International Journal of Pharmaceutics, 30 (1986) 95–99
Elsevier

IJP 01000

Targeted enteral delivery of insulin to rats

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(Received November 24th, 1985)
(Accepted December 6th, 1985)

Key words: insulin – polyacrylic polymer-coated gelatin capsules – enteral delivery of insulin – targeted administration – soft gelatin capsules

Summary

The aim of the present work was to investigate the effectiveness of a dosage form approach for monitoring both the inactivation and the absorption processes by targeting insulin delivery to the colon. The dosage form design is based on incorporating insulin into small, soft gelatin capsules coated with polyacrylic polymer (Eudragit) having pH-dependent properties. The capsules were filled with 100 mg of the following formulation: 8 units (u) porcine insulin and 20 mg of surfactant mixture (sodium laurate: cetyl alcohol 2 : 8) in arachis oil, and were coated with mixtures of various ratios of Eudragit RS, L and S. The in vitro pH-dependent release rates of coated capsules were tested by scintillation counting using [^{125}I]insulin and two formulations which released the drug the most between pHs 7.5 and 8.0 (RS1 and RS2) were chosen for further studies in rats. Insulin absorption was measured by its hypoglycemic effect. Blood glucose concentrations were determined at 610 nm using the GOD-Perid method. The oral administration of the two chosen insulin-containing formulations gave significant ($P < 0.01$) hypoglycemia when compared with controls. However, the duration, course and the intensity of effect were different for each formulation: the longest effect was obtained with formulation RS1 while the maximum glucose level reduction (up to 45% of initial value) occurred with formulation RS2. It was interesting to observe that the preadministration of a surfactant capsule did not change the glycemic profile; however, its post-administration prolonged the effect of RS2 by one hour.

Introduction

The poor compliance of diabetic patients to parenteral insulin therapy contributes to the continuing search for effective alternate routes of administration (Touitou et al., 1978, 1980; Touitou and Donbrow, 1983; Nishihata et al., 1981; Ritschel and Ritschel, 1984). Two main insulin properties impair its oral effectiveness: (1) being a pancreatic hormone peptide it is subject to proteolytic inactivation during its passage through the gastrointestinal tract, mainly in the upper region; and (2) it has a high tendency of self-association into high molecular weight oligomers (the MW of the monomer is 6000). Thus, practically, the extent of biologically active insulin transported by passive diffusion through the enteral membranes is insufficient to achieve therapeutic effects. In a previous investigation (Touitou et al., 1980), we have shown that significant hypoglycemia was induced in rats when insulin was injected intrajejunally in the presence of a non-ionic surfactant, $\text{Cetamacrogol}_{1000}$, as an enhancing absorption agent. The results of this previous work indicated

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that progress towards oral insulin therapy may be achieved by interfering with the processes of drug inactivation and absorption.

The aim of the present study was to investigate the feasibility of an oral dosage form approach for monitoring these processes, inactivation and absorption, by targeting the delivery of insulin in an enhancing absorption formulation to the colon where the proteolysis is relatively low.

Materials and Methods

Materials

The drugs and additives used for the dosage form formulation were: porcine insulin Leo Neutral 100 $u \cdot ml^{-1}$ (Nordisk Gentofte, Denmark) and [^{125}I]porcine insulin (NEN) with a spec. act. of 99 $\mu Ci \cdot \mu g^{-1}$ and a radiochemical purity of > 98%. Sodium laurate and cetyl alcohol (Sigma) were "chemically pure" substances and arachis oil conformed to the B.P. requirements.

Dosage form design

The oral dosage form design is based on the incorporation of an insulin formulation into small (5/7 mm) soft gelatin capsules coated with polyacrylic polymer — Eudragit (Rohm Pharma, F.R.G.) — having pH-dependent solubility properties. The soft capsules were filled with various compositions according to their use during the experiment. The compositions are presented in Table 1. Organic solvent solutions of Eudragit RS, L and S at various ratios (Touitou and Rubinstein, 1985) were used to coat the capsules.

