Cyclosporine A-loaded lipid nanoparticles in inflammatory bowel disease

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A B S T R A C T

Cyclosporine A (CsA) is a well-known immunosuppressive agent used as rescue therapy in severe steroid-refractory ulcerative colitis (UC). However, toxicity issues associated with CsA when administered in its commercially available formulations have been reported in clinical practice. Since nanotechnology has been proposed as a promising strategy to improve safety and efficacy in the treatment of inflammatory bowel disease (IBD), the main purpose of this study was to evaluate the effect of oral administration of CsA-loaded lipid nanoparticles (LN) in the dextran sodium sulfate (DSS)-induced colitis mouse model using Sandimmune Neoral® as reference. The results showed that the formulations used did not decrease colon inflammation in terms of myeloperoxidase activity (MPO), tumor necrosis factor (TNF)-α expression, or histological scoring in the acute stage of the disease. However, further studies are needed in order to corroborate the efficacy of these formulations in the chronic phase of the disease.

The term “inflammatory bowel disease” (IBD) covers various chronic, relapsing-remitting inflammatory disorders of the gastrointestinal tract. Ulcerative colitis (UC) and Crohn’s disease (CD) are the two major forms of IBD (Alhouayek and Muccioli, 2012). The pharmacological strategy for IBD treatment depends on the severity of the illness and the patient’s progress (Talaei et al., 2013). Conventional therapies for UC and CD include aminosalicylates, corticosteroids, thiopurines, methotrexate, and anti-tumor necrosis factor agents (Burger and Travis, 2011). Although corticosteroids are used as first line therapy for the severe stage of the pathology, approximately 30–40% of the patients do not respond to intravenous steroid treatment, and may require hospitalization for intensive health care or even colectomy if clinical enhancement is not observed. Over the last few years, cyclosporine A (CsA) has been used as rescue therapy in clinical practice owing to its rapid onset of action in severe steroid-refractory UC. However, the potential adverse effects associated with this immunosuppressant, including nephrotoxicity, hypertension, seizures and neurotoxicity, along with the need for careful monitoring of the drug during the treatment to prevent toxicity, restrict its use (Eun and Han, 2015). Given the lack of a safe and effective curative therapy of IBD, medical care is focused on minimizing complications by the induction and maintenance of IBD remission (Talaei et al., 2013).

Nanotechnology has demonstrated promising outcomes in IBD therapy thanks to the ability of nanoparticles to selectively target the inflamed tissue when taken orally (Beloqui et al., 2013, 2014). In this regard, nanomedicine could achieve increased efficacy, specifically in intestinal inflammatory cells (Viscido et al., 2014). Among the nanocarriers described so far, lipid-based nanocarriers may provide a promising improvement in the safety and efficacy of anti-inflammatory drugs (Lim et al., 2012).

Therefore, the aim of this work was to investigate the in vivo efficacy of orally administered CsA-loaded lipid nanoparticles (LN) in the dextran sodium sulfate (DSS)-induced colitis mouse model using Sandimmune Neoral® as reference, which is the most popular marketed formulation for CsA oral administration.

For this purpose, three CsA LN formulations were produced differing in the stabilizer system used (Guada et al., 2015). The lipid phase consisted of Precirol® ATO 5 (Gattefosse, Lyon, France) and CsA (Roig Farma S.A., Barcelona, Spain) and the aqueous phase contained 2% (w/v) of: (i) Tween® 80 (Tw, Roig Farma S.A., Spain).
Barcelona, Spain), (ii) L-α-phosphatidylcholine from egg yolk (Lec) and taurocholic acid sodium salt hydrate (TC) at ratio 3:1, and (iii) Pluronic® F127 (PL) and TC at ratio 1:1 (Sigma-Aldrich, St. Louis, MO, USA). The nanoparticles were prepared using the method of hot homogenization followed by ultrasonication and were further characterized in terms of particle size, polydispersity index (PDI), zeta potential and drug entrapment efficiency (EE). Blank LN were formulated following the same process without CsA incorporation. An in vivo experiment was performed using C57BL/6 female mice (18–20 g, 8 weeks; Javier Laboratories, FR). Animals were kept in standard conditions with free access to food and water. Protocols were approved by the Université catholique de Louvain’s animal

**Table 1** Characterization of the cyclosporine A loaded and unloaded lipid nanoparticles.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LN Lec:TC-CsA</td>
<td>205.42 ± 10.22</td>
<td>0.212 ± 0.015</td>
<td>−28.4 ± 1.9</td>
<td>96.30 ± 6.93</td>
</tr>
<tr>
<td>LN Lec:TC-Blank</td>
<td>204.91 ± 12.24</td>
<td>0.210 ± 0.026</td>
<td>−29.8 ± 1.1</td>
<td>–</td>
</tr>
<tr>
<td>LN PL:TC-CsA</td>
<td>114.50 ± 2.23</td>
<td>0.173 ± 0.015</td>
<td>−20.2 ± 2.2</td>
<td>97.52 ± 4.31</td>
</tr>
<tr>
<td>LN PL:TC-Blank</td>
<td>107.79 ± 1.90</td>
<td>0.182 ± 0.021</td>
<td>−23.0 ± 2.6</td>
<td>–</td>
</tr>
<tr>
<td>LN Tw-CsA</td>
<td>126.70 ± 8.66</td>
<td>0.164 ± 0.012</td>
<td>−15.4 ± 2.1</td>
<td>96.48 ± 2.51</td>
</tr>
<tr>
<td>LN Tw-Blank</td>
<td>118.03 ± 6.70</td>
<td>0.156 ± 0.017</td>
<td>−16.0 ± 2.2</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviation: PDI, polydispersity index; EE, entrapment efficiency; LN, lipid nanoparticles; Lec, L-α-phosphatidylcholine; TC, taurocholic acid sodium salt hydrate; CsA, cyclosporine A; PL, Pluronic® F127; Tw, Tween® 80.

