International Journal of Pharmaceutics, 86 (1992) 1–7

IJP 02792

Research Papers

A new approach for insulin delivery via the pulmonary route: Design and pharmacokinetics in non-diabetic rabbits

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(Received 17 February 1990)
(Modified version received 15 January 1992)
(Accepted 6 February 1992)

Key words: Bioavailability; Diabetes; Insulin; Insulin delivery system; Nebulizer; Pharmacokinetics; Pulmonary route; Subcutaneous route

Summary

A mini-mist medication air compressor fitted nebulizer was used to deliver insulin in the form of a fine mist via the rabbit's pulmonary system. An insulin immune test was first performed in order to ensure the rabbit's respiratory compliance and then the bioavailability was examined as a function of insulin dose concentration and dose volume. An increase in dose concentration from 2 to 5 U kg^{-1} rbw was found to reduce the percentage minimum plasma glucose concentration (%MPGC) from 71 to 38% without affecting the time T required to attain these values (i.e., T%MPGC = 20 min). The values of the percentage total reduction in plasma glucose from 0 to 3 h (%$TRPG_{0-3\,h}$) were also found to increase from 15 to 37% as insulin concentration increased. On the other hand, an increase in dose volume from 1 to 3 ml did not lead to marked changes in both %MPGC and %$TRPG_{0-3\,h}$ but did increase the value of T%MPGC from 20 to 50 min. Comparative study between pulmonary administered insulin (PAI) and subcutaneously administered insulin (SAI) showed that the T%MPGC for PAI was about 1/5 of that for SAI with an acceptable duration for maintaining the lowering of plasma glucose. The apparent values of %MPGC and %$TRPC_{0-3\,h}$ for PAI were about 36% of those for SAI and related to incomplete dose consumption as part of PAI was always retained inside the nebulizer. The corrected bioavailability for PAI was therefore calculated and found to be greater than 50% of that for SAI.

Introduction

Treatment of diabetes by insulin injections is known to be the most effective route of administration. The drawbacks of this route are numerous and include pain, irritation, itching, redness, swelling and stinging at the site of injections, moreover, atrophy of subcutaneous fat tissue may also occur (Larner, 1985; Gillies et al., 1986).

Several approaches aimed at improving the treatment of diabetics are currently under active study. Some of these resulted in the introduction of new delivery systems to be transplanted in patients, such as insulin pumps (Irsigler et al. 1981; Rupp et al., 1982; Brange and Havelund, 1983), microcapsules (Sun and O'Shea, 1986), biodegradable and non-degradable matrices (Sie-

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gel and Langer, 1983; Crommelin, 1987) and the interesting glucose-sensitive devices (Albin et al., 1986; Jeong et al., 1986). Although the results obtained via these approaches are indicative, an imminent solution is not yet available for problems such as (i) limited amounts of insulin entrapped in the delivery system, (ii) uncontrolled release, (iii) surgery to recover non-degradable matrices, (iv) poor biocompatibility of the matrices and (v) instability of the entrapped insulin.

Other approaches were initiated to use routes of administration other than the parenteral pathway. These are the oral (Crommelin, 1987), buccal (Nagai, 1986; Crommelin, 1987), rectal (Yamasaki et al., 1981; Nishihata et al., 1987) and nasal routes (Nagai, 1986; Crommelin, 1987), the latter being considered to be the most promising. In most cases, the bioavailability of insulin was reported to be as low as 1.5%, however, this value could be increased up to 20% by incorporating a suitable absorption enhancer in the administered dose (Siegel and Langer, 1983; Nishihata et al., 1987). Data on the reproducibility of the rate and extent of insulin absorption, the acceptability of some of these routes by the patients and the safety of the incorporated enhancers are scarcely available.

It was therefore considered appropriate in the present work to carry out further investigation of other new techniques and routes of administration with the objective of providing diabetic patients with an acceptable, inexpensive, self-administered and comparatively highly effective insulin dose avoiding most of the disadvantages and complications, mentioned above.

The pulmonary route was found to be a possible choice, since it is readily used for local and systemic applications of many drugs (Pharmaceutical Codex, 1979; American Hospital Farmalary Service, Drug Information, 1989). Its mucosa is thin and very rich in blood vessels (Scothorne, 1987) in addition to having a large surface area to facilitate the absorption of drug.

