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Research paper

Comparative pharmacoscintigraphic and pharmacokinetic evaluation of two new formulations of inhaled insulin in type 1 diabetic patients

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ABSTRACT

In this open, single-dose study, we compared the lung deposition and bioavailability of two newly developed insulin formulations for pulmonary delivery. Twelve type 1 diabetic patients were administered the two insulin products (2 U/kg b.w.), which had been radiolabelled with ^{99m}Tc. The formulations were either microparticles of insulin without excipients (F1) or lipid-coated insulin microparticles (F2). Lung deposition was assessed by γ-scintigraphy imaging performed immediately after administration. Bioavailability was evaluated by quantifying serum insulin levels over a period of 6 h.

Lung deposition was found to be 50 ± 9% and 24 ± 8% for the F1 and F2 formulations, respectively. The insulin AUC_{0-360} ratio of F1/F2 was 188%, which was consistent with scintigraphic imaging. The concordance between imaging and biological results suggests that the lower bioavailability of F2 is due to its lower lung deposition and not to a reduced absorption into the blood stream. Additional in vitro experiments indicated that the lower performance of F2 was most probably related to a lower disaggregation efficiency of the powder when administered at a sub-optimal flow rate.

The two formulations showed interesting pharmacokinetic profiles (T_{max} of 26 and 16 min for F1 and F2, respectively) that mimic the physiological insulin secretion pattern. The bioavailability of the developed formulations was within the range of other DPI insulin formulations that have reached the final stages of clinical development.

1. Introduction

The number of diabetic patients with poor glycaemic control is very high. As a consequence, they develop short- and long-term complications responsible for increased morbimortality. A recent report [1] indicates that, in the United States, only 57% of diabetic adults achieve the treatment goal of less than 7% glycosylated haemoglobin (HbA1c), and the percentage of patients achieving all three treatment goals (HbA1c <7%, blood pressure <130/80 mmHg and total cholesterol <200 mg/dl) is even lower, reaching only 12% [1]. An important cause of this poor glycaemic control is the difficulty in achieving plasma insulin profiles that resemble the natural prandial insulin secretion, despite the broad variety of subcutaneous (s.c.) insulins available [1,2]. Current s.c. insulin therapies do not provide the optimal pharmacokinetic profile. The absorption of regular human insulin is too slow, and its action is excessively prolonged to effectively mimic early insulin release. In an attempt to overcome some of the limitations of regular insulin, rapid-acting insulin analogues have been developed, but their onset of action is still relatively slow compared with prandial glucose absorption [3].

In addition, these injected formulations present an intra-patient variability in starting time of insulin action of up to 30% [3]. This is mainly due to the absorption rate from the s.c. depot, which depends on the insulin concentration, injection volume and injection site, as well as on endogenous factors such as skin temperature, local blood flow, muscle activity and thickness of the subcutaneous fat layer [4].

The ideal prandial insulin would therefore have a pharmacokinetic profile that closely matches the body's normal insulin response to a meal and would be highly reproducible. In recent years, several attempts have been made to develop insulin

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formulations that more closely meet these clinical requirements. Inhaled insulins seem particularly promising, as many formulations for pulmonary delivery exhibit pharmacokinetic profiles with absorption rates at least as fast as that of the rapid-acting insulin analogue lispro [5–9].

The first commercially available inhaled insulin (Exubera®, Nektar Therapeutics/Pfizer) was taken off the market in October 2007 after 1 year of commercialisation, because of unexpectedly low sales [10]. In April 2008, a potentially increased risk of bronchial carcinoma in ex-smokers treated with Exubera® was reported compared to patients treated with injections [11]. This increased risk of cancer could be related to the fact that insulin acts as a weak growth factor by its binding to the IGF-1 receptor, even if its efficiency is only 1/100 of IGF-1 [11]. Because all six newly diagnosed cases of primary lung malignancies among Exubera®-treated patients had a history of cigarette smoking, the association with insulin therapy was regarded as inconclusive. In addition, there is to date no evidence for a significant action of inhaled insulin on IGF-1 receptors in the lung [10,11].

After the withdrawal of Exubera®, only one company pursued the work on inhaled insulin, even though several other formulations had reached phase III of clinical development (AIR®, Alkermes/Eli Lilly, and AERx® iDMS, Aradigm/Novo Nordisk). MannKind (Valencia, CA, USA) developed Afrezza®, which is an insulin dry powder formulation produced by precipitation followed by freeze-drying [12]. Studies are currently conducted on Afrezza® and its new delivery device (Dreamboat®), but the Food and Drug Administration's final decision is not expected before 2012.

In spite of the commercial failure of Exubera®, inhaled insulin might get a place in the therapeutic arsenal. In addition to the pharmacodynamic considerations mentioned above, this type of formulation might address compliance problems observed with s.c. insulin treatments, in particular when multiple daily injections are required, or in type 2 diabetic patients, who often require insulin later in life [13]. Depending on the outcome for Afrezza®, it would thus not be surprising if other pharmaceutical companies regained interest in developing an inhaled insulin formulation.

The objective of this study was to clinically evaluate two new dry powder formulations of insulin previously developed in our laboratory [14]. The formulations present a very simple composition with few or no excipients, which are potentially acceptable for inhalation. This is of interest given the limited number of excipients currently accepted for inhalation [15]. The formulations were produced using high-pressure homogenisation (HPH) followed by spray-drying. These production techniques and lipid excipients were chosen in order to limit the degradation of the active ingredient [14].

An uncoated formulation and lipid-coated insulin formulation were chosen. These have an interesting aerodynamic behaviour in vitro, with high fine particle fractions (FPF) of 63 ± 4% and 59 ± 4%, respectively [14].

Based on these promising in vitro results, we carried out a scintigraphic and pharmacokinetic evaluation of the formulations after inhalation of a single oral dose in twelve type 1 diabetic patients. Gamma scintigraphic imaging provides information on the level and site of drug deposition in the lungs while the pharmacokinetic evaluation informs on the absorption of insulin from the deposition site and on its systemic bioavailability.

