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Modified-chitosan nanoparticles: Novel drug delivery systems improve oral bioavailability of doxorubicin

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ABSTRACT

The efficacy of most anticancer drugs is highly limited in vivo due mainly to poor pharmacokinetics behavior including poor bioavailability after extravascular administration. We have developed novel chitosan-modified polymeric nanoparticles for oral as well as i.v. administration. Nanoparticles were developed utilizing the double emulsion solvent evaporation technique for sustained delivery of various anticancer drugs. Chitosan diacetate (CDA) and chitosan triacetate (CTA) polymers were previously modified in our laboratory and used as novel matrix. Nanoparticles, loaded with various anticancer drugs, were characterized for particle size using dynamic light scattering as well as transmission electron microscopy and net surface charge using dynamic light scattering. Particles size was below 100 nm in diameter and zeta potential ranged − (25–30). Encapsulation efficiency of anticancer drugs varied considerably and was dependent on the physicochemical characteristics of the encapsulated drug. However, chitosan triacetate nanoparticles showed relatively higher encapsulation efficiency than chitosan diacetate nanoparticles. In vitro release of encapsulated drugs was sustained over a period of 14 days. Nanoparticles enhanced cellular accumulation of encapsulated drugs, compared to the free drugs, in vitro in MCF-7 and Caco-II tumor cell lines. In conclusion, diacetate and triacetate chitosan are novel polymers that can be used to formulate nanoparticles which efficiently encapsulated anticancer drugs, and sustained the release and enhanced tumor cellular uptake of these drugs. Further, chitosan triacetate nanoparticles enhanced oral bioavailability of doxorubicin. CDA and CTA nanoparticles can be used to efficiently deliver anticancer drugs and improve their in vivo profile.

1. Introduction

The efficacy of anticancer drugs is highly limited by various factors including tumor drug resistance to these drugs (Tomasetti, 2014). Tumor drug resistance is a wide term that includes several mechanisms such p-gp glycoprotein efflux transporters, unfavorable acidic tumor microenvironment, and entrapment of anticancer drugs in intracellular acidic compartments (Tomasetti, 2014; Breier et al., 2013). Single or multiple mechanisms of drug resistance renders the anticancer drug less effective and contributes to the low cure rate and failure of many drugs in clinical application. In addition, poor bioavailability limits the use of many anticancer drugs to the parenteral route.

Development of innovative drug delivery systems has been always a target of many researchers and pharmaceutical companies (Pérez-Herrero and Fernández-Medarde, 2015). In addition to serving their classical role as delivery systems, many have shown promising benefits to enhance the effect of anticancer agents, increase oral bioavailability, enhance patient's compliance, as well as overcoming tumor drug resistance.

Nanoparticulate drug delivery systems have been investigated for the past three decades and many have shown advantages in cancer treatment over conventional dosage forms (Ediriwickrema and Saltzman, 2015). The most potential target is enhancing anticancer effectiveness by increasing accumulation of these drugs through "enhance and retention effect".

In addition, oral route is the most convenient method of drug administration. However, poor oral bioavailability and unfavorable pharmacokinetics profile especially non-specific accumulation of the drug in sites other than the tumor tissues are major limitations to the clinical use of anticancer drugs (Doak et al., 2014; Widmer et al., 2014; Bergsbaken et al., 2015). For example, oral bioavailability of the classic and potent chemotherapeutic drug doxorubicin does not exceed 5%. Delivery of anticancer drugs using nanoparticles can significantly enhance oral bioavailability and favorably modify their in vivo biodistribution

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(Pérez-Herrero and Fernández-Medarde, 2015; Chavanpatil et al., 2006).

In the current work, we developed innovative nanoparticulate drug delivery systems using modified chitosan polymers and AOT (aerosol-OT) surfactant and investigated the use of these nanoparticles in encapsulating various drugs including anticancer drugs. We characterized these nanoparticles for physicochemical properties and investigated their use to enhance oral bioavailability of poorly oral bioavailable anticancer drugs.

2. Materials

Doxorubicin, vinblastine, imatinib, and pemetrexed were purchased from LC laboratories (Woburn, MA). AOT, chitosan, polyvinyl alcohol, calcium chloride, methanol, methylene chloride, ammonium acetate, RPMI 1640 cell culture medium, and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO). MCF-7 and Caco-II tumor cell line was obtained from ATCC (Manassas, VA).

