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Comparative evaluation of *in vitro* parameters of tamoxifen citrate loaded poly(lactide-co-glycolide), poly(ε-caprolactone) and chitosan nanoparticles

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Tamoxifen (TAM), the clinical choice for the antiestrogen treatment of advanced or metastatic breast cancer, was formulated in nanoparticulate carrier systems in the form of poly(lactide-co-glycolide) (PLGA), poly-ε-caprolactone (PCL) and chitosan (CS) nanoparticles. The PLGA and PCL nanoparticles were prepared by a nanoprecipitation technique whereas the CS nanoparticles were prepared by the ionic gelation method. Mean particle sizes were under 260 nm for PLGA and PCL nanoparticles and around 400 nm for CS nanoparticles. Polydispersity indices were less than 0.4 for all formulations. Zeta potential values were positive for TAM loaded nanoparticles because of the positive charge of the drug. Drug loading values were significantly higher for PCL nanoparticles when compared to PLGA and CS nanoparticles. All nanoparticle formulations exhibited controlled release properties. These results indicate that TAM loaded PLGA, PCL and CS nanoparticles may provide promising carrier systems for tumor targeting.

1. Introduction

Tamoxifen citrate (TAM), a non-steroidal antiestrogen, has potential applications in the treatment of breast cancer. It is the oldest and most frequently prescribed antineoplasic nonsteroidal selective estrogen receptor modulator. TAM competitively inhibits the binding of estradiol to estrogen receptors, thereby preventing the receptor from binding to the estrogen-response element of DNA. The result is a reduction in DNA synthesis and cellular response to estrogen (Memisoglu-Bilensoy et al. 2005; Vural et al. 2005). It was approved by the U.S. Food and Drug Administration (FDA) in 1977 for the adjuvant therapy of advanced breast cancer which is the most common type of cancer in women.

The polymers poly-ε-caprolactone (PCL), poly(lactide-co-glycolide) (PLGA) and chitosan (CS) are used extensively in drug delivery and pharmaceutical nanotechnology. PLGA is the copolymer of polylactide and polyglycolide. It is typically made by ring-opening polymerization of their cyclic diester dimers. PLGA is the most widely used and studied class of biodegradable polymer for pharmaceutical use due to its biocompatibility and biodegradability. The degradation of PLGA proceeds with the formation of low molecular weight carboxylic acid oligomers and monomers (Cirpanli et al. 2005; Jeon et al. 2000; Shive and Anderson 1997).

PCL is a biodegradable, biocompatible and semicrystalline polymer. Its glass transition temperature is −60 °C and its melting point ranges between 59 and 64 °C, depending upon its crystalline nature. The numerical average molecular weight (M_n) of PCL samples can vary from 10000 to 42500 Da and it is graded according to the molecular weight. The slow degradation of PCL has led to its application in the preparation of different delivery systems in the form of microspheres, nanospheres and implants (Cirpanli et al. 2009; Sinha et al. 2004).

CS is obtained by deacetylation of α-chitin. CS is a nontoxic, biodegradable and biocompatible product due to the availability of free amino groups in its structure. CS is positively charged and thus reacts with many negatively charged surfaces or polymers. This explains its mucoadhesive properties and the formation of nanoparticles by the ionotropic gelation method, which is a reaction between CS and controlled amounts of multivalent anion (tripolyphosphate), resulting in cross-linking between CS molecules (Aktas et al. 2005; Calvo et al. 1997; Vila et al. 2004). Recently, there have been great advances in the use of surface-modified CS in drug targeting, such as brain drug delivery (Aktas et al. 2005 a,b; Cetin et al. 2007; Karatas et al. 2009; Pinarbasli et al. 2009).

The objective of this study was to develop nanoparticulate drug delivery systems for the anticancer drug TAM by loading it in PLGA, PCL and CS nanoparticulate drug delivery systems in order to compare the *in vitro* characteristics of the nanoparticles. TAM was chosen as a model anticancer drug with bioavailability problems to compare the technological parameters of CS, PCL and PLGA nanoparticles in the development of an optimum formulation for tumor targeting.

2. Investigations, results and discussion

In this study, nanoparticulate drug delivery systems for the anticancer drug, TAM, were designed and developed. Nanoparticles were characterized by particle size distribution, morphology, zeta potential, electron microscopy, drug entrapment efficiency and *in vitro* release studies.

