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Research Article

Flixotide^{TM}-pressurized metered-dose inhalers loaded with [^{18}F]fluticasone propionate particles for drug deposition studies in humans with PET–formulation and analysis

Maria Constantinou[1,*], Stephen L. Waters[1], Colin J. Steel[1], Keith G. Poole[1], Phil S. Marino[2], Phillip W. Ind[2], Christopher G. Rhodes[1], Franklin I. Aigbirhio[3], Alison Moore[4], Victor W. Pike[5] and Sajinder K. Luthra[1]

[1] *Hammersmith Imanet Ltd., Cyclotron Building, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK*

[2] *MRC Clinical Sciences Centre, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK*

[3] *Wolfson Brain Imaging Centre, Cambridge University Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK*

[4] *GlaxoSmithKline PLC, Berkeley Avenue, Greenford, Middlesex, UK*

[5] *Molecular Imaging Branch, National Institute of Mental Health, Building 10, 10 Center Drive MSC 1003, Bethesda, MD 20892-1003, USA*

Summary

Fluticasone propionate (FP) is a potent anti-inflammatory synthetic steroid, used for the treatment of asthma. Flixotide^{TM} is a formulated pressurized metered-dose inhaler (pMDI) that contains small-micronized FP particles in a blend of CFC propellants. Our objective was to develop a radiotracer method for accurately measuring the regional deposition of FP within the human lung using positron emission tomography (PET), which would be of important clinical interest. Flixotide^{TM} pMDIs were used to prepare [^{18}F]FP pMDIs labeled isotopically with the positron emitter, fluorine-18 ($t_{1/2} = 109.7$ min). FP particles from Flixotide^{TM} pMDIs were mixed with [^{18}F]FP formulated into a pMDI and sonicated at room temperature. The drug delivery of [^{18}F]FP pMDI (250 μg of FP per actuation dose) was assessed for particle size distribution and dose uniformity. The distributions of FP and [^{18}F]FP across particle size in such preparations were measured with an Andersen cascade impactor. This procedure was shown to provide an emitted dose from a [^{18}F]FP pMDI of 246 ± 19 μg/per metered dose. The particle size distribution as measured by mass

*Correspondence to: M. Constantinou, Hammersmith Imanet Ltd., Cyclotron Building, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK. E-mail: maria.constantinou@csc.mrc.ac.uk

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median aerodynamic diameter (MMAD) (The mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) for each distribution were calculated. MMAD is defined as the aerodynamic diameter around which the mass of particles is equally distributed and the GSD is a measure of the dispersion of these particle diameters around the MMAD) from a commercial Flixotide™ pMDI was $2.6 \pm 0.2\,\mu m$ and agreed well with that from an [^{18}F]FP pMDI ($2.8 \pm 0.1\,\mu m$). The MMAD and geometric standard deviation (GSD) of newly formulated [^{18}F]FP pMDIs were unaffected by the formulation procedure. [^{18}F]FP was distributed with good uniformity with respect to the mass of FP for particles greater than $0.43\,\mu m$. Hence, the radiolabeled pMDI is a suitable source of radiotracer for the regional measurement of lung deposition for inhaled FP in human subjects with PET.

Key Words: fluticasone propionate; pressurized metered-dose inhaler; fluorine-18; [*S-fluoromethyl*-^{18}F]Fluticasone propionate; particle-size analysis; PET

Introduction

Fluticasone propionate (FP) [(*S*)-fluoromethyl-6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyl-oxy)androsta-1,4-diene-17β-carbothioate];[1] a synthetic steroid (Figure 1), behaves as a high-affinity agonist at the human glucocorticoid receptor and has potent anti-inflammatory action *in vitro* and *in vivo*.[2,3] Flixotide™, marketed by GlaxoSmithKline (GSK), contains micronized FP particles in a blend of CFC propellants (CFC11, CFC12), formulated in a pressurized metered-dose inhaler (pMDI). It is indicated for the treatment of inflammation associated with airway diseases; for the

