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Alginate/chitosan microparticles for tamoxifen delivery to the lymphatic system

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ABSTRACT

Oral administration of the nonsteroidal anti-estrogen tamoxifen (TMX) is the treatment of choice for metastatic estrogen receptor-positive breast cancer. With the aim to improve TMX oral bioavailability and decrease its side effects, crosslinked alginate microparticles for the targeting to the lymphatic system by Peyer's patch (PP) uptake were developed and *in vitro* characterized. TMX was molecularly dispersed inside the microparticles and an electrostatic interaction involving the TMX tertiary amine was detected by rheological and FT-IR assays. Microparticles showed a size less than 3 μm, then suitability to be taken up by M cells in PP and a positive surface charge. Moreover, TMX loading level as well as *in vitro* release behaviour was affected by the polymer network connected with the mannuronic/guluronic ratio of the alginate chains.

1. Introduction

Oral administration of the nonsteroidal anti-estrogen tamoxifen citrate salt (TMX) is the treatment of choice for metastatic estrogen receptor-positive breast cancer. It is employed for the long-term prophylactic therapy in high-risk and post-menopausal women as well as for the treatment of advanced or metastatic breast cancer (Buckley and Goa, 1989; 11th Report on Carcinogens, 2005). TMX is administered as tablets or oral solutions at the dose of 10 mg twice a day or 20 mg once a day. However, following long-term therapy, TMX increased the incidence of vaginal symptoms, thromboembolic events, stroke and endometrial cancer (Shin et al., 2006) and the development of drug resistance (Maillard et al., 2005). Moreover, TMX showed a fairly good oral biovailability combined with large interindividual variations and extensively liver metabolization leading to increased dose and, consequently, side effects (McVie et al., 1986; Tukker et al., 1986).

Therefore, TMX formulation in micro or nanoparticles aiming to increase the oral biovailability or to achieve the desired dose at tumour site for a longer period were studied. Polyethylene glycol-coated nanospheres were proposed, resulting in an immediate drug release (Brigger et al., 2001). Poly(ε-caprolactone) nanoparticles were rapidly internalized in MCF-7 cells and they increased the local concentration of TMX in estrogen receptor-positive breast cancer cells (Chawla and Amiji, 2003). More, a formulation of microspheres of poly lactide-co-glycolide was designed to sustain the TMX action (Shera and Dhake, 2005) and the drug dosing by hydroxybutenyl-β-cyclodextrin provided a very significant increase in TMX oral bioavailability (Buchanan et al., 2006).

The targeting to the gut-associated lymphoid tissue (GALT), through M cells in Peyer's patches (PP), by a microparticulate carrier represents another possible strategy to overcome TMX limitations. Peyer's patch M cells, which are specialized cells staying over mucosa-associated lymphoid tissue (MALT), interspersed by enterocytes in the follicle-associated epithelium (FAE), can transport via endocytosis a variety of microparticulate matter from the gut lumen to intra-epithelial lymphoid cells and subsequently through the lymphatic system into the blood stream (Florence, 1997; Clark et al., 2001; Hussain et al., 2001). Therefore, TMX delivery to the GALT by the oral administration of microparticles could increase the drug bioavailability, avoid the enzymatic degradation from enterocytes as well as the first-pass metabolism so decreasing the dose and, consequently, the toxic effects. Furthermore, since the lymph system is one of the ways in which breast cancer can spread (stage 2), the targeting to the GALT could represent an advantageous approach to prevent the stage 2 of the illness.

Calcium alginate/chitosan (CaA/CHT) microparticles were previously designed exhibiting resistance to gastro-intestinal media, mucoadhesiveness and ability to be taken up by PP, even if the involvement of transport pathways across villous enterocytes was noticed (Coppi et al., 2006).

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Thus, calcium alginate/chitosan microparticles loaded with TMX were prepared by crosslinking processes with calcium ions and chitosan carried out on alginate microparticles obtained by spray-drying sodium alginate/TMX water solutions. To evaluate the suitability of the delivery system for the TMX GALT targeting, the crosslinked microparticles were *in vitro* characterized for shape, size, charge surface, IR and DSC patterns, alginate/TMX interaction, TMX loading and release. Moreover, the influence of alginate mannuronic/guluronic ratio, affecting a possible interaction with cationic drugs, on the above properties was examined by using two types of sodium alginate in the microparticle preparation.

2. Materials and methods

2.1. Materials

The following chemicals were obtained from commercial suppliers and used without further purification. Sodium alginate (Relative Molecular Mass, M_r about 147,000, extracted from *Laminaria digitata*, containing 62% mannuronic acid and 38% guluronic acid) (high M/G ratio, NaA-H) was donated by Kelco International (Bagnolex Cedex, France). Sodium alginate (M_r about 115,000, extracted from *Laminaria hyperborea*, containing 30% mannuronic acid and 70% guluronic acid) (low M/G ratio, NaA-L) and chitosan (CHT) (low MW ≅ 70,000) were purchased from Fluka Chemie (Buchs, Switzerland). Tamoxifen, (Z)-2-[4-(1,2-diphenyl-1-butenyl)phenoxy]-N,N dimethylethylamine, citrate (TMX) was kindly donated by Solmag (Mulazzano, Lodi, Italy). All the other chemicals were of analytical grade and they were purchased from Carlo Erba (Milan, Italy).

