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International Journal of Pharmaceutics 171 (1998) 177–183

international journal of pharmaceutics

Use of sodium salt of Carbopol 934P in oral peptide delivery

Takeshi Nakanishi, Fusao Kaiho, Masahiro Hayashi *

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12 Ichigaya, Funagawara-Machi, Shinjuku-Ku, Tokyo 162-0826, Japan

Received 27 November 1997; received in revised form 27 April 1998; accepted 20 May 1998

Abstract

When insulin was orally administered as a capsule containing Carbopol 934P (CP), freeze-dried sodium salt of CP (FNaCP), or lactose to diabetic rats, FNaCP improved the intestinal absorption of insulin, whereas CP and lactose did not. In the in vitro experiments, FNaCP and CP in solution increased the mucoadhesion of the model compound, fluorescein isothiocyanate-dextran (FD) 40000 (FD-40), and inhibited the enzymatic degradation of insulin to almost the same extent. FNaCP and CP in solution changed neither the membrane resistance nor the permeability of FD 4000 (FD-4) in the rat jejunum, indicating that an improvement of the paracellular peptide delivery did not take place in the jejunum. CP formed a swollen gel layer at the boundary between the medium and the capsule, which was a barrier for the drug release, but FNaCP did not, as described in a previous paper (Nakanishi et al., 1998. *Chem. Pharm. Bull.*171–173). Since the improving effects of FNaCP and CP in solution were almost the same, the difference in the effects of these two polymers on insulin release is thought to be due to the existence of the barrier to the insulin release from the capsules. In conclusion, FNaCP is a useful adjuvant for enabling the intestinal absorption of peptide drugs in a solid formulation such as capsules.

Keywords: Carbopol 934P; Sodium salt of Carbopol 934P; Mucoadhesion; Proteolytic degradation; Membrane resistance; Peptide absorption

1. Introduction

In recent years, many bioactive peptides have been produced due to developments in biotechnology. In most cases such peptide drugs are administered only by an injectable route, since most of them are poorly absorbable across the mucosa and are highly proteolytically degradable. Considering the quality of life of the patients, however, the oral route is more convenient and desirable for peptide drugs.

Bioadhesive drug delivery is a very desirable system and has been applied to various administration routes, for example, nasal (Nagai et al.,

* Corresponding author.

0378-5173/98/$ - see front matter
PII: S0378-5173(98)00176-8

1984), transdermal (Mura et al., 1989), rectal (Morimoto et al., 1987), vaginal (Lee and Chien, 1996), ocular (Ünlu et al., 1992) and oral routes (Lueßen et al., 1994). For oral peptide delivery, mucoadhesive polymers present the potential to prolong both the residence time in the gastrointestinal (GI) tract and the contact time for absorbing mucosa, resulting in an enhancement of drug absorption.

Carbopol 934P (CP, average molecular weight 3000000) is a mucoadhesive polymer which has been extensively investigated in the pharmaceutical field due to its high viscosity at low concentrations and its low toxicity. The application of CP to oral peptide drug delivery by solid formulation, however, is difficult, since CP showed little effect on the GI transit of such formulations, the delay of which is necessary to increase the oral availability of the peptide drug (Harris et al., 1990). We prepared a freeze-dried sodium salt of CP (FNaCP) to overcome this difficulty, and have succeeded in improving the drug release rate from the capsule of FNaCP, which dispersed without a swollen gel layer (Nakanishi et al., 1998). Akiyama et al. (1996) examined the efficiency of capsules containing both FNaCP and CP for oral peptide drug delivery in vitro. In their studies, it was shown that the swelling of FNaCP was more rapid than that of CP, but CP was more efficient in inhibiting the hydrolysis of peptide by trypsin compared to FNaCP.

In the present study, FNaCP capsules were orally administered to rats for an evaluation of the effectiveness of FNaCP as an adjuvant for oral peptide delivery in vivo. The adhesiveness of CP and FNaCP on the intestinal mucosa was compared. In addition, the improving effect of FNaCP on the membrane permeability of the peptide and the inhibitiory effect on proteolytic enzyme activity were examined with the use of small intestinal fluid and brush border mebrane vesicles (BBMV) in vitro.

