Treating Diabetes With Aerosolized Insulin*

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Because of the pain, inconvenience, and disruption of lifestyle associated with the injection of insulin, many patients with diabetes are noncompliant in terms of treatment regimens that require daily multiple injections. To eliminate the pain and to improve treatment outcome, there has been increasing interest in the development of aerosolized insulin to replace subcutaneously (SC) delivered formulations. Recent studies in human volunteers have shown that when aerosolized insulin is effectively delivered to the alveolar region of the lung, absorption rates and decreases in glucose levels are similar to those achieved with SC-delivered insulin during the fasting state. Other human trials have shown that inhaled insulin also effectively controls postprandial glucose levels. Aerosolized insulin is well-tolerated, and there is no evidence of irritation, hypoglycemia, or changes in pulmonary function when administered over short periods. At present, limitations in the delivery device result in less efficient administration of insulin aerosol compared to SC dosing. However, new devices and different formulations of insulin, which are currently under development, should improve the efficiency. It is likely that the treatment of diabetes with aerosolized insulin will provide an effective alternative means for controlling plasma glucose levels in diabetic individuals. Aerosolized insulin also will serve as a developmental model for this route of administration for a number of other therapeutic peptides that are currently administered by injection only.

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Key words: aerosol; diabetes; insulin; intrapulmonary delivery

Abbreviations: NIDDM = non-insulin-dependent diabetes mellitus; SC = subcutaneous, subcutaneously

Of the 8 million individuals in the United States with diagnosed diabetes mellitus, between 500,000 and 1 million have type I, or insulin-dependent diabetes mellitus. Type I patients require injections of insulin for glycemic control from the time of diagnosis, and it is now recommended that they self-inject three or more times each day for optimal glucose control. An additional 7.5 million patients have type II, or non-insulin-dependent diabetes mellitus (NIDDM). Initially, their diabetes can be controlled with oral hypoglycemic drugs, changes in diet, and exercise. However, as their disease progresses, they too will require the injection of insulin to control their glucose levels. Thus, there is a large population of patients with diabetes who require, or will require, insulin by injection at some point in their lives. Many patients, particularly those who must inject themselves several times each day, find this regimen inconvenient, disruptive, and painful. As a result, patient compliance with treatment regimens that require insulin injection is often compromised, leading to potentially suboptimal treatment outcomes. Since inhaling an aerosolized drug is not associated with pain, beginning as early as 1925, a number of investigators have examined the possibility of administering insulin by aerosol as an alternative to injection for delivery to the systemic circulation.

While the delivery of insulin by inhalation has been most frequently and thoroughly studied, the same rationale of improving patient compliance, convenience, and comfort has led to research into inhalational therapy for other drugs that can be administered only by injection. These include the following proteins and peptides: calcitonin for Paget's disease and osteoporosis; leuprolide acetate for prostate cancer, breast cancer in postmenopausal women, and infertility; growth hormone-releasing factor for the treatment of pituitary short stature; and morphine for analgesia. Along with insulin, these peptides appear to be good candidates for aerosol delivery and systemic treatment of diseases because of their relatively high bioavailability after inhalation compared to other proteins, and the degree of bioavailability seems to be associated with molecular weight. This relationship is demonstrated in Figure 1, which shows the bioavailability of a number of therapeutic peptides in terms of their molecular weight. Bioavailability in the plasma is shown as a percentage of the dose of the drug that is deposited in the lungs of various animals and humans after inhalation, relative to subcutaneous (SC) injection. In the human studies, bioavailability decreases as molecular weight increases, such that the bioavailability of leuprolide is greater than that of calcitonin, which is greater than that of insulin.

INTRANASAL VS INTRAPULMONARY ADMINISTRATION OF INSULIN AEROSOL

From a historical point of view, investigators have focused on the possibility of developing aerosolized drugs that could be delivered by either intranasal or intrapulmonary administration. Intranasal administration often appears particularly promising because of the accessibility of the nasal cavity. The disadvantage to intranasal delivery for systemic absorption is that the surface area for absorption is relatively small (approximately 150 cm²) compared to that of the alveolar region of the lung, which has a resorbptive surface of approximately 75 m². The other disadvantage to intranasal administration is that the drug needs to be absorbed quickly (in approximately 15 to 20 min) or it will be removed to the back of the nasopharynx by the rapid mucociliary clearance mechanisms in the nose and swallowed. This is again in contrast to the fate of drugs that are deposited in the alveolar region of the lungs where

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drugs have a longer residence time because mucociliary clearance mechanisms are minimal. Although suboptimal in terms of the systemic administration of most drugs, some investigators have reported significant reductions in the plasma glucose levels of patients with diabetes following intranasal delivery of insulin aerosol. Moses et al and Salzman et al showed that a dose of 0.5 to 1.0 U/kg insulin aerosol delivered to the nose lowered plasma glucose levels by approximately 50%. However, because of the need to overcome the rapid mucociliary clearance mechanisms of the nose, these doses were effective only when a surface-active material was added to the aerosolized solution. These compounds enhanced absorption but also resulted in patient complaints of irritation to the nose and nasal congestion. For this reason, intranasal administration does not appear to be the route of choice for delivering aerosolized insulin.

As noted above, optimal systemic delivery of aerosolized drugs by the intrapulmonary route requires that the aerosol be targeted for deposition in the alveolar region of the lungs. In addition to having minimal mucociliary clearance mechanisms and comprising 95% of the resorptive surface of the lungs, the alveolar region has an extremely thin (0.1 μm) and vesiculated cell barrier to promote the absorption of drugs that are deposited there. Targeting of the alveolar region is best accomplished by oral inhalation of an aerosolized medication. However, the oral administration of aerosolized drugs also has disadvantages because particles > 5 μm typically impact at the back of the mouth and do not penetrate to the lower airways. In addition, particles associated with high aerosol velocities, such as those that are generated by propellants, and particles that are inhaled during high inspiratory flow

**Figure 1.** The bioavailability of several therapeutic peptides of increasing molecular weight (MW) relative to SC injection is shown. Bioavailability is expressed as a percentage of the dose of the drug that is deposited in the lungs of various animal species (solid symbols) and human volunteers (open symbols). The solid squares and circles represent data obtained from rodents. The solid triangles represent data obtained from monkeys. G-CSF = granulocyte-colony-stimulating factor; hGH = human growth hormone; PTH = parathyroid hormone. Source: R.K. Wolff, PhD; personal communication (July 11, 2000).
rates (ie, > 30 L/min) also will impact at the back of the mouth and be lost to the lower airways. These hurdles to drug delivery to the lung could explain why early studies involving the delivery of insulin by the intrapulmonary route demonstrated little in the way of efficacy. For example, in 1987 Elliott et al10 found that insulin aerosol was absorbed through the respiratory epithelium of human volunteers and was biologically active because plasma insulin levels increased and glucose levels decreased. However, the effect on plasma glucose levels was not statistically significant, and only one subject demonstrated a therapeutic effect. These findings were similar to those reported in earlier studies by Gaensslen2 and Wigley et al.3 However, the effect on plasma glucose levels was not statistically significant, and only one subject demonstrated a therapeutic effect. These findings were similar to those reported in earlier studies by Gaensslen2 and Wigley et al.3 Again, in each of those studies, only one subject achieved normal plasma glucose levels following the intrapulmonary delivery of aerosolized insulin.

It is likely that these failures to achieve euglycemia with intrapulmonary delivery of insulin were due to underdosage, probably arising from the loss of drug in the oropharynx or in the delivery device. For technical reasons, none of these studies quantified the dose of insulin that was available for inhalation at the mouth of the patient, or the amount of insulin that deposited within the respiratory tract. In the study by Wigley et al,11 250 U insulin was nebulized to dryness and was delivered via a traditional jet-type nebulizer. The researchers assumed at that time that they were delivering 100% of the drug to the lungs. However, it later became apparent that only about 10% of a nebulized drug is actually delivered and deposited in the lungs.12-14 Elliott and coworkers10 quantified the drug dosage that was available for inhalation at the outlet of the nebulizer but did not account for possible losses between the nebulizer and patient (ie, losses in the spacer device they used or in the delivery system tubing).

By the late 1980s, it was clear that aerosol particle size, aerosol velocity, and inspiratory flow rate were major determinants of aerosol delivery to the lungs. Taking these factors into account, we decided to reexamine the possibility of normalizing plasma glucose levels in patients with diabetes by intrapulmonary delivery of aerosolized insulin.15 In that study, aerosolized insulin or placebo was generated by a raindrop nebulizer (Puritan Bennett; Lenexa, KS) and was administered by oral inhalation following a 12-h fast. The raindrop nebulizer was chosen because it produced particles small enough to avoid impaction in the mouth, thus minimizing oropharyngeal deposition and maximizing delivery to the lung. The outlet of the nebulizer was attached to a spacer device that increased the distance between the subject’s mouth and the nebulizer. This reduced aerosol velocity and additional losses in the mouth. The insulin dose was approximately 1.0 U/kg of 500 U/mL regular pork insulin. Patients inspired the aerosol from residual volume to total lung capacity to promote deposition in the lung periphery. The inspiratory flow rate was regulated to approximately 17 L/min to further minimize impaction and loss of insulin in the mouth and larger conducting airways.

Prior to inhaling aerosolized insulin, six patients with NIDDM inhaled a saline solution aerosol containing the radioisotope technetium-99m, which was generated and inhaled as described above. This procedure provided a method for quantifying how much aerosol deposited in the oropharynx and lungs with this delivery system. Gamma camera scans of the oropharynx and lungs indicated that losses in the oropharynx were low and that most of the drug penetrated into the lung (Fig 2). Deep penetration of the drug into the smaller airways and alveoli was evidenced by well-defined lung margins. For these study subjects, the deposited fraction below the larynx ranged from 50 to 93% of the inhaled dose, and the mean (± SD) deposition was 79 ± 17%.15

A total of 10 patients with NIDDM then inhaled a dose of 1 U/kg insulin aerosol that was generated and delivered in the same way as the radiolabeled saline solution aerosol. The mean age of the NIDDM patients was 52 years, and their mean body mass index was 26.90. All patients were naive to insulin. None of the patients were receiving oral hypoglycemic agents, which were discontinued 4 days prior to the patients’ arrival at the laboratory. Patients fasted for 12 h before each study visit. All subjects were nonsmokers and had normal pulmonary functions. Blood was collected for approximately 3 h after the inhalation of the insulin in order to measure changes in plasma insulin and glucose levels. Figure 3 shows a typical time-response curve for one of the study subjects. At baseline, the glucose level for this subject was approximately 300 mg/dL. The level declined and was within the normal range (ie, < 120 mg/dL) at 160 min after drug administration. In this subject, insulin levels reached their peak in the plasma at approximately 20 min postinhalation.

Figure 2. Gamma camera scans showing the deposition of the radioaerosol in the oropharynx (top) and lungs (bottom) of a patient with NIDDM. Reprinted with permission from Laube et al.15
The mean maximal decrease from baseline in plasma glucose levels for all 10 patients was 52 ± 9% (Table 1). This was significantly greater than the decrease that was observed in seven of the patients who inhaled a placebo aerosol. The average maximal decrease in glucose following placebo inhalation was 14 ± 7%. Nine of the 10 patients also demonstrated normal glucose levels after inhaling this dosage of insulin aerosol (Table 1). No patients showed any signs or symptoms of airway irritation after either placebo or insulin aerosol inhalation, and there were no signs or symptoms of hypoglycemia.

**Control of Postprandial Glucose With Inhaled Insulin**

The results indicated that fasting glucose levels could be normalized by the intrapulmonary delivery of 1.0 U/kg insulin aerosol in patients with NIDDM. Nevertheless, the dose of aerosolized insulin needed to regulate postprandial glucose levels below diabetic levels (i.e., < 200 mg/dL) was unknown. For this reason, we undertook another study that tested the hypothesis that 1.5 U/kg aerosolized insulin administered when glucose levels are within the normal range and 5 min before a meal will control postprandial glucose levels below diabetic levels.

Seven nonsmoking patients with NIDDM and normal pulmonary functions participated in the study. After blood glucose levels had been normalized by the inhalation of 1.0 U/kg insulin aerosol, subjects inhaled 1.5 U/kg insulin aerosol 5 min before ingesting a test meal containing 681 calories (Table 2). Aerosol was generated from 500 U/mL regular pork insulin and was delivered to the mouth (Medicator delivery system; Healthline Medical; Baldwin...
During aerosolization, subjects inspired slowly and deeply to promote penetration of the aerosol into the lung periphery. Blood samples were collected for 3 h after inhalation and were analyzed for plasma glucose, insulin, and C-peptide levels. On another visit, subjects inhaled a placebo aerosol, which was generated and delivered in the same way as the insulin aerosol, 5 min before the same test meal.

Figure 4 shows that a dose of 1.5 U/kg aerosolized insulin was significantly more effective in controlling postprandial glucose levels at 1, 2, and 3 h after the test meal than placebo aerosol. The greatest disparity between placebo and inhaled insulin occurred at 2 h. At that time point, six of the seven patients had achieved glucose levels of <200 mg/dL, which is considered to be below the diabetic level, while four of the seven patients achieved glucose levels within the normal postprandial range (ie, <145 mg/dL).16

It is likely that these decreases in glucose level were attributable to the exogenous administration of insulin aerosol rather than to postprandial pancreatic stimulation for the following two reasons: 1-h insulin levels were significantly higher following aerosolized insulin administration compared with placebo (Fig 5); and C-peptide levels at 1, 2, and 3 h postinhalation were significantly lower after inhalation of insulin aerosol compared to placebo (Fig 6). Since insulin and C-peptide are cosecreted by pancreatic β-cells in equimolar amounts, the C-peptide level is considered to be an indicator of endogenous insulin secretion.17 The reduction in C-peptide levels following aerosolized insulin administration indicated that endogenous insulin had been suppressed by the intrapulmonary delivery of insulin aerosol in these patients.

**Intrapulmonary vs SC Delivery of Insulin**

A few investigators18,19 have measured the time to peak insulin level in healthy fasted volunteers after intrapulmonary delivery of nebulized insulin. In those studies, the average time to peak insulin levels varied between 50 and 60 min18 and 15 to 20 min.19 When compared with the results from a different group of healthy subjects who were injected SC with insulin, it appeared that insulin was absorbed more rapidly through the lungs, as the average time to peak insulin levels with SC delivery was 144 min.20 Heinemann and colleagues21 extended those earlier findings by comparing the time to peak insulin levels after the inhalation of a dry powder insulin aerosol vs SC injection of insulin in matched healthy subjects. Confirming what had been reported previously in unmatched groups of subjects, they found that the time to maximal insulin levels averaged 24 min with the inhalation of the powder vs an average of 106 min for SC injection.21 A similar comparison was recently completed22 in matched healthy volunteers using a liquid formulation of insulin aerosol. Twelve volunteers inhaled the insulin aerosol (Humulin Regular U-500; Eli Lilly and Company; Indianapolis, IN). On another day, the volunteers were injected SC with insulin (Humulin U-100 Ultralente; Eli Lilly and Company). The time to peak insulin levels was earlier with inhaled insulin, averaging approximately 50 min compared to SC injection, which averaged 85 min. Taken together, these data suggest that insulin absorption through the lungs may be faster than absorption after SC injection in healthy subjects.

We have examined the time to peak insulin levels after intrapulmonary delivery of nebulized insulin aerosol in patients with diabetes.15 In one study,15 we found that the time to the peak insulin level in patients with NIDDM averaged 40 min. That time was similar to what has been reported by Jendle and Karlberg23 in another group of patients with NIDDM. The time to peak insulin levels after SC injection of insulin was not quantified in either of

<table>
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<tr>
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<td>681 calories</td>
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<td>50 g corn flakes cereal</td>
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</tr>
<tr>
<td>90 g banana</td>
<td>88 g carbohydrate (49%)</td>
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<tr>
<td>100 g skim milk</td>
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<td>70 g American cheese</td>
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**Figure 4** Mean (± SD) glucose levels at 1, 2, and 3 h postmeal for seven patients with NIDDM who inhaled 1.5 U/kg insulin aerosol (solid bars) or placebo aerosol (open bars) 5 min before ingesting the test meal. Glucose levels were significantly lower at each of the time points after inhaling insulin compared to placebo aerosol.

**Figure 5** Mean (± SD) insulin levels at 1, 2, and 3 h postmeal for seven patients with NIDDM who inhaled 1.5 U/kg insulin aerosol (solid bars) or placebo aerosol (open bars) 5 min before ingesting the test meal. Insulin levels were significantly higher at the 1-h time point after inhaling insulin compared to placebo aerosol.

**Table 2—Standardized Test Meal**

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these studies. However, we have completed that comparison in another group of patients. 24 Seven fasting patients with NIDDM inhaled 1.0 U/kg nebulized regular pork insulin aerosol by the intrapulmonary route on one occasion and were injected SC with 0.1 U/kg insulin on another occasion. 24 SC and aerosol administration of insulin resulted in similar decreases in glucose levels. The average maximum decrease in glucose levels was 54 ± 16% after insulin inhalation and 40 ± 20% after SC injection. Although there was a trend toward a faster time to peak insulin level with the inhaled drug, the mean times to peak insulin levels were not statistically different for the two modes of insulin administration, averaging 43 ± 16 min for the aerosol and 64 ± 40 min for SC dosing. 24 These data suggest that insulin absorption through the lungs may not be faster than absorption after SC injection in patients with NIDDM.

It is unclear why the time to peak insulin levels after the two modes of administration appears to be similar in patients with NIDDM, whereas the peak in plasma insulin seems to occur earlier following inhalation of insulin in healthy subjects. One explanation could involve intersubject variability. It has been shown that intersubject variability in terms of time to peak insulin level is high for both inhaled and injected insulin. 25 Therefore, comparisons between different groups of subjects who are dosed by different modes of delivery may not be appropriate. Another explanation may be that absorption of regular pork insulin (which was used in the studies of patients with diabetes) across the respiratory epithelium differs from that of recombinant human insulin (which, presumably, was used in the studies of healthy volunteers). Alternatively, it may be that the number of patients with NIDDM in the most recent study (n = 7) was too small to detect a significant difference between the two treatment modalities. 24 Finally, it could be that the disease affects the absorption mechanism such that the absorption of insulin across the lung epithelium takes longer in patients with the disease than in healthy individuals. Additional studies in matched patients with NIDDM are necessary to clarify these findings.

\[ \text{Relative Bioavailability of Aerosolized Insulin} \]

Three of the studies 10,19,24 of aerosolized insulin in human subjects have reported mouth-to-blood efficiencies ranging from 15 to 25%. This means that the average bioavailability of the inhaled dose of aerosolized insulin in these studies was approximately 20%, relative to the dose delivered by SC injection. It is important to note that these results reflect the relative bioavailability of nebulized insulin. It is not known how bioavailability is affected by changes in formulation (e.g., reformulating liquid insulin as insulin powder). These bioavailability studies also were conducted in nonsmokers. This is important because it appears that smoking significantly affects the bioavailability of nebulized insulin aerosol. Kohler et al 19 reported that the bioavailability of nebulized insulin was significantly enhanced in smokers compared to nonsmokers. This may have been due to damage to the lung mucosa as a result of smoking, making the lung more "leaky" and allowing more drug to enter the systemic circulation. It is also interesting to note that although the relative bioavailability was increased as a result of smoking, the time to peak insulin levels did not differ between smokers and nonsmokers. 19

