Materials Science and Engineering C 51 (2015) 362–368

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

Nanostructured delivery system for Suberoylanilide hydroxamic acid against lung cancer cells

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ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form 8 January 2015
Accepted 23 February 2015
Available online 25 February 2015

Keywords:
Histone deacetylase inhibitor
Suberoylanilide hydroxamic acid
Poly-D, L-lactide-co glycolide
Nanoprecipitation
A549 cells
Antineoplastic

ABSTRACT

With the objective to provide a potential approach for the treatment of lung cancer, nanotechnology based Suberoylanilide hydroxamic acid (SAHA)-loaded Poly-D, L-lactide-co glycolide (PLGA) nanoparticles have been formulated using the nanoprecipitation technique. The acquired nanoparticles were characterized by various throughput techniques and the analyses showed the presence of smooth and spherical shaped SAHA-loaded PLGA nanoparticles, with an encapsulation efficiency of 44.8% and a particle size of 208 nm. The compatibility between polymer and drug in the formulation was tested using FT-IR, Micro-Raman spectrum and DSC thermogram analyses, revealing a major interaction between the drug and polymer. The in vitro drug release from the SAHA-loaded PLGA nanoparticles was found to be biphasic with an initial burst followed by a sustained release for up to 50 h. In experiments using the lung cancer cell line A549, SAHA-loaded PLGA nanoparticles demonstrated a superior antineoplastic activity over free SAHA. In conclusion, SAHA-loaded PLGA nanoparticles may be a useful novel approach for the treatment of lung cancer.

1. Introduction

Cancer is the second most frequent cause of death in developed countries and the foremost cause of death in developing countries. Lung cancer is the most common cancer-related death, accounting for 13% (1.6 million) of the total cases and 18% (1.4 million) of the deaths in 2008 [1]. The equilibrium between histone acetylation and deacetylation appears to be essential for normal cell growth, and perturbation of the histone acetylation status has been associated with several diseases including lung cancer [2]. The acetylation status of histone is governed by two opposite enzymatic activates, histone acetyltransferases [3] and histone deacetylases (HDACs) [4]. Carcinogenesis has been associated with histone acetyltransferase (HATs) inactivation, whereas aberrant HDAC activity has been linked to the development and maintenance of the transformed state of human tumors, which is at least in part due to transcriptional repression of tumor suppressor gene expression [5]. In addition to histones, HDAC enzymes are also known to deacetylate numerous non-histone targets, such as transcription factors and proteins involved in cell cycle progression, indicating a complex multifunctional role for HDACs in health and disease [6]. Given these insights into the function of HDACs, it is not surprising that HDAC inhibitors are emerging as promising new agents for cancer therapy [7–11]. Indeed, HDAC inhibitors have been shown to induce potent anti-cancer effects including apoptosis, cytostasis, differentiation and inhibition of tumor angiogenesis in cultured cell lines [12,13]. Consequently, clinical trials with various HDAC inhibitors have been conducted and Suberoylanilide hydroxamic acid (SAHA; also known as vorinostat) has received FDA approval for the treatment of cutaneous T-cell lymphoma [14]. SAHA is a second generation polar–planar compound, which induces cell cycle arrest, differentiation and apoptosis in several transformed cells, including mouse xenograft models [2,5,14]. It has also been shown to be effective against chemotherapy-resistant patient-derived cancer cells [15]. Recently, SAHA drug is loaded with solid lipid nanoparticles to target the multidrug-resistant cancer cells [16].

Nanoparticles have recently gained considerable interest in pharmaceutical applications [17,18]. Meanwhile, nanoparticles synthesized by chemical methods are widely used for target drug delivery, likewise nanoparticles synthesized by some physical methods, such as electroporation are also used for drug/gene delivery [19]. Polymeric nanoparticles-based drug delivery is being increasingly investigated as delivery route to overcome many obstacles associated with the delivery of free drugs. Several drugs have been successfully encapsulated with biodegradable polymer to enhance their bioavailability, bioactivity and controlled delivery [20,21]. Poly-D, L-lactide-co glycolide (PLGA) [22] is one of the typically used biodegradable polymers for the development of nanomedicines. The human organism effectually hydrolyzed PLGA

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http://dx.doi.org/10.1016/j.msec.2015.02.037
0928-4931/

polymer and produced two degradable monomeric metabolites like lactic acid and glycolic acid [23]. In our study, PLGA polymer was chosen as drug delivery vehicle, due to their better encapsulation efficiency, bioavailability, less toxicity and controlled release.

In our current approach, SAHA-loaded PLGA nanoparticles were formulated by the nanoprecipitation method and characterized for their encapsulation efficiency, yield percentage, drug release kinetics, particle size, surface properties, morphology, stability and drug polymer interaction. Furthermore, the antineoplastic effect of SAHA-loaded PLGA nanoparticles and SAHA were compared in the human lung cancer cell line A549.

2. Materials and methods

2.1. Materials

Poly-D, L-lactide-co-glycolide (PLGA, L/G = 50:50) was a gift sample from Prof. Dr. Dragan Uskokovic, Institute of Technical Sciences of SASA, Serbia. SAHA was purchased from Sigma Aldrich, India. Tween 80, Span 80, acetone, methanol (HPLC grade) and DMSO (cell culture grade) were purchased from Merck, India. Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin/streptomycin, MTT (dimethyl thiazolyltetrazolium bromide), acridine orange and ethidium bromide were purchased from Hi Media Laboratories, Mumbai, India. Double-distilled deionized water was used throughout the experiments.

