

Review article

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INHALED INSULIN – DOES IT BECOME REALITY?

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After more than 80 years of history the American and European Drug Agencies (FDA and EMEA) approved the first pulmonary delivered version of insulin (Exubera®) from Pfizer/Nektar early 2006. However, in October 2007, Pfizer announced it would be taking Exubera® off the market, citing that the drug had failed to gain market acceptance. Since 1924 various attempts have been made to get away from injectable insulin. Three alternative delivery methods were always discussed: Delivery to the upper nasal airways or the deep lungs, and through the stomach. From these, the delivery through the deep lungs is the most promising, because the physiological barriers for the uptake are the smallest, the inspired aerosol is deposited on a large area and the absorption into the blood happens through the extremely thin alveolar membrane. However, there is concern about the long-term effects of inhaling a growth protein into the lungs. It was assumed that the large surface area over which the insulin is spread out would minimize negative effects. But recent news indicates that, at least in smokers, the bronchial tumour rate under inhaled insulin seems to be increased. These findings, despite the fact that they are not yet statistically significant and in no case found in a non-smoker, give additional arguments to stop marketing this approach. Several companies worked on providing inhalable insulin and the insulin powder inhalation system Exubera® was the most advanced technology. Treatment has been approved for adults only and patients with pulmonary diseases (e.g., asthma, emphysema, COPD) and smokers (current smokers and individuals who recently quit smoking) were excluded from this therapy. Pharmacokinetics and pharmacodynamics of Exubera® are similar to those found with short-acting subcutaneous human insulin or insulin analogs. It is thus possible to use Exubera® as a substitute for short-acting human insulin or insulin analogs. Typical side effects of inhaled insulin were coughing, shortness of breath, sore throat and dry mouth. Physical exercise increases the transport of inhaled insulin into the circulation and in

This article is dedicated to Prof. Dr. J. Meier-Sydow, former head of the Pulmonary Department of the University Hospital of the Johann-Wolfgang-Goethe University in Frankfurt/Main, Germany in/at the occasion of his 80th birthday.

consequence the likelihood of hypoglycemia. Other problems were the inability to deliver precise insulin doses, because the smallest blister pack available contained the equivalent of 3 U of regular insulin and this dose would make it difficult for many people using insulin to achieve accurate control, which is the real goal of any insulin therapy. For example, someone on 60 U of insulin per day would lower the blood glucose about 90 mg/dl (5 mmol) per 3 U pack, while someone on 30 U a day would drop 180 mg/dl (10 mmol) per pack. Precise control was not possible, especially compared with an insulin pump that can deliver one twentieth of a unit with precision. Another disadvantage was the size of the device. The Exubera® inhaler, when closed, was about the size of a 200 ml water glass. It opened to about twice the size for delivery. To our information also other companies (Eli Lilly in cooperation with ALKERMES, Novo Nordisk (AERx®, Liquid), Andaris (Powder)) stopped further development and it is unclear whether an inhaled form of insulin will ever be marketed, because of the problems that have occurred. Only Mannkind (Technosphere®, Powder) is still working on a Phase III trial. However, our review will briefly summarize the experience regarding inhalant administration of insulin and will describe potential future developments for this type of therapy focussing on the lung.

Key words: *aerosol therapy, inhalation, insulin, systemic treatment*

PRINCIPLES OF INHALATION THERAPY FOR SYSTEMIC TREATMENT

In the last 20 years the techniques for protein production by means of recombinant DNA technology have been well refined and it is now possible to produce sufficient quantities, e.g., of hormones, growth factors, monoclonal antibodies and cytokines under good manufacturing practice conditions for commercial use (1). However, because of their large molecular weight, hydrophilia, instability against chemicals and proteases, and poor intestinal absorption rates these macromolecules cannot be administered orally and must be given parenterally. Unfortunately, techniques for parenteral drug application (e.g., subcutaneous, intravenous, or intramuscular injection) are invasive and require the compliance, especially in patients with chronic diseases (e.g., diabetes mellitus). In consequence, a number of methods for controlled injection or alternative routes of drug administration have been developed (1, 2). Inhalation is an important tool for non-invasive administration of low molecular weight pharmaceuticals and macromolecules for systemic treatment. This way of drug administration has the benefit of a large alveolar absorption area of 70 m² - 140 m² which is about the half of a tennis court (compared with 180 cm² in the nose cavity), a good perfusion of the absorptive area (about 5 l/min), a very low thickness of the alveolar epithelium (only between 0.1 and 0.2 µm) and a short total distance between epithelial surface and blood in the alveolar area (between 0.5 and 1.0 µm compared with 30-40 µm distance between mucus surface and blood in the bronchial system), a low presence of local proteases and peptidases, a marginal variance in the amount of mucus production, a rapid dissolution of the administered insulin in the alveolar mucus layer after its deposition, and the absence of a hepatic first-pass effect (1-6). In

consequence, pharmaceuticals are rapidly absorbed after deep inhalation and deposition in the alveolar region of the lung. Another advantage is that these drugs are not subject of a hepatic first pass effect after their absorption (1, 7).

However, pulmonary application of drugs by means of aerosols is influenced by a number of physical, physiological and individual factors which are described elsewhere (2-4, 6-11). A biopharmaceutical must have a sufficient physical and chemical stability to persist the process of nebulization without loss of its functional properties and without relevant aggregation within or after the nebulization process. The aerosol must be homogenous with respect to the produced particle size and the particle diameter should be optimized (aerodynamic diameter: 1-3 μm) for deposition in the alveolar region of the lung. Particles with aerodynamic diameters $<1 \mu\text{m}$ are not deposited in the lung but expired. On the other hand, larger particles ($>3 \mu\text{m}$) are deposited in the tracheobronchial airways and do not reach the alveolar region. The breathing maneuver is another critical parameter for pulmonary drug application. An optimal pulmonary deposition is achieved with a slow and deep inhalation procedure. In addition, variations in lung morphology and ventilation due to diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD)) and individual factors (e.g., smoking) have an influence on the alveolar deposition of inhaled particles. Finally, the absorption of the biomolecules after alveolar deposition is affected by structure and function of the physiological pulmonary defence mechanisms (e.g., proteases/peptidases, alveolar macrophages, physiological absorbance barriers) and specific properties of the biopharmaceuticals (e.g., molecular weight, lipophilicity, solubility in water and lipids).

The application of biomolecules by means of different inhalation approaches has been investigated in a large number of studies (4, 7, 8, 12, 13). In principle, some of the biomolecules can be given without additives. Other large molecules, especially peptides and proteins, require stabilisers and inhibitors of phagocytosis (e.g., protease inhibitors, microspheres, liposomes) or absorption enhancers (e.g., detergents, bile acids, cyclodextrins) which can cause tissue irritation (1, 2, 4, 8, 11, 14-16). Compared with absorption enhancers, the use of carrier-based systems (e.g., liposomes and microspheres) has some more specific advantages for sustained and targeted drug delivery as compiled in *Table 1* (17).

In the last years, a large number of studies on pulmonary application of metabolically active hormones (e.g., insulin, calcitonin, growth hormone, somatostatin, thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH)), growth factors (e.g., granulocyte-colony stimulating factor (G-CSF) and granulocyte monocyte-colony stimulating factor (GM-CSF)), distinct interleukins (e.g., IL-2) and heparin (unfractionated and low molecular weight heparin (LMWH)) have been performed (1, 4, 8, 9, 13). However, most experience is available for the inhalation of insulin. In addition, from the large number of substances insulin is the one with the greatest relevance because of the large number of diabetic patients worldwide. In our review we describe the current status and problems of devices for pulmonary administration of insulin.

Table 1. Advantages of carrier-based systems for sustained drug delivery; according to (17).

- Increase of the proportion of protein reaching its site of action
- Improvement of the drug transport to the site of action
- Possibility of the combined administration of the drug with other excipients (e.g., absorption enhancers, protease inhibitors)
- Improvement of drug stability *in vivo*
- Prolongation of the residence time of the drug at its site of action due to a reduction of the clearance mechanisms
- Decrease of the non-specific delivery of the drug to non-target tissues
- Decrease of drug-induced tissue irritation
- Decrease of drug toxicity even after administration of high initial drug doses
- Alteration of the immunogenicity of the protein
- Improvement of the taste of the pharmaceutical
- Improvement of the shelf-life of the product

HISTORY OF INSULIN INHALATION

Insulin, a peptide hormone (MW: 6000 Da) consisting of 2 chains (α und β) linked by three disulfide bonds, has been isolated 1921 by Banting and Best and was introduced into clinical treatment on January 11th 1922 (18-20). At the beginning, it was exclusively administered by intramuscular injection. However, because of the lower traumatization of the patient subcutaneous application was rapidly established (20). Other techniques for drug application (transdermal, ocular, oral, buccal, nasal, pulmonal, rectal, vaginal, and transuterine) were also investigated and some of them are currently under further investigation (3, 18, 20, 21) (Table 2).

