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Original article

Poly-carboxylic acids functionalized chitosan nanocarriers for controlled and targeted anti-cancer drug delivery

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ABSTRACT

The present study evaluates the in-vitro cisplatin (CDDP) release from four different poly oxalates cross-linked chitosan (CS) nanocomposites. The poly oxalates were synthesized from the reaction of four different dicarboxylic acids with ethylene glycol (EG). The encapsulation of CDDP on CS cross-linked with Oxalic acid-EG, Succinic acid-EG, Citric acid-EG and tartaric acid-EG carriers were carried out by the ionic gelation technique. The poly-oxalate nanocarriers were characterized by scanning electron microscopy, atomic force microscopy, X-ray diffraction studies and zeta potential analysis. The stability of poly-oxalates was calculated by the density functional theory (DFT) using Gaussview 05. Excellent drug release kinetics and good biocompatibility of nanocomposites were observed for the in-vitro analysis. The unloaded poly oxalate nanocomposites perform to have a low inherent cytotoxicity, whereas the loaded nanocomposites were as active as free CDDP in the MCF-7 cancer cell line. The tumor growth inhibitions of CDDP-loaded nanocomposites are more or equal to that of free CDDP. Taken together, these two poly oxalate nanocomposites are established as promising drug carriers for the delivery of CDDP.

1. Introduction

Nanotechnology approaches, where a constant dose of drugs is transported directly to the diseased sites or cells for a prolonged period, may result in a substitute therapeutic opinion for patients. The challenge lies in the strategy of nanocarriers that are effectively delivered to the targeted cells and release their contents over an extended period to achieve a clinical response. Advances in nanocarriers technologies have allowed the development of multifunctional materials for cancer detection, therapy and treatment monitoring. Their numerous advantages include cell-targeted drug delivery, which can reduce the amount of the drug required for a therapeutic dose, bio-availability [1], especially for hydrophobic drugs, lower drug toxicity [2], increased mucosal delivery that decreases first-pass metabolism [3], controllable timing of drug delivery [4] (slow, sustained, pulsatile, or stimulus-response), and the capacity to combine drugs and imaging agents in the same particle [5–7]. Scalability, security, and cost remain the almost formidable challenges hindering the multifunctional drug carriers' utilization from advancing to clinical trials.

Cancer is the large bio-disaster of today. It is respectively the first and second leading cause of death in economically developed and developing countries. One in eight deaths in the world is owing to cancer. It is reported that the global cancer burden will shoots up to 21.4 million new cases and 13.2 million cancer deaths by 2030 [8]. In spite of great effort, there has been less significant progress made in cancer treatment in the past two decades. It is the need of the hour to develop biocompatible anticancer drugs with greater efficiency to cure the disease since the problems related to drug delivery such as low drug tolerance, no specificity, multi-drug resistance; undesired pharmacokinetics and bio-distribution still remain. Nanomedicine provides a functional platform for multi-modality treatment of several health problems. Many of the anticancer drugs based on nanomaterials have been developed [9,10]. However, the high efficacy of anticancer drugs is always associated with severe side effects to normal cells. In particular, one prominent adverse effect is the dose-dependent cardiotoxicity induced by anticancer drugs is cumulative and life-threatening

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[11]. Platinum-based drugs are the most regularly used among all available chemotherapeutic agents [12,13]. In the rare situations where these two classes of medications have been combined, enriched tumor reactions were detected in patients with ovarian cancer [14], advanced breast cancer [15], and endometrial carcinoma [16], thus emphasizing the potential of this drug combination in the treatment of cancer.

Cisplatin (CDDP) has been widely used as a powerful therapeutic agent against numerous solid tumors by interacting with DNA to form intra-strand cross-link adducts and interfering with the cell transcription mechanism [17]. Though the mechanism of anticancer activity of CDDP is not completely understood, the general view of CDDP-DNA crosslink formation is believed to result in defective DNA templates and arrests DNA synthesis and replication [13,14]. However, the CDDP's therapeutic applications have been restricted as it targets tumor and healthy cells, chemically unstable, its poor water solubility, and its low lipophilicity [18–20]. Furthermore, tumor cells may develop intrinsic resistances to CDDP that results in even fewer platin uptake and more DNA repair [21]. In order to alleviate these limitations and to enhance its therapeutic ability, CDDP is frequently added with non-hydrophobic polymers or inserted in liposomes or other types of polymeric materials [22,23]. Such modifications have proven to be effective in enhancing cellular uptake as well as the shielding effectiveness of CDDP from fast degradation en route to the nuclear region. In addition various pH or intracellular chemical stimuli independent of the enzyme have been applied to control the release and cellular distribution of CDDP [24].