This clinical study was combined with the assessment of the in vitro influence of several airflow rates on the aerodynamic properties of the formulations. Finally, a dissolution test specifically designed for pulmonary formulations [16] was performed to investigate whether the differences appearing in the pharmacokinetic profiles of the 2 formulations could be related to differences in their dissolution rate.

2. Materials

Two dry powder insulin formulations were selected. The first formulation (F1) consisted of a powder that was micronised without excipient. The formulation was obtained by HPH of a 2% w/v insulin (Incelligent™ AF, Millipore®, MA, USA) suspension in isopropanol, followed by spray-drying, as previously described [14]. The second formulation (F2) consisted of lipid-coated micronised insulin particles. It was also obtained by HPH, followed by spray-drying. The lipids (20% of the insulin mass) were dissolved in a small volume (<3 ml) of hot isopropanol (55 °C) and added to the suspension just before spray-drying. These lipids were cholesterol (Fagron, The Netherlands) and Phospholipon®90H (Nattermann Phospholipids GmbH, Germany) in a ratio of 75:25 [14].

Size 3 HPMC capsules (Qualicaps, Spain) were hand-filled and loaded with 2 U insulin/kg b.w. (corresponding to 4.86 mg and 5.83 mg of powder for a 70-kg person for the F1 and F2 formulations, respectively). The dry powder inhaler (DPI) device used was the Aerolizer® (Novartis, Switzerland), a passive, breath-actuated, single-dose DPI.

3. Methods

3.1. Study design

The study was conducted at the Erasme Hospital (Brussels, Belgium) in accordance with the principles stated in the declaration of Helsinki. Approval was obtained from the ethics committee of the Erasme Hospital (Ref.: P2009/154/2009-012578-10) and from the Belgian Federal Agency for Medicines and Health Products (FAMHP, Ref.: EudraCT No. 2009-012578-10).

The study design is an open, single-dose, two-treatment, two-period cross-over study with a wash-out period of at least 6 days between the two phases of the study. Long-standing type 1 diabetic patients were chosen for the study because of the absence of endogenous insulin production, which allows easy and accurate measurement of the exogenous insulin reaching the systemic circulation after inhalation. This population of patients was preferred to healthy volunteers, in whom the circulating exogenous insulin concentration would need to be calculated based on simultaneous insulin and C-peptide measurements [17] or by suppressing endogenous insulin secretion through a continuous infusion of insulin, leading to a stable (and known) insulin serum concentration [18].

A dose of 2 U/kg b.w. of insulin was chosen for both formulations, as the bioavailability of insulin by inhalation was expected to be around 10% of the s.c. bioavailability [19,20]. Both formulations were radiolabelled with up to 0.014 mCi/kg or 0.53 MBq/kg of technetium-99m (^{99m}Tc). The maximum effective dose of radioactivity administered to the subjects in this study was estimated to be 8.29 μSV/kg.

The patients using multiple insulin injections did not inject their long-acting insulin on the evening preceding a study day to ensure that no residual s.c. exogenous insulin was present at the time of dosing. To limit night and morning hyperglycaemia, patients were asked to delay dinner to 10:30 PM and to inject short-acting human insulin (Actrapid® 100 IU/ml) 30 min before, at the usual dose for the meal. For patients with continuous subcutaneous insulin infusion (CSII), the pump was stopped 6 h prior to administration of the inhaled insulin. The subjects fasted for at least 10 h (food) and 3 h (drink) before dosing.

The morning of the study, patients received a 2-h intravenous insulin infusion of Actrapid® to control glycaemia prior to insulin inhalation. Glycaemia was assessed every 20 min using a glucometer, and the infusion rate was adapted on a case-by-case basis to

achieve a target glucose level of 100–200 mg/dl at the end of the infusion period. The infusion was stopped 30 min before dosing to avoid the presence of residual exogenous insulin.

Administration of the formulations took place as follows: after a forced exhalation, subjects were asked to inhale the formulation as fast and deep as possible and to hold their breath for 10 s before exhaling through a filter. This filter was used to trap any aerosol particles present in the expired air. The patients were taught to perform this procedure during the 30-min period preceding the formulation administration.

A standardised meal was provided 30 min after dosing. Alcohol-, caffeine-, grapefruit- and xanthine-containing food or drink were not allowed during the study period.

3.2. Patients

Twelve type 1 diabetic patients (plasma C-peptide concentration below 0.2 nmol/l) were recruited (seven men and five women; 44 ± 10 years; body weight, 88 ± 14 kg; BMI between 23.2 and 33.6 kg/m^2). Patients were physically and psychologically well, based on medical history, clinical examination and routine laboratory tests performed shortly before experiment, and had stable insulin requirements for at least 6 months before inclusion. Patients with poor diabetes control (requiring hospital admission or presenting repeated serious hypoglycaemic episodes in the past 6 months) were excluded.

Pulmonary function was recorded using a disposable spirometer (EasyOne, NDD Medizintechnik AG, Zürich, Switzerland). The values for FEV_1 (forced expiratory volume in 1 s), FVC (forced expiratory vital capacity) and PEF (peak expiratory flow) were >80% of the predicted value for all patients. Because of the modified absorption of inhaled insulin in smoking subjects [21], all patients had to be non-smokers for at least 1 year before the study (assessed by medical history and a cotinine urine test). Excluded from this study were patients with any past or active, acute or chronic pulmonary disorder, patients with a total daily insulin dose exceeding 150 U, patients with drug addiction or excessive use of alcohol (daily intake in excess of the equivalent of 25 g pure alcohol) or xanthines (tea, coffee, cocoa), and pregnant or lactating patients. Subjects participating in another study within 4 weeks of this study were also excluded. Before starting the study, the nature of the clinical trial was explained and written consent was obtained from all patients.

3.3. Safety assessment

Patients were closely monitored during the whole study period for possible hypoglycaemia (by checking physical symptoms and blood glucose levels using a glucometer). If necessary, glucose tablets (Dextro Energy) or a 50% dextrose IV injection (2 cc/kg) was administered. Pulmonary function (PEF, FVC, FEV_1) was recorded before and 30 min after dosing. Patients were monitored for adverse events throughout the study days. Subjects underwent a control visit (physical examination, pulmonary function testing, routine clinical biology and urine analysis) in the 4 weeks preceding the start and in the 10 days following the end of the study. During this end medical date, they were also asked about the occurrence of any adverse effect in the hours and days following experiment.

