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Research Article

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Characteristics of pegylated niosomal letrozole

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ABSTRACT

Letrozole is one of the hormonal anticancer drugs which can be employed to treat aromatase dependent breast cancer. Aromatase is an enzyme that catalyses biosynthesis of estrogen from testestrone (androgen).Letrozole was loaded onto pegylated niosomes through reverse phase evaporation technique. Letrozole pegylated niosomes was characterized by dynamic light scattering spectroscopy (195.2 nm pegylated niosome and 234.1 nm letrozole pegylated niosomes), scanning electron microscopy and its entrapment efficiency was calculated to be 66.6% . The drug released pattern (in vitro) from the pegylated niosomes was studied through zero order, first order, Higuchi and Hixson – Crowell kinetics models. It was found that the release pattern followed first order and Hixson-Crowell models. Finally, the IC50 values for pure letrozole and letrozole pegylated niosomes were found to be 0.051 μM and 0.0287 μM, this indicated that the toxicity of drug pegylated niosomes is more than the pure drug.

Keywords: Letrozole, pegylated niosome, drug delivery, release kinetics.

INTRODUCTION

Deaths and mortality caused by cancerous diseases continue with a growing trend so that the carcinomas are considered as major causes of death in the world and one of the cancers causing death is breast cancer mostly in women. There are several ways to treat breast cancer and one of them is hormone therapy. One of the hormonal anticancer drugs is known to be letrozole which can be employed to treat aromatase dependent breast cancer. Aromatase is an enzyme that catalyses biosynthesis of estrogen from testestrone (androgen). Furthermore, letrozole is an aromatase inhibitor of third generation. It is highly potent and selective inhibitor of aromatase [1]. It inhibits the production of estrogen in postmenopausal women. It works by blocking cytochrome P-450 (CYP) which turns the hormone androgen to small amount of estrogen in the body. This means that less estrogen is available to stimulate the growth of hormone receptor positive breast cancer cells. It does not stop the ovaries from making estrogen, therefore, aromatase inhibitors affects only on postmenopausal females [2]. Now investigators are looking for vehicles through which drugs can be delivered to the specific target .One of the vehicles which can be employed to deliver the drug to specific site is noisome [3]. Niosomes can be used to vesiculize both hydrophilic and lipophilic drugs. Niosomal vesicles are composed of non-ionic surfactant with/without cholesterol or other lipids. Niosomes have lower toxicity due to non-ionic nature of the surfactant and act to improve the therapeutic index of the drug [4]. Srinivas et al. (2010) studied the preparation and evaluation of niosomated aceclofenac . In such study, the effects of various compounds such as non ionic surfactants and cholesterol on encapsulation efficiency, particles size and drug release were evaluated. They concluded that in the presence of non-ionic surfactants, the rate of drug release and also with increasing surfactant concentration, the drug trapping efficiency in all formulations will increase [5]. Evaluation of drug delivery based on kinetic model describes the dissolution profile. Modeling by using the following parameters will provide the closest fit between experimental observations and the non-linear function. The model best showing the fit between release data and correlation coefficient will be chosen as the main model. Sanjoy K D et al. (2009) conducted a research on development and experimental

evaluation of biodegradable nanoparticles of letrozole in the treatment of breast cancer . The purpose of this study was to prepare and evaluate the biodegradable nanoparticles system of letrozole at different concentrations of letrozole, encapsulation efficiency, particles size and drug release. The results included increased encapsulation and particles size with increasing concentrations of letrozole. The drug release also followed the zero-order kinetic model [6]. In this article attempts were made to study and characterize letrozole pegylated niosomes .

EXPERIMENTAL SECTION

Materials

Letrozole (purchased from Fanavaran Parsian pharmaceutical company), span60 (sorbitan mono laurate), tween 80, cholesterol, polyethylene glycol 3350 (PEG 3350), MTT reagent (0.5 mg/ml) (Sigma, USA), acetonitrile (Merck), RPMI 1640 Medium (Invitrogen), MCF-7 cell line (National Cell Bank Department, Pasteur Institute of Iran). Other reagents used were of analytical grade.

