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Anticancer drug-loaded quantum dots engineered polymeric nanoparticles: Diagnosis/therapy combined approach

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ABSTRACT

Primary Effusion Lymphoma (PEL) is an HHV-8-related non Hodgkin lymphoma localized in body cavities (as pleural, peritoneal and pericardial) presenting lymphomatous effusion that, until now, lack of an effective therapy. Curcumin was reported to display pro-apoptotic effect *via* the inhibition of the JAK/STAT pathway, that is overexpressed in PEL cells, as consequence of virus infection. The administration of curcumin is severely restricted by its physicochemical properties, mainly its low solubility in biological fluid and consequently low bioavailability. Encapsulation into biocompatible and biodegradable PLGA nanoparticles (NPs) could be a strategy to overcome biological limits of curcumin, offering a valuable step forward for its clinical application. In this study we described single-emulsion process for curcumin loading into NPs (encapsulation efficiency about 35%). We applied a post-formulation strategy (NHS/EDC reaction) to decorate the surface of the curcumin-loaded NPs with quantum dots (QDs) as imaging agents (QDs-NPs-Cur, 24 pmol of QDs per 100 mg of NPs) obtaining tools useful for possible application in theranostic approach.

Bifunctionalized NPs were tested *in vitro* on two PEL's cell line (BCBL-1 and HBL-6). The efficacy of the treatment was evaluated by cytofluorimetric assay by measuring both cell viability and cell density. We found that the NPs significantly improve the cellular effect of curcumin (respect to free drug). Moreover, by means of confocal microscopy, both the localization of bifunctional NPs and of the released drug were easily detectable.

Thus, we conclude that the delivery of curcumin using bifunctional traceable NPs is a promising future approach for the diagnosis and the treatment of PEL.

1. Introduction

Theranostic nanomedicine is based on the combination of both therapeutic and diagnostic functions in one single drug delivery system (Jo et al., 2016). Relating to the cancer treatment, theranostic systems are usually based on biocompatible and biodegradable polymers assembled to form nanocarriers, able to co-encapsulate/conjugate chemotherapeutic drugs, contrast agents and selective ligands, specifically designed to target cells in order to improve diagnosis, evaluation and resolution of pathologies (Yu et al., 2012; Peer et al., 2007). These multifunctional systems should demonstrate (1) high drug loading capacity, (2) ability to be specifically accumulated in the diseased tissues/cells, (3) capability to deliver the therapeutic agents, (4) safety (5) ability to undergo biodegradation into nontoxic products and (6) traceability. Several multifunctional nanocarriers can be designed to this scope and after drug loading, their surface can be further modified by conjugating biochemical moieties for selective detection by means of different imaging modalities (Jin et al., 2010; Huang et al., 2006; Fan et al., 2014) as optical imaging (fluorescent and confocal imaging), magnetic resonance imaging, computed tomography, ultrasound and positron emission tomography or single photon emission computed tomography. These modalities have been combined with therapy to label target sites, to obtain biological information, to observe the distribution of nanocarriers or drugs, and to monitor the results of therapy (Lee et al., 2012; Kim et al., 2009).

Recently, our research group demonstrated the potentiality of nanocarriers as liposomes, polymeric nanoparticles and hybrid lipidic/polymeric nanosystems in delivering gene material or drugs for the treatment of a Human herpesvirus 8 (HHV-8) related lymphoma (Primary Effusion Lymphoma, PEL) (Belletti et al., 2011, 2015; Riva et al., 2015), a non-Hodgkin lymphoma localized in body cavities, as pleural, peritoneal and pericardial, presenting lymphomatous effusion.

The etiology and cellular mechanisms involved in this disease were recently identified (Kim et al., 2014; Osawa et al., 2016). Curcumin (Cur), a phenolic compound isolated from *Curcuma longa*, was shown to act as anti-inflammatory, antioxidant and anti-cancer drug (Ramadan

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et al., 2011; Araújo and Leon, 2011) especially due to the inhibition of JAK/STAT pathway, a kind of cellular signalling proteins overexpressed in PEL cells as consequence of virus infection (Uddin et al., 2005).

However, Cur is poorly soluble in the biological fluids, showing a very low bioavailability. The stable loading into NP carriers could allow the administration of Cur by the parenteral route, offering a valuable step forward for its clinical application.

Among imaging agent, QDs are a novel class of inorganic nanocrystals of semiconducting materials that are gaining widespread recognition owing to their exceptional photo-physical properties. QDs are considered useful devices for nanomedicine being sensitive and fast responsive fluorescent imaging agents able to provide a detailed and dynamic tracking of labeled nanocarriers in cells and body tissues. Some concerns were risen regarding their toxicity, in particular for the QDs that are cadmium-containing. However, previous *in vitro* experiments demonstrated that QD labeling could occur without significant toxicity as long as the QDs are coated and designed to be biologically inert. In particular PEG coating of QDs lead to a decrease in cell death, as reported in previous studies (Chang et al., 2006; Derfus et al., 2004).

The aim of this study was to optimize bifunctional NPs loaded with Cur (therapeutic agent) and decorated in their surface with PEG-coated QD (imaging agent) in order to obtain an ideal theranostic system able to track both the nanocarrier accumulation and the released drug.

Firstly we optimized step by step the formulation process in order to combine imaging and therapeutic agents in the same carrier through 1) the production of drug loaded nanoparticles (NPs-Cur), 2) the conjugation of QDs on the carboxylic-end of PLGA after the drug loading (post-formulative technique) (QDs-NPs-Cur).