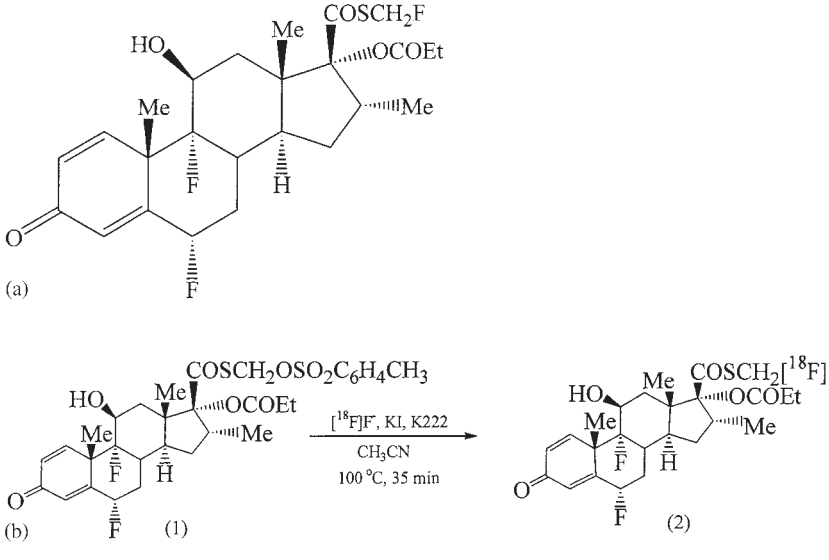

Figure 1. (a) Structure of fluticasone propionate (FP); (b) nucleophilic substitution reaction of the tosylate precursor with [^{18}F]Fluoride

preventative treatment of asthma in both adults and pediatric patients, for allergic rhinitis and in chronic obstructive pulmonary disease (COPD).[4] The pMDI remains the most commonly prescribed device for the delivery of asthma medications. For delivery of FP to the respiratory tract, an aerosol of micron-sized drug particles is discharged from a pMDI and inhaled.[4,5] However, the effectiveness of this type of therapy depends on sufficient drug reaching compromised regions of the lung. Drug deposition is likely to depend on many factors, including particle size, nature of propellant, the configuration of the pMDI, inhalation manoeuvre and disease state.[6] Therefore, any method capable of directly measuring regional deposition of FP within the human lung and subsequent pharmacokinetics would be of important clinical interest.[5]

Radionuclide imaging is useful for studying the deposition of therapeutic aerosols in the human lung. γ-Scintigraphy with technetium-99 m ($t_{1/2} = 6$ h) has been used most extensively[7–9] but only produces two-dimensional information. For this purpose, a radioactive aerosol is prepared by physical incorporation of the technetium-99 m into a product formulation.[7,10,11] In order for the drug to act as its own tracer, a more rigorous approach would be to label the drug molecule itself[9] and formulate the radioactive product as part of a commercial pMDI. However, this has rarely been done; Short *et al.*[12] prepared a pMDI containing the anticholinergic bronchodilator, ipratropium bromide labeled with γ-emitting bromine-77 ($t_{1/2} = 57$ h). This formulation was used to assess drug deposition in human subjects.[13]

Labeling with a positron-emitter, rather than a γ-emitter, enables drug deposition investigations to exploit the full advantages of state-of-the-art three-dimensional PET,[14] namely high sensitivity, high spatial resolution, high temporal resolution, absolute quantification and a large axial field of view that may include most of the upper respiratory tract or thorax. Using the positron-emitter, carbon-11 ($t_{1/2} = 20.3$ min), Berridge *et al.*[15–18] labeled the anti-asthmatic corticosteroid, triamcinolone acetonide. Formulation was achieved by adding a small quantity of the radiolabeled drug, through a rubber septum into the pMDI containing triamcinolone acetonide (Nasacort™ which normally includes ethanol as a constituent). PET was used to study the labeled drug deposition and kinetics after nasal administration.[16,17]

Previous methods for introducing trace amounts of radioactive drug into micronized drug particles have involved either grinding them together in a mortar and pestle[12] or adding an ethanolic solution of radioactive drug to the drug suspension.[16] However, the former method alters the size of the micronized particles to some extent, whereas the latter method may add a foreign substance to the standard formulation. The deposition of the drug in the lung after discharge from a pMDI prepared by one of these methods risks altering the characteristics of the drug aerosol (pMDI).

The purpose of this work was to develop a formulation of [^{18}F]FP/FP in a pMDI with minimal disturbance of the drug's characteristics to be used for clinical PET studies of drug deposition by inhalation.

The objective of the PET study was to use this radiotracer to determine the deposition of FP particles discharged from a formulated pMDI containing FP and [^{18}F]FP into the human lung. Hence, we had to establish a reliable and reproducible method for rapidly incorporating the radiotracer into the standard pMDI (micronized FP particles, with a controlled size distribution, suspended in a blend of chlorofluorocarbon propellant). This involved depositing [^{18}F]FP onto the internal surface of a standard aluminum pMDI can, adding cooled FP particles in a suspension from a standard Flixotide™ pMDI, sealing the can and then sonicating the contents. Furthermore, we had to establish that the devised method gave acceptably efficient and uniform labeling of drug particles without significantly affecting the particle-size distribution and thus the pharmaceutical formulation. Here, we describe further development and validation of a simple method for radiolabeling a Flixotide™ formulation that meets these requirements.

