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Research paper

Pharmacoscintigraphic and pharmacokinetic evaluation of tobramycin DPI formulations in cystic fibrosis patients

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Abstract

Tobramycin dry powder formulations were evaluated by gamma scintigraphy and pharmacokinetic methods. In an open single-dose, three-treatment, three-period, cross-over study, nine cystic fibrosis patients received both the two test products and the reference product Tobi® (nebulizer solution) in order to assess lung deposition and systemic comparative bioavailability of the two investigational inhaled products versus the marketed inhaled comparator product. The percentage of dose (mean ± SD) in the whole lung was 53.0 ± 10.0% for the tobramycin Form 1, 34.1 ± 12.4% for the tobramycin Form 2 and 7.6 ± 2.7% for the comparator product Tobi®. Lung deposition expressed as a percentage of the nominal dose was thus estimated to be 7.0 and 4.5 times higher for the Tobra Form 1 and Tobra Form 2 than for the Tobi®, respectively. Furthermore, the systemic bioavailability (adjusted to correspond to the same drug dose as that of the comparator product deposited in the lung) was found to be 1.6 times higher for the comparator product Tobi® than for the two DPI formulations.

The principal advantages of the DPI formulations include reduced systemic availability and thus, side effects, and higher dose levels of the drug at the site of drug action.

Keywords: Dry powder inhaler; Cystic fibrosis; Tobramycin; Gamma scintigraphy; Pharmacokinetics

1. Introduction

Cystic fibrosis (CF) is the most common lethal hereditary disorder with autosomal recessive heredity in the Caucasian population [1]. CF is caused by mutation in the cystic fibrosis transmembrane regulator (CFTR) gene and early alterations are primarily found in the small airways [2,3]. This disease is characterized by secretions of extremely high viscosity from exocrine glands in the airways. Among other factors, the increased viscosity of the mucus leads to a reduced clearance of microorganisms from the respiratory tract and to chronic bacterial infection of the airways [4]. Pulmonary infection caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been recognized as having the greatest role in morbidity and mortality leading to premature death in 90% of patients [5–7].

Pulmonary administration of various antibiotics has been found to improve lung function in CF patients with chronic pulmonary infection with *P. aeruginosa* and to reduce the frequency of hospital admission. When given

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by inhalation, the antibiotic is delivered directly to the target organ which increases the therapeutic index of drug [8]. Inhalation is recommended in the European consensus document on antibiotic treatment against *P. aeruginosa* [7]. Several antibiotics have been investigated for administration by inhalation and, at present, nebulized tobramycin and colistin are used in daily practice by CF patients in many countries [9].

However, tobramycin and, in general, all antibiotic powders are difficult to formulate in an inhaled form because the required lung dose is very high and the micronized drug is highly hygroscopic, sticky and cohesive and it is thus problematic to produce a redispersable formulation.

In a previous work [10], we developed lipid coated and uncoated tobramycin dry powder formulations for inhalation. These formulations were characterized in terms of aerolization properties, surface composition and physical state. These powders have been shown to deliver drugs efficiently to the lower respiratory tract using relatively simple and inexpensive DPIs. When tested in vitro with multistage liquid impinger (MsLI), these powder formulations produce a fine particle fraction (FPF) of at least 68% [10] and 53% of the nominal dose, for the lipid coated and uncoated tobramycin formulations, respectively.

In order to confirm these encouraging results, two formulations were selected on the basis of their aerodynamic behaviour and fine particle dose (FPD) values and compared to Tobi® (nebulizer solution) (Chiron Corporation; Seattle, WA) by performing a combined in vivo scintigraphic and pharmacokinetic evaluation of tobramycin after inhalation of a single oral dose in nine CF patients. Gamma scintigraphic imaging provides direct information on the amount and site of drug deposition in the lung after inhalation and the pharmacokinetic evaluation gives valuable information on the rapid elimination of the drug from the site of deposition by absorption and on the systemic bioavailability of the drugs.

2. Materials and methods

2.1. Dry powder formulations

Two tobramycin DPI formulations were selected.

The first formulation (Tobra Form 1) consisted of lipid coated micronized tobramycin particles. The powder was obtained by spray drying a suspension of isopropanol containing micronized tobramycin (Plantex Chemicals B.V., The Netherlands) 5% w/v and lipids 0.25% w/v in an appropriate ratio of 75% of cholesterol (Merck, Belgium) and 25% of Phospholipon® 90H (Nattermann Phospholipids GmbH, Koln, Germany). This method has been previously described [10].

The second formulation (Tobra Form 2) consisted of micronized tobramycin without excipient.

Size #3 HPMC capsules (Capsugel, Bornem, Belgium) were filled with 25 mg of these powders, which contained nominally 95% w/w tobramycin for the first formulation and 100% w/w tobramycin for the second formulation. The fill-mass specification was set at 25 ± 0.5 mg, and this target was met for all hand-filled capsules.

The DPI device used was the aerolizer® (Novartis, Switzerland), a passive breath-actuated, single-dose dry powder inhaler.

The third formulation was the comparator product Tobi® which contains 300 mg of tobramycin free base in 5 ml of sodium chloride (2.25 mg/ml) adjusted at pH 6.0. Nebulized tobramycin was administered as directed using a hand-held Pari LC Star nebuliser (PARI GmbII; Starnberg, Germany) in combination with the Pari Turbo Boy N compressor (PARI GmbII; Starnberg, Germany), as recommended by the manufacturer, until dry running.