TABLE 1

COMPOSITION FOR SOFT GELATIN CAPSULES

Materials	Caps Ins. *	Caps Ins. **	Caps. Surf. **
Porcine insulin	8 u	8 u	–
[^{125}I]insulin (porcine)	5 μCi	–	–
Sodium laurate	4 mg	4 mg	4 mg
Cetyl alcohol	16 mg	16 mg	16 mg
Arachis oil to:	100 mg	100 mg	100 mg

* Tested in vitro.

** Administered in vivo.

TABLE 2

No. of rats	No. of caps. administered per rat		
	Caps. Ins. (RS1)	Caps. Ins. (RS2)	Caps. Surf. (RS2)
5	2	–	–
5	–	2	–
5	–	2	1 *
5	–	2	1 **
4	–	–	2

Given 30 min * after ** before insulin capsules' administration.

In vitro insulin release measurements

The coating effecteness was tested in vitro using the USP disintegration apparatus (USP XIX, 1975). The release media used were artificial gastric juice (60 ml 1 N $HCl \cdot l^{-1}$) and buffer phosphate solutions pHs 6.0, 6.5, 7.0, 7.5 and 8.0. In each experiment six capsules were tested for 1 h in gastric juice then briefly rinsed with distilled water and transferred to the phosphate buffer solution.

The in vitro pH-dependent release course was tested by scintillation counting using [^{125}I]insulin diluted with cold insulin (Table 1), the USP dissolution basket and 400 ml phosphate buffer solution. Each value given is the mean of 3 experiments.

Animal experimental design

Hebrew University strain male rats (270 g) were starved for 20 h before the experiment. During the experiment the rats received water ad libitum. The capsules were administered to the rats according to the study design presented in Table 2. The absorption of the intact insulin was evaluated measuring the hypoglycemic effect. Blood was collected from the rats' tails immediately before capsule administration and at 0.5, 1, 2, 3, 4 and 6 h afterwards. The rats were ether-anesthesized during blood collection. Blood glucose concentrations were determined at 610 nm using the GOD-Perid method (Boehringer, F.R.G.).

Results and Discussion

The results obtained by direct injection of insulin into selected regions of the gastro-intestinal lumen (Touitou et al., 1980; Crane et al., 1968) suggested that it would be interesting to investigate the effectiveness of oral dosage forms designed to deliver insulin in the presence of an absorption promoter in that part of the intestine where the proteolysis is relatively low. The rationale of choosing gelatin capsules as dosage forms is based on the wide formulation possibilities offered by this form: (1) incorporation of oily compositions in which insulin and promoter are molecularly dispersed; and (2) coating for targeting the drug release into the colon.

The formulation presented in this report was selected from a number of compositions screened for the effects of: chain length (C_{10}–C_{16}) of the anionic surfactant, composition of the mixed emulsifiers (Touitou and Rubinstein, 1985) and viscosity. The capsules were coated with mixtures of various ratios of Eudragit RS, S and L (Table 3) and tested for disintegration and insulin release properties by a procedure described in the Methods section. Some of the relevant release profiles are presented in Figs. 1,2.

Fig 1 shows the time release course at pHs 7.5 and 8.0 of two formulations, RS1 and RS2, selected to be orally administered to rats. The drug percent released was estimated from the [^{125}I]insulin counted by scintillation. It can be observed that the time required for 95% of the drug to be released is relatively short, 15–40 min, and depends on coating and pH. Although for both formulations the time is shorter at pH 8.0 than at pH 7.5, the rate of release from RS1 is much slower than from RS2; thus, the percent released in the first 15 min was 95% versus 53% for RS2 and RS1, respectively. A lag time of 2 min could be detected at pH 8.0; whereas at pH 7.5, the release process was instantaneous. These release properties of RS1 and RS2 are convenient for the colon content milieu. Moreover, their choice was based on the release behavior in a wide pH range (6–8) as presented in Fig. 2. The pH-dependent release courses indicate that formulations RS1 and