The reduced bioavailability for aerosolized insulin relative to SC delivery may increase the cost of aerosolized insulin delivery. However, increases in cost may be offset by an increased demand for aerosolized insulin compared to SC delivery because of the elimination of the pain and discomfort associated with injection and because of potentially better control of blood glucose levels. Moreover, insulins with a higher bioavailability than regular pork insulin, which are also suitable for inhalation, should become available over time.

\[ \text{Safety of Inhaled Insulin} \]

To my knowledge, there have been no reports of toxicity due to inhaled insulin either in patients treated acutely or for short periods. In the treatment studies cited above, no respiratory or hypoglycemic adverse events were reported in patients who inhaled one or two doses of insulin. Also, Jendle and Karlberg 18,21 have examined the effect of the short-term administration of aerosolized insulin on the pulmonary functions of both healthy volunteers and patients with NIDDM. They found that postadministration measurements of peak expiratory flow did not differ significantly from values obtained before aerosol treatment.

In terms of treatment with inhaled insulin for longer periods of time, Ogden and colleagues 26 reviewed data from studies completed before 1996 and found no reports of adverse lung effects that were attributable to inhaled insulin that was administered in the long term for up to several weeks. In addition, the abstracts of three phase II studies, 27-29 each lasting 3 months, indicate that there were no changes in pulmonary function test results for the users of inhaled insulin in those studies.

Further studies will be needed to assess the possible development of immunogenicity and toxicity from prolonged exposure to the lung. However, it should be noted that long-term intrapulmonary delivery of insulin by the

\[ \text{Figure 6. Mean (± SD) C-peptide levels at 1, 2, and 3 h postmeal for seven patients with NIDDM who inhaled 1.5 U/kg insulin aerosol (solid bars) or placebo aerosol (open bars) 5 min before ingesting the test meal. C-peptide levels were significantly lower at each of the time points after inhaling insulin compared to placebo aerosol.} \]
Aerosolized insulin may address the issue of reducing the frequency of injections. However, the inhalation route has several challenges that require further investigation.

**Future Research**

Several issues must be addressed in order to make intrapulmonary delivery of insulin aerosol a feasible alternative to SC delivery. One of the most significant is that of the delivery system. The current generation of delivery devices, which includes conventional jet-type nebulizers with and without spacer devices or holding chambers, limits the efficient delivery of aerosolized insulin because most of the drug remains in the device and is not delivered to the lungs. In addition, these devices require compressors and electricity to generate the aerosol particles. Portable devices that do not require electricity and that deliver a high percentage of the drug to the lung will significantly enhance patient convenience and demand for this route of delivery.

New formulations for insulin aerosols are currently under development by several companies. Typically, these are either dry powder formulations or new formulations of solubilized insulin. Both formulations can be generated as particles of the optimal size for absorption into the alveoli (i.e., 1 to 3 μm in diameter). The dry powder or liquid insulin is loaded into unit packets and is released either mechanically or electronically into new portable and compact delivery systems. With the mechanical system, the insulin is released prior to inspiration into a small holding chamber from which the patient then inhales slowly and deeply. With electronic delivery, the insulin is released early during the patient’s inspiration at a predetermined flow rate of inspired air.

The effect of the patient’s baseline pulmonary functions on the deposition of aerosolized insulin within the lungs is another factor that will require further study. So far, most studies have enrolled subjects with normal pulmonary functions. Further data are needed regarding the delivery of aerosolized therapeutics to patients with compromised lung function since the degree of obstruction may determine how much of the inhaled dose deposits in the alveolar region and how much impacts and deposits in obstructed airways. This issue is likely to be of particular importance in patients who have both diabetes and cystic fibrosis, COPD, or asthma, since these diseases and the possibility of exacerbations may compromise drug delivery.

**Summary**

Aerosolized insulin is an important model for the systemic delivery of other types of therapeutic proteins. Like insulin, successful treatment outcomes with other peptides and proteins will hinge on the determination of the correct dosage and formulation for aerosolized delivery of each agent, as well as the development of optimal devices for maximizing the delivery of aerosolized medication to the alveolar region of the lung.

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