III. 11 Preparation of 2-Deoxy-2-[\(^{18}\)F]Fluoro-D-Glucose Powder Inhalation for Diagnosis of Lung Diseases

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It has been recognized that there are some differences in conditions of mucus on inner surface of air tract and clearance of mucociliary transport between normals and patients who have chronic bronchitis, bronchial asthma and other lung diseases.\(^1\)–\(^3\) Therefore, quantitative measurement of deposition, clearance of the air tract and absorption from lung after inhalation of drug is suggested to be useful for diagnosis of lung diseases. Nonsurgical measurements of mucociliary clearance with inhalation of \(^{99}\)mTc particles micronized by nebulizer have been reported.\(^4\),\(^5\) Since the images of it are plane despite of lung being three-dimensional, it cannot show the details in area including main bronchi and alveoli. Positron emission tomography (PET) can analyze the deep area in whole body exactly and quantitatively, and distinguish trachea, bronchi and alveoli. Clearance in air tract and absorption can be analyzed by periodical measurement. \(^{18}\)FDG is frequently used for clinical study. As \(^{18}\)FDG is soluble in water, it is absorbed from the alveoli into the blood. Therefore by analyzing in vivo dynamics of \(^{18}\)FDG it is assumed to be able to diagnose some lung diseases. And it is presumed that analysis of deposition of \(^{18}\)FDG particles inhaled as powder aerosol on the inner surface of respiratory tract will reveal the conditions of mucus. Since particle size is well controlled in powder rather than nebulizer, powder inhalation aerosol is adequate for diagnosis. So we investigated the preparation of \(^{18}\)FDG fine powder suitable for inhalation.

Materials and Methods

Materials

\(^{18}\)FDG was synthesized with the automated synthesis system.\(^6\) Sodium-N-acetylneuraminic (Neu5Ac-\(\text{Na}\)) reported nontoxic in inhalation\(^7\) was obtained from Mect corporation (Tokyo). Lactose was purchased from De Melkindustrie Veghel (Holland) and used after screening with 105 \(\mu\)m mesh.

Preparation of \(^{18}\)FDG fine particles

To prepare the fine particles \(^{18}\)FDG and small amount of sugars were dissolved in methylalcohol and diethylether was dropped into this solution with ultrasonication to crystalize sugars simultaneously. Methylalcohol in
the crystals was eliminated by washing twice with diethylether. The crystals was mixed with lactose and dried in vacuo (60°, 10 min). Approximately 40 mg of powder was filled into hard gelatin capsules for inhalation experiment.

Dispersion analysis of 18FDG powder

Multistage liquid impinger8,9 as shown in Fig. 1 was used for assessment of 18FDG powder inhalation dispersion at 60 l/min airflow for 3 sec. Effective cut-off diameters for 50 % collection efficiency (ECD50) of each stage of our apparatus were shown in Table 1.

Preclinical positron emission tomography (PET) study

An adult dog (Beagle, 16 kg) was anesthetized with pentobarbital (25 mg/kg) and 18FDG powder was inhaled by force using an air bag through intratracheal tube. Just after forced inhalation, PET (ECAT II, EG and G, Ortec) scannings were repeated and venous blood was taken periodically.

Clinical study

Two tomographic planes chosen by X ray CT were taken for a 46-year-old male normal volunteer after inhalation of 18FDG powder. One of them was an area including trachea and the other was primary bronchi area at mid-heart level. PET scan was repeated twice at an hour interval and venous blood was taken twice at intervals of 15 min and five times at intervals of 30 min for 3 hours.

