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International Journal of Pharmaceutics 237 (2002) 71–76

international journal of pharmaceutics

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Oral delivery of insulin from enteric-coated capsules containing sodium salicylate: effect on relative hypoglycemia of diabetic beagle dogs

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Received 25 June 2001; received in revised form 15 October 2001; accepted 16 January 2002

Abstract

The hypoglycemic effect of Eudragit S100 enteric-coated capsules containing sodium salicylate as an absorption promoter formulated with insulin in various ways: as physical mixture, by wet granulation or in suppository bases (polyethylene glycol 4000 or Witepsol W35) was studied in hyperglycemic beagle dogs. The capsules containing insulin formulated with sodium salicylate (50 mg) and prepared by either physical mixing or wet granulation using 10% polyvinyl pyrollidone gave almost the same results producing a maximum reduction in plasma glucose level (C_{max}) of 81.53 ± 8.21 and $79.59 \pm 5.75\%$, T_{max} of 6 and 5 h, area under the curve (AUC) of 69.37 ± 48.64 and $57.98 \pm 23.15\%$ reduction hour (% red. h) and resulting in relative hypoglycemia (RH) of 8.73 ± 6.12 and $7.29 \pm 2.91\%$, respectively. Formulation of insulin with sodium salicylate in PEG 4000 produced a lower AUC of $37.30 \pm 10.36\%$ red. h and RH of $4.69 \pm 1.3\%$. While, formulation in Witepsol W35 (0.5, 1.0 and 2.0 g) that was sieved to produce particle size of 180–315 μm and filled in enteric-coated capsules showed that formulating insulin and sodium salicylate in 1 g base is the best formulation. It produced 25% reduction in plasma glucose levels of the hyperglycemic beagle dogs at T_{max} of 4 h and the largest AUC of $100.10 \pm 25.72\%$ red. h, resulting in the highest RH of $12.59 \pm 3.23\%$. In conclusion, 25–30% reduction in plasma glucose levels and RH of about 12.5% relative to subcutaneous injection of regular soluble insulin can be achieved by formulating insulin in Witepsol W35 (1 g) with sodium salicylate (50 mg) as an absorption promoter, reducing the resulting mass into particle size 180–315 μm, packing into hard gelatin capsules and coating with Eudragit S100.

Keywords: Oral insulin capsules; Sodium salicylate; Diabetic beagle dogs

1. Introduction

The delivery of insulin by the non-parental routes has gained significant attention over the last 2 decades. There are several limitations of

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0378-5173/02/$ - see front matter
PII: S0378-5173(02)00024-8

traditional routes of insulin delivery. These limitations includes low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity (Paulsen et al., 1979; Freidenberg et al., 1980; Maberly et al., 1982). A large number of attempts have been made to deliver insulin orally using enteric-coated capsules (Hosny et al., 1995, 1997, 1998a), protected in liposomes (Choudhari et al., 1994), in cyclodextrin (Shao et al., 1994), in erythrocytes (Bird et al., 1983) and in conjunction with absorption promoters (Hosny et al., 1995, 1997, 1998a). Sodium salicylate has been used before in our studies for oral (Hosny et al., 1995, 1998a; Hosny, 1999) and rectal (Hosny et al., 1994, 1998b) delivery of insulin and produced significant hypoglycemic response in diabetic rats and rabbits. The aim of the present study is to formulate insulin mixed with sodium salicylate in various ways (physical mixing, wet granulation using 10% polyvinyl pyrollidone (PVP) and in suppository bases PEG 4000 and Witepsol W35) in enteric-coated capsules. Also, study the effect of these capsules upon the relative hypoglycemic effect in diabetic beagle dogs in comparison to that produced after subcutaneous (SC) injection of regular insulin.

2. Materials and methods

2.1. Materials

Alloxan monohydrate was purchased from Winlab (Middlesex, Wilfrid Smith limited, UK), Crystalline insulin HM (ge) was a generous gift from Novo Nordisk A/S (Novo Alle, 2880 Bagsvaerd, Denmark). Eudragit S100 was from Rhome Pharma (GmbH, Darmstadt, Germany). Acetone and sodium salicylate, were from BDH Chemicals, Ltd (Poole, England). Witepsol W35 from Dynamit Nobel (Northvale, NJ). PEG 4000 was from Sigma Chemical Co. (St. Louis, MO). Glucose GOD-PAP, Randox was from Randox Laboratories Limited (Antrim, UK).

