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The pharmacokinetics of pulmonary nebulized insulin and its effect on glucose tolerance in streptozotocin-induced diabetic rabbits

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Abstract

The bioavailability of insulin administered by a modified mini-mist air compressor fitted nebulizer into the pulmonary route of streptozotocin-induced diabetic rabbits was investigated. The glucose tolerance was measured by an intravenous glucose tolerance test (IVGTT) prior and after the induction of diabetes to establish diabetic control for each rabbit. An increase in nebulized dose concentration from 2 to 6 U/kg. rbw (rabbit body weight) was found to produce proportional dose response showed by the increased values of the fractional glucose clearance (FGC) from 3.8 $\pm$ 0.45 to 10 $\pm$ 1.4, the increased values in serum insulin peaks above the basal levels from 115 $\pm$ 27 to 330 $\pm$ 46 μU ml^{-1} with corresponding AUCs between 105 $\pm$ 26 and 347 $\pm$ 47 μU h ml^{-1}, and also the increased rabbits glucose tolerability in term of percentage total reduction in plasma glucose (%TRPG$_{0-2h}$) between 21.75% and 43.17% compared with a value of 14.61% for diabetic control rabbits. The rabbits' glucose tolerability after pulmonary administration was found to reach a value of up to 65% of comparable subcutaneously insulin dose; however, the AUC serum/insulin time profile of the former was about 50% of that of the latter. The results also demonstrated that insulin delivered by the nebulizer produced more rapid peaks serum insulin and minimum serum glucose concentrations in contrast to the slower peaks produced by subcutaneously administered insulin.

Keywords: Bioavailability; Diabetes; Glucose; Insulin; Nebulizer; Pharmacokinetics; Pulmonary administration; Streptozotocin; Subcutaneous administration

1. Introduction

Simulation of endogenous insulin profiles seen in normal subjects can not be reached by conventional parentral therapy without suffering the multiple daily injections required by diabetic patients (Larner, 1984; Gillies et al., 1986; Sakr, 1992). In order to preclude the problems of the parentral applications, much research was initiated to search for other routes of administration. Oral administration (Crommelin, 1987) was limited by gastrointestinal proteolytic digestion and poor absorption and whilst insulin may be delivered by buccal (Nagai, 1986; Crommelin, 1987),

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vaginal (Richardson et al., 1992), rectal (Yamasaki et al., 1981; Nishihata et al., 1985; Nishihata et al., 1987) and nasal (Nagai, 1986; Crommelin, 1987) routes, the long-term use of enhancers required to facilitate absorption was the cause of much concern. The use of the pulmonary pathway as a possible route for insulin delivery has been evaluated by some authors (Jones et al., 1988; Colthorpe et al., 1992). Results reported on insulin administered in the form of aerosol or instillate via endotracheal intubation using rabbits demonstrated rapid time to reach peak plasma insulin and minimum plasma glucose concentrations in contrast to the slower peaks produced after subcutaneous administration of the peptide (Jones et al., 1988; Colthorpe et al., 1992). The bioavailability fraction for the aerosolised insulin was also reported to be 10-fold greater than for the instilled insulin (Colthorpe et al., 1992). Sakr (1992) has proposed a new approach for insulin delivery via the pulmonary route of fasting healthy rabbits in the form of a fine mist using a modified mini mist air compressor fitted nebulizer as a delivery tool. The author showed that the new technique was safe (in terms of lungs tolerability to the peptide), rapid (in terms of time to produce minimum reduction in plasma glucose concentration) and effective with bioavailability of up to 50% of comparable subcutaneously-administered insulin dose.

The present work was therefore initiated with the aim to study the applicability of the proposed technique on diabetic rabbits whose glucose tolerability is only a function of the externally nebulized insulin. The previously used modified mini-mist medication air compressor fitted nebulizer (Sakr, 1992) was employed for insulin delivery via the pulmonary routes of streptozotocin-induced diabetic rabbits. Serum insulin concentration and blood glucose tolerability were examined as functions of the nebulized dose concentrations. Corresponding pharmacokinetic data were also obtained in terms of (FGC), onset and duration of action. The results were then compared with data on subcutaneously administered insulin (SAI) so that information about the effective applicability of the new technique in the management of diabetes mellitus could be provided.

