Journal of Controlled Release 222 (2016) 86–96

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Colonic gene silencing using siRNA-loaded calcium phosphate/PLGA nanoparticles ameliorates intestinal inflammation *in vivo*

Annika Frede [a,b], Bernhard Neuhaus [c], Robert Klopfleisch [d], Catherine Walker [b], Jan Buer [a], Werner Müller [b], Matthias Epple [c], Astrid M. Westendorf [a,*]

[a] Infection Immunology, Institute of Medical Microbiology, University Hospital Essen, University Duisburg-Essen, Germany
[b] Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom
[c] Institute for Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University Duisburg-Essen, Germany
[d] Institute of Veterinary Pathology, Freie Universität Berlin, Berlin, Germany

ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 3 December 2015
Accepted 12 December 2015
Available online 14 December 2015

Keywords:
siRNA
Nanoparticles
Delivery
Intestinal inflammation

ABSTRACT

Cytokines and chemokines are predominant players in the progression of inflammatory bowel diseases. While systemic neutralization of these players with antibodies works well in some patients, serious contraindications and side effects have been reported. Therefore, the local interference of cytokine signaling mediated by siRNA-loaded nanoparticles might be a promising new therapeutic approach. In this study, we produced multi-shell nanoparticles consisting of a calcium phosphate (CaP) core coated with siRNA directed against pro-inflammatory mediators, encapsulated into poly(D,L-lactide-*co*-glycolide acid) (PLGA), and coated with a final outer layer of polyethyleneimine (PEI), for the local therapeutic treatment of colonic inflammation. In cell culture, siRNA-loaded CaP/PLGA nanoparticles exhibited a rapid cellular uptake, almost no toxicity, and an excellent *in vitro* gene silencing efficiency. Importantly, intrarectal application of these nanoparticles loaded with siRNA directed against TNF-α, KC or IP-10 to mice suffering from dextran sulfate sodium (DSS)-induced colonic inflammation led to a significant decrease of the target genes in colonic biopsies and mesenteric lymph nodes which was accompanied with a distinct amelioration of intestinal inflammation. Thus, this study provides evidence that the specific and local modulation of the inflammatory response by CaP/PLGA nanoparticle-mediated siRNA delivery could be a promising approach for the treatment of intestinal inflammation.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn's disease (CD), are inflammatory disorders of the gastrointestinal tract, characterized by a relapsing-remitting state of chronic inflammation. It is widely accepted that both diseases are triggered by an inappropriate immune response to antigens of commensal gut bacteria in genetically prone persons [1]. An efficient intestinal mucosal barrier is critical for protection against invading microorganisms; therefore impairments in this system have serious adverse effects on health. Defects in the intestinal epithelial barrier function are a characteristic feature of IBD. The perturbations can lead to an increased epithelial barrier permeability, and cause an inadequate protection against microbial adherence and invasion. In consequence, compensatory immune reactions are excessively triggered, a process that is believed to finally result in chronic intestinal inflammation [2]. The abnormal influx of immune cells into the mucosa is dependent on an increased expression of cytokines and chemokines and their receptors during IBD [3,4]. Thus, most biological therapy strategies are aimed at neutralization and reduction of these cytokines using specific monoclonal antibodies or soluble receptors *via* systemic administration [5]. TNF-α (tumor necrosis factor-alpha) was identified as one of the key players in the onset of IBD [6]. While a systemic anti-TNF-α therapy works well in some patients, serious contraindications and side effects including opportunistic infections or decreased efficiency of the therapy have been reported [7, 8].

Recent advances in the understanding of the pathogenesis have caused a shift towards novel biological approaches for the treatment of this disease, including therapeutic gene silencing by RNA interference [9]. RNA interference mediated by small interfering RNAs (siRNAs) of 19–23 base pairs is a powerful tool for post-transcriptionally silencing gene expression [10]. The use of siRNA against pro-inflammatory cytokines like TNF-α was suggested to be an efficient approach in the therapy of IBD [11]. However, the main limitation of this therapeutic approach is the successful and local delivery of siRNA into target cells in the colon. The transport of siRNA into the cytosol is challenging. Due to its small size and negative charge it has only poor access into the cell. Additionally, the nucleotides must be stabilized against their