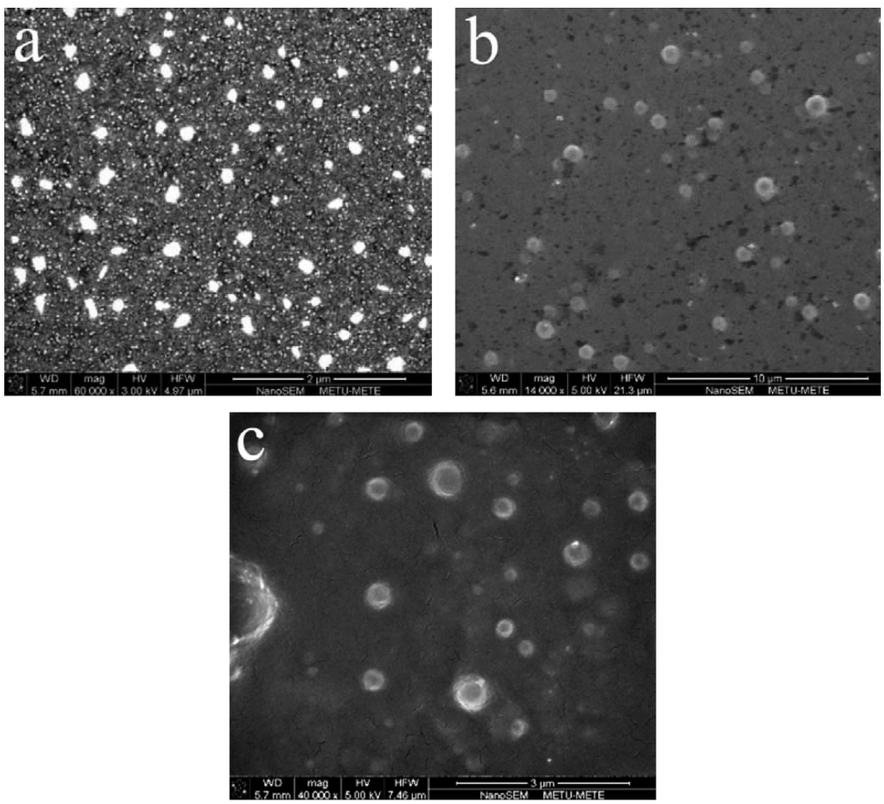

Fig. 1: Scanning electron micrographs of (a) chitosan nanoparticles (x60000), (b) poly(lactide-co-glycolide) nanoparticles (x14000), (c) poly(ε-caprolactone) nanoparticles (x40000).

All nanoparticle formulations exhibited narrow and nanoscale (<400 nm) particle size distributions (polydispersity index < 0.40) which are suitable for tumor targeting in terms of enhanced permeation and retention (EPR) effects. PCL nanoparticles yielded the smallest particles, approximately 197 nm in size, although all the nanoparticles were smaller than 400 nm. Nanospheres in particular were around 150–250 nm, suggesting favorable properties for injectable systems, the possibility of escape from reticuloendothelial system (RES) uptake, and leakage from the vasculature surrounding the tumor site resulting in an eventual passive targeting of the system.

The surface charge of the nanoparticles was evaluated and CS nanoparticles were shown to have the highest zeta potential values, highly positive and negative zeta potential values corresponding to better colloidal stability (Cirpanli et al. 2005). In addition, the difference in zeta potential values between blank and TAM loaded nanoparticles indicates that TAM, a positively charged compound, is loaded not only within the nanoparticles but also on to them, since zeta potential is an expression of the surface charge as seen in Table 1.

Imaging of nanoparticles by scanning electron microscope (SEM) would be expected to provide information on nanoparticle morphology and size. Examination of SEM photographs of the nanoparticles revealed that the surfaces were smooth and spherical, as seen in Fig. 1.

Table 2 shows the drug loading capacity and encapsulation efficiency of nanoparticle formulations. The highest values for both parameters were observed with PCL nanoparticles.

In vitro drug release studies have shown that the hydrophilicity of polymers affects the release of TAM in aqueous release mediums. Nanoparticles based on PCL, a significantly more hydrophobic polymer, released approximately 2% of the loaded TAM whereas those based on CS, a hydrophilic polymer, released almost all the loaded TAM (Fig. 2). It can be seen that PLGA and PCL nanoparticles liberate the drug with a considerably slower release profile than CS nanoparticles. For CS nanoparticles 60–70% of the drug was released within 0.5 h and

Table 1: Particle size distribution and surface charge of poly(lactide-co-glycolide), poly(ε-caprolactone) and chitosan nanoparticles ($n = 3$, mean ± SD)

Nanoparticle Formulation	Particle size (nm)	Polydispersity index	Zeta potential (mV)
Blank CS	368.18 ± 36.01	0.31	38.72 ± 7.60
CS-TAM400	375.14 ± 34.16	0.38	40.11 ± 6.32
CS-TAM800	395.67 ± 41.23	0.40	35.68 ± 7.12
Blank PLGA	224.87 ± 3.05	0.02	−4.89 ± 0.56
PLGA-TAM	260.60 ± 4.75	0.13	11.57 ± 0.55
Blank PCL	205.10 ± 2.01	0.20	−17.35 ± 5.26
PCL-TAM	197.33 ± 4.04	0.09	17.07 ± 1.43

Table 2: Drug loading capacity and encapsulation efficiency of poly(lactide-co-glycolide), poly(ε-caprolactone) and chitosan nanoparticles ($n = 3$, mean ± SD).