2. Materials and methods

2.1. Materials

Insulin crystals of porcine origin (27.2 U mg^{-1}; from Nordisk Gentofte, Gentofte, Denmark), streptozotocin (Sigma Chem. Co., St. Louis, MO USA), polysorbate 80 (Atlas Chem. Ltd., USA), 20% dextrose for injection (Nile Pharm. Co, Egypt) and purified water for injections were used. Other reagents were of analytical grade.

2.2. Construction of the 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 rabbits pulmonary system. Details of construction, modification of the nebulizer and precaution required to minimise mist condensation on rabbits nostrils were discussed previously (Sakr, 1992); however, a smaller narrower base reservoir was used to minimise the amount of retained insulin in the nebulizer.

2.3. Preparation of the nebulized doses

The nebulized doses at concentrations from 2–6 U kg rbw. were prepared, standardised by radio immunoassay kit (Diagnostic Product Corp., Los Angeles, CA, USA) and a gamma counter (Cobra Auto-Gamma, Model B 5010, Packard Instrument Co. Inc., Downers Grove, IL, USA) and stored as before (Sakr, 1992).

2.4. Animal model

Groups of white New Zealand rabbits each weighing 3–3.5 kg were used during the study. The rabbits were preconditioned for laboratory use and given a diet of dry rabbit food. Each rabbit evaluated served as its own non-diabetic and diabetic control prior to nebulization procedure with insulin. In the non-diabetic phase of the study, each rabbit was given an intravenous glucose tolerance test (IVGTT) after overnight fasting to determine the normal glucose response

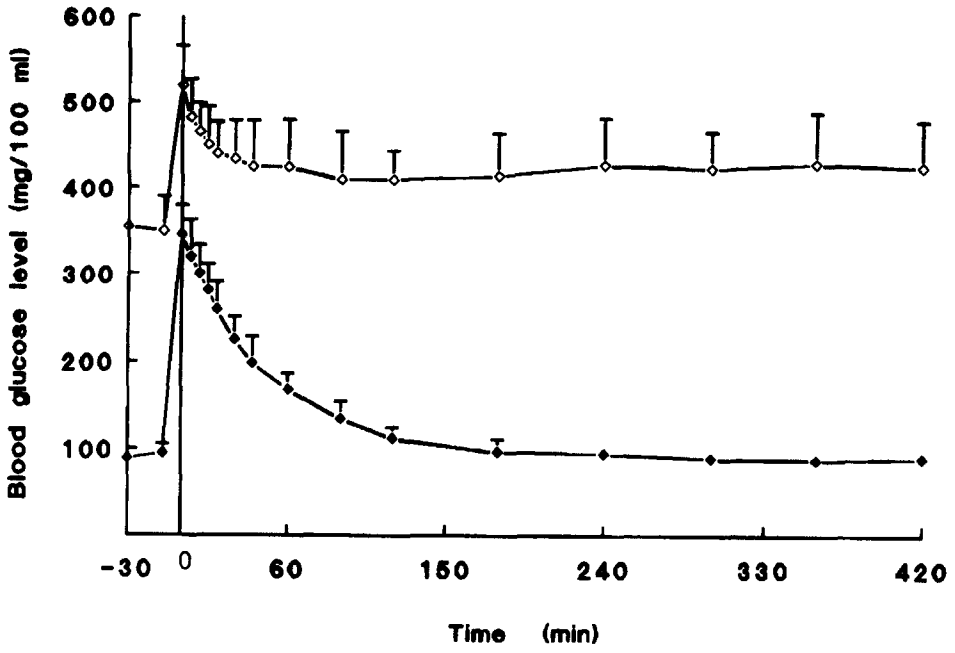

Fig. 1. Peripheral blood glucose profiles of rabbits administered bolus dextrose (500 mg/kg) during an intravenous glucose tolerance test (IVGTT). ◆ Normal rabbits; ◇ streptozotocin-induced diabetic rabbits.

