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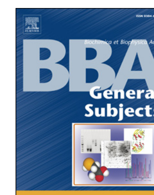

Evaluation of chitosan nanoformulations as potent anti-HIV therapeutic systems

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ABSTRACT

Background: Antiretroviral Therapy (ART) is currently the major therapeutic intervention in the treatment of AIDS. ART, however, is severely limited due to poor availability, high cytotoxicity, and enhanced metabolism and clearance of the drug molecules by the renal system. The use of nanocarriers encapsulating the anti-retroviral drugs may provide a solution to the aforementioned problems. Importantly, the application of mildly immunogenic polymeric carrier confers the advantage of making the nanoparticles more visible to the immune system leading to their efficient uptake by the phagocytes.
Methods: The saquinavir-loaded chitosan nanoparticles were characterized by transmission electron microscopy and differential scanning calorimetry and analyzed for the encapsulation efficiency, swelling characteristics, particle size properties, and the zeta potential. Furthermore, cellular uptake of the chitosan nanocarriers was evaluated using confocal microscopy and Flow cytometry. The antiviral efficacy was quantified using viral infection of the target cells.
Results: Using novel chitosan carriers loaded with saquinavir, a protease inhibitor, we demonstrate a drug encapsulation efficiency of 75% and cell targeting efficiency greater than 92%. As compared to the soluble drug control, the saquinavir-loaded chitosan carriers caused superior control of the viral proliferation as measured by using two different viral strains, NL4-3 and Indie-C1, and two different target T-cells, Jurkat and CEM-CCR5.
Conclusion: Chitosan nanoparticles loaded with saquinavir were characterized and they demonstrated superior drug loading potential with greater cell targeting efficiency leading to efficient control of the viral proliferation in target T-cells.
General significance: Our data ascertain the potential of chitosan nanocarriers as novel vehicles for HIV-1 therapeutics.

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1. Introduction

Acquired Immune Deficiency Syndrome (AIDS) is a dreadful immune disorder caused by the human immunodeficiency virus (HIV) that has resulted in the death of millions across the globe. Currently, more than 36 million people are infected with HIV globally, with a large percentage living in the developing countries [1]. The current treatment employs a combination regimen known as Highly Active Anti-Retroviral Therapy (HAART) [2]. There are five major classes of anti-retroviral drugs currently approved for treatment of AIDS. These are reverse transcriptase inhibitors that include the nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs) and non-nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors and integrase inhibitors [3]. These drugs act at different phases of the viral life cycle [4]. Protease inhibitors target the viral protease that is involved in the cleavage of the GAG-POL precursor responsible for the production of viral enzymes necessary for the viral proliferation [5]. Inhibition of the viral proliferation at the later stages is critical to prevent viral maturation resulting in the formation of the immature and non-infectious virions [6]. Currently, there are eight protease inhibitors approved by the FDA among which saquinavir is the most potent.

Conventional saquinavir therapy is not quite effective due to its poor bioavailability [7]. The poor bioavailability of saquinavir is mainly attributed to a group of MDR1 (multi-drug resistant 1) proteins, the P-gp mediated efflux system, which causes the elimination of the drug due to its resemblance with the substrate analog of the P-gp mediated system [8,9]. The accelerated drug efflux results in the low intracellular concentration of the drug in the target cells. Yet another mechanism proposed for the low bioavailability of saquinavir is its metabolism by the hepatic system and small intestine leading to the rapid clearance of the drug

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[10]. It is reported that the cytochrome P450 isoenzymes, especially the CYP3A4, are involved in the biotransformation of the drug leading to its rapid clearance and low availability thus affecting its efficacy. The poor bioavailability of saquinavir requires an increase in the dosage, and in the absence of which rapid emergence of drug resistance is a possible outcome [11]. Apart from the bioavailability issues, the use of saquinavir is also accompanied by several adverse effects including dizziness, nausea, arrhythmia, etc. The nanotechnology enabled platforms such as dendrimers [12], liposomes [13], polymeric nanoparticles [14], etc., could alleviate the limitations of the use of saquinavir, thereby enhancing the therapeutic potential of saquinavir.

Only a few studies attempted to enhance the bioavailability of saquinavir using the nanocarrier-mediated delivery strategies. A solid lipid nanoparticle was used to improve the bioavailability of saquinavir although its efficacy on reducing the viral load was not investigated [15]. A poly(ethylene glycol) modified poly(caprolactone) (PEO-PCL) nanoparticle system prepared using the solvent displacement method was found to enhance the intracellular concentrations of saquinavir suggesting improved cell uptake of the drug [16]. Using an in vitro cell model for the blood–brain barrier and HIV-1 infection, the saquinavir-loaded and transferrin-conjugated quantum rods were shown to cross the barrier alluding to improved efficacy of the drug [17]. Oil in water emulsions of saquinavir were found to exhibit superior oral bioavailability [18]. Similarly, hydroxylpropyl-β-cyclodextrin and poly(alkyl cyanoacrylate) nanoparticles have also shown improvement in the bioavailability of saquinavir [19]. Cationic submicron emulsions of saquinavir were shown to improve the oral absorption of saquinavir although its effect on the viral proliferation was not studied [20]. We previously developed a liposomal delivery system for saquinavir that exhibited excellent cell uptake properties [21].

