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International Journal of Pharmaceutics 252 (2003) 271–274
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Note

Hypoglycemic effect of surfactant-coated insulin solubilized in a novel solid-in-oil-in-water (S/O/W) emulsion

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Received 8 September 2002; received in revised form 27 November 2002; accepted 27 November 2002

Abstract

A novel solid-in-oil-in-water (S/O/W) emulsion for oral administration of insulin has been developed using surfactant-coated insulin. The S/O/W emulsion prepared by a shirasu porous glass (SPG) membrane provided a sharp size distribution and was stable. Leakage of insulin from the S/O/W emulsions was not observed for several days. The S/O/W emulsion showed the hypoglycemic activity for a long period after oral administration to rats.

Keywords: Insulin; S/O/W emulsion; Oral administration; Surfactant; Drug delivery

The bioavailability of insulin administered orally is limited by enzymatic degradation in the gastrointestinal tract and low absorption at the mucous site due to its high molecular weight and low lipophilic properties. To overcome these problems, there have been many strategies for chemical modification of insulin, administration of protease inhibitors or adsorption enhancers with a drug carrier (Hinds and Kim, 2002; Morishita et al., 1998; Yamamoto and Muranishi, 1997). However, such additional agents and the modified insulin have sometimes been toxic.

In the present study, we have developed a novel emulsion microcapsule as an insulin carrier. Although the water-in-oil-in-water (W/O/W) emulsion has been used as a carrier for hydrophilic drugs, we have improved it and created a solid-in-oil-in-water (S/O/W) emulsion as an effective carrier for oral administration. Insulin, which is a hydrophilic macromolecule ($M_W = 5800$), was converted into a lipophilic complex by coating with surfactant molecules. As a result, the surfactant-coated insulin can be dispersed in the oil phase of oil-in-water (O/W) emulsions. A better absorption of the surfactant-coated insulin from the small intestine is expected because it is known that lipophilic compounds are absorbed as micelles, which are formed by metabolic products of soybean oil.

Recently, it has been reported that the membrane emulsification method could control the particle size and enhance the stability of emulsions (Mine et al., 1996; Nakashima et al., 2000). In this article, the stability of S/O/W emulsions prepared by the shirasu porous glass (SPG) membrane is also discussed.

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doi:10.1016/S0378-5173(02)00674-9

Furthermore, the biological effect of the insulin solubilized in the S/O/W emulsions was investigated by oral administration to rats.

The novel preparation method for the S/O/W emulsion including the surfactant-coated insulin is as follows: a 15 ml aqueous solution of insulin (60 U/ml, 0.01 M HCl) and a 30 ml hexane solution containing the lipophilic surfactant ER-290 (5 wt.%) were mixed with a homogenizer at 26,000 rpm for 1 min. Stable W/O emulsions were formed. The emulsions were poured into a round-bottom flask (100 ml), followed by rapid freezing in liquid nitrogen and lyophilization in a freeze-drying machine for 24 h. A light orange paste was obtained. The surfactant-coated insulin was added to 15 ml soybean oil, and dispersed thoroughly by ultrasonication. The soybean oil containing the insulin complex and a 30 ml aqueous solution containing the hydrophilic surfactant L-1695 (1 wt.%), sodium cholate (1 wt.%) and D-glucose (5 wt.%) were mixed with a homogenizer at 26,000 rpm for 1 min. The S/O/W emulsion obtained was then adjusted to a constant particle size through a SPG membrane. The size distribution of the emulsion droplets was measured using a laser-diffraction particle analyzer (SALD-200, Shimazu Co.). The leakage of insulin from the emulsions was measured by employing an FITC-labeled insulin (FITC-INS). Samples of emulsions (1.8 ml) were withdrawn at the time indicated and treated by centrifugal filtration. The FITC-INS in the filtrate was detected using a fluorometer (Ex: $\lambda =$ 494 nm, Em: $\lambda = 517$ nm).

Male Wistar rats weighing 210–230 g were used in the present study. Diabetes in the rats was induced by an intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in a citrate buffer at pH 4.5. Two weeks after the injection of streptozotocin, the rats were fasted for at least 16 h before experiments but allowed water ad libitum. Under ether anesthesia, 1 ml of S/O/W emulsion containing the surfactant-coated insulin or 1 ml of an aqueous insulin solution (100 U/kg body weight) was orally administered to rats. Blood samples were collected from the jugular vein at 0.083, 0.5, 1, 1.5, 2, 3, 4 and 6 h after the dose. The assay of the glucose levels in the plasma was performed using a Glucose-test kit (GL-NEW TEST, Mizuho Medy Co.). All the protocols for using the animals were in accordance with the recommendations of the Miyazaki Medical College guidelines entitled “Guide for the care and use of laboratory animals”.

