Drug Development and Industrial Pharmacy, 24(3), 307–311 (1998)

COMMUNICATION

Hypoglycemic Effect of Oral Insulin in Diabetic Rabbits Using pH-Dependent Coated Capsules Containing Sodium Salicylate Without and With Sodium Cholate

E. A. Hosny,[1,*] N. M. Khan Ghilzai,[2] T. A. Al-Najar,[2] and M. M. A. Elmazar[3]

[1]*Department of Pharmaceutics,* [2]*Department of Clinical Pharmacy, and* [3]*Department of Pharmacology, College of Pharmacy, King Saud University, P.O. Box: 2457, Riyadh 11451, Saudi Arabia*

ABSTRACT

The hypoglycemic effect of oral insulin (20 U and 40 U) capsules coated with a pH-dependent soluble polymer (Eudragit S100) and containing sodium salicylate (50 mg) without and with sodium cholate (50 mg) was studied in alloxan-hyperglycemic rabbits and compared with that of s.c. insulin injection (20 U). The capsules containing 20 U insulin + sodium salicylate (50 mg) produced a significant reduction in plasma glucose level to 82 and 73% of initial values at 2 and 3 hr after administration, respectively. The blood glucose level slowly returned to normal values at 5 hr. The $AUC_{0-5\ hr}$ *was 73.7 ± 43.5 mg · hr/dl compared to 242 ± 70.5 mg · hr/dl for insulin (20 U, s.c.) with a relative hypoglycemia of 30.4%. A higher dose of oral insulin (40 U) + sodium salicylate (50 mg) was more effective in reducing plasma glucose level which steadily decreased and reached 56% of the initial value by 5 hr (*$AUC_{0-5\ hr}$ *= 132 ± 41.5 mg · hr/dl and relative hypoglycemia = 27.3%). Sodium cholate (50 mg), however, slightly improved sodium salicylate effect producing an* $AUC_{0-5\ hr}$ *of 139 ± 37.3 mg · hr/dl with relative hypoglycemia of 28.7%. The relative hypoglycemia of pH-dependent coated capsules reached in the present experiment is the highest found so far.*

*To whom correspondence should be addressed.

INTRODUCTION

Several causes may be responsible for the incomplete bioavailability of oral insulin, e.g., resistance of the mucosal membrane to insulin penetration because of its large molecular size, which is a major factor limiting its diffusion across the biological membranes; susceptibility to breakdown by proteases in the luminal cavity and the cells lining the mucosa; and rapid clearance of the administered dose from site of deposition (1). All of these factors left little doubt that administration of insulin by mouth is deemed to be a failure. For the development of oral insulin, Loehry et al. (2) studied the permeability of small intestine to substances of high molecular weight. They found that intestinal permeability was inversely proportional to the molecular weight. The permeability of macromolecules could also be increased by using surfactants (3). Others had tried to protect oral insulin from proteolytic degradation by its inclusion within liposomes to be absorbed intestinally (4–8). However, the stability and effectiveness of insulin-containing liposomes have been found to be unpredictable. Cyclodexrins have also tried to enhance enteral absorption of insulin in the lower jejunal/upper ileal segments of rats by means of an in situ closed-loop method (9). The insulin bioavailability was increased from a negligible value (approximately 0.06%) to 5.63% using 10% dimethyl-β-cyclodextrin while 10% hydroxypropyl-β-cyclodextrin did not improve the enteral insulin uptake, giving bioavailability of only 0.07%. Soft (10) and hard (11) gelatin capsules coated with Eudragits with pH-dependent properties and containing in the soft capsules surfactant mixture and in the hard capsules sodium salicylate produced maximum glucose level reduction up to 45 and 64%, respectively.

The present study was designed to test the hypoglycemic effect of Eudragit S100 coated capsules containing insulin and sodium salicylate when given orally to alloxan-hyperglycemic rabbits and compared with insulin suspension given s.c. The influence of sodium cholate to enhance sodium salicylate effect was also studied.

