

Intrapulmonary administration of insulin to healthy volunteers

J. H. JENDLE & B. E. KARLBERG

From the Department of Internal Medicine, Faculty of Health Sciences, Linköping University, S-58185 Linköping, Sweden

Abstract. Jendle JH, Karlberg BE (Department of Internal Medicine, Faculty of Health Sciences, Linköping University, S-58185 Linköping, Sweden). Intrapulmonary administration of insulin to healthy volunteers. *J Intern Med* 1996; **240**: 93–98.

Objectives. To study the biological effects of nebulized insulin, administered intrapulmonary, to healthy volunteers.

Design. A double-blind, randomized, controlled intervention study.

Setting. The department of Internal Medicine, University Hospital, Linköping, Sweden.

Subjects. Eight healthy, non-smoking volunteers, with a mean age of 28 (range 22 to 56) years.

Interventions. Regular human insulin 100 U mL⁻¹ (Actrapid®) or 0.9% saline was given randomly as an oral inhalation. Insulin was given in three different doses (40, 80 and 160 U). Aerosol was generated by a new jet nebulizer.

Main outcome measures. Blood glucose, serum insulin, and serum C-peptide.

Results. After the 160 U insulin dose the blood glucose concentration (mean $\pm$ SE) fell from 4.3 ± 0.2 to 2.8 ± 0.2 mmol L⁻¹ ($P < 0.001$), concomitant with an increase in mean serum insulin concentrations, rising from 9.5 ± 1.5 to 26.1 ± 2.5 mU L⁻¹ ($P < 0.001$). Serum C-peptide concentrations simultaneously decreased from 0.48 ± 0.03 to 0.12 ± 0.02 mmol L⁻¹ ($P < 0.001$). All changes were dose dependent. No adverse reactions were noted and no significant changes in lung function tests.

Conclusions. Intrapulmonary insulin administration to healthy subjects can induce a significant hypoglycaemia and cause a clinically relevant increase in serum insulin concentrations. If similar results can be obtained when administering insulin to diabetic subjects, this insulin administration route can be a future complement to certain groups of patients.

Keywords: aerosol, C-peptide, insulin, nebulization, oral inhalation.

Introduction

The direct delivery of drugs to the respiratory tract using nebulized aerosols has become an important component in drug therapy. During the past 20 years several investigators have reported in both animal and human studies that various peptide drugs pass through the respiratory epithelium with their bioactivity intact [1–4]. In fact, systemic absorption of insulin given by aerosol into the respiratory tract was first described as early as 1925 [4]. Previous investigations have shown this regime of drug delivery to have a low efficacy with an estimated retention, in the peripheral areas of the lungs, of only 10% of the amount delivered [2, 3]. The development

of new, high-efficacy nebulizers provided a much higher degree of drug retention, around 36% [5, 6] thus making the oral inhalation route for peptide drugs feasible.

The purpose of the present study was to evaluate the biological effects of intrapulmonary insulin administration to healthy volunteers using the new nebulizer.

Subjects and methods

Subjects

In this blind, randomized study eight healthy volunteers were randomly assigned to treatment with

active substance with three different doses of regular human insulin (Actrapid®, Novo Nordisk A/S, Bagsvaerd, Denmark) and placebo. Every volunteer received all four treatments in random order. Randomization was made using the Latin square design according to the statistics program Med Stat® (Astra A/S, Copenhagen, Denmark). Inclusion criteria for the study was non-obese, non-smoking volunteers with no current medication apart from contraceptives, nor any history of heart- or lung disease, nor of any atopy. A normal chest X-ray was required. The volunteers were aged 22 to 56 years (mean $\pm$ SD = 28.1 ± 11.5 years). Body mass index ranged from 19 to 23 with a mean $\pm$ SD of 22.0 ± 1.3 (kg m⁻²). On arrival, subjects had their baseline lung function tested. Measurements were performed using the Jaeger Masterlab, version 1.5 (Erich Jaeger GmbH, Würzburg, Germany). Vital capacity (VC), forced expiratory volume during the first second (FEV 1.0), forced expiratory volume (FEV %), peak expiratory flow (PEF), total lung capacity (TLC), and diffusion capacity (TLCO) were all assessed. These parameters were once more analysed after completion of the study. PEF was also analysed before and after each treatment using a Mini Wright® peak flow meter (Air Med, Clement Clark Int. Ltd., Harlow, UK). Insulin administrations were performed between 08.00 and 11.00 hours after a fasting period of 12 h. Each volunteer was given the four treatments on different occasions, with a minimum interval of 48 h between two treatments. They thus acted as their own control.

The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the Local Ethics Committee and the Swedish Medical Products Agency. Every volunteer gave informed consent before participation.

Insulin aerosol generation and delivery

Aerosol was generated by a jet nebulizer (MAXIN®, Clinova Medical AB, Malmö, Sweden) with a mean mass diameter (MMD) of nebulized insulin particles of 4.2 ± 0.2 μ m [6]. Regular human insulin, 100 U mL⁻¹ (Actrapid®, Novo Nordisk A/S, Bagsvaerd, Denmark) was used and diluted to appropriate concentrations (20, 40 or 80 U L⁻¹). Insulin was diluted with 0.9% saline solution to a total volume of 2 mL. Dilution and masking was made by the hospital pharmacy. The amount of

aerosol released from the nebulizer was estimated by weighing the nebulizer before and after nebulization.

The subjects inhaled the aerosol, in a sitting position, during normal tidal breathing, for 7–8 min. Nostrils were occluded by a nose clip and the subjects inhaled the aerosol directly from the nebulizer through a mouthpiece.

Laboratory procedures

Blood samples were drawn from an indwelling catheter in a cubital vein and extracted at regular intervals, from the start of nebulization (0, 10, 20, 30, 40, 50, 60, 75, 90, and 120 min).

Venous blood glucose concentrations were measured by a photometric reaction (glucose dehydrogenase method) using a Hitachi 717 chemical analyser (Boehringer Mannheim, Scandinavia AB, Stockholm, Sweden). In concurrent samples, blood glucose concentration was also analysed at bedside by the Accutrend® glucose meter (Boehringer Mannheim, Scandinavia AB, Stockholm, Sweden).

The serum was separated by centrifugation at 160 *g* for 10–15 min and stored at -20°C pending analysis [7]. Serum insulin concentrations were measured with a commercially available radioimmunoassay (RIA), ¹²⁵I-(TYR A19)-Human insulin (Novo Nordisk A/S, Bagsvaerd, Denmark) [8]. Serum C-peptide concentrations were prepared prior to analysis as for the insulin samples and measured with a commercially available RIA-kit, ¹²⁵I-Human-TYR-C-peptide (Novo Nordisk A/S, Bagsvaerd, Denmark) [9].

Statistical analyses

Data are given as mean $\pm$ standard deviation (SD) for clinical variables, and as mean $\pm$ standard error (SE) for error bars in graphs. Differences were analysed by two-way repeated measures analysis of variance (ANOVA) and paired two-tailed Student's *t*-test. Wilcoxon sign rank test was used when comparing the lung parameters before and after treatment. Differences were considered to be statistically significant when the *P*-value was < 0.05 . All calculations were performed on a Macintosh LC computer with the statistics program Abacus Concepts, Statview® 4.02 (Abacus Concepts, Inc., Berkeley, USA).