In 1924 and 1925 – only two years after the start of the therapeutic insulin era – the first studies on insulin inhalation were published. Laqueur and Grevenstuck (22) published her investigation on intratracheal administration of insulin in 1924 and reported a more rapid onset of action after intratracheal administration compared with subcutaneous administration. A first study on inhalation of insulin in patients was performed by Heubner *et al* (23) also in 1924. These investigators reported a dose-dependent effect of insulin inhalation on blood glucose. However, a 30-times higher dose for inhalation was required than that for subcutaneous administration and the authors assumed a problem in the requirement of high amounts of insulin, even though they also emphasised the advantage of this type of administration for the patients (23). At the same time and independently from the investigations of Heubner *et al* (23), Gänsslen (24) performed the investigations in patients and reported that the inhalation of insulin was well tolerated and caused a significant decrease of the blood glucose concentration, and that the amounts of insulin required for inhalation in relation to subcutaneous application were not as high as described by Heubner *et al* (23). However, because of the large number of unsolved problems, it took 46 years more until Wigley *et al* (20, 25) published their pivotal study of insulin

Table 2. Methods for non-invasive administration of insulin; according to (18, 20).

Way of absorption	Method	Principle	Advantages/disadvantages and specific aspects
Transdermal	Jet injection	Needle free high pressure injection into subcutaneous tissue	Partially different activity profile of the administered insulin; in particular cases advances in patients having problems with other types of application
	Iontophoresis	Amelioration of the absorption by means of electric current	Experimental studies in animals only; absorption dependent on skin structure (depilation, creams), type of the insulin (modified insulin, monomers), intensity and type of current flow (continuous, pulsed)
	Low frequency ultrasound	Amelioration of the absorption by means of ultrasound	Experimental studies only; distinct amelioration of the absorption by means of sonophoresis; possibly the uptake is too low for clinical use (3•1 hour sonophoresis for 36 U insulin)
	Transferosomes	Ameliorated absorption of phosphatidylcholine containing vesicles	Little number of recent data; bioavailability $\geq 50\%$ compared with subcutaneous administration; skin area of 40 cm ² required in a typical patient with diabetes mellitus type 1
Intranasal	Nasal application	Absorption via the mucosa of the nose Amelioration of the absorption by means of absorption enhancers (e.g., lecithin, bile acids, laureth-9)	Method established for some proteins (e.g., oxytocin, desmopressin, calcitonin); absorption enhancers are mandatory for insulin administration; nevertheless bioavailability often <20 %; irritation and mucosal damage caused by these substances; different pharmacokinetics of nasally administered insulin (similar to intravenously administered insulin); problems caused by tolerance and therapeutic failure; interesting data of recent animal studies (e.g., lyophilised insulin as powder aerosol, bioadhesives, packaging with chitosan, starch microparticles)
Oral	Enteral	Absorption via the mucosa of the gastrointestinal tract Amelioration of the absorption by means of absorption enhancers (e.g., salicylate) Inhibition of degradation by means of protease inhibitors, stabilisers (e.g., capronic acid, chitosan) and microcapsulation	Mostly experimental studies in animals; insulin molecule too large and too hydrophilic for mucosal absorption; slow absorption because there is no physiological mechanism for uptake of macromolecules in the intestinal mucosa; rapid chemical and proteolytic degradation of insulin; bioavailability only 0.5 %; in recent studies bioavailability improved to 5 %; recent development: Improved uptake by complexation with non-acylated aminoacids (Emisphere Technologies) and modification of insulin by means of amphiphile oligomers (hexyl-insulin-monoconjugate-2 (HIM-2); Nobex Corporation)
	Buccal	Absorption of a liquid aerosol via the mucosa of the mouth Amelioration of the absorption by means of absorption enhancers Inhibition of degradation by means of protease inhibitors and stabilisers	Little number of mostly experimental studies in animals with conflicting results and low reproducibility; advantages: Good accessibility and vascularisation of the mouth cavity; disadvantages: Multilayered epithelium, salivation, proteases; new developments: Oralin (Generex Biotechnology) with recombinant human insulin, absorption enhancers, stabilisers and metered dose inhaler (MDI)

Rectal	Enteral	Absorption of insulin from suppositories via the mucosa of the rectum Amelioration of the absorption by means of absorption enhancers and gels	Uptake of insulin via the lymphatic system; bypassing of the liver excludes the hepatic extraction of insulin from circulation; low and inconsistent absorption of insulin; bioavailability of 4 % - 10 % compared with subcutaneous administration; more rapid onset of action and shorter duration of action than after subcutaneous administration
Pulmonal	Inhalation	Absorption of an aerosol (liquid or powder) deposited in the alveoli after inhalation via the alveolar epithelium Amelioration of the absorption by means of absorption enhancers Inhibition of degradation by means of protease inhibitors, stabilisers and microcapsulation	Large alveolar surface and high permeability are ideal prerequisites for absorption; rapid absorption of insulin after inhalation (similar to rapid acting insulins for subcutaneous application); effect on glucose metabolism in patients with diabetes mellitus comparable to that of subcutaneously administered insulin; a number of different systems for drug application under investigation and one of them received market approval

inhalation offering the proof of principle of this therapy. They investigated three subjects without diabetes mellitus and four patients with diabetes and they were able to demonstrate that pork-beef insulin administered by a nebulizer caused a prompt increase in plasma immunoreactive insulin and that hypoglycemia showed a temporal relationship with the increase in plasma immunoreactive insulin (25). However, even after the investigation of Wigley *et al* (25) inhalant insulin therapy was far away from its introduction into clinical therapy and in the next two decades several studies ruled out the basics of insulin inhalation (8, 26-28). In these years, it was observed that the bioavailability of inhaled insulin in case of improved application procedures was only about 20 to 25% of that after subcutaneous administration, but also that inhalation might be an important alternative administration route (4, 8, 20). However, the methods under investigation were not able to administer sufficient drug doses in a reproducible way, because their particle spectrum was optimized for aerosol deposition in the bronchial system and not in the alveoli (20, 29).

MODERN DEVICES FOR INSULIN INHALATION

Based on the advances in asthma therapy by means of aerosols, nebulizers, metered dose inhalers (MDIs) and dry powder inhalers (DPI) at about 1990 the scientific and technical prerequisites for the inhalant application of insulin were established and a number of studies on the inhalant application of insulin were

initiated in the following years (4, 20). Several companies developed devices for inhalant administration of insulin, which are very different in respect to the technical and pharmacological principles (e.g., manual or semi-automated systems for inhalation, powder aerosol or liquid aerosol) and briefly described in *Table 3* (20). The most advanced method was Exubera® from Pfizer/Nektar, which received the approval from the American and European Drug Agencies (FDA and EMEA, respectively) in early 2006 for patients with diabetes mellitus types 1 and 2 and was marketed since September 2006. Exubera® was based on recombinant human insulin which was spray-dried and supplemented with the excipients mannitol, glycine, and sodium citrate. The insulin content of the final product, a large low-density particle, packed into small blisters was 60% (30). However, in October 2007 Pfizer announced it would be dropping Exubera®, citing that the drug had failed to gain market acceptance. Another device for insulin inhalation in advanced developmental status was AERx® iDMS (Aradigm Corporation, Novo Nordisk) (*Table 3*) (20). Shortly after the decision of Pfizer, Novo Nordisk also stopped all investigations on inhaled insulin.

PACKING OF INSULIN INTO MICROSPHERES AND LIPOSOMES

In some of the novel techniques for insulin inhalation the protein is formulated into microspheres (liposomes, particles, large porous particles). Even though the majority of these methods up to now was investigated in animal studies only, they may play a role for inhalant drug administration for insulin and other biomolecules in the future (*Table 4*) (1, 17, 37). The use of microparticles is based on the observation that smaller particles are phagocytosed more rapidly than larger ones (49). Biomolecules (e.g., insulin) can be packed into the inner part of biologically degradable polymers and lipids (microparticles and liposomes, respectively) (1, 7, 11, 15, 50). In consequence, the physiological alveolar clearance mechanism and the degradation of proteins and peptides after phagocytosis by alveolar macrophages are slowed which results in increased bioavailability. Another advantage is the alteration of the pharmacokinetic properties of the administered substances due to their slow release from these particles (7, 11, 15, 50). However, prerequisites for the use of these excipients are their rapid degradation after inhalation, readily elimination after inhalation and drug release, and immunological and toxicological inertness (37). In the last years, distinct procedures have been developed for the packing of proteins (e.g., insulin) into liposomes and solid particles (*Table 4*).

