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Reduction of the *in vivo* burst release of insulin-loaded microparticles

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ABSTRACT

The main objective of this work was to evaluate the behavior of composite microparticles (microparticles containing nanoparticles) for parenteral delivery with a view to reducing the initial burst release of short half-lives peptides/proteins (using insulin as a model drug). These composite microparticles were prepared with hydrophobic and biodegradable polymers [Poly (ε-caprolactone), poly (lactic-co-glycolic) acid] by the double emulsion extraction technique. Particles were administered subcutaneously (1 IU/kg of insulin) as a single dose to diabetic rats (streptozotocin-induced) and serum insulin levels were monitored. The results show a significant reduction of insulin release from composite microparticles both *in vitro* (around 19% after 15 min) and *in vivo* (around 582 μU/mL) with a progressive and controlled release profile up to 24 h when compared to simple microparticles (without nanoparticles inside) and nanoparticles. These experiments demonstrate that a desirable insulin injection formulation with negligible burst effect *in vivo* has been developed. Such effect was attributed to the double polymer barrier that the drug has to diffuse through before reaching the external medium either *in vitro* or *in vivo*.

1. Introduction

Bioactive proteins and peptides are a rapidly growing class of therapeutic agents. Injections of peptides/proteins have to be renewed frequently because their *in vivo* half-life is generally rather short. Therefore, sustained delivery of proteins and peptides is still of major interest for parenteral administrations [1].

However, parenteral peptides or proteins controlled release products based on biodegradable polymers (nano/microparticles and hydrogels) or lipid-based systems (liposome and solid lipid nano/microparticles) often present an inconsistent release profile characterized by a high initial burst effect defined as the initial release of a large bolus of drugs [2–4]. This initial burst phase is then followed by a plateau frequently associated with an incomplete release [4]. In most cases, the burst release corresponds to a significant loss of drug from both therapeutic and economic standpoints. Indeed, high initial drug release is not suitable for parenteral peptides and proteins products because drug released in this period is no longer available for prolonged release and may lead to a high risk of side effects (due to elevated serum levels) especially for drugs with narrow therapeutic index and short half-life. For this reason, one of the goals of protein microencapsulation is to reduce the initial burst and achieve a constant release rate thereafter.

For many parenteral therapeutic proteins, achieving acceptable high drug content into particles while maintaining satisfactory release kinetics (*i.e.* minimal burst together with acceptable duration), represents a formulation challenge.

Protein release from biodegradable polymeric particles during the initial release stage depends on diffusion escape of the protein through channels existing in the polymer matrix. Given that the time required until the onset of polymer degradation ranges from weeks to months, drug release during the first few days (or hours) depends on how successfully the diffusion is controlled. In most cases, the burst release is due to poor control over the diffusion based release in this stage. The degree of initial burst from the nano/microparticles depends on the ability of the polymer matrix to encapsulate the protein, thereby making it unavailable for immediate diffusion [4,5]. For this reason, efforts to reduce the initial burst have followed in the same track as those to increase drug encapsulation efficiency.

Initial burst release of peptides and proteins from polymeric matrix may depend on their molecular weight. Peptides and proteins with small molecular weights (for example triptorelin, calcitonin) may escape from the polymeric matrices through the pores and cracks that form during the particles manufacturing process [3,6,7]. On the other hand, burst release may also occur with high

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molecular weight proteins (immunoglobulin G [8], Glial cell line-derived neurotrophic factor (GDNF) [9], α-interferon [10]) due to i) their heterogeneous distribution in the matrix ii) their loosely association with the surface or iii) their embedding in the surface layer [3,4].

In a first report [11], we have prepared composite microparticles corresponding to drug loaded poly(ε-caprolactone) (PCL) nanoparticles encapsulated into Eudragit® RS or ethyl cellulose microparticles. This study showed high encapsulation efficiency and low initial burst effect of triptorelin (hydrophilic drug model peptide). However, the concept of controlling the burst effect by encapsulating nanoparticles into microparticles was only demonstrated *in vitro*. In addition, the tested microparticle polymers (Eudragit® RS and ethyl cellulose) are not acceptable for parenteral administration (subcutaneous or intramuscular). Thus, we decided to use a biodegradable polymer such as poly (lactic-co-glycolic) acid (PLGA) to replace Eudragit® RS or ethyl cellulose in the microparticles matrix. We first tested the influence of this new composition, *in vivo*, on the burst release of a lipophilic drug, i.e. ibuprofen [12]: as a matter of fact, an important reduction of the burst release was demonstrated. In the present work, we aimed to further extend this concept to the field of peptides/proteins delivery. Indeed, most multiparticulate-marketed systems concern peptides or proteins material. Contrary to ibuprofen, peptides/proteins are highly water-soluble molecules whose burst effect is more difficult to control. Due to its broad interest and its easy serum assay, not mentioning its relatively low cost, insulin was chosen as the peptide drug model. Indeed, patients with type 1 diabetes mellitus depend on external insulin (most commonly subcutaneously injected) for their treatment. Patients with type 2 diabetes mellitus have insulin resistance, which is progressively associated with a reduced production of insulin; thus, some type 2 diabetic patients require insulin when oral antidiabetic drugs become insufficient in controlling blood glucose levels. It is also well known that insulin-dependent patients need a relatively constant basal insulin supply to attain a near-normal physiological pattern of insulin.