MATERIALS AND METHODS

Materials

Alloxan monohydrate was purchased from Winlab (Wilfrid Smith Limited, Middlesex, UK). Crystalline insulin 23 U/mg was from Fluka Chemicals (Ag, CH-9470 Buchs, Switzerland). Eudragit S100 was from Rohm Pharma (GmbH, Darmstadt, Germany). Sodium salicylate was from BDH Chemicals Ltd. (Poole, England). Cholic acid-Na-salt was from Serva Feinbiochemica GmbH & Co. (Heidelberg, Germany). Glucose GOD-PAP, Randox was from Randox Laboratories Limited (Antrim, UK).

Induction of Hyperglycemia

Twenty-one overnight food-deprived male white rabbits of crossed strains of Chinchilla, France and Branco, France, bred in Experimental Animal Care Centre (College of Pharmacy, King Saud University, Riyadh, Saudi Arabia), weighing 4.09 ± 0.53 kg, were rendered hyperglycemic with a single intravenous injection of 8% solution of alloxan monohydrate in normal saline (60 mg/kg).

Capsule Design

Insulin 20 U or 40 U (23 U/mg) was mixed with sodium salicylate (50 mg). Insulin 40 U was also mixed with sodium salicylate and sodium cholate (50 mg each). The mixing was carried out by the geometric dilution method and the mixed powders were shaken for 5 min using a turbula mixer from Erweka-Apparatebau GmbH (Frankfurt, Germany). The equivalents of 20 or 40 U of insulin with the adjuvant(s) were filled into hard gelatin capsules (5/12 mm). The capsules were then coated by spraying with 10% solution of Eudragit S100 in acetone in a coating pan rotated at 50 rpm. The coated capsules were then air-dried. The intact nature of the film coating was confirmed by the release of its contents in vitro at pH 7.

Capsule Administration

Cage restrained hyperglycemic rabbits, food-deprived for 16 hr before the experiments, were given the Eudragit S100 coated capsules containing the equivalent of 20 or 40 U of insulin and the stated adjuvants amount by oral intubation. The capsule fitted in one end of a soft polyethylene cannula of a suitable internal diameter (3 mm) was passed into the rabbit stomach through a central hole in a gag which was used to press the rabbit's tongue and hold the jaws open. The capsule was pushed in the stomach by the help of a plunger lubricated with a thin film of paraffin liquid.

Subcutaneous Injection of Insulin

Insulin was suspended in sterile normal saline to prepare a solution of 10 U/ml. Two ml (20 U) was injected subcutaneously into each rabbit.

Blood Sampling

Blood samples (1 ml) were taken into heparinized tubes before and every 1 hr after capsule administration for 5 consecutive hr by inserting Terumo cannula (22 G × 1 in. i.d., 0.60 × 25 mm) in the central ear artery of the rabbit. The tubes were centrifuged immediately at 4000 rpm for 15 min at 4°C using a Beckman centrifuge (Model J-6B, Palo Alto, CA). The plasma was then aspirated and stored at −20°C pending analysis at the end of the experiment.

Plasma Glucose Measurement

Plasma (10 μl) was added to 1 ml glucose reagent (prepared by reconstituting the content of GOD-PAP reagent bottle with phosphate buffer). After tube contents were mixed, 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 the reagent blank at 500 nm using Spectronic 21D spectrophotometer (Milton Roy, Rochester, NY). The plasma glucose concentration was calculated as mg/dl.

Calculations of AUC and Relative Hypoglycemia

The area under the plasma glucose (expressed as percent reduction of the initial value)–time curve ($AUC_{0\text{-}5\ hr}$) following the oral administration of the Eudragit S100 coated insulin capsules containing the adjuvants and also after the s.c. injection of insulin suspension (20 U) in normal saline to food-deprived rabbits was calculated using the linear trapezoidal rule. The relative hypoglycemia of insulin oral capsules was determined by comparing their AUCs with those after s.c. injection.