Results and discussion

Radiosynthesis

The *S*-fluoromethyl group present in FP (Figure 1a) provides the simplest opportunity for isotopic labeling with fluorine-18 by nucleophilic displacement,[19] whereas Berridge *et al.* prepared the fluorohaloalkane[20] [^{18}F]Fluoroiodomethane[21] and used a carbothio acid precursor in a two-step radiosynthesis to prepare [^{18}F]FP. For deposition studies the position of the covalently incorporated radiolabel in the FP molecule has no experimental relevance.

[^{18}F]FP (2) was labeled with no-carrier-added [^{18}F]fluoride in the *S*-fluoromethyl group from the corresponding tosylate precursor (1) *via* a nucleophilic substitution reaction as shown in Figure 1(b).

The procedure was modified from that of Aigbirhio *et al.*[22] Briefly, no-carrier-added [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction on oxygen-enriched water (97%). The tosylate precursor in anhydrous acetonitrile was reacted with anhydrous [^{18}F]fluoride in the presence of potassium iodide and aminopolyether 2.2.2[23] for 35 min at 100°C in a sealed glassy carbon vessel[24] using an automated synthesis system. Water was added to the reaction mixture and loaded onto a reverse-phase HPLC column that was eluted with a mixture of acetonitrile and water [45:55 v/v] at a flow rate of 4.5 ml min^{-1}. [^{18}F]FP eluted between 55 and 60 min. The automated procedure provided between 140.6 and 466.2 MBq of [^{18}F]FP in 2 h after EOB.

[^{18}F]FP was then diluted with water and passed through a C_{18} Sep-Pak. The retained radioactivity was eluted with methanol through a filter into an empty

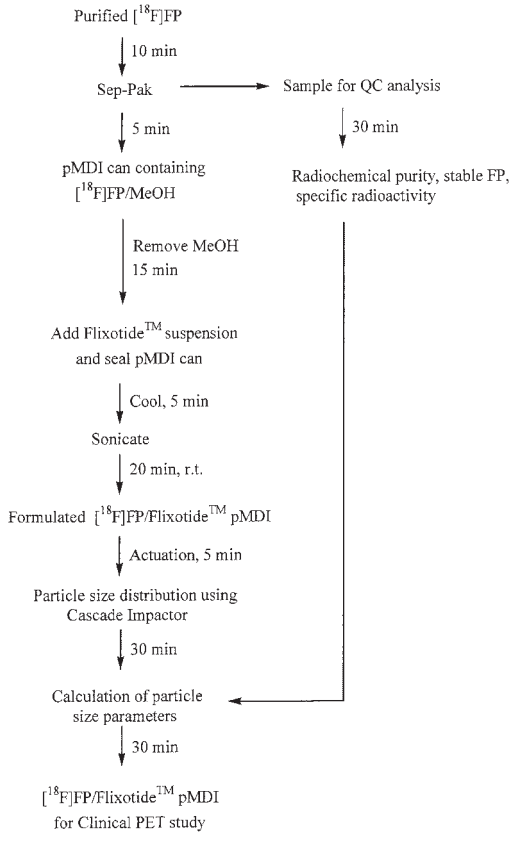

Figure 2. Process flow for the preparation of pMDIs formulated with [^{18}F]FP + Flixotide™

pMDI can, as shown in the process flow (Figure 2). An aliquot of this solution was analyzed using reverse-phase analytical HPLC to determine the radiochemical purity and amount of carrier FP. The radio-chromatogram showed a single radioactive peak having the same retention time as reference FP (*ca.* 9 min). The UV absorbance trace showed FP and several low-level non-radioactive contaminants. The total amount of carrier FP was typically $<26\,\mu g$ and unknown impurities were in the range of $98 \pm 87\,\mu g$ (assuming that they had the same extinction coefficients as FP). [^{18}F]FP was produced in high radiochemical purity (99.0% $\pm$ 2.1, $n = 16$) with a specific radioactivity of 13.3–52.9 GBq μmol^{-1} at the end of synthesis (EOS) (Table 1). The radiochemical yield was in the range 0.5–1.4% at EOS.

Formulation process

The next stage in the process (Figure 2) was to prepare a pMDI inhaler can containing [^{18}F]FP and commercial Flixotide™ suspension. Factors that

Table 1. Quality control analysis of no-carrier-added [^{18}F]FP before formulation of [^{18}F]FP pMDI with Flixotide^{TM}

Parameter	Measured (Mean ± SD; $n = 16$)
Radiochemical purity (%)	99.0 ± 2.1
Amount of carrier FP (μg)	6.0 ± 6.1
Amount of chemical impurities (μg)[a]	98 ± 87
Radiochemical yield at EOB[b] (%)	2.6 ± 1.4
Radioactivity at EOS (MBq)[c]	245.1 ± 120.5
Synthesis time	120 min
Formulation time[d]	120 min

[a] It was assumed that these unknown contaminants were derived from FP and would have similar UV extinction coefficients to that of FP.
[b] EOB = end of beam.
[c] EOS = end of synthesis; $n = 9$.
[d] Formulation time refers to the time taken to carry out all the quality control tests, e.g. particle size distribution and content uniformity.

Table 2. Association of [^{18}F]FP with FP particles in Flixotide^{TM} pMDI

Amount of FP[a] dispensed into can (g)	Sonication time (min)	Association of [^{18}F]FP with FP particles (%) (Mean ± SD)
1.98–2.04	15	76 ± 9 ($n = 3$)
1.94 ± 0.61 (1.58–3.66)[b]	20	97 ± 3 ($n = 12$)
3.04	30	96 ± 6 ($n = 2$)

[a] Total weight of Flixotide^{TM} (contains *ca.* 2.9 mg FP per g of suspension).
[b] Mean ± SD (range).

might affect this formulation were taken into consideration. Previous work by Aigbirhio *et al.*[25] had shown that in order to achieve consistently high incorporation (> 97%) of [^{18}F]FP into the pMDI Flixotide^{TM} suspension, sonication was required for at least 20 min (Table 2). Also, it had been shown that the incorporation of [^{18}F]FP was unaffected by the amount of commercial Flixotide^{TM} suspension (1.58–3.66 g) the amount of carrier FP, or the accompanying levels of unknown contaminants (Table 1).[25] These factors were taken into consideration and used in the formulation process described here. Therefore, the pMDI can containing [^{18}F]FP was dried at 50°C in a heater and a known proportion of Flixotide^{TM} suspension was added to the dried [^{18}F]FP and sealed. The pMDI can containing both [^{18}F]FP and Flixotide^{TM} suspension was placed into a water bath and sonicated for 20 min. Following this the particle size distribution analysis was carried out.

Content uniformity and particle size distribution analysis

In order to establish that the formulated pMDI [^{18}F]FP/Flixotide^{TM} suspension was comparable and within the specification required for the commercial pMDI Flixotide^{TM} suspension, the content uniformity (i.e. the accuracy and uniformity of the dose from the pMDI) and particle size

distributions were determined. This involved the analysis of the formulated pMDI [^{18}F]FP/Flixotide™ suspension ($n = 11$) and commercial Flixotide™ ($n = 13$, sampled from five batches) using an Andersen cascade impactor.[26,27] This is a well-established sampling device consisting of eight stages with pores of decreasing size, which collects particles based on their size and mass. Penetration of a therapeutic aerosol down the impactor is then taken to infer deposition within differing levels of the respiratory tract (Table 3). The particle size distributions and content uniformity of both the pMDI [^{18}F]FP/Flixotide™ suspension and commercial Flixotide™ were calculated according to standard methods as defined in the European Pharmacopoeia.[27] This involved the determination of the following parameters; weight per actuation (i.e. the total material discharged per actuation), MMAD, GSD, FPM (fine particle mass, the-distribution of particles in the discharged aerosol that can be expected to relate to the degree of drug penetration in the respiratory tract), ex-actuator content (amount of FP, μg) and amount of pMDI [^{18}F]FP/Flixotide™ deposited on the throat.

Assessing the accuracy and uniformity of dosing from the pMDI (i.e. the content uniformity) and the particle size distribution data for assembled pMDI [^{18}F]FP/Flixotide™ ($n = 11$) and a range of commercial Flixotide™ (FP) pMDIs ($n = 13$; sampled from five batches) are given in Table 4. The results show that the content uniformity as measured by the mean delivered ex-actuator content from the prepared pMDI [^{18}F]FP/Flixotide™ were higher (246 ± 19 μg) compared to the commercial Flixotide™ (207 ± 9.4 μg). However, the calculated ranges for both the commercial pMDIs Flixotide™

Table 3. The relationship between particle size and particle penetration into the airways and stages of the Andersen cascade impactor (ACI)[a]

Stage	Particle size (μm)	Deposition in respiratory tract
0	9.00–10.00	
1	5.80–9.00	
2	4.70–5.80	Pharynx
3	3.30–4.70	Trachea and primary bronchi
4	2.10–3.30	Secondary bronchi
5	1.10–2.10	Terminal bronchi
6	0.65–1.10	Alveoli
7	0.43–0.65	Alveoli
Filter	<0.43	

[a]The ACI has 8 stages (Aluminum plates, not coated with silicone) held together by airtight seals and clamps. The pMDI is placed in an induction port (throat) and actuated as a stream of air at 28 l min^{-1} draws the aerosol particles through the ACI. Each stage contains a specified number of accurately drilled holes, which decrease on size from stages 0 to 7. Finally, there is a filter to catch all the remaining smaller particles. Therefore particles of a defined size range are collected at each stage. The ACI has a pre-separator (throat stage) for the collection of particles greater than 10 μm. As the particles in the inhaler may not be spherical, their size is described by the term 'aerodynamic diameter'. This is defined as the diameter of a unit density (1 g/ml) sphere having the same terminal settling velocity as the aerosol particle in question, regardless of the aerosol particles shape and density. A particle with the effective cut-off diameter (ECD) for a particular stage of the Andersen instrument has a 50% probability of deposition in that stage.[26,27]

