Biocompatible Microemulsion Modifies the Tissue Distribution of Doxorubicin

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ABSTRACT: The incorporation of doxorubicin (DOX) in a microemulsion (DOX-ME) has shown beneficial consequences by reducing the cardiotoxic effects of DOX. The aim of this study was to determine the distribution of DOX-ME in Ehrlich solid tumor (EST) and the heart, and compare it with that of free DOX. The distribution study was conducted with female Swiss mice with EST ($n = 7$ per group; 20–25 g). Animals received a single dose (10 mg/kg, i.p.) of DOX or DOX-ME 7 days after tumor inoculation. Fifteen minutes after administration, the animals were sacrificed, and the tumor and heart tissues were taken for immediate analysis by ultra-performance liquid chromatography. No difference was observed in DOX concentration in tumor tissue between DOX and DOX-ME administration. However, the most remarkable result in this study was the statistically significant reduction in DOX concentration in heart tissue of animals given DOX-ME. Mean DOX concentration in heart tissue was 0.92 ± 0.54 ng mg^{-1} for DOX-ME and 1.85 ± 0.34 ng mg^{-1} for free DOX. In conclusion, DOX-ME provides a better tissue distribution profile, with a lower drug concentration in heart tissue but still comparable tumor drug concentration, which indicates that antitumor activity would not be compromised. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:3297–3301, 2014

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INTRODUCTION

Doxorubicin (DOX) is an anthracycline antibiotic with a broad antitumor spectrum used both as a single agent and in combination regimens.[1] DOX is currently on the market as the free drug and liposome-encapsulated drug, but research continues with DOX. For instance, it is in clinical trials with some forms of polymeric drug[2] and in animal studies with a biocompatible microemulsion.[3]

The main problem associated with the use of the free form is cardiomyopathy.[4] Acute cardiomyopathy may occur immediately after or during administration of a single dose and is a result of drug accumulation in heart muscle.[4] Acute cardiomyopathy occurs in up to 40% of the patient population, where the prognosis is generally good, may be completely asymptomatic, and usually resolves spontaneously.[5] Chronic cardiomyopathy is more common and is characterized by congestive heart failure unresponsive to digoxin, as a result of successive deleterious effects on cardiac tissue with repeated doses.[5] According to Octavia et al.,[4] the mortality rate in patients with chronic DOX-induced cardiotoxicity is 50% after 5 years.

The encapsulation of DOX in liposomes has been associated with decreased cardiotoxicity[6,7] and changes in its pharmacokinetic profile. These formulations were developed with the aim of achieving a better clinical response and less toxicity. However, these drugs are often discontinued due to severe side effects.[8] The toxicity of encapsulated DOX depends on the lipids used in liposome formulation.[5] For instance, the inclusion of phosphatidylserine or polyethylene glycol (PEG) in the formulation has been related to changes in the mononuclear phagocyte system (MPS).[5,8,9] This occurs because the liposomes are taken up by cells of the MPS, and DOX exerts its toxic effect within these cells. MPS toxicity may be of concern in immunosuppressed patients, such as those with AIDS.[5] Another liposomal formulation containing PEG causes palmar-plantarerythrodysesthesia (PPE) due to the accumulation of DOX in the skin.[5] PPE is a painful scaly dermatitis that primarily affects the hands and feet. PPE was the most common adverse effect related to liposomal DOX and occurred in 49% of patients in a phase II trial in ovarian cancer.[9] Although PPE is attributed mainly to pegylated formulations, there are reports of cases with non-pegylated formulations.[10]

According to Patel,[1] "the tumor tissue is a dynamic microenvironment and efforts to improve therapeutic efficacy might be achieved by modifying these dynamic processes to enhance drug delivery." The delivery of the drug to normal tissue is the cause of occurrence of various adverse effects, as well as its limited distribution in tumor tissues resulting in tumor drug resistance and treatment failure. Accordingly, in previous works, we described different microemulsion formulation specially designed for DOX incorporation,[11–14] we found that the pharmacokinetic profile of DOX in a microemulsion (DOX-ME) showed significant differences compared with that for free DOX in Wistar rats, with beneficial consequences regarding cardiotoxic effects.[3]

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The administration of DOX-ME in Wistar rats has been shown to result in higher plasma concentration of DOX and lower volume of distribution when compared with the administration of DOX in the free form, and increased serum creatine kinase MB (CKMB) activity in the DOX group but unchanged CKMB activity in the DOX-ME group.[3] These results suggest modifications in drug access to susceptible sites using DOX-ME. The aim of this study was to determine the distribution of DOX-ME in Ehrlich solid tumor (EST) and heart in female Swiss albino mice to compare it with free DOX. This information is essential for the assessment of product safety for future clinical application.

