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Development of albumin-based nanoparticles for the delivery of abacavir

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ABSTRACT

The study was designed to prepare and evaluate albumin nanoparticles containing antiviral drug abacavir sulphate. Various batches of albumin nanoparticles containing abacavir sulphate were prepared by desolvation method. The abacavir loaded particles were characterized for their yield, percentage of drug loading, surface morphology, particle size, surface charge, pattern of *in vitro* drug release and release mechanism studies. Drug loading ranged from 1.2 to 5.9%w/w. The mean particle size and the surface charge were 418.2 nm and −40.8 mV respectively. The *in vitro* drug release varied between 38.73 and 51.36%w/w for 24 h. The *n* value for Korsmeyer–Peppas was 0.425 indicating Fickian type drug release. The preliminary findings indicated that albumin nanoparticles of abacavir can be prepared by desolvation method with good yield, high drug loading and sustained release.

1. Introduction

Human immunodeficiency virus (HIV) is a lentivirus belongs to the family Retroviridae. The HIV causes acquired immunodeficiency syndrome (AIDS) [1]. In humans two different types of HIV, HIV-1 and HIV-2 are known to cause infection and disease [2]. Among these, HIV-1 is highly virulent, easily transmittable and more prevalent and responsible for majority of HIV infections [1]. The human immune system is compromised by HIV infection by the destruction of cells such as T-helper cells, dendritic cells, macrophages as well as other components of cells associated with cell-mediated immunity [3,4]. Therefore, HIV-infected patients are highly susceptible for other infections. HIV-infection triggers the host to produce abnormal immune responses which causes complications like neurological problems [4]. It is estimated that 33.3 million people were infected with HIV-1 worldwide in 2007 [5]. The immunopathogenesis of HIV is well documented [6]. Even though human body has many HIV reservoirs in organs such as brain, spleen and lymph nodes, the CNS is one of the important reservoirs for replicating HIV-1 virus [7]. The AIDS patients may be affected by various opportunistic infections such as pneumonia, TB, HIV associated dementia and cancer [8].

One of the greatest challenges for treating AIDS is to develop an ideal drug delivery system to target drugs into the HIV reservoirs. Even though the conventional systems enhance the life span of AIDS patients, the eradication of virus is not completely achieved with current drug delivery approaches. For effective anti-retroviral therapy, the therapeutic concentration of drug should be available in the HIV reservoirs for an extended period of time. Introduction of nanotechnological approaches such as nanoparticles for drug targeting give tremendous hope to deliver drugs into the HIV reservoirs and treatment, which improve the lifestyle of patients. Nanoparticles are colloidal, submicron and sub-cellular sized particles prepared by using a variety of biocompatible and biodegradable natural, synthetic and semisynthetic polymers. The size of nanoparticles range between 1 and 1000 nm, but particles with 10–100 nm have potential pharmaceutical applications. Nanoparticles (both polymeric and solid lipid particles), micelles, magnetic nanoparticles, ceramic nanoparticles, nanotubes, polymer-drug conjugates, dendrimers, nanocages and nanowires are the different nano-sized carriers studied for drug delivery [9,10]. A variety of biologically active agents such as drugs, DNA, RNA, phytochemicals, vaccines, peptides, proteins, probiotic organisms and neutraceuticals can be delivered by using nanoparticles [10,11].

Nanoparticles have advantages such as protecting the entrapped/encapsulated drug from degradation, ability to penetrate across biological barriers, release the entrapped or adsorbed drug in a controlled and predetermined rate and the ability to reach intracellular level. Further, nanoparticles have high surface

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area, gastrointestinal retention time and oral bioavailability of drugs. In addition, nanoparticles have many advantages over conventional drug delivery systems such as sustain therapeutic activity, target drugs to the brain, macrophages, gastric mucosa, overcome manufacturing problems like stability, solubility, drug loading and release kinetics [12–14]. Nanoparticles may reduce the dose required for the therapy as they deliver needed amount of the payload to the target organs which in turn reduce dose related side effects and improve patient compliance to a greater extent. Nanoparticle can be administered by any route including parenteral injection. Nanoparticles can be prepared by using synthetic polymers like poly(cyanoacrylates) [15,16] and natural polymers such as polysaccharides [17,18] and proteins [19]. Proteins have the advantage of biocompatibility, biodegradability and low toxicity of the degraded products. Various proteins studied for the preparation of nanoparticles include albumin, gelatin, whey protein, casein and collagen [11,20,21]. Among the various biocompatible and biodegradable natural polymers, albumin is one of the most commonly used polymers for nanoparticle preparation.

