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Bovine serum albumin-based nanoparticles containing resveratrol: Characterization and antioxidant activity

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ABSTRACT

In this study, bovine serum albumin (BSA) nanoparticles containing resveratrol (RVT) were developed to improve the biopharmaceutical characteristics of the RVT. BSA nanoparticles containing RVT (RVT-NP) were obtained by the coacervation technique and presented mean diameter of 175 ± 6 nm, polydispersity of 0.130 ± 0.01, zeta potential of −37.4 ± 1.3 mV and encapsulation efficiency about 60%. Morphological analysis revealed spherical and smooth nanoparticles. In vitro release profile was prolonged and after 144 h about 26% of RVT were released. The release kinetic followed the second order model, characterized by a rapid initial release followed by a slower RVT release diffusion-controlled. Nanoencapsulation process resulted in RVT amorphization and chemical interactions between RVT and BSA. Nanoparticles were stable over 90 days at room temperature and under refrigeration (2–8 °C). In the hypochlorous acid scavenging activity, RVT-NP presented inferior activity than free RVT, probably due to the prolonged RVT release. Blank BSA nanoparticles also presented scavenging activity, thus, antioxidant activity of RVT-NP was an addition of the effects of the RVT and BSA. Thus, BSA nanoparticles are promising carriers for prolonged RVT release and application in oxidative stress-based diseases.

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1. Introduction

Free radicals are atoms or molecules present in many biochemical processes in the human organism and they are involved in energy production, phagocytosis, cell growth regulation, intracellular signaling and synthesis of important biomolecules [1]. However, their excess can cause deleterious effects to the organism, such as DNA damage, proteins, organelles and also on the membranes, resulting in changes in the structures and cellular functions. The serie of damage exerted by free radicals is called oxidative stress and its development is involved in several diseases, including cancer and neurodegenerative diseases [2–4].

Resveratrol (3,5,4'-trihydroxystilbene) (RVT) is a polyphenol of the stilbene class, and it is produced to protect the plants against photosynthetic stress, reactive oxygen species and herbivorous plants [5]. There is a growing interest in this compound, due to the several pharmacological effects exerted by it, such as cardioprotective [6,7], neuroprotective [8], anti-inflammatory [9] and antitumor [10–12]. Many of these effects are linked to its high antioxidant activity [13].

However, RVT properties are compromised when it is exposed to biological environment, due to the high metabolism that the molecule undergoes at a systemic level [14,15]. Therefore, for its in vivo application, it is necessary to develop a carrier system capable of transporting it across biological membranes and thereby increase its bioavailability. Thus, resveratrol can reach sufficient plasmatic levels to exert its biological effects [16].

In recent decades, several drug delivery systems have been developed and special attention has been given to the nanometric carrier system such as nanoparticles, dendrimers, liposomes, among others [17–19]. Nanoparticles improve drug physicochemical properties compared to conventional systems, and present several advantages, including: drug protection, gradual and controlled drug release; improved pharmacokinetics and significant decrease of drug toxicity [20]. Several studies have shown the improvement of the physicochemical and biological properties of RVT through nanoencapsulation [21–26].

Bovine serum albumin (BSA) is a protein with several drug binding sites, low-cost, non-immunogenic and naturally biodegradable, that has been applied as a matrix for nanoparticles-based drug delivery [27] [28]. The BSA nanoparticles properties could

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reflect on new therapeutic opportunities for various drugs, like RVT, which could not be effectively used in conventional formulations, due to problems related to their biopharmaceutical characteristics [17,29,30]. In this work, we propose the development and characterization of BSA nanoparticles containing RVT and evaluate their in vitro antioxidant activity.

2. Materials and methods

2.1. Materials

Trans-resveratrol was obtained from Pharmanostra (Brazil). Bovine serum albumin (BSA), 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate (dipotassium peroxydisulfate), 3,3−5,5-tetramethylbenzidine (TMB), polysorbate 80 and polyvinyl alcohol (PVA) were obtained from Sigma-Aldrich (USA). Ethanol 99.8% was purchased from Vetec—Química Fina (Brazil), glutaraldehyde solution (8%) was purchased from Vetec (Brazil), 0.1 M NaOH was purchased from Biotec (Brazil), phosphate buffered saline (PBS) and phosphate buffered (PB) pH 7.4 were obtained from Sigma reagent (USA). Water was purified using a Milli-Q Plus system (Millipore) with a conductivity of 18 MΩ.