2. Materials and methods

2.1. Materials

Carbopol 934P (CP) was a kind gift from BF Goodrich, Cleveland, OH. FNaCP was prepared by freeze-drying an aqueous dispersed solution of 0.5% CP which was neutralized with 10 M NaOH to pH 7.0. Human recombinant insulin (h-insulin), fluorescein isothiocyanate dextran 4000 (average molecular weight 4400, FD-4) and 40000 (average molecular weight 40500, FD-40) were purchased from Sigma, St Louis, MO. Hard gelatin capsules (Size 9, volume 25 mm^3) were obtained from Japan Elanco, Nara, Japan. Sodium pentobarbital (Nembutal, 50 mg/ml) was purchased from Abbott Laboratories, Chicago, IL. All other reagents were of analytical grade or better.

2.2. Preparation of capsules

Three kinds of capsules containing the model peptide drug, h-insulin (20 U) and FNaCP, CP or lactose (average 7 mg) as an adjuvant were prepared. Lactose was used as a representative excipient.

2.3. Oral administration

Diabetic rats were prepared from male Wistar rats (200–220 g, 7 weeks old) by the intraperitoneal (ip) injection of streptozotocin (SZ, 60 mg/kg). After a single injection of SZ, the plasma glucose level increased from the normoglycemic to the hyperglycemic level ($>$ 250 mg/dl) within 24 h. The rats whose plasma glucose levels exceeded 250 mg/dl 48–72 h following the i.p. injection of SZ served as the diabetic animals in the in vivo experiments. To each diabetic rat fasted for 12 h, a capsule including h-insulin (100 U/kg) with CP, FNaCP or lactose was orally administered. Plasma glucose levels were determined with a glucose test kit (Glucose B Test, Wako, Osaka, Japan).

2.4. In vitro mucoadhesion study

Unfasted rats (male Wistar rats weighing 200–220 g, 7 weeks old) were anesthetized with sodium pentobarbital (30 mg/kg i.p.). The jejunum was isolated and washed with ice-cold isotonic phosphate buffer (pH 6.4). The jejunum segment (5 cm length) was placed on an inclined polyethylene support, the angle of which was kept at 30° from the horizontal position, according to the mucoad-

hesion test by Rao and Anovel (1989). Briefly, isotonic phosphate buffer (pH 6.4) containing 0.025% FD-40 and 0.1% CP, FNaCP or lactose was perfused in the jejunal mucosa (3.75 cm^2 surface area) by the single-pass method (0.5 ml/min) for 30 min. After the mucosa was homogenized and then centrifuged at $1000 \times g$, the adhesive amount of FD-40 was assessed by its fluorescence intensity remaining in the precipitate.

2.5. In vitro electrophysiology and membrane permeation study

The detailed method was previously reported (Tomita et al., 1992). Briefly, the jejunal or colonic mucosa of male Wistar rats (200–220 g, 7 weeks old), which was stripped of underlying muscle, was mounted as a flat sheet in the Ussing-type chamber. As the mucosal and serosal solutions, Ringer solution was used. Electrical potential differences across the jejunal mucosa were measured under the constant electric current (0.1 mA), and the transepithelial electrical resistances were determined by Ohm's law. To the mucosal solutions, 0.1% FD-4 was added in the presence or absence of CP. The apparent permeation clearance was obtained from the permeation rate of FD-4 from the mucosal to the serosal side divided by its initial mucosal concentration.

2.6. Inhibitory effect of CP or FNaCP on the activity of proteolytic enzymes

Small intestinal fluid and BBMV of rats were prepared as described previously (Asada et al., 1994; Kessler et al., 1978). Briefly, for the preparation of small intestinal fluid, the small intestine was perfused with isotonic phosphate buffer (pH 6.4) from the duodenum to the ileum by the single-pass method (1 ml/min) for 50 min, and then the perfused solution was centrifuged. The protein concentration of the supernatant was adjusted to 5 mg protein/ml with the above buffer. BBMV were prepared by the $CaCl_2$ precipitation method (Kessler et al., 1978). The final protein concentration of the purified BBMV was adjusted to 5 mg protein/ml with the above buffer. The degradation of h-insulin was examined by the incubation of h-insulin (50 μmol/ml) in the small intestinal fluid or in the BBMV solution in the presence or absence of CP or FNaCP. The concentration of h-insulin remaining in the reaction medium was determined by HPLC (Asada et al., 1994).