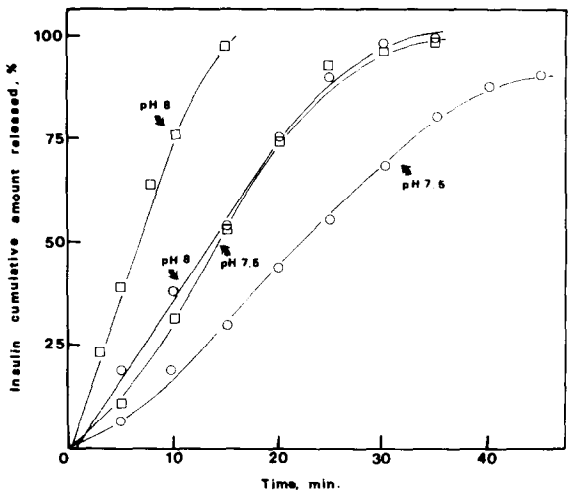

Fig. 1. Release profiles of insulin from capsules coated with Eudragit mixtures tested at pH 7.5 and pH 8. Formulations: ○, RS1; □, RS2.

TABLE 3

THE EUDRAGIT RS, S AND L RATIOS USED FOR COATING THE CAPSULES *

Formulation	Eudragit		
	RS	S	L
RS	2	–	8
RS1	4	6	–
RS2	2	2	6
RS3	1	–	9
LS	–	7	3

* Solvents: acetone and isopropyl alcohol.

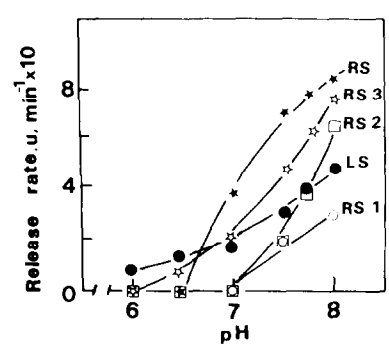

Fig. 2. Effect of pH on the release rate of insulin from soft capsules coated with various mixtures of Eudragit S, L and RS.

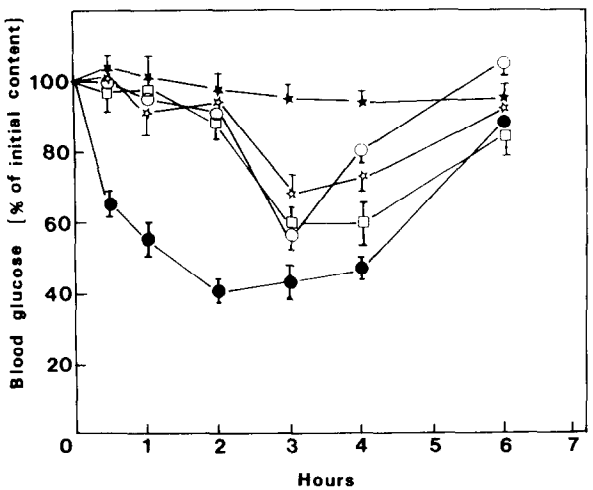

Fig. 3. Hypoglycemic effect of insulin administered orally to normal rats by means of coated soft capsules containing an absorption enhancing formulation (for formulations see Tables 1 and 2): ☆, 2 capsules RS1; ○, 2 capsules RS2; □, 2 capsules RS2 + 1 capsule. Surfactant post-insulin administration: ●, insulin i.p. 4 u; ★, 2 capsules surfactant (no insulin). Each point is the mean ± S.D. of 5 animals for insulin administration and of 4 animals for controls.

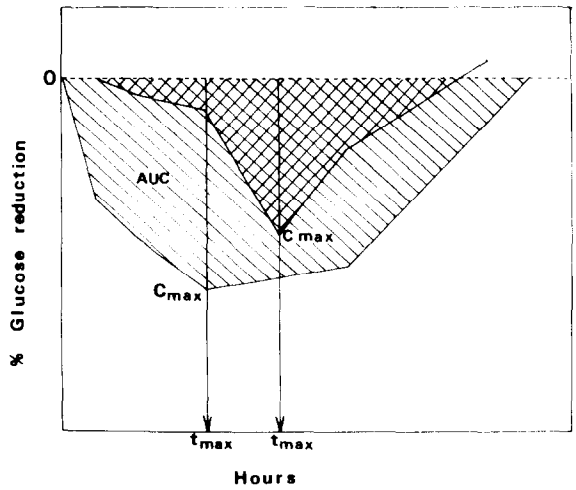

Fig. 4. Area under the curve (AUC) of the % blood glucose reduction versus time profile upon oral administration of 16 u insulin in coated capsules (RS2), as compared with intraperitoneal administration of 4 u insulin.

