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Encapsulation of Acyclovir in new carboxylated cyclodextrin-based nanosponges improves the agent's antiviral efficacy

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ABSTRACT

Cyclodextrin-based nanosponges (NS) are solid nanoparticles, obtained from the cross-linking of cyclodextrins that have been proposed as delivery systems for many types of drugs. Various NS derivatives are currently under investigation in order that their properties might be tuned for different applications. In this work, new carboxylated cyclodextrin-based nanosponges (Carb-NS) carrying carboxylic groups within their structure were purposely designed as novel Acyclovir carriers. TEM measurements revealed their spherical shape and size of about 400 nm. The behaviour of Carb-NS, with respect to the incorporation and delivery of Acyclovir, was compared to that of NS, previously investigated as a drug carrier. DSC, XRPD and FTIR analyses were used to investigate the two NS formulations. The results confirm the incorporation of the drug into the NS structure and NS-Acyclovir interactions. The Acyclovir loading into Carb-NS was higher than that obtained using NS, reaching about 70% (w/w). *In vitro* release studies showed the release kinetics of Acyclovir from Carb-NS to be prolonged in comparison with those observed with NS, with no initial burst effect. The NS uptake into cells was evaluated using fluorescent Carb-NS and revealed the nanoparticle internalisation. Enhanced antiviral activity against a clinical isolate of HSV-1 was obtained using Acyclovir loaded in Carb-NS.

1. Introduction

Acyclovir, a synthetic nucleoside analogue derived from guanosine, is a widely used antiviral agent due to of its efficacy in the treatment of herpes simplex virus infections (O'Brien and Campoli-Richards, 1989). However, neither the parenteral nor the oral administration of the currently available formulations of Acyclovir is able to result in suitable concentrations of the agent reaching at target sites. Acyclovir's absorption in the gastrointestinal tract is slow and incomplete; of consequence, its pharmacokinetics following oral medication are highly variable and its oral bioavailability ranges from just 10 to 30%. In general, around 80% of the administered dose is not absorbed and current therapies therefore require the administration of high doses, up to 1.2 g/day. As a consequence, the presence of systemic toxicity and adverse reactions is frequent with its administration.

Many technological approaches, such as pro-drug preparations and innovative formulations, have been proposed for improving the efficacy of Acyclovir treatment and decreasing its adverse side effects. In recent years, the design of new delivery systems for the administration of antivirals has attracted much research attention (Lembo and Cavalli, 2010). A number of Acyclovir nanoparticulate systems have been developed, including nanoparticles (Giannavola et al., 2003; Kamel et al., 2009; Jin et al., 2006; Cavalli et al., 2009; Bertino Ghera et al., 2009; Elshafeeya et al., 2010), liposomes (Pavelic et al., 2005; Chetoni et al., 2004), niosomes (Mukherjee et al., 2007; Attia et al., 2007) and microemulsions (Ghosh et al., 2006; Shishu et al., 2009), all of which aim at improving the bioavailability of Acyclovir for either systemic or topical administration.

The present work focuses on the potential use of new β-cyclodextrin-based nanosponges (NS) as specifically prepared novel Acyclovir carriers. Cyclodextrin-based NS are solid nanoparticles consisting of highly cross-linked cyclodextrins, and were

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recently developed as a novel nanoparticulate delivery system (Trotta and Cavalli, 2009; Trotta et al., 2012). This new nanostructured material is prepared by reacting cyclodextrin (CD) with several cross-linking agents: generally, activated carbonyl compounds (*e.g.* carbonyldiimidazole), pyromellitic dianhydride and carboxylic acids. The reaction produces nanoparticles with a rather spherical shape that possess the capacity to form stable nanosuspensions when dispersed in water under stirring. NS show good biocompatibility and negligible biotoxicity. For example, the acute systemic toxicity of nanosponges was evaluated in mice following the injection of doses that varied between 500 mg and 5000 mg/kg; the mice showed no signs of toxicity or any adverse reactions. Their oral administration has also been tested in mice, with no apparent side effects noted (Trotta et al., 2012).

Nanosponges are highly efficient at entrapping different types of molecules (both organic and inorganic), and they can achieve this by means of inclusion or non inclusion complex formation. NS are able to complex with drug molecules due to their highly cross-linked structure and their many CD cavities, which can cooperate in forming inclusion complexes. Moreover, the polymer mesh forms a network with nano-channels able to entrap the guest molecules. This peculiar structural organisation favours molecule complexation and might be responsible for the increased solubility, stabilisation and protection capacities of nanosponges in comparison with its parent cyclodextrins.

Cyclodextrin-NS have been exploited as carriers for various types of drugs, but in particular for molecules with poor aqueous solubility (Cavalli et al., 2006; Swaminathan et al., 2007, 2010; Ansari et al., 2010; Mognetti et al., 2012). Recently, loading NS with paclitaxel was found to increase the bioavailability of the drug when orally administered to rats compared to that obtained for free paclitaxel (Torne et al., 2010). Further studies demonstrated the ability of NS to increase the solubility and the oral bioavailability of other molecules, including resveratrol and tamoxifen (Ansari et al., 2011; Torne et al., 2013). Based on these findings, the current work investigates the potential for cyclodextrin-NS to increase the oral bioavailability of Acyclovir.