3.4. Radiolabelling of the formulations

A classical method for the radiolabelling of dry powders is by adsorbing the radiolabel onto the formulation particles. This is achieved by wetting the particles with a non-solvent containing the radiolabel, followed by the evaporation of the solvent, leaving the radiolabel on the surface of the drug particles [22–24]. ^{99m}Tc is the most commonly used γ-ray-emitting radionuclide for the radiolabelling of pharmaceutical aerosols. The γ-ray of ^{99m}Tc has sufficient energy (140 keV) to penetrate body tissues, and the half-life of ^{99m}Tc (6 h) is sufficient for handling and imaging, while limiting the risks of lengthy exposure of subjects to radiation [22,25,26].

The ^{99m}Tc isotope used in the study was first eluted as sodium pertechnetate, in a dedicated area of the Erasme Hospital (authorisations no. AFCN 2349/BHA-2402-B and AFCN 2348/BH-2402-A) using a ^{99}Mo–^{99m}Tc generator from Covidien (MA, USA). The ^{99m}Tc was then extracted into methylethylketone (MEK) by shaking the pertechnetate solution with an approximately equal volume of MEK. The aqueous and MEK phases were separated in a separating funnel, and the MEK phase containing the pertechnetate was evaporated to dryness. After that, the pertechnetate was redissolved in a small volume of isopropanol (<2 ml) and then added to the insulin suspension (containing dissolved lipids, when applicable) before spray-drying using a Büchi Mini Spray Dryer B-191 (Büchi Laboratory Techniques, Switzerland) with constant stirring.

3.5. Validation of the radiolabelling process

A validation procedure was performed for each formulation to ensure that the radiolabel was effectively adsorbed onto the surface of the drug particles, that it had the same aerodynamic behaviour as the drug powder (no dissociation) and that it did not alter the size distribution of the drug. The technique employed is widely used and described in the literature [27,28]. The aerosols were fractionated using a Multistage Liquid Impinger (MsLI) (Copley Scientific, UK). Measurements (n = 9) were taken at 100 l/min for 2.4 s using an Aerolizer® inhaler.

The aerodynamic fine particle fractions (FPF, expressed as a percentage of the total metered dose) and aerodynamic particle size distributions (PSD) of the following three products were compared: the unlabelled drug, to which no radiolabel had been added; the labelled drug, following the addition of the ^{99m}Tc radiolabel (determined by HPLC method [14]); and the radiolabel itself (determined by a γ-counting technique using a Cobra γ-counter [Packard bioscience, UK]). This comparison was made to show the similarity of the products' behaviour. The repeated-measures ANOVA test was used to validate the radiolabelling method.

The insulin content in the formulations was also measured using an HPLC method [14] to detect any possible degradation of the insulin molecule or any impurity that could be due to the radiolabelling process. The overall quality of the formulations was assessed by measurement of several features (see [14]), such as the presence of aggregates and residual solvents, measurement of the uniformity of the delivered dose and assessment of their stability. Microbial quality was also evaluated (data not shown), which allowed us to conclude that the formulations conformed to the requirements of the European Pharmacopoeia for microbiological quality of non-sterile pharmaceutical preparations for inhalation use.

3.6. Scintigraphic analysis

Immediately following administration of the radiolabelled aerosol, scintigraphic images were recorded in order to estimate the total amount of drug aerosol reaching the lungs and its deposition pattern. The following views were taken using a DST-XLi dual-headed γ-camera (DHD-SMV, Sopha Medical, France): posterior and anterior views of the upper body (chest and right-turned head) using a 3-min static acquisition; full scan of the whole body (rate: 14 cm/min).

A 2 mCi flat flood source was interposed between the lower detector and the patient's body, and the activity was recorded with the upper detector. This was done to define regions of interest in which activity was measured [24,29]. A background count was also recorded for each camera head.

Four regions of interest were outlined: the oropharynx and exhalation filter, the oesophagus and trachea, the stomach, and the lungs. In regions where both anterior and posterior images were recorded, the geometric mean of counts in both images was calculated. The lungs were subdivided into perihilar (or central) and peripheral regions of interest, corresponding approximately to large and small airways, respectively [22]. The ratio of peripheral to central lung deposition (P/C ratio) was calculated as an index of regional lung deposition or lung penetration index [22].

The dose in each defined area was expressed as a percentage of the loaded dose. Calculations were made as follows: the counts in each area were first expressed as a percentage of total body counts, which represented the emitted dose. This emitted dose was quantified by subtracting the insulin remaining in the Aerolizer® device and in the emptied capsule (HPLC method [14]) from the insulin dose initially loaded in the capsule. The dose fraction in each region of interest was then adjusted to correspond to the loaded dose in place of the emitted dose.

A two-tailed paired *t* test was used to determine whether differences between the deposition patterns for the two test formulations were significant.

3.7. Pharmacokinetic analysis

For the quantification of serum insulin levels, blood samples were collected according to the following sampling schedule: 25, 15, and 5 min before inhalation, and 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 135, 150, 165, 180, 210, 240, 270, 300, and 360 min after inhalation. After collection, blood samples were left to coagulate at ambient temperature for at least 30 min. The tubes were then centrifuged for 10 min at 1500 g, and serum samples were rapidly stored at −80 °C in an upright position until analysis was completed. Insulin was assayed in all relevant serum samples using a commercial ELISA kit for the determination of human insulin (Mercodia® Insulin Elisa, Sweden).

The following pharmacokinetic parameters were calculated: peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}) and area under the curve from administration to the last quantified concentration (AUC_{0-360}), calculated using the linear trapezoidal rule.