2.7. Statistical analysis

Student's *t*-test was used to examine the significance of the effect of FNaCP or CP. Differences were considered significant at the 5% level.

3. Results

The time courses of the decrease in the plasma glucose level following the administration of the three kinds of capsules containing h-insulin to diabetic rats are shown in Fig. 1. A significant decrease in the blood glucose level compared to the placebo group was observed continuously for 12 h only in the FNaCP capsule group, but not in the CP capsule group. In the lactose capsule, the blood glucose level tended to decrease at 8 and 10 h, but the level did not significantly differ from that of the placebo group. With respect to the degree of decrease in the plasma glucose level, the FNaCP capsules were the most effective of the three kinds of capsules.

Fig. 2 shows the ability of 0.1% CP and FNaCP to increase the adhesion of FD-40 on intestinal mucosa. This concentration was found to be effective for the increase in the membrane permeation of FD-40 and insulin in situ (data not shown). FD-40 was used as a model compound because this macromolecular compound is water-soluble and has a molecular weight similar to that of insulin which exists in hexamer in the neutral solution. Both CP and FNaCP increased the adhesive amount of FD-40, and a significant difference between these polymers was not observed.

Fig. 3 illustrates the effects of CP on membrance resistance and the permeation clearance of FD-4 across the jejunal membrane. CP at the

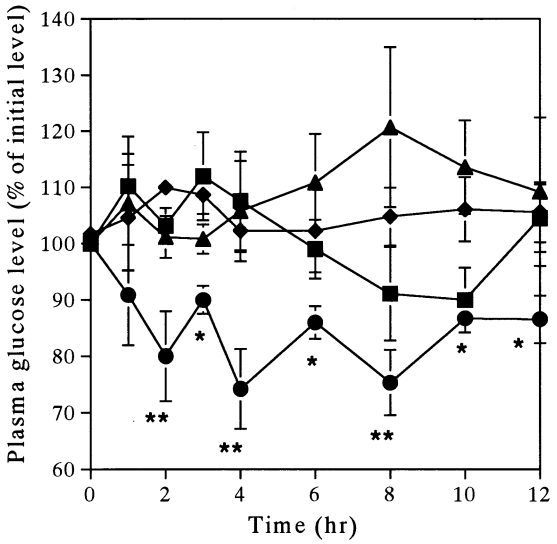

Fig. 1. Time course of the decrease in the plasma glucose level following the oral administration of h-insulin capsules to diabetic rats. ●, FNaCP; ▲, CP; ■, lactose; ◆, Placebo. Values represent the mean ± S.E. ($n = 3$–4). ** $P < 0.01$ versus Placebo, * $0.01 < P < 0.05$ versus Placebo.

concentrations of 1 and 5% did not decrease the membrane resistance and did not increase the permeation clearance of FD-4, which is considered to permeate mainly through the paracellular route (Hosoya et al., 1993).

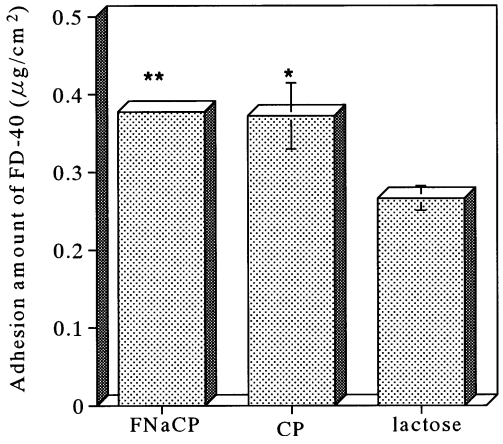

Fig. 2. Adhesion amount of FD-40 on the intestinal mucosa following the perfusion of FD-40 and 0.1% CP, FNaCP or lactose in rat jejunal mucosa. Values represent mean ± S.D. ($n = 3$). ** $P < 0.01$ versus lactose, * $0.01 < P < 0.05$ versus lactose.