(Rottiers et al., 1981). Two milliliter blood samples were taken 30 and 10 min before the intravenous bolus injection of the rabbits with 500 mg/kg dextrose solution to determine the fasting glucose levels (value = 89 ± 7.2 mg/100 ml). After, the blood samples were taken at 5, 10, 20, 30, 40, 60, 90, 120, 180, 240, 360 and 420 min. The blood glucose levels were determined by an assay kit (Beckman Glucose analyser 2, Beckman, USA) based on an enzymatic method. Serum insulin levels were determined with gamma counter.

2.5. Induction of diabetes by streptozotocin

Three groups of rabbits were injected intravenously with 50 mg kg rbw streptozotocin in citrate buffer (pH 4.5) after overnight fasting. The rabbits were then fed with standard rabbit food containing 90% whole barley flour and 10% sucrose in the form of granules. Blood glucose concentrations were measured and regulated with sufficient intermediate-acting insulin (Novo Nordisk Gentofte Denmark) in doses between 1–2 units/kg as single morning subcutaneous injection for a period of 7 days. All rabbits demonstrated severe insulin deficiency (marked hyperglycaemia with overnight fasting glucose level = 355 ± 40 mg/100 ml on insulin withdrawal). After adequate glucose/insulin control had been established, IVGTT was performed as described above to determine glucose tolerance in the diabetic phase and to ensure that the rabbits were indeed diabetic with no self-control of glucose and insulin concentration.

2.6. Effect of nebulized dose concentration on bioavailability

Determination of initial glucose and insulin serum concentrations after the bolus glucose injections (time zero) were performed on the diabetic rabbits immediately before nebulization of the animals with 2 ml of insulin solutions containing 2, 4 and 6 U kg^{-1} rbw. Blood samples were taken from the marginal ear veins at designated times during and after nebulization. Assay for