The geometric mean ($Mean_{geom}$), geometric standard deviation (SD_{geom}) and geometric coefficient of variation ($CV\%_{geom}$) of insulin concentrations were calculated for each time point. These were used in place of arithmetic descriptors because concentration data usually follow a skewed distribution that is best described according to a log-normal rather than a Gaussian statistical model [30]. A Mixed Procedure (REML approach, [31]) was applied to the log-transformed parameters C_{max} and AUC_{0-360} to calculate the F1/F2 ratios of these parameters as well as the associated 90% confidence intervals (CI) and CV%. The difference between the T_{max} of the two formulations was evaluated using the Hodges–Lehmann estimate of shift parameter, with calculation of the associated 90% CI [32].

3.8. In vitro influence of the airflow rate on the aerodynamic behaviour of the formulations

Aerodynamic features of the two formulations, such as the Mass Median Aerodynamic Diameter (MMAD) and FPF, were described previously [14]. These measurements were taken using an air flow rate of 100 l/min, which corresponds to a pressure drop of 4 kPa in the Aerolizer® device. This standardised pressure drop is recommended in the European Pharmacopoeia 6.0 for the testing of all dry powder inhaler devices. However, this assumes optimal lung function and optimal inhalation procedure from the patient to obtain a good match between in vitro and in vivo data.

Additional in vitro impaction measurements were therefore taken for the two formulations at 30 l/min and 60 l/min to determine the influence of a sub-optimal air flow rate on their aerodynamic properties. These were performed using an MsLI with an acquisition time of 8 s and 4 s for the 30 l/min and 60 l/min flow rates, respectively. The detailed procedure of the impaction measurements has been described previously [14].

3.9. Dissolution test

A dissolution experiment was performed to compare the dissolution profiles of the two formulations, which might influence their pharmacokinetics.

As no method has yet been established for the testing of inhaled drugs, we used a method adapted from the standard "Paddle over disc" described in European Pharmacopoeia 2.9.4.-2. for the testing of transdermal patches [16].

Because only a fraction of the emitted dose of the inhaled formulations enters the lungs and is accessible for dissolution, the whole insulin dose was first fractioned using a Next Generation Impactor (NGI, Copley Scientific Ltd., Nottingham, UK) in order to select only those particles that would deposit in the lungs for the dissolution test. The cup in position 3 of the NGI was chosen to collect particles and was equipped with a removable disc insert in the impaction area. This choice was based on the amount of powder impacted on the different stages of the impinger. Stage 3 was found to contain the largest impacted fraction for both formulations.

Moreover, at the selected operating flow rate (60 l/min for 4 s), the aerodynamic diameter of the particles collected at stage 3 was between 2.82 μm and 4.46 μm, values that are compatible with deep lung deposition.

An Aerolizer® inhalation device was first filled with a single no. 3 HPMC capsule loaded with 50 mg and 60 mg powder for the F1 and F2 formulations, respectively. This difference in powder load was necessary to collect the same insulin dose in the collection cup for the two formulations (8.2 ± 0.1 mg and 8.4 ± 0.2 mg, respectively).

Following size fractionation in the NGI, the disc insert was removed from the collection cup and covered with a polycarbonate membrane with a pore size of 0.4 μm (Millipore, MA, USA), presoaked in dissolution fluid and locked in the membrane holder. The disc was then placed at the bottom of the vessel of a paddle dissolution apparatus (Erweka DT6, Heusenstamm, Germany) for solid dosage forms (European Pharmacopoeia 6.0) containing 300 ml of phosphate buffer saline (PBS) as dissolution medium. Simulated lung fluid (SLF) was not used in this case as its pH varies significantly with time [33] and because the dissolution of insulin is pH dependent [34,35]. The use of PBS instead of SLF was found to give results that were similar to the dissolution profiles of budesonide-containing lipid microparticles [36].

The operating conditions were as follows: paddle rotation speed, 75 RPM; temperature of the bath, 37.0 ± 0.2 °C; distance between the blade and the upper face of the disc, 2.5 cm.

During dissolution testing, 1-ml samples were withdrawn from the dissolution vessel and replaced with an equivalent volume of fresh dissolution medium at regular intervals up to 180 min.

The percentage of dissolved insulin at each time point, and the residual insulin on the membrane and the membrane holder were quantified using HPLC analysis [14]. The total insulin dose considered in the calculation was obtained by adding the insulin dose

obtained at 180 min and the dose collected on the membrane and the membrane holder.

The similarity factor f_2 was used to compare the two dissolution profiles [37]. It was calculated using only the first nine time points ($P = 9$) in order to limit the number of points with a dissolution of the product of over 85% and to avoid bias (f_2 values are sensitive to the number of dissolution time points [37]).

4. Results

4.1. Validation of the radiolabelling process

In vitro evaluation of the aerodynamic behaviour of the two insulin formulations with the Aerolizer® device was performed to ensure that the radiolabelling method did not significantly modify the PSD of the aerosol generated by the device. It was also performed to confirm that the distribution of the ^{99m}Tc reflected that of the drug and that ^{99m}Tc therefore acted as a suitable marker for the two powder formulations.

The MsLI in vitro deposition profiles were compared for unlabelled insulin, labelled insulin and ^{99m}Tc. Fig. 1 shows the results obtained for the FPF values of both formulations. The results obtained for the F1 formulation before labelling (65 ± 4%), after labelling (66 ± 4%) and for the radiolabel (70 ± 3%) were not significantly different (repeated-measures ANOVA test, $p > 0.05$). There was also a good match between the FPF values of the F2 formulation before labelling (59 ± 4%), after labelling (59 ± 4%) and for the radiolabel (56 ± 2%) ($p > 0.05$). The results were also not significantly different at any stage of the MsLI (data not shown), demonstrating that the PSD of the radiolabel and the F1 and F2 formulations were well matched, with no alteration of the aerosol properties of the formulations after radiolabelling.

4.2. Adverse events

The lung function (PEF, FVC, FEV_1) of patients was evaluated just before dosing and 30 min after dose using a disposable spirometer. For both formulations, mean values for these parameters (expressed as a percentage of the predicted value for each subject) were not significantly different before and after dosing (two-tailed paired t test, $p > 0.05$). Measurements were always above 80% of the predicted value for both formulations. Two episodes of mild coughing occurred just after inhalation of the drug (one episode for each formulation), which resolved within 1 min and did not affect the results of the lung function test.

