Colloids and Surfaces B: Biointerfaces 144 (2016) 1–7

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Colloidal mesoporous silica nanoparticles enhance the biological activity of resveratrol

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ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form 16 February 2016
Accepted 25 March 2016
Available online 26 March 2016

Keywords:
Resveratrol
Mesoporous silica
Anticancer
Nanoparticles
Colorectal cancer
Anti-inflammatory

ABSTRACT

The naturally occurring polyphenol resveratrol (RES) has attracted increasing attention in recent years due to its antioxidant, anti-inflammatory, and anticancer activity. However, resveratrol's promising potential as a nutraceutical is hindered by its poor aqueous solubility, which limits its biological activity. Here we show that encapsulating resveratrol in colloidal mesoporous silica nanoparticles (MCM-48-RES) enhances its saturated solubility by ~95% and increases its *in vitro* release kinetics compared to pure resveratrol. MCM-48-RES showed high loading capacity (20% w/w) and excellent encapsulation efficiency (100%). When tested against HT-29 and LS147T colon cancer cell lines, MCM-48-RES-mediated *in vitro* cell death was higher than that of pure resveratrol, mediated via the PARP and cIAP1 pathways. Finally, MCM-48-RES treatment also inhibited lipopolysaccharide-induced NF-κB activation in RAW264.7 cells, demonstrating improved anti-inflammatory activity. More broadly, our observations demonstrate the potential of colloidal mesoporous silica nanoparticles as next generation delivery carriers for hydrophobic nutraceuticals.

1. Introduction

Whilst research and development into cancer therapies has intensified in recent decades and significant advancements in cancer treatments have been made, cancer remains the leading cause of disease burden in Australia [1,2]. Key challenges in treatment include poor solubility of drugs and lack of site specific delivery to cancer cells, development of drug resistance in cancer cells, and adverse effects due to poor drug targeting [3,4]. Consequently, the search continues for novel drug delivery technologies with better targeting and reduced toxicity. Colorectal cancer is the second most common cause of cancer in males and third most common in females, accounting for 8% of all cancer deaths [5]. Diet is associated with risk of development of colon cancer, hence there is great interest in dietary factors that may exert chemopreventive and chemotherapeutic actions [6]. Dietary components contain many bioactive ingredients that are able to regulate multiple molecular pathways involved in cancer development and progression.

Resveratrol (3,5,4′-trihydroxystilbene) (RES) is a naturally occurring polyphenol and phytoalexin, which is produced by plants in response to environmental stress such as fungal infections, injury and UV irradiation [7–10]. RES can also be found in many foods commonly consumed in the human diet such as red wine, grapes and peanuts [11,12]. In recent years RES have attained significant attention due to its various therapeutic effects including antioxidant, cardioprotective, anti-inflammatory and anticancer activity, however, RES suffer from many pharmacokinetic limitations [13–15].

RES is a Biopharmaceutics Classification System (BCS) class II drug with poor aqueous solubility (0.03 g/L) and a partition coefficient (log $P_{o/w}$) of 3.1 [16–18]. RES exist in two geometric isomers, *cis*- and *trans*-, with the latter being more abundant and biologically active [19–21]. However, when exposed to light *trans*-resveratrol undergoes photoisomerization to *cis*-resveratrol [22,23]. Whilst the oral absorption of RES in humans is high (≈75%), the drugs bioavailability is less than 1% as a result of erratic absorption in the gut and extensive first pass metabolism in the intestine and liver [24,25]. Walle et al. [25] observed RES had a half life of 9.2 h in humans with two peak plasma concentrations, after one and six hours.

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http://dx.doi.org/10.1016/j.colsurfb.2016.03.076
0927-7765/

Together these poor pharmacokinetic properties severely hinder resveratrol's potential as a therapeutic agent. [26]

Nanocarriers are advantageous in cancer therapy due to the enhanced permeation and retention effect (EPR) whereby nanosize particles accumulate preferentially in cancer tissue due to wider than usual capillary fenestrations [27,28]. This phenomenon enables lower drug concentrations to be used, hence reducing the potential for adverse effects. Healthy cells are less affected due to drug targeting, which ensures localized drug action in tumors. Additionally, nanocarriers increase bioavailability of poorly soluble drugs by increasing solubility and peak plasma concentration of the drug in the blood following uptake via M-cells [29]. Several studies have attempted to improve RES's physicochemical properties by incorporating it into various nanocarriers including liposomes [30,31], cyclodextrins [32,33], solid lipid nanoparticles [34], polymeric micelles [35] and polymeric nanoparticles [36]. However, these formulations suffer from drawbacks such as poor stability (e.g. liposomes [37]), low drug loading (e.g. polymers and liposomes 2–10% [36,37]) and high production costs (e.g. cyclodextrins and polymers [38,39]). Hence, there is a pressing need for superior cost effective delivery carriers for nutraceuticals such as RES [26].

Mesoporous silica nanoparticles (MSNs) are biocompatible, have large surface areas and pore volumes and are able to be functionalized making them an ideal shuttle for poorly water-soluble drugs [40–44]. MSNs are regarded as next generation pharmaceutical carriers due to their ability to enhance the efficacy of drugs by improving their aqueous solubility, altering release kinetics, and by targeting via functionalisation. Many recent studies have shown that MSN's improve the aqueous solubility, bioavailability and cell cytotoxicity of hydrophobic drugs such as curcumin, griseofulvin and cyclosporine A [40,41,45–48]. However, to the best of our knowledge there is no report on successful encapsulation of RES in MSNs with high drug loading and enhanced biological activities.

