDTA and 0.25% (w/v) trypsinase. The cell suspension was fixed with ice-cold 4% paraform for 20 min and washed with ice-cold PBS. Then cell suspension was permeabilized with 0.3% Triton X-100 and washed with ice-cold PBS, stained with fluorochrome dye DAPI (Santa Cruz, U.S.A) and observed under a fluorescence microscope (OlympusIX51, Japan) with a peak excitation wave length of 340 nm.

2.4. Colorimetric MTT-assay

The viability of cells was measured by MTT-assay according to our previous work [11]. The IC_{50} value was taken as the concentration that caused 50% inhibition of cell growth and was calculated by Bless statistical method.

2.5. Annexin-V/PI double-staining assay

SMMC-7721 and L02 cells were treated with GA as above for 2 h. Then they were harvested and washed and resuspended with PBS. Apoptotic cells were identified by double supravital staining with recombinant FITC (fluorescein isothiocyanate)-conjugated Annexin-V and