Mild hypoglycaemic episodes (plasma glucose level <60 mg/dl) occurred in 3 patients for the F1 formulation, and in 1 patient for the F2 formulation. These events were easily resolved using glucose tablets. Hypoglycaemia occurred around 1 h after dosing. Four and five patients for the F1 and F2 formulations, respectively, were hyperglycaemic (plasma glucose level >200 mg/dl) at the end of the study period. When needed, s.c. insulin was administered to correct glycaemia after collection of the last blood sample.

One patient presented headache the day after administration of the two formulations, which could possibly be linked to the taking of the inhaled insulin. No other adverse events were noted in any subject for either formulation.

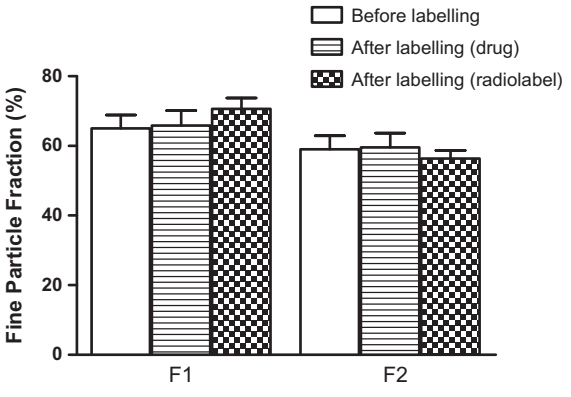

Fig. 1. In vitro aerodynamic evaluation (FPF) (mean ± SD, $n = 9$) of the two formulations: before labelling, after labelling (drug labelled) and after labelling (radiolabel) with ^{99m}Tc.

4.3. Scintigraphic results

Analysis of the scintigraphic results was performed on 11 subjects as one of the patients was withdrawn because of poor compliance with the instructions for the inhalation procedure (deep and slow inspiration in place of deep and fast).

The percentage of radiolabelled insulin formulation deposited in the device (determined by HPLC) and in the whole lung, oropharynx, trachea (inseparable from the oesophagus), and stomach, as determined by γ-scintigraphy, is shown in Table 1.

The F1 and F2 formulations exhibited a mean lung deposition of 50 ± 9% and 24 ± 8%, respectively (two-tailed paired t test, $p < 0.0001$). Similar percentages of the insulin dose were deposited in the device ($p > 0.05$), the trachea ($p > 0.05$), and the stomach ($p > 0.05$) for the F1 and F2 formulations, respectively (see Table 1). The dose impacted in the oropharynx was significantly higher for the F2 formulation than for F1 (35% vs. 18% ($p < 0.01$)).

A representative comparison of the scintigraphic images obtained for the two formulations is shown in Fig. 2. The powder is homogeneously distributed in the peripheral region of the lungs for the two formulations, and more powder is impacted in the oropharynx and trachea for the F2 formulation. These observations are in accordance with the distribution of the dose obtained in Table 1.

Regional deposition results are shown in Table 1. The relative distribution (P/C ratio) of the radiolabel within the central and peripheral airways was higher for the F1 formulation ($p < 0.001$), indicating a greater deposition in small-diameter airways and alveoli.

4.4. Pharmacokinetic results

Analysis of the pharmacokinetic results was also performed on 11 subjects. The mean concentration–time curves obtained after inhalation of the two formulations are shown in Fig. 3, and the pharmacokinetic parameters C_{max}, T_{max} and AUC_{0-360} in Table 2.

The pharmacokinetic data for the two formulations correlated well with the mean percentage of insulin deposited in the lungs as determined by γ-scintigraphy. The AUC_{0-360} and C_{max} values were significantly higher for the F1 formulation than for the F2 formulation, with a F1/F2 ratio for AUC_{0-360} of 188% (90% CI: [148; 240], CV% = 32%). In comparison, the F1/F2 ratio of the mean

Table 1
Distribution of the insulin dose (%, mean ± SD) in the lungs, oropharynx, trachea, stomach and device for the F1 and F2 insulin formulations in type 1 diabetic patients ($n = 11$).

	F1	F2
Device	2.5 ± 1.0	5 ± 2
Oropharynx	18 ± 9	35 ± 11
Trachea/oesophagus	4.9 ± 1.3	6 ± 2
Stomach	3 ± 2	5 ± 3
Whole lung	50 ± 9	24 ± 8
1/3 Perihilar	25 ± 5	14 ± 7
2/3 Peripheral	25 ± 4	10 ± 2
P/C Ratio	1.05 ± 0.15	0.80 ± 0.18

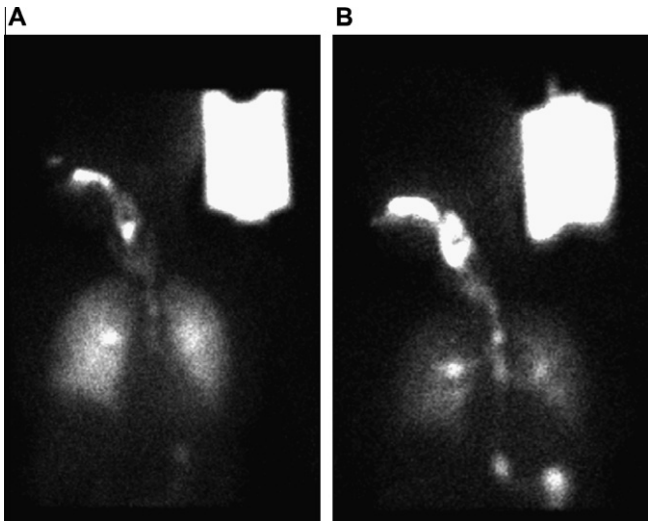

Fig. 2. Scintigraphic images obtained for one representative patient after administration of the F1 formulation (A) and F2 formulation (B).