In the present work, we report a chitosan-based nanodelivery strategy for saquinavir. The choice of chitosan, a natural polymer, is due to its immunogenicity and its anti-microbial properties [22]. The cationic surface of chitosan is expected to contribute to its superior targeting efficacy to negatively charged cells. Furthermore, the polycationic nature of chitosan is expected to favor the deposition of the complement proteins on the nanoparticles resulting in their uptake by the macrophages through complement receptors [23]. The macrophages serve as HIV-1 reservoirs thus efficient drug delivery to these cells via chitosan could be an added advantage. The drug release from the chitosan carriers could be accentuated in the acidic endosome due to extensive protonation and destabilization of the polymer matrix [24].

2. Materials & methods

2.1. Materials

Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck Chemicals, India. Chitosan (M.W. = 140 kDa, 85% deacetylated) was a kind gift from India Sea Foods, Kochi, India. Saquinavir was a kind gift from M/s Hetero drugs, India. Sodium tripolyphosphate (TPP) was purchased from Lobachemie, India. RPMI 1640media and fetal bovine serum were procured from Sigma-Aldrich, USA.

2.2. Preparation of blank and saquinavir loaded nanoparticles

Chitosan nanoparticles were prepared by the ionic gelation technique. A 1000 μg (w/v) solution of chitosan dissolved in 1% acetic acid was stirred with 0.1% (w/v) TPP. The appearance of the turbidity was taken as an indicator of the formation of the chitosan nanoparticles [25]. For the preparation of saquinavir loaded chitosan, 1 mg/ml of the chitosan solution and 1 mg/ml of the drug were dissolved in the phosphate buffered saline (PBS) (1:1 chitosan:saquinavir) followed by the addition of the TPP until the appearance of a turbid solution with constant stirring. The samples were centrifuged at 18,000 rpm and washed thrice to remove the unencapsulated saquinavir. The samples were lyophilized and stored in a cool dry place before use. 1% w/v sucrose was used as the cryoprotectant during lyophilization. The Alexa Fluor 647 loaded chitosan nanoparticles were prepared following the same protocol employed for preparation of saquinavir-loaded nanoparticles.

2.3. Morphology of chitosan

The ultra fine structure of the blank chitosan and saquinavir loaded chitosan was observed using field emission transmission electron microscopy (JEM2100 F, JEOL, Japan). 500 μg of the lyophilized drug-loaded and blank chitosan nanoparticles was taken and dispersed in 500 μl of PBS and placed on a copper grid coated with carbon. The sample was left overnight for drying and then imaged.

2.4. Physico-chemical characterization

The saquinavir loaded chitosan solution was centrifuged at 20,000 rpm for 20 min to separate the drug loaded with chitosan as pellet and the supernatant containing the unencapsulated saquinavir. The blank corrections were carried out using TPP and the absorbance of the unencapsulated drug was measured using the UV–vis spectrophotometer (Lambda 25, Perkin Elmer, USA) at 239 nm [26]. The absorbance was converted to concentration using a standard curve.

The encapsulation efficiency was calculated as:

$$\text{Encapsulation Efficiency} = \frac{Total\ Drug - Unencapsulated\ Drug}{Total\ Drug} * 100.$$

1000 μg of blank and drug loaded chitosan nanoparticles dispersed in 1000 μl PBS was used for the particle size and zeta potential measurements. The particle size and zeta potential of the samples were measured using the Zeta sizer (Nano-ZS, Malvern, UK). The blank chitosan and drug loaded chitosan were dispersed in 1 ml PBS and the mean particle size and zeta potential were measured.

The glass transition temperature of the blank chitosan and drug loaded samples was measured using a differential scanning calorimeter (DSC, Q20, TA Instruments, USA). Six milligrams of the sample was kept in an aluminum pan along with the standard reference aluminum and the glass transition temperature was measured between the range of 10 °C and 180 °C at a scan rate of 10 °C/min under nitrogen atmosphere.

100 mg of placebo chitosan was placed in a dialysis bag and the dialysis bag was immersed in the 200 ml of buffer solution that was maintained at 37 °C. The swelling studies were carried out at different pH values (5.5, 7.4 and 9.0). After 1 h of incubation, sample was removed from the buffer medium and the excess moisture was removed gently using tissue paper followed by weighing. The swelling ratio was calculated using the following formula:

$$\text{SW} = \frac{\text{WT} - \text{WO}}{\text{WO}} * 100$$

where SW represents the swelling ratio, WO represents the original dry weight of the blank chitosan and WT represents the weight of the sample at time t.

2.5. Release kinetics

1000 μg of the drug-loaded chitosan was taken and dispersed in 4 ml of PBS and then the entire sample was placed in a dialysis bag, which was then sealed at both the ends and immersed in 4 ml of PBS buffer solution. Each experiment was carried out in triplicate. The PBS was removed at a predetermined interval of time and replaced with the fresh media of the same volume. The procedure was repeated for different pH values (5.5, 7.4 and 9.0) and the absorbance of the collected PBS was measured at 239 nm using a UV–visible spectrophotometer

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