2.2. Methods

2.2.1. Induction of hyperglycemia

Male beagle dogs weighing between 9.5 and 16.5 kg were overnight food-deprived and rendered diabetic with an intravenous injection of a cocktail containing alloxan and streptozotocin (70 mg/kg, in a ratio of 1:1) dissolved in sterile normal saline. This cocktail was injected in two occasions. On the first day, as a dose of 40 mg/kg, and 2 days later, as 30 mg/kg. Daily SC injections of 2 U/kg of regular insulin and 1 U/kg of NPH insulin (Eli Lilly and Company, Indianapolis, IN) managed the diabetic dogs.

2.2.2. Subcutaneous injection of insulin

Regular human insulin injection, USP (100 U/ml) was injected subcutaneously as 3 U of insulin per each kg of the body weight to hyperglycemic overnight food-deprived beagle dogs.

2.2.3. Preparation of insulin capsules

The oral dosage form design is based on the incorporation into hard gelatin capsules of insulin granules obtained by formulation with sodium salicylate by physical mixing, wet granulation (using 10% PVP) or in suppository bases PEG 4000 or Witepsol W35. The capsules were coated in a coating pan rotated at 50 rpm by spraying with a 10% solution of Eudragit S100 in acetone. Eudragit S100 is an anionic polymer synthesized from methacrylic acid and methacrylic acid methyl ester and has a pH-dependent solubility. It is insoluble in buffer solutions below pH 6 and also in gastric juices. It is slowly soluble in the region of the digestive tract where juices are neutral to weakly alkaline (Rhom Pharma, 1982; Mehta et al., 1986; Li et al., 1991). When used as a film to coat insulin capsules, it is expected to protect the insulin from degradation by the gastric juice and allows insulin to be released in the region of the GIT of pH >7 where proteolytic enzymes are in low concentrations.

2.2.4. Capsule administration

Eudragit S100 enteric-coated insulin capsules each containing the required amount of insulin were administered orally to fasted hyperglycemic

dogs by placing the capsule at the end of the mouth and flushing it down the dog's throat by 50 ml of water.

2.2.5. Blood sampling

Over a 6-h period, blood samples (1 ml each) were collected into heparinized tubes for plasma glucose measurement. Samples were collected before and every hour after oral or SC administration via a disposable I.V. cannula (20G-O.D. 1×32 mm luer lock, Casnate Co., Italy) inserted into the cephalic vein. Blood samples were immediately centrifuged and aliquots of plasma aspirated and stored at 4 °C for subsequent glucose measurement at the end of the experiment.

2.2.6. Plasma glucose measurement

Plasma (10 μl) was added to 1 ml glucose reagent (GOD-PAP). After vortexing for 10 s, the tubes were incubated for 25 min at room temperature. The absorbance of the standard and plasma glucose samples was measured within 60 min against reagent blank at 500 nm using Spectronic 21D Spectrophotometer (Milton Roy, Rochester, NY). The plasma glucose concentration was calculated as milligrams per deciliter (mg/dl) using the equation:

$$\text{Glucose concentration} = (A_{\text{sample}}/A_{\text{standard}}) \times 100$$

where A, is the absorbance.

2.2.7. Calculations of the hypoglycemic effect

The maximum reduction in plasma glucose concentration ($C_{\max}$) was obtained from the plasma glucose concentration–time curves (% change of initial) of each dog using the equation:

$$\%\text{change} = [(F - P_t)/F] \times 100$$

where, F is the fasting plasma glucose level. P_t is the plasma glucose level at time (t) after oral administration of the capsules.

The time to reach this reduction ($T_{\max}$) was obtained from the mean data. The area under % glucose reduction–time profile ($AUC_{0-6\ h}$) was determined using the linear trapezoidal rule. The relative hypoglycemia (RH) of insulin formulations was calculated by comparing their AUC's relative to that after SC injections and taking dose differences in consideration. All the data are expressed as mean ± S.D.

2.2.8. Statistical analysis

Plasma glucose levels (0–6 h) after oral capsules administration or SC injection of insulin were compared in each group with the respective initial values using repeated measures analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. Absolute differences between groups in $C_{\max}$, AUC and RH were carried out using one way ANOVA followed by Tukey–Kramer multiple comparison test. These statistical calculations were performed using the Graph Pad Instat computer program (1990–1993; Graph Pad Software, V2.04, San Diego, CA).