Table 4. Content uniformity and Particle size distribution data for Flixotide™ and prepared [^{18}F]FP pMDIs

Parameter[a]	Commercial[b] Flixotide™ ($n = 13$)	Literature[c] Flixotide™	Prepared [^{18}F]FP pMDI Chemical FP ($n = 11$)	^{18}F radioactivity ($n = 11$)
MMAD (Stages 2–5, μm)	2.6 ± 0.2 (2.4–3.0)	3.2	2.8 ± 0.1 (2.5–3.0)	2.7 ± 0.2 (2.5–3.2)
GSD (Stages 2–5, μm)	1.6 ± 0.1 (1.6–1.7)		1.7 ± 0.1 (1.6–1.8)	1.8 ± 0.1 (1.7–2.0)
FPM (Stages 3–5, μg)	102 ± 9.7 (86–117)	106	112 ± 10 (95–129)	Not applicable
FPM (ex-act, %)	49 ± 5 (42–57)		46 ± 4 (36–51)	47 ± 8 (34–56)
Ex-actuator content (μg)	207 ± 9.4 (192–222)	223	246 ± 19 (216–274)	Not applicable
Throat (ex-act, %)	43 ± 7 (34–52)		44 ± 6 (33–53)	47 ± 7 (33–55)
Throat (μg)	90.1 ± 16.7 (69.5–115.2)	98	120.4 ± 44.5 (63.7–259.5)	Not applicable
Weight per actuation (mg)	85 ± 2 (83–87)[d]		87 ± 6 (75–94)	Not applicable

[a] Mean ± SD (range).
[b] Five different batches.
[c] Reference 28.
[d] $n = 3$. MMAD is defined as that aerodynamic diameter around which the mass of particles is equally distributed. The GSD is the measure of dispersion of particle diameters and is defined as the ratio of the median diameter to the diameter at ± one standard deviation (σ) from the median diameter. For each distribution, MMAD and GSD were calculated for particles between 4.7 and 1.1 μm ECD (i.e. particles collected on stages 2–5).

(192–222 μg) and the assembled pMDIs [^{18}F]FP/Flixotide™ (216–274 μg) were within manufacturing specifications (i.e. close to the mean literature[28] value of 221 μg and almost within 10% label claim 198–242 μg as defined in the literature).[28] The particle size distribution as measured by the MMAD values for FP (stages 2–5 inclusive) was similar for commercial (2.6 μm) and assembled pMDIs (2.8 μm). The MMAD of [^{18}F]FP within the assembled pMDIs [^{18}F]FP/Flixotide™ was also similar (2.7 μm). The corresponding GSD values were close at 1.8 μm for [^{18}F]FP within the assembled pMDIs [^{18}F]FP/Flixotide™ and 1.6 μm for assembled pMDIs (Table 4).

The FPM (stages 3–5 inclusive) for commercial and the assembled pMDIs were similar, 112 μg for prepared [^{18}F]FP pMDI and 102 μg for assembled and 106 μg for the literature value whereas the FPM (ex. act) parameter calculated as a mean percentage showed good correlation between the commercial Flixotide™ (49 ± 5%), FP in the assembled [^{18}F]FP pMDI of 46 ± 5% and [^{18}F]FP 47 ± 8%. Some difference was observed in the amount of FP deposited on the throat for commercial (69.5–115.2 μg) and assembled (63.7–259.5 μg) pMDIs. The mean value for the amount of FP deposited on the throat (120 *vs* 90 μg) showed a 33% increase but when compared to the literature value of 98 μg[28] the increase was about 19%, whereas, the mean value of FP deposited on the throat as a percentage (throat, ex. act.) showed a good correlation between commercial 43 ± 7% and FP in assembled [^{18}F]FP pMDIs (44 ± 6%) and [^{18}F]FP (47 ± 7%).

The weight per actuation (Table 4) for a commercial pMDI Flixotide™ with the type of valve used in this study (Neotechnic Spraymiser™ [Mk II]) is 85 ± 2 mg (range 83–87 mg, $n = 3$). The mean weight calculated for the assembled pMDI [^{18}F]FP/Flixotide™ was 87 ± 6 mg (range 75–94 mg, $n = 11$). Although the range for pMDI [^{18}F]FP/Flixotide™ was slightly greater than for commercial pMDI Flixotide™, the means for both were very similar.

The particle size distribution of assembled pMDI [^{18}F]FP/Flixotide™ ($n = 11$) and commercial Flixotide™ pMDIs ($n = 13$; sampled from five batches) over all stages of the Anderson impactor are shown in Figure 3 as a percentage of total deposition. The stages together with particle size and their relation to the human respiratory tract is given in Table 3. Although the distribution of non-radioactive FP, radiolabeled [^{18}F]FP in the assembled pMDI with that in commercial pMDI Flixotide™ was variable (throat compared to stages 0–2), the distribution was similar within each individual stage. The deposition in the throat, stages 3–5 were higher than that for stages 0–2, 6,7 and the filter. The profile observed for the particle size distribution minus the throat was similar to the findings in the literature.[28]

The distribution of the relative specific radioactivity of the assembled [^{18}F]FP/Flixotide™ for each of the particle size fractions (throat, stages 0–7

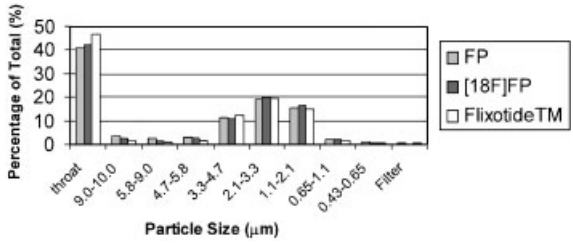

Figure 3. Representative example of the particle size distribution of FP in (a) commercial (blue columns) Flixotide^{TM}; (b) [^{18}F]FP in assembled radioactive [^{18}F]FP pMDIs (maroon columns) and (c) FP in the same [^{18}F]FP pMDIs (yellow columns)

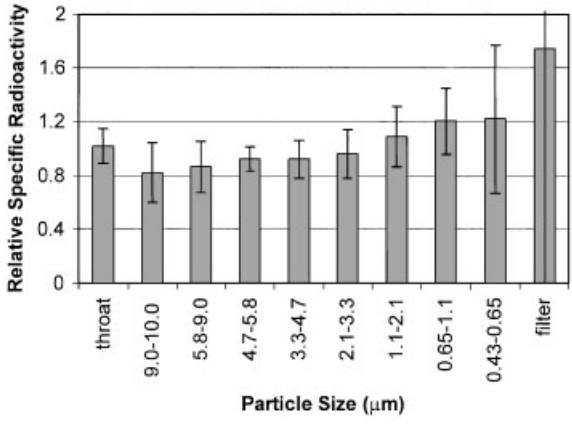

Figure 4. The relative specific radioactivity of particle size fractions (normalisation made using specific radioactivity of total material on cascade). Error bars are mean $\pm$ SD ($n = 16$). The error bar for the filter stage has been truncated to give a better appreciation over all the stages

and the filter) is shown in Figure 4. The mean relative specific radioactivities of the various particle fractions were fairly uniform and close to unity (Figure 4, $n = 16$) except the filter. For the throat and stages 3–5 where deposition is high, the mean for relative specific radioactivity was very close to 1. The radiolabeling of the very small fraction of radioactive particles on the filter was extremely variable but is inconsequential in terms of yield (0.1%, Figure 3). A slight trend of increasing relative specific radioactivity was observed with decreasing particle size. However, the consistency of relative specific radioactivity for the throat and across stages 0–6 argues in favor of relative uniform labeling. A degree of surface labeling of the FP particles by [^{18}F]FP cannot be ruled out. The collected data do not provide any information on the distribution of radioactivity within individual radioactive FP particles.

Other workers have used another statistical analysis $f2$[29] to evaluate the distribution match between parameters and this figure is found to be more sensitive to variations in size and distributions. In this case, the calculated mean value of $f2$ between the distribution of unlabeled FP and [^{18}F]FP is 85.7 ± 8.7 (range 71.9–95.8; $n = 8$). When a value of $f2$ is between 50 and 100, then similarity of the distribution profiles could be claimed.[29]

The above analysis and the calculated data have shown that the assembled pMDI [^{18}F]FP/Flixotide™ was comparable to the commercial pMDI Flixotide™.

The assembled pMDI [^{18}F]FP/Flixotide™ cans after decay were tested for sterility by a microbial limit test and found to be free of bacterial contamination. The radiosynthesis including formulation takes *ca.* 4 h from the end of radionuclide production. The formulation process itself including subsequent particle size distribution analysis takes *ca.* 2 h.

Conclusion

The devised protocol for radiolabeling fluticasone propionate with fluorine-18 followed by formulation as an [^{18}F]FP/Flixotide™ pMDI results in an essentially unchanged formulation which has a relatively uniform distribution of radioactivity with respect to particle mass. The radiolabeled particles were therefore considered to an appropriate tracer of particulate FP deposition in lung. Pilot clinical PET studies with the assembled pMDI [^{18}F]FP/Flixotide™ as a surrogate for commercial Flixotide™ have been published by Marino *et al.*[30]

This methodology could become very important for drug development, in view of the variety of inhaled drugs currently used in the treatment of airway diseases and also the potential for the development of the inhaled route for administration of a range of other drugs (e.g. insulin, heparin, etc). This technology may permit further study of the various factors that affect deposition and clearance of inhaled drugs. Furthermore it may assist the development of new and better inhaler devices.

