Temperature-modulated noncovalent interaction controllable complex for the long-term delivery of etanercept to treat rheumatoid arthritis

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ABSTRACT

The clinical applications of etanercept (Enbrel), an emerging therapeutic protein for rheumatoid arthritis (RA), are limited by its instability and low bioavailability. In this study, a long-term and efficient therapeutic nanocomplex formulation for RA treatment was developed in the form of a temperature-modulated noncovalent interaction controllable (TMN) complex based on a temperature-sensitive amphiphilic polypelectrolyte (succinylated pullulan-g-oligo(ε-lactide)-SPA). The TMN complexes were prepared by simply mixing the negatively charged SPL copolymer and the positively charged etanercept via electrostatic interaction at 4 °C below the polymer’s clouding temperature (CT), and the resulting complex demonstrated significantly improved salt and serum stability with increased hydrophobic interactions at temperatures (physiological condition, 37.5 °C) above the CT. An in vitro study of the bioactivity of etanercept indicated that the TMN complex improves the long-term stability of etanercept in an aqueous environment because of the exposure of the functional active site and the molecular chaperone-like effect of the hydrophobic copolymer. This formulation possessed prolonged in vivo pharmacokinetic parameters. In a collagen-induced arthritis (CIA) rat model, we verified the outstanding therapeutic effect of the TMN complexes. These results imply that this approach would be widely applied to proteins and peptide delivery systems.

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1. Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by chronic, progressive inflammation and gradual joint destruction [1,2]. Although the root cause of RA remains unknown, it is a well-established fact that the proinflammatory cytokine tumor necrosis factor-α (TNF-α) plays a critical role in the pathogenesis of RA by orchestrating the inflammatoryimmune-response in the synovium [3]. Accordingly, inhibiting the production and/or biological activity of TNF-α is considered a promising therapeutic approach. Most of the available therapeutic agents are therapeutic proteins such as etanercept (Enbrel), infliximab (Remicade), and adalimumab (Humira), which specifically bind to proinflammatory cytokines and other proteins [3,4]. Among these immunosuppressant proteins, etanercept, which is a genetically engineered fusion protein consisting of two identical chains of the recombinant human tumor necrosis factor receptor (TNFR) p75 monomer fused with the Fc domain of human IgG1, has the largest market due to safety and efficacy benefit [5]. However, despite their high activity and specificity, the therapeutic application of protein drugs suffers from poor biophysical stability (i.e., high clearance rates) and low bioavailability [6,7]. These hurdles have led researchers to develop various vehicles that can carry proteins to prolong their half-life with reduced undesirable effects [8–11].

Recently, the polypelectrolyte (PE) complex has received a lot of attention in this field because it is formed easily via ionic interactions between charged proteins and counter-charged polymers in aqueous phase [12–14]. However, the lack of physical stability still remains unresolved; the polymer/protein complex dissociates during the fabrication process and readily releases proteins under physiological conditions (i.e., physiological serum and salt concentrations) due to weak ionic interactions [15,16]. To overcome this limitation, several approaches have been reported to enhance the stability of PE complexes by covalent crosslinking of the core or shell [17,18]. However, the chemical reactions involved in the covalent interaction in the core or shell may induce side effects due to undesirable crosslinking [19].

Herein, we describe a sophisticated approach to stabilise etanercept and prolong its therapeutic effect against RA by using temperature-induced noncovalent interaction controllable (TMN) complex without covalent crosslinking. A temperature-sensitive amphiphilic polypelectrolyte was utilised to form PE complexes with etanercept, as well as to control hydrophobic interactions depending on the temperature (Scheme 1): this complex exhibited mainly electrostatic interactions between the negatively charged temperature-sensitive polymer and positively charged etanercept (pI = 8.1) at temperatures below the polymer’s clouding temperature (CT). As temperature increased to over the polymer’s CT (at physiological temperature, 37.5 °C), new noncovalent interactions (hydrophobic) are formed within...