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Review Article

POLYMERIC NANOCARRIERS: A PROMISING RESEARCH AVENUE FOR THE DELIVERY OF ANTI-HIV DRUGS

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ABSTRACT

Human immunodeficiency virus (HIV) is a lentivirus that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. An estimated 40 million people globally are HIV infected, with the majority from the developing world. It is established that effective antiretroviral therapy requires long-term treatment using higher dosage regimen to reduce and maintain the viral suppression. However, the therapy using conventional formulation such as tablets, capsules, and suspension, does not eliminate the viral reservoirs in anatomical and intracellular sites. Moreover, the unfavorable physicochemical and biological properties make the delivery of anti-HIV drugs a challenging job to the pharmaceutical scientists. In this context, polymeric nanocarrier system could be useful which will not only reduce the frequency of administration, also target the infected cells and release the drug at a controlled manner for a prolong period of time. Indirectly, this could improve the patient compliance and adherence to the HIV treatment. This article focuses on the hurdles toward the development of an effective anti-HIV drug delivery system, recent developments in the field of polymeric nanocarrier system and the challenges standing ahead.

Key words: HIV life cycle, polymeric nanocarriers, antiretroviral therapy, anti-HIV drugs

INTRODUCTION

The human immunodeficiency virus (HIV) infects cells of the immune system, destroying these cells as well as the immune system's ability to fight off the invaders. The aim of antiretroviral therapy (ART) is to keep the amount of HIV in the body at a low level. This stops any weakening of the immune system and allows it to recover from any damage that HIV might have caused already. The High Activity Antiretroviral Therapy (HAART) introduced in 1996 combines at least three antiretroviral (ARV) drugs and, for over a decade, is being used to extend the lifespan of HIV-infected patients[1]. Though attempts have been made to eradicate HIV, an estimated 40 million people globally are virus infected, with the majority from the developing world[2]. Chronic intake of HAART is mandatory to control HIV infection[3]; without it, viral replication resumes several weeks after withdrawal. Epidemiology reveals that optimal therapeutic results are attained when treatment adherence levels are greater than 95% (no more than two doses missed monthly in a twice-a-day regime); adherence levels below 95% could diminish therapeutic effectiveness by 50%[4]. Organoleptic drawbacks that lead to avoidance and reduced adherence to antiretroviral therapeutic regimes are often neglected[5]. Moreover, high adherence to HAART does not lead to complete HIV elimination from the host. Intracellular and anatomical viral reservoirs are responsible for the perpetuation of infection[6]. Body compartments with blood-tissue barriers prevent drug penetration, thus preventing the eradication of latent viral pools. Active transport mechanisms involving proteins of the ATP-binding cassette (P-glycoprotein) that are present, for example, in central nervous system (CNS) prevent the penetration of anti-HIV drugs into the brain[7]. On the other hand, multiple daily dosing regimens and untoward secondary side effects diminish achievement of significant long-term HIV-1 suppression in infected people[8-10]. The well-known adverse reactions and side effects of ART are often related to the accumulation of drug at inappropriate sites[11]. Another basic requirement for the successful use of any drug against retrovirus-related diseases is sufficient bioavailability. However, many promising ARV agents are unfortunately compromised by disadvantageous physicochemical properties which lead to poor biodistribution and insufficient cellular uptake[12,13].