METHODS

Chemicals and Reagents

Soya phosphatidylcholine (SPC) (Epikuron™ 200, Germany) was purchased from Degussa Texturants Systems Deutschland GmbH & Company (Hamburg, Germany); cholesterol (CHO) and sodium oleate (SO) from Sigma–Aldrich (St. Louis, Missouri); Tris (hydroxymethyl) aminomethane from Merck (Darmstadt, Germany); DOX hydrochloride (Adriamycin™) from Eurofarma (Sao Paulo, Brazil); daunorubicin hydrochloride [DAU—internal standard (IS)] from Pfizer (Sao Paulo, Brazil), polyoxyethyleneglyceroltrihydroxystearate 40 (EU; Eumulgin® HRE 40) from Pharma Special (Sao Paulo, Brazil); and acetonitrile and methanol, HPLC grade, from J.T. Baker (Phillipsburg, New Jersey, United States). All other solvents and chemicals were analytical grade. Water was purified in a Milli-Q Plus system (Millipore, Billerica, Massachusetts, United States) with 18.2 MΩ-cm resistivity.

Microemulsion Preparation

The microemulsion preparation was carried out as described by Assumpção et al.,[3] which started with the addition of CHO, the oil phase, to the semisolid mixture of SPC/EU/SO, the surfactant phase. The aqueous phase (80%, w/w) was then added to this mixture, which was homogenized by ultrasound for 10 min and allowed to stand for 24 h at 25 ± 0.1°C to reach complete equilibrium.

The samples were centrifuged at 8500*g* for 15 min (Ultracentrifuge Hitachi Himac CP-80) to remove titanium residues that might have been released from the ultrasound tip. Finally, a suitable amount (1.5 mg mL^{-1}) of DOX was dissolved directly in the previously prepared ME.

EST Inoculation

A model of EST was used, where 2×10^6 Ehrlich ascites tumor (EAT) cells were implanted subcutaneously in the thigh of the left hind limb of mice. A solid tumor mass developed within 7 days. EAT cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation every 7 days. This procedure was carried out at the Laboratory of Clinical Immunology of the School of Pharmaceutical Sciences, Sao Paulo State University, Araraquara, Brazil. Cell viability was evaluated by the trypan blue exclusion test, and only the cell suspensions that showed 95% viability were employed.

Distribution Study of free DOX and DOX Incorporated in Microemulsion

A preclinical drug distribution study was conducted in female Swiss mice with EST ($n = 7$ per group; weighing 20–25 g). The sample size was based on the method reported by Chow and Wang.[15] The animals were housed at a constant temperature (24 ± 1°C), humidity controlled (55 ± 5%) and 12-h light cycle starting at seven a.m., with food and water *ad libitum*. The experiments were conducted during the light phase and the experimental protocol was approved by the Research Ethics Committee of the School of Pharmaceutical Sciences, UNESP, Araraquara, SP, Brazil (Process #60/2012). Animals received a single dose (10 mg kg^{-1}, i.p.) of DOX or DOX-ME. At 15 min after administration, the animals were sacrificed and the tumor and heart tissues were taken for immediate analysis. A pilot study had been conducted to select the time for extracting the heart. In that study, the organs were extracted at 15, 30, and 45 min after administration of the two DOX formulations. We observed that tissue DOX concentration peaked at 15 min, and thus, this time point was selected for the extraction.

DOX Analysis in Heart and Tumor Tissue by Ultra-Performance Liquid Chromatography

An ultra-performance liquid chromatography (UPLC) method for DOX determination in tumor and heart was developed and validated. This bioanalytical method detects free DOX, regardless of the formulation administered. The method was according to Alhareth et al.[16] Analysis was conducted using a Waters Acquity H-Class UPLC System® with fluorescence detector at $\lambda_{ex/em}$ of 480/560 nm. Chromatographic separation was carried out with a Waters Acquity CSH ®C18 column (100 × 2.1 mm^2, with 1.7 μm particle size) connected to a Waters Vanguard® C18 guard column (5 × 2.1 mm^2, with 1.7 μm, particle size). The mobile phase consisted of acetonitrile:formic acid 0.1% (40:60, v:v) in isocratic mode, with flow rate of 0.4 mL min^{-1}. The injection volume was 10 μL. The sample manager was maintained at 10°C.

The tissue samples were processed as follows. A 50-μL volume of IS (10 μg mL^{-1} daunorubicin) was added to 100 mg of tumor or heart tissue, followed by vortexing (30 s), and 100 μL 1 M Tris–HCl (pH 8.8) was then added, followed by vortexing (30 s). Afterward, 1 mL ethyl acetate was added and the sample was centrifuged for 15 min at 23,800*g*. The supernatant (900 μL) was filtered through a polyvinylidene difluoride membrane (0.22 μm) and evaporated to dryness under vacuum (Genevac mini VacSample Concentrator Range®). The residue was resuspended in 100 μL mobile phase along with 10 μL 35% perchloric acid and the solution filtered again directly into the injection vial of the chromatographic system. The blood was removed from the tissue with 10 mL saline before processing to reduce the interference of blood DOX with tissue DOX concentrations.

The bioanalytical method was validated according to the Food and Drug Administration—Guidance for Industry Bioanalytical Method Validation[17] and ANVISA—RE 899/2003[18] and RDC 27/2012.[19]

Statistical Analysis

The amount of DOX in tumor and heart tissue was expressed in ng mg^{-1} as median, mean, and 95% CI. Intergroup comparison was performed by the Mann–Whitney test (GraphPadPrism® software, version 5.0). The calibration curve and coefficient of