* Corresponding author at: Infection Immunology, Institute of Medical Microbiology, University Hospital Essen, Hufelandstrasse 55, 45122 Essen, Germany.
E-mail address: astrid.westendorf@uk-essen.de (A.M. Westendorf).

http://dx.doi.org/10.1016/j.jconrel.2015.12.021
0168-3659/© 2015 Elsevier B.V. All rights reserved.

quick degradation by nucleases [12–14]. Among other delivery strategies, calcium phosphate (CaP) nanoparticles have proven to be efficient in biomedicine [15–19] *e.g.* as carriers of small molecules across the cell membrane [20,21]. CaP nanoparticles have a high affinity to nucleic acids, and the crystal growth of CaP can be inhibited on the nanometer scale by stabilization and simultaneous functionalization with nucleic acids [22,23]. After endocytosis, the nanoparticles are dissolved at the low pH inside the lysosome [24]. The rise in osmotic pressure supports the escape of the nucleic acids into the cytosol [25–27]. Furthermore, as mineral in human hard tissue (bone and teeth), calcium phosphate is a biocompatible and biodegradable compound [15,16]. Silencing of proteins like eGFP and TNF-α has been shown with CaP nanoparticles *in vitro* [28,29].

Based on these promising results obtained so far, we investigated the potential use of biodegradable, non-toxic siRNA-loaded CaP/PLGA nanoparticles for the therapeutic modulation of intestinal inflammatory processes. First, we analyzed the uptake and the silencing capacity of siRNA-loaded CaP/PLGA nanoparticles by an intestinal epithelial cell line and primary colonic organoids *in vitro*. Then, we assessed the therapeutic efficiency of intrarectally administered siRNA-loaded CaP-PLGA nanoparticles to mice with dextran sulfate sodium (DSS)-induced acute colitis.

2. Materials & methods

2.1. Preparation of functionalized CaP-PLGA nanoparticles

The nanoparticles were prepared and functionalized as described previously [30]. In short, single shell nanoparticles were synthesized by fast mixing equal amounts of an aqueous solution of calcium-L-lactate (6.25 mM; 105 μL) and diammonium hydrogen phosphate (3.74 mM; 105 μL). Instantly after mixing, the calcium phosphate dispersion was mixed with solutions of either functional siRNA, scrambled siRNA (GE healthcare life sciences, Chalfont St. Giles, UK) or fluorescence-labeled oligonucleotides (Life technologies, Darmstadt, Germany) (4 mg mL^{-1}; 40 μL) in a 1.5 mL reaction tube to functionalize and stabilize the particles. To encapsulate the calcium phosphate nanoparticles into the biodegradable polymer poly(D,L-lactide-*co*-glycolide) (PLGA) (Resomer® RG 502 H, Evonik Industries, Darmstadt, Germany), a water-in-oil-in-water (W1/O/W2) double emulsion solvent evaporation method was applied. The dispersed polyvinylalcohol (PVA)-coated nanoparticles were purified by centrifugation and after redispersion in H_2O they were shock-frozen in liquid nitrogen and lyophilized. The freeze-dried particles were then resuspended in a solution of aqueous PEI (2 mg mL^{-1} branched 25 kD, Sigma-Aldrich, St. Louis, Missouri, USA) at a ratio (*w*/*w*) of 1:2 to obtain the final nanoparticle structure. The positively charged PEI interacts with PLGA by electrostatic interactions based on the layer-by-layer procedure by Decher et al. [31]. The particles were characterized by scanning electron microscopy (ESEM Quanta 400) after sputtering with gold-palladium. Dynamic light scattering was performed with a Zetasizer nanoseries instrument (Nano-ZS Malvern, $\lambda = 532$ nm). The concentration of encapsulated siRNA was attained from UV/Vis spectroscopic measurements of the supernatant. About 9.54 μg siRNA were encapsulated per mg of nanoparticles. Assuming a particle diameter of 152 nm, 1 mg of freeze-dried particles consisted of $3.8 * 10^{11}$ nanoparticles. Calculations were performed according to Doerdelmann et al. assuming the particles contained 5% calcium phosphate as hydroxyapatite [30].

2.2. Mice

BALB/c and BALB/cOlaHsd-Foxn1^{nu} mice were purchased from Harlan Laboratories (Harlan Winkelmann GmbH, Borchen, Germany). All animals used in this study were 8- to 10-week-old male and female mice housed under specific pathogen-free conditions in the Laboratory Animal Facility of the University Hospital Essen. Experiments were performed in accordance with state and federal guidelines.