The effects of 0.5% CP and FNaCP on the stability of h-insulin in the small intestinal fluid and the BBMV solution are shown in Figs. 4 and 5, respectively. As shown in the control, h-insulin was more stable in the BBMV solution than in the small intestinal fluid, but CP and FNaCP significantly inhibited the proteolysis of h-insulin in both reaction media at the concentration of 0.5%. Assuming that the proteolysis occurs by a first-order reaction, the decomposition rate constant is obtained as a slope of the linear line from the semilogarithmic plot of the remaining insulin concentration in the reaction medium versus time. The rate constants, which were obtained only in the intestinal fluid where the fast reaction was observed, were 0.09 min^{-1} in the control and 0.02–0.03 min^{-1} in the presence of FNaCP or CP. Accordingly, the inhibitory effect of FNaCP was almost equal to that of CP, and increased the stability of insulin against enzymatic degradation by 3- to 4-fold.

4. Discussion

We already reported that FNaCP disperses in medium faster than CP and improves the release rate of insulin from CP capsules (Nakanishi et al., 1998). Regarding the mechanism, it was shown that CP forms a swollen gel layer as a drug release barrier, whereas FNaCP disperses without forming such a barrier. Such differences in the effects of these two polymers on the dissolution rate may affect the pharmacological effects of insulin. In the present study, only when the FNaCP capsule containing insulin was orally administered to diabetic rats, a decrease in the plasma glucose level was observed 2 h following the administration, and the decreasing effect continued for 12 h (Fig. 1). This effect was not found in the case of the CP capsule. The appearance time of the pharmacological effect of insulin from the capsule coincided with the results of the transit study of the CP capsule by Harris et al. (1990), suggesting that the absorption site of insulin from the capsule is the upper part of the small intestine.

The mucoadhesion force of CP depends on the hydrogen bonding between the carboxy group in

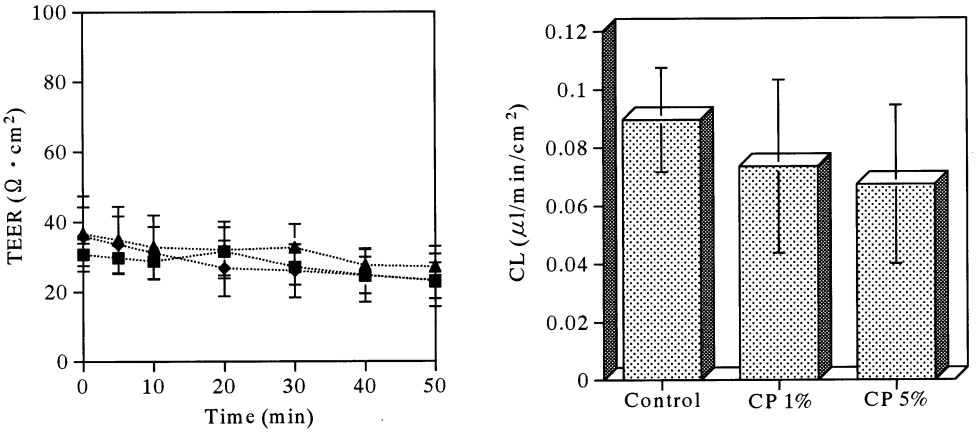

Fig. 3. Effects of 1 and 5% CP on membrane resistance (transepithelial electric resistance, TEER) and permeation clearance (CL) of FD-4 in rat jejunal membrane. ▲, CP 1%; ■, CP 5%; ◆,Control. Values represent mean ± S.D. ($n = 3–5$).

the polymer and the mucus. Although the mucoadhesion force of FNaCP is suspected to be weaker than that of CP due to the screening effect of the carboxyl group by Na^+ ions, there was no difference between FNaCP and CP in the adhesion of FD-40 on the intestinal mucosa (Fig. 2). Accordingly, this adhesive effect may be affected by the interaction of FD-40 and the polymer. Experimental conditions such as the perfusion rate, the polymer concentration and the viability of the mucosa are also thought to affect the adhesion results. Harris et al. (1990) showed that CP in the solution had a delaying effect on the GI transit time, due to the mucoadhesion, but no effect was reported for the solid formulation. The above significant effect of FNaCP from the solid formulation in vivo and in vitro is thus a major finding of the present study.