2.2. Study design

The study design was an open single-dose, three-treatment, three-period, cross-over study with wash-out period of at least six days between the three phases of the study.

All patients received the drug treatment as one capsule of 25 mg tobramycin (Tobra Forms 1 and 2) or one dose of Tobi® (nebulizer solution) on each occasion.

The amount of technetium-99m (^{99m}Tc) was adjusted so that the maximum amount of radioactivity inhaled by the subjects on any occasion did not exceed 28 MBq of ^{99m}Tc.

Furthermore, a total of 50 g of activated charcoal (Carbomix, Norit, The Netherlands) was given orally before and over the 30 min (divided in two doses of 25 mg) after each administration of drug treatment [11]. Even if anti-pseudomonas agents have low oral bioavailability, activated charcoal has been given to prevent any risk of gastrointestinal absorption of tobramycin.

2.3. Subjects

Nine patients (five men and four women; 34 ± 5 years) were recruited. Each patient was clinically well (without infective exacerbation) and had mean values for FEV_1 (forced expiratory volume in one second) and for FVC (forced expiratory vital capacity) >50% of the predicted value. Excluded from this study were patients that were receiving continuous home intravenous antibiotic therapy, pregnant or participating in another study within four weeks of the proposed study. Patients were seen before entering the study and were carefully instructed in the use of the aerolizer® and the Tobi® nebulizer set.

Before starting the study, the nature of the clinical trial was explained and written consent was obtained from all patients. The study was conducted at the Erasme Hospital (Brussels, Belgium), in accordance with the principles stated in the declaration of Helsinki, and approval was obtained from the ethics committee of Erasme hospital (Ref.: P2006/072) and the Belgian minister of social affairs and public health (Ref.: EudraCT No. 2006-000456-40).

2.4. The radiolabelling method

A method of wide application to radiolabelling dry powders is by adsorbing the radiolabel on the active particles in a suitable liquid. This is achieved by wetting the drug particles with a nonsolvent containing the radiolabel, followed by the evaporation of the solvent, leaving the radiolabel on the surface of the drug particles [12].

Firstly, there is the elution of ^{99m}Tc as sodium pertechnetate from a ^{99}Mo–^{99m}Tc generator. Then, the ^{99m}Tc is extracted into methylethylketone (MEK) by shaking the pertechnetate solution with approximately equal volume of MEK. The aqueous and MEK phases were separated in a separating funnel and the MEK phases containing the pertechnetate were collected and evaporated to dryness in a bath at 60 °C for 10 min and 80 °C for 5 min. After this, the pertechnetate was re-dissolved in isopropanol and then tobramycin (and if applicable lipids) were added to the solution, which was homogenized with a CAT high speed homogenizer X620 (CAT M. Zipperer, Staufen, Germany) at 24000 rpm for 10 min. The suspension was then spray dried (Büchi Mini Spray Dryer B-191a [Büchi laboratory-Techniques, Switzerland]) with constant stirring.

Nebulized tobramycin was radiolabelled by simply dissolving an appropriate amount of ^{99m}Tc-DTPA (diethylenetriamine penta-acetic acid) complex in the aqueous nebulized tobramycin solution [13,14].

2.5. Validation of radiolabelled powder

Before initiation of the clinical phase of the study, in vitro validation experiments were conducted to demonstrate that significant alteration of the particle size distribution (PSD) did not occur during the labelling process, and that the PSD of the radiolabel matched the PSD of the drug.

For each formulation, the FPD and the PSD of the unlabelled drug were determined then compared against those of the labelled drug and of the radiolabel ($n = 3$). The measurements were made with a Multistage Liquid Impinger (MsLI) (Copley instruments, UK) operating at an air flow rate corresponding to a pressure drop of 4 kPa (Eur. Pharm. 5th edition). The test was carried out at 100 L/min for 2.4 s.

Drug and radiolabel content at each stage of the MsLI were determined by a validated analytical HPLC method and by gamma counting (using a Cobra gamma counter, Packard bioscience, UK), respectively. Quantification of tobramycin was done by the US Pharmacopeia method that involves derivatization of the tobramycin with 2,4-dinitrofluorobenzene, and quantification by high-performance liquid chromatography.

The total dose of particles with aerodynamic diameters smaller than 5.0 μm was calculated by interpolation from the cumulative mass against cut-off diameter of respective stages and considered as the fine particle dose (FPD) (μg) or fine particle fraction (FPF), expressed in percentage of the total labelled dose and not of the emitted dose. The FPD is considered to be directly proportional to the amount of drug able to reach the pulmonary tract in vivo: consequently, the higher the value of the FPD (or FPF), the higher the lung deposition is estimated to be.

It should be noted that since the radiolabel is adsorbed onto the surface of the drug particles, its particle size distribution (as determined by a gamma counting technique) corresponds to that of the drug particles.

2.6. Gamma scintigraphy analysis

In vivo evaluation of pharmaceutical inhalation products is achieved by γ-scintigraphic imaging of the aerosol deposited in the lung. Imaging provides direct information on the amount and location of the drug deposited in the lung after inhalation. This local bioavailability, rather than the systemic bioavailability after absorption, is pertinent to reflect efficacy of drugs that act directly on the lung [12].

To measure lung deposition by imaging, the aerosol must be first labelled or tagged with a suitable radionuclide. Lung imaging is achieved using a γ-camera which creates an image of the γ-rays emitted by the radionuclide in the lung.

One of the most commonly used techniques, known as gamma scintigraphy, involves taking two-dimensional "planar" views of radionuclide distributions with a single-headed or dual-headed gamma camera. Typical radiolabelling methods involve adsorbing ^{99m}Tc as sodium pertechnetate onto the surface of drugs particles in a dry powder inhaler [15].

^{99m}Tc is the most commonly used pure γ-emitter for indirect radiolabelling of pharmaceutical aerosols. The γ-ray of ^{99m}Tc has sufficient energy (140 keV) to penetrate body tissues and when it reaches the detector of the γ-camera, it is absorbed and converted into light photons, thus optimizing γ-camera imaging. The half-life of ^{99m}Tc is 6 h, which is long enough for handling and imaging but not so long that it increases the radiation dose to the subject unnecessarily [16].

Each labelled capsule was first measured at distance from a gamma counter and the activity compared with that of a syringe containing approximately 30% of the capsule activity as ^{99m}Tc. This syringe was injected into a perfusion bag which was placed near the patient's chest. This was considered to be the standard and was used to assess radioactive decay over time.

Immediately following the administration of the radiolabelled aerosol, scintigraphic images of the chest (posterior and anterior) and lateral oropharynx were recorded (DHD-SMV, Sopha Medical, France). A flat flood source was interposed between the lower detector and the posterior aspect of the trunk of the subject, and the activity was recorded with the upper detector. This was done to take into account the attenuation of the radioactivity resulting from absorption by tissues and to allow the lung fields to

be outlined. A background count was recorded for each camera head.

The empty device and capsule (Aerolizer®), and exhalation filter were also counted [17,18].

Regions of interest were drawn around the oropharynx, oesophagus, stomach, and whole lung. The counts obtained within these regions were corrected for background radioactivity, radioactive decay, and tissue attenuation of gamma rays [19]. In regions where both anterior and posterior images were recorded, the geometric mean of counts in both images was calculated.

Determination of the percentage of the dose deposited in the oropharynx included activity adhering to the mouth and oropharynx together with any swallowed activity detected in the oesophagus, stomach and intestine. Any activity detected on the mouthpiece or the exhalation filter was deemed to be due to direct transfer from the mouth.

The edges of the lungs were delineated using a ^{99m}Tc transmission scan and the lungs were subdivided into central, intermediate and peripheral regions of interest, corresponding approximately to large, medium and small airways [20–23]. The ratio of peripheral to central lung deposition (P/C ratio) was calculated as an index of regional lung deposition [21,22].

The counts in each named area were expressed as a percentage of the nominal dose, which was determined from the sum of the total body counts and those from the Aerolizer® inhaler device and capsules, and the exhalation filter.

2.7. Pharmacokinetic analysis

Venous blood samples (7 ml) were collected at pre-dose and at 30 min, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 10 h post-dose in order to quantify plasma levels of tobramycin. After centrifugation, the plasma samples were decanted and divided into two approximately equal portions of not less than 2 ml per tube (aliquot) and rapidly stored at 80 °C, in an upright position.

The extraction of the tobramycin was performed by passing the plasma samples through a solid phase extraction column (MCX, Waters, Belgium). The sample was then evaporated to dryness and the residue was reconstructed and vortex mixed with solvents A and B 40:60 (v/v). Solvents A and B consisted of methanol:water 50:50 and 0:100, respectively, both containing 5 mM HFBA (heptofluorobutyric acid). An aliquot was injected into the validated LC/MS–MS method described below.

An HPLC system (HP 1100 series, Agilent Technologies, Belgium), coupled to an API365 quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Canada), was used to measure tobramycin in plasma samples. The separation system was a 150 × 2 mm Polar-RP Synergi column (Phenomenex, USA). Analyses were performed in a binary gradient mode. Solvents A and B consisted of acetonitrile:water 90:10 and 0:100 (v/v), respectively, both containing 2 mM NH_4Ac and 1% HCOOH. The solvent B also contained 5 mM HFBA. The HLPC gradient started at 20% A and 80% B (1 min hold) and was linearly increased to 100% A over 0.2 min (6.8 min hold) before returning to 20% A over 1 min (7 min hold). The flow was adjusted to 0.150 ml/min. Electrospray experiments used a capillary voltage set at 4.5 kV. The source temperature was kept at 100 °C and the desolvation temperature at 350 °C. The limit of quantification of the method is 20 ng/ml.

The calculation of pharmacokinetic parameters was as follows

Maximal plasma concentration (C_{max}) and time to maximal plasma concentration (T_{max}) were taken directly from the plasma concentration vs. time curve. The area under the curve (AUC) was calculated by the linear trapezoidal rule from measured data points from time of administration until the time of the last quantifiable concentration.

The AUC was also adjusted to the same drug dose of the comparator product (Tobi®) deposited in the lung (based on scintigraphic deposition values) in order to facilitate the comparison of the test and reference products.

2.8. Safety assessment

Pulmonary function (FVC, FEV_1) and vital signs were recorded before and 30 min after dose. Adverse events were monitored throughout the study. In addition, each subject underwent a physical examination, pulmonary function testing, routine clinical chemistry, haematology and urinalysis at the beginning and the end of the study.

2.9. Statistical analysis

The repeated-measures ANOVA test was used to validate the radiolabelling method and to compare the pharmacokinetic data obtained with the three formulations. The Student's t test was used to compare the lung deposition pattern between the two DPI products. For all tests, the significance level was set at $p = 0.05$.