Special drug carrier systems such as nanoparticles hold the promise of overcoming these pharmacokinetic obstacles to bring about successful therapy[14]. Nanoparticles are stable, solid colloidal particles consisting of macromolecular material and ranging in size from 10 to 1,000 nm. Drugs can be adsorbed on the particle surface or can be entrapped or dissolved in the particle matrix[15]. Various techniques for the preparation of nanoparticles have been employed for a large number of anti-HIV drugs. Following intravenous administration, nanoparticles are known to accumulate in the tissues of mononuclear phagocytic system (MPS) because of phagocytosis by monocyte/macrphages (Mo/Mac)[16]. This results in a specific enrichment of nanoparticles in macrophage-containing organs like the liver and spleen. Infection of these cells with HIV does not abrogate their phagocytic activity *in vitro* or *in vivo*[17]. Therefore nanoparticles represent an interesting carrier system for the specific transport of ARV agents. With this drug targeting technology, substances whose development has been halted because of their unfavorable pharmacokinetic properties could potentially be made available for the treatment of HIV-related diseases. Due to greater stability and easier manufacturing, polymeric nanocarriers offer advantages over other nanocarrier systems such as liposomes and niosomes. This article gives a comprehensive review on the recent developments of polymeric nanocarrier system related to anti-HIV drugs and highlights the fields of further investigation.

HIV LIFE CYCLE AND POTENTIAL TARGETS FOR ANTI-HIV DRUGS

Established HIV infection is diagnosed by finding antibodies to HIV in the plasma using various serological tests such as ELISA (Enzyme Linked Immuno Sorbent Assay), Orasure western blot, SUDS (single used diagnostic system), Orasure HIV-1[18]. People are diagnosed with AIDS when they have certain signs or symptoms defined by the U.S Centers for Disease Control and Prevention (CDC). The CDC's definition of AIDS includes: (a) CD4+ T cell count is less than 200 per cubic millimeter of blood, compared with about 1,000 CD4+ T cells for healthy people; (b) CD4+ T cells accounting for less than 14% of all lymphocytes, a type of white blood cell. The CDC recommends CD4+T testing every three to six months in all HIV-infected patients, though the need may vary by individual.

The first step of the HIV life cycle is the binding to CD4 receptor and one of two co-receptors on the surface of a CD4+ T lymphocyte, followed by membrane fusion, to get the virus particle's contents into the host cell. Then follows reverse transcription of the HIV's genome from RNA into DNA, and its integration into the host genome by an HIV enzyme integrase. Once integrated the virus can lie low in human cells, or can begin the production of new viral RNA

and proteins, turning the cell into a HIV factory. This production is followed by assembly, budding, and maturation, in which the new HIV particles are packaged up and sent out to infect new cells[19]. Each step in the retroviral infection cycle represents a potential target for antiviral therapy[20]. Various substances with different chemical structures and mechanisms of interference with the replication of HIV have already been described as antiviral agents[21,22].

HURDLES TO THE DELIVERY OF ANTI-HIV DRUGS

Majority of the currently marketed anti-HIV agents (Table 1) are formulated as either solid (tablets, capsules for oral use) or liquid dosage forms (solution, suspension for oral and parenteral use). While the oral dosage forms offer convenience, delivery of these drugs via this route suffers from significant first pass effect, variation of absorption and degradation in the gastrointestinal tract due to enzymes and extreme pH conditions. Also many compounds exhibit poor bioavailability due to low aqueous solubility and permeability. Main drawbacks of NRTIs are limited stability, first pass metabolism and systemic toxicity. For example, didanosine presents poor stability under gastric conditions (10% degrades within 2 min at pH<3 and 37°C) and undergoes hepatic first pass metabolism. This results in low bioavailability[1]. Tenofovir, the only FDA-approved nucleotide reverse transcriptase inhibitor possesses bioavailability of only 25-30%[23]. The NNRTI, Efavirenz is recommended by the WHO for the initial treatment of children above the age of three[24]. However, the very low solubility of efavirenz (3-9 µg/ml) hinders its administration, absorption and biodistribution[25]; bioavailability is around 40-45%. In addition, it produces a burning sensation upon swallowing that precludes the development of water-based liquid formulations[26]. Again, very low absorption extent is expected due to low solubility (10µg/ml) and permeability of etravirine[27]. Protease inhibitor drugs are substrates for efflux pumps and so their oral absorption is restricted[28]. Because of their affinity for removal transporters, the pharmacokinetic profiles depend on pharmacogenetic patterns and they require dose adjustment. This is a crucial issue in pediatric patients. In addition, it has been found that the taste of some extemporaneous solutions (indinavir, tipranavir) is often unbearable for many children[29]. The extreme bitterness of commercially available pediatric aqueous solution of ritonavir hampers compliance[30]. The low aqueous solubility of saquinavir restricts absorption upon oral administration and bioavailability is extremely low (4-10%). Again, due to its instability in the gastric environment, enfuvirtide is administered subcutaneously twice a day. Upon injection, local irritation and pain are observed[31]. In this framework, the development of a drug delivery system, protecting the drug from degradation and enabling efficient absorption upon oral administration appears to be a challenging task in the years to come. Moreover, HIV is able to conserve its replication machinery in anatomical and intracellular sites where the ARV drugs have restricted access. HAART does not eliminate these reservoirs, nor prevent their generation and hence, a rebound in viral plasma levels occurs upon HAART withdrawal[32]. The presence of efflux transporters that remove the absorbed drug in the basolateral-apical direction leads to the generation of one of the most challenging viral reservoirs. Accumulation in the CNS not only generates a virus pool that curtails the total elimination of the HIV from the host but also may lead to neuroinflammation, neurodegeneration and dementia (HIV-1 encephalitis, HIVE)[33].