A modified mini-mist medication air compressor fitted nebulizer was employed in an effective technique for insulin delivery via the rabbits pulmonary system. The insulin mist was first tested for possible immune effects in order to ensure the rabbit's respiratory compliance. The bioavailability attained with the proposed technique and its pharmacokinetics were examined in relation to insulin dose concentration and dose volume. The results obtained were then compared with data on subcutaneously administered insulin to pro-

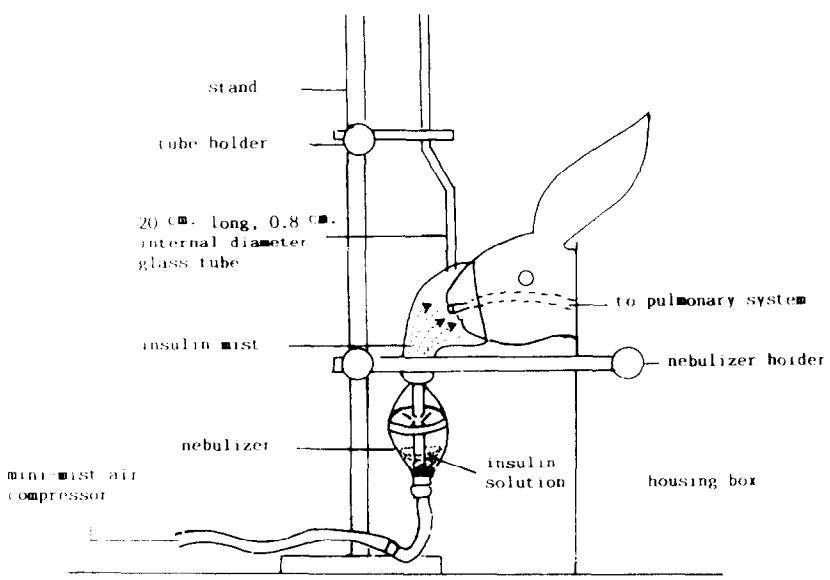

Fig. 1. An illustration of the nebulizer fittings used to deliver insulin mist via the rabbit's pulmonary system.

vide information regarding the efficacy criteria of the new system.

Materials and Methods

Materials

Insulin crystals of porcine origin (27.2 U mg^{-1}; from Nordisk Gentofte, Gentofte, Denmark), polysorbate 80 (Atlas Chemicals Ltd, U.S.A.), and purified water for injections were used. Other reagents were of analytical grade.

Construction of the mini-mist nebulizer

A mini-mist medication air compressor fitted nebulizer Bunn ® (Model 510 E, John Bunn Division, Tonawanda, NY; 240 V, 5 A, 50 Hz) was used to deliver the insulin mist via the rabbit's pulmonary system. The nebulizer was modified so that it could fit around the rabbit's nose and mouth. The upper surface of the mask was perforated and connected with glass tube (20 cm long, 0.8 cm internal diameter) so that the pressure inside the nebulizer could be maintained at around atmospheric pressure. The glass tube also served as a condenser to return any escaped mist to the reservoir of the nebulizer.

Fig. 1 illustrates the nebulizer fittings used to deliver insulin mist via the rabbit's pulmonary system.

Preparation of the nebulized doses

The crystalline insulin was dissolved in purified water for injections containing 0.001% of polysorbate 80 (the presence of surfactant helping to minimize possible adsorption of insulin molecules on the inner walls of the nebulizer and other fittings during administration) to give a known concentration of insulin of 20 U ml^{-1} detected using a radioimmunoassay kit (Diagnostic Product Corp., Los Angeles, CA, U.S.A.) and a gamma counter (Cobra Auto-Gamma, Model B 5010, Packard Instrument Co. Inc., Downers Grove, IL, U.S.A.). The desired dose concentration and dose volume could be achieved by suitable dilution using the same solvent. All prepared doses were refrigerated between 2 and 8 °C and used within 10 h of preparation.

Preparation of the rabbits

Selected healthy rabbits each weighing 3.25 ± 0.25 kg were divided into eight groups of four rabbits each. The animals were maintained on a uniform diet for a period of 10 days before tests. During this period, six groups (A) were subjected to nebulization with 2 ml of purified water twice daily in order for the rabbits to become accustomed to the new technique. The two remaining groups (B) were not subjected to nebulization and were used in the assessment of bioavailability for insulin administration via the subcutaneous route. In order to minimize mist condensation at the front of the rabbit's nostrils, a polyethylene cannula of 5 mm internal diameter was inserted into both nostrils to keep them open during the nebulization period (average duration: 12 min). The whiskers and hair around the nose and mouth were also removed. All of the rabbits (except those used for the immune reaction test denoted the Ax group) were deprived of food for a period of 8 h prior to conducting experiments.