3. Results

3.1. Validation of the radiolabelling method

In vitro assessment of the output of radiolabelled tobramycin with the Aerolizer® was used to ensure that the radiolabelling method did not significantly modify the PSD of the aerosol generated by the device and also to confirm that the distribution of ^{99m}Tc reflected that of the drug, thus acting as a suitable marker for tobramycin.

The in vitro deposition was compared on the MsLI, for unlabelled tobramycin, labelled tobramycin and the ^{99m}Tc. The PSD for the Tobra Form 1 are given in Fig. 1. The FPF values (mean ± SD) obtained for the drug before labelling (69.8 ± 1.0%), after labelling (68.4 ± 1.9%) and for the radiolabel (67.7 ± 2.3%) were not significantly different ($p > 0.05$). The data for the Tobra Form 2 are shown in Fig. 2. There was also a good match between the FPF

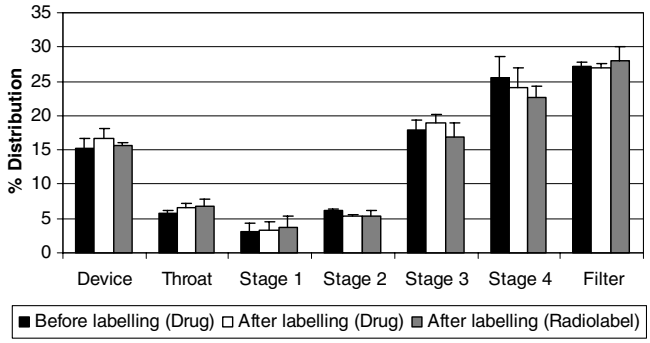

Fig. 1. In vitro comparative deposition of Tobra Form 1: before labelling, after labelling (drug) and after labelling (radioactive) ($n = 3$).

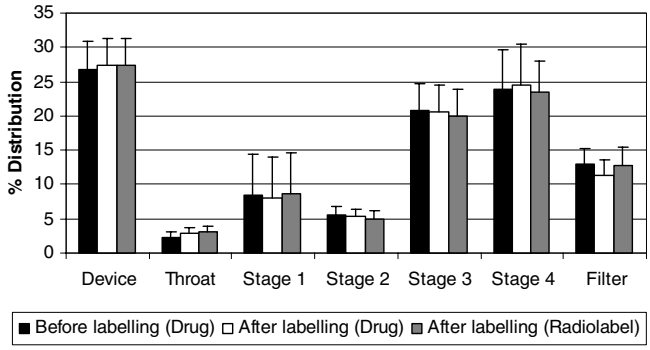

Fig. 2. In vitro comparative deposition of Tobra Form 2: before labelling, after labelling (drug) and after labelling (radioactive) ($n = 3$).

values (mean ± SD) of the drug before labelling (57.6 ± 2.3%), after labelling (56.5 ± 2.8%) and for the radiolabel (56.1 ± 2.9%) ($p > 0.05$). The results did not show any significant differences among the three measurements on any stages of the MsLI. The amount remaining in the device was also similar for the three measurements.

This validation demonstrated that the PSD of the radiolabel and the Tobra Forms 1 and 2 were well matched, with no alteration of aerosol properties of the formulations after radiolabelling. So, for both formulations a homogeneous cover of ^{99m}Tc that did not affect the size of the particles was obtained around the active drug.

For nebulized tobramycin, the radiolabel and drug are both dissolved in the aqueous phase, and hence the concentration of drug and radiolabel was uniformly distributed in a given nebulized droplet [24].

3.2. Lung function tests

The FEV_1 values, measured prior to and 30 min after dosing, were similar on each study day (data not shown). Mean FEV_1 value (as predicted value) for all subjects was 68 ± 11% (ranged from 55% to 88%).

It indicated that the inhalation of 25 mg of tobramycin in the presence of lipids or alone did not have any significant effects on lung function. Furthermore, no major or minor complications were observed during the clinical trial and there was no evidence of bronchoconstriction, suggesting good tolerance of these products.

3.3. Scintigraphic results

The percentage of the radiolabelled tobramycin deposited in the whole lung, the oropharynx, the stomach and the device as determined by gamma scintigraphy is shown in Table 1.

Mean ± SD lung deposition for Tobra Form 1 for all subjects was 53.0 ± 10.0% (range from 35.7% to 62.7%), for an overall coefficient of variation (CV) of 19%. For Tobra Form 2, the mean ± SD lung deposition was 34.1 ± 12.4% (range from 15.3% to 50.0%), for an overall CV of 36%.

The mean lung deposition for Form 1 of 53.0 ± 10.0% was significantly greater ($p < 0.01$) than that for Form 2 (34.1 ± 12.4%). These results corresponded to 13.3 mg (12.6 mg of tobramycin) and 8.5 mg tobramycin deposited in the lung, assuming a total delivered dose of 25 mg via the Aerolizer® loaded with Tobra Form 1 and Form 2 products, respectively.

Approximately the same percentage of the tobramycin dose was deposited in the oropharynx (16.0% vs. 15.2% ($p > 0.05$)) and the stomach (3.1% vs. 2.1% ($p > 0.05$)) for Tobra Forms 1 and 2, respectively.

Nevertheless, the dose retained inside the capsule and inhaler was significantly higher ($p < 0.01$) for Tobra Form 2 than for Tobra Form 1 (43.4% vs. 20.4%). The exhaled fraction was found to be less than 0.2% for both products and was assumed to be negligible.

The high variability observed in lung deposition in patients is attributable in part to the very low deposition results observed in two subjects (Patients 5 and 9) because of poor lung function. If we perform the calculation without including these subjects ($n = 7$), the lung deposition results are higher and the SD and CV is lower: the mean ± SD (CV) lung deposition for Tobra Form 1 is about 57.7 ± 4.1 (8%) and for Tobra Form 2 about 39.4 ± 7.4 (18%).

Concerning the reference product (Tobi®), after a time of 20 min, necessary to nebulize to dryness the product, only 7.6 ± 2.7% (CV of 39%) of the radiolabelled tobramycin (range from 3.6% to 13.2%) was deposited in the lung and 2.7 ± 1.3% in the oropharynx. This result

Table 1
Mean ± SD (CV) fractionation of the dose between lungs, oropharynx, stomach and device for Tobra Form 1, Tobra Form 2 and nebulized tobramycin (Tobi®) in 9 CF patients

	Tobra Form 1	Tobra Form 2	Tobi
Lungs	53.0 ± 10.0 (19)	34.1 ± 12.4 (36)	7.6 ± 2.7 (39)
Oropharynx	16.0 ± 6.0 (38)	15.2 ± 7.1 (47)	2.7 ± 1.3 (50)
Stomach	3.1 ± 3.2 (107)	2.1 ± 2.0 (93)	1.8 ± 1.2 (68)
Device	20.4 ± 8.5 (41)	43.4 ± 14.6 (33)	/

corresponded to 22.8 mg tobramycin deposited in the lung, assuming a total delivered dose of 300 mg after nebulization.

The scintigraphic images showing deposition patterns for each formulation for two patients are illustrated in Fig. 3. As can be seen in Fig. 3d, e and f, patient 9 presented a low respiratory function with a deficient lung, resulting in poor deposition results. Regional deposition results after inhalation of radiolabelled tobramycin are shown in Table 2.

The relative distribution (P/C ratio) of radiolabel within the central, intermediate, and peripheral airways was relatively similar for Tobra Form 1, Tobra Form 2 and the nebulized solution (0.9, 0.8 and 0.7). Even if the P/C ratio did not vary significantly ($p > 0.05$), it indicated that more of the dose was deposited in medium- and small-diameter airways and alveoli than in primarily large-diameter airways for the dry powder inhalation than for the nebulization. The P/C ratio results ranged from 0.7 to 1.2 for both Tobra Forms 1 and 2 while they ranged between 0.3 and 1.0 for Tobi®.

Table 2
Regional lung deposition: mean ± SD (CV) percentage deposition in peripheral, intermediate and central lung zones, and mean ± SD (CV) peripheral zone/central zone deposition ratio (P/C ratio)

	Tobra Form 1	Tobra Form 2	Tobi®
Central zone	19.7 ± 4.4 (22)	13.0 ± 4.8 (37)	2.9 ± 1.1 (36)
Intermediate zone	16.0 ± 3.7 (23)	10.1 ± 4.0 (39)	2.2 ± 0.8 (38)
Peripheral zone	17.3 ± 3.3 (19)	11.0 ± 4.2 (39)	2.1 ± 0.9 (44)
P/C ratio	0.9 ± 0.2 (20)	0.8 ± 0.2 (21)	0.7 ± 0.5 (50)

3.4. Pharmacokinetic data

All the subjects completed the study.

The mean mass of tobramycin deposited in the lung as determined by gamma scintigraphy for the DPI formulations correlates well with the pharmacokinetics data herein below. Indeed, the mean deposition in the lung was found to be 1.5 higher for Tobra Form 1 than for Tobra Form 2 and the mean AUC was also found to be 1.4 higher for Tobra Form 1 than for Tobra Form 2.

As can be seen in Table 3, the mean AUC and C_{max} of the Tobi® were approximately 3 and 4 times higher than those of Tobra Forms 1 and Tobra Form 2, respectively. Nevertheless, the dose administered of Tobi® was 12 times

Table 3
Mean ± SD (CV) pharmacokinetic parameters for tobramycin ($n = 9$)

	Tobra Form 1 (25 mg)	Tobra Form 2 (25 mg)	Tobi® (300 mg)
T_{max} (h)	1.4 ± 0.5 (35)	1.2 ± 0.5 (43)	0.9 ± 0.3 (32)
Parameters			
AUC (ng × h/ml)	1881 ± 475 (25)	1348 ± 622 (46)	5687 ± 1590 (28)
C_{max} (ng/ml)	422 ± 105 (25)	331 ± 184 (56)	1274 ± 389 (30)
Adjusted to the same drug dose of the Tobi® deposited in the lung			
AUC (ng × h/ml)	3404 ± 860 (25)	3615 ± 1669 (46)	5687 ± 1590 (28)
C_{max} (ng/ml)	764 ± 190 (25)	887 ± 494 (56)	1274 ± 389 (30)

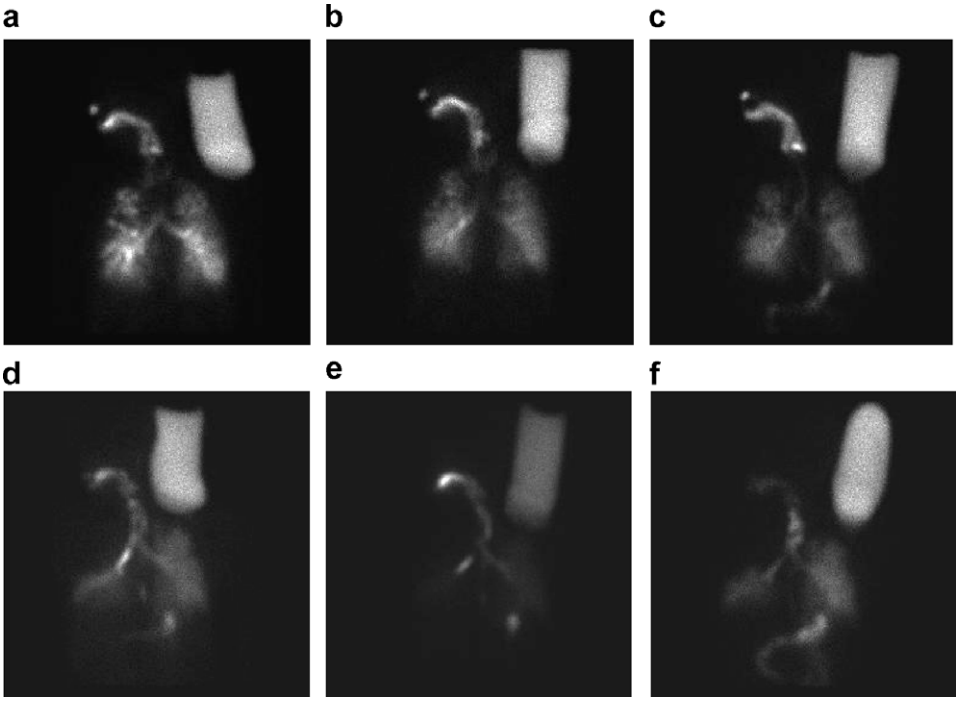

Fig. 3. Scintigraphic images obtained for the Tobra Form 1: (a and d), the Tobra Form 2 (b and e) and the Tobi® (c and f) for patients 6 and 9, respectively.

greater than for the DPI forms (300 vs. 25 mg). Only one inhalation was performed and the DPIs were loaded with about twelve-fold less drug, but there is no reason why multiple inhalations could not be performed.

The examination of Fig. 4, representing the plasma concentration-time profile adjusted to give the same drug dose deposited in the lung as that of the comparator product (Tobi®) (based on scintigraphic deposition values (22.8 mg)), showed a rapid plasma concentration peak for the DPI formulations as well as for the comparator product, followed by a progressive decrease in tobramycin plasma concentrations over 10 h. Nevertheless, Tobi® (nebulizer solution) was absorbed a little faster than the Tobra Form 1 with maximum plasma concentrations (C_{max}) occurring at 0.9 and 1.4 h, respectively. So, the droplets with their hydrophilic properties and a median diameter about 2.2 μm were dissolved more easily than the particles of tobramycin in the bronchial fluid and then in the systemic circulation.

As can be observed from the examination of the pharmacokinetic data adjusted to the same drug dose of the comparator product (Tobi®) deposited in the lung, shown in Table 3, the C_{max} and AUC values were found to be significantly higher for Tobi® than for DPI formulations ($p < 0.05$). For example, the C_{max} values were found to be 1274, 764 and 887 ng/ml for the Tobi®, Tobra Forms 1 and 2, respectively.

Consistent with the difference in C_{max} between the two methods of administration, the AUC after inhalation of the nebulized tobramycin was 1.6 times greater than after Tobra Form 1 and Form 2 (5687 ng × h/ml vs. 3404 ng × h/ml and 3615 ng × h/ml, $p < 0.01$).

The high variability observed is attributable in part to the very low lung deposition observed in two subjects (Patients 5 and 9) because of poor lung function. As the inter-subject variability was relatively high, the clinical trial should be carried out on a higher number of volunteers and with the drug dose adjusted to that of the Tobi® in order to confirm these results.

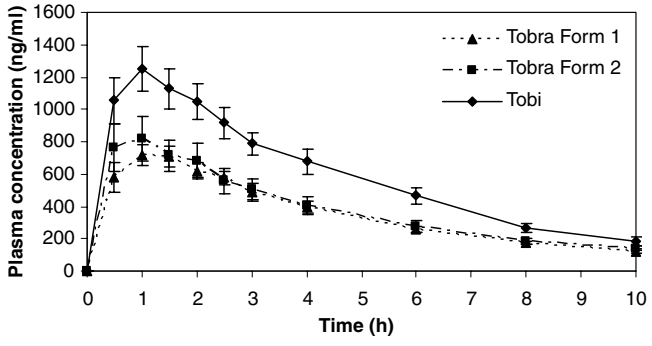

Fig. 4. Mean plasma concentrations of tobramycin adjusted to correspond to the same drug dose of the comparator product (Tobi®) deposited in the lung (based on the scintigraphic deposition values (22.8 mg)) following administration of Tobra Form 1, Tobra Form 2 and nebulized tobramycin (Tobi®) (mean ± SEM, $n = 9$).

4. Discussion

In this study, two kinds of tobramycin dry powder formulations for inhalation were compared to Tobi® (nebulizer solution) by a pharmacoscintigraphic method.

