Characterization and Evaluation of Nano-niosomes Encapsulating Docetaxel against Human Breast, Pancreatic, and Pulmonary Adenocarcinoma Cancer Cell Lines

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ABSTRACT

Background: Docetaxel (DXL) is an antineoplastic agent for cancer treatment, the therapeutic efficiency of which is limited due to low solubility, hydrophobicity, and tissue specificity.

Objective: In this study, nano-niosomes were introduced for improving therapeutic index of DXL.

Material and Methods: In this experimental study, two nano-niosomes were synthesized using Span 20® and Span 80® and a thin film hydration method with DXL loading (DXL-Span20 and DXL-Span80). Characterization, in-vitro cytotoxicity and bioavailability of the nano-niosomes was also evaluated via in-vivo experiments.

Results: DXL-Span20 and DXL-Span80 have vesicles size in a range of 84-90 nm and negative zeta potentials. DXL entrapment efficiencies were obtained as 69.6 and 74.0% for DXL-Span20 and DXL-Span80, respectively; with an in-vitro sustained release patterns. Cytotoxicity assays were performed against MDA-MB-231, Calu-6, and AsPC-1 cell lines, and the results indicated that DXL loading into nano-niosomes led to decrement in values of half-maximal inhibitory concentration (IC50) at least 2.5 times and at most 6.5 times, compared to free DXL. Moreover, the rat blood bioavailability of DXL after intraperitoneal administration and the pharmacokinetic parameters indicated higher DXL plasma level and the higher effectiveness of DXL-Span80 compared to DXL-Span20.

Conclusion: Carrying DXL by the nano-niosomes led to enhanced cytotoxicity (and lower IC50 values) and higher efficacy with enhanced pharmacokinetic parameters.

Citation: Ajdari MR, Ranjbar A, Karimian Kh, Karimi M, Heli H, Sattarahmady N. Char-acterization and Evaluation of Nano-niosomes Encapsulating Docetaxel against Human Breast, Pancreatic, and Pulmonary Adenocarcinoma Cancer Cell Lines. *J Biomed Phys Eng*. 2024;14(2):159-168. doi: 10.31661/jbpe.v0i0.2401-1708.

Keyword

Docetaxel; Taxane; Taxotere®; Niosome; Sorbitan Monolaurate; Drug Delivery Systems

Introduction

Cancer is the second most serious disease which lead to human death in the past decade. Nowadays, researchers are developing new approaches for discovering interventions for early diagnosis and treatment of cancers. The conventional cancer treatment modalities,

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Received: 14 January 2024
Accepted: 29 February 2024

including surgery, chemotherapy, and radiotherapy, have also caused side effects, and new treatment strategies are under development [1]. Targeted, photothermal, photodynamic, sonodynamic, radiation-chemo, and stem cell therapy are among the new routes of cancer treatment. Nanomedicine has introduced novel treatment approaches and represented solutions for challenges in cancer medicine [2-9].

In chemotherapy, most of the traditional drugs have had problems such as toxicity, poor aqueous solubility, and low permeability that limit their therapeutic efficiency. Nanotechnological-based drug delivery systems can resolve the main problem issues of the current drugs through introducing different types of nanosystems as drug delivery vehicles [3, 9-12]. In this regard, nano-niosomes, as self-assemble delivering systems for either hydrophilic or hydrophobic agents, are successful in pharmacology and pharmaceutics studies due to biodegradability, biocompatibility, bioavailability, low cost, stability, and diversity of suitable surfactants for their fabrication [13]. Up to now, different nano-niosomes, as vehicles of various anticancer drugs, have been designed [14].