3. Results and discussion

Fig. 1 shows the effect of SC insulin injection (3 U/kg) and the oral administration of enteric-coated insulin (6 U/kg) capsules containing 50 mg sodium salicylate and the calculated equivalent of insulin mixed in various ways: as physical mixture or physical mixture wetted by few drops of 10% PVP or prepared using suppository bases e.g. PEG 4000 and Witepsol W35, on the plasma

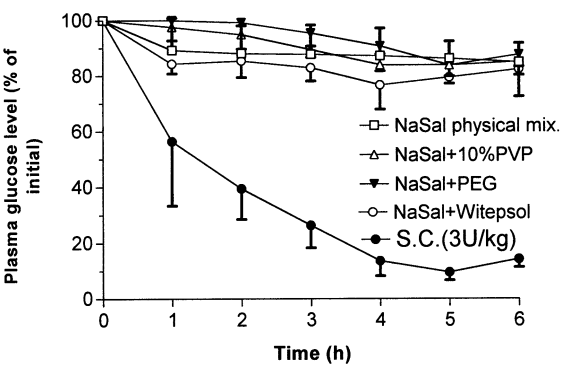

Fig. 1. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after subcutaneous insulin injection (SC, 3 U/kg) and oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg sodium salicylate (NaSal) and prepared as granules by physical mixing, wet granulation, mixed with 1 g polyethylene glycol 4000 (PEG) or mixed with 1 g Witepsol W35.

Table 1
Pharmacodynamic parameters: C_{max}, T_{max}, AUC and RH of six hyperglycemic beagle dogs after subcutaneous insulin injection (SC, 3 U/kg) and oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg sodium salicylate (NaSal) and prepared as granules by physical mixing, wet granulation with 10% PVP, mixed with 1 g polyethylene glycol 4000 or mixed with 1 g Witepsol W35

Pharmacodynamic parameters	SC (3 U/kg)	NaSal (50 mg) physical mix	NaSal + 10% PVP (wet granulation) (180–315 μm)	NaSal + PEG 4000 (pieces)	NaSal + Witepsol W35 (180–315 μm)
C_{max} (%)	13.1 ± 6.8	81.5 ± 8.2*	79.6 ± 5.8*	83.7 ± 0.4*	75.3 ± 6.3*
T_{max} (h)	5	6	5	5	4
AUC (% red. h)	379.5 ± 37.6	69.4 ± 48.6*	58.0 ± 23.2*	37.3 ± 10.4*	100.1 ± 5.7*,**
RH (%)	100	8.7 ± 6.1*	7.3 ± 2.9*	4.69 ± 1.30*	12.6 ± 3.2*,**

C_{max}: maximum plasma glucose reduction (% of initial), T_{max}: time to reach C_{max}, AUC: area under % glucose reduction–time curve, RH: relative hypoglycemia.
* $P < 0.001$ compared to that of SC injection.
** $P < 0.05$ compared to that of NaSal + PEG 4000 (pieces).

glucose level (% of initial). It should be mentioned at this point that capsules containing insulin alone or promoters without insulin gave no reduction of plasma glucose levels.

Table 1 shows that insulin capsules containing granules prepared by wet granulation with 10% PVP did not produce a significant ($P > 0.05$) change in C_{max}, AUC or RH compared to those produced by capsules containing the physical mixture. It, however significantly ($P < 0.01$) reduced the initial plasma glucose levels during 4–6 h post administration (Fig. 1).

When insulin and sodium salicylate were formulated in PEG 4000, a significant ($P < 0.001$) reduction in plasma glucose levels from the initial value was reached by 4 h and continued to 6 h. There was however, no improvement in AUC and RH compared to that produced after oral administration of insulin enteric-coated capsules containing the physical mixture or granules prepared by wet granulation.

On formulation of insulin and sodium salicylate in Witepsol W35, a significant ($P < 0.01$) reduction in plasma glucose levels was reached by 1 h. This reduction continued to the end of the experiment producing a C_{max} of $75.3 \pm 6.3\%$ by 4 h and an AUC of $100 \pm 25.7\%$ red. h resulting in RH of $12.6 \pm 3.2\%$. These values of AUC and RH are significantly ($P < 0.05$) higher than those produced by capsules containing sodium salicylate and PEG 4000.

To study the optimum amount of Witepsol W35 in which incorporation of insulin and sodium salicylate would produce the maximum reduction in plasma glucose levels, 0.5, 1 and 2 g base were used in the formulations.