Thus, the technological approach should aim to improve the effectiveness of the treatment by targeting different cellular and anatomical viral reservoirs. The use of polymeric nanoparticles has arguably become the most attractive research avenue for targeting of these viral reservoirs. Nanocarriers display a number of advantageous features: (i) poor water soluble or unstable drugs can be hosted within the particle to attain improved solubility and stability under physiological conditions; and (ii) they could be easily taken up by phagocytic cells. To mask the unpleasant taste of anti-HIV drugs, the nanoparticles could be prepared using pH-dependent polymer that is insoluble under intake conditions but dissolves fast in the stomach in order to completely release the drug. Hence, orally acceptable pediatric formulations of anti-HIV drugs could be possible.

NANOCARRIERS IN THE DELIVERY OF ANTI-HIV DRUGS: RECENT DEVELOPMENTS AND FUTURE SCOPE

In recent years, several research reports are available regarding the development of polymeric nanocarrier system with the aim to have better cellular targeting, overcoming the pharmacokinetic problems, and enhancing the activities of drugs for the treatment of HIV infection and AIDS. In early studies, Bender et al.[34] developed saquinavir-loaded polyhexylcyanoacrylate nanoparticles by emulsion polymerization technique and tested for antiviral activity in primary human Mo/Mac *in vitro*. The formulation led to a dose-dependent reduction of HIV-1 antigen production. At a concentration of 100 nM, saquinavir was completely inactive in chronically HIV-infected macrophages, but when bound to nanoparticles it caused a 35% reduction in antigen production. Lobenberg and co-workers[35] developed zidovudine-loaded hexylcyanoacrylate nanoparticles and evaluated their biodistribution upon peroral and intravenous administration in rats. The oral administration resulted in higher plasma levels than the free drug in solution and a more effective delivery to the cells of the reticuloendothelial system (RES). Moreover, following intravenous injection, drug concentrations were found to be up to 18-fold higher in the cells of the MPS with the drug-loaded nanoparticles as opposed to the control solution.

Aiming to improve the intracellular delivery of saquinavir to a THP-1 human Mo/Mac cell line, Shah and Amiji[36] investigated the encapsulation of the drug within poly (ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles prepared by means of a solvent displacement technique. A significantly higher uptake was noticed with the drug-loaded nanocarriers, compared to the free drug.

In an attempt to reduce the viral pool in CNS, some workers formulated trans-activating transcriptor (TAT) peptide-conjugated polylactic acid nanoparticles to bypass the efflux action of P-glycoprotein and increase the transport of the encapsulated ritonavir across the blood-brain-barrier to the CNS[37]. The brain drug level with conjugated particles was 800-fold higher than that with drug in solution at two weeks and TAT-conjugated particles maintained therapeutic drug levels in the brain for a sustained period.