In the latest advancements of nanoformulations, the use of biodegradable polymer-based formulations have found inevitable from controlled drug delivery to targeted, environmentally sensitive, pulsatile, and intelligent drug delivery systems [25,26]. From investigations to date, it has been observed that poly oxalate has been unique, structural design to obtain an active drug delivery system and combinations of polymer properties. Furthermore, demands for fabrication conditioning and release characteristics vary for different drugs based on their pharmacological and physicochemical profile. Therefore, the prerequisite properties of biodegradable poly oxalate polymers differ from diverse formulations as well as drugs. This in itself requires special considerations before formulation of carrier design [27–29]. The selection of polymers for a typical drug delivery design is based on the polymer properties such as chemical composition, solubility, degradation behavior, crystallinity and hydrophilicity, the polymer drug compatibility, zeta potential (ζ), surface morphology, and drug release from prepared formulations [30,31]. Moreover other factors like type of formulation, route of administration, drug properties, etc. must also be considered for polymer selection. Hence, polymer selection is the crucial step in the development of a successful drug delivery system or device.

Polymer nanocarriers, primarily based on CS, liposome, gelatin, polyethylene glycol and poly-lactic acid, have been widely used for the delivery of diverse therapeutic agents such as proteins, peptides, nucleic acids, and water insoluble small drug molecules. They are especially useful for delivering drugs requiring continuous and sustained release with a single bolus direction since their excellent biodegradability and biocompatibility over natural pathways. Recently, much attention has been focused on utilizing CS and its derivatives for drug delivery vehicles, wound healing, accelerators, and nerve regeneration agents [32]. CS, an amino polysaccharide obtained from the N-deacetylation of chitin, is known to have low immunogenicity, good biocompatibility, biodegradability, and biological activities [33]. These polyester based nanocarriers also exhibit an excellent shelf life, suitable physicochemical properties, and well-characterized degradation products. However, their applications are potentially problematic for infection and inflammation-associated diseases because of their acidic degradation products that can lead to increased pH values and cause inflammation. Slow hydrolysis kinetics is also limiting their applications for the treatment of inflammatory diseases. Consequently, there is great interest in mounting new strategies for the synthesis of non-inflammatory and biodegradable poly-oxalate polymers as drug delivery vehicles which can degrade into nontoxic compounds [34], have prepared, highly stable polymer micelles, namely core-surface crosslinked nanoparticles from amphiphilic brush copolymers. The loaded CDDP into nanoparticles with poly-caprolactone cores and hydrophilic polyethylene glycol or poly [2-(*N*,*N*-dimethylamino) ethyl methacrylate] shells possessed high loading efficiency (90%). Amphiphilic tri-block co-polymer of covalently conjugated with CDDP complexes can protect them against blood clearance [35] as well.

In this study, we have developed a new form of in-vivo compatible agglomerated CS based on EG conjugate containing various dicarboxylic acid linkages cleavable by polyoxalate. It has been shown in-vitro that the release rate of encapsulated compounds from these carriers can be studied by various parameters. Peroxalate ester linkages of this polymer can be cleaved by water hydrolysis, leading to the degradation of polymers into a nontoxic, low molecular weight substance that can be easily excreted. Herein, we report the synthesis and characterization of four different poly oxalate derived nanocarriers and demonstrate their potential delivery of CDDP as a new family of biodegradable drug delivery vehicles.

2. Materials and methods

2.1. Materials

CS, derived from crab shell, was purchased from Otta, Chemika—Biochemika reagents, Mumbai, India. Ethylene glycol (EG), sodium tripolyphosphate (TPP), oxalic acid (OA), succinic acid (SA), triethylamine, hexane, phosphate buffer saline (PBS) and acetic acid were obtained from Merck Chemical Company Inc. (Mumbai, India). CDDP drug was purchased from Sigma Aldrich, Mumbai, India. All of the chemicals were of analytical grade, and all of the solutions were prepared with triply distilled water.

2.2. Polymer synthesis

2 g of EG was dissolved in 20 ml of distilled water and then triethylamine (30 mm) was added drop wise at 4 °C. To this mixture, prepared oxalyl chloride solution was added dropwise. The conversion of dicarboxylic acid to oxalyl chloride was done separately as follows. Briefly, 2 g of oxalic acid and 2 ml thionyl chloride was taken in a 50 ml round bottom flask. Then 0.1 ml N, N-dimethyl formamide was added and attached a calcium chloride drying tube directly to the flask. The flask was heated in a water bath at 60–70 °C. The product formed a liquid layer on the bottom and the excess thionyl chloride was removed by rotary evaporation. The total reaction mixture having oxalyl chloride and EG was maintained under an inert atmosphere at room temperature for 8 h. Obtained polymer was isolated by precipitation in cold hexane. The same procedure was performed using succinic, citric and tartaric acid (TA) instead of oxalic acid. The polymers were isolated and then dried. The structure of the dried polymers obtained was confirmed by ^{1}H and ^{13}C NMR spectroscopy (Bruker 300 MHz DMX NMR spectrometer). ^{1}H NMR (300 MHz, D_2O) spectra of compound OA-EG, SA-EG, CA-EG and TA-EG (Figs. S1–S4) exhibits the chemical shift values: δ of OA-EG: 4.73, 3.45, 3.45, 3.45, 3.45, 3.44, 3.44, 3.44, 3.44, 3.43, 2.99, 2.98, 1.06, 1.04; δ of SA-EG: 4.80, 4.79, 4.78, 4.73, 4.72, 4.72, 4.71, 4.65, 4.65, 4.64, 4.57, 4.03, 3.71,