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A comparative study of curcumin-loaded lipid-based nanocarriers in the treatment of inflammatory bowel disease

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ABSTRACT

Selective drug delivery to inflamed tissues is of widespread interest for the treatment of inflammatory bowel disease (IBD). Because a lack of physiological lipids has been described in patients suffering IBD, and some lipids present immunomodulatory properties, we hypothesize that the combination of lipids and anti-inflammatory drugs together within a nanocarrier may be a valuable strategy for overcoming IBD. In the present study, we investigated and compared the *in vitro* and *in vivo* efficacy of three lipid-based nanocarriers containing curcumin (CC) as an anti-inflammatory drug for treating IBD in a murine DSS-induced colitis model. These nanocarriers included self-nanoemulsifying drug delivery systems (SNEDDS), nanostructured lipid carriers (NLC) and lipid core-shell protamine nanocapsules (NC). *In vitro*, a 30-fold higher CC permeability across Caco-2 cell monolayers was obtained using NC compared to SNEDDS (NC > SNEDDS > NLC and CC suspension). The CC SNEDDS and CC NLC but not the CC NC or CC suspension significantly reduced TNF-α secretion by LPS-activated macrophages (J774 cells). *In vivo*, only CC NLC were able to significantly decrease neutrophil infiltration and TNF-α secretion and, thus, colonic inflammation. Our results show that a higher CC permeability does not correlate with a higher efficacy in IBD treatment, which suggests that lipidic nanocarriers exhibiting increased CC retention at the intestinal site, rather than increased CC permeability are efficient treatments of IBD.

1. Introduction

Inflammatory bowel disease (IBD) can be described as chronic, relapsing-remitting inflammatory disorders of the gastrointestinal tract. The inflamed colonic areas present an increased mucus production, mucosal surface alterations, crypt distortions and ulcers associated with a disrupted intestinal barrier as well as infiltration by immune cells such as macrophages, lymphocytes or dendritic cells [1–3].

Conventional treatment of IBD involves the use of anti-inflammatory drugs such as corticosteroids, aminosalicylates, immunosuppressants and biological agents (*e.g.*, anti-TNF-α monoclonal antibodies). However, these drugs present several side effects, such as diarrhea and lymphopenia, which are mainly related to their systemic administration [4–6]. An efficient approach to IBD therapy could be the specific targeting of inflamed colonic areas *via* oral administration to decrease systemic side effects and improve therapeutic efficacy [7]. Nano-sized drug delivery systems are promising for IBD drug delivery and targeting due to their selective accumulation in the inflamed areas of the gut [8,9].

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Among the nanocarriers described so far, lipid-based nanocarriers may provide a promising improvement to the safety and efficacy of anti-inflammatory drugs [10]. Although many lipid-based formulations have been exploited for the oral administration of drugs, to date, few have been investigated in IBD treatment [8]. Because a lack of physiological lipids has been described in patients suffering IBD [11], and some lipids present immunomodulatory properties [12], we hypothesized that the combination of lipids and anti-inflammatory drugs together within a nanocarrier may represent a valuable strategy for overcoming IBD.

On this basis, the aim of this study was to comparatively analyze the behavior and efficacy of three lipid-based nanocarriers containing the anti-inflammatory drug curcumin (CC) in terms of their potential efficacy in treating IBD. CC is known for its pleiotropic effects, including its anti-inflammatory, antioxidant, anti-carcinogenic, antimicrobial, hepatoprotective and anti-hyperlipidemic properties. Moreover, the efficiency of CC in preventing and ameliorating experimental colitis in mice has been previously demonstrated [13,14]. In the present study, three different lipid nanocarriers were evaluated and compared: self-nanoemulsifying drug delivery systems (SNEDDS); nanostructured lipid carriers (NLC); and lipid core-shell protamine nanocapsules (NC). SNEDDS are clear isotropic mixtures of oils, water-soluble surfactants and, optionally, hydrophilic co-solvents, and are thus spontaneously generating oil-in-water colloidal nanoemulsions in gastrointestinal fluids. This formulation has been commonly used to improve the solubility of poorly water-soluble drugs [15]. NLC are solid lipid-based nanoparticles composed of biocompatible and biodegradable lipids. The lipid core remains solid at body temperature, protecting the encapsulated drug from degradation and making them suitable for topical applications [16]. We have previously shown the efficacy of NLC in colonic drug delivery in IBD [17]. NC are made of a lipid core containing a combination of surfactants and a surrounding protamine corona, a polypeptide that enhances cell penetration [18]. The evaluation of the CC-loaded lipid-based nanoparticulated drug delivery systems was performed (i) based on their physicochemical properties; (ii) *in vitro* by evaluating their potential in reducing TNF-α secretion from activated J774 macrophages as well as their cell association and transport across Caco-2 cell monolayers; and finally (iii) *in vivo* in a murine dextran sodium sulfate (DSS)-induced colitis model, by quantitating the neutrophil infiltration and TNF-α concentrations and by histologically evaluating the severity of inflammation.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Curcumin, *o*-dianisidine, hexadecyltrimethylammonium bromide (HTAB), bovine serum albumin (BSA), hydrogen peroxide (30%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), pepsin, sodium taurodeoxycholate, L-α-phosphatidylcholine from egg yolk (~60%), tris-maleate, dimethyl sulphoxide (DMSO), Tween® 80 and Triton-X 100 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cremophor® EL (polyoxyl 35 castor oil) and Kolliphor® P188 (poloxamer 188) were kindly provided by BASF (Burgbernheim, DE). Protamine sulfate was obtained from Yuki Gosei Ltd (YGK, JP). Labrafil®M2125CS (linoleoyl polyoxyl glycerides), Labrasol® (caprylocaproyl polyoxyl glycerides) and Precirol ATO®5 (glyceryl palmitostearate) were a kind gift from Gattefossé (Saint-Priest, FR). Miglyol 812N/F was a gift from Cremer Oleo GmbH & Co. KG (Hamburg, DE). D-l-α tocopherol (vitamin E) and phosphate salts were obtained from Merck (Darmstadt, DE). Dextran sodium sulfate (DSS) was purchased from TdB Consultancy (Uppsala, SE). Complete protease inhibitor cocktail tablets were purchased from Roche Diagnostics (Vilvoorde, BE).