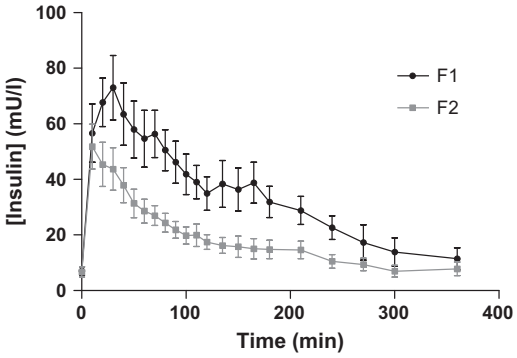

Fig. 3. Insulin mean serum concentrations following administration of 2 U/kg b.w. of the F1 and F2 formulations in type 1 diabetic patients (geometric mean ± SE, $n = 11$).

deposition in the lungs, measured by scintigraphy, was around 208%. The F1/F2 ratio for C_{max} was 138% (90% CI: [107; 177], CV% = 34%).

The rapid serum concentration peak for the two DPI formulations followed by a progressive decrease in insulin concentrations over 6 h is illustrated in Fig. 3.

The T_{max} of the uncoated formulation (F1) and lipid-coated formulation (F2) occurred at 26 and 16 min, respectively. The Hodges–Lehmann estimation of the median difference in T_{max} was 10 min with a 90% CI of [−10; 20]; hence, T_{max} was not statistically different for the two formulations. It should be noted that the first blood sample used for the measurement of pharmacokinetics was collected only 10 min after dosing, which could have slightly under-estimated the AUC_{0-360} and C_{max} for the F2 formulation.

4.5. In vitro aerodynamic evaluation

Additional in vitro deposition tests were performed at 30 l/min and 60 l/min to determine the influence of a sub-optimal air flow rate on the aerodynamic properties of the formulations. For the F1 formulation, FPF remained constant when decreasing air flow rate (Fig. 4). For the F2 formulation, however, FPF markedly decreased at a flow rate of 60 l/min compared to 100 l/min, but showed no further decrease at 30 l/min (Fig. 4). The difference in particle deposition of the F2 formulation at 60 l/min mainly consisted in an increased deposition in the throat of the impinger, whereas less powder was impacted at stage 4 and in the filter. This indicates that a sub-optimal aspiration flow rate used by the patient (below 100 l/min) results in a decreased disaggregation efficiency of the F2 formulation, whereas the aerodynamic properties of the F1 formulation remain almost unchanged.

4.6. Dissolution

The dissolution profiles of the two formulations are shown in Fig. 5. At the end of the experiment, the fraction of undissolved insulin remaining on the disc insert and membrane was 4.4 ± 0.2% and 5.3 ± 0.2% for the F1 and F2 formulations, respectively. This was most probably due to the presence of air bubbles trapped under the membrane, which prevented optimal contact between the powder and the dissolution media. F1 exhibits a faster dissolution rate than F2, with 50% of insulin mass dissolved at 4.8 min and 90% at 17.6 min for the F1 formulation, and 50% dissolved at 15.5 min and 90% at 53.3 min for the F2 formulation.

The difference between the two dissolution profiles was confirmed by the similarity factor ($f_2 = 28$), which was below the limit value of 50. This difference could be explained by the presence in the F2 formulation of a lipid-coating around insulin particles, which is made up of 75% hydrophobic cholesterol. The extent of this sustained-release effect is probably limited by the presence of 25% Phospholipon® 90H in the coating, which contains more than 90% amphiphilic phosphatidylcholine.

These results differ from the in vivo observations where insulin absorption rates were similar. This can probably be explained by the relatively thick layer of powder deposited in vitro on the disc insert, which could have caused a wettability problem for the F2 formulation because of its hydrophobic lipid-coating. This does probably not occur in vivo as the particles disperse over a much larger surface area and alveolar surfactant wet the particles.

5. Discussion

The adverse events noted during the study were mainly a mild cough, occurring just after inhalation of the drug. Cough episodes have also been reported in 25% of the patients treated with Exubera®. They were transient, disappearing with continued treatment, and did not result in discontinuation of treatment [38].

The inhalation of 2 U/kg b.w. of insulin, alone or in the presence of lipids, did not cause immediate bronchoconstriction, suggesting good tolerance of these products. The lipid excipients used in this study had already shown good tolerance in other phase I clinical trials on DPI formulations of budesonide [27] and tobramycin [23] in healthy volunteers and cystic fibrosis patients, respectively.

Other adverse events were linked to glycaemia. The mild hypoglycaemic episodes (3 patients for the F1 formulation, 1 patient for the F2 formulation) were most probably due to a delay in taking the meal. The higher number of patients having undergone hypoglycaemia after taking the F1 formulation is in accordance with the higher pulmonary deposition and bioavailability observed with this formulation.

The hyperglycaemic episodes observed in 4 and 5 patients for the F1 and F2 formulations, respectively, were probably linked to the end of action of the inhaled insulin. By study protocol, the patients could not use long-acting insulin the evening preceding the study days, which could also have contributed to these hyperglycaemic events.

Regarding potential long-term toxicity concerns for inhaled insulin, currently available studies with both Exubera® (a 3-year

Table 2
Comparison of the scintigraphic and pharmacokinetic parameters obtained for the F1 and F2 formulations, and for other inhaled insulin formulations in selected studies.

DPI formulation	Inhalation device	Scintigraphic data		Pharmacokinetic data							
		Whole lung deposition (% of loaded dose)	Ref.	*n*	Dose	T_{max} (min)	C_{max} (mU/l)	AUC_{0-360} (mU h/l)	Dose-normalised C_{max} [d] (mU/l)	Dose-normalised AUC_{0-360} [d] (mU h/l)	Ref.
F1 formulation	Aerolizer®	50 ± 9	–	11	2 U/kg b.w. ~176 U	26 ± 7[a]	73 (52) [c]	230 ± 95[a]	41(52)[c]	131 ± 54[a]	–
F2 formulation	Aerolizer®	24 ± 8	–	11	2 U/kg b.w. ~176 U	16 ± 9[a]	52 (51) [c]	125 ± 62[a]	30 (51)[c]	71 ± 35[a]	–
Exubera® (Pfizer, New York, NY)	Exubera® inhaler	~40	[54]	10	1 mg = 27.5 U	55.0[a]	9.7[c]	27.4[c]	35.3[c]	99.7[c]	[17]
Afrezza® (MannKind, Valencia, CA)	MedTone® inhaler	40 ± 9	[55]	16	100 U	19.8[b]	241 (52) [c]	219 (43)[c]	241 (52)[c]	219 (43)[c]	[51]
AIR® (Alkermes, Cambridge, MA)	AIR® inhaler	51 (18)	[56]	15	5.2 mg = 150 U	45[b]	44 (49) [c]	148 (39)[c,e]	29.3 (49)[c]	99 (39) [c,e]	[52]

