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Pharmaceutical Nanotechnology

Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations

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Abstract

The aim of this investigation was to develop novel oil-in-water (o/w) nanoemulsions containing Saquinavir (SQV), an anti-HIV protease inhibitor, for enhanced oral bioavailability and brain disposition. SQV was dissolved in different types of edible oils rich in essential polyunsaturated fatty acids (PUFA) to constitute the internal oil phase of the nanoemulsions. The external phase consisted of surfactants Lipoid®-80 and deoxycholic acid dissolved in water. The nanoemulsions with an average oil droplet size of 100–200 nm, containing tritiated [^{3}H]-SQV, were administered orally and intravenously to male Balb/c mice. The SQV bioavailability as well as distribution in different organ systems was examined. SQV concentrations in the systemic circulation administered in flax-seed oil nanoemulsions were threefold higher as compared to the control aqueous suspension. The oral bioavailability and distribution to the brain, a potential sanctuary site for HIV, were significantly enhanced with SQV delivered in nanoemulsion formulations. In comparing SQV in flax-seed oil nanoemulsion with aqueous suspension, the maximum concentration (C_{max}) and the area-under-the-curve (AUC) values were found to be five- and threefold higher in the brain, respectively, suggesting enhanced rate and extent of SQV absorption following oral administration of nanoemulsions. The results of this study show that oil-in-water nanoemulsions made with PUFA-rich oils may be very promising for HIV/AIDS therapy, in particular, for reducing the viral load in important anatomical reservoir sites.

Keywords: Oil-in-water nanoemulsions; Biodistribution; HIV/AIDS; Protease inhibitor; Reservoir sites; Oral administration; CNS delivery

1. Introduction

Acquired immunodeficiency syndrome (AIDS) is a debilitating disease caused by human immunodeficiency virus (HIV). More than 25 years have elapsed since the first discovery of HIV-1 as a causative agent for AIDS. Currently, HIV/AIDS represent one of the deadliest worldwide epidemics, with significant social, economical, and political challenge. According to the December, 2005 World Health Organization's estimates of AIDS epidemic, 38 million adults and 2.3 million children are infected with the virus across the globe. Also in 2005, over 3.0 million individuals died due to HIV/AIDS world-wide, with over 2.4 million in sub-Saharan Africa (http://www.avert.org/worldstats.htm). There have been significant accomplishments in the past 25 years in terms of greater emphasis on disease prevention, technologies for diagnosis, and development of innovative therapeutic strategies (Gallo, 2006). At present, there are over 20 different anti-retroviral drugs approved in the United States under the general classes of nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), and fusion inhibitors (FI) (Chearskul et al., 2006).

Highly active anti-retroviral therapy (HAART) strategy involves the use of combination anti-retroviral agents for synergistic therapeutic outcomes. With the adoption of HAART, the average survival of HIV/AIDS patients has increased from less than 1 year to over 10 years (Frezzini et al., 2005; Holtgrave, 2005). Despite the success of HAART in the clinics, HIV/AIDS therapy is far from optimal. One of the major problems in the chronic treatment is the fact that the viral particles are able to reside in cellular and anatomical sites in the body following replication and remain viable even when there are adequate drug concentrations in the blood (Schrager and D'Souza, 1998; Chun et al., 2000). Examples of cellular reservoirs include T-

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lymphocytes, monocytes, and macrophages, while the major anatomical reservoirs include central nervous system (CNS), lymph nodes, liver, spleen, lungs, and the genitals (Vyas et al., 2006). Poor drug availability in the cellular and anatomical reservoirs is affected by expression of efflux transporters (e.g., P-glycoprotein), presence of drug metabolizing enzymes (e.g., cytochrome P-450), poor permeability properties, non-targeted distribution, and rapid clearance. The reduced bioavailability and short residence of anti-retroviral agents at these viral reservoir sites have profound impact on the clinical management of the disease. The overall consequence is that upon discontinuation of therapy or when drug resistance develops, HIV is able to re-seed the systemic circulation and continue to propagate the infection (Clarke et al., 2000, Kulkovsky and Bray, 2006).

Saquinavir (SQV, Invirase®), the first HIV-protease inhibitor to be marketed for the treatment of HIV/AIDS, is a peptide derivative and a transition-state mimetic of the Phe-Pro peptide bond (King et al., 2004). It competitively inhibits HIV-1 and HIV-2 protease-mediated cleavage of the gag and pol polyproteins, thus preventing the post-translational processing required for virus maturation and spread. Although SQV has a very potent anti-HIV activity *in vitro* (IC_{50} of 20 nM), it is currently not indicated as a single agent. In addition, when SQV is used in combination therapy protocols, the oral daily dose ranges from 1200 to 3400 mg (Figgitt and Plosker, 2000). This is due to the fact that oral bioavailability of SQV from the conventional gelatin capsule formulation is only 4–5%. SQV is a substrate for P-glycoprotein efflux transporter on the enterocytes and is also metabolized by the cytochrome P-450 enzyme system locally in the gastrointestinal tract and upon first pass effect (Kandanearatchi et al., 2003). In addition, SQV is not adequately transported into the CNS or other anatomical reservoir sites.

