Novel Core-Shell Protein Nanoparticles for Oral Drug Delivery
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ABSTRACT SUMMARY

The aim of this study was to develop novel core-shell nanoparticles using food grade polymers. Saquinavir (SQ), an anti-retroviral drug was used as a model compound in the study. SQ is a BCS class IV drug with poor aqueous solubility and permeability. The nanoparticles were prepared using zein (Z), a hydrophobic corn protein as the core and lactoferrin (LF), a hydrophilic milk protein as the shell. The nanoparticle was prepared by phase separation method. The goal of this study is to explore the use of the protein nanoparticles for oral delivery of SQ. The SQ loaded ZLF nanoparticles had particle size of 184 ±10 nm and the particles had a high zeta potential (35.1 ±4.31 mV). The encapsulation efficiency for SQ was 85 ±8%. In vitro release of SQ from the nanoparticles was studied in simulated gastrointestinal fluids (fasted and fed conditions). The nanoparticle protected SQ in milk and sustained its release in simulated gastrointestinal fluids. The permeability of SQ loaded nanoparticles across Caco-2 monolayers was investigated. The ZLF nanoparticles significantly enhanced the absorptive permeability of SQ and more importantly the ZLF nanoparticles decreased the efflux of SQ from basolateral to apical side. The ZLF nanoparticles were found to be taken up by receptor mediated endocytosis through transferrin receptors. Overall the results from this study demonstrate the potential of ZLF nanoparticles for improving the bioavailability of drugs with poor oral absorption.

INTRODUCTION

Oral drug administration is the most preferred route of drug administration. However the oral absorption of many drugs is limited by poor solubility and/or poor permeability. Many delivery approaches have been explored for oral drug delivery. However there is a need to develop carriers that are safe and compatible with food matrices, especially for pediatric patients or patients with difficulty in swallowing medications. Saquinavir (SQ) is an anti-retroviral drug that is widely used in the treatment of HIV. SQ is a class IV drug according to the Biopharmaceutics Classification System (BCS) with poor aqueous solubility and permeability leading to low bioavailability (0.7–4%). \(^1\) In addition SQ is a bitter drug and hence has to be taste masked for oral drug administration. Given that more than 3.3 million HIV patients are children, there is a strong need for developing pediatric drug delivery system for SQ. \(^2\) Recently World Health Organization (WHO) has stressed the need for developing safe and effective medicines for Children. \(^3\) Nanoparticles are potential carriers for oral drug delivery. \(^4\) The advantages of nanoparticles formulation include increased drug solubility, increased stability of the drug in the harsh gastrointestinal environment and drug permeability. The key challenge is to identify safe and effective biodegradable polymers for preparing oral nanoparticles. To this end food proteins are potential biomaterials for preparing oral nanoparticles. Zein (Z) is a hydrophobic water insoluble protein from corn. It is widely used in the food and packaging industry to form a moisture impervious barrier. \(^5\) Given its hydrophobicity, it can be used to encapsulate water insoluble compounds and sustain the drug release. \(^6\) Lactoferrin (LF), an iron binding cationic milk protein was used as the shell to stabilize the nanoparticles. The advantages of using LF include its good proteolytic stability, high isoelectric pH (pI=9.2) that affords cationic surface charge to the nanoparticles and LF is also a substrate for transferrin receptors. \(^7\) Taken together the ZLF nanoparticles can increase the oral absorption of SQ. Hence the goal of this study is to explore the use of ZLF nanoparticles for oral delivery of SQ.

EXPERIMENTAL METHODS

The nanoparticles were prepared by phase separation method by using the differential solubility of zein and LF in hydro-alcoholic solution and buffer (pH 7.4). Particle size and Zeta potential were determined using the Malvern Zetasizer. Transmission electron microscopy and atomic force microscopy were used to characterize the morphology of ZLF nanoparticles. SQ release from the ZLF nanoparticles were tested in fed state simulated gastrointestinal fluid (FeSSGIF) and fasted state simulated gastrointestinal fluid (FaSSGIF). SQ concentration was analyzed by HPLC method. \(^8\) To characterize the cell uptake (Caco-2 cells) of ZLF nanoparticles Nile red was used as a fluorescent probe and was analyzed by flow cytometry. The permeability of SQ loaded nanoparticle was studied using Caco-2 cell monolayers. Apparent permeability coefficient in the direction of apical to basolateral (AP-BL) and basolateral to apical (BL-AP) was calculated using the formula: \(P_{app} \ (cm/s) = (\frac{dQ}{dt}) \times (1/A C_0)\). Where \(dQ/dt\) is the drug flux across caco-2 monolayer, \(C_0\) is the initial concentration of SQ in the apical chamber, and \(A\) is the absorption surface area (4.71 cm²).

RESULTS AND DISCUSSION

The particle size of SQ-ZLF nanoparticles was 184 ±10 and showed a polydispersity index of 0.25, indicating nanoparticles with a narrow size distribution. The high
positive zeta potential (35.1 ± 4.31 mV) can be attributed to the cationic charge of LF, which results in stable nanoparticles. Atomic force microscopy and transmission electron microscopy images showed the core-shell architecture of ZLF nanoparticles (Fig 1). The cationic LF is adsorbed to the negatively charged zein to form core-shell nanoparticles.

Figure 1. AFM and TEM image shows the morphology of ZLF nanoparticles. A) AFM (scale bar, 2 μm; height, 40 nm). B) TEM Scale bar, 50 nm.

Table 1: Apparent permeability coefficients (cm/s × 10⁻⁶) of SQ and SQ-ZLF nanoparticles:

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<th>Papp&lt;sub&gt;(A→B)&lt;/sub&gt;</th>
<th>Papp&lt;sub&gt;(B→A)&lt;/sub&gt;</th>
<th>Efflux ratio</th>
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<tr>
<td>SQ (20 μM)</td>
<td>0.85 ± 0.21</td>
<td>14.23 ± 5.3</td>
<td>16.7</td>
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<tr>
<td>SQ loaded ZLF</td>
<td>1.83 ± 1.2</td>
<td>1.07 ± 0.72</td>
<td>0.889</td>
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The data is shown as mean ±SEM (n=3)

In vitro release profile shows sustained release of SQ with no significant burst release. SQ release was not significantly different between FeSSGIF and FaSSGIF, implying that the ZLF nanoparticles can protect the drug from food matrices and also is stable to proteolytic degradation. In-vitro studies in Caco-2 cells demonstrated the ability of ZLF nanoparticles to increase the permeability of SQ (Table 1). ZLF nanoparticles increased the apical to basolateral permeability of SQ by 2 folds and most importantly the nanoparticles reduced the basolateral to apical permeability by 14 fold. The increased permeability is attributed to the receptor mediated endocytosis of ZLF through LF receptors. This was confirmed by the cell uptake and competitive inhibition assay (data not shown). Further the fluorescence microscopy showed that ZLF nanoparticles were mainly transported through transcellular pathway (Fig 2b).

CONCLUSION

The results from this study demonstrate that ZLF nanoparticles can be used to encapsulate SQ and increase its oral absorption. The ZLF nanoparticles protected SQ and sustained its release. Further the ZLF nanoparticles improved the permeability of SQ by receptor-mediated endocytosis and inhibiting drug efflux pump. Since the nanoparticles are made of food proteins, it can be mixed with food matrices for oral administration in pediatric patients. Future studies will focus on in-vivo preclinical pharmacokinetic studies. Overall the results from this study demonstrate that ZLF nanoparticle is a promising oral drug delivery carrier.

REFERENCES


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