Albumin is a biocompatible, nontoxic, non-immunogenic, biodegradable and water soluble polymer. The degraded products of albumin are not harmful to the body. Further, albumin nanoparticles can be easily prepared and easy to scale up [22]. Albumin molecules contain various drug binding sites, hence large amount of drug can be incorporated [23]. The primary structure and charged aminoacids content of albumin make the albumin nanoparticles to adsorb positively or negatively charged molecules [24]. It is believed that albumin is transported across the capillary *via* albondin (gp60), intercellular junctions and/or fluid phase mechanisms [25,26]. The usefulness of albumin nanoparticles is evident from the approval of first human serum albumin-based nanoparticles of paclitaxel (Abraxane®) by the FDA in 2005 for treating breast cancer [27,28]. Further, albumin nanoparticles have successfully been used to deliver anti-HIV drugs [29,30], target drugs into tumour cells [31–33], brain [34] and neurons [35].

Abacavir sulphate is chemically (1S, cis)-4-[2-amino-6-(cyclopropylamino)-9H-purin-9yl]-2-cyclopentene-1-methanol sulphate. It is a nucleoside reverse transcriptase inhibitor used for the treatment of AIDS caused by HIV [36]. Carbocyclic guanosine triphosphate is the active moiety of abacavir which is produced by phosphorylation of abacavir by a unique intracellular pathway [37]. The present study was undertaken to develop and characterize albumin nanoparticles of abacavir.

2. Materials and methods

2.1. Materials

Abacavir sulphate was a kind gift by Strides Arco Lab, Bangalore, India. Bovine serum albumin was purchased from Himedia Lab, Mumbai, India. All other chemicals were of analytical grade and used as purchased.

2.2. Methods

2.2.1. Preparation of nanoparticles

Desolvation technique [38] was used to prepare the albumin nanoparticles of antiviral drug abacavir sulphate. Briefly, bovine serum albumin was dissolved in 2 ml of 10 mM sodium chloride solution. The drug abacavir was dissolved in the albumin solution. The drug-polymer solution was adjusted to pH 7. By using a syringe, ethanol was added at the rate of 1 ml/min under magnetic stirring until turbidity appeared. The formed particles were cross-linked with 100 μl of 4% aqueous solution of gluteraldehyde. The stirring was continued at room temperature for 2 h and 1% anhydrous glucose was added as cryoprotectant to improve the redispersibility after lyophilization [39]. The unbound drug in the nanosuspension was removed by centrifugation at 10,000 rpm for 30 min. The supernatant was carefully removed and the nanoparticles were freeze dried (ModulyoD, Thermo, Milford, MA). Five batches were prepared by keeping the drug concentration constant and varying the polymer concentration (Table 1).

Table 1
Formula for the preparation of abacavir loaded albumin nanoparticles.

Ingredient	Batch				
	F-1	F-2	F-3	F-4	F-5
Abacavir	10 mg	10 mg	10 mg	10 mg	10 mg
Albumin	10 mg	20 mg	30 mg	40 mg	50 mg
10 mM sodium chloride solution	2 ml	2 ml	2 ml	2 ml	2 ml
Glutaraldehyde solution (4%v/v)	100 μl	100 μl	100 μl	100 μl	100 μl

2.2.2. Process yield

The percentage yield was determined after freeze drying with respect to the initial quantity of drug, polymer and other (solid) materials used for preparing nanoparticles [40].

2.2.3. Determination of drug loading capacity

Weighed quantity (50 mg) of abacavir-loaded nanoparticles was taken from each batch and the drug was completely extracted using pH 7.4 phosphate buffer. The drug concentration was determined by using a UV spectrophotometer (Shimadzu 160A, Japan) at a wavelength of 216 nm against blank.

2.2.4. Particle size analysis

The mean size of the drug-loaded particles (F-1) was determined by dynamic laser scattering technique using Zetasizer NanoZS (Malvern Instrumets, Malvern, UK). For particle size measurement, samples were analyzed at 25 °C at an angle of 90°. Polydispersity index was used to characterize the size distribution of particles.

2.2.5. Zeta potential measurement

Zeta potential (surface charge) of the particles (F-1) was also determined at 25 °C using Zetasizer NanoZS (Malvern Instrument, Malvern, UK) after making suitable dilutions with deionized distilled water to obtain required concentration.

2.2.6. In vitro *drug release*

Abacavir release from the particles was studied by dialysis bag method [20]. Nanoparticles (equivalent to 1 mg of drug abacavir) were suspended in 2 ml of donor medium (pH 7.4 phosphate buffer) in a dialysis bag and was dialyzed against 50 ml of pH 7.4 phosphate buffer (receptor medium). The medium was kept under stirring at 100 rpm at 37 °C. Samples were (2 ml) drawn at different time intervals and the same volume was replaced with fresh medium. The abacavir concentration in the samples was measured using UV spectrophotometer (Shimadzu 160A, Japan) at 216 nm against blank. The mechanism of abacavir release from the albumin nanoparticles was studied by fitting the *in vitro* release studies data (F-1) to kinetic models such as first order, Higuchi and Korsmeyer–Peppas model [41].

3. Results

3.1. Preparation and characterization of nanoparticles of abacavir

Five different batches of albumin nanoparticles of abacavir sulphate were prepared by desolvation method. The drug loaded particles were characterized for their percentage yield, percentage