2.1.2. Cell culture reagents

J774 murine macrophages were kindly donated by Prof. M.-P. Mingeot (UCL, LDRI, BE). Caco-2 cells (clone 1) were kindly provided by Dr M. Rescigno, University of Milano-Bicocca (Milano, IT) [19].

Roswell Park Memorial Institute (RPMI) 1640 medium, Dulbecco's Modified Eagle's Minimal Essential Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin (PEST), Hanks balanced salt solution (HBSS), phosphate buffered saline (PBS), non-essential amino-acids (NEAA) and trypsin-EDTA (0.05%) were purchased from Gibco™ (Invitrogen, UK). Lipopolysaccharide (LPS, E. coli O111:B4) was purchased from Sigma-Aldrich.

2.2. Preparation and characterization of the formulations

2.2.1. CC SNEDDS preparation

Curcumin-loaded SNEDDS (CC SNEDDS) were prepared by first mixing 300 mg of Labrafil® M2125CS, Labrasol® and Cremophor® EL under agitation (400 rpm, 30 min, 20 °C). Thirty milligrams of CC were then added per 1 g of SNEDDS pre-concentrate (drug loading = 30 mg of CC/g of excipients) and mixed until dissolution under agitation (400 rpm, 120 min, 20 °C) under protection from light [20].

2.2.2. Preparation of CC NLC

Curcumin-loaded NLC (CC NLC) were prepared using the high pressure homogenization technique [21,22]. Briefly, Precirol ATO®5 (5 g), Miglyol 812N/F (0.5 mL) and CC (165 mg) (drug loading = 30 mg of CC/g of lipid) were blended and melted at 75 °C until a uniform oil phase was obtained. The aqueous phase was prepared by dispersing Tween 80 (2%) (w/v) and Kolliphor® P188 (1%) (w/v) in water (50 mL) and heating to the same temperature as the lipid phase. The hot aqueous phase was then added to the oil phase and the mixture was sonicated for 15 s to form a hot pre-emulsion, which was subsequently homogenized at 80 °C and 500 bar using a Stansted nG12500 homogenizer (SFP, Essex, UK) for ten homogenization cycles. Untrapped CC was isolated by ultrafiltration, as previously described [17], and was quantified by HPLC (Section 2.2.7).

2.2.3. Preparation of CC NC

Curcumin-loaded protamine nanocapsules (CC NC) were prepared by the solvent displacement technique as previously described [23]. In brief, the organic phase was composed of 75 μL of an ethanolic Tween 80 solution (160 mg/mL) and 75 μL of an ethanolic vitamin E solution (800 mg/mL). Additionally, 600 μL of a CC-solution (2.5 mg/mL in ethanol) were incorporated into the organic phase. Finally, 4.25 mL of acetone were added over the organic phase, and the mixture was immediately poured over 10 mL of an aqueous phase containing 0.5 mg/mL protamine. The organic solvents were then evaporated under vacuum (Rotavapor Heidolph, DE) to a constant volume of 5 mL. CC NC were isolated by ultracentrifugation (Optima™ L–90 K Ultracentrifuge Beckman Coulter, Rotor type 70.1 Ti, 1 h, 30,000 rpm, 15 °C).

Table 1 summarizes the compositions of the formulations.

2.2.4. CC suspension preparation

A CC suspension (CC sus) was prepared as a control by dispersing CC (125 mg) in PBS (10 mL). The median particle diameter of the CC sus in the primary volume was measured by laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, DE). Before sizing, the sus-