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On the design of in situ forming biodegradable parenteral depot systems based on insulin loaded dialkylaminoalkyl-amine-poly(vinyl alcohol)-g-poly (lactide-co-glycolide) nanoparticles

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Abstract

The feasibility to generate in situ forming parenteral depot systems from insulin loaded dialkylaminoalkyl-amine-poly(vinyl alcohol)-g-poly (lactide-co-glycolide) nanoparticles, was investigated. Biodegradable nanoparticles formed polymeric semi-solid depots upon injection into isotonic phosphate buffered saline (PBS) with no additional initiators. Nanoparticles (NP) prepared from the different amine-modified polyesters displayed a pronounced positive ζ-potential of >25 mV. Diethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAPA (68)-PVAL-g-PLGA(1:20)), diethylaminoethyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAEA(33)-PVAL-g-PLGA(1:20)), and dimethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DMAPA(33)-PVAL-g-PLGA(1:20)), formed in situ depots by an ion-mediated aggregation with subsequent fusion of nanoparticles, related to a decreased glass transition temperature in the presence of PBS.

Moreover, two factors, namely, polymer and insulin-nanocomplex concentration, were evaluated using a response surface design with respect to nanoparticles formation and insulin loading. Nanoparticles and implants were investigated by atomic force microscopy (AFM). The in vitro release from implants loaded with 2% insulin was carried out in a flow trough cell and quantified by high performance liquid chromatography (HPLC). The release showed a triphasic profile with an initial burst, pore diffusion and diffusion from the swollen matrix over more than two weeks. Insulin distribution in the implants during the release was followed by confocal laser scanning microscopy (CLSM). These findings combined with the protection of the model peptide against competitive macromolecules and the possibility to get dry powders by lyophilization make these nanoparticles-based depots suitable candidates for the design of controlled release devices for bioactive macromolecules.

Keywords: In situ forming parenteral depots; Nanoparticle networks; Ion-mediated aggregation; Protein delivery; Insulin release

1. Introduction

Progress in recombinant DNA technology during the past two decades has yielded a host of therapeutically relevant proteins. Thus, new strategies to retain the stability of the proteins during storage on one hand and to overcome the barriers for effective systemic delivery on the other hand have been the focus of intensive research efforts [1–4].

Proteins are known to be unstable in the gastrointestinal tract and show low permeability across biological membranes. Therefore, oral administration is not feasible requiring parenteral administration. Apart from designing protein analogues with improved therapeutic efficacy [5] and stabilization of proteins by PEGylation [6,7], much work was devoted to drug delivery systems [8]. Polymeric drug delivery systems such as microspheres, nanoparticles and hydrogels have been investigated as well as lipid-based systems such as liposomes and solid lipid nanoparticles [9]. In situ forming parenteral drug delivery systems, ISFPD, i.e. liquid injectable systems forming a (semi)-solid depot at the injection site could be advantageous for the delivery of therapeutic proteins [9–12]. Their simple manufacturing, constant "infusion-like" plasma level time profiles, and therefore, the reduction of application frequency are attractive features.

ISFPD based on (biodegradable) polymers can be classified into four categories, according to their mechanism of depot formation [12]: *Thermoplastic pastes* are injected as a melt and

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form a depot upon cooling [13]. Formation of a solid polymer system or hydrogel by *in situ cross-linking* can be achieved by various mechanisms such as photo-initiation, ionic interaction or radical-initiated polymerization [14–16]. The removal of the solvent by body fluids leads to *polymer precipitation* at the injection site [17] and several polymers showed *thermally induced gelling* when injected as highly concentrated solutions [18]. Other systems rely on water-soluble amphiphilic lipids which form various types of lyotropic liquid crystals [19] or sugars to obtain in situ depots [20]. While some ISFPD have become commercially available [21,22], a trend to avoid organic solvents in the ready-to-use formulations has become apparent [23,24]. This approach was supported by controversial findings concerning tissue tolerability of organic solvents used for in situ forming systems, under both in vitro and in vivo conditions [25,26].

