Effects of Extract from Rhizoma Alismatis on Three Kinds of Tumor Cell Lines

Zhengming Huang, Xinbo Yang, Wenbin Cao, Hongyan Chen

Department of Pharmacology, Beijing Medical College of PLA, Beijng 100071 P.R.China

Abstract **Objective** to observe the effects of liquor-ethanol extract from Rhizoma Alismatis (RAE) on three kinds of tumor cell lines (human Leukemia HL-60 , human carcinoma Bel-7402 and human cervical carcinoma (Hela). **Methods** Inhibitions of proliferation of tumor cells with MTT and SRB methods. **Results** The growth of human Leukemia HL-60 cells was significantly inhibited by RAE. The maximum inhibition was 51%. But it only showed a slight inhibition for other two tumor cell lines to be treated with RAE. **Conclusion** RAE has the significant effect of inhibiting the growth of human HL-60 cells.

Key Words Rhizoma Alismatis; antitumor in vitro; human Leukemia HL-60 cells

Rhizoma alismatis (RA) is one of Traditional Chinese Medicines. It has been reported to have many pharmacological actions in modern researches, such as hypo-lipid[1], anti-platelet[2], anti-diabetes[3], and so on. In recent years, some researches showed that RA could regulate immuno-reaction[4]. But up to now, there has been no report about action of RA on tumor cells. The purpose of this investigation was to observe the effects of extract from rhizoma alismatis(RAE) on human tumour cells in vitro.

MATERIALS AND METHODS

Preparation of RAE

RA was purchased from a Herbal Medicine shop in Beijing. The extracts of RA was prepared by present department. RA was decocted 1 h with water for twice. The crude preparation was filtered through gauze and then clarified by centrifugation at 2000 rpm for 10 min. The upper part was retained and blended with 95% ethanol for 1 day. The soak was depressurized and distilled, then yielding a clear solution. At last the solution was dried into powder.

Reagents and instrument

CO_2 incubator (American NAPL, Model 5410), enzyme linked immunosorbent instrument (Bio-Rad, Model 1550), RPMI 1640 (Gibco), calf serum (FCS, Shanghai Huamei Company) and trypsin.

Cell culture and cytotoxicity in vitro

Correspondence: Zhengming Huang , Professor.
Tel: 010-66947721;
E-mail: huang_zhengming@sohu.com

Human leukemia HL –60 cell lines Human leukemia HL-60 cell lines kept in the cell collection of Shanghai Institute of Materia Medicine, Chinese Academy of Sciences was used. Cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated calf serium, streptomycin 100mg.L-1, benzylpenicillin 100 kU.L-1 in a 5% CO_2 humidified incubator at 37℃。 The cytotoxicity of RAE on HL-60 cells was tested by MTT assay. Exponentially growing HL-60 cells were seeded in 96-well. RAE (10μ L per well) was added immediately to achieve desired concentration of 1mg.L-1. The plates were incubated for 24 h and tested in triplicate wells. At the end of each exposure, MTT (5g.L-1) 20 μ L was put into each well and the plates was incubated at 37℃ for 4 h. After that 150μ L dimethyl sulfoxide was added and the cells were incubated at 37℃ for an additional 1 h. The absorbance A570 was read with a plate reader. The cell viability (percentage of growth) was calculated for each well: % viability = A570 treated cells/A570 control cells × 100%.

Human hepatocarcinoman Bel-7402 and human cervical carcinoma (Hela) The growth inhibition of these two tumor cell lines were determined by SRB methods[5]。

RESULTS

Growth inhibition on HL–60 cells

HL-60 cells exposed to different concentration of RAE revealed evident anti-proliferative action after 48 h in a concentration-dependent manner(Tab 1), especially in the concentration of 1000μ g.ml-1 .

Tab. 1 Growth inhibition on HL-60 cells by RAE

concentration (μ g.ml^{-1})	inhibition (%)
1	3.63
10	19.54
100	31.67
1000	51.68

Growth inhibition on other two tumor cell lines

RAE at the different concentration were demonstrated a slight cytotoxic activity against other two tumor cell lines, human hepatocarcinoman Bel-7402 (Tab 2) and human cervical carcinoma(Hela) (Tab 3).

Tab. 2 Growth inhibition on human hepatocarcinoman Bel-7402 cells by RAE

concentration (μ g.ml^{-1})	inhibition (%)
1	7.71
10	10.12
100	15.43
1000	25.67

Tab. 3 Growth inhibition on human cervical carcinoma (Hela) cells by RAE

concentration (μ g.ml^{-1})	inhibition (%)
1	4.3
10	6.75
100	12.97
1000	27.85

DISSCUCION

In this study, we first observed the anti-tumor effect of the extract of RA (RAE). The results showed that the growth of HL-60 cells was significantly inhibited by RAE in vitro. Surprising was the finding that RAE only had the slight inhibition on other two kinds tumour cells. This phenomenon revealed that the cytotoxic effect of RAE was specific in a degree.

To the knowledge, the development of tumor was associated with low immunologic function. The chemical compound which possesses immuno-regulative action may be beneficial to cure cancer, making the tumor reduce or increasing the survival rate. It was pointed that some researches have depicted immuno-regulation of RA alone, complex prescription, or active component of RA in recent years. Liuwei Dihuang Tang, a famous Chinese complex prescription, enhanced the immuno-function, as well as increased the weight of thymus gland and spleen in mice. There was a research reported that Alisman SI, a component from RA, was able to boost the activities of reticuloendothelial system and complement [6]. But another report show that methanol extract from RA manifested a inhibition on producing NO from macrophage-stimulated by LPS[7].

Any how, the present study only observed the actions of anti-tumour in vitro . It need further research in vivo in order to make sure whether it has the utility value for the treatment to cancer.

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