Docetaxel (DXL), as an antineoplastic agent from taxoid family, is a semisynthetic derivate of paclitaxel that binds particularly to the beta-tubulin subunit of microtubules and stabilizes them by inhibiting depolymerization, resulting in arrest of cell cycle and cell apoptosis [15, 16]. It has 1.9 fold higher potency than paclitaxel as an inhibitor of microtubule depolymerization [17]. DXL has significant effects in treating various solid tumors, such as locally advanced or non-small cell lung cancer, metastatic breast cancer, ovarian cancer, gastric adenocarcinoma, and head and neck cancers [18-21]. Although DXL is more soluble than paclitaxel in aqueous media, its hydrophobicity and low tissue specificity result in adverse side effects and limitation in clinical applications [22]. Taxotere®, the only commercial formulation of DXL in which tween 80 and anhydrous ethanol are used to solubilize DXL, has been found to induce several severe adverse side effects including hypersensitivity reaction [23], peripheral neuropathy [24], fluid retention [25], and skin, nail and vascular toxicities [26-28]. Thus, developing a better-tolerated vehicle for DXL administration with higher solubility, stability, increased entrapment efficiency, better biological compatibility, and lower systemic toxicity is of great necessity.

Nanocarriers are among the effective ways to improve therapeutic index and reduce toxicity and side effects of antineoplastic drugs [29]. They overcome several limitations of these drugs, such as nonspecific biodistribution, low bioavailability, poor water solubility, and limited half-life [30, 31]. Among the lipid-based nanocarriers, although liposomes (as phospholipid-based vesicles) have wide applications in drug delivery systems, they face some problems such as high cost, complex preparation method, and limited shelf-life due to lipid rancidification [32]. On the other hand, niosomal formulations (as nonionic surfactant-based vesicles) are of promising nanocarriers [33]. They are made of amphiphilic molecules and nonionic surfactants that are surrounded by an aqueous compartment [34]. Niosomes have similar behavior to liposomes; however, many advantages such as having easy and low-cost production method, high chemical stability, low toxicity, biodegradability and biocompatibility, non-immunogenic stimulation, and wide applications for lipophilic and hydrophilic drugs make them proper alternatives to liposomes [35]. Nano-niosomes are a suitable choice for unstable drugs and drugs with low oral bioavailability, and are useful for inhibition of rapid metabolism and degradation. They can improve the therapeutic efficacy and availability, decrease resistance by protecting from biological environment, and improve penetration into the tumor tissue of antineoplastic drugs. Controlled drug release and targeting into a specific tissue/organ are

other interesting abilities of niosomal carriers that play an important role in decreasing toxicity and side effects. Niosomal formulations have been developed for delivery of some antineoplastic drugs such as methotrexate, doxorubicin, and paclitaxel [13, 14, 29, 34, 36].

Based our knowledge, no study on the preparation of DXL-loaded nano-niosomes has been reported. In this study, the results of niosomal carriers for DXL are presented for the first time. The nano-niosomes were prepared and then characterized. Cytotoxicity assays against MDA-MB-231, Calu-6, and AsPC-1 cell lines were done for efficacy evaluation of DXL upon loading into nano-niosomes.

Material and Methods

Materials

In this experimental study, DXL was received from Arasto Pharmaceutical Chemicals Inc. (Iran). Chloroform, dimethyl sulfoxide (DMSO), ethanol absolute, and high-performance liquid chromatography (HPLC). Grade acetonitrile were purchased from Scharlau (Spain). Span 20 and Span 80 were received from Daejung (Korea). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and trypan blue were purchased from Sigma (USA). Cholesterol was from MedChem Express (China). MDA-MB-231 (epithelial, human breast cancer, National Center for Biotechnology Information (NCBI) C578), Calu-6 (renin-expressing human pulmonary adenocarcinoma, NCBI C431) and AsPC-1 (human pancreatic tumor, NCBI C558) cell lines were received from Pasteur Institute Cell Bank of Iran. Roswell Park Memorial Institute-1640 (RPMI 1640) was received from Shell max (Iran). Fetal bovine serum (FBS) was purchased from Gibco (USA). Penicillin-streptomycin solution was from Danesh Azma Cell (Iran).