2.2. Microparticle preparation

The microparticles were prepared by two steps, using both alginates (NaA-L and NaA-H).

2.2.1. Spray-drying step

TMX methanol solution (0.1%, w/v) was added to NaA-H or NaA-L aqueous solution (1%, w/v) and the 10/1 alginate/TMX mixture was spray-dried (Buechi 190 Mini-Spray Dryer, Buechi Laboratorium, Technik AG, Flawil, Switzerland) to obtain uncrosslinked microparticles in the following operating conditions: inlet temperature 140 °C, outlet temperature 60–65 °C, pump setting 5 ml min^{-1}, spray flow 600 Nlh^{-1}, aspirator setting 15 and nozzle cap diameter 0.5 mm.

2.2.2. Crosslinking step

The uncrosslinked microparticles were suspended in 10% (w/v) $CaCl_2$ aqueous solution (0.9 M), in a 0.1/1 microparticles/$CaCl_2$ ratio, under mechanical stirring (Ultraturrax, IKA, Labortechnik, Staufen, Germany) at 9000 rpm for 5 min. Subsequently, an equal volume of 1% (w/v) CHT, solubilized in pH 5.5 acetic acid solution, was added to the microparticle suspension under mechanical stirring and maintained for 10 min. The crosslinked microparticles were then recovered by centrifugation, rinsed with water and freeze-dried (Lyovac GT2, Leybold Heraues, GMBM, Köln, Germany).

2.3. Morphological and size analysis

Microparticle morphological structure was examined by a scanning electron microscope (SEM, XL-40, Philips, Eindhoven, The Netherland). Microparticle size was determined by computerized image analysis (IMG-VIEW, CIGS, University of Modena and Reggio Emilia, Italy) of at least 200 microparticles on SEM micrographs.

2.4. Zeta potential measurement

Zeta potential measurements of TMX loaded and unloaded crosslinked microparticles suspended in deionized distilled water were performed using a PALS zeta potential analyser (Ver. 3.29, Brookhaven Instruments Corp., Holtsville, NY).

2.5. Rheological analysis

To investigate the interaction occurring between alginates and TMX, rheological measurements were carried out on alginate/TMX water solutions by evaluating the reduced viscosity owing to an "electroviscous effect" produced by dilute solutions of polyelectrolytes. A 0.5% (w/v) solution of sodium alginate is considered a diluted polyelectrolyte solution (Stockwell et al., 1986). Therefore, rheological measurements were carried out by using both the sodium alginates and sodium alginate/TMX solutions, in a 10/1 ratio, prepared by mixing 1% (w/v) alginate solutions with an equal volume of 0.1% (w/v) TMX water solution containing 1% (w/v) Tween 80. After 24 h, the rheological behaviour was determined at 25 °C by placing 9 ml of the solutions in a coaxial cylinder (radii ratio = 1.02) rheometer (Rotovisco RV 12 Haake, Karlsruhe, Germany) and measuring the shear stress as a function of the shear rate. In the same conditions, a solution containing pure 0.05% (w/v) TMX was examined. The rheological experiments were carried out in triplicate.

2.6. Thermal analysis

Thermal analysis was performed on TMX in bulk, melted TMX, TMX in physical mixture with both alginates (10:1 alginate/TMX ratio), uncrosslinked and crosslinked microparticles. Thermograms were recorded on a differential scanning calorimeter (DSC-4, PerkinElmer, Norwalk, CT, USA) coupled with a computerized data station (PerkinElmer). Samples were heated in crimped aluminium pans at a scanning rate of 10 °C/min using nitrogen flow (30 ml min^{-1}).

2.7. FT-IR spectrophotometry

FT-IR analysis was performed on TMX in bulk, melted TMX, TMX in a physical mixture with both alginates (10/1 alginate/TMX ratio), uncrosslinked and crosslinked microparticles. An FT-IR spectrophotometer (Model FT-IR 1600, PerkinElmer) was used for recording the spectra of the samples in nujol mull.

2.8. TMX content determination

Microparticle TMX content was determined by dissolving exactly weighed amounts of microparticles in 6% (w/v) sodium citrate aqueous solution containing 1% (w/v) Tween 80 enhancing TMX solubility. Such a medium was selected because it contains calcium chelating ions as well as ions displacing the drug associated to alginate. After centrifugation at 12,000 rpm for 10 min, TMX concentrations in the supernatant were assayed spectrophotometrically (λ = 278 nm) (Lambda 35, PerkinElmer) in comparison with standard solutions. The reported data were averaged on three determinations and statistically analyzed by ANOVA one-way.

2.9. TMX in vitro release

TMX release from microparticles was performed under sink condition for 1 h in saline solution at pH 3.0 and for 2 h in simulated intestinal fluid at pH 7.4 containing 1% (w/v) Tween 80, enhancing TMX water solubility (0.5 mg/ml) (Memisoglu-Bilensoy et al.,