In general, the W/O/W emulsion is used as a capsule for loading proteins. However, it was reported that insulin which was loaded into emulsions lost its activity at the interface of the emulsions (Sah, 1999). It is also difficult to control the size of the W/O/W emulsions on a nanometer scale because they contain relatively large inner W/O droplets. Recently, it was reported that solid-state proteins solubilized in S/O/W emulsions retained their activity (Morita et al., 2000; Putney and Burke, 1998). These proteins were coated by an amphipathic polymer. In the field of enzyme technology, there have been many studies of enzymes expressing their activity in organic solvents by complexing with an amphipathic compound (Kamiya et al., 2000; Okazaki et al., 2000). In this study, we have prepared a novel S/O/W emulsion by employing surfactant-coated insulin. The lipophilic surfactant ER-290, a food additive, was used as the modifier to coat the insulin. After the S/O/W emulsion was prepared by homogenization, a constant particle size (1.0 mm) was adjusted through a SPG membrane. The time course of the particle diameters of S/O/W emulsions is shown in Fig. 1. The S/O/W emulsions maintained a constant diameter for 30 days. Furthermore, no coalescence or breakdown of the droplets were observed during the storage. The average inner diameter of the emulsions was almost equal to

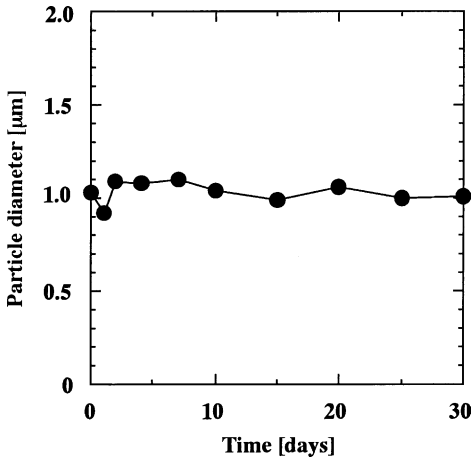

Fig. 1. Time courses of inner diameter change of S/O/W emulsions. Each value represents the mean of two experiments.

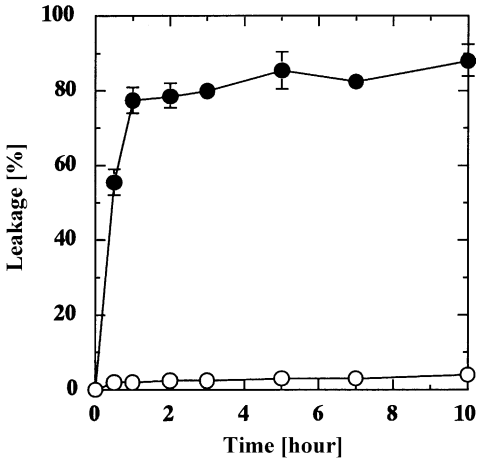

Fig. 2. Leakage of insulin from S/O/W emulsions: (○) surfactant-coated FITC-insulin, (●) lyophilized FITC-insulin. Each value represents mean ± S.D., $N = 3$.

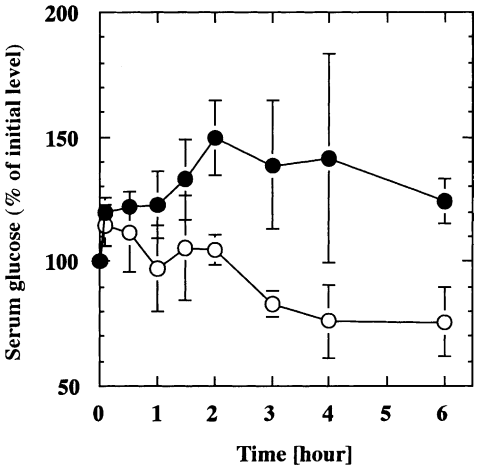

Fig. 3. Changes in serum glucose levels after oral administration of insulin emulsion or solution: (○) S/O/W emulsion, (●) insulin solution. Each value represents mean ± S.D., $N = 4$.

the pore size (1.1 μm) of the SPG membrane. It is known that emulsions having a sharp size distribution can depress the Ostwald ripening effect resulting in high stability for a long time. It was confirmed that the O/W emulsions prepared through a hydrophilic SPG membrane exhibited a sharp particle size distribution.

Next, the encapsulation of insulin in the S/O/W emulsions was investigated. Fig. 2 shows the time course of leakage of the lyophilized FITC-labeled insulin (FITC-INS) and FITC-INS coated with the lipophilic surfactant from the emulsions. The surfactant-coated FITC-INS dispersed well in the soybean oil and the solution was clear. On the other hand, the soybean oil solution containing the lyophilized FITC-INS showed white turbidity. The lyophilized FITC-INS rapidly leaked from the S/O/W emulsions, whereas the surfactant-coated FITC-INS did not leak from the emulsions. Since the surfactant-coated insulin is a lipophilic complex, it did not distribute to the aqueous phase of the emulsions.

Finally, the effect of oral administration of the S/O/W emulsions was investigated using the diabetic male Wistar rats. The insulin solution alone was used as the control. The hypoglycemic activity is shown in Fig. 3. The level of glucose in the serum increased in a few hours after administration of the insulin solution. However, the S/O/W emulsions containing the coated insulin showed a slowly decreasing effect on the glucose level. The hypoglycemic effect continued for a long time. In general, W/O/W emulsions, which are used as a protein carrier, do not express the hypoglycemic activity except for co-administration with a protease inhibitor or an absorption enhancer. The triglyceride, which is the main constituent of soybean oils, is degraded by lipases in the small intestine. The degraded fatty acids form micelles. Since the coated insulin is a lipophilic complex, it is readily soluble in the micelles, that is, the coated insulin was slowly absorbed with micelles from the mucous sites.

In the present study, a novel S/O/W emulsion was prepared using surfactant-coated insulin. This emulsion resulted in enhanced absorption of insulin after oral administration. It is expected that the S/O/W emulsions will become widely used in the treatment of diabetes.

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