[a] Arithmetic mean ± SD.
[b] Median.
[c] Geometric mean (geometric CV%).
[d] Dose-normalised C_{max} and dose-normalised AUC_{0-360} are calculated on the basis of a 100 U dose.
[e] AUC_{0-600} in place of AUC_{0-360}.

study) and Afrezza® (a 1-year study) indicate no apparent safety issue, although reversible, non-progressive and non-pathological effects on lung function were noted [39,40]. The assessment of the long-term safety requires more extensive and longer evaluation, including further studies on the manner inhaled insulin goes through the alveoli [10].

The γ-scintigraphy results of this study showed that the F1 uncoated insulin formulation leads to an in vivo pulmonary deposition around 50%, which corresponds to the in vitro impaction results (FPF = 63 ± 4%) [14]. However, the F2 lipid-coated formulation showed an in vivo deposition around 24%. This last result was expected neither from the validation experiments (see Fig. 1) nor from previous in vitro data, as the FPF of F1 and F2 formulations obtained in MsLI experiments performed at 100 l/min were comparable (FPF of 63 ± 4% and 59 ± 4%, respectively) [14].

The inter-patient variability observed in lung deposition is attributable in part to the fact that very few of the selected patients had prior experience with drug inhalation. It is therefore assumed that many of them did not perform the inhalation procedure optimally, despite brief prior training. The variability observed for the whole lung deposition (CV% of 18% and 33% for the F1 and F2 formulations, respectively) is however in the range of that observed in other pharmacoscintigraphic studies performed with DPI formulations [41].

The pharmacokinetic analysis was in keeping with the scintigraphic results, with F1/F2 ratios of the lung deposition and AUC_{0-360} of 208% and 188%, respectively. This concordance indicates that if an equal dose of each formulation had reached the lungs, they would probably have shown comparable bioavailability. This also shows that the higher P/C ratio value obtained with F1 has probably not caused a significant increase in the bioavailability of this formulation.

The variability observed in the pharmacokinetic data can, again, be partly attributed to the patients' lack of experience with an inhaled medication.

The results of additional in vitro aerodynamic evaluation showed that the F2 lipid-coated formulation, unlike the F1 formulation, had decreased disaggregation efficiency at a flow rate between 60 and 100 l/min. This resulted in an increased powder impaction in the throat of the impinger, and a decreased deposition at stage 4 and in the filter. This effect was also observed in vivo (see Fig. 2 and Table 1), with higher deposition of F2 in the oropharynx resulting in a smaller deposition in the peripheral region of the lung. As this decreased disaggregation efficiency was not observed for the F1 formulation, this could at least partly explain the higher lung deposition observed for this formulation, leading to a higher bioavailability.

However, the difference in the in vitro FPFs measured at 60 l/min (67% and 47% for the F1 and F2 formulations, respectively) cannot fully explain the in vivo difference observed in lung deposition (50% vs. 24%). Although the correlation between FPFs and lung deposition was reasonable in many DPI studies, poor agreement was also observed from a variety of drug delivery systems [42]. A number of potential causes have been investigated. Firstly, the geometry of the MsLI throat may not be optimal as it typically takes the form of a right angle tube and cannot adequately mimic the anatomical complexity of the human upper airway, or the interaction of the aerosol cloud with it. Differences in particle deposition were indeed shown between the traditional throat model and several human-cast models [43,44]. Secondly, the constant airflow profile used in the impactor may not be representative of the clinical inspiratory flow profile, since patients instead supply a time-varying flow rate through the device. This unsteady flow can result in enhanced mouth–throat deposition that can be explained by the higher velocity reached by the particles due to the accelerating flow rate [45]. Peak Inspiratory Flow (PIF) and Flow Increase Rate (FIR) have been shown by various researchers to be important parameters for DPI performance [45].

Based on these considerations, future pharmacopoeial recommendations for in vitro DPI testing should take into consideration realistic inhalation profiles. The development of new-generation induction ports that more closely fits physiological features would also be highly valuable.

In view of the above-mentioned in vitro/in vivo mismatch, the present study points out the importance to perform human lung deposition studies in addition to in vitro studies. This provides a much better understanding of the drug delivery process and avoids further misinterpretation of pharmacokinetic and pharmacodynamic data.

The sticky behaviour of lipid-coated powders had already been observed in previous papers [46,47] and is the function of the percentage of lipid-coating and of the cholesterol/Phospholipon®90H ratio. This phenomenon is mainly attributed to the presence of phospholipids in the coating. Indeed, if a small proportion of these

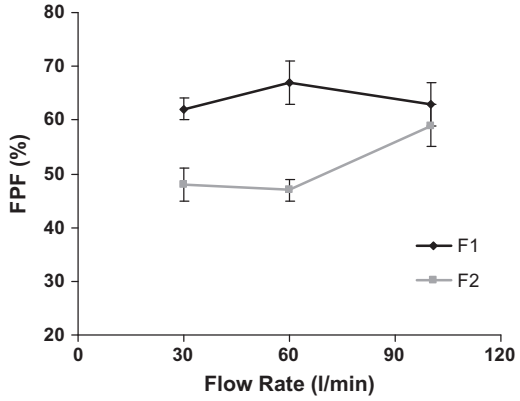

Fig. 4. In vitro FPF obtained for the F1 and F2 formulations at 30, 60 and 100 l/min (mean ± SD, $n = 3$).