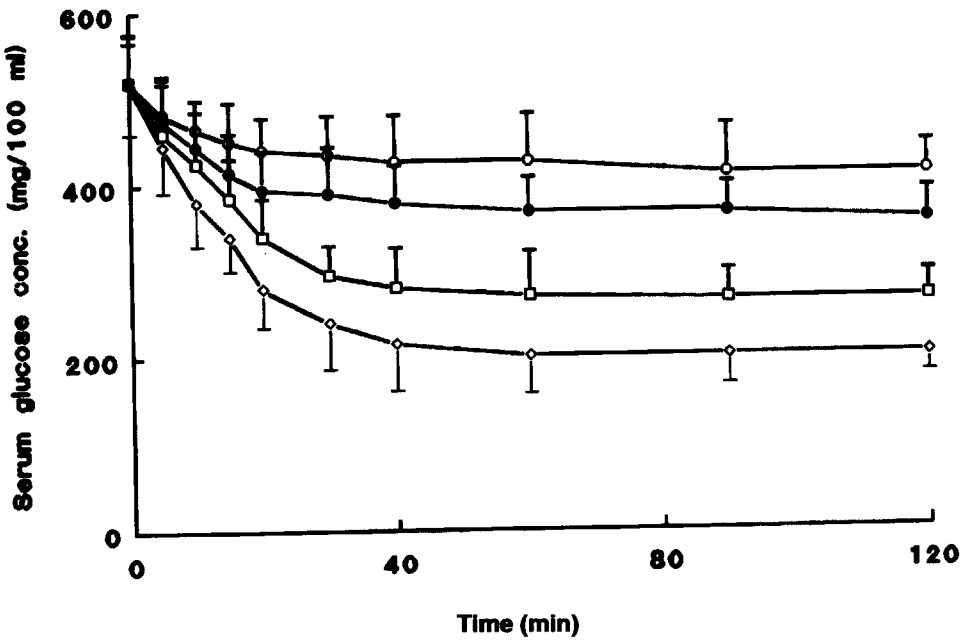

Fig. 2. Effect of nebulized insulin on plasma glucose-time profiles of diabetic rabbits administered bolus dextrose (500 mg/kg). ○ blank diabetic control; ● 2 U kg^{-1} rbw; □ 4 U kg^{-1} rbw; ◇ 6 U kg^{-1} rbw.

serum insulin and glucose levels were measured as before.

2.7. Calculation of the non-utilised insulin

It was observed that considerable proportions of the nebulized doses were retained inside the nebulizer and condensed on the rabbits nostrils without entering their pulmonary system (Sakr, 1992). These amounts were calculated in the present study and found to reach a value of about 15% of the initial nebulized concentration.

2.8. Subcutaneous administration of insulin

The increases in serum insulin and the reductions in plasma glucose concentrations after SAI were determined. In the present investigation, a dose of 2 U kg^{-1} rbw insulin was found appropriate (on the bases of preliminary trials) for producing a suitable reduction in plasma glucose without the incidence of hypoglycaemic convulsion (Sakr, 1992).

3. Results and discussion

Two aspects of primary interest were the determination of the amount of insulin reaching the blood compartment (bioavailability) and how this would affect the biological response (glucose tolerance) in relation to the administered dose concentration.

As seen in Fig. 1, the mean blood glucose level for the normal rabbits rises from a fasting value of below 100 mg/100 ml to about 350 mg/100 ml after the bolus glucose injection at time zero then gradually falls to just above the fasting value in 180 min, whereas the mean fasting blood glucose level for the diabetic control rabbits rises from 355 mg/100 ml to over 500 mg/100 ml and remained at a level of about 450 mg/100 ml for 420 min, indicating that the rabbits were indeed diabetic. The effect of nebulized insulin concentration on blood glucose profiles measured during IVGTT time of the study are seen in Fig. 2 and summarized in terms of pharmacokinetic data in Table 1. It is apparent that the nebulized rabbits exhibited blood glucose profiles that go steeper in

Table 1
Effect of insulin dose concentration on pharmacokinetics after pulmonary and subcutaneous administration

Phase of study (rabbits group) (n = 4)	Fraction glucose clearance rate (FGC)	Minimum reduction plasma glucose (PG mg/100 ml)	AUC plasma glucose time (mg h/100 ml)	AUC serum insulin time μU h ml^{-1}	Peak insulin concentration (μU/ml)
Diabetic control[a]	2 ± 0.39	410 ± 56	888 ± 112	-	-
PAI-treated diabetic at insulin concentration[a] (U kg^{-1} rbw)					
2	3.8 ± 0.45	355 ± 35	813.88 ± 114	105 ± 26	115 ± 27
4	7.2 ± 1.10	265 ± 34	691.66 ± 105	229 ± 32	242 ± 39
6	10 ± 1.40	200 ± 23	591.12 ± 98	347 ± 47	330 ± 46
SAI-treated diabetic at insulin concentration[b] (U kg^{-1} rbw)					
2	-	110 ± 15	2202 ± 178	353 ± 46	150 ± 30
PAI-treated diabetic at insulin concentration[b] (U kg^{-1} rbw)					
6	-	200 ± 23	1843.3 ± 198	346.7 ± 51	330 ± 46

[a]$AUC_{0\text{-}2h}$ plasma glucose time and $AUC_{0\text{-}2h}$ insulin time.[b]$AUC_{0\text{-}7h}$ plasma glucose time and $AUC_{0\text{-}7h}$ insulin time.

