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α-Tocopherol succinate improves encapsulation and anticancer activity of doxorubicin loaded in solid lipid nanoparticles

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ABSTRACT

This work aimed to develop solid lipid nanoparticles (SLN) co-loaded with doxorubicin and α-tocopheryl succinate (TS), a succinic acid ester of α-tocopherol that exhibits anticancer actions, evaluating the influence of TS on drug encapsulation efficiency. The SLN were characterized for size, zeta potential, entrapment efficiency (EE), and drug release. Studies of in vitro anticancer activity were also conducted. The EE was significantly improved from 30 ± 1% to 96 ± 2% for SLN without and with TS at 0.4%, respectively. In contrast, a reduction in particle size from 298 ± 1 to 79 ± 1 nm was observed for SLN without and with TS respectively. The doxorubicin release data show that SLN provide a controlled drug release. The in vitro studies showed higher cytotoxicity for doxorubicin-TS-loaded SLN than for free doxorubicin in breast cancer cells. These findings suggest that TS-doxorubicin-loaded SLN is a promising alternative for the treatment of cancer.

1. Introduction

Doxorubicin is one of the most-used antitumor drugs, being highly active against an extensive variety of neoplastic diseases [1,2]. However, cell resistance to this drug and its side effects, such as cardiotoxicity, are the major limitations for a successful treatment [3–6].

The superiority of combination therapy with two or more drugs to improve cancer treatment has been well known. So, the combination of doxorubicin, an anthracycline, with other cytotoxic agents is an interesting alternative to maximize its antitumor efficacy. Anthracycline-based combination chemotherapy regimens have shown improvement in activity compared to a single anthracycline, including doxorubicin combined with fluorouracil, ifosfamide, dacarbazine, cyclophosphamide, and taxol [7–9].

Drug nanocarriers such as liposomes, solid lipid nanoparticles (SLNs), and polymeric micelles are also an alternatives to increase activity of doxorubicin. These systems present the potential to enhance concentration of drugs in the tumor, a mechanism known as the enhanced permeability and retention (EPR) effect, which is improved by smaller particles (100–200 nm) [10]. Compared with other drug nanocarriers, SLN present some advantages, most notably the fact that their production is easily transposable to industrial scale since they do not require the use of organic solvents [11–13].

However, the encapsulation of hydrophilic drugs such as doxorubicin in SLN is usually low and the formation of an ion pairing with a lipophilic contra-ion has been proposed as an alternative to increase the drug encapsulation [14–17]. Using a lipophilic anion that also presents antitumor activity represents an interesting alternative for the design of SLN loaded with doxorubicin. In fact, our previous studies demonstrated that docosahexaenoic acid (DHA), a polyunsaturated fatty acid that enhances the activity of

Abbreviations: SLN, solid lipid nanoparticles; EPR, enhanced permeability and retention; DHA, docosahexaenoic acid; TS, α-tocopheryl succinate; TEA, Triethanolamine; DMEM, modified eagle medium; CTB, Blue assay kit; OP, oily phase; AP, aqueous phase; NTA, nanoparticle tracking analysis; DLS, dynamic light scattering; PI, polydispersity index; THF, tetrahydrofuran; MeOH, methanol; EE, encapsulation efficiency; DL, drug loading; C_T, total doxorubicin concentration in SLN; C_{AP}, doxorubicin concentration in aqueous phase; W_{DL}, mg of drug loaded in nanoparticles; W_{NP}, g of nanoparticles (lipids); TG, Thermogravimetric analysis; DSC, differential scanning calorimetry; CR, released fraction; Doxo, doxorubicin.

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the doxorubicin, improved the doxorubicin encapsulation in SLN [18].

Recently, a vitamin E derivative, the α-tocopheryl succinate (TS), a succinic acid ester of α-tocopherol, was suggested to have promise as a material with antitumor activity. TS is the most effective form of vitamin E analogues causing inhibition of proliferation and apoptosis of cancer cells [19]. TS has been shown to suppress tumor growth in several preclinical animal models, including mice with experimental breast, bladder, prostate, and neck carcinomas [20–23]. In addition, TS enhances anticancer efficiency of doxorubicin [24].

We hypothesized that TS could be an interesting alternative to improve doxorubicin encapsulation in SLN, while enhancing its cytotoxicity. Therefore, this study aimed to develop an SLN co-loaded with doxorubicin and TS, evaluating the physicochemical characteristics, drug release, and the cytotoxicity against breast cancer cell lines in a monolayer model. The in situ formation of an ion pairing between cationic doxorubicin, which has a basic amino group, and the anionic TS, was also evaluated.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride was purchased from ACIC Chemicals (Ontario, Canada). α-Tocopherol succinate (TS) was purchased from Sigma–Aldrich (St. Louis, USA). Triethanolamine (TEA) was obtained from Merck (Darmstadt, Germany). Glyceryl behenate (Compritol 888 ATO) and monooleate of sorbitan ethoxylated (Super refined Polysorbate 80™; Tween 80™) were kindly provided by Gattefossé (Saint Priest, France) and Croda Inc. (Edison, USA), respectively. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, penicillin, streptomycin, and trypsin EDTA 0.25% were purchased from Gibco-Invitrogen (Grand Island, USA). Cell Titter Blue assay kit (CTB) was purchased from Promega (Wisconsin, EUA). All other chemicals were of analytical grade.