In detail, drug carrying capacity, drug release rate, toxicity, and pulmonary deposition of liposomes depend on their size, drug/lipid ratio, the properties of the used phospholipids (chain length, electrical charge, composition by neutral or anionic lipids), and the chosen method of delivery (1, 15, 17, 50). Most frequently they are made from lecithins (phosphatidylcholines), phosphatidylethanolamines,

Table 3. Devices for inhalant administration of insulin; modified according to (18, 20, 31-36).

Trade name (developer/partner)	Status of development in 2006	Principle or pharmaceutical form	Selected clinical data
Exubera [®] (Nektar Therapeutics/ Pfizer)	Market approval 2006	Dry powder insulin packed into blisters of 1 mg (≈ 3 U insulin) or 3 mg Dosing <i>via</i> the number of blisters Pneumatic release of the aerosol out of the blister in an inhalation chamber Particle diameter of $<5 \mu\text{m}$	Normal short acting insulin More rapid onset of action than subcutaneously administered insulin or lispro Proven reproducibility for all pharmacokinetic and pharmacodynamic parameters Applicability for monotherapy or combined therapy in patients with diabetes mellitus type 2 insufficiently treated with oral antidiabetics Similar quality of metabolic adjustment (HbA_{1c}) in patients with diabetes mellitus types 1 and 2 and subcutaneous administration of insulin Bioavailability: 10 - 16 % Most data regarding long time efficiency and safety available
AERx [®] iDMS (Aradigm/Novo Nordisk)	Phase III	Liquid insulin packed into single strips and dosed in single units Regulation of the breathing maneuver by means of microprocessors and electronic optimisation of insulin release within the inspiratory flow Particle diameter of 1 – 3 μm	More rapid absorption and onset of action than subcutaneously administered insulin Variability of pharmacodynamic parameters in patients with diabetes mellitus type 1 similar to those after subcutaneous administration of insulin Similar quality of metabolic adjustment in patients with diabetes mellitus type 2 and subcutaneous administration of insulin Bioavailability: 10 - 16 %
HIIP [®] (Alkermes/Eli Lilly)	Phase III	Dry powder insulin packed into blisters Mechanical system with breath activated release of particles Porous particles of low density with a geometric diameter of 5 μm – 30 μm (aerodynamic diameter of $<5 \mu\text{m}$)	Studies in patients with diabetes mellitus types 1 and 2 Development of a formulation with rapid release (pharmacokinetic similar to Humulin R) and a formulation with sustained release (pharmacokinetic similar to Humulin L) Bioavailability: 10 - 16 %
Technosphere [®] (Pharmaceutical Discovery Corporation/ Mannkind Pharmaceuticals)	Phase III	Dry powder recombinant insulin combined with a derivative of diketopiperazine for absorption enhancement Self-assembly into an ordered lattice array at low pH-value; mass median aerodynamic diameter 2 – 4 μm ; dissolution of particles and insulin release at neutral pH- value on alveolar surface Formulation developed for administration by means of a dry powder inhaler and passive desagglomeration (MedTone [®])	Studies in healthy individuals and patients with diabetes mellitus types 1 and 2 Low interindividual variability of the therapeutic effect of insulin Rapid absorption and onset of action and the short duration of the therapeutic effect indicate a potential use for treatment of postprandial hyperglycemia Bioavailability: 16 - 46 %

Microdose DPI [®] (Microdose Technologies/Elan Corporation)	Phase II	Dry powder insulin packed into blisters Desaggregation of drug powder by means of a piezo vibrator Mass median aerodynamic diameter approximately 1.5 µm, 84 % of the particles <4.7 µm	Few clinical data; well tolerated in clinical studies, bioavailability about 18 % compared with subcutaneous administration; more rapid absorption than subcutaneously administered insulin
Unknown (Abbott (formerly Kos Pharmaceuticals)/-)	Phase II	Dry crystals of a recombinant insulin formulation Administration by means of a hand held breath actuated inhaler (BAI) driven by a propellant	Comparison to Lantus [®] (insulin glargine) revealed a comparable effectiveness in controlling blood glucose concentrations
Aerodose [®] (Aerogen/-)	Phase II (stopped in 2003)	Liquid insulin Administration by means of a breath activated multiple dose inhaler Mean particle diameter of 3.2 µm, 87 % of the particles between 1 and 6 µm	More rapid onset of action than subcutaneously administered insulin Reproducibility of pharmacokinetic parameters similar to that after subcutaneous administration Linear dose-response-relationship of pharmacodynamic parameters Important effect of nebulization time on biological properties Bioavailability: 10 - 22 %
Bio-Air [®] (BioSante Pharmaceuticals/-)	Phase I	Coated dry particles based on calcium phosphate nanoparticle carriers Administration by means of a calcium phosphate nanoparticulate delivery system	Few data available; preclinical studies demonstrated an extension of the hypoglycemic effect after insulin inhalation
Alveair [®] (CoreMed/Fosun and Xuzhou)	Phase I	Liquid insulin Administration by means of a generic handheld device delivering inhaled insulin with the same units as injected insulin	Few clinical data; the manufacturer states a very high level of bioavailability of the compound
Unknown (Epic Therapeutics/-)	Phase I	Microspheres of recombinant human insulin (PROtein MATriX microspheres; ProMaxx [®]) Administration by means of a dry powder inhalation device (Cyclohaler) > 90 % insulin 95 % of the microspheres with mass median aerodynamic diameters 0.95 – 2.1 µm (mean: 1.5 µm), 95 % of the particles < 4.7 µm	Few clinical data; well tolerated in a phase I trial, bioavailability > 12 % compared with subcutaneous administration; more rapid absorption than subcutaneously administered insulin
Spiros [®] (Elan Pharmaceuticals (formerly Dura Pharmaceuticals)/-)	Phase I (stopped in 2004)	Dry powder insulin packed into blisters Release by means of a hand held battery driven multiple dose inhalator Development of a novel powder dispersion system (Spiros-S2) without the electromechanical components of the Spiros for administration at low inspiratory flow rates (15 – 30 l/min)	Small number of studies only in healthy individuals Administered doses consistent over a wide range of inspiratory flow rates

sphingomyelins, phosphatidylserines, phosphatidylglycerols and phosphatidylinositols (17). According to this structure, both hydrophobic and hydrophilic compounds can be packed into liposomes prior to the delivery to the lungs. Hydrophilic compounds (e.g., pharmaceuticals and larger biomolecules) are entrapped into the vesicle in the inner of the liposome whereas lipophilic (hydrophobic) compounds are encapsulated into the membrane bilayer. Small liposomes are unilamellar bodies with a hydrophilic core, whereas larger multilamellar liposomes have an onion-like structure with several layers of phospholipids and aqueous compartments. Because of their strong chemical and structural similarity, liposomes deposited in lung alveoli merge with cell membranes and facilitate the absorption of the carried biomolecule (e.g., insulin). Advantages of liposomes are sustained drug release, prevention of local irritation, reduced toxicity, improved stability in the large aqueous core, and the possibility for manipulation of release and targeting by variation of the bilayer constituents.

Solid particles (microspheres or large porous particles) are chemically and physically more stable than liposomes and allow higher drug loading (17). Pharmacological properties of microparticles (size range: <500 nm) depend on the used material, preparation technique, particle size, porosity, surface structure, and the delivery device (1, 15, 17, 50). Most frequently, the synthetic polymers polylactic acid (PLA) and polylactic-co-glycolic acid (PLGA) are used for their production. However, a number of other synthetic and natural polymers have been investigated (*Table 4*) (17). Up to now little is known about the pharmacological properties of most of the particles listed in *Table 4*, although some of these polymer-based systems might have toxicologically relevant effects especially after administration of high doses and/or for a longer time period (37). Microspheres can be produced by a number of distinct methods based on supercritical fluid technology, emulsion-solvent evaporation, spray-drying and phase separation. The encapsulation of peptides/proteins (e.g., insulin) by means of these techniques is affected by a number of physical and chemical properties (e.g., effect of solvents, heat, moisture, pH-value, oxygen and mechanic stress). Additionally, new techniques for production of microspheres from pure proteins have been developed (17). The release rate of the drug depends on many properties of the drug itself (concentration, solubility, molecular weight, nature of the peptide or protein) and of the polymer (e.g., nature, molecular weight, porosity, tortuosity, size, and uniformity) (17). Modification of the latter, e.g., by coating procedures, can be used to reduce the uptake by alveolar macrophages and, in consequence, to alter the pharmacological properties of the administered biomolecule (increase of pulmonary residence time and bioavailability) (17). Examples for the clinical use of microspheres for insulin inhalation are: ProMaxx®, Epic Therapeutics; Technosphere®, Pharmaceutical Discovery Corporation and calcium phosphate-polyethylene glycol particles, BioSante Pharmaceuticals (*Table 4*).

Table 4. Microparticle and liposome formulations for delivery of insulin to the lungs; modified according to (17, 37). Note that some types of microparticles are also subject of clinical studies described in *Table 3*.

Polymer material (manufacturer)	Drug load (%)	Particle size (μm)	Effect	Reference
PLGA ¹⁾	3.0	0.4 ²⁾	In guinea pigs significant reduction of blood glucose concentration; prolonged hypoglycemia over 48 h compared with the nebulised aqueous solution of insulin (6 hours)	(38)
Large porous PLGA ¹⁾ particles (AIR [®]) [Alkermes]	20.0	2.2 ³⁾	In rats high concentrations of insulin achieved systematically within 1 h after aerosolization; concentrations remained high for 96 hours; method is also subject of clinical investigation	(39)
PEG ⁴⁾ (ProMaxx [®]) [Epic Therapeutics]	> 90.0	0.95 – 2.1	In non-diabetic dogs rapid glucose depression; method is also subject of clinical investigation	(40)
Sodium hyaluronate	10.0	1-4 ³⁾	In dogs altered pharmacokinetic profile with increased MRT ⁵⁾ (9-fold), increased AUC ⁶⁾ /dose (2.5-fold) and increased t _{max} ⁷⁾ (3-fold)	(41)
FDKP ⁸⁾ (Technosphere [®]) [Pharmaceutical Discovery Corporation]	18.0	3.3 ³⁾	In healthy humans and patients with diabetes mellitus type 2 rapid onset and short duration of action and greater metabolic effect than subcutaneous injection over 3 hours; method is also subject of clinical investigation	(42, 43)
Calcium phosphate-PEG particles [BioSante Pharmaceuticals]	58.0	2-4 ³⁾	In rats prolonged t _{1/2} ⁹⁾ and MRT ⁵⁾ , slower elimination than insulin solution; increased bioavailability (1.8-fold) compared with subcutaneous injection; method is also subject of clinical investigation	(44)
DPPC ¹⁰⁾ coated insulin microspheres	n.a.	n.a.	Extension of the biological effect in proportion to the amount of lipid present	(45)
Oligosaccharide ester derivative DPPG ¹¹⁾ (SoliDose [®]) [Elan Pharmaceuticals - Quadrant]	10.0	n.a.	Improvement of the pharmacokinetic profile and prolongation of the duration of action (8 hours)	(46)
Material undisclosed (AIR [®]) [Alkermes]	NA	1-3 ³⁾	Long acting insulin microspheres	(47)
HPC/Cholesterol ¹²⁾	52	1	In mice prolonged reduction of blood glucose concentration (6 hours)	(48)

¹⁾PLGA: Polylactic-co-glycolic acid; ²⁾Geometric diameter; ³⁾Aerodynamic diameter; ⁴⁾PEG: Polyethylene glycol; ⁵⁾MRT: Mean residence time; ⁶⁾AUC: Area under the curve; ⁷⁾t_{max}: Time to reach the maximum serum concentration (C_{max}); ⁸⁾FDKP: 3,6-bis(N-fumaryl-N-(n-butyl)amino-2,5-diketopiperazine; ⁹⁾t_{1/2}: Plasma half-life time; ¹⁰⁾DPPC: Dipalmitylphosphatidylcholine; ¹¹⁾DPPG: Dipalmitylphosphatidylglycerol; ¹²⁾HPC: Hydrogenated egg yolk phosphatidylcholine.

Large porous particles are characterized by geometric diameters $>5\ \mu\text{m}$, low particle density (generally $<0.1\ \text{g/ml}$), and aerodynamic diameters $<5\ \mu\text{m}$. In consequence, these particles have good flow and aerosolization properties due to their low aerodynamic diameter and they are able to evade phagocytosis because of their large size (17). Aerosolized large porous particles deposit homogeneously and reproducibly without relevant toxicity on the alveolar cell surface. However, further toxicological and pharmacological studies are required also for this excipient. Currently, only one system for insulin inhalation is based on large porous particles (AIR[®], Alkermes) (*Table 4*).

EFFECT OF ABSORPTION ENHANCERS ON THE BIOAVAILABILITY OF INHALED INSULIN

Bioavailability of inhaled molecules after pulmonary deposition can be enhanced by a number of compounds increasing the absorption or inhibiting proteolytic degradation. Some of them are introduced into clinical treatment (e.g., Exubera[®]). However, most of the substances have been subject to studies in animals only and some of them can damage lung epithelium, especially after administration of higher doses and prolonged duration of treatment, necessary for patients with diabetes mellitus (2, 13, 15, 18, 36, 51).

The mode of action of absorption enhancers, which differ strongly with respect to their chemical structure and properties, is not yet completely understood. For example, bile acids presumably increase the absorption by alteration of the mucus layer, protection of proteins against enzymatic degradation, desaggregation of protein multimers, opening of epithelial tight junctions and solubilization of phospholipids and proteins out of the cell membrane followed by formation of micelles, whereas cyclodextrins, which are cyclic polymers of glucose, additionally form complexes with molecules fitting into their lipophilic inner structure (15, 50). *Table 5* compiles the absorbance enhancing effect of various compounds and demonstrates that the intensity of their pharmacological effect depends on their type (e.g., different cyclodextrins and lanthanides) and their administered dose (e.g., sodium taurocholate and sodium glycocholate). However, it should be considered that the toxicity of absorption enhancers often correlates with the strength of their pharmacological effect limiting their clinical use (15, 36). The majority of data was obtained in rats only, whereas results from other mammals or human studies are available for few compounds only. For example, sodium citrate, mannitol and glycine are excipients used in Exubera[®] (30). The effect of bile acids was investigated in humans by Heinemann *et al* (36, 56), who found only a small increase in bioeffectivity if a powder aerosol of insulin was administered in combination with an endogenous bile acid in healthy individuals ($12.0 \pm 3.5\%$ vs. $7.6 \pm 2.9\%$). In contrast, Johansson *et al* (15, 22) observed a strongly increased bioavailability

Table 5. Effect of absorption enhancers on pulmonary insulin absorption; modified according to (15, 51). Note that the experiments in rats were often in situ studies.

Substance	Species	Dosage form	Concentration or pH-value	Efficacy ¹⁾	Reference
Sodium tarocholate	Beagle dog	Solution	8 mM, 16 mM, 32 mM	2.5, 6.1, 8.9	(52)
Sodium glycocholate	Rat	Solution	10 mM, 50 mM	2.4, 5.1	(53, 54)
Sodium glycocholate	Rat	Solution	10 mM	4.2	(55)
Bile salt	Human	Dry powder	1.028 µg/87.2 U insulin	1.6	(56)
Surfactin	Rat	Solution	1 mM, 10 mM	1.1, 6.1	(54)
Span 85	Rat	Solution	1 %	1.1, 6.1	(54)
Span 85	Rat	Solution	1 %	7.2	(57)
Span 85	Rat	Dry powder	160 µg/dose	0.7	(57)
N-Lauryl-β-D-maltopyranoside	Rat	Solution	5 mM	7.1	(55)
MM ²⁾	Rat	Solution	10 mM	2.5	(55)
Liposomes	Rat	Solution	14 mg/ml	1.8	(58, 59)
Cyclodextrins	Rat	Solution	Hydroxypropyl-β-cyclodextrin: 5% γ-cyclodextrin: 5% β-cyclodextrin: 1% α-cyclodextrin: 5% Dimethyl-β-cyclodextrin: 5%	Hydroxypropyl-β-cyclodextrin: 1.2 γ-cyclodextrin: 1.7 β-cyclodextrin: 2.0 α-cyclodextrin: 2.3 Dimethyl-β-cyclodextrin: 2.8	(60)
Lanthanides (CeCl ₃ , GdCl ₃ , LaCl ₃ , LuCl ₃)	Rat	Solution	0.2 mg/kg	CeCl ₃ : 4.2, GdCl ₃ : 4.4, LaCl ₃ : 2.3, LuCl ₃ : 1.2	(61)
EDTA ³⁾	Rat	Solution	100 mM	0.6	(54)
Salicylate	Rat	Solution	100 mM	0.5	(54)
Citrate	Rat	Solution	pH 3.0; pH 5.0	4.5, 3.4	(57)
Citrate	Rat	Dry powder	36 µg/dose citric acid	2.1	(57)
Citrate	Rat	Solution	pH 3.0	3.2	(53)
Citrate	Rat	Dry powder	0.5 mg/dose citrate	2.7	(53)
Carboxy methylcellulose	Rat	Solution	0.5 %	1.1	(54)
Gelatin	Rat	Solution	1.0 %	0.4	(54)
HMAP ⁴⁾	Rat	Solution	16 mg/kg	2.2	(62, 63)

¹⁾Efficacy: Ratio of the area under the curve (AUC) or biological response between the dosage form with absorption enhancer and that without absorption enhancer; ²⁾MM: Mixed micelles of linoleic acid and HCO60 (hydrogenated castor oil); ³⁾EDTA: Ethylene diamine tetraacetic acid; ⁴⁾HMAP: Hydroxymethyl amino propionic acid.

of insulin in dogs, if the substance was administered as a fluidic aerosol containing also taurocholate (taurocholate vs. control; 23.2 ±4.4% vs. 2.6 ±0.3%).