proportion to dose concentrations compared with the untreated rabbits. The FGC calculated on a semi-log paper from the slopes of blood glucose concentration versus time interval between 5–30 min indicated that the response behaviour for the nebulized insulin with value between 3.8 and 10 was dependent on the nebulized dose concentration and were higher than that for the diabetic group (FGC = 2). Likewise, by comparing the values of percentage total reduction in plasma glucose from 0 to 2 h (%$TRPG_{0-2h}$) for the different nebulized concentration, determined from the AUC in Fig. 2 and applying the equation,

$$\%TRPG_{0-2h} = 100(1 - AUC_{0-2h}/1040) \quad (1)$$

it can be seen that increasing dose concentration were able to achieve normalized glucose utilizations of 21.75, 33.49 and 43.17% with respective minimum plasma glucose concentrations (MPGC) of 355, 265 and 200 mg/100 ml. The diabetic control rabbits, however, showed lower values of %$TRPG_{0-2h}$ (14.61%) and MPGC of 410 mg/100 ml in about 120 min. The FGC, MPGC and %$TRPG_{0-2h}$ for the diabetic control rabbits were statistically different ($P < 0.01$) from the treated rabbits. The peaks serum insulin were found to increase substantially with the increase in the administered dose. Fig. 3 and Table 1 show that an increase in nebulized dose from 2–6 U kg^{-1} was followed by corresponding increase in maximum peak insulin concentration from 115 ± 27 to 330 ± 46 μU/ml^{-1} and AUC values from 105 ± 25.6 to 347 ± 47.3 μU h ml^{-1} with increased duration from 40–60 min for maintaining insulin concentration > 30 μU ml^{-1}.

The serum insulin and glucose time profiles and data obtained on pulmonary administered insulin (PAI) were compared with those after SAI. The serum glucose concentration time profile in Fig. 4 for pulmonary nebulized insulin at a dose concentration of 6 U kg^{-1} (chosen for convenience) was compared with that for 2 U kg^{-1} injected subcutaneously. It is clear that nebulized insulin has produced rapid onset of action to reduce blood glucose concentration from 520 to 200 mg/100 ml with no further reduction in about 60 min. In contrast, SAI showed longer time to reach this value (120 min); however, the value continued to

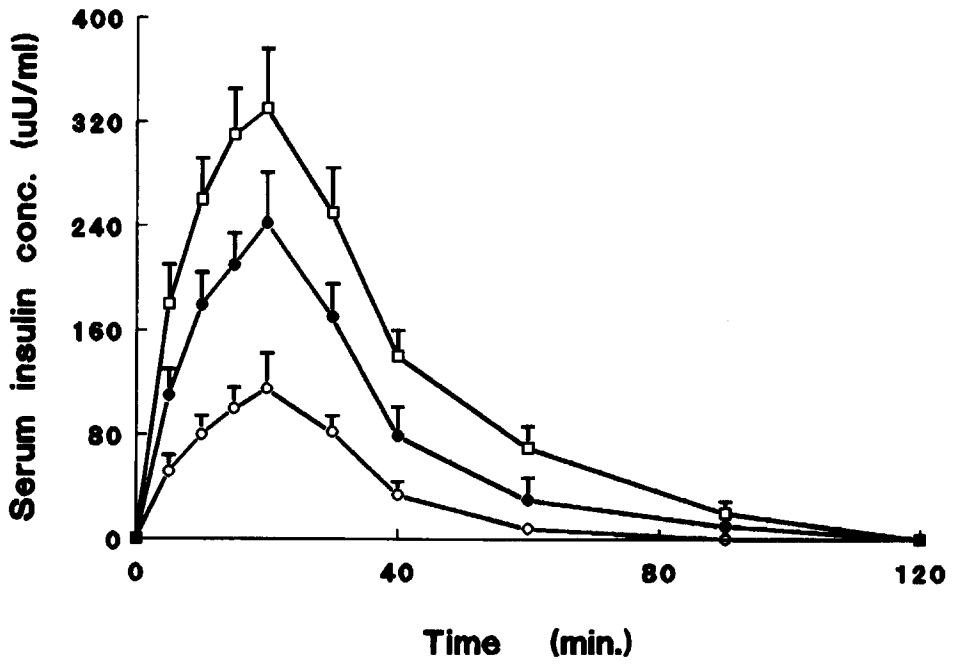

Fig. 3. Effect of nebulized insulin on peripheral insulin levels of diabetic rabbits administered bolus dextrose (500 mg/kg). ○ 2 U kg^{-1} rbw; ● 4 U kg^{-1} rbw;□ 6 U kg^{-1} rbw.