Experimental

Materials and apparatus

Allen & Hanbury Ltd supplied Flixotide™ FP pMDIs (nominal 8 ml can to deliver 120 doses of 250 μg drug per actuation), containing micronized drug particles suspended in a blend of propellants [CFC 11 (CCl_3F: b.p. 23.8°C), CFC 12 ($CC1_2F_2$: b.p. −29.8°C)] plus surfactant. Established methods were used to prepare reference FP (2) and the tosylate precursor (*S*)-tosylmethyl-6α, 9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)-androsta-1,4-diene 17β-carbothioate[22,25] precursor. The precursor was produced free of

organic solvents, apart from diethyl ether (<0.7% w/w) and ethyl acetate (<2.7% w/w), and stored desiccated at −20°C and used within 3 months. Sterile pyrogen-free water was obtained as Steripak Pourpak™ (Baxter Healthcare Ltd). Methanol and acetonitrile (HPLC grade) were purchased from Fisher Chemicals. 4,7,13,16,21,24-hexaoxa-1,10-diazacyclo[8.8.8]hexacosane (Kryptofix® 2.2.2.; aminopolyether 2.2.2; APE 2.2.2) and potassium iodide were purchased from Aldrich Chemical Co. Ltd. C_{18} Sep-paks were obtained from Alltech Associates Inc. Primesphere C-18 and Bondclone C-18 columns were obtained from Phenomenex Ltd. Minisart filters were obtained from Sartorius Ltd. GSK provided components of standard pMDIs [aluminum canisters (5 ml internal volume) and Neotechnic Spraymiser™ valves (Mk II) and a hand crimper (Pamasol P2016) for assembly of the pMDIs. An 8-stage Andersen cascade impactor[26,28] ACI (Mk 2) was purchased from Graseby Andersen Ltd.

Radioactivity measurements

Fluorine-18 was measured with a high-pressure re-entrant ionization chamber (IG 12: Centronics Ltd) filled with argon (20 bar) with accompanying picoammeter (CP 10, Cooknell Electronics Ltd) or by a sodium iodide well counter (EG & G Instruments). Both instruments had been pre-calibrated with standard sources of fluorine-18 measured at the National Physical Laboratory (Teddington, Middlesex, UK). Radioactivity measurements were corrected for background and for physical decay to a set time.

Preparation of [^{18}F]FP

[^{18}F]FP was prepared in remotely controlled semi-automatic apparatus for safety from radiation, as reported previously[22,25] with some modifications. Briefly, the tosylate precursor (1, 10 mg) in anhydrous acetonitrile (1.0 ml) was reacted with cyclotron-produced no-carrier-added [^{18}F]fluoride in the presence of potassium iodide (3.3 mg) and aminopolyether 2.2.2 (8 mg) for 35 min at 100°C in a sealed glassy carbon vessel. [^{18}F]FP was separated from the crude reaction mixture by reverse-phase HPLC (Primesphere C18 column; 5 μ, 250 × 10 mm i.d.) with a mixture of acetonitrile and water [45:55 v/v] at a flow rate of 4.5 ml min^{-1} (FP retention time, 55–60 min).

Analysis of [^{18}F]FP

An undiluted sample (100 μl) of the methanol solution of [^{18}F]FP (after C_{18} Sep-Pak) was analyzed for radiochemical purity, chemical purity and specific radioactivity by HPLC on a reverse-phase column (150 × 3.9 mm i.d.; 10 μm particle diameter; Bondclone C-18) eluted with acetonitrile-water (1:1 v/v) at 1.5 ml min^{-1}. The eluate was monitored for absorbance at 240 nm (Waters 486

detector) and for radioactivity (Bioscan detector; EG and G Instruments). The data were acquired and analyzed with a Turbochrom™ 3 system (Perkin Elmer Ltd). The retention time for FP was *ca.* 9 min. The HPLC system was calibrated by injection of standard solutions (100 µl) of FP in methanol–water (3:2 v/v) over the concentration range 25–3000 ng ml^{-1} (0.05–6.25 nmol ml^{-1}). Linear curves ($r^2 = 0.999$) were fitted to the peak area data for FP and used for subsequent quantification of the amount of FP in analytes. Other unidentified UV absorbing peaks in the chromatogram were integrated and summed by assuming that they gave the same response (extinction coefficient) as FP in the analysis. The specific radioactivities of [^{18}F]FP samples were calculated from the measurements of mass of FP and the radioactivity of associated [^{18}F]FP, and then decay corrected to a set time.