2.2. Methods

2.2.1. Preparation of SAHA-loaded PLGA nanoparticles

SAHA-loaded PLGA nanoparticles were prepared by the nanoprecipitation method with modifications [24]. In brief, individual 10 mg of PLGA polymer was dissolved in 5 ml of acetone; 2 mg of SAHA was dissolved in 1 ml of ethanol and then mixed together for 10 min. Fifty milligrams of Span 80 was added over it and vortexed for 5 min. The formed solution was poured in 20 mg of Tween 80 solution under magnetic stirring for 12 h; the resulting nanoemulsion was evaporated overnight to remove the organic solvents. Further it was centrifuged at 28,000 rpm for 15 min and the nanoparticulate solution was freeze dried using 5% glucose as a cryoprotectant, stored in 4 °C until further use.

2.2.2. Encapsulation efficiency

SAHA entrapped in PLGA nanoparticles was estimated using RP-HPLC (Shimadzu prominence consisting of LC 20AD). The chromatographic separation was achieved on Phenomenex Luna C18 (250 × 4.6 mm, 5 μ) analytical column. Accurately 0.1 ml (25 μg) of SAHA-loaded PLGA nanoparticles were dissolved in acetone:ethanol (1:1) and further dilution made with 0.1% Tween 80 containing phosphate buffered saline (PBS) pH 7.4 buffer. The mobile phase of methanol:0.1% trifluoro acetic acid (50:50% v/v) was used at a flow rate of 1 ml/min. The UV detection was carried out at 242 nm. The retention time of SAHA was found to be 4.2. The method was found to be linear at 5–25 μg/ml.

2.2.3. In vitro release studies

The in vitro release study of SAHA-loaded PLGA nanoparticles was carried out using the dialysis bag method. One milliliter of nanoparticulate suspension was instilled in dialysis bag (cellophane molecular weight cutoff), tied and placed into 100 ml of 0.1% Tween 80 containing PBS pH 7.4 buffer solution at 37 °C with continuous stirring. At predetermined time intervals, aliquots were withdrawn and replaced with the same amount of (0.1% Tween 80 containing PBS pH 7.4) fresh buffer. The samples were analyzed using RP-HPLC using the same chromatographic condition of the encapsulation efficiency test.

2.2.4. Characterization of SAHA-loaded PLGA nanoparticles

2.2.4.1. Size and zeta potential. The particle size and zeta potential of SAHA-loaded PLGA nanoparticles were measured by dynamic light scattering (Malvern Instruments Ltd., Malvern, UK). 10 μg of particles was dispersed in 1 ml of deionized water sonicated for 5 s before measurement and performed triplicate.

2.2.4.2. Scanning electron microscopy (SEM). The size and surface morphology changes of the SAHA-loaded PLGA nanoparticles were achieved using scanning electron microscopy (Hitachi, Model: S-3400N). The nanoparticles were prepared on aluminum stubs and coated with gold prior to examination.

2.2.4.3. Transmission electron microscopy (TEM). SAHA-loaded PLGA nanoparticles were imaged using transmission electron microscopy performed on a Philips, Tecnai 10, Japan. The nanoparticles were placed on a copper grid and dried under vacuum, and the image was recorded at an accelerated voltage of 80 kV.

2.2.4.4. Differential scanning calorimetry (DSC). DSC thermogram of SAHA, PLGA polymer and SAHA-loaded nanoparticles were recorded. The physical status of SAHA inside the nanoparticles was investigated by differential scanning calorimetry (DSC 200F3 Maia). The samples were purged with dry nitrogen. The heating rate of 10 K/min was applied.

2.2.4.5. Fourier transform infra red spectroscopy (FTIR). FTIR studies were carried out to confirm the nanoparticles. The spectra were recorded on a Perkin Elmer FTIR spectrometer scanned over a range of 500–4000 cm^{-1}. Test samples were mixed with KBr pressed into a disk. FTIR spectra of drug, polymer and drug-loaded nanoparticles were recorded.

2.2.4.6. Micro-Raman spectroscopy. The Micro-Raman spectra of drug, polymer and drug-loaded nanoparticles were recorded at room

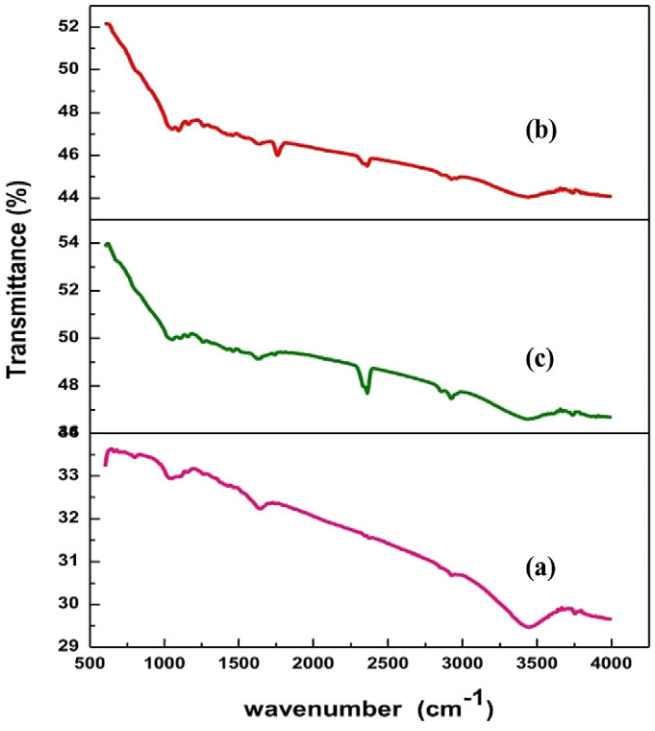

Fig. 1. FTIR spectra of (a) SAHA, (b) PLGA polymer and (c) SAHA-loaded PLGA nanoparticles.