Biomaterials 34 (2013) 1327–1336

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Dual ionic interaction system based on polyelectrolyte complex and ionic, injectable, and thermosensitive hydrogel for sustained release of human growth hormone

Mi-Ran Park [a,1], Bo-Bae Seo [a,b,1], Soo-Chang Song [a,*]

[a] Center for Biomaterials, Korea Institute of Science & Technology, Seoul 130-650, Republic of Korea
[b] Department of Biomolecular Science, University of Science and Technology (UST), Daejeon 305-350, Republic of Korea

ARTICLE INFO

Article history:
Received 21 August 2012
Accepted 11 October 2012
Available online 11 November 2012

Keywords:
Human growth hormone (hGH)
Protamine sulfate (PS)
Polyelectrolyte complex (PEC)
Anionic, injectable, and thermosensitive hydrogel
Poly(organophosphazene)
Sustained release

ABSTRACT

A dual ionic interaction system composed of a positively charged polyelectrolyte complex (PEC) containing human growth hormone (hGH) and anionic thermosensitive hydrogel has been suggested for sustained delivery of bioactive hGH. The PEC was prepared by ionic interaction between negatively charged hGH and positively charged protamine sulfate (PS) to suppress diffusion of hGH. Moreover, we loaded the positively charged PEC into an anionic, injectable, and thermosensitive poly(organophosphazene) hydrogel to enhance sustained release of hGH by dual ionic interactions. PS formed a spherical complex with hGH, and their ionic interaction grew stronger with increasing amounts of PS. From a weight ratio of 0.5, the PS/hGH complex had a size and zeta-potential that were constantly maintained around 500 nm and +8 mV, respectively, in 0.9% NaCl. The PEC-loaded hydrogels suppressed the initial burst release of hGH and extended the release period *in vitro* and *in vivo*. In a pharmacokinetic study in rats, the PEC-loaded anionic hydrogel extended half-life 13-fold with similar area under the curve (AUC) compared to hGH solution. Furthermore, single injection of PEC-loaded anionic hydrogel showed a more increased growth rate than daily injection of hGH solution for 7 days in hypophysectomized rats, demonstrating its potential as an injectable, sustained delivery system that can release bioactive hGH.

1. Introduction

Human growth hormone (hGH), which stimulates growth and differentiation of target tissue such as muscle and bone, is one of the major protein drugs [1,2]. Its clinical applications include not only growth hormone deficiency in children and adults, but also growth failure due to Turner syndrome or chronic renal failure [3,4]. However, like other protein drugs, it requires frequent administration via parenteral injection due to its short plasma half-life; parenteral injection results in poor patient compliance, high dose, non-specific toxicity and increased cost [4–7]. Therefore, development of a sustained hGH delivery system that can provide reduced frequency of injection, adverse effects, and cost is very attractive, and many delivery systems have been suggested to overcome this obstacle.

Among them, microparticles are the most investigated delivery system. The representative example is a biodegradable poly(lactic acid-*co*-glycolic acid) (PLGA) microsphere. The advantage of a PLGA microsphere is long-term delivery of loaded hGH [8,9]. Nutropin depot®, the first launched sustained delivery system of hGH, uses the PLGA microsphere. It was launched as a once every 2 weeks to 1 month formulation, although it was withdrawn from the market due to the high cost of manufacturing [10]. However, this system also has several disadvantages as a delivery carrier of protein including low loading efficiency, use of a thick needle for injection, high initial burst release, protein aggregation by the hydrophobic surface of the microsphere, denaturation from using organic solvent and inflammation from acidic degradation products [11–13]. To date, the only commercially available sustained release formulation of hGH is the hyaluronate microparticle formulation. It was launched as a once-a-week injection formulation in Korea by LG Life Sciences in 2007 [4]. However, because the serum hGH level was maintained for 30 h in cynomolgus monkeys, development of a new sustained delivery system of hGH was still required for more improved patient convenience [4,14].

* Corresponding author. Tel.: +82 2 958 5123; fax: +82 2 958 5308.
E-mail address: scsong@kist.re.kr (S.-C. Song).
[1] Both authors contributed equally to this work.

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2012.10.033

Injectable and thermosensitive hydrogel is one of the promising carriers for proteins. It has some desired properties as a protein delivery carrier compared to other delivery systems, such as high water content and a temperature-dependent gelation without organic solvents or chemical cross-linkers [15,16]. The drug can be easily formulated by simple mixing without any loss of drug content. Patient convenience can also be improved by a simple injection using a fine needle without surgery [15,17]. Generally, however, the loaded protein drugs are rapidly released by diffusion during the initial swelling of hydrogel due to their hydrophilic nature and small hydrodynamic sizes compared to the pore size of hydrogel [16,18,19]. This initial burst release of loaded proteins was also observed in the thermosensitive poly(organophosphazene) hydrogel, which has been developed by our group [20,21].

Incorporation of another delivery system such as microparticles or implants to a hydrogel can be methods to achieve controlled and sustained release of protein from hydrogel [19]. As the another delivery system, PLGA microparticles [18,22], cholesteryl group-bearing pullulan (CHP) nanogels [23], or poly(propylene fumarate) (PPF) implants [24] have been incorporated into hydrogels. These composite delivery systems succeeded to retard protein release compared to hydrogel only. However, preparation of an additional delivery system can complicate the manufacturing process, and some problems can be newly generated by the inherent disadvantages of another delivery system, such as low loading efficiency of protein.