Macrophages of the RES and brain act as major reservoir for HIV because of their long term survival after HIV infection and ability to spread virus particles to bystander CD4+ lymphocyte cells. Mannan-coated gelatin nanoparticles have been fabricated using the double desolvation method for macrophage targeting of didanosine[38]. Results of *ex vivo* cellular uptake study indicated 5-fold higher uptake of didanosine from this formulation (62.5 ± 5.4%) by the macrophages in comparison with didanosine solution in pH 7.4 phosphate buffer saline (12.1 ± 2.3%). In a later work, Jain and his co-researchers[39] mannosylated the gelatin nanoparticles (MN-G-NPs) using a two-step desolvation technique for the selective delivery of didanosine to the target organs. The unconjugated nanoparticles (G-NPs) released a comparatively higher percentage of drug than MN-G-NPs. Cellular uptake by MN-G-NPs was 2.7 times higher than G-NPs. Coupling of the nanoparticles with mannose significantly enhanced the lung, liver, and lymph nodes uptake of drug following administration of MN-G-NPs in comparison to uncoupled G-NPs or free drug. In a study, it has been demonstrated that Pluronic® P85 could increase the biodistribution of a zidovudine/lamivudine/ /nelfinavir cocktail in the brain due to the inhibition of P-glycoprotein in blood-tissue barriers[40].

In recent years, the particles coated with hydrophilic polymer such as poly (ethylene glycol) (PEG) are commonly known as long-circulating nanoparticles and have been used as potential carriers because of their ability to circulate for a prolonged period time and to enable active drug targeting[41]. *In vitro* release studies showed that PEGylation and transferring-anchoring slightly lowered the drug release from albumin nanoparticles. While the free drug was quickly cleared from plasma, all the nanoparticles prolonged the circulation times. *In vivo* drug distribution study showed a significant increase in the percentage of drug recovery from the brain for transferrin-anchored nanoparticles[42].

Combination antiretroviral therapy continues to be the mainstay for HIV treatment. However, antiretroviral drug non-adherence can lead to the development of resistance and treatment failure. In a recently published work,[43] poly-(lactic-co-glycolic acid) nanoparticles containing ritonavir, lopinavir and efavirenz were fabricated using multiple emulsion-solvent evaporation technique. Fluorescence microscopy and flow cytometry demonstrated phagocytosis of nanoparticles into monocytes-derived macrophages. The results showed high drug levels in peripheral blood mononuclear cells until day 28 without cytotoxicity.

To improve the solubility of saquinavir in water, Boudad and his coworkers[30] produced a hydroxypropyl-β-cyclodextrin-saquinavir inclusion complex. At a concentration of 10%, cyclodextrin increased the apparent solubility to 15.8 and 9.3 mg/ml at pH values of 7.0 and 2.0, respectively. Incorporation of the complex into poly (alkylcyanoacrylate) nanoparticles significantly improved the drug entrapment efficiency of the nanoparticles. To alleviate the drawbacks and limitations of lamivudine sustained release formulations, polymethacrylic acid nanoparticles containing lamivudine was developed using different drug to polymer ratio by nanoprecipitation method. All drug-loaded particles provided sustained release over a period of 24 h[44].

Based on the above literature reports, we observed that much attention has been paid toward the development of nanocarrier system using synthetic polymers. However, natural polysaccharides have been neglected in the fabrication of anti-HIV nanocarrier system; though several natural polysaccharides such as sodium alginate, chitosan, pectin, guar gum have been investigated for a large variety of drug substances because of their non-toxic, biodegradable nature.