Nanoparticle Formulation	Drug loading capacity (%)	Encapsulation efficiency (%)
CS-TAM400	0.026 ± 0.001	71.19 ± 3.26
CS-TAM800	0.049 ± 0.002	66.37 ± 4.01
PLGA-TAM	2.89 ± 0.670	38.99 ± 1.18
PCL-TAM	14.53 ± 0.990	72.67 ± 4.93

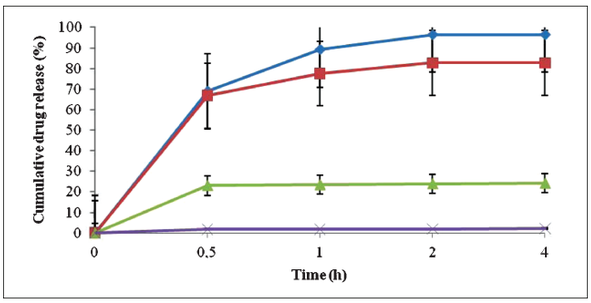

Fig. 2: Cumulative in vitro drug release (%) profiles of CS, PLGA and PCL nanoparticles ($n = 3$) CS-TAM400; CS-TAM800; PLGA-TAM; PCL-TAM

release was completed in 24 h. For PLGA nanoparticles, 24% of the drug was released within 24 h, and 2% of the drug was released within 24 h for PCL nanoparticles.

3. Experimental

3.1. Materials

CS (Protasan UP CL 113 with a deacetylation degree between 75 and 90%) was purchased from FMC Biopolymers (Haugesund, Norway). PCL ($M_W = 65$ kDa), penta sodium tripolyphosphate (TPP), polyvinyl alcohol (PVA) and Pluronic F-68 (PF-68) were purchased from Sigma-Aldrich, Inc. (St. Louis, USA). PLGA (50:50, $M_W = 34$ kDa) was purchased from Medisorb (Wilmington, USA). Chromatography grade octane-1-sulfonic acid sodium salt, methanol, acetone and dichloromethane, analytical grade glacial acetic acid and disodium hydrogen phosphate anhydrous were supplied by Merck KGaA (Darmstadt, Germany). Sodium chloride and potassium dihydrogen phosphate (Carlo Erba, Italy) were extra pure. Water was purified to 18.2 MΩ cm of electrical resistivity in-house using a Millipore Simplicity® UV system (Bedford, USA). TAM was donated by TEVA Pharmaceuticals (Petah Tikva, Israel).

3.2. High performance liquid chromatography (HPLC) equipment and conditions

An Agilent Technologies 1200 Series HPLC system, with a degasser, a quaternary pump, an auto-sampler, a thermostatted column compartment, and a variable wavelength detector was used, with ChemStation B.02.01 software installed on a PC (Hewlett-Packard Waldbronn, Germany) for data processing. The column was a Clipeus C_{18} (250 x 4.6 mm i.d., 5 µm; Higgins Analytical, Inc., California, USA). The flow rate of mobile phase was 1.0 ml min^{-1} with isocratic elution. The injection volume was 20 µl, the column thermostat temperature was maintained at 25 °C and the detector wavelength was set at 232 nm. Since TAM is a polar compound, normal phase HPLC was preferred. The mobile phase composition was as follows: a solution of 1.08 g of octane-1-sulfonic acid sodium salt and 2 ml of glacial acetic acid in water:methanol [322:678]. The mobile phase was degassed via sonication for 30 min before use. The HPLC method has been characterized and validated by our group, as previously described (Yerlikaya et al. 2009).

3.3. Formulation of poly(lactide-co-glycolide), poly(ε-caprolactone) and chitosan nanoparticles

The formulation of the CS nanoparticles is based on the ionic gelation method using two different TAM loading levels (400 ng ml^{-1}, 800 ng ml^{-1}), as previously described (Aktas et al. 2005). For this 1.75 mg of CS and 800 (CS-TAM400) or 1600 ng (CS-TAM800) of TAM were dissolved in 1 ml of water and 0.4 mg TPP was also dissolved in 1 ml water. While stirring the CS and TAM solution, 1 ml of TPP solution was added drop-by-drop to give a total volume of 2 ml. After 10 min, the dispersion was centrifuged at 13500 rpm for 30 min. The supernatant was removed and the nanoparticles were resuspended using 1 ml of water.

