Modified Glycol Chitosan Nanocarriers Carry Hydrophobic Materials into Tumours

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ABSTRACT

Etoposide is a hydrophobic cytotoxic drug with activity in a range of cancers. In order to reduce side effects of the current formulation we have formulated the drug in nanosized carriers prepared from amphiphilic derivatives of glycol chitosan which dramatically improve bioavailability of hydrophobic drugs [1]. To this end a 13 kDa glycol chitosan was modified by palmitoylation and quaternisation. Micelles prepared from this polymer encapsulate up to 3.42 mg/ml etoposide. Using the hydrophobic fluorescent dye nile red we show that micelles efficiently deliver their payload to MCF-7 cancer cells in vitro and to A431 xenograft tumours in vivo, suggesting these systems could also deliver hydrophobic anti - cancer drugs such as eto poside to tumours .

Key words: Glycol chitosan, nile red, micelles, etoposide

BACKGROUND

Etoposide (Figure 1), an epipodophyllotoxine derivative, is extensively used in anticancer therapy,

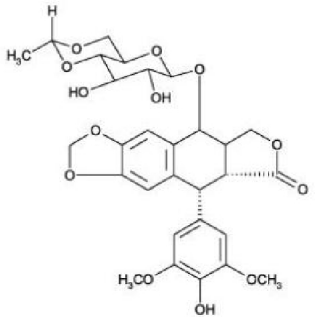

Figure 1 Chemical structure of Etoposide

both of solid tumours and haematological malignancies [2-4]. Because of its poor water-solubility and lipophilicity, the pharmaceutical formulation of etoposide is quite challenging requiring various excipients which have been associated with adverse effects such as hypotension, anaphylaxis, and bronchospasm [5, 6].

One promising alternative approach for the efficient delivery of etoposide to tumors relies on the use of nanocarriers to encapsulate such hydrophobic drugs and then deliver them to the target site, thus potentially reducing undesired side effects.We have recently reported the use of an amphiphilic derivative of glycol chitosan for the preparation of nano-sized

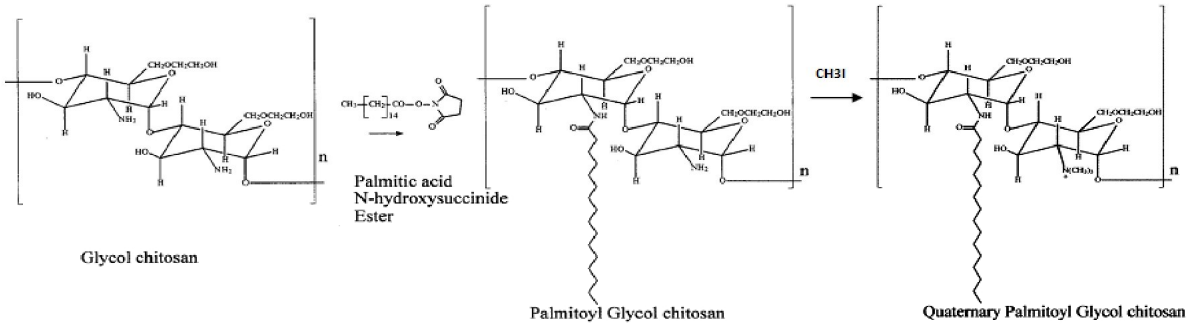

Figure 2 Stepwise synthesis of quaternary ammonium palmitoyl glycol chitosan

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micellar clusters that efficiently encapsulate hydrophobic drug molecules, leading to an up to tenfold increase in the drug bioavailability across barriers such as the blood-brain barrier [1]. The aim of the current project was to explore the use of this technology for the delivery of etoposide so as to eliminate the need for problematic excipients and potentially increase delivery to the tumour.