Neither a decrease in the membrane resistance nor an increase in the permeability of FD-4 was produced in the jejunum by CP, even at the concentration of 5% (Fig. 3). Thus, the effect of

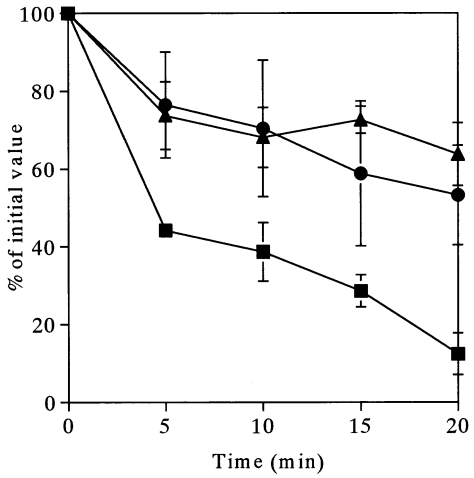

Fig. 4. Degradation of h-insulin in rat small intestinal fluid in the presence or absenceof 0.5% CP and FNaCP. ●, FNaCP; ▲, CP; ■, control. Values represent mean ± S.D. ($n = 3$).

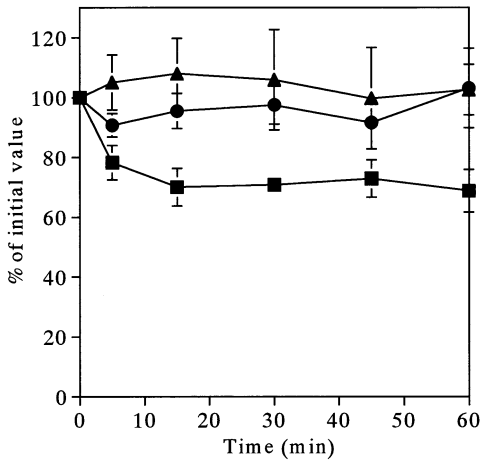

Fig. 5. Degradation of h-insulin in rat BBMV solution in the presence or absence of 0.5% CP and FNaCP. ●, FNaCP; ▲, CP; ■,Control. Values represent mean ± S.D. ($n = 3$).

CP on the paracellular route, i.e., the opening of the tight junction, was ruled out. The effect of FNaCP on the paracellular route was not observed, in the same manner as that of CP (data not shown). Lueßen et al. (1994) reported a widening effect on the intercellular space by a pH-dependent calcium depletion by CP and the reduction in membrane resistance. The widening effect on the paracellular routes was found at pH 4, but not at pH 7.4. Our results obtained at pH 6.4 are not contradictory with the above results.

Since we previously found that FNaCP capsules degrade and release enclosed drugs rapidly in the first and second fluids of the 13th Pharmacopeia of Japan (Nakanishi et al., 1998), in the present study we examined the inhibitory effects of FNaCP in solution on the proteolysis of insulin. The inhibitory effect of CP on the degradation of *N*-α-benzoyl-l-arginine ethyl ester (BAEE) by trypsin was reported to be greater than that of FNaCP (Akiyama et al., 1996). For the degradation of h-insulin in the rat small intestinal fluid and BBMV solution, however, the inhibitory effects of CP and FNaCP were almost the same (Figs. 4 and 5). The degradation rate constant of h-insulin was reduced by three to four times compared to the control in the intestinal fluid. Regarding the inhibitory mechanism of CP on the insulin degradation in the BBMV solution, the membrane destruction by CP was ruled out (Lueßen et al., 1996). The difference between the above finding by Akiyama et al. and ours may be due to the use of different substrates. In additional support of our result, our investigation on the degradation of insulin by trypsin and α-chymotrypsin showed that the inhibitory effects of CP and FNaCP were almost the same (data not shown). By these results, CP and FNaCP in solution were shown to be useful for enabling the intestinal absorption of insulin to the almost same extent, from the stand point of improving the stability against enzymatic degadation.

5. Conclusion

FNaCP was found to be useful as an adjuvant for peptide drug absorption from a solid formulation. FNaCP significantly improved the small intestinal absorption of insulin after the oral administration of a capsule, but CP did not. The effects of FNaCP were due to the adhesion on the intestinal mucosa and the inhibition of the enzymatic degradation of insulin. Since the above adhesive effect and enzymatic inhibitory effect of FNaCP and CP in the solution were almost the same, the difference between these two polymers is thought to be due to the rapid dissolution of insulin observed in the FNaCP capsule but not in the CP capsule, as shown in our previous study (Nakanishi et al., 1998).

Acknowledgements

The authors thank Yuichiro Imamura for his technical assistance.

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