Preparation of nano-niosomes

Two blank (b-Span20, b-Span80) and DXL-loaded nano-niosomes (DXL-Span20, DXL-Span80) were prepared via thin-film hydration method from cholesterol and non-ionic surfactants (Span 20 and Span 80). For this purpose, appropriate amounts of the surfactants and cholesterol with mole ratios of 1:1 were dissolved in chloroform, with or without 10 mg of DXL. Then, chloroform was evaporated by a rotary evaporator at 60 °C. The thin layer of film that as formed on the inner wall of the flask was hydrated with phosphate-buffered saline (PBS, pH=7.4) for one hour at 55 °C, and then hydration was continued at room temperature for 24 h. The nano-niosomes were finally sonicated using a microtip probe sonicator for 30 min to reduce the size of the vesicles. The nano-niosomes were kept at 4 °C for further use. For free DXL analysis, DXL was dissolved in a mixture of ethanol and tween and then diluted with DI water.

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Characterization of the nano-niosomes

The droplet size and zeta potential of the

synthesized nano-niosomes were determined by a Scatterscope, Qudix (South Korea) particle size analyzer.

In-vitro cytotoxicity

The cytotoxicity of DXL-loaded nano-niosomes was investigated by the MTT assay and compared to free drug against MDA-MB-231, Calu-6, and AsPC-1 cell lines. The seeded cells in 96-well plates (2×10^4 per well, in Dulbecco's Modified Eagle Medium (DMEM) contained 10% FBS, and 1% streptomycin and penicillin) were treated with different concentrations of DXL-loaded nano-niosomes (1, 2, 5, 10, 20, 50 and 100 nmol L^{-1}). Untreated cells were considered as a control. The plates were incubated for 48 h at 37 °C in a humid atmosphere with 5% CO_2. Then, 10 µL of a MTT solution (5 mg mL^{-1} in PBS) was added to the wells, and the plates were placed for 4 h in dark. After that, the media were separated and 100 µL of DMSO was added to the wells for dissolving the formazan crystals. The optical density was read at 570 nm using an Awareness Technology (USA) ELISA reader. All experiments were repeated three times at sterile conditions.

Quantitation of DXL by HPLC

The concentration of DXL in the nano-niosomes was determined using a Waters HPLC (USA), a UV-vis detector and a Eurospher C18 column (5 µm, 4.6 mm × 250 mm). A mixture of water:acetonitrile:methanol (5:30:65% V/V) was employed as a mobile phase at an isocratic condition with a 1.0 mL min^{-1} flow rate. The mobile phase was passed through a 0.22 µm membrane filter and degassed by an ultrasonic cleaner for 5 min before run. The separation/detection was done at ambient temperature with a run time of 5 min and a retention time of 3.2±0.2 min. The flow rate was 1.0 mL min^{-1}. 60-µL volumes of samples were injected with a detection wavelength of 244 nm. Standards were prepared with the mobile phase as a serial dilution in 25 to 1000 µg mL^{-1} of DXL and a standard calibration curve were plotted for DXL quantitation. The DXL concentration in the samples was found by the calibration curve.

Entrapment efficiency measurement

1.0 mL of DXL-Span20 and DXL-Span80 (1.0 mg mL^{-1}) were centrifuged at 13000 g for 15 min, and the supernatants that contained free drug were collected. The amount of DXL in supernatants was determined using HPLC. Then, entrapment efficiency (EE) was determined using the following equation:

EE=Weight of drug loaded/Weight of drug added (1)

In-vitro drug release study

The amounts of released DXL from DXL-Span20 or DXL-Span80 were measured using dialysis tubes containing 2.0 mL of nano-niosomes dispersed into 25 mL of PBS (pH=7.4) containing 0.1% (V/W) tween 80 at 37 ºC with agitation at a rotation speed of 100 rpm. Samples collected at predetermined times were analyzed by HPLC. All experiments were done in triplicate.