SYNTHESIS OF BIONANOCARRIERS

In the present work, hydrophobically modified glycol chitosan (GCP) was obtained by covalently coupling of palmitic acid *N*- hydroxysuccinimide with glycol chitosan of a defined molecular weight. Additionally, the solubility of the polysoap in aqueous media was improved by partial quaternization of the free chitosan amino groups as described previously [7] Figure 2. The molecular weight of the acid degrade starting material glycol chitosan (GC) and of the GCPQ product were determined by Quasi-Elastic Light Scattering after gel permeation chromatography (WyattQELS™; ASTRA software, Dawn EOS, Wyatt USA).

The level of palmitoylation was calculated using NMR by comparing the ratio of palmitoyl methyl protons (δ=0.89 ppm) to sugar protons (δ =3.5–4.5 ppm), the level of quaternisation was calculated by comparing the ratio of quaternary ammonium (δ=3.45 ppm) to sugar protons Figure 3. The selected GCP characteristics are given in the Table 1

The modified GCPQ polymer was characterized for its ability to form self assemblies. In aqueous media, the amphiphilic polymers self-assembled into polymeric micelles at or above the critical micellar

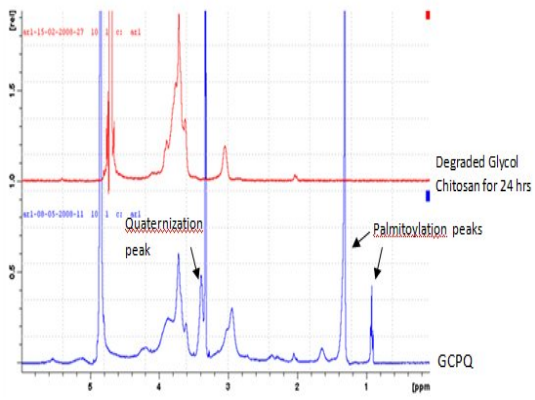

Figure 3 ^{1}H NMR spectra of degraded Glycol chitosan (red) and GCP (blue) showing the levels of palmitoylation and quaternization.

concentration (CMC). CMC was calculated using methyl orange as probe. A hypsochromic shift in the wavelength (350–550 nm) was recorded as we go towards increase in polymer, being the CMC 0.183 ± 0.04 mg/ml. (UV-1650PC UV-Visible spectrophotometer Shimadzu using UV Probe 2.21 version)

Furthermore, the size of micelles was studied using photon correlation spectroscopy (Nanosizer, Malvern Instruments) followed by transmission electron microscopy Figure 4. Micelles of 154.5 ±2.7 nm were obtained for at a concentration of GCP of 0.6 mg/ml.

Table 1 Charctersistics of the nanocarrier used in the study.

	GCPQ nanocarrier
Mol. Wt of glycol chitosan starting material (kDa)	7.91± 0.3956
Palmitoylation Degree (%age)	21.9 ± 0.6245
Quaternization level (%age)	24.3 ± 1.808
Molecular wt of GCPQ (kDa)	13.72 ± 1.3917
Cmc (gL^{-1}/µM)	0.183 ± 0.04

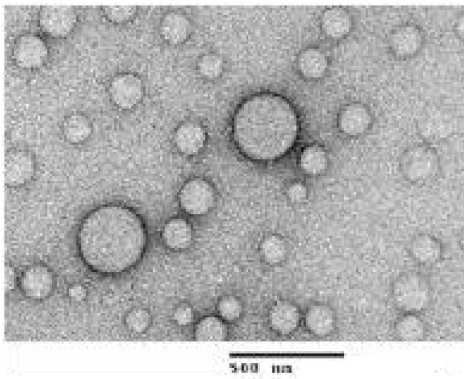

Figure 4 Micelles under transmission electron microscope

ETOPOSIDE ENCAPSULATION

The resulting micelles (5 mg/mL) were successfully loaded with the anticancer drug etoposide by probe sonication method, to a level of 3.42 ± 0.116 mg/ml. The etoposide-loaded GCPQ (Et-GCPQ) micelles had a mean size distribution of 217 ± 20.1 nm, with a low polydispersity index, and were found to be stable for up to 3 months at 4°C. Exposure to salts and serum lead to a limited size increase (348 ± 40nm).

