Solid Lipid Nanoparticles Improved The Targeted Delivery of Phosphodiesterase 5 Inhibitor For The Treatment of Pulmonary Hypertension

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ABSTRACT SUMMARY
Management of pulmonary hypertension necessitates frequent administration of specific vasodilators with short plasma half life such as sildenafil citrate (SC). Encapsulation of SC in solid lipid nanoparticles (SLNs) and its application as inhalation therapy is thought to enhance the control of pulmonary hypertension by extending duration of action, reducing both the dose and the cost of treatment course.

SC-loaded SLNs prepared using different types of lipids (glycerides and waxes) were < 200 nm in diameter with narrow size distribution and negative surface charge. SC was efficiently entrapped in the nanocarriers (EE 88-99%). SLNs provided sustained SC release (30-50% over 6h). Waxes showed more controlled release compared to glycerides after 24h. SC-loaded SLNs retained their colloidal stability, entrappment efficiency of the payload and output rate following nebulization.

Results suggest sildenafil-loaded SLNs as promising approach for the treatment of pulmonary hypertension.

INTRODUCTION
Pulmonary hypertension (PH) refers to a progressive increase in the mean pulmonary arterial pressure. The recent years were a breakthrough for patients with PH as several specific vasodilators came on the market. These include endothelin-1 receptor antagonists (ERA; bosentan, sitaxentan, ambrisentan); phosphodiesterase-5 inhibitors (PDE5I; sildenafil, tadalafil, vardenafil) and prostacyclin derivatives that act specifically on pulmonary resistance vessels. Sildenafil is a selective and potent inhibitor of PDE type 5 which specifically degrades cyclic guanosine-monophosphate (c-GMP) and is found in high concentrations in pulmonary arteries [1]. In addition, sildenafil inhibits calcineurin/NFATc2-mediated cyclin A expression in pulmonary artery smooth muscle cells [2]. It is used in combination with other therapies for pulmonary hypertension, such as inhaled iloprost nebulizer solution [3].

Being water-soluble drug with relatively short plasma half life of 3-5 h, frequent administration of SC is a serious drawback. Encapsulation of SC in SLNs is intended to offer: first, targeted delivery of SC as a PDE5I to the pulmonary arteries via inhalation; second, sustained drug release; and third, avoidance of possible drug interactions that may take place in case of co-administration of PDE5I and other antihypertensive medications especially nitrates [4].

EXPERIMENTAL METHODS
Preparation of SLNs:
Different solid lipids with GRAS status were used including glycerides: Glyceryl behenate (GB, Compritol® 888ATO), glyceryl distearate (GDS, Precirol® ATO5), glyceryl monopalmmitostearate (GMPS), and waxes: beeswax. SLNs were prepared by hot melt homogenization. Briefly, SC was mixed with the molten lipid. Aqueous emulsifier solution (polyvinyl alcohol, PVA, or poloxamer 188) was added dropwise to the molten lipid during homogenization. The emulsion produced was further homogenized in an external aqueous phase with adjusted pH value to maximize drug entrapment.

Characterization of SLNs:
The effect of the drug entrapment on the size, polydispersity index (PDI), ζ-potential was investigated using Malvern Zetasizer Nano ZS, Malvern Instruments, Malvern, UK. Particle morphology was examined by SEM.

Entrapment of Sildenafil in SLNs:
The entrapment efficiency of SC in different SLNs was determined both directly and indirectly by measuring the drug concentration in the particles and in the supernatant, respectively. SC was assayed spectrophotometrically at 291 nm (UV-160A; Shimadzu, Koyoto, Japan). The effect of formulation variables (eg. initial drug loading, pH, lipid type,..) on the entrapment efficiency was studied.

In vitro release study:
Appropriate amounts of SC-loaded SLNs were added to the release medium (pH 7.4 PBS supplemented with 0.5% SLS) and shaken at 37°C. At predetermined time intervals, samples were withdrawn and the amount of drug released in the supernatant was analyzed spectrophotometrically.
Nebulization of SC-loaded SLNs:
Different SC-loaded SLNs were nebulized using jet nebulizer (Microlux, Italy). The effect of nebulization on the colloidal stability of SLNs was verified. The aerosol output rate was determined gravimetrically.

RESULTS AND DISCUSSION

Particle size measurement:
Encapsulation of SC in SLNs relatively increased the particle size confirming drug entrapment, Figure 1. SC-loaded precirol SLNs were 192±1.12 nm in diameter compared to 175.8±0.5 nm for the corresponding plain particles. In all cases, SLNs were monodisperse with PDI values of 0.01 to 0.2.

ζ-potential measurement:
SC-loaded SLNs showed lower ζ-potential compared to plain SLNs. This demonstrates the entrapment of the positively charged drug in the lipid matrix. For instance, the ζ-potential of plain GB SLNs was -16.1 ±1.87 while that of the loaded SLNs was -6.9±2.76 mV.

Entrapment efficiency
In general, SC was highly entrapped in SLNs. EE varied according to the lipid and emulsifier type, Table 1. SLNs stabilized with poloxamer showed higher entrapment (>95%) compared to PVA stabilized SLNs.

Table 1: the effect of emulsifier type on the entrapment efficiency of SC

<table>
<thead>
<tr>
<th>Lipid type</th>
<th>Emulsifier type</th>
<th>PVA</th>
<th>Poloxamer 188</th>
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<tbody>
<tr>
<td>Beeswax</td>
<td>84±0.98%</td>
<td>95.6±0.23%</td>
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<tr>
<td>GMPS</td>
<td>88.68±1.64%</td>
<td>99.99±0.05%</td>
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In-vitro release profile:
SLNs provided sustained release of SC; more sustained release was obtained with lipids with longer carbon chain length therefore more hydrophobicity eg. GMPS. Beeswax SLNs showed the slowest release profile. About 80% of drug was released after 24 h in PVA-stabilized precirol SLNs, Figure 2.

Figure 2: The effect of lipid type on the release profile of PVA stabilized SLNs

Effect of nebulization
Testing stability of SC-loaded SLNs after nebulization including size, ζ-potential, PDI and EE illustrated tolerance of the formulated nanoparticles to nebulization process. Drug entrapment didn’t affect the aerosol output rate.

CONCLUSION
Sildenafil-loaded SLNs provided high loading of the drug, sustained release over 24h and efficient application via inhalation. These results represent a promising approach to the currently unmet pharmaceutical needs for pulmonary delivery of sildenafil for the treatment of pulmonary hypertension.

REFERENCES