3. Methods

3.1. Nanoparticles formulation

Nanoparticles individually loaded with doxorubicin, vinblastine, imatinib, or pemetrexed were fabricated using previously published method of double-emulsion solvent-evaporation and cross-linking technique with slight modifications (Khdair et al., 2008). Briefly, 1 mL of chitosan diacetate or chitosan triacetate aqueous solution (1% w/v) containing 5 mg of doxorubicin, vinblastine, imatinib, or pemetrexed was emulsified into 2 mL of AOT organic solution (2.5% w/v in methylene chloride). The (w/o) nanomulsification was enhanced by sonication for 1 min over an ice bath (UP200Ht ™, Hielscher Ultrasonics GmbH, Germany). The secondary (w/o/w) was formed by further emulsification into 15 mL of polyvinyl alcohol solution (2% w/v in water) by sonication for 1 min over an ice bath. Nanoparticles were formed by continuous stirring using a magnetic stirrer with drop-wise addition of 5 mL of aqueous calcium chloride (60% w/v). For complete evaporation of methylene chloride, the emulsion was further stirred overnight at room temperature and with additional 1 h under vacuum. Excess drug, methylene chloride, and polyvinyl alcohol were removed from the nanoparticles suspension by three cycles of centrifugation and washing the collected pellet (nanoparticles) in between with deionized water. At the end of third wash, nanoparticles were resuspended in 1–2 mL of deionized water and the suspension was frozen for 48 h at —80C. The frozen nano-suspension was lyophilized for 3 days ((FreeZone 4.5®, Labconco, Kansas City, MO) to collect dry nanoparticles. Dry nanoparticles were weighted and yield was calculated. In addition to anticancer drugs, three model non-anticancer drugs were used in the formulation development; methylene blue (a model water-soluble positively charged drug), diclofenac sodium (a model water-soluble negatively charged drug), and clopidogrel bisulfate (a model pH-dependent water-soluble drug with limited water solubility at physiologic pH). Blank nanoparticles were formulated using above method without the addition of any drug.

3.2. Particles size, morphology, and surface charge characterization

Nanoparticles size and charge (zeta potential) were measured using dynamic light scattering (Zetasizer ZS, Malvern, United Kingdom). Briefly, a nanoparticle suspension in deionized water was prepared by sonicated and then particle size was measured and analyzed by the nonnegatively constrained least square algorithm. Particles size was confirmed and particles morphology was studied using transmission electron microscope (TEM; Philips/FEI, Inc., NY). Briefly, a dry sample of nanoparticles was added to the TEM grid and visualized and images from various fields were then captured. At least fifty particles in the TEM images in various fields were used to determine particle size using ImageJ software (National Institutes of Health, Bethesda, Maryland). As an additional method to verify size and morphology, nanoparticles were examined using scanning electron microscopy (SEM). Nanoparticles were metalized with gold/palladium and visualized with the SEM at an accelerating voltage of 30 kV and then images were captured and particles in various fields were analyzed for size and morphology, Inspect™ F50 equipped with Field Emission gun (Oregon, USA).

3.3. Drug loading and encapsulation efficiency

Amount of the drug loaded in nanoparticles was determined using UV–Vis absorbance methods. In brief, 10 mg of nanoparticles was extracted for 3 h in the dark with 10 mL methanol in an orbital shaker and then centrifuged for 8 min at 10,000 rpm. About 2 mL of the supernatant was further diluted and absorbance was measured at 440, 250, 280, 420 nm, 670 nm, 276 nm, and 220 for doxorubicin, pemetrexed, imatinib, and vinblastine, methylene blue, diclofenac sodium, clopidogrel bisulfate, respectively, against blank. Drug loading in nanoparticles was calculated as the amount in milligrams of each anticancer agent per 100 mg of nanoparticles, and was represented as % w/w. Encapsulation efficiency of the nanoparticulate system was defined as the actual amount of the loaded drug in the total yield of nanoparticles to the initial amount of the drug incorporated during formulation, and was represented as % w/w.

3.4. In vitro drug release

Under sink condition, the release of nanoparticle-encapsulated drug was studied over a period of 3 weeks for doxorubicin nanoparticles. In brief, 5 mg of the loaded nanoparticles were suspended in 0.5 mL of PBS in Float-A-Lyzer® G2 dialysis tubes (8–10 kDa MWCO, Spectrum Europe B.V., Netherlands). Each dialysis tube was individually placed in a 15-mL centrifuge tube containing 10 mL PBS as sink-condition release medium. The 15-mL centrifuge tubes were placed in an orbital shaker at 100 rpm and 37 °C. At 0.5, 1, 3, 6, 12 h, and 2, 3, 5, 7, 14, and 21 days post incubation, 0.5 mL of the external release medium was removed from the centrifuge tube, and was immediately replaced with fresh pre-warmed PBS. Collected samples were analyzed for drug concentration using HPLC, Smartline™ UV detector 2520 (Knauer, Wissenschaftliche Geräte GmbH, Germany). A system consisted of a C18 reversed-phase HPLC column, a mobile phase composed of acetonitrile: water (30:70, at pH 3.0 adjusted with ammonium acetate) and a flow rate of 1.0 mL/min, and detection at 240 nm using a UV detector at ambient conditions. To verify the release pattern from nanoparticles loaded with a drug which has low drug loading and probably weak entrapment, a similar release study was performed on CTA nanoparticles loaded with diclofenac sodium.

3.5. In vitro cellular uptake studies

Cellular accumulation of nanoparticle-encapsulated drug was studied in MCF-7 and Caco-II cells using nanoparticles containing clopidogrel bisulfate as a model non-cytotoxic drug. Cells were seeded in 24-well plates for 36 h at 50,000 cells/well and 37 °C in CO2 incubator. Prior to starting accumulation study, attachment of cells and formation monolayer to the plate was confirmed by examining cells using light microscope. Following that, cells were incubated with a suspension of nanoparticles containing clopidogrel bisulfate (100 μg/mL in RPMI medium) or equivalent dose of free drug dissolved in RPMI medium for 2 h at 37 °C. Cells were washed thrice with cold PBS and then lysed with 300 μL Triton-x. The amount of protein in cells was determined in the lysate using Pierce protein assay reagents (Rockford, IL). Clopidogrel content in the cells were measured in the cell lysate using