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Original article

Doxorubicin-loaded nanocarriers: A comparative study of liposome and nanostructured lipid carrier as alternatives for cancer therapy

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ABSTRACT

Nowadays cancer is one of the most common causes of deaths worldwide. Conventional antitumor agents still present various problems related to specificity for tumor cells often leading to therapeutic failure. Nanoscale particles are considered potential alternative to direct access of drugs into tumor cells, therefore increasing the drug accumulation and performance. The aim of this study was to evaluate the antitumor activity of doxorubicin (DOX)-loaded nanostructured lipid carriers (NLC) *versus* liposomes against a breast cancer animal experimental model. NLC-DOX and liposomes-DOX were successfully prepared and characterized. Tumor-bearing mice were divided into five groups (blank-NLC, blank-liposome, DOX, NLC-DOX, liposome-DOX). Each animal received by the tail vein four doses of antitumoral drugs (total dose, 16 mg/kg), every 3 days. Antitumor efficacy was assessed by measuring 1) tumor volume, calculating the inhibitory ratio (TV-IR, see after) and 2) acquiring scintigraphic images of the tumor using doxorubicin radiolabeled with technetium-99m as an imaging tumor probe. Liposome-DOX and free DOX did not showed differences in the tumor mean volume, whereas NLC-DOX proved to be the best treatments in controlling the tumor growth. NLC-DOX showed an inhibition ration (TV-IR) of 73.5% while free DOX and liposome-DOX decreased TV-RI of 48.8% and 68.0%, respectively. Tumor was clearly visualized in controls, DOX, and liposome-DOX groups. Yet, regarding the NLC-DOX group, tumor was barely identified by the image, indicating antitumor efficacy. Moreover, both NLC and liposomes proved to be able to delay the occurrence of lung metastasis. In conclusion, results of this study indicated that NLC-DOX might be an alternative strategy to achieve an efficient antitumor activity.

1. Introduction

Cancer is becoming a major cause of morbidity and mortality worldwide [1]. Conventional chemotherapeutic agents are distributed non-specifically in the body where they affect both cancerous and normal cells. Consequently, many undesirable effects have been reported using these drugs, often reducing their use [2]. Moreover, it is well known that inherent and acquired resistance pathways frequently developed in tumor cells account for the high rate of failure in cancer treatment [3].

Most solid tumors possess unique pathophysiological characteristics, such as extensive angiogenesis and hence increased blood flow due to increased vascularisation architecture, and reduced lymphatic drainage. This is known as the enhanced permeability and retention (EPR) effect and is one of the main tumor characteristics that become a gold standard in antitumor drug delivery [4,5].

The use of nanoparticles in cancer treatment is receiving even more attention once these systems may provide a new model for drug delivery to cancer reducing side effects to normal tissues. Nanoscale particles are allowed to pass through the porosity presents in tumor vasculature allowing a direct access to tumoral

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cells [6]. Furthermore, nanoparticles might show additional advantages, such as a) improve the solubility of hydrophobic compounds, b) protect molecules from undesirable interactions with biological components and thus improving antitumor drug stability, provide controlled release of drugs, and favorably modify pharmacokinetics and biodistribution [7].

Among these nanoparticles, liposomes are the most studied class being defined as spherical lipid vesicles composed of amphiphilic phospholipids with a bilayer membrane structure having central aqueous space. This system is able to incorporate both hydrophilic and lipophilic drugs [8]. Currently, there are some liposomal products commercially available for cancer therapy, that include the liposomal formulations of doxorubicin (Doxil), daunorubicin (DaunoXome), cytarabine (DepoCyt), and vincristine (Onco-TCS) [9]. However, the number of products is far behind the expectations due to the relatively high costs of preparation [10].

Alternatively, nanostructured lipid carriers are a class of particulate drug carriers derived by a mixture of lipids that remain solid and liquid at room and body temperatures [11]. Lipids utilized for NLC are typically physiological lipids and a wide variety of biocompatible surfactants are characterized by high stability. Therefore, NLC have the advantage of physical stability with low toxicity. NLC are becoming increasingly used for the protection of labile drugs from degradation in the body and for increase the release in the target tumoral tissue [11–13]. Moreover, the absence of organic solvent in their preparation along with the relatively low cost make this NLC are even more taken into account as an alternative to overcome the difficulties above faced with liposome translational researches.