Insulin immune reaction on rabbit's pulmonary system

Rabbits of group Ax were nebulized twice daily with 2 ml of 5 U kg^{-1} rbw insulin solution for a period of 3 consecutive days. The behaviour of the rabbits including any changes in respiratory functions was recorded for another 3 days after the last nebulization.

Effect of dose concentration on bioavailability

Determinations of fasting plasma glucose levels were performed on three groups of rabbits (A) immediately before nebulization of the animals with 1 ml of insulin solutions of 2, 3 and 5 U kg^{-1} rbw. Blood samples were then taken from the marginal ear veins at designated times during and after nebulization for the determination of plasma glucose concentrations using an assay kit (Beckman Glucose Analyser 2, Beckman, U.S.A.) based on an enzymatic method.

Effect of dose volume on bioavailability

Two groups of rabbits (A) were individually nebulized using a fixed concentration of insulin of 5 U kg^{-1} rbw in 2 and 3 ml solutions. Their

fasting plasma glucose levels and plasma glucose concentrations throughout the experiments were evaluated as described above.

It was observed that considerable proportions of the nebulized doses were retained inside the nebulizer and in contact around the rabbit's noses and hence did not reach the pulmonary system. These amounts are referred to as unconsumed insulin in calculations.

Calculation of unconsumed insulin

The nebulizer including the reservoir, glass tube and mask were rinsed with three portions each of 5 ml purified water containing 0.001% polysorbate. The exposed area around the rabbits' nose was also washed with another 10 ml of the same solvent and added to the previously collected portions. After adjusting the volume, the unconsumed insulin could be determined as before.

Subcutaneous administration of insulin

The reduction in plasma glucose concentration after subcutaneous injections of designated concentrations of the prepared insulin was examined. Dose concentrations of 1 and 2 U kg^{-1} rbw were found to be appropriate (on the basis of preliminary trials) for producing a suitable reduction in plasma glucose at 1 and 2 h after injection without the incidence of convulsion. These doses are

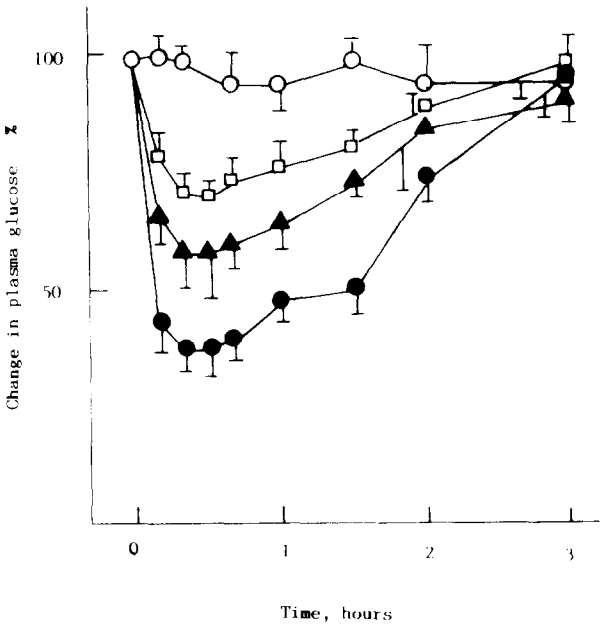

Fig. 2. Effect of insulin dose concentration on plasma glucose-time profiles after nebulization. (○) Blank test, (□) 2 U kg^{-1} rbw, (▲) 3 U kg^{-1} rbw and (●) 5 U kg^{-1} rbw. Each value represents the mean ± S.D. ($n = 4$).

comparatively higher than those stated in most pharmacopoeias for insulin assay and could be attributed to differences in the properties of the solutions employed (i.e., adjustment to acidic pH and the presence of phenol or cresol in the stated

TABLE 1

Effect of insulin dose concentration and dose volume on pharmacokinetics after pulmonary and subcutaneous administration