The results in Table 2 and Fig. 2 show that the optimum amount of Witepsol W35 base to be used is 1 g where increasing the amount of the base resulted in a significant reduction ($P < 0.05$) in C_{max}, AUC and RH. Decreasing the amount of Witepsol resulted, also in a marked reduction in C_{max} ($P < 0.001$), AUC and RH ($P < 0.01$) from values of $68.4 \pm 4.0\%$, $97.3 \pm 23.8\%$ red. h and $12.2 \pm 3.0\%$ to $88.2 \pm 2.0\%$, $48.6 \pm 6.0\%$ red. h and $6.11 \pm 0.76\%$, respectively.

In this work the sodium salicylate was chosen to be included in insulin capsules with Witepsol W35 base as this additive in insulin suppositories produced a RH of 50–55% compared to SC insulin injection but the results here were not as high as in rectal delivery formulations. This could be due to difference in enzyme content and activity between the upper GIT and the rectum, gastric emptying and the extent of first pass liver metabolism.

Salicylate was found to promote absorption by acting on both the apical cell membrane and the tight junctions between cells (Szabo et al., 1981). Salicylate may also act on protein components of plasma membranes, red blood cell membranes and small intestine brush border membranes (Nishihata et al., 1984; Kajii et al., 1986), the

Table 2
Pharmacodynamic parameters: C_{max}, T_{max}, AUC and RH of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg sodium salicylate (NaSal) and different amounts of Witepsol W35 base and sieved into particle size 180–315 μm

Time (h)	Plasma glucose levels mg% ± S.D.		
	NaSal+0.5 g Witepsol	NaSal+1 g Witepsol	NaSal+2 g Witepsol
C_{max} (%)	88.20 ± 1.96*,**	68.42 ± 4.02	76.40 ± 3.33*
T_{max} (h)	5	4	4
AUC (% red. h)	48.55 ± 6.04*	97.27 ± 23.81	59.55 ± 11.12*
RH (%)	6.11 ± 0.76*	12.23 ± 2.99	7.49 ± 1.4*

C_{max}: maximum plasma glucose reduction (% of initial), T_{max}: time to reach C_{max}, AUC: area under % glucose reduction–time curve, RH: relative hypoglycemia.
* $P<0.05$ compared to that of NaSal+1.0 g base.
** $P<0.01$ compared to that of NaSal+2.0 g base.

non-protein thiols (Susuka et al., 1988) which are believed to play an important role in maintaining cell integrity and in preventing uptake of hydrophilic compounds.

Nishihata et al. (1984) and Susuka et al. (1988) showed that salicylate decreased the levels of non-protein thiols in intestinal tissues and isolated enterocytes.

These results show that oral administration of enteric-coated insulin capsules can produce gradual and reproducible lowering in plasma glucose level. It can be concluded that insulin absorption might be accompanied by oral administration of a suitably designed product containing insulin and sodium salicylate provided that insulin is protected against degradation by a suitable coating during its passage to the absorption site. The studies presented here, indicate that problems of delivering insulin by the oral route can be minimized; although delivery by this route is not bio-equivalent to the parental routes, the convenience to the patient will, in this case outweigh the demand for complete bio-equivalence.

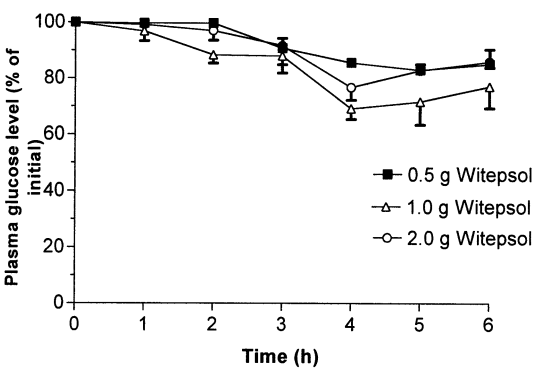

Fig. 2. Plasma glucose level (% of initial) of six hyperglycemic beagle dogs after oral administration of enteric-coated insulin capsules (6 U/kg) containing 50 mg sodium salicylate (NaSal) and different amounts of Witepsol W35 base and sieved into particle size 180–315 μm.

Acknowledgements

The investigators would like to acknowledge the financial support and the valuable suggestions of King Abdulaziz City for Science and Technology (KACST) to carry out this project through their grant No. AT/17/11. We are also grateful to Mr Abdullah A. Abdulrazig for his expert technical assistance throughout this work.

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