Insulin-Carrying Microspheres, in Vitro Studies

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Loading and release characteristics of insulin-carrying albumin and starch microspheres have been studied in vitro. The sorption characteristics of 125I-labelled insulin onto albumin microspheres were studied and found to be completed within 5h, and the loading capacity was found to be 0.14% w/w. Insulin did not show any sorption into the matrix of the starch microspheres. The release characteristics were analyzed by high performance liquid chromatography. About 80% was released within 5-10 min from albumin microspheres and starch microspheres, respectively.

Keywords: adsorption; insulin; microsphere; release; sorption

Introduction

Growing attention has been paid to the preparation of delivery systems based on drug-loaded colloidal carriers such as microspheres. To attain the desired therapeutic effects in these systems, the release characteristics of a drug to the surrounding medium over a certain period of time is very important. More than 90 drugs have been incorporated into albumin microspheres and their release profiles have been reported, as reviewed.23

Bioadhesive microspheres, which form gel-like structures in contact with a mucous surface such as the nasal membrane, thereby prolonging the contact between the delivery system and the surface, have a potential for releasing the drug in a sustained and controlled manner, possibly increasing the absorption efficiency of the drug. In vitro studies of insulin loaded microspheres have been investigated to achieve the prolonged release of insulin5-6 and to increase absorption across the nasal membrane in rats.7,8a The release of drugs from microspheres is generally biphasic, with an initial rapid release phase (called the "burst effect"), where the drug adsorbed on the surface is released, followed by a slower release phase where the drug incorporated into the matrix is released over a long period.

The purpose of the present paper is to study, in vitro, the sorption and release characteristics of insulin from produced albumin and commercially available starch microspheres.

Materials and Methods

Materials: Zinc-free human insulin powder and monosaccharides were obtained as a gift from Novo Industry A/S and prepared in 0.1 M Tris buffer (pH 7.4). 25% w/v 1,5-glucaraldehyde and rabbit serum albumin (RSA), fraction V, (RIA grade) were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Petroleum ether, highly refined olive oil and ethanol were obtained from Mecobencon (Copenhagen, Denmark). Distilled water was used throughout and all other chemicals were of a reagent grade.

Microspheres: Albumin microspheres were produced by an emulsification technique described by Burger et al.9 with slight modifications. 500 ml of highly purified olive oil was mixed with 750 ml of petroleum ether and presaturated for 15 min in a 3000 ml flat-bottomed glass beaker (equipped with four 10mm deep baffles positioned against the wall of the beaker) using a Heidolph mixer (Heidolph Electro GmbH, Kelheim, Germany). In order to crosslink the microspheres, 20 ml of a 25% w/v aqueous solution of RSA in phosphate buffer (pH 7.4) was added in aliquots of 2.0 ml, and stirring was continued at 600 rpm for 15 min. The microspheres were isolated by centrifugation, washed with petroleum ether, filtered through a Millipore filter (Miteq C311 621 A6) by washing with petroleum ether and ethanol and then freeze-dried overnight.

Freeze-dried starch microspheres (SphereX®) (swelling factor 8-10) were obtained as a gift from Pharmacia AB (Uppsala, Sweden), and used as obtained.

Analytical Methods: The microspheres were sized in normal saline using a Coulter Counter model TA III, Coulter Counter Electronic Ltd. (Herts, U.K.). The particle size was expressed as a mean volume diameter. The mean particle diameters of the swollen microsphere systems were found to be about 58 ± 13 and 40 ± 8 μm (swelled size) for the albumin and the starch microspheres, respectively.

Sorption Characteristics: The sorption characteristics of insulin to the albumin and starch microspheres was evaluated by suspending the microspheres (70 mg) into 2.5 ml of an insulin solution containing 125I-labelled insulin at about 20.8 mg/ml and about 180 μCi/ml. At selected time intervals (1, 3, 8, 16, 18, 22 and 46h), about 10 mg samples of microspheres were taken from the suspension, filtered (no significant adsorption was found to occur to the filters) and washed with ethanol, and the activity detected by the γ-counter. The quantities measured at 24h sorption time were taken as equilibrium values.

Release Characteristics: The in vitro release of insulin was evaluated using about 10 mg of microspheres, carrying about 0.10-0.13 mg insulin/mg. The sorption of insulin to the microsphere systems was performed by suspending 100 mg of microspheres in 5 ml of an insulin solution containing about 20.8 mg/ml. After storage at ambient room temperature for 24 h, the albumin microspheres were separated from the solution by filtration, resuspended in water and then freeze-dried. In these experiments the starch microspheres were freeze-dried directly after storage without separation of the microspheres from the insulin solution. The microspheres were suspended in an 11 ml Tris-buffer at pH 7.4 (sink condition) and kept at 25°C. At selected time intervals samples of 25 μl were taken from the top of the suspension and injected directly into the high performance liquid chromatography (HPLC) system. Prefiltration was not possible, since the HPLC-microfiltration was designed to absorb about 14% (0.14 mg/cm²) of the insulin. 25 μl of the buffer were added to the suspension after each sampling to maintain a constant volume.

Calculations: The maximal sorption capacity was calculated by HPLC determination of the decrease in the insulin concentration after the microspheres (t=24h).

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Results and Discussion

Figure 1 gives the sorption profile of insulin to albumin microspheres. It shows that the sorption on and into the microspheres is completed within 3 h, as measured by labelled insulin, and the maximal sorption capacity was found to be 0.14 mg/mg microspheres, from an insulin solution containing 20.8 mg/ml. Since the loading of insulin to starch microspheres was performed by suspending the microspheres in insulin solution, followed by freeze-drying, the amount of insulin per weight microspheres could be adjusted as wanted. The sorption of insulin to albumin microspheres was found to be in accordance with Langmuir adsorption isotherm (data not shown).

In contrast to the albumin microspheres, the analysis of the insulin concentration, before and after addition of starch microspheres, showed that the concentration of insulin increased significantly from 16.7 to >20.8 mg/ml. This indicates that, during the swelling of the starch microspheres, water is released from the insulin solution without allowing insulin molecules into the matrix. Hence, in all further experiments the "loading" of the starch microspheres with insulin was performed by freeze drying the microsphere-insulin suspension without prior separation.

Figure 2 shows the release profiles of insulin from albumin (about 0.14 mg/mg) and starch (about 0.11 mg/mg) microspheres as the percentage of insulin released with time. For both the albumin and starch microspheres, an initial fast release phase was observed. After the first 5—10 min about 80% of all insulin was released. In starch microspheres only, the initial phase tended to be followed by a second slower release phase, where about 20% of the insulin was released within 3 h. The rate and extent of release was not dependent on the amount sorbed to albumin or starch microspheres.

In conclusion, it has been demonstrated that in contrast to starch microspheres, albumin microspheres show the ability to sorb and carry native peptides such as insulin. The release profiles from albumin as well as from starch microspheres show a fast initial release phase which can be used clinically e.g. for the delivery of peptides to mucosal surfaces such as the nasal mucosa. Microspheres are able to stick to the mucosal surface, thereby prolonging the duration of e.g. insulin at the absorption site. This could be preferable for increasing the amount absorbed in a transnasally, but clinically relevant sustained release, from the studied microspheres, is not likely due to the fast in vitro release observed.

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References and Notes
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