2.3. Preparation of single cell suspensions

Intestinal epithelial cells from the colon were isolated as described previously [32]. In short, colons were isolated, rinsed with PBS and cut open longitudinally. Samples were treated with PBS containing 1 mM Dithiothreitol and culture media under constant shaking. Single epithelial cells were gained by vigorous shaking and subsequent filtering through 70 μM cell strainers. Cells were stained with α-CD45 antibody to label hematopoietic cells. IECs were sorted by size and negative selection for CD45 with the FACS ARIA II sorter (BD Bioscience, Franklin Lakes, New Jersey, USA). Lymphocytes from the lamina propria of the colon were isolated as described previously [33]. In brief, colons were washed with ice-cold PBS and cut into small pieces. The pieces were washed in PBS plus 2 mM EDTA and cell culture media under constant stirring. The tissue was then subjected to digestion with collagenase IV (Sigma-Aldrich) for 90 min at 37 °C. Single-cell suspensions were obtained by passing suspensions through 70 μm cell strainers. Spleens and mLNs were squashed through 70 μm cell strainers, and washed with erythrocyte lysis buffer or PBS containing 2% FCS and 2 mM EDTA, respectively.

2.4. Isolation and stimulation of DCs and CD4$^+$ T cells

For isolation of DCs spleens were cut into small pieces and treated with 1 mg/mL collagenase type D (Roche, Mannheim, Germany) and 10 μg mL^{-1} DNase type II (Sigma-Aldrich) diluted in PBS with 2% FCS and 2 mM EDTA for 45 min at 37 °C. The remaining tissue was filtered through a 70 μm cell strainer and cells were washed in PBS containing 2% FCS and 2 mM EDTA. CD11c$^+$ cells were selected positively and CD4$^+$ T cells negatively with AutoMACS technology according to the manufacturer's instructions (Miltenyi Biotec, Bergisch-Gladbach, Germany). DCs or CD4$^+$ T cells were stimulated over night with either 100 ng mL^{-1} LPS (Sigma-Aldrich) or 1 mg mL^{-1} α-mouse α-CD3 (145-2C11, BD Bioscience), respectively, or siRNA-loaded CaP/PLGA nanoparticles at a concentration of 0.1 μg siRNA mL^{-1} and analyzed by flow cytometry.

2.5. Antibodies and flow cytometric analysis

Flow cytometric analysis was performed using antibodies against α-mouse, CD4 (RM4-5), CD8 (53-6.7), CD40 (3/23), B220 (RA3-6B2), CD80 (16-10A1), CD86 (GL1, all BD Bioscience), CD11c (N418), CD45 (30-F11, both Biolegend, San Diego, CA, USA), F4/80 (BM8, Invitrogen, Carlsbad, CA, USA), CD69 (H1.2F3), CD62L (MEL-14) and Ki-67 (SolA15, all eBioscience, San Diego, CA, USA) conjugated with pacific blue, phycoerythrin, phyocerythrin-cyanine7, fluorescein isothiocyanate, brilliant violet 510 or peridinine-cyanine5.5 conjugates.

Oligonucleotides were labeled with AlexaFluor647 or AlexaFluor750 (Life technologies) and dead cells were excluded by staining cells with 7-AAD or the fixable viability dye eFluor780 (both eBioscience). TNF-α expression was analyzed by incubating cells in the presence of 5 μg μL^{-1} Brefeldin A (Sigma Aldrich), treating them with 2% paraformaldehyde and 0.1% NP-40, and staining them with α-mouse TNF-α conjugated with allophycocyanin (eBioscience). Analysis was performed on a LSR II or Canto II flow cytometer using FACS DIVA software (BD Bioscience).

2.6. RNA isolation, quantitative real time PCR and Luminex assay

Isolation of RNA from biopsy samples from colons and mLNs was performed using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany). The NucleoSpin XS Kit (Macherey-Nagel, Düren, Germany) was used for RNA isolation from single cells according to

Download English Version:

https://daneshyari.com/en/article/1423604

Download Persian Version:

https://daneshyari.com/article/1423604

Daneshyari.com