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Liposomal delivery vehicles of docetaxel

Naresh Kalra[1,*], Manju Nagpal[2], G.jeyabalan[1] and Suresh Choudhary[1]
[1]Department of Pharmaceutics, Alwar Pharmacy College, Alwar India.
[2]Chitkara School of Pharmaceutical Sciences, Chitkara University, Barotiwala (HP), Nims University Jaipur.

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ABSTRACT
Docetaxel stabilizes microtubules while inhibits mitotic spindle formation. It has been found to be effective in treating several solid cancers. The aim of this study was to in cooperate docetaxel in conventional and Chitosan coated liposomes, and to evaluate the antiproliferative effects of different formulations on MCF-7 and HepG-2 cell lines. MTT assay was used to determine the growth inhibition of the cell line by docetaxel. A significant dose-dependent inhibition of proliferation was found after the cells were exposured to certain liposomal docetaxel preparations, suggesting the possible use of liposomes as effective docetaxel delivery devices.

Introduction

Breast carcinoma cells grow in situ as soild tumor masses. The MCF-7 and HepG-2 cell line, derived from breast carcinomas, grow in tissue culture in monolayers with an epithelial sheet like morphology. Recently, docetaxel has been introduced as a novel anticancer agent showing activity against a broad range of human tumors especially drug-resistant ovarian and breast carcinomas. The commercial formulation of docetaxel consists of a micelle solution of the drug in lipids containing 50% ethanol. The aim of present investigation is to analyze the ant proliferative effects of different docetaxel formulation on MCF-7 and HepG-2 cells. and to compare the efficiencies and the time course of their effects.

Material Methods

Cell Culture

MCF-7 and HepG-2 breast cancer cells were obtained from the Institute of Toxicology Research center (ITRC). and they were routinely cultured in modified Eagle's Medium (MEM) containing 10% heat inactivated fetal bovin serum (FBS), 1% L- glutamine ,50µ/ ml penicillin and 50µ /ml streptomycin, using a standard protocol. Cells were maintained at 37° C in humidified atmosphere of 95% air and 5% CO_2. Cells were in logarithmic phase of growth at the time of the drug sensitivity assays.

Preparation of docetaxel-containing liposome

The lipids were dissolved in ethanol, docetaxel in ethanol, mixed properly by vertex. Chitosan heated in water a make a solution for this. To obtain a small and homogeneous vesicles, the liposomes suspension was extrudes 10 cycles each through polycarbonate filters with 0.2 and 0.1µm pores. Ethanol injection method is used to prepare liposomes[3,4,5].

To assay for incorporation efficiency the liposome were pass through Sephadex G-50 coloum and supernatant was analyzed by The HPLC system was equipped with 10 ATVP binary isocratic pumps (Shimadzu), a Redone (Cotati, CA, USA) model. The column was eluted acetonitrile: water (50:50). Detection was by UV absorption measurement at 227 nm (flow rate 1 ml/ min).

Determination of Cytotoxicity

Drug sensitivity was determined using a standard colorimetric MTT (3-4, 5-dimethylthiazol-2-yl-2, 5-diphenyl-tetrazolium bromide) assay. Briefly, cells were plated out at a density of 10^4 cells/100µl/well in 96-well micro litre plates and allowed an overnight period for attachment. Then the medium was removed and fresh medium, along with various concentrations of docetaxel, control cultures containing no docetaxel were set up in conditions otherwise identical. Triplicate plates of each of the treated and control cultures in three separate independent experiments were incubated for 5 days at 37^0 C in a humidified 5% CO_2 incubator. Following treatment, cells were fed with MTT (10µl/ well, 5mg per ml in PBS) and incubation was prolonged for 3h at 37^O C. After removing the supernatants, The MTT- formazan crystals were dissolved in DMSO (100µl/well) and the absorbance was measures at 570nm in a multi-well plated reader(Model Anthos Labtec 2010.7 reader). The percent viability of each well was calculated from the following[6,7]:

Percent viability = A-B/C-B *100% (1)

A- absorbance of test;
B- absorbance of blank;
C- absorbance of control.

The data presented are the mean +_ standard derivation from three replicated wells per micro well plate and three replicate micro well per cell line. Data from the MTT assays were analyzed means of student's t-test. AP-value less than 0.05 were considered to be significant.

The 50% inhibitory drug concentration (IC_{50} value) was statistically determined using SSPS (probit-analysis).

Results

Docetaxel liposome formulation were prepared by extruding of multilamellar liposomes using different phospholipids mixtures Hydration of the drug-lipid film, followed by 10 cycles

Tele:
E-mail addresses: neshu07@gmail.com

each of extrusion through 0.2μm, 0.1μm polycarbonate filters, was found to be feasible preparation method for homogeneous small unilamellar vesicles.

Different liposomal formulations were used to entrap docetaxel, varying liposome properties such as membrane fluidity and surface modification. The formulations are to substantial entrapment of docetaxel in liposome formulations were generally achievable despite the lipid composition both chemically and physically stable under physiological conditions for at least 1 month. The difference in MTT utilization between control and treated MCF-7 and HepG-2 cells were determine to calculate cell viability. Beginning with 1 μg/ml docetaxel. There is about 50 %in MCF-7 and 45%in HepG-2 cell line cells left after 96h. Such profile changed significantly with various liposome formulations.

Discussion

Liposomal formulations of docetaxel have been suggested to have many advantages over current commercial formulations. Various lipid compositions were tested for better loading efficiency and storage stability. Further more, for in vivo stability and tumor-targeting effect; PEGylated lipids may also be included. Certainly, such improvements in formulation will definitely change various pharmacokinetic aspects of the drug. There have been several extensive studies about it. But at the same time, we think it is also important to look into the change of their interaction mode with cells.

Here we established a model system using breast cancer cultured cells. The interactions between free docetaxel molecule and cells and between docetaxel containing liposomes and cells are considerably different. In this report, we focus on the antiproliferative efficiencies of the various liposome formulations and the time courses of their effect.

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