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Rutin-loaded chitosan microspheres: Characterization and evaluation of the anti-inflammatory activity

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ABSTRACT

Rutin was microencapsulated in a chitosan matrix using the spray-drying technique and the resulting system was investigated. High amounts of rutin were efficiently entrapped within polymeric microspheres, and these microparticles were characterized by a smooth surface and afforded a controlled release of the active compound. The anti-inflammatory activity of rutin-loaded microspheres was investigated in *in vitro* models of NCTC 2544 and C-28 cells treated with LPS by determining the levels of IL-1β and IL-6. The rutin-loaded microspheres showed an increase of *in vitro* anti-inflammatory activity with respect to the free active compound. Confocal laser scanning microscopy demonstrated that massive intracellular uptake of the chitosan microspheres took place after a few hours of incubation and that the drug was localized in the cytosol compartment of the treated cells. The improved anti-inflammatory activity of the rutin-loaded microspheres was further confirmed by an *in vivo* model of carrageenan-induced paw edema.

1. Introduction

Microencapsulation is a useful tool in modern pharmaceutical technology, and is used to modulate the physico-chemical and biopharmaceutical properties of a number of active compounds (Ma, 2014). To this end, biocompatible polymers able to encapsulate bioactives have been used in the food, cosmetic, cosmeceutical and pharmaceutical fields, in order to increase the stability of entrapped compounds, improve miscibility in aqueous food products, mask off-flavours and provide site-specific targeted delivery (Augustin & Sanguansri, 2015). In particular, a growing interest is being shown in the use of safe polymeric nano- and microdevices in order to achieve innovative nano- and microformulations of natural bioactives, respectively (Mohammadi, Jafari, Assadpour, & Faridi Esfanjani, 2016; Nazzaro, Orlando, Fratianni, & Coppola, 2012).

Scientific investigation has demonstrated that some flavonoids such as rutin, luteolin, and apigenin carry on anti-inflammatory activity by means of scavenging ROS and the reduction of pro-inflammatory cytokines. Rutin is a flavone glycoside present in many plants; it has notable anti-inflammatory and anti-oxidant properties and exerts antitumor, antidiarrheal and antimutagenic effects, affords myocardial protection and possesses immunomodulation traits (Chua, 2013; Jang et al., 2014).

A number of drug delivery systems were proposed for the entrapment of this active compound with the aim of improving its pharmacological activity. Namely, vesicles made up of sorbitan monostearate and polyethylene glycol fatty acid esters were investigated for the rectal delivery of rutin (Kamel, Basha, & Abd El-Alim, 2013) while rutin-loaded ceramide liposomes embedded in a hydrogel were used to obtain an efficacious transdermal delivery (Park, Lee, Kim, & Yu, 2013). Other devices were also used for the encapsulation of rutin, for instance mesoporous silica nanoparticles (Berlier et al., 2013), nanoemulsions (Macedo, Quelhas, Silva, & Souto, 2014), micelles (Löf, Schillén, & Nilsson, 2011) and nanocrystals (Mauludin, Müller, & Keck, 2009).

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Few investigations in literature have dealt with the use of biopolymers for the encapsulation of rutin and no in-depth investigations have been carried out. For this reason, microspheres made up of a bio-polymer (such as chitosan) were investigated in this paper, specifically for the entrapment of rutin and the enhancement of its anti-inflammatory properties.

Chitosan is a biopolymer made up of β1→4 linked 2-amino-2-deoxy-glucopyranose and 2-acetamido-2-deoxy-β-D-glucopyranose residues, obtained by the *N*-deacetylation of chitin, an abundant biopolymer isolated from the exoskeletons of crustaceans such as crabs and shrimps (El Kadib, Bousmina, & Brunel, 2014). Chitosan has been widely used for the microencapsulation of bioactive compounds due to its biocompatibility and bio- and mucoadhesion features, which recommend this biopolymer as a functional material for many applications, such as pulmonary, intestinal and trans-dermal drug delivery (Cordeiro, Alonso, & de la Fuente, 2015; da Silva, Ferreira, Pintado, & Sarmento, 2016; Grabowski et al., 2013; Matos, Reis, Gratieri, & Gelfuso, 2015) or as useful compound able to coat preformed magnetic nanotubes (Wang, Zhang, & Wang, 2013; Wang, Zhang, Mu, Fan, & Wang, 2014). The positive charge of chitosan also allows efficient interaction with negatively-charged substrates such as genetic materials, thus favoring their complexation, protection and intracellular delivery (Cosco et al., 2014; Cosco, Cilurzo et al., 2015).

The properties of chitosan can be used to improve the pharmacological activity of rutin thus achieving a microformulation boasting long-term activity. To this end, rutin-loaded chitosan microspheres were experimentally prepared using the spray-drying technique and then characterized. Their physico-chemical properties were investigated as a function of the amount of rutin used during the preparation and then their *in vitro* and *in vivo* anti-inflammatory effects were evaluated in comparison with the free drug. The interaction rate of fluorescent chitosan microspheres with different cell lines was investigated by confocal laser scanning microscopy as a function of the incubation times.