For these reasons, our objective was to investigate the feasibility of an ISFPD based on an aqueous colloidal system forming parenteral depots after intramuscular or subcutaneous injection. We hypothesized that on contact with isotonic fluids aqueous suspensions of positively charged nanoparticles could offer the possibility to form a semi-solid depot at the injection site due to ion-mediated nanoparticle aggregation. Nanoparticles were prepared from amphiphilic polyesters, namely diethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAPA(68)-PVAL-g-PLGA(1:20)), dimethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DMAPA(33)-PVAL-g-PLGA(1:20)), and diethylaminoethyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAEA(33)-PVAL-g-PLGA(1:20)), composed of PLGA chains grafted onto an amine-substituted poly(vinyl alcohol) backbone [27]. The insulin loaded nanoparticles (NP) were generated using a solvent displacement technique [29]. Here we report the formation and preloading of the nanoparticles with insulin, the formation of in situ generated depots, and the release profile of insulin from these biodegradable depots under in vitro conditions.

2. Materials and methods

2.1. Chemicals

Five different polymers were used in this study: (1) the polymer backbone diethylaminopropyl-amine-poly(vinyl alcohol) (DEAPA(68), MW 17,800), (2) water-soluble diethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAPA(26)-PVAL-g-PLGA(1:2), MW 51,700) for the formation of insulin-nanocomplexes according to [28], (3) water-insoluble dialkyllaminoethyl-amine-poly(vinyl alcohol)-g-poly (lactide-co-glycolide) (DEAEA(33)-PVAL-g-PLGA(1:20), MW 350,200), (4) dimethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DMAPA(33)-PVAL-g-PLGA(1:20), MW 358,300), and (5) diethylaminopropyl-amine-poly(vinyl alcohol)-g-poly(lactide-co-glycolide) (DEAPA(68)-PVAL-g-PLGA(1:20), MW 323,700) for the preparation of nanoparticles, as described earlier [27]. Pluronic™ F68 was purchased from BASF (Ludwigshafen, Germany). Acetonitrile (HPLC grade) was from JT Baker (Deventer, Netherlands). Human recombinant insulin powder (26.2 I.U./mg) was a gift from Aventis Pharma AG (Frankfurt, Germany). Deoxyribonucleic acid sodium salt from herring testes (HT-DNA) was from Sigma (Munich, Germany). All other chemicals were of the highest analytical grade commercially available.

2.2. Labelling of insulin with tetra-methyl-rhodamine isothiocyanate (TRITC)

Insulin was dissolved at a concentration of 4.5 mg/ml in Na_2CO_3 buffer, pH 9.30. A solution of TRITC (1.0 mg/ml) in dimethyl sulfoxide (DMSO) was quickly added (molar ratio of insulin/TRITC, 1:2.9) and the mixture stirred for 18 h at 4 °C while protected from light. The reaction was quenched with an excess of ammonium chloride and stirred for another 4 h. Separation was performed on a PD-10 Sephadex G-25 column (Amersham Bioscience, Munich, Germany) with PBS pH 7.40. For further experiments, the insulin stock solution for nanocomplex (NC)/nanoparticle (NP) preparation was a mixture of labelled and non-labelled insulin in a 1:10 (v/v) ratio.

2.3. Preparation of insulin-nanocomplexes

Nanocomplexes (NC) were prepared according to [28]. Briefly, insulin stock solution (20 mg/ml) was mixed with an equal volume of DEAPA(68) stock solution (9.2 mg/ml) resulting in a defined mass ratio of insulin:polymer of 1:0.46 (m/m) in the final NC-solution. The colloidal polymer–insulin complexes formed immediately after mixing by spontaneous self-assembly.

2.4. Preparation of nanoparticle suspensions

2.4.1. General procedure

Nanoparticle (NP) suspensions were produced by using a modified solvent displacement method [29]. Briefly, polymer solutions of different concentrations in acetone (see statistical design below) were slowly injected into magnetically stirred (500 rpm) aqueous solutions containing 0.1% of poloxamer 188 as stabilizing agent. Insulin or an insulin containing NC-suspension was added to the polymer solution immediately before NP preparation. After removal of the organic solvent by stirring the NP-suspensions for at least 8 h under reduced pressure, the evaporated water was replaced.

2.4.2. Statistical design and analysis

A response surface design in the form of a rotatable central composite design: 2^2 + star ($\alpha_{cc}=1.414$) was chosen to study the effect of 2 factors in 10 runs. The number of trials (N) was based on the number of the design factors ($k=2$).

The two most important factors namely, “polymer concentration in O-Phase”, where the O-phase is a polymer solution in acetone, and “insulin [mg] amount in NC” were determined in preliminary experiments. The intervals between levels were chosen to maintain the rotatable values of the design, resulting in polymer concentrations in O-phase of 10.0, 23.0, 45.0, 87.0 or 100.0 mg/ml and insulin amounts in NC of 0.000, 0.375, 1.250,