Expert Opinion

informa
healthcare

Non-invasive routes for insulin administration: current state and perspectives

Claudio González, Diego Kanevsky, Rubén De Marco, Guillermo Di Girolamo† & Silvina Santoro
†*Department of Pharmacology, School of Medicine, Universidad de Buenos Aires, Paraguay 2215, Piso 16, CP:C1121ABG, Ciudad Autónoma de Buenos Aires, Argentina*

Diabetes mellitus is a chronic disease that usually requires multiple insulin injections to achieve adequate glycaemic control. This represents a major cause of reduced compliance to treatment. Consequently, other routes for insulin administration have been explored. During recent years, much progress in the development of inhaled insulin has been made. Inhaled insulin has shown favourable properties, such as a rapid onset of action, improved bioavailability and good tolerability; thereby providing satisfaction and ease of administration. However, long-term safety of inhaled insulin needs to be assessed, and the cost would be higher than injectable insulin. Nasal, oral and transdermal insulins are undergoing early phases of pharmacological development. The purpose of this review is to describe the latest developments in the area of non-invasive routes for insulin delivery.

Keywords: diabetes therapy, nasal insulin, oral insulin, pulmonary insulin

Expert Opin. Drug Deliv. (2006) 3(6):763-770

1. Introduction

The Diabetes Control and Complications trial (DCCT) [1] has demonstrated that intensified metabolic control delayed the progression of chronic complications in patients with Type 1 diabetes. The Kumamoto [2] and UKPDS (United Kingdom Prospective Diabetes Study) [3] trials have also consistently proven that optimised glycaemic control reduces the incidence of microvascular end points in patients with Type 2 diabetes. In many patients with diabetes, adequate glycaemic control often requires multiple insulin injections per day.

Subcutaneous insulin injection has become a standard treatment for both Type 1 and 2 diabetes. A better knowledge of insulin kinetics within the subcutaneous tissue has allowed for the development of long-acting insulin preparations by co-crystallisation with protamine, or through complexation with zinc. As a result of an evolutionary process, insulin analogues that are rapid (e.g., aspartic, lyspro, glulysine) and long acting (e.g., glargine, detemir) were developed by modifying the insulin self-association behaviour, its precipitation or its albumin binding in the subcutaneous compartment. Long-acting analogues could simplify insulin treatment, and would induce less variable effects than native protracted preparations, such as NPH (Neutral Protamine Hagedorn) insulin. Insulin analogues that act faster and shorter than the human regular hormone seem to be at least as effective, and are probably safer, than the native-based compounds [4,5], allowing a more convenient and flexible control of prandial glucose excursions. Nevertheless, insulin injections can result in a barrier for adequate glycaemic control at times, and in many patients this is a cause of reduced compliance. Thus, many other routes for insulin administration have been explored to improve the patients' compliance, and to provide an acceptable delivery profile.

Preparations for oral, nasal or transdermal administration have been tried, and other routes have also been studied. Despite the preliminary results being erratic, some significant progress has been achieved in the last years. Pulmonary insulin delivery has been intensively studied since 1996, and the development of the concept is impressive, driving towards preparations that are very close to reach the market in several countries.

The purpose of the present review is to describe the latest developments in the area of alternative, non-invasive routes for insulin delivery, focusing on their rationale, potential advantages and their inconveniences.

2. Explored non-invasive routes for insulin delivery

2.1 Pulmonary insulin

The concept of pulmonary insulin delivery was explored soon after the discovery of this hormone. In effect, this route was evaluated with no success in 1924 [6]. Knowledge of this route of administration has improved since then.

Once inhaled, the lung surface allows an appropriate absorption of peptides. Alveolar surface (~ 100 m^2 [7]) is larger than many other absorptive areas, with the exception of the intestine (which is nearly 200 m^2 in area). The alveolar surface is well perfused, and the absorption across its thin membranes is rapid and probably less variable when compared with subcutaneous injection of regular insulin. Nevertheless, deposition in the deep lung is affected by the inhaled volume, the flow rate and the end-inspiration breath-holding pause [8] (high inhalatory flow rates are associated with an enhanced pharynx and tracheal deposition). It has also been clearly demonstrated that to effectively achieve deposition in the deep lung, the size of the particles containing the molecules to be absorbed is crucial. Large particles, with an aerodynamic diameter of > 10 µm, will be deposited in pharynx, and particles exhibiting an aerodynamic diameter < 0.5 µm could be exhaled with the air flux, not reaching the alveoli surface [9]. It should be noted that the aerodynamic diameter results from multiplying the geometric diameter by the square root of the particle's density; less dense particles have a reduced aerodynamic diameter. Optimal particle-aerodynamic diameter ranks between 1 and 5 µm. The aerosolisation time constitutes an additional factor that has an impact on the metabolic effect of inhaled insulin [8]. Thus, delivery technology is important to provide size and uniformity to the carrier particles.