In-vivo drug release (in-vivo bioavailability) study

In-vivo drug release of DXL from DXL-Span20 or DXL-Span80 was evaluated in Sprague Dawley rats. The experimental procedures were confirmed by the Research Ethics Committees of the National Institute for Medical Research Development. In addition, a minimum number of animals necessary to achieve scientific and reliable data were employed, and high attempts were followed to reduce animal suffering. The rats were randomly separated into three groups (three animals in each group) that received 2.0 mL (2.5 mg kg^{-1}) of DXL in normal saline, DXL-Span20 and DXL-Span80, via intraperitoneal injection (I.P.). The animals were anaesthetized in a chamber saturated by ether. Blood samples of 0.5 mL from each animal were collected at certain times via heart puncture

(0.5, 1, 2, 4, 8, 12, 24 and 30 h after injection). Blood samples were immediately centrifuged at 4000 rpm for 15 min and the sera were refrigerated at -20 °C. Before determination of DXL in the sera, acetonitrile was added and vortexed for 10 min to separate the plasma proteins, and the supernatant was taken.

Statistical analysis

All quantities were expressed as mean±standard deviation after three separated measurements. Differences between the experimental values were evaluated with one-way analysis of variance (ANOVA) followed by the Turkey-Kramer multiple comparison test. All statistical analyses were performed using SPSS 22 software, and *P*-values of <0.05 were considered as statistically significant.

Results

Physicochemical characterization of the nano-niosomes

Figure 1 shows a presentation of sizes of different nano-niosomes in a comparison manner. The hydrodynamic diameter of all the formulations was nearly the same in a range of 84-90 nm.

Zeta potential of the nano-niosomes was measured; it was -36, -31, -29 and -27 mV for b-Span20, b-Span80, DXL-Span20, and DXL-Span80, respectively.

To measure EE of DXL into DXL-Span20 and DXL-Span80, a standard curve for DXL quantitation was first plotted, as shown in Figure 2. The calibration plot represented a good linearity with a regression equation as y=(0.106±0.002)x+(2.24±0.687) and a square correlation coefficient equal to 0.999. Then, DXL content of the nano-niosomes was measured, and EE of DXL was obtained as 69.6 and 74.0% for DXL-Span20 and DXL-Span80, respectively.

In-vitro DXL release analysis

Drug release patterns into the administration environment are important for selection and evaluation of a carrier. For this purpose, dialysis experiments were performed for a DXL solution, DXL-Span20, and DXL-Span80 to evaluate drug release at different times. The in-vitro release patterns of DXL-Span20 and DXL-Span80 were determined and presented in Figure 3.

Cytotoxicity analysis

Cytotoxicity estimation of free drug and drug loaded in the synthesized nano-niosomes

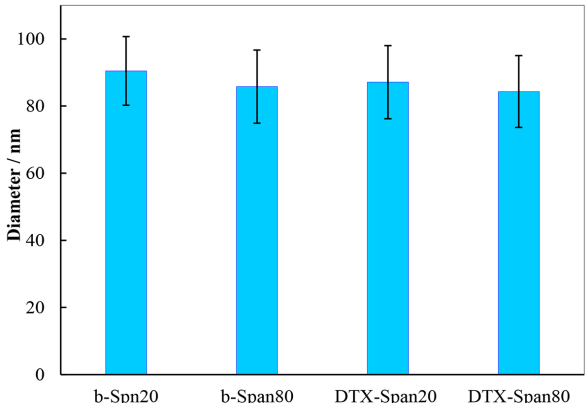

Figure 1: Presentation of sizes of docetaxel (DXL)-free (b-Span20, b- Span80) and DXL-loaded (DXL- Span20, DXL- Span80) nano-niosomes.

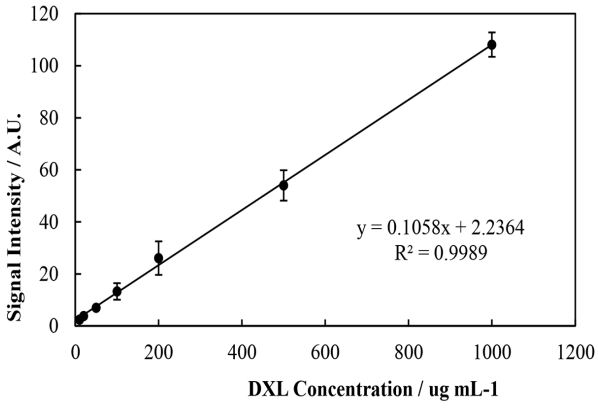

Figure 2: A standard plot for docetaxel (DXL) quantitation by high-performance liquid chromatography (HPLC).

was evaluated using the MTT assay after 48 h of incubation in three tumor cell lines of MDA-MB-231, Calu-6 and AsPC-1. Groups of each cell without treatment with DXL or nano-niosomes were considered as controls with a viability of 100%. The results of cytotoxicity evaluation are presented in Figure 4.