2.2. Preparation of solid lipid nanoparticles (SLN)

SLN were prepared by the hot melting homogenization method using an emulsification-ultrasound. The composition of the SLN was based on previous studies [18]. Briefly (batch 10 ml), the oily phase (OP) was composed of 150 mg of Compritol,100 mg of Tween 80, TS (0, 0.2, 0.4 or 0.8% w/v), 10 mg of doxorubicin, and 6 mg of TEA. The aqueous phase (AP) was composed of purified water. First, the OP without ST was heated to 80 °C while the AP was also heated separately to the same temperature. TS was added after OP melting. With the temperature maintained at 80 °C, AP was gently dropped onto the OP under constant agitation, at 8000 rpm, with an Ultra Turrax T-25 homogenizer (Ika Labortechnik, Germany). This emulsion was immediately submitted to the high intensity probe sonication for 10 min [18]. The pH of the SLN was adjusted to 7.0 with a solution of 0.1 M NaOH and the formulations were stored at 4 °C, protected from light in a nitrogen atmosphere.

The influence of the formation of an ion pairing between doxorubicin, a cationic amphiphilic drug, and anionic lipophilic TS was investigated. Fig. 1 shows the reactions proposed, where (A) represents the conversion of doxorubicin hydrochloride into free base doxorubicin with TEA (doxorubicin/TEA molar ratio was 1:2), (B) corresponds to the formation of the ion pairing between doxorubicin and TS (doxorubicin:TS molar ratio was of 1:0; 1:2; 1:4, and 1:8), and (C) is the reaction of the TS when added in excess to the TEA.

2.3. Measurements of particle size and zeta potential

The mean particle diameter and zeta potential were measured by dynamic light scattering (DLS) and electrophoretic mobility, respectively, using the Zetasizer Nano-ZS90 (Malvern Instruments; England) with a fixed angle (90°) laser beam at 25 °C. The SLN dispersions were diluted in distilled and filtered water (cellulose ester membrane, 0.45 mm, Millipore, Billerica, MA, USA). The data reported were particle size, evaluated as the intensity obtained from three repeat measurements, and the polydispersity index (PI).

2.4. Nanoparticle tracking analysis (NTA)

Nanoparticle tracking analysis (NTA) experiments were performed using NanoSight LM 10 & NTA 2.2 Analytical Software (Nanosight Ltd., Amesbury, UK). After appropriate dilution of the SLN in ultrapure water, the sample was introduced into the Nanosight sample chamber with a disposable syringe. The samples were measured at room temperature for 60 s with automatic detection. The particles suspended in a fluid were irradiated by a laser source, and scattered light and the image were captured by a charge-coupled device camera.

2.5. Drug encapsulation efficiency (EE) and drug loading (DL)

The encapsulation efficiency of doxorubicin and drug loanding in SLN was determined by ultrafiltration method using centrifugal devices (Amicon® Ultra—4 100 k, Millipore, USA) with a 100 kDa molecular weight cut-off membrane [16]. The drug concentration in samples was determined by a validated UV–vis spectrophotometric method (UV-mini 1240; Shimadzu, Japan) at 480 nm [15]. Regression equation and linearity (r^2) were $y = 0.02\,x + 0.0168$ and 0.9999, respectively. The accuracy was found to be $102 \pm 4\%$ ($n = 3$) and samples of blank SLN revealed that there are no UV absorbing substances. To evaluate the total concentration of doxorubicin in SLN, an aliquot of SLN dispersion was dissolved in a mixture of tetrahydrofuran (THF)/methanol (MeOH) 40:60 v/v. This mixture, which keeps doxorubicin in solution but causes lipid precipitation, was centrifuged for 10 min at 2400 g and the supernatant was analyzed. To evaluate the non-encapsulated doxorubicin, an aliquot (2 ml) of the SLN was submitted to ultrafiltration (10 min at 2400 g) to separate the aqueous phase from particles. Next, the aqueous phase was diluted in THF/MeOH 40:60 (v/v) and analyzed by UV–vis spectrophotometer.

To eliminate the binding of doxorubicin on the devices, a pretreating of the filters was performed as previously described [18]. The devices were soaked in a passivating solution (Tween™ 20, 5% w/v), maintained overnight at room temperature, and washed with distilled water prior to use. The EE and DL were calculated using the following equation:

$$EE\% = \frac{(C_T - C_{AP})}{C_T} \times 100$$

$$DL_{(mg/g)} = \frac{W_{DL}}{W_{NP}}$$

where C_T = total doxorubicin concentration in SLN, C_{AP} = doxorubicin concentration in aqueous phase (nonencapsulated). W_{DL} = milligram of drug loaded in nanoparticles and W_{NP} = gram of nanoparticles (lipids).

2.6. Thermal analysis

Differential scanning calorimetry (DSC) patterns of the samples were collected on a DSC-60 differential scanning calorimeter (Shi-