Preparation of pMDIs containing fluticasone propionate labeled with [^{18}F]FP

The collected HPLC fraction containing [^{18}F]FP was diluted with water to a volume of *ca.* 20 ml and passed through a C_{18} Sep-Pak that had been pre-treated with acetonitrile–water (1:1 v/v; 10 ml). [^{18}F]FP (mean 245 MBq: $<$ 26 µg carrier FP at EOS) was eluted from the Sep-Pak with methanol (2 ml) through a Minisart filter (0.2 µm) into an opened empty pMDI can. The pMDI can containing the [^{18}F]FP was placed into a block heater (at 50°C) and the solvent was evaporated off under a stream of nitrogen to leave a dry residue of [^{18}F]FP. Methanol (*ca.* 0.5 ml) was added to the residue and the solvent was again evaporated. The open can and a standard Flixotide™ (containing FP) pMDI (250 µg per actuation) were chilled by dry ice (−78°C). The pMDI was then quickly clamped and the valve body removed with a tube cutter. Previously, aliquots of prepared Flixotide™ suspension were chilled and poured into a number of weighed aluminium canisters plus valves and the amount of Flixotide™ in each can was determined by re-weighing. For a clinical PET experiment, a chilled can containing a proportion of this FP suspension (*ca.* 2.0 g, containing *ca.* 5.8 mg of FP) was poured into the open can containing the [^{18}F]FP. A hand crimper was promptly used to seal a weighed Spraymizer valve onto this can. The radioactive pMDI was then immersed in a water bath at room temperature and sonicated for a set time. The assembled pMDI was weighed so that the amount of transferred FP could be calculated. Finally, the radioactive pMDI was placed in an actuator and primed for use with four actuations. The amount of radioactivity associated with FP particles was calculated from measurement of the total loss of radioactivity from the pMDI can and actuator, on venting a proportion of the pMDI contents into the Andersen cascade impactor.

Assembled pMDIs were sent for assessment of internal sterility (total viable counts) to International Laboratory Services Ltd.

Determination of the particle size distribution of FP and [^{18}F]FP pMDIs

The particle size distribution and dose uniformity in aerosol discharges of FP in an actuated dose from pMDIs were measured using an 8-stage Andersen cascade impactor (Table 4),[26,28] For each analysis of an [^{18}F]FP pMDI and a Flixotide^{TM} pMDI, the operator discharged the primed pMDI four times into the Andersen cascade impactor. The impactor was dismantled and the material was collected at each deposition stage by irrigating with methanol into a volumetric flask containing water (to achieve a final ratio of methanol–water of 3:2 v/v). The FP content of each volumetric flask was determined by calibrated reversed-phase HPLC as described above.

The total [^{18}F]FP deposited on each stage was determined from a measurement of radioactivity in an aliquot taken from the appropriate volumetric flask using a calibrated well counter. The particle size distribution of [^{18}F]FP was then calculated according to standard methods (European Pharmacopoeia[27]) from these measurements and compared with the distribution of the non-radioactive FP. Particle size distributions were also determined for standard commercial Flixotide^{TM} FP pMDIs.

The particle size distributions were compared using assessments of a number of standard parameters: mass median aerodynamic diameter (MMAD), the geometric standard deviation (GSD) of the MMAD, throat deposition, ex-actuator content or measure of content uniformity (amount of FP), weight per actuation (total material discharged per actuation) and fine particle mass (FPM; FP on stages 3–5) and percentage of FP deposited on the throat (Table 4).

The specific radioactivity (ratio of radioactivity to amount of drug) of particles in the different size ranges were compared following normalization of specific radioactivity values for individual stages to the specific radioactivity of the total material deposited (radioactive and non-radioactive) in the cascade for each [^{18}F]FP pMDI. This removed the effect of the varying absolute radioactivity of each [^{18}F]FP pMDI and allowed the important relative differences in specific radioactivity for each size range to be compared for different preparations and combined for 11 runs.

Procedure for administration of [^{18}F]FP from a pMDI to human subjects

Following the confirmation and determination of an acceptable radioactivity particle size distribution of the [^{18}F]FP pMDIs by cascade analysis as described above, subjects were asked to inhale from the [^{18}F]FP pMDI.[30] The number of actuations required varied between 4 and 8. This depended on the level of radioactivity per actuation. The total radioactivity administered to

the subject did not exceed 74 MBq. The radioactivity to be delivered by the [^{18}F]FP pMDI during the clinical dosing studies was predicted from the measurement of the total loss of radioactivity from the [^{18}F]FP pMDI following the 4 test actuations made into the cascade. This gave the expected amount of radioactivity *delivered to the subject* per actuation by the metered-dose inhaler (i.e. ex-actuator) rather than the total amount of radioactivity discharged from the canister.

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This paper is dedicated to Keith G. Poole, who died unexpectedly. He will be missed by all his friends and colleagues.

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