2. Materials and methods

2.1. Materials

Chitosan (copolymer of β (1→4) linked 2-acetamido-2-deoxy-β-Dglucopyranose and 2-amino-2-deoxy-β-D-glucopyranose; low molecular weight, <10.000 Da, degree of deacetylation 85%) (Fig. S1), rutin (Fig. S1), 3-[4,5-dimethylthiazol-2-yl]-3,5-diphenyltetrazolium bromide salt (used for MTT-tests), dimethylsulfoxide (DMSO), rhodamine and amphotericin B solution (250 μg/ml) were purchased from Sigma Chemicals Co. (St. Louis, USA). NCTC2544 and C-28 cells were provided by the Istituto Zooprofilattico of Modena and Reggio Emilia. Minimum essential medium (D-MEM) with glutamine, trypsin/ethylenediaminoacetic acid (EDTA) (1×) solution, fetal bovine serum (FBS) and penicillin-streptomycin solution were obtained by Gibco (Invitrogen Corporation, Life Technologies, Italy). All other materials and solvents used in this investigation were of analytical grade (Carlo Erba, Milan, Italy). Deionized double-distilled water was used throughout the study.

2.2. Preparation of chitosan microspheres

Empty chitosan microspheres were prepared as previously described (Ventura et al., 2011). Briefly, chitosan (0.2% w/v) was solubilized in 1% (v/v) acetic solution (350 ml), filtered with a 0.45 μm nylon millipore filter and spray-dried using a Büchi Minispray Model B190 (Büchi Laboratoriums-Tecknik AG, Flawil, Switzerland) with a standard 0.7 mm nozzle. The Rutin-loaded chitosan microspheres were prepared by adding an ethanol solution (125 ml) of rutin (at drug concentrations ranging from 100 to 500 mg) to an aqueous solution of chitosan and then spray-drying it. The fluorescent microspheres used during experimentation were obtained by co-solubilizing rhodamine (1% w/w) with chitosan just before the spray-drying process.

The manufacturing parameters were: inlet temperature 210 °C; outlet temperature 80–90 °C; pump ratio 30%; aspirator ratio 90%; and rate of spray feed 6 ml/min.

2.3. Microsphere characterization

The mean sizes of the microspheres were investigated by a laser diffractometer Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, United Kingdom) equipped with a Hydro sampler. Morphological characterization and the surface charges of the microsystems were evaluated through scanning electron microscopy (SEM) and photocorrelation spectroscopy, respectively (Ventura et al., 2011).

The prepared microspheres were then weighed and the percent yield was calculated according to the following equation:

$$\%\text{yield} = (\text{Actual weight}/\text{Theoretical Weight}) \times 100 \quad (1)$$

The drug content of the microspheres was determined by dissolving 5 mg of each sample in 4 ml of 0.1 N HCl/ethanol (65/35) solution. This solution was analyzed by a PerkinElmer Lambda 35 UV–vis spectrophotometer equipped with PerkinElmer acquisition software (Perkin-Elmer GmbH Uberlingen, Germany). The calibration curve at rutin λ_{max} 362 nm was used for its determination:

$$y = 58.37x - 6.19 \quad (2)$$

where y is the drug concentration (μg/ml) and x is the absorbance at 362 nm, and the r^2 value was 0.998.

The rutin content of the microspheres was expressed both as the entrapment efficiency percentage (EE%) and the loading capacity percentage (LC%) according to the following equations:

$$\text{EE}(\%) = [rutin]_m/[\text{rutin}]_i \times 100 \quad (3)$$

where $[rutin]_m$ is the drug amount determined to be present in the microspheres and $[rutin]_I$ the drug amount initially added to the formulation;

$$\text{LC}(\%) = [rutin]_m/[\text{M}] \times 100 \quad (4)$$

where $[rutin]_m$ is the drug amount determined to be present in the microspheres and $[M]$ is the weighed quantity of microspheres.

The release rate of rutin from the chitosan microspheres was investigated at pH 7 and pH 5, using the dialysis method (Cosco, Paolino, Maiuolo, Russo, & Fresta, 2011) with a water/ethanol (65/35 v/v) mixture as the medium; 5 mg of each formulation was placed into the dialysis bags. The medium was constantly stirred at 250 rpm by using a magnetic plate and warmed to 37 ± 0.1 °C (IKA RCT Basic, equipped with a thermometer ETS-D4 Fuzzy, IKA®-Werke GmbH & Co. KG, Staufen, Germany).

2.4. Cell cultures and evaluation of cytotoxicity

NCTC2544 (human keratinocytes) and C-28 (human chondrocytes) cells were incubated in plastic culture dishes (100 mm × 20 mm) (Forma® Series II Water-Jacketed CO_2 Incubator, Thermo Scientific, Germany) at 37 °C (5% CO_2) using DMEM medium with glutamax, penicillin (100 UI/ml), streptomycin (100 μg/ml), amphotericin B (250 μg/ml) and FBS (10%, v/v). Fresh medium was replaced every 48 h. When ~80% confluence was reached, the cells were treated with trypsin (2 ml) to separate