2.2. Preparation of BSA nanoparticles containing resveratrol

Resveratrol-loaded BSA nanoparticles (RVT-NP) were obtained by the coacervation method, as described by KAMATH et al (2014) [31] with some modifications. Initially, 100 mg of BSA was solubilized in ultrapure water under constant stirring. The addition of 10 mg RVT followed two methods: i). Formulation 1: RVT was solubilized in 200 μL of ethanol and it was added drop by drop to the BSA solution and incubated in an orbital shaking incubator at 200 rpm, for 1 h under constant agitation, protected from light and at room temperature. After incubation, 6 mL of ethanol was added under constant stirring. ii) Formulation 2: RVT was solubilized in 6 mL of ethanol and added to the BSA solution drop by drop, under agitation, without prior incubation. For both formulations, after the desolvation process, 50 μL of 8% glutaraldehyde was added and incubated in shaker for 24 h at 25 °C to full crosslinking of the amino groups of BSA. The pH of the formulation (6.5) was not altered during the process to preserve RVT molecule. After, RVT-NP were collected and centrifuged in two cycles (15500 rpm for 30 min). The supernatant was separated for further analysis and the pellet was dispersed in aqueous solution and protected from light. The same procedure was carried out for the production of blank nanoparticles, but without the addition of RVT.

2.3. Determination of particle size, polydispersity and zeta potential

Mean particle size and polydispersity of the nanoparticles were determined by Dynamic Light Scattering (DLS) (BIC 90 plus, Brookhaven Inst. Corp.). Samples were diluted in ultra-purified water and analyzed with a scattering angle of 90° at 25 °C. Zeta potential was determined from the electrophoretic mobility of the nanoparticles. Samples were diluted in KCl 0.1 M at 25 °C and potential of ±150 mV was applied (ZetaSizer ZS, Malvern). Results were expressed as mean ± standard deviation.

2.4. Morphological analysis

Morphology was verified by scanning electron microscopy field emission (FE-SEM) (Superscan SS-550, Shimadzu Corporation), with accelerating voltage of 5 kV. Films of RVT-NP were covered with a thin gold layer and examined.

2.5. Determination of encapsulation efficiency

The RVT encapsulation was determined by an indirect method. Quantitation was performed by the HPLC method described by LINDNER et al. (2013) [32]. Chromatographic analysis was performed on a Waters 2695 Alliance HPLC (Milford, MA, USA) using a reverse phase C18 column (Xterra Waters®). The analysis was conducted on the isocratic mode and with mobile phase consisting of methanol and water mixture (51:49, v/v) pumped at a flow rate of 0.9 mL/min. The sample injection volume was 20 μL and PDA detector was set at 306 nm. The method run time was 6.4 min. The reserved supernatant obtained from centrifugation process (15500 rpm for 30 min) of nanoparticles was diluted in water: methanol (50:50, v/v), filtered through 0.22 μm pore size filter and analyzed by HPLC. The encapsulation efficiency (EE) was determined following equation (1).

$$\%EE = RVT_{initial} - RVT_{free} \Big/ RVT_{initial} \times 100 \quad (1)$$

where, $RVT_{initial}$ is the amount of resveratrol initially added to the formulation and RVT_{free} is the concentration of the non-encapsulated drug quantified in the supernatant after ultracentrifugation.

2.6. X-ray diffraction (XRD)

XRD patterns of the pure RVT, RVT-NP and blank NP were obtained using the X-ray diffractometer (D2 Phaser, Bruker, Germany) using CuK α-radiation and operating at 30 KV and 10 mA. The X-ray scan was performed in open angle 2θ ranged from 10 to 70°, scanning rate of 0.05°/min, in a wavelength 1.54 Å.

2.7. Differential scanning calorimetry (DSC) and derivative thermogravimetry (DTG)

Samples (RVT-NP, blank NP, RVT and BSA) were placed on aluminum pans and analyzed (TA Instruments®, Mod SDT Q600). The capsules were heated at a scan rate of 10 °C/min from 30 at 450 °C under nitrogen atmosphere with gas flow entrainment of 100 mL/min.

2.8. Fluorescence spectroscopy

Samples (BSA, RVT, RVT-NP, blank NP and physical mixture (RVT: BSA 1:10)) were diluted in phosphate buffer (pH: 7.4) and analyzed by a Fluorescence spectrometer (Flexstation 3 - Molecular Devices®). The excitation wavelength was set at 280 nm and scan range 300−550 nm. Excitation and emission slit widths were set at 10 nm.

2.9. In vitro release profile

The in vitro RVT release profile from BSA nanoparticles was determined by the dispersion/ultracentrifugation method, as described by CASA et al. (2015) [33]. Before the assay, RVT solubility in the release medium was determined to ensure sink conditions. An excessive amount of RVT was added in phosphate buffered saline (PBS-pH: 7.4−50 mM) containing a surfactant: Tween 80 (1 or 5%) or PVA (1−5%) and incubated in a shaker for 48 h. The solubility of RVT was determined by HPLC (Waters 2695 Alliance®). Subsequently, for the in vitro release assay, RVT-NP were suspended in PBS (pH: 7.4−50 mM) containing the selected surfactant and incubated at 37 °C under constant stirring. At predetermined times, samples were centrifuged (15500 rpm−15 min) and the

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