PLGA nanoparticles were prepared by a nanoprecipitation method (Cirpanli et al. 2009). Briefly, 75 mg of PLGA and 7.5 mg of TAM were dissolved in 5 ml of acetone. This organic phase was poured into deionized water (15 ml) containing 75 mg of PF-68 at room temperature with moderate stirring. Nanoparticles were immediately formed, and acetone was then removed from the colloidal suspension by rotoevaporation under reduced pressure to obtain a nanosphere dispersion.

For the formulation of PCL nanoparticles, a nanoprecipitation method was used (Leroueil-Le Verger et al. 1998). 20 mg of PCL and 2 mg of TAM were dissolved in 8 ml of acetone to obtain the organic phase. The aqueous phase comprised PF-68 solution (0.25%, *W/V*). Aqueous phase 8 ml and 4 ml of organic phase were mixed with magnetic stirring for 15 min. Finally, acetone was evaporated under vacuum at room temperature to obtain the desired volume of nanoparticle aqueous dispersion.

3.4. Characterization of poly(lactide-co-glycolide), poly(ε-caprolactone) and chitosan nanoparticles

Particle size distribution, morphology, surface charge, drug loading capacity, encapsulation efficiency and *in vitro* drug release characteristics of blank and TAM loaded CS, PLGA and PCL nanoparticle formulations were evaluated.

3.4.1. Particle size analyses

Mean particle diameter and polydispersity index of the nanoparticles were determined by Photon Correlation Spectroscopy (PCS) with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK). Measurements were performed in triplicate at an angle of 90 ° at 25 °C under suitable dilution conditions.

3.4.2. Zeta potential measurements

Zeta potential of the nanoparticle dispersions was measured in triplicate in mV using a Malvern Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) at 25 °C and an angle of 120° to determine the surface charge and the potential physical stability of the nanosystems. The zeta potential of the nanoparticles was measured in aqueous dispersion.

3.4.3. Scanning electron microscope analyses

A SEM (Jeol-SEM ASID-10. Device in 80 KV, Japan) was used to evaluate the surface characteristics of the nanoparticles. Nanoparticles were mounted on metal stubs with conductive silver paint and then coated with a 150 Å thick layer of gold in a Bio-Rad sputter apparatus. SEM images of the samples were obtained at different magnifications.

3.4.4. Entrapment efficiency

Drug loading capacities and association efficiencies for PLGA and PCL nanoparticles were determined after centrifugation of nanoparticle suspensions for 5 min at 5000 rpm. The supernatants were removed and the resulting nanoparticles were lyophilized. The lyophilized powders (10 mg) were dissolved in methanol for PCL nanoparticles and dimethylsulfoxide for PLGA nanoparticles and filtered through 0.22 µm nylon filters, since only tamoxifen citrate is soluble in methanol and dimethylsulfhoxide. After the extraction, the solutions were analyzed by HPLC. The drug loading capacities and association efficiencies of nanoparticles were calculated according to the following equations:

$$\text{Drug loading capacity } \% = \frac{\text{Loaded } TAM \text{ amount}}{\text{Nanoparticle weight}} \times 100$$

$$\text{Association efficiency} \% = \frac{\text{Loaded } TAM \text{ amount}}{\text{Initial } TAM \text{ amount}} \times 100$$

For CS nanoparticles, a similar method to that used for PCL nanoparticles was used to evaluate encapsulation of TAM. When preparing the CS nanoparticles, the supernatant removed was filtered through 0.22 µm nylon filters to eliminate suspended nanoparticles and the resulting solution was analyzed by HPLC. In order to calculate the drug loading capacity and association efficiency of CS nanoparticles the following equations were used:

$$\text{Drug loading capacity} \% = \frac{\text{Initial } TAM \text{ amount} - \text{Free } TAM \text{ amount}}{\text{Nanoparticle weight}} \times 100$$

$$\text{Association efficiency} \% = \frac{\text{Initial } TAM \text{ amount} - \text{Free } TAM \text{ amount}}{\text{Initial } TAM \text{ amount}} \times 100$$

3.4.5. In vitro drug release

Release profiles of TAM from the nanoparticle formulations were determined in 1 ml of isotonic PBS (pH 7.4) at 37 °C providing sink conditions in a thermostated bath system (Memmert, Schwabach, Germany) in polypropylene vials with no shaking. At predetermined time intervals, 1 ml samples

were withdrawn from the system and replaced with equal volumes of fresh release medium maintained at the same temperature. The amount of TAM released was assayed by HPLC as described above.

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