Statistical Analysis

Plasma glucose level (1–5 hr after insulin administration) was compared in each group with the respective initial values using repeated measures analysis of variance followed by Bonferroni multiple comparisons test. Difference in percent reduction in plasma glucose levels between groups was carried out by unpaired Student's *t*-test and that of AUC by one-way analysis of variance followed by Dunnett's multiple comparisons test. Statistical calculations were performed by the GraphPad Instat Computer program (1990–1993; GraphPad Software, V 2.04, San Diego, CA).

RESULTS AND DISCUSSION

Subcutaneous administration of insulin suspension (20 U/rabbit) produced a progressive decrease in plasma glucose level (Table 1). The plasma glucose levels reached 35.5 and 34.1% of initial values by 4 and 5 hr after administration, respectively (Fig. 1). When insulin (20 U) was given orally in the pH-dependent coated capsule form with sodium salicylate (50 mg/capsule), a significant reduction in plasma glucose level was found 2 hr (81.7%) after administration, reaching a maximum reduction (73.4%) of initial values by 3 hr, and slowly returning to normal values (Table 1), but still significantly lower than the initial values by 16.2% (Fig. 1) by hour 5. By increasing the dose of insulin to 40 U/rabbit in the presence of sodium salicylate (50 mg/capsule), blood glucose level showed a steady decrease and reached a lower value 56% of the initial value by hour 5. This value was significantly ($p < 0.005$) lower than that produced by 20 U insulin in the capsule form, but was still significantly ($p < 0.05$) higher than that of 20 U insulin when given s.c. These results indicate the effect of sodium salicylate on promoting absorption of insulin. The coated capsules containing no salicylate and also the uncoated capsules containing salicylate did not produce any reduction in plasma glucose level (results not shown).

Salicylates were found to promote absorption by acting on both the apical cell membrane (the transcellular pathway) and the tight junctions between cells (the paracellular pathway) (12–14). Salicylates may also act on protein components of plasma membranes, red blood cell membranes, and small intestinal brush border membranes (15,16). Salicylates can also affect the nonprotein thiols (14,17) which are believed to play an important role in maintaining cell integrity (12) and in preventing uptake of hydrophilic compounds. Nishihata et al. (14) and Suzuka et al. (17) showed that salicylates decreased the levels of nonprotein thiols in intestinal tissues and isolated enterocytes. The effectiveness of sodium salicylate as enhancer varies from site to site; at 5% concentration, it was less effective in promoting

Table 1

Effect of Insulin Given by s.c. Injection (Ins., 20 U) or Orally in a pH-Dependent Coated Capsule Form (20 and 40 U) in the Presence of Sodium Salicylate (Sal., 50 mg) Without and with Sodium Cholate (Ch., 50 mg) on Plasma Glucose Level of Overnight Food-Deprived, Alloxan-Induced Hyperglycemic Rabbits

	Plasma Glucose Level mg % ± SEM (Number of Animals/Group)			
Time (hr)	Insulin 20 U s.c. (5)	Insulin + Sal. 20 U + 50 mg Capsule (5)	Insulin + Sal. 40 U + 50 mg Capsule (6)	Ins. + Sal. + Ch., 40 U + 50 mg + 50 mg, capsule (5)
0	226 ± 69.4	327 ± 60.9	166 ± 8.7	185 ± 8.5
1	144 ± 71.3	304 ± 59.7	130 ± 7.1**	152 ± 6.0*
2	120 ± 64.1*	267 ± 51.7**	119 ± 6.7**	133 ± 7.0**
3	87 ± 35.1**	240 ± 48.6**	109 ± 5.1**	121 ± 10.3**
4	77 ± 27.8**	264 ± 47.8**	99 ± 5.8**	108 ± 11.4**
5	74 ± 26.8**	274 ± 49.5*	93 ± 7.0**	91 ± 13.9**
AUC	242 ± 70.5	73.7 ± 43.5[b]	132 ± 41.5[b]	139 ± 37.3[a]
RH		30.43%	27.26%	28.66%