RS2 do not release detectable amounts of insulin at pHs lower than 7. The other formulations tested, RS, RS3 and LS, released considerable amounts of drug at pHs 6.5 and 7.0 corresponding to upper-intestinal regions. These formulations have been considered unsuitable for our purpose even though their release rate at pHs 7.5 and 8.0 were higher than that of the chosen formulations RS1 and RS2 (Fig. 2).

The selected capsules have been administered to rats following the protocol presented in Table 2, and the results were compared with those obtained by intra-peritoneal administration of 4 u neutral insulin.

The mean of the blood glucose concentration of the samples prior to dosage administration was used as a baseline for plotting the response vs time curves. Fig. 3 presents the changes in blood glucose concentration that occurred after oral and intra-peritoneal treatment. It is interesting to note the lag time of 2 h that occurred for each insulin oral regime tested. The effect of RS2 is higher (45% reduction in glycemia) but shorter (it lasted for about 1 h) than RS1.

It was suggested that one of the causes of the short duration of enteral administration of insulin with promoter may reside in a difference in the

TABLE 4

SOME PHARMACOKINETIC PARAMETERS RELATED TO THE HYPOGLYCEMIC EFFECT IN RATS OF INSULIN UPON ORAL ADMINISTRATION OF SOFT CAPSULES COATED WITH EUDRAGIT

Treatment	Loading dose (u)	Dose ($u \cdot kg^{-1}$)	AUC (% gluc. reduc. · h)	C_{max} (% gluc. reduc.)	t_{max} (h)	f * (%)
i.p.	4	15	258	58	2	100.0
p.o. RS1	16	59	110	45	3	10.6
RS2	16	59	96	32	3	9.3
RS2 + Surf.	16	59	131	42	3	12.7

* Obtained from Eqn. 1 (see text).

absorption rate, from the intestinal tract, of insulin and promoter. To test this hypothesis, capsules containing only the surfactant were administered, in one trial before and in one trial after insulin administration. No change was observed by pre-treatment. However, the surfactant given 30 min post-insulin oral treatment extended the duration of RS2 by about 1 h, improving the drug bioavailability. Similar results have been obtained by Nishihata et al. (1985), who reported that post-administration of promoter (enamine) in rectal dosage of insulin in dogs improved the bioavailability from 19.4% to 38.2%.

Pharmacological availability, f, (Ritschel and Ritschel, 1984) and other pharmacokinetic parameters were obtained from the curves of percent glucose reduction versus time and from Eqn. 1:

$$f = \frac{AUC_{0\text{-}6\ oral}}{AUC_{0\text{-}6\ i.p}} \times \frac{\left(\frac{weight}{dose}\right)_{i.p.}}{\left(\frac{weight}{dose}\right)_{oral}} \qquad (1)$$

The area under the curve (AUC), the maximum glucose reduction (C_{max}) and the time of the maximum effect (t_{max}) were estimated from the plots presented in Figs. 3 and 4. Their values are given in Table 4. The schematic comparison (Fig. 4) of the AUC of orally administered insulin (RS2) and intra-peritoneally administered insulin clearly indicated that the oral preparation is effective (C_{max} = 45% glucose reduction); however its relative bioavailability is still low. This is supported further by the values of f, which are in the range of 9.3% to 12.7%. The C_{max} and the prolongation effect obtained with formulation RS2 and the post-administration of promoter are conceptually of interest.

Current work is being undertaken in our laboratories to improve the relative bioavailability of these dosage forms.

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