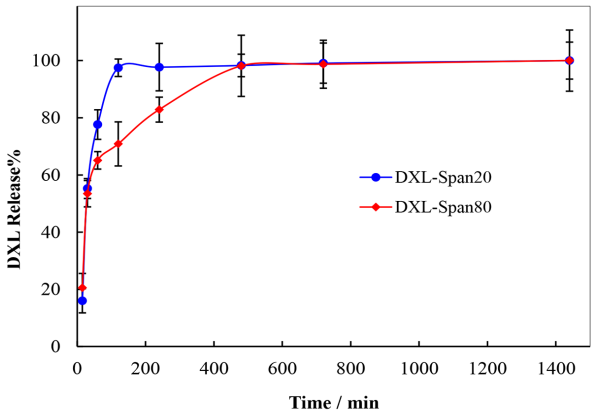

Figure 3: In-vitro release patterns of docetaxel (DXL)-Span20 and DXL-Span80

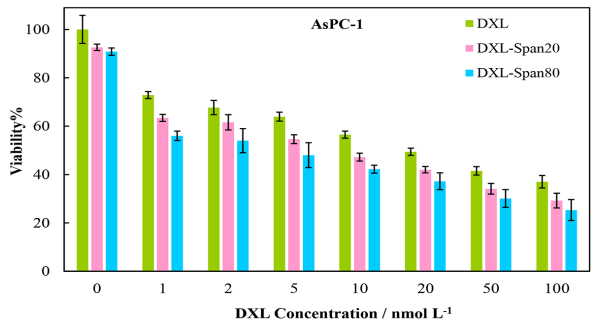

Figure 4: Viability of MDA-MB-231, Calu-6 and AsPC-1 cell lines upon treatment with different concentration of docetaxel (DXL) in different formulations.

Based on the results, b-Span20 and b-Span80 had no toxicity effect on the cell lines with viabilities >91% (with no significant difference with controls, $P>0.05$), confirming biocompatibility trait of the carriers. On the other hand, DXL loading into the nano-niosomes led to enhancement in its effectiveness to kill the cell lines with higher efficacy attained from DXL-Span80. Using these results, we calculated half-maximal inhibitory concentration (IC50) values for the formulations, as presented in Table 1.

In-vivo DXL bioavailability analysis

The bioavailability of DXL attained from DXL-Span20 and DXL-Span80 was evaluated after administration of a 2.5 mg kg^{-1} dose via intraperitoneal (IP) administration in rats. For this purpose, the plasma samples were collected after administration, and DXL content of plasma was measured by the HPLC calibration plot (Figure 2). Figure 5 shows the mean concentrations-time profiles of DXL in the plasma after IP administration of DXL-Span20 and DXL-Span80. Pharmacokinetic parameters were measured using the profiles data, as presented in Table 2.

Discussion

Physicochemical characterization of the nano-niosomes indicated that the hydrodynamic diameter of all the formulations was nearly the same. Therefore, the type of the surfactant used, as well as loading of DXL, did not have a deep effect on the vesicles size. DXL loading induced a slight positive shift in zeta potential

Table 1: Half-maximal inhibitory concentration (IC50) values for different formulations against different cell lines in nmol L^{-1}.

	MDA-MB-231 cell line	Calu-6 cell line	AsPC-1 cell line
DXL	12	8.7	20
DXL-Span20	4.7	3.4	7.2
DXL-Span80	2.4	1.7	3.1

DXL: Docetaxel

of the nano-niosomes, and the zeta potentials with negative values indicated a great stability with no significant aggregation in aqueous solution and minor carrier protein capturing.

EE of DXL indicated that DXL-Span80 carried a bit more DXL and would be a better formulation.