Bioavailability and pharmacological activity of inhaled peptides and proteins can also be improved by addition of proteinase inhibitors preventing their inactivation by proteolytic cleavage (1, 11, 15). The effect of these compounds varies strongly depending on the type of the protease and the susceptibility of the peptide or protein. For example, an *in vitro* study dealing with the effect of

selected protease inhibitors on the permeability of insulin across the rabbit trachea revealed peptidase efficacies in the order di-peptidylaminopeptidase IV > leu-aminopeptidase > cathepsin B > trypsin (15, 64). Another *in vitro* study demonstrated an inhibitory effect of the protease inhibitors bacitracin, aprotinin, soybean trypsin inhibitor, and sodium glycocholate on the degradation of insulin in lung homogenate in a descendent order (15, 65). However, the antiproteolytic properties of these compounds on insulin after tracheal or pulmonary administration were up to now only subject of few animal studies and not introduced into clinical investigations (*Table 6*).

PHARMACOKINETICS OF INHALED INSULIN IN INDIVIDUALS WITHOUT PULMONARY DISEASES

The pharmacokinetics of inhaled insulin was investigated in a large number of studies performed in healthy subjects and patients with diabetes mellitus types 1 and 2. Unfortunately, the comparison of the study results is hampered by differences of the used inhalers, administered formulations and doses of insulin, small numbers of included individuals (healthy individuals or patients), inappropriately used pharmacological models, and distinct parameters determined (36, 66). However, it has been observed that inhaled regular insulin is absorbed at least as fast as subcutaneously administered insulin (time to peak concentration in plasma (t_{max}): 7-90 min vs. 42-274 min (*Table 7*) (4, 8, 20, 36, 66-68), an observation which has also been made in one of the first inhalation studies by Laqueur and Grevenstuck (22). The pharmacokinetics of inhaled insulin seems to be a biphasic one with a first peak rapidly after inhalation, which is followed by a slow release comparable to that after subcutaneous injection (36, 67). In the first 60 min after drug administration, the area under the concentration vs. time curve (AUC) is larger for inhaled insulin than for subcutaneously administered insulin. In contrast, subcutaneously administered insulin has a larger AUC if an observation period of 6 hours is considered (36). This suggests that

Table 6. Effect of protease inhibitors on pulmonary insulin absorption; modified according to (51).

Substance	Species	Dosage form	Concentration or pH-value	Efficacy ¹	Reference
Bacitracin	Rat (in situ)	Solution	1 mM	0.9	(54)
Bacitracin	Rat (in situ)	Solution	20 mM	6.8	(55)
Bacitracin	Rat (in situ)	Solution	10 mM	7.0	(57)
Bacitracin	Rat (in situ)	Dry powder	420 µg/dose	1.0	(57)
Nafamostat	Rat (in situ)	Solution	13 mM	2.1	(54)
Aprotinin	Rat (in situ)	Solution	10 mg/ml	2.0	(55)
Soybean trypsin inhibitor	Rat (in situ)	Solution	10 mg/ml	2.5	(55)

¹Efficacy: Ratio of the area under the curve (AUC) or biological response between the dosage form with protease inhibitor and that without protease inhibitor.

inhaled insulin might have some therapeutic benefit in the treatment of prandial or postprandial hyperglycemia, when compared with conventionally administered insulin. In addition, compared with the subcutaneously administered drug, inhaled insulin shows a lower risk for postprandial hypoglycemia because of its increased clearance after inhalation (36, 78). Accordingly, determination of the glucodynamics in healthy individuals revealed a more rapid, but even shorter decrease of plasma glucose concentration than after subcutaneous administration of insulin (36).

Better conclusions on the pharmacokinetics can be obtained in studies by means of the glucose clamp technique or in patients without residual synthesis of insulin (diabetes type 1) and healthy individuals under pharmacological inhibition of insulin synthesis (*Table 7*) (36, 66). Different doses of inhaled insulin resulted in a widely linear dose-response relationship in patients with diabetes type 1 (36). However, although the maximum of the insulin concentration (C_{max}) increased with the administered dose, there was an increasing delay of the time to peak concentration in plasma (t_{max}) indicating the existence of a dose dependent pulmonary uptake mechanism (36). In addition to the large number of studies with normal insulin, a small number of investigations were performed with lispro, an insulin derivative modified by means of molecular biology. Compared with normal insulin lispro showed a better therapeutic effect (i.e., lower doses required to achieve the same serum concentration of insulin and more rapid onset of action). Probably, both effects are caused by a breakup of the hexamer into monomers followed by an increased bioavailability (19, 36).

In most studies, bioavailability was calculated by comparison of the AUC after inhalation to that after subcutaneous administration of insulin. In contrast, bioeffectivity describes the hypoglycemic effect of inhaled insulin compared with a defined insulin dose administered by subcutaneous injection (36). Therefore, the provision for the bioeffectivity can give further information. However, the parameters bioavailability and bioeffectivity result in an underestimation of the therapeutic effect of insulin, because only a small proportion of the administered drug is deposited in the lung periphery (i.e., alveolar region) from where it can be absorbed into circulation. In detail, the commercially available systems for pulmonary administration of insulin are characterized by bioavailabilities and bioeffectivities of 9-22 % and 8-16 %, respectively (*Table 7*), which is more than the 3% reported by Heubner *et al* (23) in 1924. In consequence, the insulin dose which is required to achieve the same therapeutic effect after inhalation is up to 11-times higher compared with subcutaneous administration (36). Between 50 and 80% of the insulin filled in the inhalation system does not reach the lung, but is remaining in the nebulizer, is deposited in the mouth, or the oropharynx, or is expired. Taking this into account, the bioavailability from the lung deposited fraction is about 2-5 times of the subcutaneously given insulin dose. However, from this dose more than 50% is deposited in the airways (bronchial system) and is removed from the lung by the mucociliary transport and/or degradation. Only

about 40% is rapidly absorbed into the circulatory system. If this is also considered, it is obvious that the „pulmonary extradose“ for insulin inhalation is 2-3-times of the dose required for injection (36).

Pulmonary deposition and, in consequence, bioavailability of inhaled aerosols (including insulin and other compounds) is mainly influenced by biological and physical parameters of the substance, the nebulizer, the breathing maneuver, and the oropharyngeal filter efficiency of the patient (9, 10, 18, 36, 66). An optimum deposition of the inhaled insulin is achieved if the aerosol is released at the beginning of a slow and deep inhalation maneuver. This enables the particles to penetrate deeply into the lung and they can be deposited in the alveolar region (9, 10, 18, 67). Farr *et al* (18, 67) observed in their study a later and weaker effect of insulin administered by a shallow inhalation maneuver (40% of inspiratory vital capacity (IVC)) than after a deep inspiration maneuver (80% IVC). This shows the importance of breathing pattern on alveolar deposition. The breathing maneuver does not only affect the total amount of alveolar insulin deposition, but also the intraindividual reproducibility of this therapy. However, other biological parameters (e.g., smoking, physical stress, lung perfusion) have also a strong effect on the intraindividual variability of insulin administered by inhalation. The reproducibility of pulmonary delivered insulin was investigated in several studies. In summary, these studies demonstrate a similar or even better reproducibility of insulin administration by inhalation than by subcutaneous injection (36, 68, 79-81). Obviously, the better reproducibility after inhalation is caused by the missing of some influencing parameters (e.g., physical exercise, smoking, temperature, body position and injection), which play a role after subcutaneous injection of insulin (18, 81, 82). In detail, similar intraindividual variabilities of various pharmacokinetic parameters (AUC, C_{max} , t_{max} , blood glucose and rate of glucose infusion) were observed in the studies of Gelfand *et al* (36, 83) using Exubera® in patients with diabetes type 2, Hompesch *et al* (36, 84) using the AERx® system in patients with diabetes mellitus type 1 (36, 84) and Perera *et al* (68) using the Aerodose® system in patients with diabetes type 2 (coefficients of variation (CV) of the AUC_{0h-3h} 19 and 23% after inhalation and subcutaneous injection, respectively). On the other hand, lower variabilities after inhalation than after injection were observed from Himmelmann *et al* (36, 80) using the AERx® system in healthy individuals (CV values of 13.7 and 16.5% in non-smokers and smokers, respectively) and Pfützner *et al* (36, 85) using Technosphere® in patients with diabetes mellitus type 2 (CV values 16-20%). For comparison, interindividual CV values for subcutaneous application of insulin are about 25% (79).