In order to enhance the availability and distribution of anti-retroviral agents, like SQV, to cellular and anatomical reservoir sites, we have proposed that nanotechnology-based drug delivery systems could provide a unique strategic advantage (Vyas et al., 2006). Using biodegradable poly(ethylene oxide)-modified poly(epsilon-caprolactone)-based nanoparticles of less than 200 nm in diameter, we showed enhanced delivery and prolonged residence of SQV in THP-1 monocytes/macrophage cells (Shah and Amiji, 2006). Additionally, we observed that when the nanoemulsions were made with oils rich in polyunsaturated fatty acids (PUFA), paclitaxel was efficiently solubilized in the oil droplet and there was significant enhancement in the drug absorption across the gastro-intestinal tract following oral administration (Tiwari and Amiji, 2006). Moreover, with the nanoemulsions made with pine-nut oil, which is rich in alpha- and gamma-linolenic acid, an example of omega-3 fatty acid with 18 carbon and 3 double bonds, and stabilized with Lipoid-80® and sterylamine, there was significant enhancement in the delivery of paclitaxel across the blood–brain barrier in mice (results not published).

In order to enhance delivery of SQV to anatomical reservoirs, in the present study, we have formulated the drug in different nanoemulsions made with oils rich in PUFA. These oil-in-water nanoemulsions with the oil droplet size of 100–200 nm were made either with flax-seed oil or safflower oil. Flax-seed oil contains up to 57% by weight of linolenic acid, an example of omega-3 fatty acid, and 17% by weight linoleic acid, an example of omega-6 fatty acid with 18 carbons and 2 double bonds. Safflower oil, on the other hand, contains up to 73% by weight of linoleic acid (Boles et al., 2005). To examine oral bioavailability and distribution to vital organs including the brain, SQV was incorporated in the nanoemulsions and administered orally to conscious Balb/c mice. Intravenous administration was also carried out to determine the relative bioavailability values of SQV following oral administration in different formulations. Control preparation of SQV was made as aqueous suspension containing all of the other ingredients (e.g., surfactants) except the oils.

2. Materials and methods

2.1. Materials

SQV base was purchased from Aapin Chemicals Limited (Abingdon, United Kingdom). Tritiated [^{3}H]-SQV, with an activity of 250 μCi in 250 μL ethyl alcohol, was purchased from Moravek Biochemicals (Brea, CA, USA). PUFA-containing pure flax-seed and safflower oils were kindly provided by Jedwards International, Inc. (Quincy, MA, USA). Egg phosphatidylcholine (Lipoid® E80) was purchased from Lipoid GMBH (Ludwigshafen, Germany). Deoxycholic acid was purchased from Sigma Chemicals (St. Louis, MO, USA). Deionized distilled water (Barnsted/Thermolyne, Dubuque, IA, USA) was used for the preparation of the nanoemulsions and other aqueous solutions.

2.2. Preparation of the nanoemulsions and aqueous suspension formulations

2.2.1. Preparation of [^{3}H]-SQV-containing nanoemulsions

SQV nanoemulsions, containing a final concentration of 400 μg/mL of the therapeutic agent, were prepared by adding SQV solution (50%, w/w stock solution in dehydrated ethanol) to 1 mL of either flax-seed oil or safflower oil. The weight ratio of radiolabeled (i.e., [^{3}H]-SQV) to unlabeled drug was maintained constant at 0.023:1 by weight.

The oil–drug mixture was stirred to homogenously distribute the drug and allow the ethanol to completely evaporate. The aqueous phase was prepared using deionized distilled water (4 mL) containing 120 mg of egg phosphatidylcholine (Lipoid E80®) and 40 mg of deoxycholic acid. The aqueous phase was also mixed to insure complete dissolution of all additives. Subsequently, both the oil phase and the aqueous phase were independently heated to 70 °C on a hot-plate.

The oil phase was gradually added to the aqueous phase with constant stirring. The resultant mixture containing both oil and aqueous phase was sonicated for 10 min using the Vibra Cell VC 505 probe sonicator (Sonics and Material Inc., Newtown, CT, USA). The probe sonicator was adjusted at 21% amplitude and 50% duty cycle. The resulting stable dispersion was uniform and milky-white in color. Following sonication, the nanoemulsions were kept on a hot-plate under stirring condition and the tem-