Preparation of Niosomes

Niosomes were prepared by reverse phase evaporation wherein the composition of the formulation is as per table no.1.The formulation constituents were accordingly prepared and dissolved in alcohol .They were allowed to react at room temperature on magnetic stirrer(120 rpm) for 30 minutes. The solvent was evaporated on rotary evaporator. The resultant gel was dissolved in phosphate buffer saline at pH7.2 and 40ºC. The obtained suspension was subjected to sonication. This was further passed through filter 0.22μm in order to obtain uniform niosomes.

Zeta Potential Measurement

In vivo performance of the niosomes relatively could be related to charges present on the surface of the vesicles. The stability of niosomal formulation is also related to the stability of niosomal formulation. Thus the zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system .The prepared formulation was suitably diluted in order to measure the zeta potential and the size of the vesicles by Zeta sizer (Malvern, Instruments Ltd, Worcestershire, Zen 3600 UK).

Scanning Electron Microscopy (SEM)

A drop of aqueous suspension of letrozole PEGylated niosomes was spread on a slab and dried under vacuum. The sample was coated with a 20 nm thick gold layer in a cathode evaporator. The diameter of particles in each field was calculated by using JSM-5200 operation of scanning electron microscope (Tokyo, Japan) at 15 kV.

Entrapment efficiency

The entrapment efficiency of letrozole loaded pegylated niosome was studied by rupturing the vesicles. Letrozole pegylated niosomal formulation was passed through 0.22 μm syringe filter. A volume of 1 ml SDS solution prepared in phosphate buffer was added to 0.1 ml of letrozole pegylated niosome and again passed through syringe filter. The solution was incubated at 37 ° C for 1.5 h. Then, the solution was passed through a 0.22 μm filter. The OD of the transparent solution was read at λ 240 nm against PBS containing SDS [7].The standard curve of letrozole was constructed employing different concentration of pure letrozole at the range of 2 to 7 μg/mL.

Kinetic Studies

Data of the letrozole release from pegylated niosomes were analyzed for release kinetics. Drug release kinetics from nanovesicles is analyzed by zero-order kinetics, first order kinetics, Higuchi and Hixson-Crowell models.

Zero order kinetics model follows the equation as $(Q_t/Q_0) = k_0 t$, where, k_0 is the zero order rate constant; Q denotes the amount of letrozole released at time t and Q_0 denotes the initial amount of letrozole.

First order kinetics model follows the equation as $\log (Q_t/Q_0) = -k_1 t / 2.303$, where $k_1$is the first order rate constant.

Higuchi model, $Qt/Q_0 = k_h t^{1/2}$ Q denotes amount of letrozole released at time t Q_0 denotes the amount of letrozole in nanoniosome initially, k_h is the Higuchi matrix release kinetics.

Hixson-Crowell model:

$Q_0^{1/3} - Q_t^{1/3} = \kappa t$, where Q_0 is the initial amount of drug in the niosomes, Q_t is the remaining amount of drug in the niosomes at time t,and κ (kappa) is a constant incorporating the surface-volume relation. The data were fitted into the models (zero and first orders, Higuchi and Hixson-Crowell) as reported by Chime A et al[8] and the graphical representations were plotted in order to obtain correlation coefficient (R^2).

In vitro release study

Letrozole release rate of release from niosomes is specified by membrane diffusion technique. The noisome suspension equivalent to 1 mg of letrozole and letrozole pegylated noisome was poured into a dialysis bags (cut off 12000Da, sigma) separately. The dialysis bags were immersed inside a container containing 250 ml of phosphate buffer, pH 7.4, and placed on the magnetic stirrer (37 ºC, 120 rpm) separately. At certain intervals, 2 ml of phosphate buffer was taken and replaced with an equal volume of the buffer. The ODs samples were separately measured spectrophotometrically at a wavelength of 240nm.

Evaluation of cellular cytotoxicity

Assessment of cytotoxicity on MCF-7cells was performed using MTT assay. The cells were cultured at dilution of 1×10^4 cells per well in DMEM medium containing 10% fetal bovine serum and 1% Penicillin/ Streptomycin antibiotics

under 10% carbon dioxide at 37° C. After 24 hours of cells culturing, the supernatant was poured off and the cells with pharmaceutical formulations of pure letrozole and letrozole PEGylated noisome were treated at 0.15, 0.075, 0.0375, 0.0187, 0.0094, 0.0046, 0.0023 and 0.0011 micro-molar concentrations. After 48 hours of incubation, the culture media with pharmaceutical formulations were removed and100 µL of PBS, 0.5 mg/ml MTT solution with pH equal to 4.7 was added to each well and incubation was carried out for 3 hours. The MTT solution was then removed, and 200 µL of isopropanol was added to each well and stirred to dissolve the formed Formazan crystals. In the next stage, absorption was read at λ at 570nm using ELISA reader (BioTek Instruments, VTU.SA).The cell viability rate was obtained from the ratio of treated cells absorption with different formulations of the drug to the absorption of control cells, and the results were evaluated using the Pharm program. The IC50 values were reported for each of the samples.