EFFECT OF SMOKING ON THE PHARMACOKINETICS OF INHALED INSULIN

A number of original studies and reviews describe a higher absorption (up to 3-5 times) of inhaled insulin in smokers than in non-smokers (*Table 8*) (3, 8, 12,

20, 27, 36, 66, 80, 81). For example, Köhler *et al* (27, 66) reported a higher absorption ($C_{\max}$) and bioavailability (65 vs. 25%) of inhaled insulin, which was accompanied by a more pronounced decrease of the glucose concentration in smokers compared with non-smokers. In another study Himmelmann *et al* (80) reported a higher absorption of inhaled insulin (AUC; 63.2 mU•h/l vs. 40.0 mU•h/l, $P<0.01$), a higher peak concentration ($C_{\max}$; 42.0 mU/l vs. 13.9 mU/l, $P<0.001$), and a shorter time to peak ($t_{\max}$; 31.5 min vs. 53.9 min, $P<0.001$) in smokers compared with non-smokers. In addition, the mean residence time (MRT) in smokers was less than half of that in the non-smoker group ($P<0.0001$) and, accordingly, the apparent elimination rate constant of exogenous insulin was almost twice as high in smokers compared with non-smokers ($P=0.0019$). However, the intraindividual variability was similar in both groups (80). In another study Becker *et al* (87) investigated the effect of smoking cessation and subsequent resumption on the absorption of inhaled insulin. It was found that AUC and $C_{\max}$ were higher in smokers than in non-smokers, whereas $t_{\max}$ was shorter. Smoking cessation resulted in a rapid change of the values obtained in smokers toward those of non-smokers. In contrast, smoking resumption completely reversed the effect of smoking cessation. In principle, this can be explained by several mechanisms. It is well established that chronic cigarette smoke inhalation increases the permeability of the alveolar-capillary barrier (91, 92). Postulated mechanisms for this increase are immunological modifications (93), an increase in the blood perfusion (94), surfactant antioxidant depletion due to an increased burden of inhaled reactive oxygen species (ROS) (95) and a disruption in surfactant function (96). On the other hand, an increased metabolism of drugs in smokers, e.g., due to an induction of drug-metabolising enzymes, has been reported (97). In consequence of augmented insulin metabolism, the metabolic activity of the hormone would be diminished. Importantly, the significantly higher values of AUC and $C_{\max}$ observed after insulin inhalation in smokers compared with non-smokers are not necessarily followed by a concomitant increase in insulin action. This apparent contradiction can be explained by the inhibition of metabolic insulin action followed by an induction of insulin resistance and glucose intolerance due to cigarette smoke inhalation (98-101). The increased epithelial permeability as a cause of the varied pharmacokinetics of insulin in smokers is reversible within a few days after the end of tobacco abuse (92), whereas the chronic bronchitis typically existing in long-time smokers is not reversible in this short period.

The effects of acute cigarette smoke inhalation on the absorption of inhaled insulin are largely different from that of chronic cigarette consumption, as cigarette consumption just before insulin inhalation significantly blunts the enhanced insulin absorption in smokers. However, there are no differences in $t_{\max}$ (80). The underlying mechanisms of these effects are not understood. In principle, the well established bronchoconstrictory effect of nicotine might cause changes in ventilation and distribution which are followed by variations of the particle

Table 7. Selected studies on pharmacokinetics and pharmacodynamics of inhaled insulin; according to (36).

Subjects and device (type of insulin)	n	Route	Type of insulin and dose	Serum insulin $t_{max}^{(d)}$ (min)	Serum insulin $C_{max}^{(b)}$	Serum insulin AUC ^(c)	GIR ^(d) t_{max} (min)	GIR ^(e) t_{max} (mg/kg/min) ^f	GIR AUC ^(g) (mg/kg)	Bioavailability and bioefficacy of inhaled insulin	Reference
Healthy and non-diabetic individuals											
AERx [®] iDMS	17		Regular 2 U AERx [®] Regular 4 U AERx [®] Regular 6 U AERx [®] Regular 8 U AERx [®] Regular 6 U		29.6 mU/l 33.1 mU/l 40.3 mU/l 47.1 mU/l 33.3 mU/l	168 mU•h/l (0-10h) 162 mU•h/l (0-10h) 210 mU•h/l (0-10h) 236 mU•h/l (0-10h) 204 mU•h/l (0-10h)	6.35 6.9 8.12 8.7 7.75	2170 2310 2600 3130 2590			(69)
Aerodose [®]	12	s.c.	Regular 1.5 U/kg Regular 0.15 U/kg	50 85	360 pmol/l 364 pmol/l	48900 pmol•min/l (0-6h) 56400 pmol•min/l (0-6h)			1520 (0-6h) 1750 (0-6h)	Bioavailability 9.3 %, bioefficacy 10.3 %	(70)
Spiros [®]	6	s.c.	Regular 2.31 mg (emitted)	30	1.13 µg/l		112	235 mg/min		Not dependent on the inhalation technique	(71)
Spiros [®]	12		Regular 2 blisters Regular 3 blisters Regular 4 blisters Regular 5 blisters Regular 8 U Regular 14 U Regular 20 U	30 20 45 20 120 105 120	24 mU/l 31 mU/l 38 mU/l 46 mU/l 31 mU/l 34 mU/l 59 mU/l		187 129 161 162 227 241 241	2.0 3.3 3.4 4.2 4.1 5.1 6.1			(72)
Inhalator M [®] (Dry powder inhaler)	5	s.c. i.v.	Technosphere 100 U Regular 10 U Regular 5 U	13 121 5	2225 pmol/l 204 pmol/l 3692 pmol/l	96.4 nmol•min/l (0-3h) 104.2 nmol•min/l (0-6h) 41.6 nmol•min/l (0-3h) 71.5 nmol•min/l (0-6h) 35.2 nmol•min/l (0-3h) 36.0 nmol•min/l (0-6h)	39 163 14	16.7 10.6 18.9	1940 (0-3h) 2940 (0-6h) 1150 (0-3h) 2170 (0-6h) 1330 (0-3h) 1700 (0-6h)	Bioavailability 26 % (0-3h), 16 % (0-6h) (relative); 15 % (0-3h), 16 % (0-6h) (absolute); bioefficacy 19 % (0-3h), 14 % (0-6h) (relative), 8 % (0-3h), 10 % (0-6h) (absolute)	(43, 73)
Turbuhaler [®] DPI	11	s.c. i.v.	Regular 99 U Regular 10 U Regular 5 U	24 106 1	305 pmol/l 254 pmol/l 5692 pmol/l	27.0 nmol•min/l (0-6h) 37.1 nmol•min/l (0-6h) 25.8 nmol•min/l (0-3h)	108 147 14	6.2 9.1 17.6	1440 (0-6h) 1900 (0-6h) 830 (0-6h)	Bioavailability 7.8 % (relative), 5.6 % (absolute); bioefficacy 7.6 % (relative), 9.5 % (absolute)	(74)

deposition of inhaled insulin (80). In addition, nicotine has a vasoconstrictory activity which might cause a delay of insulin absorption after subcutaneous administration (102). Likely, this vasoconstrictory effect inhibits also the absorption of insulin if the drug is administered directly after acute cigarette consumption (80). Furthermore, pulmonary neutrophils activated by components of cigarette smoke might cause an enzymatic degradation of insulin deposited in the alveoli (93). Finally, it should be noted that even acute passive cigarette smoke exposure may affect the pharmacokinetics of inhaled insulin. However, the effect of acute passive cigarette smoke exposure is just the opposite of that of active chronic smoking, because it causes a modest decrease of the bioavailability of inhaled insulin due to reduced lung permeability (103).