In comparison with Tobi® (nebulizer solution), it was estimated that lung deposition expressed as a percentage of the nominal dose was 7.0 and 4.5 times higher for Tobra Forms 1 and 2, respectively. The difference in deposited dose values between Tobra Forms 1 and 2 could be explained by the fact that the presence of a lipid coating around tobramycin particles allowed a decrease in deposition in the device of the inhalator, and allowed a more complete redispersion of tobramycin as individual particles, thus increasing deposition in the lung, which is beneficial in terms of drug targeting efficiency. In accordance with the previously published in vitro results [10], lipids improve particle dispersion properties during inhalation with efficient reduction of their inherent agglomeration tendency. Moreover, they may reduce local irritation, offering a good tolerance in the pulmonary tract as they are mainly constituted of biocompatible and biodegradable material. Finally, the hydrophobic nature of neutral lipids (cholesterol) reduces the absorption of the ubiquitous vapour leading to a reduction of the aggregation and the adhesion of particles.

Furthermore, the absence of carrier particles enables a much greater drug dose to be loaded into capsules. These formulations are more particularly useful for drugs that are active at relatively high doses, such as antibiotics, as they permit the delivery of a high concentration of antibiotic directly to the site of infection while minimizing systemic exposition.

This technique indicated that 53.0% of the nominal dose was deposited in the lungs for Tobra Form 1, which was found to be 1.5 times higher than the 34.1% total lung deposition for Tobra Form 2. These results therefore corresponded with the in vitro fine particle assessment in which the FPF of tobramycin from Tobra Form 1 was 1.2 times higher than that from Tobra Form 2. The lung deposition in patients was lower than the in vitro FPF, indicating, as expected, that the nature of the disease and, probably, its severity play key roles in the deposition of drugs in the lungs.

These deposition results are especially elevated and very promising comparing to the values of the clinical evaluation of dry powder tobramycin in healthy volunteers of Newhouse et al. using lipid-based Pulmosphere technology and given a mean whole lung deposition of 34 ± 6% [24].

The deposition results of the Tobi® are in accordance with other studies of nebulized antibiotic in CF patients where the mean pulmonary deposition varied between 5% and 11% [6,24–26]. The other 90% of active drug either remained attached to the wall of the nebulizing system or impacted in the oropharynx and was swallowed or exhaled into the surrounding atmosphere. It should be noted that because of the shape of the nebulizer system, the activity

of the drug remaining inside the device could not be counted with the gamma counter.

It should be noted that there is no correlation between the P/C ratio and the $FEV_1$and FVC results. Patients with low or high respiratory capacity show relatively similar regional deposition results. In fact, deposition of drug depends upon a complex interaction between the device, the formulation, and the patient, who controls the rate of flow of inhaled air through the system. These P/C ratio results below 1 demonstrate the thickening of the peripheral airways in CF causing a reduction in maximal expiratory flow. Indeed, it has been described that the airway disease in CF begins in the small peripheral airways and progresses to the development of widespread bronchiectasis. It has been reported that the inflammatory process, and geometrical airway changes resulting from that, are significantly more severe in the peripheral than in the central airways [4].

On the other hand, when adjusted to give the same drug dose deposited in the lung as that of the comparator product (Tobi®), the proportion of the drug delivered to the lung that reached the systemic circulation was not the same for the nebulization form as for the DPI forms. This can be explained by differences in the physical state (solution vs. solid form) and the lipophilicity of the two forms. In the lungs of CF patients especially, where secretions are of extremely high viscosity, the solid particles of tobramycin from the DPI forms seem to present greater retention in the pulmonary tissue, leading to a much slower rate of dissolution in the bronchial fluid (delay in the T_{max}), than the tobramycin droplets. This is beneficial for the treatment of the CF patient, as it decreases systemic exposure.

Moreover, aerosol administration via nebulization takes much longer, approximately 30 min if set-up, drug administration and cleaning are taken into account [24]. This imposes a significant time burden on patients with CF, who are commonly treated with multiple inhaled medications. Thus, further improvement in aerosol delivery systems with greater efficiency and portability and shorter administration time could improve patient quality of life and compliance.

Such an evaluation, in accordance with the in vivo scintigraphic deposition, confirms the superiority of dry powder formulation Tobra Forms 1 and 2 in terms of drug deposition and reduced systemic exposure in comparison with the conventional comparator product Tobi® (nebulizer solution).

5. Conclusion

This study has demonstrated that Tobramycin DPI formulations containing high drug concentrations allow high lung deposition in patients. The inhalation of one capsule of 40 mg or two capsules of 20 mg of Tobra Form 1 or two capsules of 30 mg of Tobra Form 2 could be an advantageous substitute for the administration of Tobi®. Thus, these new tobramycin DPI formulations based on the use of very low excipient levels and presenting very high lung deposition properties offer very good prospects for improving the delivery of drugs to the pulmonary tract and to the widest possible CF patient population.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejpb.2007.05.005.

