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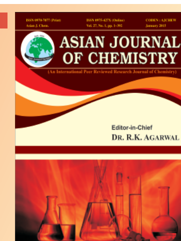

Preparation of Paclitaxel Liposome and its Anticancer Activity

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The aim of the study was to study the preparation process of paclitaxel liposome and to determine its anticancer effect. Orthogonal experiment was used to study the preparation process of paclitaxel liposome and MTT assay was used to determine its anticancer effect. Factors influencing the encapsulation efficiency of paclitaxel liposome in order of importance were: B > A > C, *i.e.* the ratio of hydrogenated phospholipid to paclitaxel > the weight ratio of phospholipid to cholesterol > rotary evaporation temperature. It can be seen from the results of range analysis that under the experimental conditions, the optimal preparation process of paclitaxel liposome was A3B1C3, *i.e.* the phospholipid to cholesterol weight ratio of 20:4.28, hydrogenated phospholipid to paclitaxel weight ratio of 20:1 and rotary evaporation temperature of 60 °C. Different concentrations of paclitaxel liposomes (0.05, 0.1, 0.5 and 1 μmol/L) all had significant inhibitory effects on SGC-7901 cells and within a certain dose range, the inhibitory effects gradually increased with the increase of drug concentration in culture medium and the extension of incubation time, of which 1 μmol/L paclitaxel liposome had the strongest inhibitory effect on SGC-7901 cells at 72 h, reaching 75.9 %. Paclitaxel liposome can effectively inhibit the proliferation of SGC-7901 cells.

Keywords: Paclitaxel, MTT assay, SGC-7901 cell.

INTRODUCTION

Taxus chinensis (Pilger) Rehd. is a plant in the family Taxaceae, there are about 11 species of the plant known worldwide, of which 4 species and 1 variant are identified in China[1,2]. The main active constituent of Taxus chinensis (Pilger) Rehd. is paclitaxel, which is a tetracyclic triterpenoid. Modern pharmacological experiments have shown that paclitaxel can block tumor cell growth through induction and promotion of tubulin polymerization and has a significant curative effect on gastric cancer, liver cancer, ovarian cancer, breast cancer, non-small cell lung cancer and prostate cancer[3-6]. Because the paclitaxel content in plants is very low, extraction from plants alone is far from meeting the clinical needs, domestic and foreign scholars have increased the yield of paclitaxel and improved its bioavailability by chemical synthesis, liposomes and other bioavailability improving methods[7-9], in the hope of meeting clinical needs.

This experiment aims to improve the bioavailability of paclitaxel and to further enhance its clinical application through studying the liposomal encapsulation technology of paclitaxel and determination of the growth inhibitory effect of paclitaxel liposome on human gastric cancer SGC-7901 cells.

EXPERIMENTAL

Rotary evaporator, RE-52CS (Shanghai Zhenjie Experimental Equipment Co., Ltd.); UV-visible spectrophotometer, UV-2550 (SHIMADZU CORPORATION); CO_2 incubator (model CO-150, NBS, USA); clean bench, SW-CJ-SF (Suzhou Purification Equipment Factory, Sujing Group).

Drugs: RPMI 1640 medium, Gibco; newborn calf serum, Hangzhou Sijiqing Biological Co., Ltd.; MTT, Sigma; human gastric cancer SGC-7901 cells, purchased from China Medical University.

Preparation of paclitaxel liposome by thin-film hydration and ultrasonic dispersion: The required paclitaxel sample, hydrogenated phospholipid, cholesterol and lipid globule were accurately weighed according to the proportion of prescription and dissolved in appropriate amount of chloroform. The solution was then rotary evaporated in a water bath, after uniform thin film was formed on the flask wall, the thin film was hydrated at 65 °C for 1 h and then ultrasonically dispersed for 0.5 h to give paclitaxel liposome.

Determination of encapsulation efficiency of paclitaxel liposome

UV spectral scanning: Paclitaxel novel liposome and blank novel liposome were dissolved and diluted with anhydrous ethanol, respectively and scanned between 200-900 nm; paclitaxel had maximum absorbance at 230 nm, while blank novel liposome had no absorbance peak within that wavelength range.

Determination of encapsulation efficiency: Appropriate amount of pretreated Sephadex G-50 was taken and gel column was prepared. 2 mL of paclitaxel liposome was accurately pipetted and loaded on the column, then eluted with phosphate buffer (pH = 7.4) at a flow rate of 0.5 mL/min, eluents (3 mL per fraction) were collected and changes in absorbance of each fraction were measured at a wavelength of 230 nm. The results showed that the liposomes were completely eluted between about 6-24 mL and the drugs between about 30-60 mL, the degree of separation between the two was relatively good. The isolated liposome and non-isolated liposome solution were added with ethyl ether-anhydrous ethanol-water solution, fully shaken to break emulsion and then evaporated to dryness in a water bath. The residue was dissolved in anhydrous ethanol and diluted to 5 mL. The absorbance was measured at 30 nm by colourimetry and liposome encapsulation efficiency was calculated as follows:

Encapsulation efficiency (En) % = $C_{encapsulation}/C_{total} \times 100$ %, where $C_{encapsulation}$: amount of paclitaxel encapsulated into the liposome; C_{total}: total amount of paclitaxel in liposome.