CELL UPTAKE EXPERIMENTS

For uptake experiments, nile red was selected as probe dye on the basis of its similarity with etoposide in size and hydrophobicity. The particle size and stability of nile red-loaded GCPQ micelles (NR-GCPQ), demonstrated their similarity with Et-GCPQ micelles.

NR-GCPQ or a solution of nile red were incubated with MCF-7 cells. After 2h, the cells were fixed and observed under the confocal microscope. Results showed an enhanced uptake from nile red-loaded GCPQ nanoparticles compared to the compound in solution Figure 5. Similar results were obtained with A2780 cells (not shown).

INVIVO TUMOUR MODELS

The ability of these carriers to deliver their payload to solid tumours after i.v. administration was evaluated in established A431 xenograft tumours. Tumour bearing mice were given NR-GCPQ micelles intravenously and sacrificed after 4 hours. Tumours were excised, freezed and mounted sections were observed with laser scanning confocal microscopy

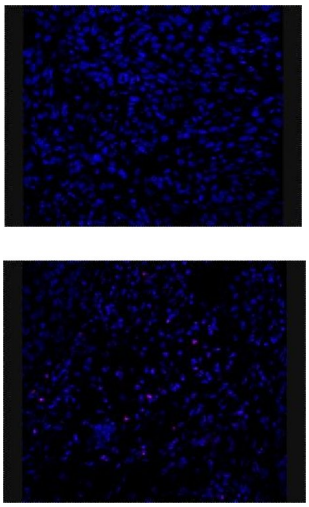

Figure 6 Confocal images of A431 mouse xenograft sections after intravenous injection of 5% Nile Red-loaded GCPQ micelles (bottom) compared to untreated mice (top). Blue channel: cell nuclei counterstained with DAPI. Red channel: Nile red.

Nile red fluorescence was detected in the treated tumour but not in the untreated control suggesting that GCPQ micellar clusters are able to deliver hydrophobic payload to solid tumours.

CONCLUSION

In conclusion, the capacity of hydrophobically modified glycol chitosan for the preparation of colloidal micelles and the efficient encapsulation of hydrophobic drugs has been demonstrated. Moreover, we were able to show that these micelles can deliver the encapsulated compounds to solid tumours.

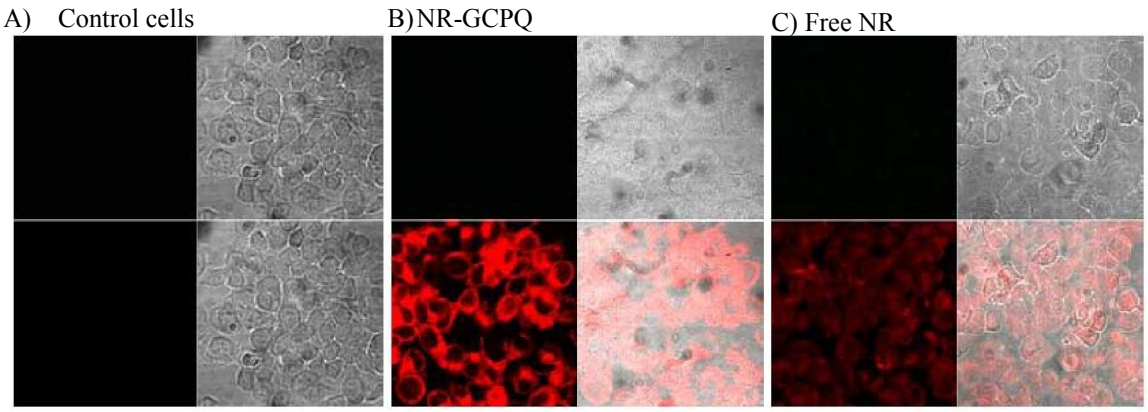

Figure 5 Nile red uptake in MCF7 cells- A- control cells, B-cells treated with nile red-loaded micelles, C- cells treated with nile red in solution. A, B & C- maximum projections. Red channel: Nile red.

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