International Journal of Pharmaceutics 503 (2016) 196–198

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Cyclosporine A-loaded lipid nanoparticles in inflammatory bowel disease

Melissa Guada[a,b], Ana Beloqui[c], Mireille Alhouayek[d], Giulio G. Muccioli[d], Maria del Carmen Dios-Viéitez[a], Véronique Préat[c], Maria J. Blanco-Prieto[a,b,*]

[a] Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, C/Irunlarrea 1, E-31008 Pamplona, Spain
[b] Instituto de Investigación Sanitaria de Navarra, IdiSNA, C/Irunlarrea 3, E-31008 Pamplona, Spain
[c] Université catholique de Louvain, Louvain Drug Research Institute, Advanced Drug Delivery and Biomaterials, 1200 Brussels, Belgium
[d] Université catholique de Louvain, Louvain Drug Research Institute, Bioanalysis and Pharmacology of Bioactive Lipids Research Group, 1200 Brussels, Belgium

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form 8 March 2016
Accepted 9 March 2016
Available online 10 March 2016

Keywords:
Cyclosporine A
Immunosuppressant
Nanoparticles
Colitis
Dextran sodium sulfate
Inflammatory bowel diseases

ABSTRACT

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 (Gattefossé, 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.,

* Corresponding author at: Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, C/Irunlarrea 1, E-31008 Pamplona, Spain.
E-mail addresses: mjblanco@unav.es, maria.blanco@nanomedicinas.es (M.J. Blanco-Prieto).

http://dx.doi.org/10.1016/j.ijpharm.2016.03.012
0378-5173/

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

Formulation	Size (nm)	PDI	Zeta potential (mV)	EE (%)
LN Lec:TC-CsA	205.42 ± 10.22	0.212 ± 0.015	−28.4 ± 1.9	96.30 ± 6.93
LN Lec:TC-Blank	204.93 ± 12.24	0.210 ± 0.026	−29.8 ± 1.1	–
LN PL:TC-CsA	114.50 ± 2.23	0.173 ± 0.015	−20.2 ± 2.2	97.52 ± 4.31
LN PL:TC-Blank	107.79 ± 1.90	0.182 ± 0.021	−23.0 ± 2.6	–
LN Tw-CsA	126.70 ± 8.66	0.164 ± 0.012	−15.4 ± 2.1	96.48 ± 2.51
LN Tw-Blank	118.03 ± 6.70	0.156 ± 0.017	−16.0 ± 2.2	–

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.

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

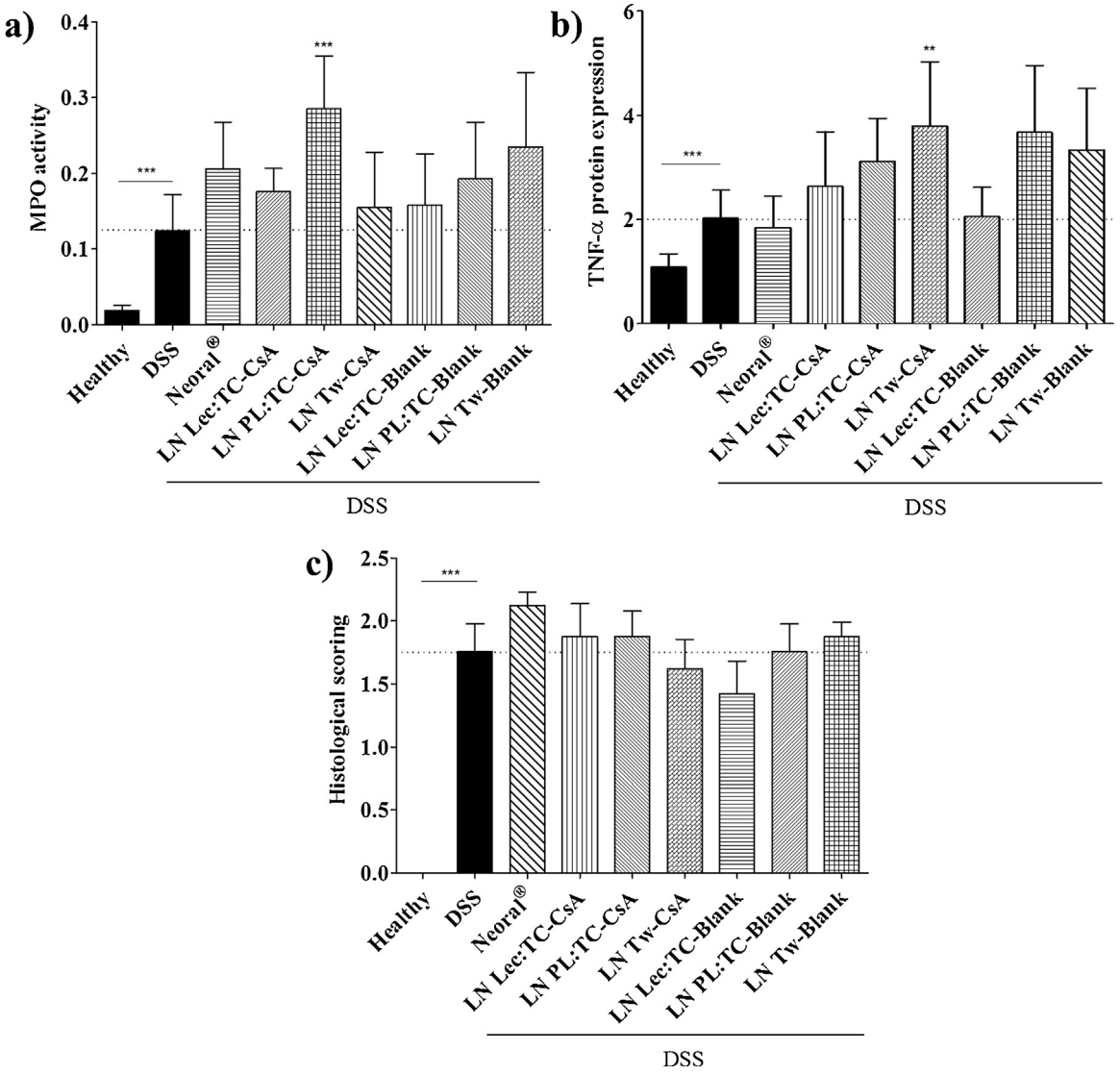

Fig. 1. Evaluation of colon inflammation in mice with dextran sodium sulfate (DSS)-induced colitis orally treated with lipid nanoparticles (LN) and Sandimmune Neoral® for 7 days in terms of: a) myeloperoxidase (MPO) activity; b) expression of tumor necrosis factor (TNF)-α; c) colonic histological scoring. 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.