Variables			% MPGC	% $TRPG_{0-3h}$	T%MPGC (min)	Time during which less than 70% of plasma glucose is held fixed
	(apparent)	(calculated)				
Insulin dose	2	1.4	71 ± 5	15	20	20–30
concentration	3	2.1	58 ± 9	25	20	8–80
(U kg^{-1} rbw) (PAI)	5	3.5	38 ± 6	37	20	4–114
Insulin dose	1		38 ± 6	37	20	4–114
volume	2		44 ± 5	38	40	8–120
(ml) (PAI)	3		42 ± 6	40	50	10–136
Insulin dose	1		65 ± 10	23	90	75–130
concentration	2		42 ± 11	45	120	40–240
(U kg^{-1} rbw) (SAI)						

pharmacopoeial doses could enhance their absorption and consequent bioavailability) and also to be possible binding effect of the added surfactant. The two remaining groups of rabbits (B) were individually injected with a 1 ml volume containing the designated concentrations and the fasting blood glucose level before and plasma glucose concentration after injections were determined as above.

Results and Discussion

Nebulization of insulin into the rabbits' pulmonary system twice daily for 3 successive days did not result in any marked immune response by the rabbits' respiratory system. The only distinct difference was that the animals appeared to be less active (probably due to the expected fall in plasma glucose), however, they recovered quickly and behaved normally as did those nebulized with purified water.

The plasma glucose-time profiles and pharmacokinetic data for the various concentrations used in nebulization are shown in Fig. 2 and Table 1. The results show that an increase in dose concentration produced a significant decrease in percentage minimum plasma glucose concentration (%MPGC) from 71 to 38%. Although the time T required to attain each %MPGC value remained the same (i.e., T%MPGC = 20 min), the period during which plasma glucose concentrations were maintained at below 70% of the initial levels (taken as a suitable value for comparison) was found to increase with concentrations of insulin. These data together with the values of the percentage total reduction in plasma glucose from 0 to 3 h ($\%TRPG_{0-3\ h}$), as determined from the AUC in Fig. 2 and applying the equation.

$$\%TRPG_{0-3\,h} = 100(1 - AUC_{0-3\,h}/300) \qquad (1)$$

indicate that greater bioavailability was attained with increase in the extent of absorption of insulin into the blood circulation and, consequently, a greater decrease in plasma glucose concentration could be maintained over a longer period of time.

The effects of increasing dose volume on the plasma glucose-time profiles are depicted in Fig. 3 while the pharmacokinetic data are listed in Table 1. The finding that increasing dose volume

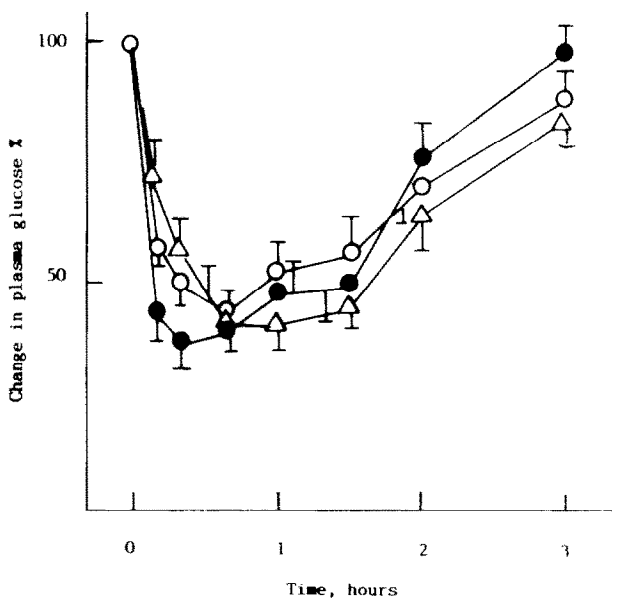

Fig. 3. Effect of insulin dose volume on plasma glucose-time profiles after nebulization. (●) 1 ml, (○) 2 ml and (△) 3 ml. Each volume contains the same amount of insulin equivalent to 5 U kg^{-1} rbw and each value represents the mean ± S.D. ($n = 4$).

produced a delay in the time T required for attaining %MPGC (i.e., T%MPGC from 20 to 50 min) is consistent with the longer period of time required for the larger volume to be nebulized into the pulmonary system. However, the values of %MPGC and $\%TRPG_{0-3\,h}$ in Table 1 indicate little effect, if any, of dose volume on insulin bioavailability after pulmonary administration.