The quality of the insulin formulation, of the efficiency of the device (inhaler performance), the proportion of the aerosol deposited in the deep lung and the extent of absorption are four main factors that are closely related to the clinical efficacy of pulmonary insulin administration.

Many different insulin preparations and delivery systems are under study, and at least one of these is very close to the market. These preparations have been formulated to deliver the hormone (regular human insulin) to be delivered as a dry powder or as an aerosol of liquid insulin.

2.1.1 *Dry powder formulations of insulin*

The method closest to market launch is based on dry powder insulin, which is stable, facilitates storage and avoids microbial contamination. The spray-drying process preserves the secondary structure of insulin [10]; thus, the pharmacological activity is not affected. The powder hygroscopicity was reduced to avoid deterioration by humidity. This system, constituted by a spray-dried insulin powder formulation, is called Exubera® (Pfizer), which is enclosed in a blister packet that is placed within the inhalation device. The patient presses a button that sets off the device to deliver an insulin steam into the main compartment. The patient inhales this insulin steam. Another mechanism employing a dry powder formulation of insulin is Technosphere® (MannKind Corporation). This technology involves microencapsulation of self-assembled insulin plus fumaryl (which is a secure compound that carries the hormone into the lungs), facilitating the absorption of insulin [11]. The Technosphere inhaler is simpler than other devices, but it is still in a less advanced stage of development compared with Exubera. Finally, other systems using a dry powder insulin formulation are AIR (Alkermes Inc.) and Spiros (Spiros Development Corporation).

2.1.2 *Liquid formulations of insulin*

Other systems are based on the aerosolisation of liquid formulations of insulin. One of these also possesses an electronically guided inhaler (the AERx mechanical insulin delivery system: AERx iDMS® [Novo Nordisk and Aradigm Corp.]). AERx iDMS is at an advanced stage of clinical development. In this formulation, liquid insulin is contained in disposable strips (stabilised by polysorbate, phenol and zinc chloride). The insulin has to be stored in a refrigerator between +2 and +8°C. The device detects patient inspiratory parameters and insulin is delivered only if the inhaled volume and air flow are in the appropriate range. The equipment supervises patient inhalation and, through indicator lights, alerts patients to whether they are breathing at the correct rate to set off the device. The AERx insulin device is portable and is easy to handle. It includes a pressure sensor that regulates volume and flow rate, and has a temperature-control component. The device actually warms the air steam generated to avoid unwanted reactions and to facilitate lung penetration (limiting the effect of the physical conditions of the air as a source of variation). Patients are not required to hold their breath while using this device. The optimal dose (as selected by the patient) is shown via a liquid crystal display screen.

Aerodose® (Aerogen) is another system that uses a liquid formulation, which is activated by inspiration [12]. Other liquid formulated systems are in development. **Table 1** shows a comparison between dry powder, liquid and injectable formulations of insulin.

In general, the bioavailability of inhaled insulin, when compared with subcutaneous injection, ranks between 10 and 20%, and time-to-peak concentrations are close to 45 – 60 min (faster than regular subcutaneous insulin) [13]. These

Table 1. Comparison between insulin formulations: pulmonary liquid, pulmonary dry powder and injectable insulin.

Parameter	Injectable insulin	Inhaled insulin	
		Liquid formulation	Dry powder formulation
Bioavailability*	100% (reference)	15 – 20%	10 – 20 (50)%*
T_{max}	60 min (rapid-acting analogues)	30 – 50 min	15 – 60 min
Stability	Unstable in storage, cooling necessary (+2 to +8°C), no freezing	Storage conditions are required: room temperature (+2 to +8°C), no freezing	Stable at room temperature, freezing is possible
Inhalation device properties	NA	Detects patient's inspiratory parameters to deliver insulin only if inhaled volume and air flow are in the appropriate range	Delivers an insulin steam that would be inhaled by the patient (without detection of inhalatory parameters)
Risk for microbacterial growth	None	Existent (but limited in presence of preservatives)	Low
Absorption area	Small	Large	Large
Pharmacokinetics	Variable: relatively slow absorption	Fast absorption	Fast absorption
Pain	Yes/no (subjective, depending on device)	No	No
Acceptance to multiple applications	Low	High	High
Psychological impact	Yes	No	No

Adapted from [13].