*$p < 0.05$; **$p < 0.01$ compared with the respective initial blood glucose level (0 hr).
[a]$p < 0.05$.
[b]$P < 0.01$ the $AUC_{0\text{-}5\ hr}$ (mg · hr/dl) is compared with that of insulin (20 U, s.c.)
RH : the relative hypoglycemia as a percent of that of insulin (20 U s.c.).

insulin absorption from buccal as well as nasal mucosa than from the rectal mucosa, while it had no effect on promoting the sublingual absorption of insulin (18,19). Sodium salicylate 1.5 M has been shown to increase the insulin solubility 7875 times (20), permitting the preparation of an aqueous solution of 630 mg/ml of insulin. Thus the interference between sodium salicylate and insulin self-association behavior, by increasing drug

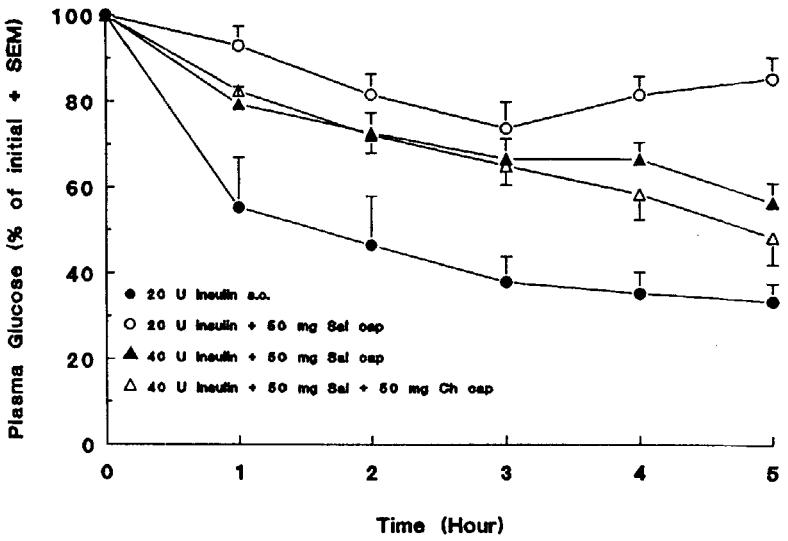

Figure 1. Effect of insulin given by s.c. injection (20 U) or orally in a pH-dependent coated capsule (Cap) form (20 and 40 U) in the presence of sodium salicylate (Sal., 50 mg) without and with sodium cholate (Ch., 50 mg) on plasma glucose level (percent of initial ± SEM) of overnight food-deprived, alloxan-induced hyperglycemic rabbits.

solubility, may substantially contribute to the improved drug bioavailability mediated by salicylate.

In order to study the effect of sodium cholate on the hypoglycemic effect of insulin/sodium salicylate coated capsules, sodium cholate (50 mg) was mixed with sodium salicylate and insulin. Sodium cholate is known to increase absorption of high molecular weight polar drugs such as insulin when given as aerosol intranasally (21). It also inhibits the intestinal proteolytic activity (22). The addition of sodium cholate, however, did not significantly improve salicylate-induced enhancement of intestinal absorption of insulin in the first 3 hr after administration. Thereafter a slight nonsignificant reduction of plasma glucose level was observed where this formulation produced an area under the plasma glucose (% reduction)–time curve of 139 ± 37.3 mg · hr/dl compared to 132 ± 41.5 mg · hr/dl without sodium cholate. These AUCs produced a relative hypoglycemia of 27.3 and 28.7%, respectively, compared to s.c. injection of 20 U of insulin suspension that gave an AUC of 242 ± 70.5 mg · hr/dl. The insulin capsule containing 20 U insulin and sodium salicylate (50 mg) gave an AUC of 73.7 ± 43.5 mg · hr/dl and relative hypoglycemia of 30.4%.