The aim of this study was to develop new β-CD nanosponges purposely tuned for the formulation of Acyclovir, a drug with medium polarity and solubility. To this end, a new type of NS-derivative, containing dissociable carboxylic groups, was considered for the encapsulation of Acyclovir and its behaviour compared to the β-cyclodextrin-based NS that has previously been investigated in relation to more lipophilic drugs. The synthetic rationale consisted of increasing drug incorporation by increasing complexation due to electrostatic interactions between the acid groups belonging to the NS structure and the Acyclovir amino group. The physico-chemical characterisation of the Acyclovir NS formulations along with their *in vitro* antiviral activities are herein reported.

2. Materials and methods

2.1. Materials

Acyclovir, fluoresceine isothiocianate, carbonyldiimidazole and ammonium acetate were purchased from Sigma–Aldrich (USA). Cyclodextrin was a kind gift from Roquette (Lestrem, France). All solvents used are of HPLC grade. All reagent used are of analytical grade. Milli Q water was used for all the experiments.

2.2. Synthesis of carbonate and carboxylate nanosponges

In this work, two different types of cyclodextrin-based NS, namely carbonate and carboxylate NS, were synthesised.

Carbonate NS were prepared as previously reported (Trotta and Cavalli, 2009; Trotta et al., 2012). Briefly, an amount of anhydrous cyclodextrin was dissolved in anhydrous DMF and allowed to react with carbonyldiimidazole at 90 °C for at least 5 h. Once the reaction was over, a large excess of water was added to destroy the excess of carbonyldiimidazole and the solid recovered by filtration. Then, the solid was ground in a mortar and Sohxlet-extracted with ethanol to remove residual reaction by-products. The reaction was carried out using a molar excess of crosslinker (*e.g.* 1:4 of β CD:cross-linker). Following purification, NS were stored at 25 °C.

New carboxylated nanosponges (Carb-NS) were obtained by reacting succinic anhydride on preformed NS in DMSO at 90 °C for 3 h. The solid nanosponges was recovered by filtration and washed with a large amount of water. The presence of carboxylic groups in the structure was assessed by titrimetry with NaOH solution and by FTIR analysis (Perkin Elmer System 2000).

For titrimetry determination, a known amount of Carb-NS was dispersed in a KCl solution. After standing for a long time (*i.e.* 24 h) the suspension was titred with NaOH 0.1 M.

NS surface charge modification, determining the Zeta potential value, was also used to ascertain the presence of the acidic groups.

Finally, fluorescent Carb-NS were also synthesised for cellular trafficking studies. For this purpose, pre-formed NS were added to a fluorescein isothiocyanate solution in DMSO and incubated at 90 °C for 3 h. Then, the solid was recovered by filtration and washed with ethanol. The dried product was reacted with the succinic anhydride as previously described to obtain fluorescent Carb-NS.

2.3. Preparation of Acyclovir-loaded nanosponges

A weighed amount of Acyclovir was dispersed in aqueous suspensions (pH = 5.5) of both NS and Carb-NS in a weight ratio of 1:4 and magnetically stirred for 24 h. Suspensions were then centrifuged at 2000 rpm for 10 min to separate out the non-complexed drug as a residue below the colloidal supernatant. The colloidal supernatants were freeze-dried using a Modulyo freeze-drier (Edwards, UK) to obtain the drug-loaded nanosponges. The two drug-loaded NS formulations were stored in a covered vacuum desiccator at ambient temperature until further use. Nanosponge nanosuspensions were sterilised by autoclaving (121 °C, 2 bar) for the biological studies.

2.4. Physical mixture preparation

Binary physical mixtures were prepared by mixing Acyclovir and the two dried NS types in a glass mortar (4:1 nanosponge:Acyclovir weight ratio).

2.5. Acyclovir quantitative determination

The quantitative determination of Acyclovir was achieved by HPLC analysis using a Perkin Elmer instrument (L2 Binary Pump, Perkin Elmer) with a UV–vis spectrophotometer detector (LC 95, Perkin Elmer, USA) with an external standard method. A reverse-phase hypersil ODS column (25 cm × 4.6 mm Varian, USA) was used with a mobile phase consisting of a 12:88 (v/v) ratio of acetonitrile:20 mM ammonium acetate buffer pH = 3.5 and a flow rate of 1 ml/min. The UV detector wavelength was set to 250 nm. The calibration curve is linear in the range 0.5–15 μg/ml with a r^2 of 0.9997. For cellular studies, the HPLC method for Acyclovir determination was tuned by changing the mobile phase to a ratio of water (adjusted to pH 2.5 with orthophosphoric acid):methanol (92:8); the same flow rate of 1 ml/min was used and UV detection was carried out at 252 nm.