*Reported for Technosphere® (MannKind Corporation).

NA: Not applicable.

time-to-peak values resemble the kinetic behaviour of the fast-acting analogues. Inhalation is recommended 10 – 15 min before a meal. The duration of action may be marginally longer than that of aspart or lyspro [14]. Nevertheless, there are few studies that directly compare inhaled insulin with short-acting analogues [12]. One of such studies was carried out by Rave *et al.*, in which pharmacokinetics and effectiveness of human insulin inhalation powder delivered via AIR particle technology was compared with insulin lyspro, concluding that, although the time–action profile was longer for inhaled insulin, both treatments showed rapid initial absorption and similar overall pharmacokinetic exposure and effectiveness [15].

Optimal metabolic control can only be obtained by combining inhaled insulin with a subcutaneous injection of long-acting formulations in Type 1 diabetes. An increased bioavailability and a shorter time-to-peak has been reported with a particular dry powder formulation and delivery system (Technosphere), but this preparation is just entering Phase III trials, and comparison with other formulations would still be premature [13].

Several trials have been conducted to evaluate inhaled insulin, both in patients with Type 1 and 2 diabetes. In a study carried out in 335 patients with Type 1 diabetes, a dry powder formulation of inhaled insulin plus a single injection of long-acting insulin were compared with 2 – 3 insulin injections. Glycosylated haemoglobin was similar in both groups, as well as the number of patients who achieved a value < 8% [16]. A 6-month randomised trial examined whether a basal/bolus insulin regimen with a rapid-acting, dry powder, inhaled insulin could offer glycaemic control that would be comparable to a subcutaneous regimen in patients with Type 1 diabetes. It was concluded that inhaled insulin may provide an option for the management of Type 1 diabetes, as mean glycosylated haemoglobin decreased comparably in the inhaled and subcutaneous insulin groups, with a similar fraction of subjects achieving haemoglobin A_{1c} levels < 7%. Furthermore, fasting plasma glucose levels declined more in the inhaled than in the subcutaneous insulin arm of the trial [17].

Regarding Type 2 diabetes mellitus, several methods were applied to carry out studies evaluating inhaled insulin, such as monotherapy with inhaled insulin in patients without any other pharmacological treatment for their Type 2 diabetes [18]; monotherapy and add-on therapy in patients using oral antidiabetics [19]; and monotherapy in patients who were previously treated with insulin [20]. In all of these different methods, a comparable rate of effectiveness, compliance to treatment and adverse events was reported when comparing inhaled insulin with conventional treatments to Type 2 diabetes. For example, in 106 patients with Type 2 diabetes, a 12-week study was carried out, comparing a preprandial dose of a liquid formulation of inhaled insulin to subcutaneous insulin, both combined with NPH. The results showed that inhaled insulin was as effective as the subcutaneous injection, regarding to glycaemic control and tolerability [21]. Another

trial, including poorly controlled patients with Type 2 diabetes, compared an arm receiving a dry powder inhaled insulin plus an oral antidiabetic drug and another receiving a combination of oral antidiabetic drugs. Glycaemic control was achieved in both groups with a similar rate of hypoglycaemic events. At 2 years, there was no statistical difference between the groups in lung functional tests [22,23]. Furthermore, the compliance to treatment with a liquid formulation of inhaled insulin was tested in a multi-centre open trial, demonstrating that the dosing, timing and technique were very well accepted by the patients [24].

An improved compliance to treatment was reported in several trials comparing inhaled insulin to conventional therapies for diabetes mellitus: constituting one of the most relevant aspects of evaluating satisfaction level and quality of life in patients with Type 1 diabetes [16,20].

Intrasubject variability for insulin half-life, elimination and mean residency time, seems to be lower in patients receiving pulmonary insulin. Several trials that were carried out in smokers showed that total insulin absorption is higher than for non-smokers (50% greater $AUC_{0\text{-}6}$, higher C_{max} and half the time to peak). Nevertheless, at least for liquid formulations, intrasubject variability is similar in smokers and non-smokers, and lower than that obtained through subcutaneous injection [25]. Some patients with bronchial asthma may require higher doses than non-asthmatic controls, but intrasubject variability does not seem to be different [26]. It has been reported that patients suffering from acute upper respiratory tract infection who received a liquid formulation of inhaled insulin did not show any change in insulin pharmacokinetics or dynamics, and the dose adjustment was equal to individuals with upper respiratory tract infection receiving subcutaneous insulin [27]. One study compared the pharmacological profile of a liquid formulation in patients with Type 2 diabetes who were < 45 years of age versus subjects who were > 65 years of age. Glucose reduction was smaller in older patients, probably because of their higher insulin resistance [12]. Nevertheless, further information is needed to confirm inhaled insulin requirements in older patients with Type 2 diabetes.

On the topic of adverse events, studies comparing dry powder formulations of inhaled insulin to subcutaneous insulin reported that the frequency and nature of these in both groups were comparable, although mild-to-moderate cough was more common in patients taking inhaled insulin. The rates are probably slightly higher for dry powders. This effect decreased over the period of the study [16]. In addition, in another study, an audible wheeze attributed to a liquid formulation of inhaled insulin was described, but no clinical change in lung function was noticed [9]. In conclusion, despite the reductions in forced expiratory volume in 1 s (FEV_1) and carbon monoxide-diffusing capacity of the lung in the first months, these findings seem to be reversible without any clinical relevance.

The formation of antibodies has been a topic of concern in patients treated with insulin. However, in individuals treated with inhaled insulin (dry powder formulation), it was reported that their formation did not increase over the time, or, if it did, insulin performance was not affected [8,13]. With regards to hypoglycaemia, it has been reported that fewer hypoglycaemic events were suffered in patients using a dry powder formulation of inhaled insulin plus subcutaneous insulin, when compared with subcutaneous injections alone [16]. Although the same reports were found in a study using a liquid formulation regarding mild-to-moderate hypoglycaemia, in this trial three major hypoglycaemic events were reported in the group taking inhaled insulin, and no events in the group who were treated with subcutaneous insulin [21]. However, most of the studies have reported a similar rate of hypoglycaemia in groups treated with inhaled insulin or with subcutaneous insulin [22,23].

Overall, although the drug was reported to be safe with regards to pulmonary function and well tolerated in the majority of the trials, further studies are needed to assess the long-term safety of this route of administration [9,13,16].

2.2 Oral insulin

Peroral insulin administration is a non-invasive method that could deliver insulin in a more physiological way, and also improving patient compliance. This method would also re-establish the physiological ratio of the portal vein to peripheral blood insulin concentration, providing a more complete activation of insulin-dependent metabolic pathways of the liver. As a result, an improved glycaemic control and a reduction in the complications related to diabetes could be accomplished [28]. The main difficulty observed in attaining a satisfactory insulin profile by this route of administration has been the instability of the insulin to enzymatic degradation in the gastrointestinal tract.

There are two main pathways for delivering oral insulin: oral–buccal (Oralin®; Generex) and oral–enteric (hexyl insulin monoconjugate 2 [HIM2]). The first delivers an aerosol of uniform-sized droplets enclosing regular human insulin into the oropharyngeal cavity for local transmucosal absorption. In one study, a higher C_{max}, shorter T_{max} and faster time-to-peak was reported when compared with subcutaneous insulin [29]. Furthermore, the metabolic effects of this method in patients with Type 2 diabetes were studied, concluding that Oralin could be used as a meal insulin in place of short-acting insulin injections to regulate the postprandial glucose excursion [30].

HIM2 is composed of an oligomer that is covalently linked to the free amino group on the Lys-B29 residue of recombinant insulin. This leads to resistance to enzymatic degradation, facilitation of absorption and reduction of the clearance of insulin from circulation [31].

A randomised, controlled trial in healthy volunteers showed the safety and efficacy of single escalating doses of HIM2. In this study, all of the doses were well tolerated, and a dose-dependent increment in serum insulin was observed. Hypoglycaemia was reported in four subjects, two of which needed glucose infusion [32].

Another randomised trial conducted in six patients with Type 1 diabetes demonstrated that, when administered 15 min before a meal, HIM2 was maximally absorbed. It was minimally absorbed when given after a meal, and the absorption decreased ~ 80% when taken immediately before a meal. This correlated with postprandial glucose levels [33]. Furthermore, in a study carried out in 16 patients with Type 1 diabetes, HIM2 was well tolerated, and no hypoglycaemic events were reported. During the post-dose period, ~ 70% of the patients presented plasma glucose levels that were 150% lower than pre-dose values [34]. In addition, a study carried out in 29 patients with Type 1 diabetes using Oral-Lyn® (an oral liquid insulin formulation; Generex) showed that the replacement of subcutaneous insulin for oral insulin was associated with adequate glycaemic control and similar haemoglobin A_{1C} levels [101].

In one randomised study in 18 patients, the efficacy and safety of HIM2 was compared with subcutaneous insulin and placebo. Single, oral doses of HIM2 were safe and well tolerated. Similar mean glucose AUC values were observed for 0.5 and 1.0 mg/kg of HIM2 and regular subcutaneous insulin. Peripheral insulin concentrations in patients receiving HIM2 were lower than in individuals receiving subcutaneous insulin, suggesting that HIM2 may control glycaemia without inducing hyperinsulinaemia in patients with Type 2 diabetes [35].

Other Phase II studies are ongoing, evaluating the treatment of Type 1 and Type 2 diabetes with HIM2, and a Phase III study is planned for the future.

2.3 Nasal insulin

This route of administration is one of the most interesting means for delivering drugs to the systemic circulation, for several reasons. First, the nasal epithelium has a rich vascularisation and is highly permeable, which guarantees rapid absorption and a quick onset of action of compounds. Second, the ease of administration ensures a better compliance. Finally, the circumvention of first-pass metabolism may represent another advantage [36].

There are two main formulations for intranasal delivery of peptide compounds: solutions or dry powders. The latter has the advantage that the drug accumulates in the nasal mucosal surface [37]. The major strategy that has been used to achieve an adequate residency time and absorption of nasal insulin is to add absorption enhancers, such as polyacrylic acid, polymer ethyl and chitosan gels [38]. These carriers exhibit bioadhesive properties and absorption-enhancing effects. Therefore, aminated gelatin was explored as an alternative for the delivery of nasal insulin in rats, with favourable outcomes, which depend mostly on the molecular weight and number of amino groups of the insulin [37]. Another preclinical study examined the use of chitosan gel as a carrier for nasal insulin. It was reported that the gel, of 2% medium molecular weight of chitosan with EDTA, produced an increment in insulin absorption and a 46% reduction in glycaemia with the intravenous route, suggesting it could be a suitable formulation for delivering nasal insulin [36].

Intranasal formulations of insulin are being developed in preclinical trials.

2.4 Rectal and vaginal insulin

In one preclinical study, carried out in rabbits, insulin penetrated well through rectal and vaginal membranes. Chitosan gel was used as a carrier, providing a longer insulin release when compared with other transporters [39].

2.5 Insulin eyedrops

The main limitation of the ocular route is that the amount of insulin absorbed would not be enough to have an impact on glycaemia control. However, it has been reported in a study conducted in rabbits that the absorption via the ocular route was improved at a pH of 8, with the use of glycocholate and fusidic acid. It was concluded that, although further studies are needed, achieving normoglycaemia could be viable by this means [40].

Furthermore, in one study it was theorised that the topical application of insulin to the eye may be beneficial in treating diabetic retinopathy, even in patients with sustained hyperglycaemia. As there are a vast number of insulin receptors in the retina, not only on the retinal microvasculature, but in all cellular layers of the retina, it is suggested that there is an important role for insulin in retinal function. Therefore, the pharmacokinetics of topical insulin applied in rats has been investigated, concluding that topically applied insulin accumulates in the retina and optic nerve in normal and diabetic rats. However, it did not appear that systemically absorbed insulin, resulting from ocular drainage, contributed to this effect [41].

Further studies are required to investigate the role of insulin eyedrops in both diabetic retinopathy and glycaemic control.

2.6 Transdermal insulin

Although there are several studies on transdermal insulin delivery, such as those investigating the synergistic effect of electroporation and iontophoresis on the *in vivo* percutaneous absorption of human insulin [42], they are in early phases of development, and further studies are needed to investigate the role of transdermal insulin in the future.

3. Conclusion

Diabetes is a chronic and, so far, incurable disease. Due to this, in most patients with diabetes, an adequate glycaemic control often requires multiple insulin injections per day, causing pain and a psychological impact, which, in turn, leads to a poor compliance to treatment.

During recent years, there has been much progress in the development of non-invasive methods of insulin administration that has been made.

Inhaled insulin has shown favourable properties, such as a rapid onset of action and a rapid T_{max}, short duration of action, an adequate bioavailability and an absence of significant side effects. It also enables ease of administration;

and all of these properties ensure vast patient compliance [13]. However, development is still in Phase II/III trials, and the long-term efficacy and safety need to be assessed. Moreover, inhaled insulin does not eliminate the use of at least one long-acting insulin application [43].

Another possible limitation of this compound would be the economical cost. As the bioavailability of pulmonary insulin is ~ 10 – 20% of that of subcutaneous insulin, it may be expected that inhaled insulin would be more expensive than even insulin analogues [13].

Other attempts, such as nasal, oral or rectal/vaginal routes of administration, are still far from being launched into the market as alternative means to deliver insulin, although preclinical, and even Phase II studies (for oral insulin), are taking place. Thus, at present, inhaled insulin would be the first available alternative to the subcutaneous injection [21].

In conclusion, the major benefit of non-invasive routes of insulin administration is the elimination of multiple injections as the only means to achieve an adequate glycaemic control. This leads to a considerably improved compliance to therapy, with the subsequent delay of diabetes-related complications. The aforementioned benefits ought to be balanced with the greater cost and possible long-term side effects of alternative routes of insulin delivery.

4. Expert opinion

Regarding alternative routes for insulin administration in the near future, the landscape is dominated by inhaled insulin, in both dry and liquid forms. Until now, the developed preparations are providing rapid effects, comparable to those of rapid-acting analogues. Nevertheless, further approaches could include some projects exploring slow-acting inhalatory formulations.

Although inhaled insulin seems to be convenient and easy-to-accept for the patient, allowing more flexible insulinisation regimes, long-term safety and health economics-related issues are still a matter of concern. Further administration routes are being explored, but they are far from satisfying the requirements for more expanded clinical development programmes. Their real potential would be probably defined within the next decade.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. THE DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP: The effect of intensive treatment of diabetes in the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* (1993) **329**:977-1036.
2. SHICHIRI M, KISHIKAWA H, OHKUBO Y *et al.*: Long-term results of the Kumamoto Study on optimal diabetes control in Type 2 diabetic patients. *Diabetes Care* (2000) **23**(Suppl. 2):B21-B29.
3. UK PROSPECTIVE DIABETES STUDY (UKPDS) GROUP: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with Type 2 diabetes (UKPDS 33). *Lancet* (1998) **352**:837-853.
4. BOLLI GB, MARCHI RD, PARK GD *et al.*: Insulin analogues and their potential in the management of diabetes mellitus. *Diabetologia* (1999) **42**:1151-1167.
5. Insulin pharmacology: In: *Textbook of Diabetes (3rd edn)*. Pickup J, Williams G (Eds), Blackwell Publishing, Oxford, UK (2003):8-11.
6. VON HEUBNER W, DE JONGH SE, LAQUER E: Uber inhalation von insulin. *Klin. Wochenschrift* (1924) **51**:2342-2343.
7. HEINEMANN L: Alternative delivery routes: inhaled insulin. *Diabetes Nutr. Metab.* (2002) **15**(6):417-422.
8. MANDAL TK: Inhaled insulin for diabetes mellitus. *Am. J. Health Syst. Pharm.* (2005) **62**:1359-1364.
9. KAPITZA C, HEISE T, FISHMAN R *et al.*: Impact of particle size and aerosolization time on the metabolic effect of inhaled insulin aerosol. *Diabetes Technol. Ther.* (2004) **6**:119-127.

•• **Pharmacokinetic properties of inhaled insulin.**

10. WHITE S, BENNETT, DB, CHEU, S *et al.*: EXUBERA®: Pharmaceutical development of a novel product for pulmonary delivery of insulin. *Diabetes Technol. Ther.* (2005) 7:896-906.
11. SKYLER J: Pulmonary insulin update. *Diabetes Technol. Ther.* (2005) 7:834-839.

•• **Comprehensive review of pulmonary insulin.**

12. HARSCH IA: Inhaled insulins. Their potential in the treatment of diabetes mellitus. *Treat. Endocrinol.* (2006) **4**(3):131-138.
13. PFUTZNER A, FORST T: Pulmonary insulin delivery by means of the Technosphere drug carrier mechanism. *Expert Opin. Drug Deliv.* (2005) **2**(6):1097-1106.
14. PETERSEN A, PLANK J, BOCK G *et al.*: Onset of action of inhaled insulin via the AERx® iDMS was faster than subcutaneous human regular insulin aspart. *65th Annual Meeting of Scientific Sessions of American Diabetes Association Annual Meeting, San Diego, CA, USA, 10 – 14 June* (2004):479. Abstract.
15. RAVE KM, NOSEK L, DE LA PENA A *et al.*: Dose response of inhaled dry-powder insulin and dose equivalence to subcutaneous insulin lyspro. *Diabetes Care* (2005) **28**(10):2400-2405

•• **Comparision of inhaled insulin and lyspro.**

16. QUATTRIN T, BELANGER A, BOHANNON N *et al.*: Efficacy and safety of inhaled insulin (Exubera) compared with subcutaneous insulin therapy in patients with Type 1 diabetes: results of a 6 month, randomized, comparative trial. *Diabetes Care* (2004) **27**:2622-2627.

•• **Complete comparison of inhaled and subcutaneous insulin in Type 1 diabetes.**

17. SKYLER JS, WEINSTOCK RS, RASKIN P: Use of inhaled insulin in a basal/bolus insulin regimen in Type 1 diabetic subjects: a 6-month, randomized, comparative trial. *Diabetes Care* (2005) **28**(7):1630-1635.
18. DEFRONZO RA, BERGENSTAL RM, CEFALU WT *et al.*: Efficacy of inhaled insulin in patients with Type 2 diabetes not

controlled with diet and exercise: a 12-week, randomized, comparative trial. *Diabetes Care* (2005) **28**(8):1922-1928.

•• **Large randomised trial of inhaled insulin in Type 2 diabetes.**

19. ROSENTOCK J, ZINMAN B, MURPHY LJ *et al.*: Inhaled insulin improves glycemic control when substituted for or added to oral combination therapy in Type 2 diabetes: a randomized, controlled trial. *Ann. Intern. Med.* (2005) **143**(8):I28.

•• **Trial describing inhaled insulin as add-on therapy to oral antidiabetics.**

20. HOLLANDER PA, BLONDE L: Efficacy and safety of inhaled insulin (exubera) compared with subcutaneous insulin therapy in patients with Type 2 diabetes: results of a 6-month, randomized, comparative trial. *Diabetes Care* (2004) **27**(10):2356-2362

•• **Large randomised trial on inhaled insulin in Type 2 diabetes.**

21. HERMANSEN J, RONNEMAA T, PETERSEN A *et al.*: Intensive therapy with inhaled insulin via the AERx insulin diabetes management system. *Diabetes Care* (2004) **27**:162-167.

• **Complete comparison between inhaled and subcutaneous insulin in Type 2 diabetes.**

22. WEISS S, CHENG S, KOURIDES I *et al.*: Inhaled insulin provides improved glycemic control in patients with Type 2 diabetes mellitus inadequately controlled with oral agents: a randomized controlled trial. *Arch. Intern. Med.* (2003) **163**:2277-2282.

23. DREYER M: Efficacy and 2 year pulmonary safety of inhaled insulin as adjunctive therapy with metformin or gliblenclamide in Type 2 diabetes patients poorly controlled with oral monotherapy. *40th Annual Meeting European Association Study Diabetes, Munich, Germany, 5 – 9 September* (2004):865. Abstract.

•• **Inhaled insulin as adjunctive treatment in Type 2 diabetics.**

24. CRAMER BS, OKIKAWA MS, BELLAIRE MS *et al.*: Compliance with inhaled insulin treatment using the AERx® Idms insulin diabetes management system. *Diabetes Technol. Ther.* (2004) **6**:800-807.

•• **Compliance to treatment with inhaled insulin.**

25. HIMMELMANN A, JENDLE J, PETERSEN A *et al.*: The impact of smoking on inhaled insulin. *Diabetes Care* (2003) **26**:677.

• **Inhaled insulin in smokers.**

26. HENRY R, MUDALIAR S, HOWLAND W *et al.*: Inhaled insulin using the AERx insulin diabetes management system in healthy and asthmatics subjects. *Diabetes Care* (2003) **26**:764.

• **Inhaled insulin in asthmatics.**

27. McELDUFF A, MATHER L, KAM P *et al.*: Influence of acute upper respiratory tract infection on the absorption of inhaled insulin using the AERx insulin diabetes management. *Br. J. Pharmacol.* (2005) **59**:546-551.

28. SURGULADZE D, ANDERSON W, FILBEY J *et al.*: Insulinization of the liver in normal mice by oral delivery of HIM2, a novel modified insulin. *Diabetes* (2002) **51**:1284.

29. CERNEA S, KIDORN M, WOHLGELERNTER J: Comparison of pharmacokinetic and pharmacodynamic properties of single-dose oral insulin spray and subcutaneous insulin injection in healthy subjects using the euglycemic clamp technique. *Clin. Ther.* (2004) **26**(12):2084-2091.

30. GUEVARA-AGUIRRE J, GUEVARA M, AGUIRRE J *et al.*: Oral spray insulin in treatment of Type 2 diabetes: a comparison of efficacy of the oral spray insulin (Oralin) with subcutaneous (SC) insulin injection, a proof of concept study. *Diabetes Metab. Res. Rev.* (2004) **20**(6):472-478.

•• **Comparision of oral and subcutaneous insulin.**

31. RIGGS SAUTHIER J: Strategies toward the oral delivery of insulin: using molecular modification to solve drug delivery challenges. *227th ACS National Meeting, Anaheim, CA, USA, March 27 – April 1* (2004*)*:MEDI 202. Abstract.