Design of factors and levels of orthogonal experiment: There are quite many factors that influence the encapsulation efficiency of liposomes. According to the preliminary experiment and relevant literatures, the ratio of hydrogenated phospholipid to cholesterol, the ratio of hydrogenated phospholipid to paclitaxel and rotary evaporation temperature were selected as three factors, three levels were selected for each factor and screening was performed using orthogonal table L9(3^3), the factors and levels were shown in Table-1.

Cell culture: SGC-7901 cell lines were cultured in DMEM containing 10 % fetal bovine serum and incubated *in vitro* in a 37 °C, 5 % CO_2 incubator, cells in logarithmic growth phase were used for the experiment.

Grouping: Experimental groups: paclitaxel liposomes, 0.05, 0.1, 0.5 and 1 μmol/L; control group: blank negative control.

Determination of growth inhibitory effect of paclitaxel liposome on SGC-7901 cells by MTT assay[10]: Exponential growth phase cells were taken, digested with 0.25 % trypsin and prepared into a cell suspension with a concentration of 1×10^5 cells mL^{-1}, the cells were then seeded in 96-well plates at 5×10^4 cells per well and acted by different concentrations (0.05, 0.1, 0.5 and 1 μmol/L) of paclitaxel liposomes, five replicate wells were set up for each concentration, on the 3rd day, 30 μL of 5 mg/mL MTT solution was added to each well, after the incubation was continued for an additional 4 h, the culture ended, then the supernatant was carefully aspirated and discarded and each well was added with 100 μL of DMSO, after shaken well, absorbance (A) of each well was measured at a 570 nm wavelength on an automatic ELISA reader and growth inhibition rate was calculated.

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

RESULTS AND DISCUSSION

Orthogonal experiment results of optimization of paclitaxel liposome preparation process: As can be seen from the results in Table-2, under the factors and levels used in this experiment, the factors influencing the encapsulation efficiency of paclitaxel liposome in order of importance were: B > A > C, *i.e.* the ratio of hydrogenated phospholipid to

TABLE-1
FACTORS AND LEVELS OF ORTHOGONAL EXPERIMENT

Level	Phospholipid to cholesterol weight ratio (A)	Hydrogenated phospholipid to paclitaxel weight ratio (B)	Rotary evaporation temperature (°C) (C)
1	20:3.15	20:1	40
2	20:3.84	20:2	50
3	20:4.28	20:2.5	60

TABLE-2
ORTHOGONAL EXPERIMENT RESULTS OF OPTIMIZATION OF PACLITAXEL LIPOSOME PREPARATION PROCESS

Experiment No.	A	B	C	Encapsulation efficiency (%)
1	1	1	1	73.8
2	1	2	2	72.4
3	1	3	3	78.5
4	2	1	2	85.9
5	2	2	3	72.5
6	2	3	1	77.3
7	3	1	3	87.2
8	3	2	1	74.1
9	3	3	2	79.4
k_1	224.7	246.9	225.2	
k_2	235.7	219.0	237.7	
k_3	240.7	235.2	238.2	
K_1	74.9	82.3	75.1	
K_2	78.6	73.0	79.2	
K_3	80.2	78.4	79.4	
R	5.3	9.3	4.3	

paclitaxel > the weight ratio of phospholipid to cholesterol > rotary evaporation temperature. It can be seen from the results of range analysis that under the experimental conditions, the optimal preparation process of paclitaxel liposome was A3B1C3, *i.e*. the phospholipid to cholesterol weight ratio of 20:4.28, hydrogenated phospholipid to paclitaxel weight ratio of 20:1 and rotary evaporation temperature of 60 °C.

Growth inhibitory effect of paclitaxel liposome on SGC-7901 cells: Different concentrations of paclitaxel liposomes (0.05, 0.1, 0.5 and 1 μmol/L) all had significant inhibitory effects on SGC-7901 cells and within a certain dose range, the inhibitory effects gradually increased with the increase of drug concentration in culture medium and the extension of incubation time, of which 1 μmol/L paclitaxel liposome had the strongest inhibitory effect on SGC-7901 cells at 72 h, reaching 75.9 % (Fig. 1).

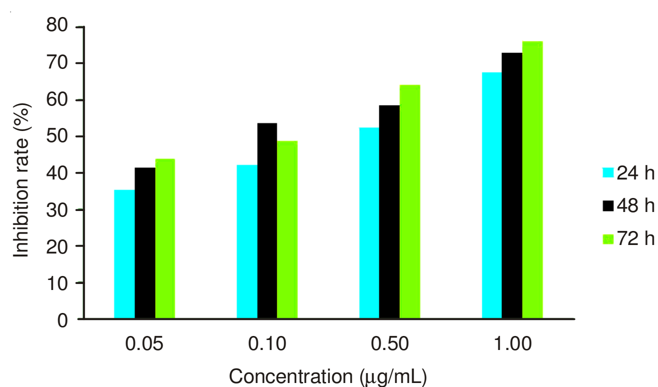

Fig. 1. Growth inhibitory effect of paclitaxel liposome on SGC-7901 cells

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