RESULTS AND DISCUSSION

Letrozole was loaded into nanovesicle which was prepared according to table 1 by reverse phase evaporation technique. Bhaskaran and Lakshmi dissolved surfactant in a mixture of ether and chloroform to which an aqueous phase containing the drug was added. The resulting two-phase system was then homogenized and the organic phase evaporated under reduced pressure to form stable and uniform niosomes containing lansoprazole [9]. Guinedi et al. also used this method to develop niosomes containing acetazolamide and reported that spherical vesicles were produced with less drug entrapment than in multilamellar vesicles [10]. Furthermore the zeta potential and size distribution of the prepared pegylated niosomal letrozole and pegylated niosomes were studied. Zeta potential is an important physicochemical parameter, which can influence factors like stability of pegylated niosomal preparation. Table 2 depicts the size and zeta potential of nano formulated letrozole. Extremely positive or negative zeta potential values cause larger repulsive forces, while electrostatic repulsion between particles with the same electric charge prevents aggregation of the spheres [11]. The size of pegylated niosomal letrozole was increased which could be due to location of letrozole between water layers of the niosomes. Morphologically, it was observed that the pegylated niosomal form of letrozole is crystalline. (Fig. 1a and 1b). The values of released letrozole from two formulations of pure letrozole and letrozole pegylated noisome in phosphate buffer were obtained at intervals of 1, 2, 4, 6, 8, 23, 26, 29 and 46 hours using the letrozole standard curve (Fig 2).Data obtained from in vitro release (both letrozole and letrozole pegylated nanoniosomes) were employed to study the kinetics of the drug released from the pegylated nanovesicles (Fig. 2). The data were fitted into the models (as they are explained in methods) in order to find out K_0,k_1, k_h and *K*.(Kappa), zero and first orders, Higuchi and Hixson –Crowell coefficients respectively. Thus through their correlation coefficients (R^2)(Table 3). it was found that the drug release obeys first order kinetics and Hixson –Crowell kinetic model. Dissolution occurs at smooth and plane surfaces parallel to the drug level, revealing that the drug dimensions decrease proportionately, the initial geometry remains constant at all times [8].ThusIC_{50}values for the pure drug and pegylated noisome formulations (Fig.3) indicate the higher effectiveness of the letrozole pegylated noisome formulation. In conclusion, as compared to conventional formulation, the drugs carried by vehicles such as niosomes at nano scale can increase the effectiveness and decrease the side effects of the drug under medication.

Table 1 Composition used to prepare pegylated niosomal letrozole

Span 60 (mM)	Tween (mM)	Cholesterol (mM)	PEG 3350 (mM)	Letrozole (mM)
6	0.2	1	0.9	1
6	0.2	1	0.9	----------

Table2 Size and zeta potential of pegylated niosomal letrozole as compared to pegylated niosome devoid of letrozole

Formulation	Encapsulation efficiency (%)	Mean diameter (nm)	Zeta Potential (mV)
Pegylated niosomal letrozole	66.6	231.4	-8.71
Pegylated niosome	-----	195.2	-8.04

Table 3 Correlation coefficients obtained for each model by in vitro release studies

Model	R^2 Pegylated niosomal letrozole
Zero order kinetics	0.84
First order kinetics	0.92
Higuchi model	0.94
Hixson and Crowell model	0.95

Figure 1a: SEM image of control PEGylated noisome. Figure 1b: SEM image of drug PEGylated noisome