In order to achieve blood glucose control in diabetes, intensive insulin treatment is used. This treatment involves the daily injection of one or more doses of intermediate- or long-acting insulin to satisfy basal insulin requirement, as well as injection before each meal. The multiple daily injections regimen leads to poor patients' compliance, pain and even mental stress. Theoretically, injectable polymeric or lipidic delivery systems (microspheres, microcapsules, microemulsion, liposomes, and gel) could be used for controlled release of insulin continuously for a desired period of time [13,14]. Such a release pattern would satisfy continuous low-level of basal insulin requirement. Basal insulin delivery from these novel systems could partly relieve patients from multiple daily injections. Such formulations would contribute not only to an improvement in the patient's compliance, but also to a reduction of developing additional diabetes complications [15]. In the literature, there are a lot of attempts to prepare satisfactory parenteral sustained insulin formulation without burst release [14,16–19].

The purpose of the present study was to develop and test *in vivo* composite microparticles based on biodegradable polymers only, thus adapted to the subcutaneous injection and *in vivo* administration of insulin.

2. Materials

Regular and fast human insulin (Actrapid®) was from Novo Nordisk (Bagsvaerd, Denmark): concentration of the solution was 100 IU/mL of human insulin. Excipients of Actrapid® are metacresol, zinc chloride, glycerol, sodium hydroxide and/or hydrochloric acid and water for injectable preparation. Poly (ε-caprolactone) (*Mw* 40,000 Da) and D-L poly(lactic-co-glycolic) acid 50:50 (m/m) Resomer RG 504S end-capped (M_W 48,000; viscosity: 0.47 dL/g) were purchased from Aldrich, (Saint Quentin Fallavier, France) and Boehringer Ingelheim (Ingelheim, Germany), respectively.

Polyvinylalcohol (PVA, *Mw* 30,000, 88% hydrolyzed) was supplied by Sigma (Saint Quentin Fallavier, France). Ethyl acetate (water solubility = 8.3 g/100 mL at 20 °C) was purchased from Fluka Chemie GmbH (Buchs, Switzerland). Methylene chloride (water solubility = 1.3 g/100 mL at 20 °C) was supplied by Prolabo (Paris, France). Acetonitrile and orthophosphoric acid were obtained from Carlo-Erba (Val de Reuil, France) and Prolabo (Paris, France), respectively. All other chemicals were of analytical grade and used without further purification.

3. Methods

3.1. Preparation of particles

3.1.1. Nanoparticles

Insulin loaded PCL nanoparticles were prepared by the water-in-oil-in water (W/O/W) solvent evaporation method [20]. Briefly, 1 mL of Actrapid® aqueous solution was emulsified for 15 s in 5 mL of methylene chloride (containing 125 mg of PCL) with the help of an ultrasound probe (Vibra cell 72 434, BioBlock Scientific, Strasbourg, France) at 80 W output. This primary emulsion was poured into 40 mL of a 0.1% PVA aqueous solution and sonicated again with the same ultrasound probe for 1 min in the same conditions in order to create the water-in-oil-in-water (W/O/W) emulsion. Three mL (±1 mL) of nanoparticles suspension were obtained after solvent evaporation under reduced pressure.

For incorporation determination, nanoparticles were separated from the bulk suspension by centrifugation (Biofuge Stratos; Heraeus Instruments. GmbH&Co., Hanau, Germany) at 42,000 × g for 20 min. The supernatant was kept for drug assay according to the methods described later and the sedimented nanoparticles were then redispersed in 3 mL of purified water before freeze-drying. After lyophilization, a dry powder of nanoparticles was obtained: the freeze-dried nanoparticles powder was used for dissolution purposes only. The nanoparticles preparation method was slightly modified for manufacturing of composite microparticles (nanoparticles in microparticles). Indeed, the only difference was that the solvent evaporation process was continued till 1.5 mL (±0.5 mL) of nanoparticles were obtained: this suspension was used directly (without freeze-drying) as the internal aqueous phase in the preparation of the composite microparticles in order to prevent any issue with regards to particle size change between manufacturing and freeze-drying process. Blank nanoparticles were prepared under the same conditions but without drug.

3.1.2. Microparticles

Microparticles containing insulin PCL nanoparticles (so-called composite microparticles) or not were prepared by the W/O/W solvent extraction method [21]. In the first step (W/O emulsion), the PCL nanoparticles suspension (1.5 ± 0.5 mL as mentioned before) was used as the internal aqueous phase, which was emulsified (ultrasound probe at 80 W output for 15 s) in the organic solution of ethyl acetate (10 mL) containing PLGA (150 mg).

This primary emulsion was poured into 30 mL of 0.1% PVA aqueous solution in order to obtain a W/O/W pre-emulsion. After magnetically stirring for 20 s (600 rpm) at room temperature, this pre-emulsion was added to 1 L of purified water and stirred mechanically (three-bladed propeller, 600 rpm) for 10 min to form the final W/O/W emulsion.

Upon solvent extraction, the polymers precipitated and the