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RESEARCH PAPER

Deposition of Nacystelyn from a Dry Powder Inhaler in Healthy Volunteers and Cystic Fibrosis Patients

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ABSTRACT

The aim of this study was to compare, using gamma scintigraphy, the lung deposition of a novel mucoactive agent, Nacystelyn (NAL), administered as a dry powder inhaler (DPI) in six healthy volunteers, six adult patients with cystic fibrosis (CF), and six children and adolescents patients with CF. The correlation between in vitro and in vivo results was also tested. It was first demonstrated that the method of labeling of NAL with ^{99m}Tc was reliable as tested by three in vitro methods (multistage liquid impinger, multistage cascade impactor, and 2-stage glass impinger). The deposition of unlabeled NAL, labeled NAL, and the radiolabel was similar in all stages of each device. Furthermore, the fine particle fraction (FPF) was the same on all apparatuses. The mean lung deposition obtained in volunteers was 27.5 ± 13.5%. The results are approximately three times higher than the results obtained previously in healthy volunteers with NAL metered-dose inhalers (MDIs). As expected, the lung deposition observed in patients with CF was lower, e.g., 23.5 ± 7.0% for adults and 16.5 ± 5.9% for children and adolescents. A significant correlation was found between lung deposition and both the patient

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weight ($p < 0.02$) and height ($p < 0.04$). Surprisingly, the peripheral:central (P:C) ratio was similar for the three populations, indicating that the presence of mucus in moderately ill patients with CF does not modify the lung distribution of NAL. The FPF measured in vitro was similar to that obtained in volunteers but higher than that found in both patient populations. The DPI formulation of NAL developed will probably improve patient compliance and comfort in future clinical trials and postmarketing use of the drug.

KEY WORDS: Cystic fibrosis; Deposition; Dry powder inhaler; Gamma scintigraphy; Healthy volunteers; Nacystelyn.

INTRODUCTION

The inhaled route has several well recognized advantages over other routes of administration of drugs to the respiratory tract (1). By inhaling the drug directly to their receptor site within the bronchial tree, drug doses can be held as small as possible. Therefore, undesirable side effects can be minimized, and drug response can be detected rapidly (2,3). Gamma-scintigraphy is a well known technique of assessment of in vivo deposition of inhaled drugs (4,5). Briefly, the drug is labeled using an acceptable radiolabel (usually ^{99m}Tc) administered to the patient and its distribution in the body imaged with a suitable gamma-camera (6). L-lysine-*N*-acetylcysteinate (ACC) or Nacystelyn (NAL) is a novel mucoactive agent possessing mucolytic, antioxidant, and anti-inflammatory properties (7–9). It is currently being tested in phase 3 clinical trials for the treatment of cystic fibrosis (CF). Chemically, it is a salt of ACC. This drug appears to present an activity superior to its parent molecule ACC because of a synergistic mucolytic activity of L-lysine and ACC. Furthermore, its almost neutral pH (6.2) allows its administration in the lungs with a very low incidence of bronchospasm, which is not the case for the acidic ACC (pH 2.2) (10).

NAL is difficult to formulate in an inhaled form because the required lung dose is very high (approximately 2 mg) and the micronized drug is sticky and cohesive and it is thus problematic to produce a redispersable formulation.

NAL was first developed as a chlorofluorocarbon (CFC) containing metered-dose inhaler (MDI) because this form was the easiest and the fastest to develop to begin the preclinical and the first clinical studies. NAL MDI delivered 2 mg per puff, from which approximately 10% was able to reach the lungs in healthy volunteers (11). One major inconvenience of this formulation was patient compliance because as many as 12 puffs were necessary to obtain the required dose. Futhermore, the progressive removal of CFC gases from medicinal products combined with the problems of coordination met in a large proportion of the patient population (12) have led to the development of a new galenical form of NAL. A dry powder inhaler (DPI) formulation was chosen to resolve the problems of compliance with MDIs and to combine it with an optimal, reproducible, and comfortable way to administer the drug to the widest possible patient population, including young children.