In this study, we report the first example of RES encapsulation within MSNs and study its saturated solubility, drug release, anti-inflammatory and anticancer efficacy against colon cancer cells. MCM-48 type MSNs were chosen for this study since they displayed a number of desirable features, including: mono dispersed particle size (150–200 nm), high surface area (~1200 m^2/g), large pore volume (0.9 cm^3/g) highly ordered three dimensional cubic structure of bidirectional mesoporous channels and their ability to encapsulate large amounts of drug [49,50]. Additionally, our group and others have used MSNs effectively as nanosuspensions and orally delivered tablets, showing their applicability to multiple dosage forms [51]. In this report we describe RES encapsulation in mesoporous silica nanospheres (MCM-48) achieving 20% w/w drug loading, and test their efficacy in a variety of biological assays. We hypothesise that these particles can be used both intravenously as nanosuspensions and/or orally by forming tablets to enhance the overall nutraceutical efficacy and applicability of RES.

2. Experimental

2.1. Materials

Cetyltrimethylammonium bromide (CTAB), tetraethylorthosilicate (TEOS), phosphate buffered saline (PBS), Pluronic F127 (PF127), hydrochloric acid 32% (HCl), ammonium hydroxide 28% (NH_4OH), ethanol 100% and methanol were purchased from Sigma-Aldrich Australia. Resveratrol was provided as a gift sample by MegaResveratrol, USA. CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) was purchased from Promega. DMEM: F12 media was purchased from Sigma and fetal calf serum was purchased from Life Technologies.

2.2. Physicochemical characterisation

Particle size, polydispersity index and zeta potential were measured using a Malvern Zetasizer Nano-ZS. Transmission Electron Microscopy (TEM) images were obtained using a JEOL 1010 microscope operated at 100 kV. Thermogravimetric analysis (TGA) was performed using a Mettler Teledo instrument with a heating rate of 5 °C/min in airflow. RES concentration was determined using a UV–vis spectrophotometer (Varian Cary 50 Bio) at 305 nm. X-ray diffractograms were recorded using a Rigaku Miniflex X-ray diffractometer with Fe-filtered Co radiation (λ = 1.79 Å). Nitrogen physisorption measurements were performed at −196 °C using a Micromeritics Tristar II 3020 system.

2.3. Synthesis of MCM-48

MCM-48 synthesis was performed with slight modifications to the method reported by Kim et al. [52]. In a typical synthesis, 425 mL of NH_4OH (2.8%) and 170 mL of ethanol were transferred into a 1 L glass bottle to which, 2.0 g of CTAB and 8.0 g of PF127 was added and stirred at 500 rpm until dissolved. The speed of stirring was increased to 850 rpm and 7.203 g of TEOS was quickly added to the solution whilst stirring at this speed for 1 min. The solution was then left in static condition for a further 24 h at room temperature. The resulting white product was isolated using high-speed centrifugation (15,000 rpm for 15 min), redispersed once in Milli-Q water and then twice in ethanol and dried at 60 °C overnight. The MCM-48 was then calcined (air, 550 °C, 1 °C/min) for 5.5 h.

2.4. Resveratrol loading

Resveratrol loading was performed using a rotary evaporation technique with slight modifications to the procedure reported by Jambhrunkar et al. [41]. 40 mg of RES was placed in a rotary evaporation flask with 10 mL of ethanol and sonicated for 2 min 160 mg of MCM-48 was then added and sonicated for a further 5 min. The solvent was slowly evaporated using rotary evaporator at 50 °C to obtain RES loaded MCM-48 (MCM-48-RES) with 20% w/w theoretical drug loading. The evaporation process was continued until all ethanol was removed and a dry powder was observed in the flask. This sample was collected, stored covered in aluminum foil to protect it from photo degradation and used for further studies.

2.5. Solubility and in vitro release

We performed saturated solubility studies by adding an excessive quantity (0.5 mg equivalent) of RES, MCM-48 and RES physical mixture (PM) and MCM-48-RES to 0.5 mL of Milli-Q water. This mixture was kept shaking for 48 h at 37 °C whilst protected from light. The suspension was then centrifuged, diluted with deionized water and the supernatant was analysed for RES content using UV–vis spectroscopy ($r^2 = 0.999$) at 305 nm.

The *in vitro* release of RES from MCM-48-RES was conducted by suspending RES and MCM-48-RES equivalent to 1 mg of RES in 1 mL of PBS (pH 7.4) This suspension was placed in a snakeskin dialysis bag with a 10 kDa molecular weight cut-off and immersed into 9 mL of PBS at 37 °C whilst continually stirring. At predetermined time intervals 1 mL of the sample was withdrawn and immediately replaced with an equal volume of PBS to maintain sink conditions. The removed samples were analysed for RES content using UV–vis spectroscopy ($r^2 = 0.997$) at 305 nm.

2.6. In vitro cytotoxicity assay

HT-29 and LS174T cell lines were propagated in monolayers to sub-confluency at 37 °C in 75 cm^2 flasks containing 10 mL of DMEM: