Biomaterials 122 (2017) 10–22

Contents lists available at ScienceDirect

Biomaterials

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

Targeting NF-kB signaling with polymeric hybrid micelles that co-deliver siRNA and dexamethasone for arthritis therapy

Qin Wang, Hao Jiang, Yan Li, Wenfei Chen, Hanmei Li, Ke Peng, Zhirong Zhang, Xun Sun*

Key Laboratory of Drug Targeting and Drug Delivery Systems, Ministry of Education, West China School of Pharmacy, Sichuan University, Chengdu 610041, China

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form
4 January 2017
Accepted 7 January 2017
Available online 11 January 2017

Keywords:
NF-kB
p65 siRNA
Macrophage polarization
Arthritis

ABSTRACT

The transcription factor NF-kB plays a pivotal role in the pathogenesis of rheumatoid arthritis. Here we attempt to slow arthritis progression by co-delivering the glucocorticoid dexamethasone (Dex) and small-interfering RNA targeting NF-kB p65 using our previously developed polymeric hybrid micelle system. These micelles contain two similar amphiphilic copolymers: polycaprolactone-polyethylenimine (PCL-PEI) and polycaprolactone-polyethyleneglycol (PCL-PEG). The hybrid micelles loaded with Dex and siRNA effectively inhibited NF-kB signaling in murine macrophages more efficiently than micelles containing either Dex or siRNA on their own. In addition, the co-delivery system was able to switch macrophages from the M1 to M2 state. Injecting hybrid micelles containing Dex and siRNA into mice with collagen-induced arthritis led the therapeutic agents to accumulate in inflamed joints and reduce inflammation, without damaging renal or liver function. Thus, blocking NF-kB activation in inflammatory tissue using micelle-based co-delivery may provide a new approach for treating inflammatory disease.

1. Introduction

In rheumatoid arthritis, the synovial tissue is infiltrated by various immune cells and activated macrophages, which degrade cartilage and destroy the synovium via a complicated inflammatory cytokine signaling network [1,2]. Activated macrophages in inflamed joints, termed M1 macrophages, drive the progression of rheumatoid arthritis by secreting large amounts of inflammatory cytokines such as TNF-α and IL-1β, making them prime therapeutic targets in strategies to treat inflammatory disorders [3–6]. Conversely, macrophages involved with anti-inflammatory activity and tissue repair are considered to be M2 type [7,8]. Therefore, switching predominant M1 type macrophages in arthritic joints to the M2 type may be an effective treatment of rheumatoid arthritis [4,9]. Since NF-kB signaling is essential for macrophage polarization [10,11], blocking such a signaling might reverse the polarization from M1 to M2. We are unaware of published attempts to address this question.

Under normal conditions, NF-kB localizes to the cytoplasm as an inactive complex with inhibitory kB proteins. In rheumatoid arthritis, stimulus signals such as TNF-α or IL-1β trigger phosphorylation and degradation of the inhibitor kB proteins, liberating NF-kB subunits to translocate to the nucleus, where they turn on several pro-inflammatory genes. This leads ultimately to progressive damage of the extracellular matrix and destruction of cartilage [12–14]. Several small-molecule inhibitors of NF-kB signaling have been developed in an effort to halt this process, but none has been approved for clinical use in rheumatoid arthritis, in some cases because of serious adverse effects [15,16]. An alternative strategy is to down-regulate inflammatory cytokines such as TNF-α and IL-1β using small-interefering RNA (siRNA), and this approach has shown potential in vivo [17–19]. In an attempt to inhibit NF-kB directly, here we used p65 siRNA to target the NF-kB family member p65. So far, siRNA against p65 has not been extensively investigated, particularly not in rheumatoid arthritis.

Here we examined the ability of siRNA-mediated down-regulation of p65 to re-polarize M1 macrophages into the M2 state and to alleviate rheumatoid arthritis in an animal model. We reasoned that a single siRNA agent was unlikely to be able to block the complex inflammatory signaling underlying rheumatoid arthritis, so we planned to co-deliver the siRNA drug with the glucocorticoid dexamethasone (Dex), which inhibits transcription of NF-kB [20] and which is widely used to suppress inflammation [21,22]. When internalized into target cells, Dex binds to the glucocorticoid

* Corresponding author.
E-mail address: sunxun@scu.edu.cn (X. Sun).