The polyelectrolyte complex (PEC) has several advantages compared to other delivery systems, mentioned above. It can be prepared by simple mixing between charged protein and oppositely charged materials, and the use of water as a solvent helps to maintain protein stability [25]. Recently, we confirmed the potential of the PEC incorporated hydrogel as a controlled and sustained delivery system of protein [20]. The PEC was induced between negatively charged hGH (p*I*: 5.27) and positively charged poly-L-arginine (PLA, >70 kDa, p*I* of arginine: 10.76) and loaded to the thermosensitive poly(organophosphazene) hydrogel. The composite system suppressed the initial burst release and extended hGH release compared to hGH solution *in vivo*. However, the area under the curve (AUC) of released hGH was decreased more than 2-fold compared to hGH solution due to difficult dissociation of hGH from PLA.

In this study, we designed dual ionic interaction system composed of a slightly positively charged PEC containing anionic hGH and anionic thermosensitive hydrogel to improve the bioavailability of hGH while keeping a sustained release profile (Fig. 1). To facilitate dissociation of hGH from PEC, a protamine sulfate (PS) was used as a cationic material, to make a PEC. It is an FDA approved, arginine rich peptide used as an additive for subcutaneous injection and it has been used clinically as a long-acting formulation of insulin [26]. We hypothesized that its lower molecular weight (5 kDa), existence of other amino acids (except arginine), and sulfate groups might help to dissociate hGH compared to PLA. The characteristics of the PS/hGH PEC were examined by electrophoresis, transmission electron microscopy (TEM), particle size, and zeta-potential. Moreover, we also synthesized anionic poly(organophosphazene) hydrogel and loaded the PEC into that to induce an additional ionic interaction between the positively charged PEC and anionic hydrogel. The effect of a dual ionic interaction on the release profile and bioactivity of hGH was then examined *in vitro* and *in vivo* by comparing it

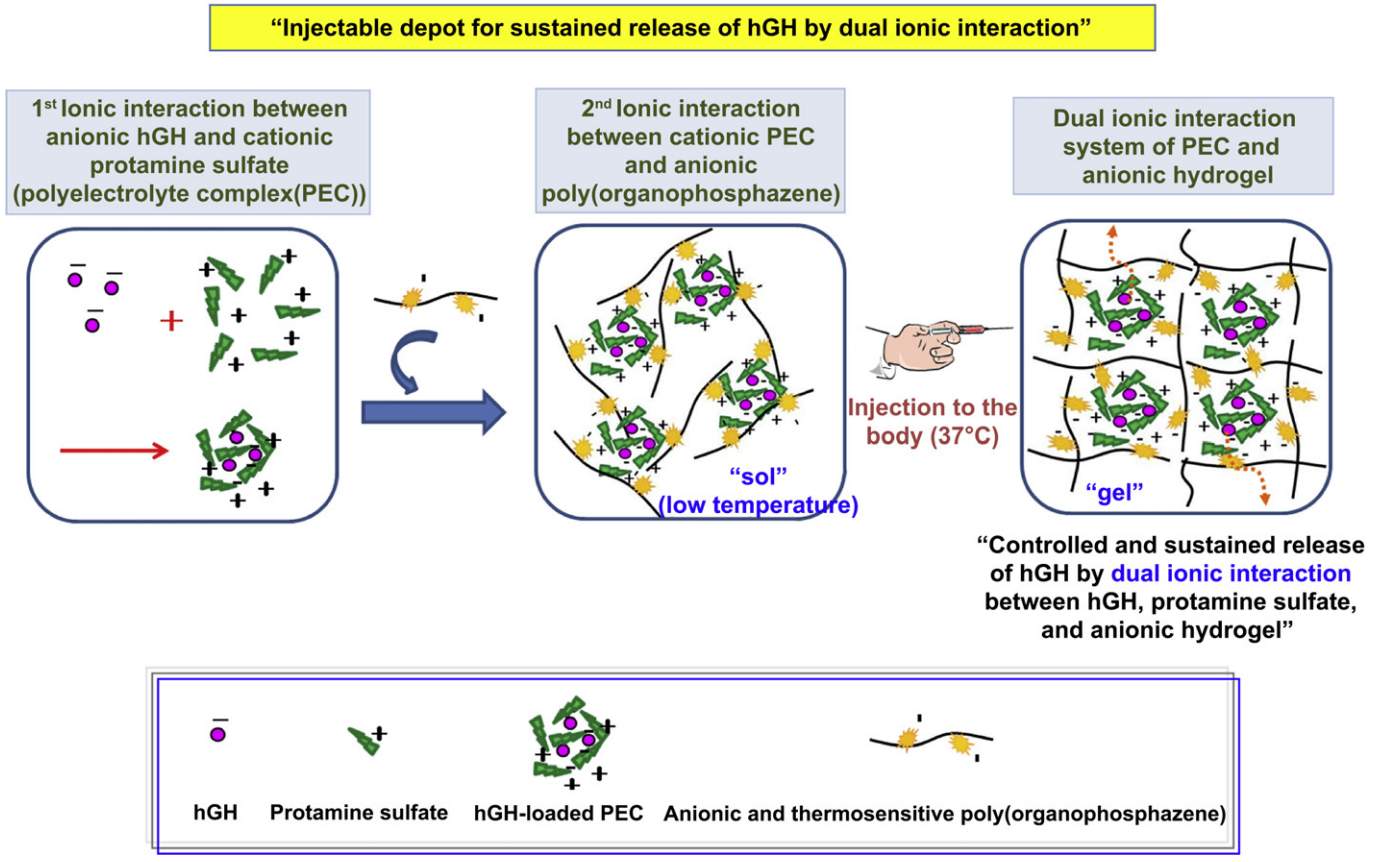

Fig. 1. A schematic illustration cartoon for proposed dual ionic interaction system based on hGH loaded PEC and anionic hydrogel for sustained delivery of hGH.