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Maximizing the Therapeutic Efficacy of Imatinib Mesylate–Loaded Niosomes on Human Colon Adenocarcinoma Using Box-Behnken Design

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ABSTRACT

This research purposed to formulate an optimized imatinib mesylate (IM)–loaded niosomes to improve its chemotherapeutic efficacy. The influence of 3 formulation factors on niosomal vesicular size (Y_1), zeta potential (Y_2), entrapment capacity percentage (Y_3), the percentage of initial drug release after 2 h (Y_4), and the percentage of cumulative drug release after 24 h (Y_5) were studied and optimized using Box-Behnken design. Optimum desirability was specified and the optimized formula was prepared, stability tested, morphologically examined, checked for vesicular bilayer formation and evaluated for its *in vitro* cytotoxicity on 3 different cancer cell lines namely MCF-7, HCT-116, and HepG-2 in addition to 1 normal cell line to ensure its selectivity against cancer cells. The actual responses of the optimized IM formulation were 425.36 nm, −62.4 mV, 82.96%, 18.93%, and 89.45% for Y_1, Y_2, Y_3, Y_4, and Y_5, respectively. The optimized IM-loaded niosomes confirmed the spherical vesicular shape imaged by both light and electron microscopes and further proven by differential scanning calorimetry. Moreover, the optimized formula exhibited improved stability on storage at $4 \pm 2^{\circ}C$ and superior efficacy on MCF7, HCT-116, and HepG2 as IC_{50} values were 6.7, 16.4, and 7.3 folds less than those of free drug, respectively. Interestingly, IC_{50} of the optimized formula against normal cell line was ranged from 3 to 11 folds higher than in different cancer cells indicating a higher selectivity of the optimized formula to cancer cells. In conclusion, the incorporation of IM in niosomes enhanced its efficacy and selectivity toward cancer cells, presenting a promising tool to fight cancer using this approach.

Introduction

Several techniques were developed and well recognized for defeating cancer. One of the most rapidly progressive disciplines is nanomedicine and nanotechnology that proved successful returns and mechanisms for fighting cancer.[1] Many benefits were recorded from applying nanomedicine over the conventional medicine in either cancer diagnosis or therapy with a very good prognosis results such as metabolism reduction, better pharmacokinetic profile, and improved selectivity on a particular tissue of interest. Another recent and interesting advantage is nanoparticles (NPs) role in cancerous autophagy pathway interruption. Autophagy is substantially involved in many different mechanisms of cancer treatments and even cancer progress. Nanocarriers (NCs) application is considered an exploited tool in cancer remediation.[2]

NCs and NPs are particles which generally not exceeding 100 nm in length.[3] This standard definition was definitely approved and well recognized for many earlier studies in nanotechnology field.[2,4,5] From nanotechnology, NCs have been assembled from inorganic and organic materials to boost remedies efficacy, decrease their adverse events, and reinforce their therapeutic effectiveness. NCs could deliver drugs as adsorbed on or attached to their surfaces or encapsulated within their cavities.[6] NCs used for medicinal purposes are synthesized from biocompatible materials which apart them a minimum toxicity and also offer high characteristic uptake adequacy in the affected cells rather than healthy

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ones. In addition, they are characterized by their expanded half-life with reduced agglomeration rate and prolonged shelf life.[7] The most popular and widely used NCs systems are niosomes, liposomes, dendrimers, micelles, nanofibers, and NPs.[8]

Niosomes, in specific, are known as vesicles mainly consisting of nonionic surfactant possessing a unilamellar or multilamellar bilayer composition which is formatted by aqueous hydration of the surfactant dried films and so can be used for entrapping either lipophilic or hydrophilic drugs within the vesicular membrane or in the aqueous core, respectively.[9] Niosomal NCs are one of the prominent vesicular systems, being the most focused as efficient drug delivery systems for various administration routes as niosomes do not have the disadvantages of other NCs that probably countered. Besides, they have an immense entrapping capability of diverse types of remedies, vaccines, genes, and proteins.[10]

For anticancer drug delivery, niosomes provided a powerful and an effective delivery vehicle for many tumor medicaments. They efficiently overcame such problems as insufficient drug access through the blood brain barrier,[11] low stability and bioavailability,[12] and multiple dosing with risk of high incidence of side effects and toxicity.[13,14]

Imatinib mesylate (IM) is renowned as a protein-tyrosine kinase inhibitor that prevent the action of tyrosine kinase formed abnormally by the anomaly of Philadelphia chromosome in many aggressive cancers such as gastrointestinal stromal tumor and chronic myeloid leukemia.[15] IM has been shown to compete with adenosine triphosphate and inhibit specific tyrosine kinases such as Bcr-Abl kinase, c-kit receptor kinases activation, as well as inhibit platelet-derived growth factor (α, β) receptor.[16] Several studies were designed to explore the growth inhibitory effect of IM either *in vitro* (cancer cell lines) or *in vivo* (tumor-bearing experimental animals) on variety of cancer cell types, such as of breast,[17,18] liver,[19] prostate,[20] and colorectal origin[21] as well as many other types of malignant tumors.[22,23] Besides IM great effectiveness, patients treated with IM may suffer from its side effects and long-term toxicity as renal, cardiac, and hepatotoxicity.[24,25]

In relation to autophagy pathway of cancer cells, the inhibition of autophagy was proved to increase the effect of IM and thus, by using NCs with IM treatment, the autophagy hindrance effect of NCs will give a synergistic action, which can substantially reveals a potential formulation of IM in the era of cancer treatment.[26-29]

This study main objective was to develop an optimized formula of a niosomal anticancer drug (IM) for increasing the activity and prolonged release characteristics of IM, finding new indications for resistant cancer types, decreasing the dose in therapy, and thus increasing safety of the drug.