Inhalant insulin therapy was not approved in current smokers and individuals who quitted smoking less than 6 months before therapy until marketing was stopped by the manufacturers. In consequence, the number of diabetic patients which can be treated with insulin is strongly reduced, because about 20-25% of these patients are tobacco smokers (30, 104). By the time the inhalant therapy with insulin will be reintroduced into the market, further systematic investigations on the effect of cigarette consumption on insulin bioavailability in smokers should be performed. It might well be that insulin doses for smokers may be adapted (3, 36, 80).

EFFECT OF PULMONARY DISEASES ON THE PHARMACOKINETICS OF INHALED INSULIN

The lung is a dynamic organ, strongly exposed to environmental factors and at risk for very different diseases. Patients with manifest pulmonary diseases affecting drug absorption were excluded from inhalant drug therapy in order to ensure a sufficient and reproducible deposition and bioavailability of the inhaled insulin. However, the effect of respiratory diseases on the pulmonary absorption of inhaled insulin was subject of a small number of studies. For example, infections of the upper respiratory tract have obviously no relevant effect on the bioavailability of inhaled insulin as it was shown by McElduff *et al* (36, 88) who observed no differences of pharmacokinetics and pharmacodynamics in otherwise healthy individuals within the period of an acute respiratory infection (*Table 8*). Another respiratory disease, asthma bronchiale, is characterized by hyperreactivity with bronchospasm, inflammation and airway remodelling. There are two primary concerns regarding the inhalation of insulin in asthma patients. Firstly, drug inhalation especially by means of DPI can induce bronchospasm. Secondly, in asthma exacerbation respiratory effort and bronchospasm limit the deposition of inhaled insulin in the lung alveoli. This is due to a variation of pulmonary convective gas transport, a smaller airway diameter and in consequence the higher rate of particle deposition in the central airways of these

patients. Henry *et al* (36, 89) investigated the pharmacokinetics of inhaled insulin in asthma patients and reported a mild decrease of $C_{\max}$ and a distinct decrease of AUC (bioavailability) and plasma glucose concentration (bioeffectivity) after insulin inhalation in asthma patients compared with healthy individuals. Furthermore, patients with asthma showed a higher variability of $C_{\max}$ and AUC, but not of the glucose lowering effect than healthy controls after insulin inhalation (Table 8). Presumably, these inappropriate effects can be improved by administration of bronchodilators in these patients (105). Data regarding the pharmacokinetics of inhaled insulin in patients with chronic obstructive pulmonary disease (COPD) are limited and conflicting. COPD patients demonstrated a variable (higher or lower) absorption of insulin compared with subjects without COPD. It is not clear whether this variability is secondary to differences in inhalation devices or different study populations (105, 106). In consequence, the effect of COPD on insulin absorption should be subject of further studies prior to the reintroduction of inhalant insulin therapy into the market, because COPD is a frequent and often not or not correctly diagnosed pulmonary disease.

EFFECT OF THE AGE ON THE PHARMACOKINETICS OF INHALED INSULIN

Lung morphology and function change as a function of age. Elder individuals show a decrease of the alveolar surface, a variation of lung elasticity, a decrease of the alveolar capillary volume combined with a decline of the ventilation/perfusion ratio, a decrease of the pulmonary diffusion capacity for carbon monoxide (DLCO), and an increase of the pulmonary residual volume (RV) (21). Therefore, the age is another important parameter influencing the pharmacokinetics of inhaled insulin. Henry *et al* (36, 90) reported similar values of $C_{\max}$ and AUC in patients with diabetes type 2 aged >65 years and young individuals of the age between 18 and 45 years. The variability in these parameters was not different between both study groups either. However, the observed decrease of plasma glucose concentrations was more pronounced in younger individuals than in elder patients indicating a requirement of higher doses in aged patients (Table 8).

SAFETY OF INHALED INSULIN

The experience of the last 80 years in millions of patients has shown that the treatment of diabetes mellitus with subcutaneously administered insulin is relatively safe. However, beside the specific aspects of bioavailability and bioeffectivity discussed before, the aspects of tolerability and toxicity must be once more investigated for the inhalant therapy with insulin. In principle, not only insulin, but also absorption enhancers might cause adverse effects in the lung. The

Table 8. Studies investigating factors influencing the pharmacokinetics of regular human insulin inhaled by devices developed for insulin inhalation; according to (36).

Subjects/System	n	Type of insulin and dose	Serum insulin t_{max} ^{a)} (min)	Serum insulin C_{max} ^{b)}	Serum insulin AUC ^{c)}	Additional comments	Reference
Non-smokers (NS) vs. smokers (S) Exubera®	30 (NS) 38 (S)	Regular 2 mg Regular 2 mg	53 31	16 mU/l 72 mU/l	1410 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h) 4847 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h)	Partially reversible on smoking cessation	(86)
Non-diabetic non-smokers (NS) vs. smokers (S) AERx® iDMS	13 (NS) 23 (S)	Regular 33.8 U Regular 33.8 U	53.9 31.5	13.9 mU/l 42.0 mU/l	40.0 mU•h/l (0-6h) 63.2 mU•h/l (0-6h)	Significant difference in t_{max} , C_{max} and AUC	(80)
Non-smokers (NS) vs. smokers (S) Exubera®	10 (NS) 20 (S) ^{d)} 20 (S) ^{e)} 20 (S) ^{f)}	Regular 1 mg Regular 1 mg Regular 1 mg Regular 1 mg	52.5 20.0 37.5 30.0	9.7 $\mu\text{U}/\text{ml}$ 26.8 $\mu\text{U}/\text{ml}$ 15.8 $\mu\text{U}/\text{ml}$ 29.2 $\mu\text{U}/\text{ml}$	1645 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h) 2583 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h) 1887 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h) 3156 $\mu\text{U}\bullet\text{min}/\text{ml}$ (0-6h)	Significant difference in t_{max} , C_{max} and AUC between NS and S, values of S approaching to those of NS after smoking cessation, smoking resumption reversed the effect of smoking cessation	(87)
Non-diabetic individuals with upper respiratory tract infection (URTI) vs. post-upper respiratory tract infection (PU) AERx® iDMS	20 (URTI) 20 (no URTI)	Regular 45 U Regular 45 U	59 80	15.4 mU/l 17.3 mU/l	44.0 mU•h/l (0-6h) 47.7 mU•h/l (0-6h)	Significant difference of t_{max} , but no effect of URTI on the other parameters of pharmacokinetics	(88)
Non-asthmatic (NA) vs. asthmatic (A) non-diabetic individuals AERx® iDMS	28 (NA) 16 (A)	Regular 45 U Regular 45 U	50 45	9872 pmol/kg 8310 pmol/kg	1.45•10 ⁶ pmol•min/l•kg (0-6h) 1.07•10 ⁶ pmol•min/l•kg (0-6h)	Significant difference in AUC	(89)
Younger (Y, 18-45 years) vs. older (O, >65 years) patients with type 2 diabetes AERx® iDMS	27 (Y) 28 (O)	Regular 45 U Regular 45 U	40 30	219 pmol/l 221 pmol/l	38055 pmol•min/l (0-6h) 37892 pmol•min/l (0-6h)	Comparable pharmacokinetic profiles in both groups, but lower glucose lowering effect in elderly individuals	(90)

a) t_{max} : Time to reach C_{max} .

b) C_{max} : Maximum concentration of insulin in serum

c) AUC: Area under the serum insulin concentration-time curve (between specified limits)

d) Before smoking cessation

e) 7 days after smoking cessation

f) 9-10 days after smoking resumption

latter are variations in lung function, local inflammation, allergic reaction, formation of antibodies against insulin, pulmonary fibrosis, and lipodystrophy (3, 20, 36). Most data regarding the long-term tolerability are published for the Exubera® system and the AERx iDMS® system for study periods of up to 2 years and more in patients with diabetes types 1 than 2 (18, 33, 36, 81, 107-113).