The relative hypoglycemia of (27–30%) reached in the present experiment was the highest obtained for an oral insulin preparation so far. It is nearly double that previously obtained with a similar preparation in the rat model (11), in which capsules coated with Eudragit S100 containing comparable doses of insulin (as U/kg), and sodium salicylate was inserted surgically into rat stomach under ether anesthesia. The difference in experimental design and animal species could account for the higher relative hypoglycemia of oral insulin capsules observed in the present experiment. Enteric insulin capsules coated with pH-dependent soluble polymers and containing absorption promoters such as sodium salicylate seems a promising approach for the development of oral insulin preparations. Further experiments are in progress to improve the relative hypoglycemia of oral insulin formulations.

REFERENCES

1. V. H. L. Lee, Pharm. Int., 7, 208 (1986).
2. C. A. Loehry, A. T. R. Axon, P. J. Hilton, R. C. Hider, and B. Creamer, Gut, 11, 466 (1970).
3. K. Taniguchi, S. Muranishi, and H. Sezaki, Int. J. Pharm., 4, 219 (1980).
4. H. M. Patel, H. G. L. Harding, F. Logue, C. M. Kesson, A. C. Mac Cuish, J. F. McKenzie, B. E. Ryman, and I. N. Scobie, Biochem. Soc. Trans., 6, 784 (1978)
5. J. F. Arrieta-Molero, K. Aleck, M. K. Sinha, C. M. Brownscheidle, L. J. Shapiro, and M. A. Sperling, Horm. Res., 16, 249 (1982).
6. A. V. Stephanon, V. K. Lishko, A. V. Shenchenka, A. S. Efimov, and V. V. Khovaka, Ukr. Biokhim. Zh., 58, 58 (1986).
7. N. Das, M. K. Basu, and M. K. Das, Biochem. Int., 16, 983 (1989).
8. K. B. Choudhari, V. Labhasetwar, and A. K. Dorle, J. Microencapsulation, 11, 319 (1994).
9. Z. Shao, Y. Li, T. Chermok, and A. K. Mitra, Pharm. Res., 11, 1174 (1994).
10. E. Touitou and A. Rubinstein, Int. J. Pharm., 30, 95 (1986).
11. E. A. Hosny, N. M. Khan Ghilzai, and A. H. Al-Dhawalie, Drug Dev. Ind. Pharm., 21, 1583 (1995).
12. S. Szabo, J. S. Trier, and P. W. Frankel, Science, 214, 200 (1981).
13. H. Kajii, T. Horie, M. Hayashi, and S. Awazu, Life Sci., 37, 523 (1985).
14. T. Nishihata, B. T. Nghiem, H. Yoshitomi, C. S. Lee, M. Dillsaver, T. Higuchi, T. Choh, T. Suzuka, A. Furuay, and A. Kamada, Pharm. Res., 3, 345 (1986).
15. T. Nishihata and T. Higuchi, Biochim. Biophys. Acta., 775, 269 (1984).
16. T. Nishihata, T. Higuchi, and A. Kamada, Life Sci., 34, 437 (1984).
17. T. Suzuka, N. Yata, K. Sakai, and T. Nishahata, J. Pharm. Pharmacol., 40, 469 (1988).
18. B. J. Aungst and N. J. Rogers, Pharm. Res., 5, 305 (1988).
19. B. J. Aungst, N. J. Rogers, and E. Shefter, J. Pharmacol. Exp. Ther., 244, 23 (1988).
20. E. Touitou, F. Alhaique, P. Fisher, A. Memoli, F. M. Riccieri, and E. Santucci, J. Pharm. Sci., 76, 791 (1987).
21. A. C. Moses, G. S. Gordon, M. C. Carey, and J. S. Flier, Diabetes, 32, 1040 (1983)
22. E. Ziv, O. Lior, and M. Kidron, Biochem. Pharmacol., 36, 1035 (1987).