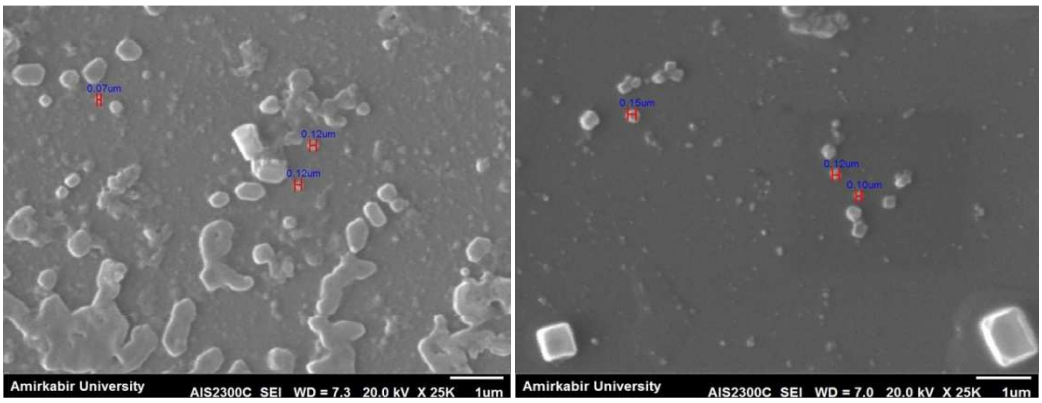


Figure 2: Drug release from two formulations, pure letrozole and PEGylated noisomal letrozole

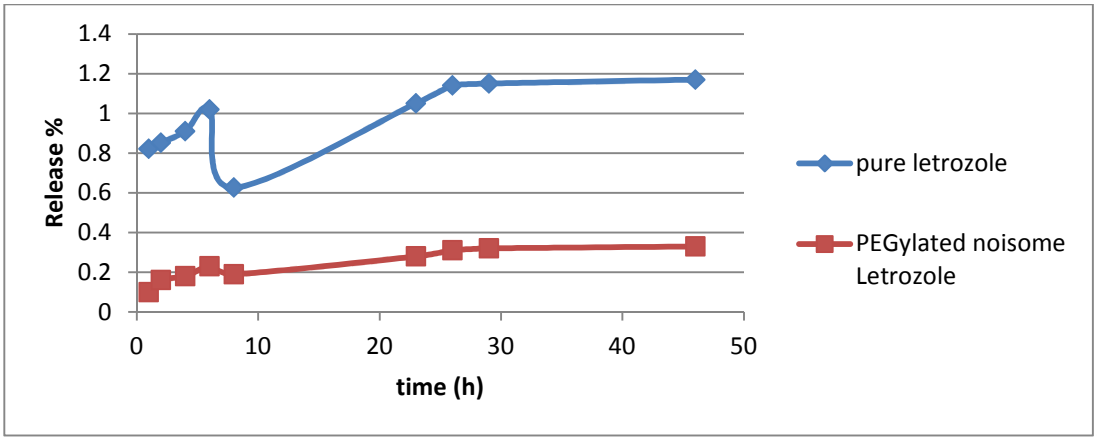
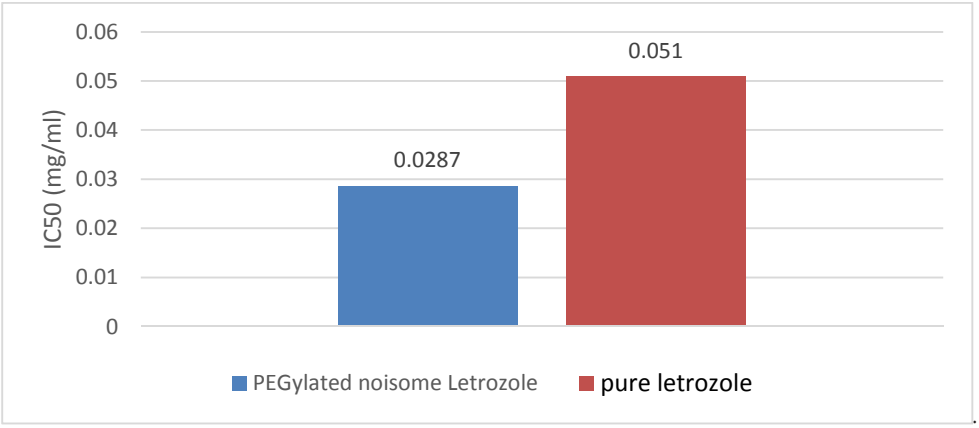


Figure 3: Evaluating cellular cytotoxicity of PEGylated noisomal letrozole formulation as compared to letrozole



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