decrease up to 110 mg/100 ml in about 360 min. On the other hand, the $\%TRPG_{0-7h}$ determined from the AUCs in Fig. 4 and applying the equation,

$$\%TRPG_{0-7h} = 100(1 - AUC_{0-7h}/3640) \quad (2)$$

indicated that the value for PAI was about 41.66% of that for the SAI calculated on dose-respond bases. Fig. 5 illustrates that insulin peak of a maximum 330 μU/ml was reached from the PAI in about 20 min with a duration from 5–60 min for maintaining >30 μU/ml. A comparatively smaller insulin peak of 150 μU/ml was obtained after subcutaneous administration in about 60 min with a longer duration from 20–240 min for maintaining >30 μU/ml. It is clear that $>50\%$ of the IVGTT was utilized by the nebulized insulin indicating that not only the duration but also the onset for maximum peak insulin concentration is responsible for the greater reduction in plasma glucose.

The comparatively smaller peak and slower onset of insulin concentration observed in the SAI was apparently compensated by the longer duration of action and hens the increase in the total area under the curve (AUC = 353 μU h/ml for 2 U kg^{-1} rbw) compared to that after pulmonary administration (AUC = 346.7 μU h/ml for 6 U kg^{-1} rbw). The AUC serum/insulin profile obtained from the PAI was then calculated for a dose concentration equal to that from the SAI and found to be about 35% of the later value. The major effect of insulin on carbohydrate homeostasis was reported to follow its binding to specific cell-surfaces receptors on insulin-sensitive tissue (Reynolds, 1993). The rapid onset of action and the higher insulin concentration after pulmonary administration would therefore allow for the specific cell-surface receptors to be saturated with the available insulin in a shorter period of time. This will be followed by a quicker enhancement of peripheral glucose disposal, thereby a quicker reduction in blood glucose concentration. By considering the amount of insulin retained inside the nebulizer reservoir and condensed on the rabbits nostrils (unconsumed insulin = 15% of the ad-