Material and Methods

Materials

IM was purchased from Shaanxi Pioneer Biotech Co. (Xi'an, China). Chloroform for HPLC was obtained from Honeywell Riedel-de Haën (Seelze, Germany). Absolute ethyl alcohol and sodium chloride 0.9% for injection were provided from El-Nasr Pharmaceutical Chemicals Co. (Abuzaabal, Cairo, Egypt). Sorbitan laurate (Span 20), sorbitan monopalmitate (Span 40), and Sorbitan monostearate (Span 60) were procured from Merck Schuchardt OHG (Hohenbrunn, Germany). Disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen orthophosphate (KH_2PO_4), and sodium chloride (NaCl) were purchased from Oxford Laboratory (Mumbai, India) to prepare phosphate-buffered saline (PBS). Potassium phosphotungstate was obtained as a gift from National Research Center, Pharmaceutical Technology department (Cairo, Egypt). Cholesterol from lanolin, dicetyl phosphate (DCP), dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salt (MTT), and stearylamine (SA) were procured from Sigma-Aldrich Company (St. Louis, MO).

Methodology

Design of Experiments

Preliminary study was carried out using Span 60 along with cholesterol for the preparation of IM-loaded niosomes with 2 molar ratios; namely, Span 60: cholesterol (1:1) and Span 60: cholesterol (2:1) for the preparation of neutral niosomes. Dicetyl phosphate was used for the negatively charged niosomes formulation, whereas SA was used for the elaboration of positively charged niosomes. For both negatively and positively charged niosomes, 3 molar ratios were used as follows, Span 60: cholesterol: charge inducing agent (1:1:0.1), (2:1:0.5), and (2:1:0.2).[30] By evaluation of the prepared niosomes, the obtained results from this preliminary study were used for determination of the factor levels that will be implemented in the optimization of IM-loaded niosomes.

For designing the layout of the experiential work for the elaboration of IM niosomes, Box-Behnken design (BBD) was applied.[31-33] Three factors, namely the surfactant hydrophilic lipophilic balance (HLB) values (X_1), cholesterol molar ratio concentration (X_2), and DCP molar ratio concentration (X_3), were specified to inspect their impacts on the niosomal mean vesicle size (Y_1), zeta potential (Y_2), entrapment efficiency percentage (Y_3), the initial release percentage of IM after 2 h (Y_4), and the cumulative release percentage of IM after 24 h (Y_5). The goals to be achieved and the studied dependent variables (responses) are presented in Table 1. In fully randomized order, 15 different formulae were prepared as demonstrated in Table 2. By using the statistical package Statgraphics® Centurion XV software, version 15.2.05 (StatPoint, Inc., Warrenton, VA), the mathematical relevancies among the observed responses and the designated factors were explicated as a polynomial equations format and elucidated for their significance by ANOVA.

Elaboration of IM-Loaded Niosomes

Drug-free niosomes as well as the 15 IM-loaded niosomes formulations based on Box-Behnken design were formulated using thin-film hydration method with some adjustments.[34-37] In 10-mL chloroform, a specified amount of charge-inducing agent, cholesterol, and Span 60 were dissolved completely in 100-mL pear-shaped phial of Büchi-M/HB-140, (Flawil, St. Gallen, Switzerland) rotary evaporator, and rotated, 100 rpm, on a 58°C-60°C water bath (temperature above the transition temperature of cholesterol-surfactant mixture). For the thin film to be established on the wall of the flask, the chloroform was removed under vacuum. The thin film was then hydrated with 10-mL PBS (pH 5.5) containing 5-mg IM.

Table 1
Imatinib Mesylate Niosomal Formulation Independent Variables Levels and the Studied Responses Used in Box-Behnken Design

Independent Variables (Factors)	Levels			Units
	Low	Medium	High	
X_1: HLB of surfactant	4.7	6.7	8.6	Value
X_2: cholesterol conc.	0.5	1.0	1.5	Molar ratio
X_3: DCP conc.	0.05	0.075	0.1	Molar ratio
Dependent Variables (Responses)		**Units**		**Goal**
Y_1: mean vesicle size		nm		Minimize
Y_2: zeta potential		mV		Maximize
Y_3: entrapment efficiency		%		Maximize
Y_4: initial release		%		Maximize
Y_5: cumulative release		%		Maximize