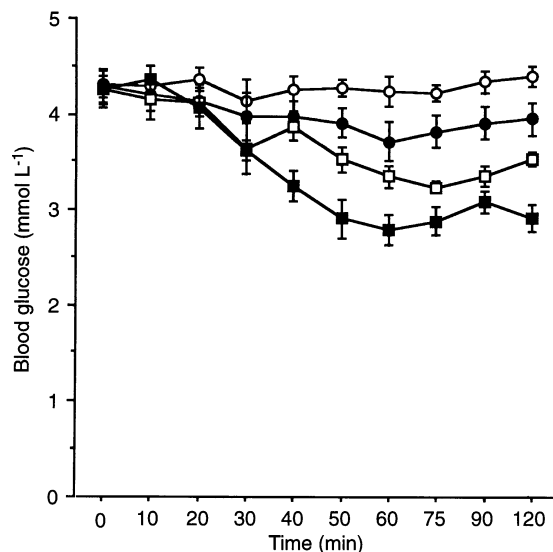


Fig. 1 Blood glucose concentrations after intrapulmonary administration of insulin (●, 40; □, 80; ■, 160 U respectively) or placebo (○). Data are given as mean $\pm$ SE.

Results

Blood glucose

Mean blood glucose concentrations fell significantly in a dose dependent fashion. The blood glucose concentration fell from 4.3 ± 0.2 to 2.8 ± 0.2 mmol L⁻¹ ($P < 0.001$), reaching the lowest level at 60 min after administering 160 U of nebulized insulin. After inhaling the 80-U insulin dose, the blood glucose concentration fell from 4.3 ± 0.2 to 3.2 ± 0.1 mmol L⁻¹ ($P < 0.001$) reaching the lowest level at 75 min and after 40 U of insulin, the blood glucose level fell from 4.3 ± 0.2 to 3.7 ± 0.2 mmol L⁻¹ ($P < 0.001$) reaching the lowest level at 60 min. The highest dose (160 U) lowered mean blood glucose concentration $34.5 \pm 0.5\%$ below baseline, as shown in Fig. 1.

Insulin

Concordantly with the blood glucose fall, a significant and dose-dependent rise in the mean serum insulin concentrations was noted after treatment with the different insulin doses. Serum insulin levels increased from 9.5 ± 1.5 to 26.1 ± 2.5 mU L⁻¹ ($P < 0.001$) reaching a plateau value after 30 min and the peak value after 60 min when administering the 160 U dose. After inhaling the 80-U insulin dose, serum insulin levels increased from 8.5 ± 0.9 to 17.3 ± 1.4 mU L⁻¹ ($P < 0.001$), with the peak value

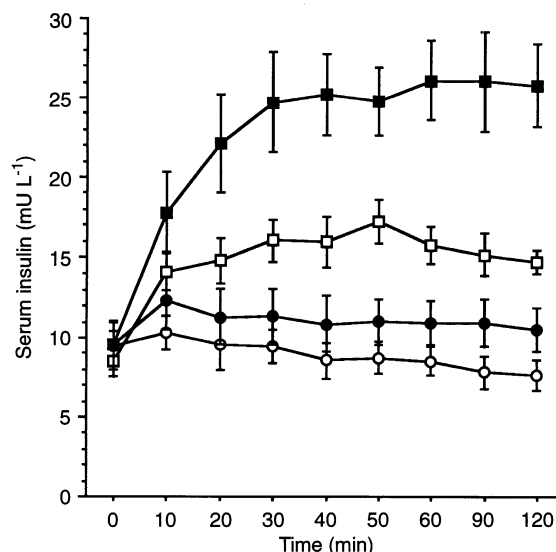


Fig. 2 Serum insulin concentrations after intrapulmonary administration of insulin (●, 40; □, 80; ■, 160 U respectively) or placebo (○). Data are given as mean $\pm$ SE.

being attained after 50 min. After the lowest insulin dose, 40 U, the serum insulin levels increased from 9.5 ± 1.4 to 12.3 ± 1.8 mU L⁻¹ ($P < 0.001$) (see Fig. 2).