The drug release results indicated that after about 30 min, burst releases were started for both formulations (DXL-Span20, and DXL-Span80), with a significant slower rate for DXL-Span80. The release patterns showed complete DXL release after about 480 min, and DXL-Span80 represented a sustained DXL release.

The IC50 values of the formulations that obtained from cytotoxicity measurement, are in the ranges reported elsewhere [37, 38], indicating that DXL loading into the nano-niosomes led to decrement of at least 2.5 times and at most 6.5 times in the DXL toxicity against the cell lines. It should also be noted that the higher effectiveness of DXL-Span80, compared to DXL-Span20, was in line with the release patterns of the formulations presented above.

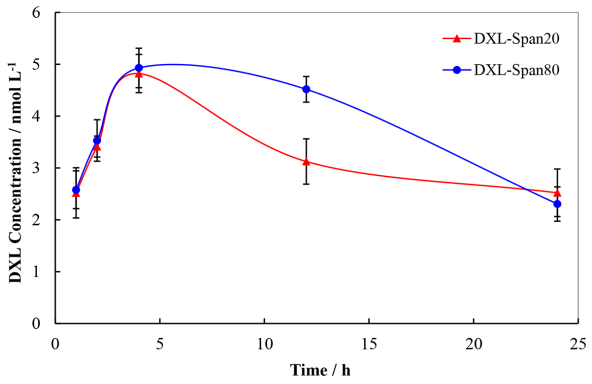

Figure 5: Mean concentrations-time profiles of docetaxel (DXL) in plasma after intraperitoneal (IP) administration of DXL-Span20 and DXL-Span80 in rat.

The bioavailability of DXL attained from DXL-Span20 and DXL-Span80 after administration of a single dose via IP administration in rats. The profiles of DXL content of plasma indicated that DXL-Span80 represented higher DXL plasma level, in accordance with the results obtained for EE determination, in-vitro DXL release analysis, and cell cytotoxicity assessment. The results of pharmacokinetic parameters confirm the higher effectiveness of DXL-Span80, compared to DXL-Span20.

Conclusion

Extensive research is being conducted for development of drug nanovehicles aiming at improvement permeability, delivery and efficacy, and decrement of the side effects. Here, nanoniosomal formulations for DXL were prepared using simple, low-cost components, and a simple method, and evaluated in terms of size, EE, release patterns, cytotoxicity and bioavailability. Carrying DXL by the nano-niosomes led to enhanced cytotoxicity (and lower IC50 values) and higher efficacy with enhanced pharmacokinetic parameters. Preclinical studies would provide more data and information regarding the utility of the presented formulations in animal models.

Acknowledgment

Research reported in this publication was supported by Elite Researcher Grant

Table 2: Pharmacokinetic parameters obtained for docetaxel (DXL), DXL-Span20 and DXL-Span80 upon intraperitoneal (IP) administration in rat.

	DXL-Span20	DXL-Span20
Maximum concentration (C_{max} (nmol L^{-1}))	4.8±0.4	4.9±0.4
Maximum time (T_{max} (h))	4	4
Area under the curve (AUC_{0-24} (nmol h L^{-1}))	11.5±0.9	12.1±0.8

DXL: Docetaxel

Committee under award number [987893] from the National Institute for Medical Research Development (NIMAD), Tehran, Iran. We would also like to thank the Research Council of Shiraz University of Medical Sciences.

Authors' Contribution

N. Sattarahmady, H. Heli and Kh. Karimian conceived the original idea. N. Sattarahmady and H. Heli supervised the project. M. Karimi played a pivotal role in designing the procedure and fabrication of the nano-niosomes. MR. Ajdari and A. Ranjbar actively participated in data collection and writing the manuscript. All the authors read, modified, and approved the final version of the manuscript.

Ethical Approval

The national ethics committee confirmed the study with the ethical code of IR.NIMAD.REC.1399.037. We did not perform any intervention in therapeutic procedures. Therefore, gathering the consent forms was waived due to the nature of this study.

Conflict of Interest

None

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