**Fig. 1.** Evaluation of colon inflammation in mice with dextran sodium sulfate (DSS)-induced colitis orally treated with lipid nanoparticles (LN) and Sandimmune Neoral® for 7 days in terms of: a) myeloperoxidase (MPO) activity; b) expression of tumor necrosis factor (TNF)-α; c) colonic histological scoring. Results are normalized by protein content in the colon samples and represented by mean values ± standard deviation (n = 8). Statistical differences are represented by **p < 0.01 and ***p < 0.001 compared to DSS group.
committee (2014/UCL/MD/033). Mice were randomly divided into 9 groups (n = 8): (i) LN LeC:TC-CSA + DSS, (ii) LN PL:TC-CSA + DSS, (iii) LN Tw-CSA + DSS, (iv) LN LeC:TC-Blank + DSS, (v) LN PL:TC-Blank + DSS, (vi) LN Tw-Blank + DSS, (vii) Neoral® + DSS, (viii) DSS group and (ix) healthy mice. For colitis induction, DSS (TDB Consultancy, Uppsala, Sweden) was added to the drinking water at a 3% concentration (w/v) during the first 5 days of the experiment. All the formulations were dispersed in drinking water and administered orally once a day by gavage from day 1 to day 7 at a dose equivalent to 2 mg/kg CsA (approximately 276 mg/kg LN). DSS group and healthy mice received drinking water instead of the formulations. Animals were sacrificed on day 7 and colon samples were collected and properly stored for further analysis. The severity of the inflammation in the colonic tissue was investigated by measuring the degree of neutrophil infiltration (intestinal myeloperoxidase (MPO) assay) and the pro-inflammatory cytokine tumor necrosis factor (TNF)-α (ELISA) as well as the histological evaluation of the colon samples, as previously described (Alhouayek et al., 2011; Beloqui et al., 2013, 2014). The total protein concentration was determined using the Lowry method protein assay.

Table 1 summarizes the characteristics of the LN used for the in vivo studies. In all cases, particle sizes were below 300 nm, appropriate for oral delivery, with homogeneous size distribution (PDI < 0.212) and negative surface charge. High drug EE, close to 100%, was achieved. These CsA LN have demonstrated immuno-suppressive activity in vitro by the inhibition of IL-2 levels secreted from stimulated Jurkat cells (Guada et al., 2015). Also, CsA LN have exhibited benefits in the pharmacological response over Neoral® that was proven by the earlier decrease of lymphocyte count in peripheral blood in mice as indicator of immunosuppression (Guada et al., 2016b).

Fig. 1 represents a) the MPO activity, which is a measure of neutrophil infiltration; b) the concentrations of the pro-inflammatory cytokine TNF-α in the colon; and c) the colonic histological score.

In the in vivo experiment CsA was administered at a 2 mg/kg concentration, as reported by Fukata et al. (2011). The authors reported an in vivo efficacy of CsA in the reduction of inflammation at this concentration in a DSS-induced colitis model. Surprisingly, in our study, Neoral® did not decrease inflammation in DSS-induced colitis model in terms of MPO activity (Fig. 1a), TNF-α expression (Fig. 1b), or histological scoring (Fig. 1c) at the administered CsA dose. The various formulations had no effect either (Fig. 1). In a previous in vivo study on CsA LN oral permeability in mice, we showed that CsA LN were highly permeated exhibiting a similar or even enhanced drug bioavailability compared to Neoral® (Guada et al., 2016a). On the contrary, Fukata et al. (2011) reported significantly decreased CsA serum concentrations when the drug was encapsulated within polyactic and polylactic-co-glycolic acid (PDLLA) microspheres (MS) compared to CsA alone. However, these authors reported a significant decrease in DSS-induced colitis inflammation in animals treated with PDLLA MS (P < 0.05). The difference in particle size between the cited microspheres (~4 μm) and the nanoparticles evaluated in the present study (100–200 nm) should be noted. Altogether, one might hypothesize that nano- and microparticles exhibiting increased colonic retention and decreased CsA permeability are more effective in IBD treatment, at least within the acute phase of the disease (Fukata et al., 2011). On the other hand, nanoparticles exhibiting a high permeability and a reduced colonic retention, as in the present study, are less effective in reducing in-situ inflammation in a murine DSS-induced colitis model. Further studies should be undertaken in order to corroborate the efficacy of the formulations during the chronic phase of the disease, considering the advantages of the new CsA delivery systems in terms of more predictable pharmacokinetics that facilitate dose adjustment (Guada et al., 2016b), and therefore minimize the risk of side effects, which represents one of the major concerns for ensuring patient safety and compliance in clinical practice.

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