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The synthesis of tamoxifen-loaded albumin nanoparticles by homogenizers: Optimization and *in vitro* characterization

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ABSTRACT

The aim of this study was to develop mechanical homogenization processes to fabricate protein-based nanoparticles. The high-pressure homogenizer (HPH) and high-speed homogenizer (HSH); were used to encapsulate the hydrophobic drug, tamoxifen, in albumin nanoparticles. The results revealed that the rotational speed with HSH and the pressure with HPH were the main factors affecting the size, while increasing the residence time led to more homogenous nanoparticles. Seven homogenization cycles at 14917 psi and 8.24 min of mixing at 17360 rpm ensured a drug loading of 14.2 ± 1.9% and 11.6 ± 2.3% for HPH and HSH, respectively. We found a direct correlation between the obtained size and energy input and retention time with both homogenizing devices. The characteristics of the optimized nanoparticles were within the desired range to meet the requirements of intravenous injection. The surface morphology of the nanoparticles determined by transmission electron microscopy showed semi-spherical nanoparticle shapes. Further, the secondary structure of albumin in nanoparticles was determined via circular dichroism, which showed only slight structural changes versus native albumin, making it a promising, self-targeted drug delivery system. Finally, BT474 viability assays and western blot analysis showed the effectiveness of the tamoxifen-loaded albumin nanoparticles prepared via homogenization.

1. Introduction

The application of nanotechnology to treat cancer has produced promising new therapeutic strategies [1]. It is predicted that nanoparticle-based therapeutics will become the predominant delivery method for cancer treatment due to their higher selectivity, lower toxicity, enhanced efficacies, and longer clearance times compared to that of conventional systematic treatments [2,3]. Because many chemotherapeutic agents are hydrophobic, there has been great interest to encapsulate, protect, and release poorly soluble therapeutic agents in various nanocarriers, especially protein-based carriers [4,5]. The application of protein-based carriers has been extensive owing to their potential advantages over synthetic carriers, including low toxicity, high drug-binding capacity, remarkable uptake into the target cells, easy preparation, and scale-up capability [6].

Tamoxifen (TMX) is a poorly water-soluble drug used to treat estrogen receptor-positive breast cancers in pre- and post-menopausal women, which can reduce breast cancer death rate to one-third with its application [7,8]. Its use for breast cancer prevention in healthy women has also been reported [9]. TMX can interfere with DNA and alters or blocks subsequent mRNA transcription, leading to cellular apoptosis [10].

Studies have been conducted on TMX encapsulation in

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nanocarriers for intravenous administration using various methods [7,11–13]. Each method has its advantages and disadvantages, and many studies have developed and modified existing methods to fabricate protein-based nanoparticles (NPs) [14–16]. Nanoparticle albumin-bound (NAB) technology made with a high-pressure homogenizer (HPH) is the first nano-based, FDA-approved commercial drug carrier [1,17,18]. The FDA-approved, albumin-bound paclitaxel, Abraxane®, uses physical forces to encapsulate the lipophilic drug, paclitaxel, in an albumin carrier, which leads to fewer protein structural changes compared with that of solvent-based formulations. Moreover, loading of curcumin, a water insoluble anti-malarial agent, and paclitaxel in human serum albumin (HSA) with homogenization has been reported [19–22]. The use of a high-speed homogenizer (HSH) has also been proposed as a physical method to prepare NPs. Minimizing the use of chemical materials by using physical forces to fabricate NPs is a promising approach that needs to be studied in more detail. Homogenization at elevated pressures or speeds can be applied to prepare very small particles [1,18,20,23,24]. During the homogenization process, two main factors influence the loading of hydrophobic drug into albumin in the aqueous phase, the energy input and retention time [25]. The energy input in an HPH is the operational pressure, forcing the material through a very narrow gap, which creates the high acceleration and turbulence that are vital to make nanoemulsions. In addition, the number of cycles a crude emulsion passes the main valve could be considered the retention time [26,27]. In an HSH, the energy input is controlled by the rotor speed, and the mixing time at the desired speed is equal to the retention time. Understanding the effect of the HPH and HSH mechanical parameters is important for optimizing the energy efficiency of the homogenization devices to efficiently fabricate drug delivery systems that can improve pharmacokinetic and pharmacodynamic profiles of conventional therapeutics.

