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The intracellular effects of non-ionic amphiphilic cyclodextrin nanoparticles in the delivery of anticancer drugs

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ABSTRACT

The aim of this study was to develop nanoparticles made of the amphiphilic cyclodextrin heptakis (2-*O*-oligo(ethyleneoxide)-6-hexadecylthio-)-β-CD (SC16OH) entrapping docetaxel (Doc) and establish their *in vivo* potential. Doc-loaded SC16OH nanoparticles were prepared by the emulsion–solvent evaporation technique and fully characterized for size, zeta potential, amount of entrapped drug, release rate and degradation rate. Spherical vesicular nanoparticles displaying a hydrodynamic radius of about 95 nm which did not change upon storage as an aqueous dispersion, a negative zeta potential and entrapment efficiency of Doc very close to 100% were produced. DSC study highlighted the crystalline nature of SC16OH, unloaded and Doc-loaded SC16OH nanoparticles which resulted in their very slow dissolution during release stage and well-modulated release of entrapped Doc for about 8 weeks. Doc-loaded SC16OH nanoparticles were not hemolytic toward red blood cells as compared to a commercial Doc formulation (Taxotere®) which shows a dose-dependent toxicity. After exposure of HEp-2 cells to equivalent doses of free Doc and Doc-loaded SC16OH nanoparticles, superior cell killing and cell damage were observed for nanoparticles. Finally, cell damage was attributed to aberrant mitosis which was found to be significantly higher for HEp-2 cells treated with Doc-loaded SC16OH nanoparticles as compared to free Doc likely due to the ability of nanoparticles to slowly release the drug allowing prolonged cell arrest in mitosis. Taken together, these results highlights a great potential of nanoparticles based on SC16OH in solid tumors therapy.

1. Introduction

Hydrophilic cyclodextrins (CD) have been widely used as pharmaceutical excipients to formulate poorly water soluble drugs with the aim to increase drug apparent solubility in biological media and in some cases enhance bioavailability. Besides soluble complexes, CD were demonstrated to form supramolecular aggregates with drugs [1] although their high external hydrophilicity can represent a drawback for cell internalization and result in a lack of affinity of the included molecule for biological membranes [2]. This constitutes one of the reasons why researchers have been interested in developing cyclodextrin derivatives with a modulated external hydrophobicity. Numerous chemical modifications have been carried out on CD by grafting substituents to different positions (primary face, secondary face or both faces) thus obtaining non-ionic or cationic derivatives able to form supramolecular nanoaggregates suitable for pharmaceutical applications [3]. However, the potential of these molecules in engineering nanocarriers able to deliver and especially target a drug is largely unexplored. In fact, supramolecular nanoaggregates, either spontaneously obtained or structured through a specific preparation method, have a size compatible with i.v. injection and could be used to optimize drug distribution in the body. Strategies involving nanocarriers are well suited for the delivery of highly toxic anticancer drugs to solid tumors making use of their potential to extravasate at level of tumor defective capillary bed and deliver the drug at the site of action. The most relevant studies in this sense have been produced from Bilensoy and coworkers who demonstrated that 6-*N*-CAPRO-β-CD and β-CDC6 (modified on the primary and secondary face, respectively, with 6C aliphatic chains) could give

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nanoparticles or nanocapsules able to entrap anticancer drugs such as tamoxifen citrate [4] and paclitaxel [5] with good efficiency. Furthermore, nanoparticles based on amphiphilic cyclodextrins were demonstrated non-hemolytic and non-cytotoxic [6,7].

In this context, some of us have recently synthesized several neutral and cationic amphiphilic derivatives of β-CD which form supramolecular aggregates such as micelles and vesicles with different host molecules [3]. This new generation of amphiphilic CD provides more water soluble and adaptable nanoparticles through modulation of the balance between hydrophobic and hydrophilic chains at both CD sides. The grafting of small portions of polyethylene glycol at the secondary rim of CD can increase drug bioavailability and potentially decrease their immunogenicity. Furthermore, the use of long hydrophobic chains (C16 or C12) at CD primary side may allow to preserve the affinity for the biological membrane. Amphiphilic CD can be conveniently tailored by covalently appending receptor-targeting glycosyl groups [8,9] in order to build nanocarriers with increased drug selectivity toward specific cell lines. Supramolecular aggregates of amphiphilic cyclodextrins are thereby versatile systems toward the encapsulation of both hydrophobic and hydrophilic guests [10–12]. Photosensitiser (SENS) drugs embedded in cationic CD nanoassemblies were effective in inducing photodynamic damage in cancer cells [13,14]. Furthermore, multilayer films obtained by electrostatically assembling cationic CD and anionic porphyrins were found to behave as useful photoresponsive materials, yielding singlet oxygen upon irradiation [15].

Amid anticancer drugs, Docetaxel (Doc) is being used in clinical trails for the treatment of prostate and breast cancers as well as lung carcinoma. Doc was chosen as a model drug since it is sparingly soluble in water and thus difficult to formulate in dosage forms intended for intravenous delivery. Furthermore, in analogy to many other anticancer drugs, the lack of drug specificity for tumor site can be strongly improved by incorporation in a nanocarrier with the final aim to increase drug efficacy and limit side effects. Regarding its biological activity, Doc has high affinity for tubulin and causes the inhibition of several cellular functions such as endosomal uptake, secretion, transport and mitotic cell division [16,17].