http://dx.doi.org/10.1016/j.biomaterials.2017.01.008
0142-9612/

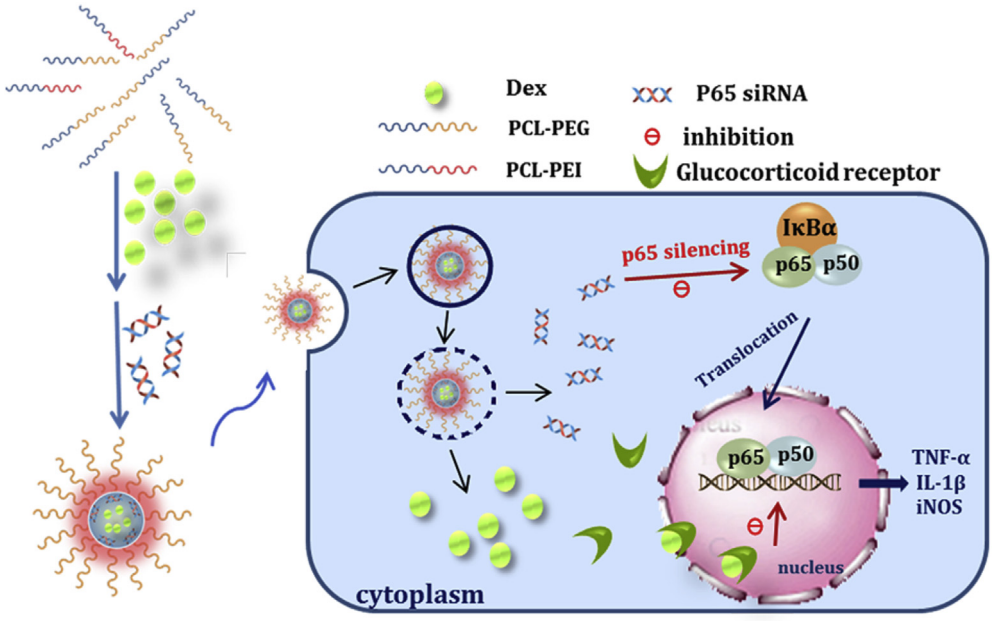

Fig. 1. Schematic of Dex and siRNA co-encapsulation in micelles and the intracellular fate of micelles.

receptor in the cytoplasm, whereupon it dimerizes and translocates to the nucleus. Subsequently, the glucocorticoid receptor represses NF-kB transcription, leading to overall anti-inflammatory effects [20]. This combination of siRNA and chemotherapy, while common in cancer treatment [23,24], is only beginning to be explored in inflammatory disorders. The present work is, to our knowledge, the first investigation of systemic, simultaneous co-delivery of siRNA and glucocorticoid to treat rheumatoid arthritis.

Since siRNA shows short half-life in serum and is poorly internalized into cells [25,26], therapeutic siRNA must be delivered in appropriate vehicles [27]. To ensure delivery of both our therapeutic agents, we exploited a versatile hybrid micelle system previously developed in our laboratory [28]. This system consists of two amphiphilic diblock copolymers, PCL-PEI and PCL-PEG, the ratio of which can be adjusted to optimize the physicochemical properties of the micelles. The hydrophobic core of PCL components can encapsulate hydrophobic drugs via hydrophobic interaction, while cationic hydrophilic PEI segments can form polyplexes with siRNA (Fig. 1).

Here we examined the ability of micelles loaded with Dex and siRNA to inhibit NF-kB signaling in macrophages as well as switch macrophages in the arthritic synovium from the M1 to M2 state. We also evaluated therapeutic efficacy and safety in a mouse model of collagen-induced arthritis.

2. Material and methods

2.1. Material

PCL2k and PEI2k were obtained from Sigma Aldrich (Saint Louis, MO, USA); PEG5k, from JenKem (Beijing, China); 4-nitrophenyl chloroformate (NPC), from Aladdin (Shanghai, China); and p65 siRNA, from Riobobio (Shanghai, China), sense; 5'GCGACAAGGUGCAGAAAGAdTdT3, antisense; 3'dTdTCGCUGUUCCACGUCUUUCU5, target sequences; GCGACAAGGTGCAGAAAGA. Other biochemical reagents were purchased from Sigma Aldrich, including dexamethasone (Dex), lipopolysaccharide (LPS), bovine serum albumin (BSA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), TRITC, DiD and coumarin. All other reagents were of analytical purity and were obtained from Kemiou (Tianjin, China).

The primer sequences were as follows:

Primer	Forward	Reverse
β-actin	CTACAATGAGCTGCGTGTGG	CAGGTCCAGACGCAGGATGGC
TNF-α	GCTCCCTCTCATCAGTTCCA	GCTTGGTGGTTTGCTACGAC
IL-1β	GCCAACAAGTGGTATTCTCCA	TGCCGTCTTTCATCACACAG
Arg-1	GCAGAGGTCCAGAAGAATGG	AGCATCCACCCAAATGACAC
CD206	GGAGGCTGATTACGAGCAGT	CATAGGAAACGGGAGAACCA
iNOS	AGCCAAGCCCTCACCTACTT	CTCTGCCTATCCGTCTCGTC
IL-12	CACGGCAGCAGAATAAATA	CTTGAGGGAGAAGTAGGAATG

All the sequences of primers were designed by Primer Premier 5.0.

2.2. Preparation and characterization of polymeric micelles co-loaded with Dex and siRNA

Using the solvent exchange method, we dissolved 12 mg copolymer containing 2% (w/w) cationic segment PCL_{2000}-PEI_{2000}, 98% (w/w) neutral segment PCL_{2000}-PEG_{5000}, and 0.25 mg Dex in 1 mL tetrahydrofuran. This solution was then added dropwise to 10 mL ddH2O while stirring, and the resulting solution was rotary-evaporated under vacuum at 45 °C, eliminating the tetrahydrofuran and leaving behind Dex-loaded hybrid micelle in solution. The micelle solution was then mixed in an equal volume of p65 siRNA in RNase-free water, vortexed for 15 s, and left to stand a further 15 min to allow formation of M-Dex/siRNA micelles.

Micelle size and zeta potential were measured using dynamic light scattering (ZetasizerNano ZS90, Malvern, UK), while morphology was examined using transmission electron microscopy. Samples were overlaid on a copper grid and negatively stained with phosphotungstic acid (1%, w/v) for 10 s. The entrapment efficiency and drug loading was determined by an ultrafiltration method using a Molecular Weight Cut-Off (MWCO) 30kD ultrafiltration tube (Millipore, USA). Samples were centrifuged for