C-peptide

Mean serum C-peptide concentrations fell significantly and in a dose-dependent fashion, from 0.48 ± 0.03 to 0.12 ± 0.02 nmol L⁻¹ ($P < 0.001$) after inhaling the 160 U dose, and from 0.49 ± 0.05 to 0.20 ± 0.02 nmol L⁻¹ ($P < 0.001$) after inhaling the 80 U dose. After inhaling the lowest insulin dose, 40 U, the fall was from 0.53 ± 0.07 to 0.25 ± 0.03 nmol L⁻¹ ($P < 0.001$), as shown in Fig. 3.

Drug output

The mean amount of drug released from the nebulizer was 1.9 ± 0.05 mL. The insulin released from the nebulizer was $95 \pm 2.5\%$ of the dose initially deposited in the nebulizer.

Adverse reactions

No significant changes in pulmonary function tests were seen, except for a small increase in total lung capacity, TLC changed (mean $\pm$ SE) from 6.11 ± 0.35 to 6.30 ± 0.34 L, $P = 0.02$.

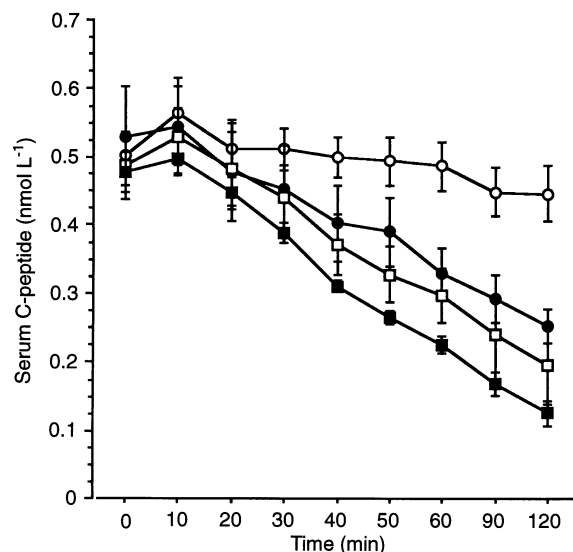


Fig. 3 Serum C-peptide concentrations after intrapulmonary administration of insulin (●, 40; □, 80; ■, 160 U respectively) or placebo (○). Data are given as mean $\pm$ SE.

The peak expiratory flow (mean $\pm$ SD) before treatment was 608 ± 105 L min⁻¹, CV 0.2, and after treatment 607 ± 108 L min⁻¹, CV 0.2.

Discussion

Our study shows that in normal subjects, insulin, administered by oral inhalation, is well absorbed and induces a significant hypoglycaemia. Serum insulin concentrations also rose concomitantly in a dose-dependent fashion. The amount of insulin released from the nebulizer was $95 \pm 2.5\%$ of the dose deposited in the nebulizer, corresponding well with previous studies using this nebulizer [6]. In an animal model, the delivery of nebulized insulin, to intubated and mechanically ventilated pigs, was $88.9 \pm 5.3\%$, when inhaling a volume of 1 mL. The fraction of insulin retained in the animals respiratory tract was calculated to be $36.1 \pm 8.8\%$ [6], using the same inhalation device as in the present study.

In contrast to our study, earlier investigations on intrapulmonary and intranasal insulin administration have not been randomized, nor blinded and the materials have also been fairly small with as few as six subjects receiving treatment on only one occasion [10, 11], except for Salzman *et al.* who investigated a total of 45 patients with insulin-dependent diabetes mellitus (IDDM) [12].