In this paper we present the first full investigation on the application of heptakis (2-*O*-oligo(ethyleneoxide)-6-hexadecylthio-)-β-CD (SC16OH) (Scheme 1) as a nanocarrier for Doc and evaluate its potential to treat solid tumors. SC16OH nanoparticles loaded with Doc were prepared by the emulsion–solvent evaporation method and characterized for surface morphology, size distribution and zeta potential, drug encapsulation efficiency and *in vitro* release. Toxicity toward red blood cells was considered too. Finally, the biological effects of Doc delivered through SC16OH nanoparticles were evaluated in terms of mitotic catastrophe induction.

2. Materials and methods

2.1. Reagents and materials

Docetaxel (Doc), potassium phosphate dibasic and potassium phosphate monobasic were from Sigma–Aldrich. Taxotere® is a Doc commercial formulation for i.v. administration. MEM and Trypan blue were purchased from Gibco, paraformaldehyde was from Merck, Triton X-100 and HOECHST 33342 were from Sigma–Aldrich, FBS was from Lonza, Fluorescein-Conjugated Monoclonal Antibody Against Human Tubulin was from ICN, FITC-conjugated goat anti-mouse IgG was from Pharmingen. Analytical grade tetrahydrofuran (THF), methylene chloride (DCM) and acetonitrile were from Carlo Erba Reagenti (Italy).

2.2. Synthesis of SC16OH

SC16OH was prepared in a four step procedure from β-CD as previously reported [9]. Briefly in the last step ca. 1 mmol of heptakis(6-hexadecylthio-6-deoxy)-β-CD (SC16), K_2CO_3 (10% by weight relative to cyclodextrin) and ethylene carbonate (50 molar equiv.) were dissolved in 10 mL of tetra-*N*-methylurea. The mixture was stirred at 150 °C under N_2 for 4 h, analysed by TLC ($CHCl_3/CH_3OH/H_2O = 50:10:1$) to assess complete conversion of SC16 ($Rf = 0$) into SC16OH ($Rf = 0.55$). Furthermore, after this period CO_2 evolution had ceased. The solvent was removed at 100 °C under reduced pressure. The crude product was purified by crystallisation from 25 mL of methanol containing 20% acetone to give SC16OH in 71% yield as a powder.

2.3. Nanoparticle preparation

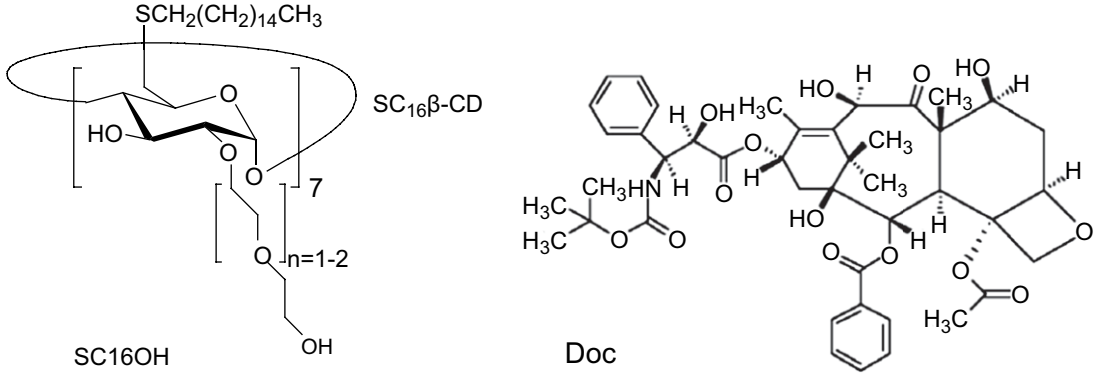
Doc-loaded or unloaded nanoparticles at theoretical loading of 9% by wt. were prepared by the emulsion–solvent evaporation method. SC16OH (10 mg) and Doc (1 mg) were codissolved in DCM (1 mL). This solution was poured in water (10 mL), and sonicated for 2 min at 3 W (Sonicator 3000, Misonix, USA) by a microtip probe under controlled temperature of 4 °C. Thereafter, the organic solvent was evaporated by mechanical stirring (300 rpm) for 4 h at room temperature. Doc-loaded nanoparticles were collected by ultracentrifugation (55000 rpm, 30 min, 4 °C) and washed twice with distilled water to remove unencapsulated drug. After redispersion in water, nanoparticles were freeze-dried for 24 h (Modulyo, Edwards, UK).

2.4. Particle size analysis

Dispersions of unloaded and Doc-loaded SC16OH nanoparticles (0.01 mg/mL) in microfiltered water were investigated along 8 weeks of preparation by quasi-elastic light scattering (QELS). QELS measurements were performed by using a He–Ne laser source ($\lambda = 632.8$ nm) at a power of 10 mW, linearly polarized orthogonally to the scattering plane. The scattered light, collected in a self-beating mode, was analysed using a Malvern 4700 correlator which builds up the normalized intensity autocorrelation function [18–20]. The measured intensity–intensity time correlation function $g_2(t)$ is related to the electric field correlation function, $g_1(t)$, by the Siegert relation [19].

$$g_2(t) = B\left(1 + f|g_1(t)|^2\right) \quad (1)$$

where B is the baseline and f is a spatial coherence factor. In the case of dilute solutions of monodispersed particles $g_1(t) = \exp(-\Gamma t)$, where the decay rate is $\Gamma = Dk^2$, D being the translational diffusion coefficient and k is the exchanged wavevector ($k = [(4\pi n)/\lambda]\sin(\theta/2)$, where n is the refractive index of the medium and



Scheme 1. Sketched structure of the investigated heptakis (2-*O*-oligo(ethyleneoxide)-6-hexadecylthio-)-β-CD (SC16OH) and docetaxel (Doc).