In this study, both HSH and HPH processes have been investigated to encapsulate TMX in HSA-NPs. Variables such as homogenizing factors and crude emulsion conditions influence the drug entrapment in albumin NPs. In this study, only the main homogenization parameters of each device, including the energy input and residence time, were selected to investigate NP fabrication. The size of the NPs and albumin structural alterations during fabrication were selected as response parameters to optimize. In addition, the polydispersity index (PDI) and yield were monitored in experiments to provide insight on the mechanism of action. It is important that NP size should be in the proper range to accumulate in the tumor via enhanced permeability and retention (EPR) effect [28]. Recent studies have shown that albumin may reach tumors by an EPR effect and by receptor-mediated transport, in which the HSA structure plays a fundamental role. According to radiolabeled drug experiments, it has been hypothesized that NAB formulation may achieve some tumor selectivity because of albumin-specific receptors in the tumor microenvironment [29]. Therefore, the preparation of albumin NPs with slight structural changes in HSA is the goal of this study. Alterations in HSA secondary structure in the obtained NPs and their cytotoxicity on the breast cancer's cell line, BT474, were investigated. Finally, western blot analysis was performed to predict the therapeutic effects of the synthesized NPs.

2. Materials and methods

2.1. Materials

Tamoxifen (*trans*-2-[4(1,2-diphenyl-1-butenyl)phenoxy]-*N,N*-dimethylethylamine, $C_{26}H_{29}NO$, Mw 371.51 Da) and human serum albumin lyophilized powder >96% (Mw 66437 Da) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, ethanol, and methanol (HPLC grade) were obtained from J.T. Baker (Philipsburg, NJ, USA). All other reagents were obtained from Sigma-Aldrich and used as received.

2.2. Design of experiments

2.2.1. Determination of the effective physical parameters

Most reports regarding the optimization of mechanical methods for microemulsions and nanoemulsions have been from the food industry [24,26,27,30]. Generally, two different categories of factors affect the final product of homogenization: 1) the device-based parameters (operational parameters), such as the energy input, residence time, fluid temperature during processing, changing of flow regime, and shear stresses due to influential forces, which complicate studies of the homogenization process [31]; and 2) the characteristics of oil/water emulsions, especially the properties and concentration of the materials.

In this study, the albumin and TMX concentrations were held constant. For the HPH, the applied pressure operating at the valve, which corresponds to the energy density, and the number of cycles, which is considered the residence time of the emulsion, were selected as the two key influential parameters. For HSH, the rotational speed and time of mixing were chosen to investigate the influence of energy and residence time, respectively.

The main mechanical factors affecting the TMX-HSA-NPs preparation process and their levels in the homogenization process are presented in Table 1. The lowest and highest pressure and rotational speed were set based on preliminary experiments and the maximum safe working power of the devices. The objective of this optimization was to synthesize TMX-HSA-NPs with desirable sizes between 100 and 200 nm. NPs less than 200 nm have low uptake by opsonization and show higher circulation times [32]. However, very small nanoparticles would cause side effects or accumulate in liver more than in the targeted tumor tissue [33].

2.2.2. Fitting the model via response surface methodology

Response surface methodology (RSM) was applied to determine the influence of physical factors on NP size and finally to determine the conditions to obtain the desired size of TMX-HSA-NPs. A central composite design was utilized to obtain the effects of homogenization pressure, the number of cycles for the HPH method, and the rotational speed and mixing time for the HSH method on the final sizes of NPs. For instance, in the HPH method, the homogenization pressure (X_1) and the number of cycles (X_2) were the independent variables and were coded according to the following equation:

$$X_i = \frac{x_i - x_i^*}{\Delta x_i} \quad (1)$$

X_i is the coded variable, x_i is the natural value of the ith parameter, x_i^* is the average of the high and low levels of the natural variable, and Δx_i is the step size. The same methodology was applied in the case of HSH, where K_1 and K_2 represent the coded values for independent variables of the rotational speed and nanoemulsion mixing time, respectively. The sizes of TMX-HSA-NPs prepared using the HPH and HSH technologies can be explained by the following second-order polynomial equations:

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{12} X_1 X_2 \quad (2)$$

$$Z = \beta_0 + \beta_1 K_1 + \beta_2 K_2 + \beta_{11} K_1^2 + \beta_{22} K_2^2 + \beta_{12} K_1 K_2 \quad (3)$$

Y and Z represent the size (nm) of TMX-HSA-NPs; α_0 and β_0 are the constant terms; α_1, α_2, β_1, and β_2 are the linear effect coefficients; α_{11}, α_{12}, β_{11}, and β_{22} are the quadratic coefficients; and