Elliott *et al.* [10] and Wigley *et al.* [13] found that only one diabetic patient in the two studies combined, achieved a normal blood glucose level after the inhalation of aerosolized insulin. The authors indicated that the amount of insulin delivered was 250 U (0.5 mL of 500 U mL⁻¹) and 50 U (50 breaths, each with 2 μ L of 500 U mL⁻¹) respectively, and concluded that inefficient absorption of insulin across the alveolar membrane could account for their poor results. Elliott *et al.* [10] used a patient-activated nebulizer when administering aerosolized insulin (conventional human semi-synthetic, 500 U mL⁻¹) to six diabetic children. This patient-activated nebulizer was rather complex, with some malfunctions, and required individual calibration. Laube *et al.* [14] normalized plasma glucose levels in five of six non-obese patients with non-insulin-dependent diabetes mellitus (NIDDM) after oral inhalation of aerosolized insulin. A jet nebulizer was being used and loaded with 2 mL of porcine insulin solution 500 U mL⁻¹. The output of the nebulizer was interfaced with a 700-mL holding chamber (spacer) to reduce oropharyngeal deposition of the drug [15]. The insulin dose was correlated to the subjects body weight (BW). A calculated dose of approximately 1.0 U kg⁻¹ body weight was administered. In our study we only used a maximum of 160 U of insulin deposited in the nebulizer and there was no need for correlation between the inspiration and the nebulizing since the nebulizer uses continuous nebulization. The use of an expandable bag connected to the nebulizer will collect aerosol during the expiratory phase of the breathing cycle and reduces drug losses. It also permits settling of larger droplets and minimizes deposition in the central airways. The nebulizer used in the study is very easy to handle and requires no individual calibration. Insulin absorption across the lung surface peaked at 60 min in the present study. Galloway *et al.* [16] showed that the average time to peak insulin concentration when administering 500 U mL⁻¹ insulin subcutaneously, at a dose of 0.25 U kg⁻¹ of body weight (BW), was 144 ± 42 min in normal fasting subjects. Salzman *et al.* [12] reported rapid insulin absorption across the nasal mucosa, using porcine insulin (500 U mL⁻¹) in combination with a surfactant. The insulin doses used were 0.5 and 1.0 U kg⁻¹ of the patient's BW. Serum insulin levels peaked after 15 min using this nasal route for insulin administration.

In the present study the serum insulin concen-

trations did not return to baseline during the 120-min study period. This could be due to hexamer formation of the insulin molecule [17] causing a slower absorption through the alveolar membrane. This molecular transport has been shown to be inversely correlated to the molecular weight of the compound inhaled [18]. An appealing approach would be to investigate the absorption of insulin when using an insulin analogue without spontaneous hexamer formation, i.e. lys(B28)pro(B29) insulin.

When administering exogenous insulin the serum C-peptide concentrations consequently fell with the rise of serum insulin concentrations. The time lag between the nadir of the C-peptide concentration and the peak concentration of insulin could be due to the longer half-life of C-peptide, which is approximately 20 min compared to that of insulin (2–3 min) [19, 20].

Sensitization to insulin, secondary to intrapulmonary administration of the drug has been proposed to be a theoretical risk. However, Patton *et al.* [21] demonstrated that homologue peptides are not prone to elicit an immunologic response in the lung.

Histological examination of porcine lungs after receiving repetitive intrapulmonary insulin administration has shown a histology similar to untreated control lungs [6].

Delivery of insulin through the lungs was very well tolerated by the volunteers with no adverse signs or subjective symptoms being reported. Nor were significant changes in lung function tests found when comparisons were made before and after treatment, except for a small increase in total lung capacity. A very good correlation between the PEF before and after treatment was also seen.

This new regime of insulin administration could, if proven safe and efficient, be a complement in the treatment of diabetic subjects, especially for subjects with a visual handicap or for the disabled with difficulties in giving subcutaneous injections. Another potential patient group is diabetic patient with a fear of needles, and perhaps children too.

Additional studies are necessary to fully elucidate the safety and clinical utility of intrapulmonary insulin administration, both *in vivo* and *in vitro*. The reproducibility of glucose responses, and the appropriate dosages of aerosolized insulin, required to normalize plasma glucose concentrations in diabetic

subjects, also needs to be explored. We believe, however, that the results obtained in the present study, performed on healthy volunteers, prompts further investigations in diabetic subjects.

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Correspondence: Johan Jendle MD, Department of Internal Medicine, University Hospital, S-58185 Linköping, Sweden.