The DPI formulation of NAL involved the use of a nonconventional lactose (usually reserved for direct compression of tablets), namely, a roller-dried (RD) anhydrous β-lactose. When tested in vitro with a monodose DPI device, this powder formulation produces a fine particle fraction (FPF) of at least 30% of the nominal dose, namely three times higher than that with MDIs. This study aimed to assess the in vivo lung deposition and distribution of NAL in healthy volunteers and in two populations of patients with CF, i.e., children and adults. It was also intended to determine any in vitro/in vivo correlation.

MATERIALS AND METHODS

Patients with CF

Six children and adolescent (6 to 15 years of age; one male) and six adult patients with CF (>18 years of age; six males) were recruited. Each patient was clinically well (without infective exacerbation) and had mean values for $FEV_1 \geq 30\%$ and for FVC $\geq 40\%$ of the predicted value. Demographic and physical data for both patients and volunteers are summarized in Table 1. Excluded from this study were patients with concomitant treatment with another mucolytic, history of significant allergic reaction of any origin, pregnancy or likelihood of pregnancy, history of asthmatic episodes, inability to use the Monodose Miat Inhaler® correctly, and participation in another study with radioisotopes within 6 months of the proposed study. Patients were seen before entering the study and were

Table 1.

Demographic and Physical Data for Healthy Volunteers, CF Adults, and CF Children

Patients	Age (Years)	Gender	Height (cm)	Weight (kg)	PEF (% of Normal Value)
Healthy volunteers					
	36	F	172		
	31	M	184		
	25	M	191		
	38	M	177		
	29	M	178		
	37	F	176		
CF adults					
	24	M	184	74.1	110
	25	M	176	63.2	96
	24	M	178	78.5	64
	34	M	170	61.3	72
	28	M	171	58.1	75
	24	M	166	62.6	87
CF children					
	10	F	138	27.3	84
	13	F	152	44.6	103
	8	M	123	21.5	95
	10	F	145	31.6	94
	12	F	153	38.9	122
	7	F	123	22.6	96

carefully instructed in the use of the Monodose Miat Inhaler. This study was approved by the Ethics Committee of Erasme Hospital (Brussels, Belgium). Informed consent was obtained from patients or from parents of children. The children who completed the study were willing participants. The maximum radioactive dose administered to each patient was 900 μCi.

Healthy Volunteers

Six healthy volunteers (25 to 38 years of age; four males) were recruited. This study was performed before the study of patients with CF to confirm the in vitro deposition results. Volunteers were excluded from this study for the following reasons: history of significant allergic reaction of any origin, pregnancy or likelihood of pregancy, inability to use the Monodose Miat Inhaler correctly, participation in another study using radioisotopes within 6 months of the proposed study. Patients were seen before entering the study and were carefully instructed in the use of the Monodose Miat Inhaler. This study was approved by the Ethics Committee of Erasme Hospital. Informed consent was obtained from volunteers. The maximum level of radiation dispensed to each subject was 900 μCi.

Radiolabeling of NAL Powder

Nacystelyn ($C_{11}H_{23}N_3O_5S$) was purchased by Freedom Chemical Diamalt (München, Germany) and micronized by SMB Technology (Marche-en-Famenne, Belgium). Radiolabeling of NAL powder was carried out using a method described previously (11,13). Briefly, ^{99m}Tc was extracted from the aqueous phase into butanone using a separating funnel. NAL was then added to the butanone solution. The butanone was evaporated to dryness in presence of NAL using a Rotavapor®. The powder obtained was mixed manually with lactose (Pharmatose DCL 21, DMV International, Netherlands) with a mortar (without crushing) in the ratio 1:4 (w/w), and 40 mg of the powder mixture was put into n°3 hard gelatin capsules (Capsugel, Bornem, Belgium). The capsule was administered to the volunteer or patient using the Monodose Miat Inhaler device.