32. WAJCBERG E, MIYASAKI Y, TRIPLITT C *et al.*: Dose response effect to a single administration of HIM2 in healthy nondiabetic subjects. *Diabetes Care* (2004) **27**(12):2868.

•• **Effects of oral insulin in healthy individuals.**

33. CLEMENT S, DANDONA P, STILL J *et al.*: Oral modified insulin (HIM2) in patients with Type 1 diabetes mellitus: results from a Phase I/II clinical trial. *Metab. Clin. Exp.* (2004) **53**:54.

34. DICOSTANZO C, MOORE M, LAUTZ M *et al.*: Simulated first-phase insulin release using Humulin or insulin analog HIM2 is associated with prolonged improvement in postprandial glycemia. *Am. J. Physiol. Endocrinol. Metab.* (2005) **289**:E46.

35. KIPNES M, DANDONA M, TRIPATHY D *et al.*: Control of postprandial plasma glucose by an oral insulin product (HIM2) in patients with Type 2 diabetes. *Diabetes Care* (2003) **26**(2):421-426.

•• **Complete trial involving HIM-2 in patients with Type 2 diabetics.**

36. VARSHOSAZ J: Nasal delivery of insulin using bioadhesive chitosan gels. *Drug Delivery* (2006) **13**:31-38.

37. TOSHINOBU S, HIROSHI K, TOMONOBU N *et al.*: Effect of aminated gelatin on the nasal absorption of insulin in rats. *Biol. Pharm. Bull.* (2005) **28**:510-514.

38. VERHOEF J, MERKUS F: *Drug Absorption Enhancement: Concepts, Possibility, Limitations And Trends*. De Boer AG (Ed.), Harwood Academic Publishers, Singapore (1994):119-153.

39. DEGIM Z, DEGIM T, ACARTUK F *et al.*: Rectal and vaginal administration of insulin-chitosan formulations: an experimental study in rabbits. *J. Drug Target.* (2005), **13**:563-572.

40. XUAN B, MCELLAN D, MOORE M *et al.*: Alternative delivery of insulin via eye drops. *Diabetes Technol. Ther.* (2005) **5**:695-698.

41. KOEVARY SB, NUSSEY J, LAKE S: Accumulation of topically applied porcine insulin in the retina and optic nerve in normal and diabetic rats. *Invest. Ophthalmol. Vis. Sci.* (2002) **43**(3):797-804.

42. PAN Y, ZHAO HY, ZHENG JM: The enhancing effect of electroporation and iontophoresis on the permeation of insulin through human skin. *Yao Xue Xue Bao* (2002) **37**(8):649-652.

43. SELAM J: Inhaled insulin for treatment of diabetes: projects and devices. *Expert Opin. Pharmacother.* (2003) **4**:1373-1377.

•• **Complete description of inhaled insulin devices.**

Website

101. http://www.generex.com/news-management/templates/press.asp?articleid=244&zoneid=2 Generex Press release (8 March 2006).

Affiliation
Claudio González[1] MD,
Diego Kanevsky[2] MD, Rubén De Marco[3] MD,
Guillermo Di Girolamo[†4,5] MD &
Silvina Santoro[6] MD
[†]Author for correspondence
[1]Professor and Head, Department of Pharmacology, Instituto Universitario, CEMIC, Galvan 4102, Ciudad Autónoma de Buenos Aires, Argentina
[2]Clinical Research Associate of Medical Department, Medical Department, Novo Nordisk Pharma Argentina, Avenida del Libertador 14099, Martinez, CP:B1640AOL, Provincia de Buenos Aires, Argentina
[3]Professor of Internal Medicine, Department of Internal Medicine, School of Medicine, Universidad de la Plata, Calle 9N175 CP 1900, La Plata, Provincia de Buenos Aires, Argentina
[4]Professor and Head of Pharmacology, Department of Physiological , Biochemistry and Pharmacological Sciences, School of Medicine, Universidad Favaloro, Solis 453, CP 1078, Ciudad Autónoma de Buenos Aires, Argentina
[5]Second Chair of Pharmacology, Department of Pharmacology, School of Medicine, Universidad de Buenos Aires, Paraguay 2215, Piso 16, CP:C1121ABG, Ciudad Autónoma de Buenos Aires, Argentina
Tel: +54 114 753 8211;
Fax: +54 114 753 8211;
E-mail: gdigirolamo@arnet.com.ar
[6]Lecturer in Pharmacology, Department of Pharmacology, School of Medicine, Universidad de Buenos Aires, Paraguay 2215, Piso 16, CP:C1121ABG, Ciudad Autónoma de Buenos Aires, Argentina