References

[1] M.E. Hodson, D. Geddes, Cystic Fibrosis, Chapman and Hall, London, 1995, 1–13.
[2] M. Griese, R. Essl, R. Schmidt, M. Ballmann, K. Paul, E. Rietschel, F. Ratjenand the beat study group, Sequential analysis of surfactant lung function and inflammation in cystic fibrosis patients, Respir. Res. 6 (133) (2005) 1–10.
[3] R.L. Gibson, J.L. Burns, B.W. Ramsey, Pathophysiology and management of pulmonary infections in cystic fibrosis, Am. J. Resp. Crit. Care Med. 168 (2003) 918–951.
[4] H.A. Tiddens, Detecting early structural lung damage in cystic fibrosis, Pediatr. Pulmonol. 34 (2002) 228–231.
[5] C. Koch, N. Hoiby, Pathogenesis of cystic fibrosis, Lancet 341 (1993) 1065–1069.
[6] D.E. Geller, W.H. Pitlick, P.A. Nardella, W.G. Tracewell, B.W. Ramsey, Pharmacokinetics and bioavailability of aerolized tobramycin in cystic fibrosis, Chest 122 (2002) 219–226.
[7] J. Döring, S.P. Conway, H.G.M. Heijerman, M.E. Hodson, N. Hoiby, A. Smyth, A.J. Touw, Antibiotic therapy against *Pseudomonas aeruginosa* in cystic fibrosis: a European consensus, Eur. Respir. J. 16 (2000) 749–767.
[8] I. Sermet-Gaudelus, Y. Le Cocguic, A. Ferroni, M. Clairicia, J. Barthe, J.P. Delauney, V. Brousse, G. Lenoir, Nebulized antibiotics in cystic fibrosis, Paediatr. Drugs 4 (2002) 455–467.
[9] P.H.P. Le Brun, A.H. de Boer, G.P.M. Mannes, D.M.I. De Fraîture, R.W. Brimicombe, D.J. Touw, A.A. Vinks, H.W. Frijlink, H.G.M. Heijerman, Dry powder inhalation of antibiotics in cystic fibrosis therapy: Part 2. inhalation of a novel colistin dry powder formulation: a feasibility study in healthy volunteers and patients, Eur. J. Pharm. Biopharm. 54 (2000) 25–32.
[10] G. Pilcer, Th. Sebti, K. Amighi, Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation, Pharm. Res. 23 (5) (2006) 931–940.
[11] L. Thorsson, S. Edsbacker, T.B. Conradsen, Lung deposition of budesonide from Turbuhaler is twice that from a pressurized metered dose inhaler P-MDI, Eur. Respir. J. 7 (1994) 1839–1844.
[12] H.K. Chan, Radiolabeling of Pharmaceutical Aerosols and γ-Scintigraphic Imaging for Lung Deposition Encyclopedia of Pharmaceutical Technology, Marcel Dekker, New York, 2002, 2365–2374.
[13] S.P. Newman, G. Woodman, S.W. Clarke, Deposition of carbenicillin aerosols in cystic fibrosis: effects of nebuliser system and breathing pattern, Thorax 43 (1988) 318–322.
[14] C.C. Kuni, W.E. Regelmann, R.P. Ducret, R.J. Boudreau, J.R. Budd, Aerosol scintigraphy in the assessment of therapy in cystic fibrosis, Clin. Nucl. Med. 17 (1992) 90–93.

[15] S.P. Newman, G.R. Pitcairn, P.H. Hirst, L. Rankin, Radionuclide imaging technologies and their use in evaluating asthma drug deposition in the lungs, Adv. Drug. Deliv. Rev. 55 (2003) 851–867.
[16] S.P. Newman, P.H. Hirst, G.R. Pitcairn, A.R. Clark, Understanding Regional Lung Deposition Data in Gamma Scintigraphy, in: R.N. Dalby, P.R. Byron, S.J. Farr (Eds.), Respiratory Drug Delivery, VI, Interpharm Press, Buffalo Grove, 1998, pp. 9–15.
[17] F. Vanderbist, F. Wery, D. Baran, B. Van Gansbeke, A. Schoutens, A.J. Moës, Deposition of nacystelin from a dry powder inhaler in healthy volunteers and cystic fibrosis patients, Drug. Dev. Ind. Pharm. 27 (3) (2001) 205–212.
[18] D.J. Ball, P.H. Hirst, S.P. Newman, B. Sonet, B. Streel, F. Vanderbist, Deposition and pharmacokinetics of budesonide from the Miat Monodose inhaler, a simple dry powder device, Int. J. Pharm. 245 (2002) 123–132.
[19] G.R. Pitcairn, S.P. Newman, Tissue attenuation corrections in gamma scintigraphy, J. Aerosol. Med. 3 (1997) 187–198.
[20] S.P. Newman, F. Morén, E. Trofast, N. Talaee, S.W. Clarke, Deposition and clinical efficacy of terbutaline sulphate from Turbuhaler, a new multi-dose powder inhaler, Eur. Respir. J. 2 (1989) 247–252.
[21] S.P. Newman, R. Wilding, Gamma scintigraphy an in vivo technique for assessing the equivalence of inhaled products, Int. J. Pharm. 170 (1998) 1–9.
[22] S.P. Newman, G.R. Pitcairn, P.H. Hirst, A brief history of gamma scintigraphy, J. Aerosol. Med. 14 (2001) 139–145.
[23] M. Dolovich, Practical aspects of imaging techniques employed to study aerosol deposition and clearance, in: A.J. Hickey (Ed.), Pharmaceutical Inhalation Aerosol technology, second edition, revised and expanded, vol. 134, Marcel Dekker, New York, 2004, pp. 171–213.
[24] M. Newhouse, P. Hirst, S. Duddu, Y. Walter, T. Tarara, A. Clark, J. Weers, Inhalation of a dry powder tobramycin pulmosphere formulation in healthy volunteers, Chest 124 (2003) 360–366.
[25] J.S. Ilowite, J.D. Gorvoy, G.C. Smaldone, Quantitative deposition of aerolized gentamycin in cystic fibrosis, Am. Rev. Respir. Dis. 136 (1987) 1445–1449.
[26] S. Mukhopadhyay, G.E. Staddon, C. Eastman, M. Palmer, E.R. Davies, F. Carswell, The quantitative distribution of nebulized antibiotic in the lung in cystic fibrosis, Respir. Med. 88 (1994) 203–211.