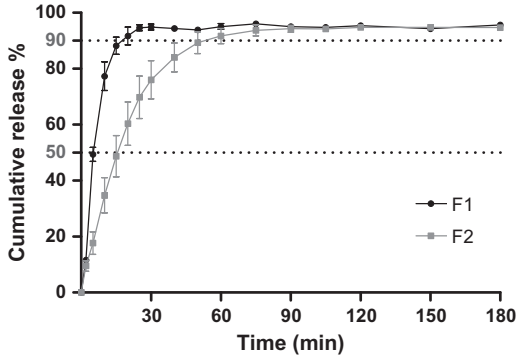

Fig. 5. Dissolution profiles obtained for the F1 and F2 formulations (mean ± SD, $n = 6$).

decreases the generation of electrostatic charges at the particle surface, an excessive proportion leads to the softening of the particles because of the low phase transition temperature (around 55 °C) of Phospholipon®90H [46,47]. However, no dependency on the flow rate had been noted for the aerodynamic properties of the coated powders in previous experiments [47]. This difference could be linked to the lower proportion of lipid-coating that was used (up to 5%).

Although the presence of a lipid-coating in the F2 formulation (20%) seems to induce a lower disaggregation efficiency at a lower inspiratory flow rate, the concept of a lipid-coating around protein particles should still be of interest. It was indeed shown to provide a low residual moisture content in the formulation [14], which is a great advantage for the long-term stability of proteins stored in the dry state, as it has been shown in several studies [48,49]. Moreover, proteins can adsorb at the air–liquid interface of droplets during spray-drying, leading to unfolding and aggregation at the droplet surface. Surfactants such as phospholipids have been shown to reduce this phenomenon by excluding the protein from the interface, which might improve protein integrity after spray-drying [50].

These properties could be of particular interest for the formulation of large biomolecules such as monoclonal antibodies, which are more likely to undergo degradation than the smaller insulin molecule.

In Table 2, the pharmacokinetic parameters of the F1 and F2 formulations are compared with those of the three inhaled insulin DPI formulations that have reached phase III clinical investigation [17,51,52]. The T_{max} of F1 and F2 (16–26 min) is comparable to that of Afrezza® (19.8 min). Although the fast absorption of Afrezza was proposed to be related to the fast dissolution of the particles in the lung, thanks to fumaryl diketopiperazine (FDKP) residues [12], a low T_{max} (24 ± 19 min) was also obtained for a pure microcrystalline insulin powder [18], indicating that short T_{max} can be achieved without the help of an excipient. On the other hand, the AIR® and Exubera® formulations presented higher T_{max} of respectively 45 min and 55 min. The presence of lipids (~60% DPPC) in the AIR® formulation causes a sustained-release effect, leading to its higher T_{max} [53]. Although the F2 formulation also contains lipids, their proportion might be too low to exhibit this sustained release. The Exubera® formulation, which mainly contains mannitol and glycine as excipients, is not intended for sustained release. Its high T_{max} might be related to its particle size distribution. Although Exubera® presents whole lung deposition comparable with the other formulations (Table 2), it might have a broader distribution leading to lower P/C ratio. Larger particles deposit in the larger bronchioles of the lung and have a thicker epithelium to go through, which could lead to slower absorption. Moreover, these large particles dissolve more slowly than smaller ones and, due to high local insulin concentration, dissolved insulin molecules might stay in an associated state (i.e. as hexamers), which also reduce the absorption rate [18].

The fast release and absorption of insulin observed for F1, F2 and Afrezza® formulations is of great interest for the control of post-prandial glucose excursions as it better corresponds to the natural insulin secretion profile. Indeed, with normal pancreatic beta-cell function, insulin secretion increases rapidly after a meal, with approximately two-thirds of the insulin response occurring in the first 2 h and a return to fasting levels over the course of 3–4 h [3]. This rapid absorption of insulin is also interesting in comparison with traditional s.c. prandial insulins. In a 1-year trial in patients with type 2 diabetes, the insulin therapy was either a twice-daily premixed biaspart insulin or a bedtime long-acting insulin glargine plus prandial Afrezza®. The two groups had similar efficacy regarding reduction of HbA1c, and Afrezza® induced slightly lower early post-prandial glucose excursions as well as less weight gain and fewer hypoglycaemic episodes [40].

The dose-normalised AUC_{0-360} (Table 2) shows that the F1 and F2 formulations seem to have a slightly higher (AUC_{0-360} = 131 mU h/l) and slightly lower (AUC_{0-360} = 71 mU h/l) bioavailability, respectively, than the Exubera® and AIR® formulations (both with an AUC_{0-360} around 99 mU h/l). Afrezza® exhibits a greater normalised AUC value (AUC_{0-360} = 219 mU h/l), indicating a higher bioavailability. Interestingly, this cannot be related to higher lung deposition of the powder (Table 2). Thanks to its narrow particle size distribution, Afrezza® might have a higher P/C ratio than other formulations, which would increase the extent of insulin absorption.

It can be noted that the F1 and F2 formulations were administered using a commercially available Aerolizer® device. Development of a dedicated DPI device could enhance their bioavailability by improving disaggregation, and decrease the sensitivity of the lipid-coated formulation to flow rate variability.

6. Conclusion

This phase I study compared the in vivo lung deposition and bioavailability of two new insulin formulations administered by inhalation to type 1 diabetic patients. The formulation with a lipid-coating around the insulin particles exhibited a lower lung deposition in comparison with the uncoated formulation. This was found to be related to a decrease in the disaggregation efficiency of the powder at a sub-optimal flow rate.

The two formulations showed interesting features with pharmacokinetic profiles that mimic the natural insulin secretion pattern, and bioavailability that is within the ranges of two of the three DPI insulins that have reached phase III clinical development.

This was achieved using fast and simple production techniques, as well as simple formulations with few or no biocompatible excipients. Moreover, the formulations were administered using a simple and passive commercial device. A new dedicated device could be developed in the future to improve formulation performance.

These observed advantages are obviously of interest for the successful use of inhaled insulin in diabetic patients, as well as for the development of DPI formulations containing proteins or monoclonal antibodies that would benefit an inhaled therapy.

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