Physicochemical and technological properties of NPs were monitored after drug loading (NPs-Cur) and after multifunctional formulation (QDs-NPs-Cur) by determining particle size, size distribution, surface charge, morphology and structure, efficacy of Cur encapsulation and QD coupling efficiency. Then, *in vitro* cellular localization of both Cur and nanoparticles (marked with QDs) was examined in PEL cell line by confocal microscopy as Cur displays a fluorescent profile with an maximum peak of emission at 550 nm while QDs show maximum emission peak at 605 nm.

Contextually, the efficacy of Cur released over time was evaluated and compared with that of free Cur by cytofluorimetric analyses.

2. Experimentals

2.1. Material

Poly-(D,L-lactic-*co*-glycolic) acid RG503H (PLGA) (50:50 m/m, inherent viscosity in 0.1% (w/v) $CHCl_3$ at 25 °C = 0.38 dL/g), was used as received from the manufacturer (Boehringer-Ingelheim, Ingelheim am Rhein, Germany). According to the experimental titration results of the carboxylic end of the polymers (4.94 mg KOH/g polymer) the molecular weight of RG-503H was calculated to be 11,000 Da. Pegylated Quantum Dots with amino terminal end (QDs) emitting at 605 nm, suspended in sodium borate buffer (Qdot605 ITK; catalog number Q21501MP) were provided from Life Technologies Italia (Monza, Italy). Curcumin [(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione; Cur] (purity > 98%, water solubility in the range of 1–10 μg/mL (Jagannathan et al., 2012) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Polyvinyl alcohol (PVA; MW near to 15,000 Da, degree of hydroxylation 86–89 Mol%, viscosity of the 4% w/w water solution at 20 °C 3 mPa ∗ s), was provided from Fluka (Milan, Italy). RPMI 1640, culture media, heat-inactivated bovine fetal serum solution (FBS) and phosphate buffered saline (PBS) pH 7.4 were purchased from Invitrogen (Milan, Italy). A Milli-Q water system (Millipore, Bedford, MA, USA), supplied with distilled water, provided high-purity water (18 Ω). All the solvents were of analytical grade, and all other chemicals [ethyl(dimethylaminopropyl)carbodiimide (EDC), *N*-hydroxysuccinimide (NHS), trehalose dehydrate, 2-(*N*-morpholino) ethanesulfonic acid (MES)] were used as received from the manufacturers.

2.2. Preparation of nanoparticles

Cur loaded PLGA nanoparticles (NPs-Cur) were prepared by an optimized single emulsion–solvent evaporation method (Zambaux et al., 1998; Vrignaud et al., 2011). In brief, 3 mL of a dichloromethane solution of 100 mg PLGA and 5 mg Cur were emulsified under sonication (55 W for 60 s at 5 °C, Branson SLPe Branson Ultrasonics Corporation, Danbury, CT, USA) in 12 mL of 1% w/v aqueous solution of PVA to form an oil-in-water emulsion. The emulsion was mechanically stirred (1500 rpm; RW20DZM, Janke & Kunkel, IKA-Labortechnik, Staufen, Germany) for at least 1 h at r.t. until the complete solvent evaporation and finally purified by Hi-Speed Refrigerated Centrifugation (Beckman J21, Milan Italy) at 17,000 rpm for 10 min at 5 °C, washed several times with water and re-suspended in water. As control unloaded nanoparticles (NPs) were prepared applying the same preparation procedure but without adding Cur.

2.2.1. Nanoparticle conjugation with QDs

NPs-Cur engineered with QDs (QDs-NPs-Cur) were prepared by applying a post formulation strategy based on exposed PLGA chain activation of NPs-Cur by using the NHS/EDC method (Nobs et al., 2003; Bondioli et al., 2010; Pederzoli et al., 2016). Briefly, 25 mg of NPs-Cur were suspended in MES buffer (0.1 M, pH 4.7) (Sigma Aldrich) and let to react with 25 mg of NHS and 75 mg of EDC (Sigma Aldrich) to activate the carboxylic group of the polymer. After 1 h, activated NPs-Cur were purified by ultracentrifugation at 17,000 rpm for 10 min at 4 °C and re-suspended in 1 mL of phosphate buffer (PBS) pH 7.4. Then, 8 μL of an 8 μM QDs solution (64 pmol02 of QDs) were added and the suspension was magnetically stirred for 1 h at r.t. Then, QDs-NPs-Cur were collected by centrifugation (17,000 rpm, 4 °C) and further washed twice with water (2 mL).

As control unloaded nanoparticles engineered with QDs (QDs-NPs) were prepared applying the same preparation procedure to the NPs.

All the nanoparticle samples (NPs, QDs-NPs, NPs-Cur, QDs-NPs-Cur) were freeze dried (LyoLab 3000, Heto-Holten, Allerod, Denmark) using D-(+)-trehalose (Fluka-Sigma Aldrich) as cryo-preservative at 1:1 w/w polymer/trehalose ratio. The yield (Yield%) was calculate as follows:

$$\text{Yield (\%)} = [\text{mg of nanoparticles recovered (excluded PVA residual and anhydrous trehalose)} / (\text{mg of PLGA} + \text{mg of Cur used for the preparation})] \times 100$$

3. Physico-chemical characterization

3.1. Size and zeta potential analysis

The mean diameter (*Z*-Average), the size distribution measured in intensity [expressed as $D_i(50)$ and $D_i(90)$] and the polydispersity index (PDI) of the samples were determined at 25 °C by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern, Malvern, UK; Laser 4 mW He−Ne, 633 nm, Laser attenuator Automatic, transmission 100–0.0003%, Detector Avalanche photodiode, Q.E. > 50% at 633 nm). For each preparation, Z-Average and PDI were calculated as the mean of three replicates of three different batches (9 measurements). The zeta potential (ζ-pot) was measured using the same equipment with a combination of laser Doppler velocimetry and phase analysis light scattering (PALS). Data of ζ-pot were collected as the average of 10 measurements.

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