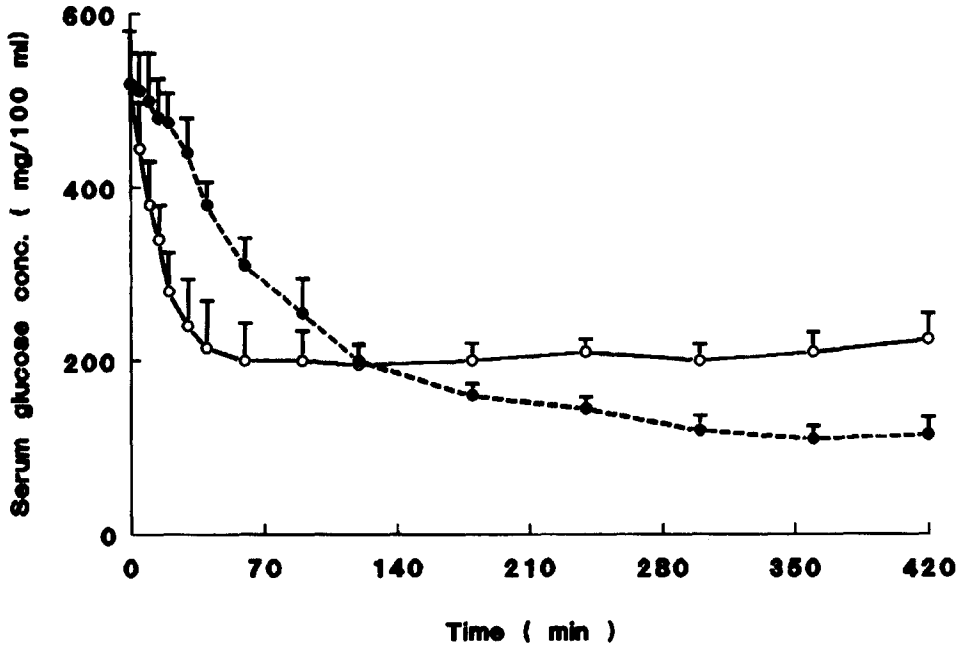

Fig. 4. Comparison of plasma glucose-time profiles for pulmonary and subcutaneously-administered insulin on diabetic rabbits given bolus dextrose (500 mg/kg). ● subcutaneous administration of insulin (2 U kg^{-1} rbw); ○ pulmonary-administered insulin (6 U kg^{-1} rbw).

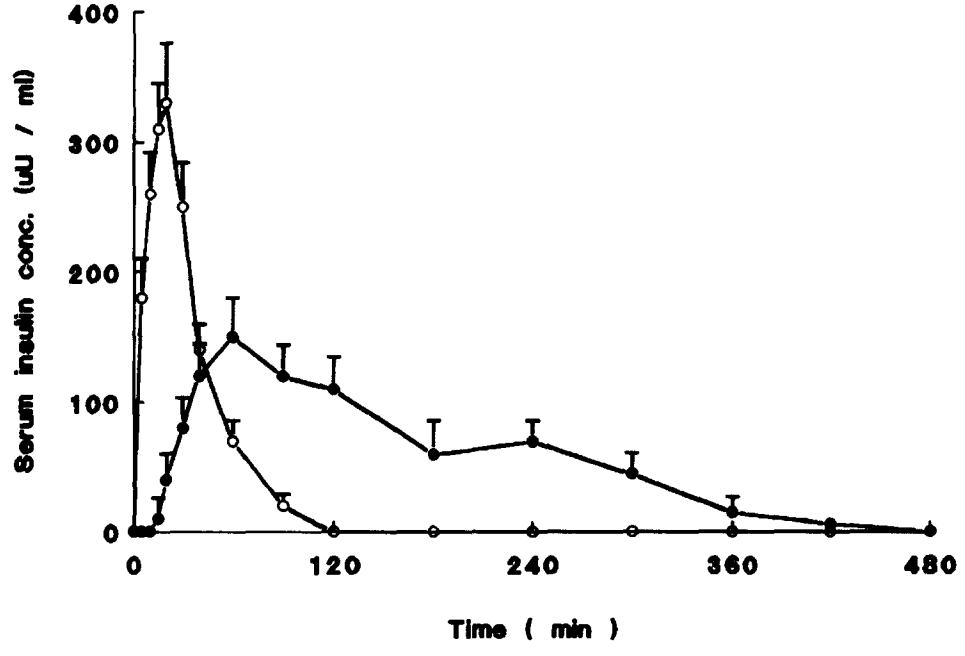

Fig. 5. Comparison of serum insulin levels for pulmonary and subcutaneously-administered insulin on diabetic rabbits given bolus dextrose (500 mg/kg). ● subcutaneous administration of insulin (2 U kg^{-1} rbw); ○ pulmonary-administered insulin (6 U kg^{-1} rbw).

ministered dose), the bioavailability and biological response compared with SAI could therefore corrected to 50% and 65%, respectively.

4. Conclusion

The study reveals that pulmonary administration of insulin is an effective rapid and safe route to reduce plasma glucose concentration in diabetic rabbits. The effect was shown to depend on the nebulized dose concentration and its ability to saturate the insulin-specific receptor sites due to the rapid onset for attaining maximum peak concentration, whereas depending on the longer duration for SAI.

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