In Vitro Assessment of Radiolabeling Efficiency

In vitro assessment of the output of radiolabeled NAL with the Monodose Miat Inhaler was used to ensure that

the radiolabeling method did not significantly modify the particle size distribution (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 NAL. PSD and FPF were measured using three in vitro apparatuses, i.e., a multistage liquid impinger (MLI), a cascade impactor (CI), and a two-stage glass impinger (GI), on 3 consecutive days.

Expression of Results

The main parameter resulting from the deposition tests were the amount of drug retrieved on each stage of the apparatus and the FPF, e.g., the fraction (%) of NAL having a diameter inferior to a fixed value (6.8 μm for the MLI, 6.2 μm for the MCI, and 6.4 μm for the GI) expressed as a percentage of the nominal dose (8 mg). All the in vivo results are expressed as percentages of the initial capsule content. On each study day, the PSD and FPF were assessed before the study to confirm that the labeling was satisfactory.

Inhalation Procedures

Each patient and volunteer inhalation technique was assessed with placebo before the study. Subjects were asked to exhale comfortably, and then to inhale with a nonmaximal inspiratory effort and to sustain the effort for approximately 4 sec. A breath-holding period of 5 sec was imposed, and then a disposable paper face mask was put on the mouth and nose of the subjects to assess the retained radioactivity in the exhaled air.

Assessment of the ^{99m}Tc Activity in Subjects

Each labeled capsule was first measured at distance from a gamma counter and the activity compared with that of a syringe containing approximately 20% of the capsule activity as ^{99m}Tc. This syringe was considered to be the standard and is used to assess the radioactivity decay during time. A flat flood source was charged with 20 mCi ^{99m}Tc. Immediately after inhalation, the subject was lying down in a supine position between two camera heads equipped with 140 KeV low-energy parallel collimators, and the activity in throat, lungs, and stomach was recorded for 3 min. Thereafter, the flat field 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. The flat field source was withdrawn and the standard placed on the trunk, and the activity was recorded by both cameras for 3 min. This was done to take into account the attenuation of the radioactivity resulting from tissues absorption. A background counting was recorded for each camera head. The activity and amount of NAL remaining in the device (+ capsule) and in the exhaled air (facial mask) were also assessed using a gamma counter.

The flat-field source-transmission image recorded from the lower camera allowed the lung fields to be outlined. Expanding centripetally from the hilar region, three regions of interest were delineated, each containing an equal number of pixels. The activity was determined for both cameras, corrected for background and radioactive decay, and the geometric mean of the anterior and the posterior count for each region of interest was calculated. The geometric mean of the standard was established in the same manner. Finally, the activity in the lung region was recorded as a percentage of the activity of the intact capsule. Likewise, an estimation (not an actual measurement because attenuation was not accurately taken into account in those tissues) of the percentage of dose deposited in the throat and in the stomach-esophagus were derived from these records.

Statistical Analysis

The main method of statistical analysis was carried out using ANOVA. The significance level was taken to be 95% ($p < 0.05$). Linear regression analysis was performed to determine significant relationships among variables.

RESULTS

In Vitro Deposition

The in vitro deposition was compared on the MLI, for unlabeled NAL, labeled NAL, and the radiolabel. The results did not show any significant differences among the three measurements on any stages of the MLI except for the throat stage, where the deposition was significantly ($p = 0.01$) lower for ^{99m}Tc than for both unlabeled and labeled drug (Fig. 1). The amount remaining in the device was also similar (NS) for the three measurements. To confirm these results and to perform a cross-comparison of the in vitro methods, the reliability of the labeling method was assessed using two other in vitro methods, the MCI and the GI. Again, no difference was observed on any stage of the apparatuses, thus further validating the labeling method. Moreover, the FPF obtained from the three apparatuses was compared because the cut-off values chosen were similar (see "Materials and Methods"). As seen

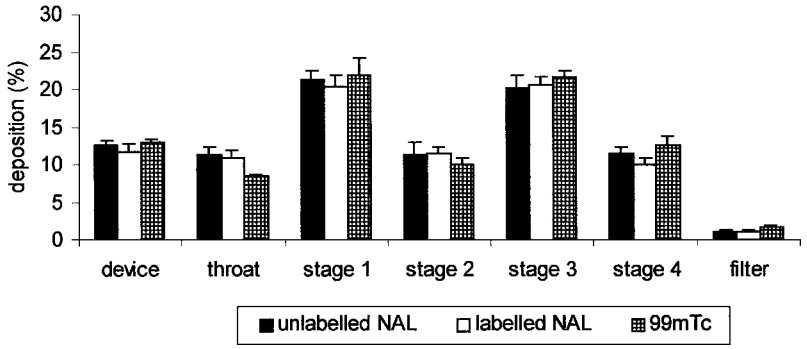

Figure 1. In vitro comparative deposition (MLI, 60 L/min) of unlabeled NAL, labeled NAL, and ^{99m}Tc.

in Figure 2, the FPF obtained with the three apparatuses were comparable (NS).

Healthy Volunteers

The complete distribution of the drug in the different tissues (lung, oropharynx, and stomach) is given in Table 2 for healthy volunteers and adults and children and adolescents with CF. The high variability observed in lung deposition in volunteers is attributable in part to the very low deposition observed in one subject because of poor device manipulation. Indeed, the capsule was incompletely pierced by the inhaler needles, resulting in a poor output of the powder from the device and consequently a very low pulmonary deposition (9.0%) and a high amount of drug remaining in the device (61.2%). An adapted Student's t test was performed to assess whether this subject had to be removed from the statistical analysis. The value is not considered as erroneous for $\alpha = 0.05$ but is for $\alpha = 0.1$ ($p = 0.08$), which makes the value suspicious but not erroneous. It was nevertheless decided to perform all the statistical analysis both with ($n = 6$) and without ($n = 5$) including this subject. As can be seen, when the subject is removed, the lung deposition results show good correlation with the in vitro results.

The partitioning of the radiolabel among central, intermediate, and peripheral areas of the lung is characterized by the so-called peripheral:central ratio (P:C ratio). The relatively low P:C ratio obtained indicates a greater deposition of the drug in the central area. But this must be carefully interpreted because the manner by which the lungs are divided into different regions strongly influences the results, making comparisons with other studies difficult (see "Discussion"). The fraction of the drug remaining in the device was similar to the data obtained in vitro (MLI) for $n = 5$, although it becomes significantly higher when subject 2 is included. The recorded activity in the exhaled air was nil.

Patients with CF

All patients were carefully trained by the investigators, and the inhalation technique was found to be good

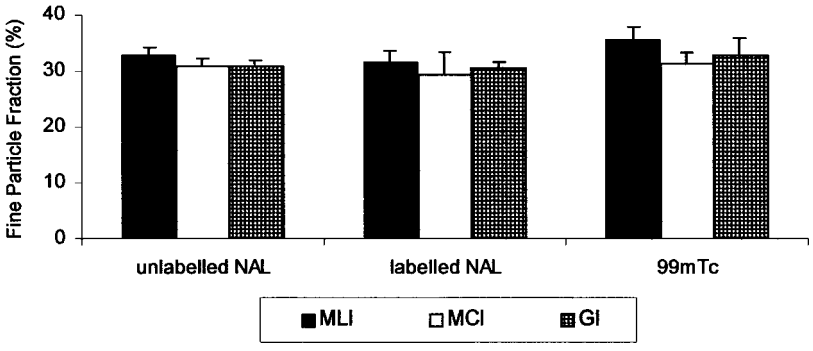

Figure 2. Comparative fine particle fraction (FPF) obtained with unlabeled NAL, labeled NAL, and ^{99m}Tc on the multistage liquid impinger (MLI), the multistage cascade impactor (MCI), and the two-stage glass impinger (GI).

Table 2.

Lung, Oropharynx, Stomach, and Device Deposition of Radiolabeled NAL in Each Treated Group Expressed as a Proportion of the Nominal Dose (Capsule Contents)

Group	*N*	Lungs	Oropharynx	Stomach	Device	P:C Ratio
Volunteers	6	27.5 ± 13.5	37.4 ± 13.4	3.4 ± 3.3	22.1 ± 19.5	0.57 ± 0.23
	5	31.0 ± 11.5	41.1 ± 11.2	4.1 ± 3.6	14.3 ± 4.0	0.50 ± 0.20
CF adults	6	23.5 ± 7.0	38.4 ± 2.8	4.5 ± 2.8	8.9 ± 1.5	0.45 ± 0.10
CF children and adolescents	6	16.5 ± 5.9	32.8 ± 11.8	14.0 ± 7.3	13.7 ± 5.5	0.42 ± 0.10

Note: For stomach and oropharynx, an estimation is given because the attenuation by tissues is not taken into account.

for all the patients. Of particularly relevance was the fact that all the children were able to empty the capsule. As expected, the drug deposition was lower for patients with CF than for healthy volunteers ($n = 5$). The p values were respectively 0.2 between healthy volunteers and adults with CF and 0.04 between healthy volunteers and children and adolescents with CF. The difference between both patient populations was not significant for $\alpha = 0.05$, but was significant for $\alpha = 0.1 (p = 0.0922)$. There was a significant positive correlation between lung deposition and both body weight ($r^2 = 0.449$; $p < 0.02$) and height ($r^2 = 0.378$; $p < 0.04$) (Figs. 3 and 4). A similar correlation was found by Devadason and al. (14) when budesonide Turbuhaler® was administered to children and adolescents with CF.

On the other hand, no significant correlation was found between lung deposition and FEV_1, and the P:C ratio was not significantly different for either CF population. Furthermore, the P:C ratio for each patient population did not differ significantly from that of healthy volunteers. The amount of drug remaining in the devices used by the patients was slightly lower (NS; $p = 0.06$) for the adults than for the children. This trend may be explained by the lower inspiratory force of children and adolescents.

On the other hand, the amount of drug remaining in the device after inhalation was significantly higher ($p = 0.01$) for healthy volunteers than for the adult patients with CF. This may be explained by the fact that the patients' inhalation technique was better than that of healthy volunteers because patients with CF are familiar with the use of inhalers. The high amount of radiolabel found in the stomach of children may be explained by the fact that they swallowed the drug more rapidly than did adults. The recorded activity in the exhaled air was nil for both CF patient populations.

DISCUSSION

This study provides the first direct information on the dose of NAL delivered to the lungs of healthy volunteers and adults, children, and adolescents with CF when administered with the monodose Miat Inhaler DPI device. The comparison among the three in vitro tests was interesting

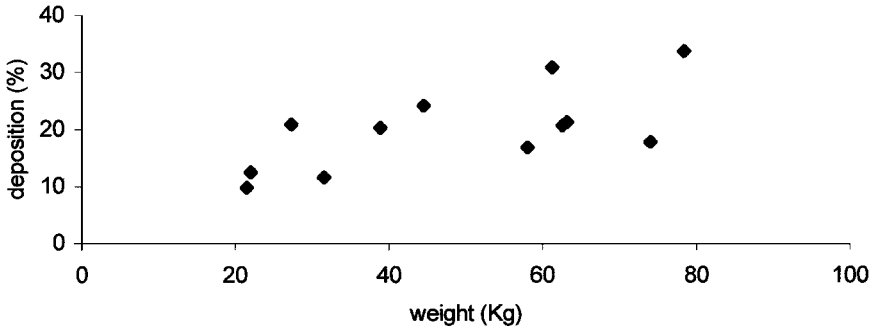

Figure 3. Correlation between lung deposition (% of the capsule content) and patient's weight ($r^2 = 0.449$; $p < 0.02$).

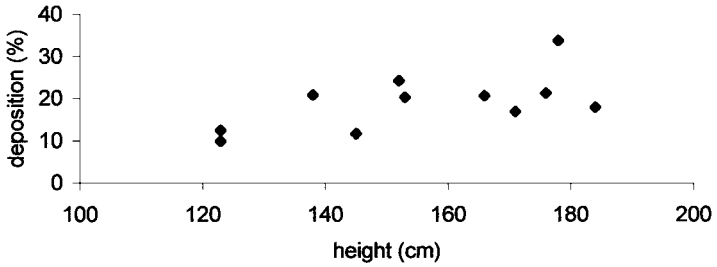

Figure 4. Correlation between lung deposition (% of the capsule content) and patient's height ($r^2 = 0.378$; $p < 0.04$).

in that the FPF was similar for the three devices. This indicates that an apparatus as simple as the two-stage GI may be used, in a reliable way, in early pharmaceutical development of a DPI for assessing the lung deposition of the drug. Of course, multistage devices must be used to obtain a more detailed PSD in later stages of development and to determine specifications of finished product. As expected, the deposition data obtained in vivo were higher in healthy volunteers than in both populations of patients with CF. Indeed, CF being a chronic disease accompanied with hypersecretion of mucus, the trapping of drug by this fluid renders the access to the lungs very difficult. But the intersubject coefficient of variation being large for the volunteer group, the difference in lung deposition in adults with CF did not reach the significance level, whereas it did for children and adolescents with CF. A lower intersubject variation was observed in patients because they are familiar with the use of inhaler and because of their better compliance. The difference between adults and young patients with CF may be mainly explained by anatomical differences. Indeed, a significant correlation between lung deposition and both patient weight and height indicates that the lung deposition is, in some way, related to the anatomical characteristics of the subject. Considering the distribution of NAL in the central, intermediate, and peripheral regions of the lungs, it was surprising to observe that there was no significant difference between the three groups studied for the P:C ratio (± 0.5). The presence of mucus in patients was indeed thought to reduce the peripheral deposition of drug. Furthermore, because the children usually produce less mucus than do adults, the P:C ratio was also expected to be inferior in this last group. Nevertheless, the mean value of the P:C ratio measured in this trial is lower than that determined in other studies involving either patients with CF or with volunteers (15,16). There are two possible explanations. First, the method used to divide the lungs into three equal regions adopted in this study is completely different than that used in other studies. For instance, the method described by O'Doherty et al., (17) in which the lungs was divided into only two regions, the central one representing half the width and one third of the total lung area, of course gives higher values of P:C because of the relatively small size of the central area. The second reason is linked to the pharmacological properties of the drug and its galenical formulation. Indeed, because the mucus of patients with CF is located principally in bronchi and bronchioles and not in the alveoli, the drug was micronized to produce particles between 2 and 5 μm so that they would deposit in the conducting airways rather than in the alveoli. The in vitro/in vivo comparison was interesting in that a good correlation was established between the in vitro FPF and the lung deposition in healthy volunteers ($n = 5$). The lung deposition in patients was significantly lower than the FPF, indicating, as expected, that the nature of the disease and probably its severity play key roles in the bioavailability of drugs in the lungs. This difference is of course more marked for children with CF.

This study indicates that gamma-scintigraphy studies are very useful in assessing lung bioavailability of inhaled drugs but must be performed on both volunteers and patients, and, ideally, in different populations affected by the disease.

In conclusion, this study has demonstrated that NAL DPI formulation allows high lung deposition in healthy volunteers and that this can be predicted by the in vitro studies. The inhalation of only one DPI capsule, which gives the same lung dose as 12 puffs of the previous MDI formulation, will undoubtedly improve patient comfort and compliance. As expected, the lung deposition was lower in both populations of patients with CF and is higher for adults than for children and adolescents. A significant correlation was found between lung dose and both weight and height of patients. The distribution of the drug in the lung (P:C ratio) was surprisingly similar for the three populations assessed.

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