The aim of this study was to evaluate the antitumor activity of both NLC and liposomes doxorubicin-loaded. To achieve this goal, doxorubicin was encapsulated into liposomes and NLC. Antitumor efficacy was assessed by measuring the tumor volume, calculating the inhibitory ratio (TV-IR) and acquiring scintigraphic images in an experimental breast cancer murine model (4T1 cell line).

2. Material and methods

2.1. Material

Doxorubicin hydrochloride (DOX) was purchased from ACIC Chemicals (Ontario, Canada). Ethylenediamine tetraacetic acid sodium (EDTA), cholesterol (CHOL) and $SnCl_2{\cdot}2H_2O$ were purchased from Sigma–Aldrich (São Paulo, Brazil). 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. The phospholipids hydrogenate soy phosphatidylcholine (HSPC), and distearoylphosphatidyl-ethanolamine polyethyleneglycol2000 (DSPE-PEG_{2000}) were supplied by Lipoid GmbH (Ludwigshafen, Germany). Technetium-99m (^{99m}Tc) was obtained from an alumina-based $^{99}Mo/^{99m}Tc$ generator from IPEN (São Paulo, Brazil). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, penicillin, streptomycin and trypsin EDTA 0.25% were purchased from Gibco-Invitrogen (Waltham, MA, USA). All other chemicals were of analytical grade.

The subcutaneous tumor model was established in 6–8-week-old female BALB/c mice purchased from CEBIO-UFMG (Belo Horizonte, Brazil). All animal studies were approved by the local Ethics Committee for Animal Experiments (CEUA/UFMG).

2.2. Liposome preparation

Aliquots of HSPC, CHOL, and DSPE-PEG_{2000} in chloroform at a lipid concentration of 40 mM (molar ratio 5.7:3.8:0.5) were transferred to a flask. The solvent was removed in a rotatory evaporator in order to form a thin lipid film. The resulting film was hydrated with ammonium sulfate solution (300 mM), at room temperature, under continuous stirring. Liposomes were submitted to a filtration through 0.4-, 0.2-, and 0.1-μm polycarbonate membranes (5 cycles for each). Empty liposomes were incubated with a solution of doxorubicin (2 mg/ml), at 60 °C, for 1 h. By this method, drug loading was achieved via ammonium sulfate gradient. Free DOX was removed by ultracentrifugation (2 h, 150000*g*, 4 °C).

2.3. NLC preparation

NLC were prepared by the hot melting homogenization method using an emulsification-ultrasound. Briefly, the oil phase (OP), composed by compritol ATO 188, sesam oil, tween 80, and the aqueous phase (AP), composed by EDTA and purified water, were heated, separately, to 80 °C. AP was gently dropped onto the OP under constant agitation (8000 rpm, 2 min) with an Ultra Turrax T-25 homogenizer (Ika Labortechnik, Germany), followed by sonication (20% amplitude, 10 min), using a high intensity ultrasonic processor (Cole-Palmer Instruments, USA). Afterwards, the suspension was cooled to room temperature and the formulations were stored at 4 °C, protected from light. Doxorubicin (0.5 mg/ml) was entrapped in the lipid matrix due to an ion-pair formation with oleic acid.

2.4. Characterization

The mean particle diameter was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments, UK), at a fixed angle of 90° and 25 °C. Zeta potential measurements were carried out by DLS associated with electrophoretic mobility, at a temperature of 25 °C. All measurements were performed in triplicate. The encapsulation efficiency (EE) of doxorubicin in NLC was determined by ultrafiltration method using centrifugal devices as describe by Mussi et al. [14]. For the liposomes, doxorubicin was quantified by HPLC, using methanol: phosphate buffer pH 3.0 (65:35) as the mobile phase, and a reversed-phase C_8 column with fluorescence detection [15].

2.5. Cell culture

4T1 cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% (v/v) fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml). Cells were kept in humidified air containing 5% CO_2 at 37 °C. The cells were grown to confluence and harvested by trypsinization. After centrifugation (5 min at 330*g*), the cells were re-suspended in phosphate buffer saline (PBS) for tumor inoculation.

2.6. Tumor cell inoculation

Aliquots (100 μl) of 2.5×10^6 4T1 cells in Phosphate-Buffered Saline (PBS) were injected subcutaneously into the right thigh of female BALB/c mice (18–22 g). Tumor cells were allowed to grow *in vivo* for 5 days before the treatment, when once the tumor volume reached about 50 mm^3.