Table 1: FDA approved medications to treat HIV infection

Class	Target of action	Drug (Brand name)	Adult dose (mg/day)
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Disable reverse transcriptase, a protein that HIV needs to make more copies of itself	Delavirdine (Rescriptor)	1200 (3 doses)
		Efavirenz (Sustiva)	600 (1 dose)
		Etravirine (Intelence)	400 (2 doses)
		Nevirapine (Viramune)	200, After14 days: 400 (2 doses)
Nucleoside reverse transcriptase inhibitors (NRTIs)	When HIV uses an NRTI as faulty versions of building blocks instead of a normal building block, reproduction of the virus is stalled	Abacavir (Ziagen ABC)	600 (1or 2 doses)
		Didanosine (Videx)	400 (1 dose)
		Emtricitabine (Emtriva)	200 (1 dose)
		Zidovudine (Retrovir)	600 (2 doses)
		Stavudine (Zerit)	80 (1 or 2 doses)
		Zalcitabine (Hivid)	2.25 (3 doses)
		Lamivudine (Epivir)	300 (1or 2 doses)
		Tenofovir (Viread)	300 (1 dose)
Protease inhibitors (PIs)	Disable protease, a protein that HIV needs to make more copies of itself	Amprenavir (Agenerase)	2400 (2 doses)
		Atazanavir (Reyataz)	400 (1 dose)
		Saquinavir (Invirase)	2000 (2 doses)
		Indinavir (Crixivan)	2400 (3 doses)
		Ritonavir (Norvir)	1200 (2 doses)
		Fosamprenavir (Lexiva)	2800 (2 doses)
		Darunavir (Prezista)	1200 (1or 2 doses)
		Nelfinavir (Viracept)	2500 (2or3 doses)
		Tipranavir (Aptivu)	1000 (2 doses)
		Lopinavir (Aluviran)	Not Available
Fusion/entry inhibitors	Block HIV entry into cells	Enfuvirtide (Fuzeon)	180 (2 doses)
		Maraviroc (Selzentry)	1200 (2 doses)
Integrase Inhibitors	Disable HIV to insert viral genetic material	Raltegravir (Isentress)	800 (2 doses)
Fixed-Dose Combination	Fixed-dose combination tablets contain two or more anti-HIV medications that can be from one or more drug classes	Lamivudine/Zidovudine (Combivir)	450 (2 doses)
		Abacavir/Lamivudine/ zidovudine (Trizivir)	750 (2 doses)
		Lopinavir/Ritonavir (Kaletra)	250 (2 doses)
		Tenofovir/Emtricitabine (Truvada)	500 (1 dose)
		Efavirenz/Emtricitabine/ Tenofovir DF (Atripla)	1100 (1 dose)
		Abacavir/Lamivudine (Epzicom)	900 (1 dose)

The preparation of nanoparticles using synthetic polymers often involves heat, organic solvent or high shear force that can be harmful to the drug stability. Moreover, some preparation techniques such as emulsion polymerization and solvent evaporation are complex and require a number of preparation steps that are more time and energy consuming. In contrast, water-soluble polymers offer mild and simple preparation methods without the use of organic solvent and high shear force.[45]Therefore, the future

work should be directed towards the development of polysaccharide nanocarrier system for anti-HIV drugs and evaluation of their cell targeting potential.

CONCLUSION

Most of the anti-HIV drugs suffer from poor aqueous solubility and bioavailability. Furthermore, antiretroviral therapy requires a long term treatment with high doses of the drugs and selective cellular targeting to reduce the HIV load. Polymeric nanoparticles could release the anti-HIV drug at the site of action in a sustained manner for a prolong period of time. Extemporaneous liquid formulations are the first choice to treat HIV infection in children. However, the physiological and organoleptic properties of anti-HIV drugs are the major obstacles in developing pediatric oral liquid formulations. The polymeric nanocarrier system could be advantageous in developing such liquid formulations. Regardless of socioeconomic status of the patients, the design and development of polymeric nanocarrier system could benefit broad portions of the infected population. However, further research is necessary in order to bring nanoparticulate formulations into the market.

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