Animal studies and investigations in diabetic patients demonstrated that diabetes and consecutive insulin treatment cause a morphological change of lung structure (e.g., thickening of the alveolar membrane and the capillary basal lamina, vascular hyalinosis, granulomas, intraseptal nodular fibrosis and emphysema-like septal obliteration) which depends on the duration and severity of the disease and on additional factors, like smoking (104, 114). Therefore, the effect of insulin inhalation on lung function has been thoroughly investigated. In most studies inhalation of insulin caused no changes of spirometric parameters of lung function (e.g., forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC)) and parameters of diffusion capacity for carbon monoxide (DLCO). and blood gas analysis (3, 18, 20, 33, 36, 81, 107-120). Changes in the DLCO which were observed in some studies could not be explained by the investigators. However, based on the results of the lung function tests and the observed variations in individual patients, the manufacturer recommended spirometric measurement of lung function before treatment, after 6 months, and thereafter at least annually in the product information for Exubera® (121). In summary, the experience regarding the effect on lung function indicates that inhaled insulin is characterized by a low pulmonary toxicity, good tolerance, and good bioeffectivity (3, 18, 20, 36). This may be explained by a relatively low toxicity of insulin itself and the distribution of the inhaled doses of 4-5 mg (three times a day) on a total alveolar surface of about 80-120 m² (3, 9, 10). The total quantity of the inhaled substance is lower than the threshold value for dust inhalation of 30 mg/day recommended by the American Council of Government Industrial Hygienists (3, 78). Furthermore, the initially high concentration of insulin in the epithelial lining fluid is rapidly decreasing due to absorption and distribution in the body fluid and proteolytic degradation. As a result, there is no evidence that pulmonary tissue is exposed to a higher insulin concentration after inhalation than after subcutaneous injection (36).

Beside its strong metabolic effect, insulin also acts as a weak growth factor (efficiency of only 1/100 of insulin-like growth factor-1(IGF-1)) after binding to the receptor for IGF-1. However, there is up to now no evidence for a relevant competitive effect of inhaled insulin at the IGF-1 receptors in the lung (36). In one of the studies on inhalant insulin application, pulmonary fibrosis had been observed, but there was obviously no relationship to the study medication (81). Recently, about 6 months after the end of the marketing of Exubera®, the American Food and Drug Administration (FDA) published a press release reporting a potentially increased risk for bronchial carcinoma in ex-smokers treated with inhalant insulin (122). In detail, there have been 6 newly diagnosed

cases of primary lung malignancies in clinical trials among Exubera®-treated patients, and 1 newly diagnosed case among comparator treated patients. There has also been 1 post-marketing report of a primary lung malignancy in an Exubera®-treated patient. All these patients had a prior history of cigarette smoking. However, even though the number of cases is too small for a final risk evaluation, the potentially increased risk for lung cancer should be subject of further investigation prior to product relaunch of Exubera® or similar products of competitors.

In a number of studies, inhalation of insulin was followed by increased serum titres of non-neutralizing IgG antibodies against insulin. However, the development of these antibodies had no therapeutic relevance, i.e., there was no correlation to the metabolic control, lung function, and adverse events (18, 20, 21, 33, 36, 81, 107, 108, 110-112, 116-118, 120, 123, 124). The occurrence of these antibodies has been observed in up to one third of patients with diabetes mellitus (especially those of type 1) and long-time subcutaneous administration of insulin, more frequently in younger individuals than in elderly and with a strong increase in frequency and titre until 6 to 12 months after start of insulin inhalation, but also in patients without diabetes mellitus (e.g., patients with autoimmune diseases). It is likely, that the induction of antibodies against insulin is caused by its formulation and dose (inhaled insulin is given in higher doses and more frequently for treatment of postprandial hyperglycemia than subcutaneous insulin) and the site of delivery (presence of macrophages, dendritic cells, and lymphocytes in the lung), whereas impurity of the peptide, a structural alteration of insulin in the powder aerosol during its preparation, a modification of the molecule by storage, and immunogenicity of excipients added to insulin are less likely, or were refuted (3, 81, 123, 124).

Lipodystrophy is a phenomenon observed in up to 30% of patients treated with subcutaneous injections of insulin developing at the site of injection and was firstly described by Lawrence 1925, i.e., soon after the introduction of insulin into clinical treatment (125-127). Lipohypertrophy is caused by the anabolic effect of insulin promoting the synthesis of protein and fat, whereas lipoatrophy is caused by an inflammatory process and is nowadays rarely observed because of the use of highly purified insulins (127). Since adipocytes are also located in the lung, inhaled insulin can also affect these cells after pulmonary deposition. However, at present it is not known if and how inhaled insulin affects pulmonary adipocytes (128).

Cough is a typical symptom in clinical treatment with inhalation of dry powder aerosols which might affect patient convenience and compliance. Therefore, cough was addressed in a number of studies investigating inhaled insulin. Mild to moderate cough was reported to occur rapidly after inhalation (seconds to minutes) in up to 20-30% of patients. However, the reported symptoms were transient, settled with continuation of the therapy and seldom resulted in treatment withdrawal (107, 108, 111, 112, 117-120).

Hypoglycemia is a common problem in patients treated with antidiabetics, especially insulin. Therefore, the evaluation of hypoglycemia incidence and severity was subject of many clinical studies investigating inhaled insulin. The data obtained in these studies are conflicting, demonstrating an increased frequency of severe hypoglycemic events in patients treated with inhaled insulin compared with patients treated with subcutaneous injections in some of these studies (115-120). However, there is no, or only little, difference regarding the risk for the occurrence of hypoglycemia between inhaled and subcutaneous insulin (129), whereas the risk is expectedly higher for patients treated with inhaled insulin when compared with treatment with oral antidiabetics (107, 108, 111, 112).

ACCEPTANCE AND COSTS OF INHALED INSULIN

In the last decades of diabetic therapy, patient convenience and compliance were improved by development of smaller and sharper needles and pen injector systems and insulin pumps for injection. However, all these systems are based on needles and, therefore, are invasive. The development of inhaled insulin was an approach for a non-invasive insulin therapy in patients with diabetes types 1 and 2. A number of studies were performed to investigate patient convenience and improvement of life quality and improvement of metabolic control (determined by measurement of glycated haemoglobin (HbA_{1c})). In these studies, it was found that patients welcome the non-invasive alternative for administration of insulin by means of an inhaler, even though its handling (use and cleaning) requires a large number of steps (30, 110). Especially patients with diabetes type 2, who fail on oral antidiabetic therapy, and whose switch to insulin treatment is often delayed, and patients with needle phobia representing at least 10% of the population should profit from insulin inhalation (32, 128, 130). However, these advantages are opposed by a number of other arguments. In detail, because of the small bioavailability of inhaled insulin much higher doses must be administered than in conventional therapy by means of subcutaneous injection. In addition, the high costs for the development of inhalant therapy and the device were considered in the price of the product. Furthermore, the patients must be thoroughly trained prior to inhalant therapy and require controls of lung function before and under therapy. All these factors result in an extra cost between £ 600 and more than £ 1000 (currently £ 1 $\approx$ 1.25 €) – depending on the required doses (114). The advantage of a non-invasive (needle free) treatment in patients is also restrained by the requirement of blood sampled by finger puncture for the measurement of glucose concentration. Furthermore, there is no relevant improvement of metabolic control as determined by means of HbA_{1c} measurement due to an inhalant insulin therapy (30, 110, 114). Based on these arguments and the results of studies investigating clinical effectiveness and cost-effectiveness the National

Institute for Health and Clinical Excellence (NICE, United Kingdom) and the Institute for Quality and Efficiency in Health Care (IQWiG, Institut für Qualität und Wirtschaftlichkeit im Gesundheitswesen, Germany) declined funding for inhaled insulin in Great Britain and Germany, respectively. It is likely that these decisions for the decline of reimbursement were substantial reasons that this therapy option failed to produce relevant sales and that the manufacturers stopped marketing of the product.

CONCLUSIONS

More than 80 years after the first experiments, insulin received the approval for inhalant administration (Exubera®). In addition, several other techniques for insulin aerosolization and aerosol application have been investigated and are in a different phases of their development. Shortly after its market launch, Exubera® has been stopped by the manufacturer (Pfizer), because of unexpectedly low sales. To our information, competitors have also put their developments on hold. During a short time on the market, Exubera® was accepted by patients (although not reimbursed by most health insurance companies) and well tolerated without adverse effects. However, up to now some questions are not completely answered, e.g., effects of long-time inhalation on lung function and effects of pulmonary diseases on deposition, absorption, and pharmacokinetics of inhaled insulin, or increased risk for lung cancer in ex-smokers. Under the current circumstances, it is unlikely that inhaled insulin will be relaunched in any formulation in the near future. However, if there is a relaunch, the unanswered questions should be subject to further investigation. The experience on insulin inhalation may help develop inhalation therapies for other compounds serving for the treatment of systemic diseases, because such type of treatment seems to be a safe and reliable technique for drug application improving the patient compliance due to its non-invasive character.

Conflict of interest: Dr. Rudiger Siekmeier has no conflicts of interest in relation to this article. Dr. Gerhard Scheuch does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Dr. Scheuch is a consultant for several pharmaceutical companies in the field of aerosol medicine and pulmonary drug delivery (e.g., Bayer-Schering, Boehringer/Ingelheim, GSK, Novartis, Talecris, Sandoz).

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