Although the above results are indicative of how insulin dose concentration and dose volume could affect bioavailability after pulmonary administration, if should be borne in mind that considerable proportions of the nebulized doses were found to be retained inside the nebulizer and around the rabbits' noses without entering their pulmonary system. These amounts were calculated and found to be about 30% of the initial concentration nebulized.

The plasma glucose-time profiles and data obtained on nebulized insulin were compared with those after subcutaneous administration, the results being shown in Fig. 4 and Table 1. It is clear that the values of T%MPGC for pulmonary administered insulin (PAI) are about 1/5 of those for subcutaneously administered insulin (SAI), demonstrating faster absorption and rapid onset of action. The results can be attributed to the smaller site of absorption in SAI in comparison to the larger surface area of the pulmonary route. In addition, it would appear from the results that one of the factors contributing to the enhancement of insulin absorption is the fact that very fine droplets of the insulin mist are delivered into the pulmonary system as compared with those in the case of subcutaneous administration. On inspection of the values of %MPGC and $\%TRPG_{0-3\,h}$ for PAI and SAI and assuming a linear dose response relationship, the apparent bioavailability for PAI was determined to be 36% of that of SAI. However, on taking the value of unconsumed insulin administered by nebulization into consideration (about 30% of the initial concentration), the corrected bioavailability for PAI could be calculated and was found to be more than 50% of that for SAI.

The contention that pulmonary administered drugs are susceptible to first-pass metabolism appears reasonable, since 25% of the cardiac output flows through the liver (Pharmaceutical Codex, 1979). Despite the fact that the liver is the major

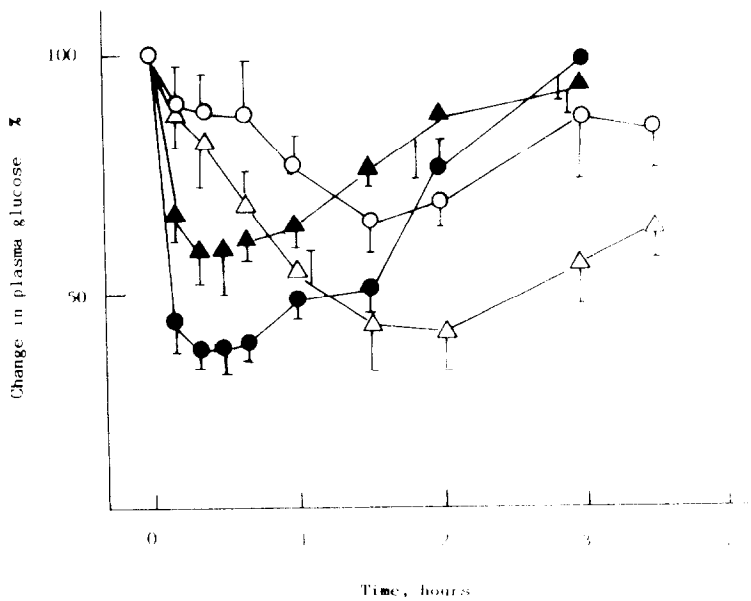

Fig. 4. Comparison of plasma glucose-time profiles for pulmonary and subcutaneously administered insulin. Subcutaneous administration of insulin at: 1 (○) and 2 (△) U kg^{-1} rbw; pulmonary administration of insulin at: 3 (▲) and 5 (●) U kg^{-1} rbw.

organ for insulin degradation, it is also the region where the greatest extent of insulin utilization occurs (Nishihata et al., 1987). An increase in liver uptake of insulin may therefore enhance the magnitude of hepatic glucose uptake so that the pulmonary route of administration could be feasible in the approach to techniques for the effective delivery of insulin in the treatment of diabetes.

Conclusions

The present study has shown that pulmonary nebulization of insulin is a safe and effective technique for lowering plasma glucose concentrations in experimental animals. The bioavailability results and pharmacokinetic data are dependent on the dose concentrations rather than the dose volumes. Accordingly, other devices including metered dose atomizers and spinhalers might be employed to deliver insulin in the form of solutions, suspensions or powders via the pulmonary route.

Acknowledgements

I would like to express my thanks to Mr A. Al Ferdan, the federal director of pharmacy, for the encouragement that made this work possible. I am also grateful to the laboratory staff of Aljazera Hospital and Mrs Carol Gosling of the Central Hospital Radioimmunoassay Department for technical assistance.

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