Results

Crystals of 18FDG could not be obtained by dropping diethylether into methylalcohol solution. Crystallization with another sugars was studied. Glucose and Neu5Ac-Na were used in this study. Smaller particles were obtained when Neu5Ac-Na was used as seed crystal rather than glucose. Therefore, Neu5Ac-Na was chosen for experiment. Twenty mg of Neu5Ac-Na and 18FDG were dissolved in 2 ml of methylalcohol and diethylether was dropped into this solution at the rate of 10 ml/min under ultrasonication. More than 90 % of crystals were smaller than 10 μm of diameter but radioactivity incorporation yield was not enough for our inhalation experiment. Then a relationship between radioactivity incorporation ratio and methylalcohol volume in crystallization was investigated. The results are represented in Table 2. When the volume of methylalcohol was decreased to 0.1 ml incorporation yield of radioactivity increased over 60 % and it was sufficient for experiment. But Neu5Ac-Na was not completely dissolved in the volume of methylalcohol. Since the crystals grew with depositing 18FDG on their surface, the crystal size was affected by a diameter of starting Neu5Ac-Na particles. Neu5Ac-Na having median diameter of 4-5 μm was used as seed. Figure 2 shows the results of 3 experiments. Eighty % of crystals were of a
particle size smaller than 10 µm. A dispersion test of $^{18}$FDG powder prepared using micronized Neu5Ac-Na was carried out with the multi stage liquid impinger and the results are represented in Table 3. Only 30% radioactivity dispersed to stage 2, 3 and 4 which were assumed as trachea and bronchi from the ECD50 in Table 1. Crystals which were smaller than 10 µm was 80%, however, for adhesion of powder to inhaler and aggregation of powder dispersion ratio decreased to 30%. Examination with fine particles (median diameter, 4−5 µm) of lactose instead of Neu5Ac-Na was carried out and powder dispersable in multistage liquid impinger test was not obtained. Consequently micronized Neu5Ac-Na was chosen as seed crystals. Since there was a possibility that an inorganic salt (NaCl) was contaminated from ion exchange resin$^{11}$ in the synthesized $^{18}$FDG, extraction of $^{18}$FDG with ethylalcohol and diethylether was performed before crystallization. The established preparation procedure of $^{18}$FDG powder inhalation is represented in Fig. 3.

Preclinical study was carried out in the dog with forced inhalation of $^{18}$FDG powder. Just after administration of powder (0.47 mCi) deposition of radioactivity in the trachea was recognized. Radioactivity in the trachea increased once and then decreased periodically (Fig. 4). Absorption of $^{18}$FDG was observed and incorporation in the heart was measured.

In the male normal volunteer, after inhalation of 0.40 mCi of $^{18}$FDG powder it was recognized that $^{18}$FDG was delivered to trachea, bronchiole and peripheral area containing terminal bronchiole and alveoli (Table 4, Fig. 5 and 6). Blood level and elimination of radioactivity in two planes could be measured (Fig. 7).

Discussion

Several measurement methods of mucosiliary clearance have been reported.$^{2-4,12,13}$ Analytical method utilizing $^{99m}$Tc has a merit that it is nonsurgical and patients feel no pain. But it has a demerit that details of the lung cannot be distinguished. And diethylenetriamine penta-acetate or other high molecular weight materials are required for $^{99m}$Tc technique. To improve it we developed $^{18}$FDG powder inhalation aerosol for PET study. Since in nebulizer particles grow larger under the circumstances of high humidity like respiratory tract$^{14}$, powder inhalation aerosol is adequate for diagnosis. In order to deliver the powder to near alveoli particle size must be smaller than 6 µm.$^{15}$

Crystallization of $^{18}$FDG with addition of diethylether to the methylalcohol solution which dissolved $^{18}$FDG and other sugars was studied. Glucose, Neu5Ac-Na and lactose were examined as sugars in this experiment. When micronized Neu5Ac-Na and 0.1 ml of methylalcohol were used we could obtain the crystals which were assumed to be delivered to the lung from the results of the multistage liquid impinger dispersion test.
In experiment of forced inhalation of $^{18}$FDG powder to the dog, radioactivity distributed in trachea mainly and was increased once and then followed by a gradual decrease. Increase was occurred by mucosiliary transport of inhaled $^{18}$FDG from a deep area of the lung to the measurement site. Since $^{18}$FDG was transferred to an upper site of throat by mucosiliary clearance, a decrease in radioactivity occurred consequently. $^{18}$FDG powder inhalation aerosol enabled to measure an ability of the mucosiliary clearance in the air tract. In the inhalation test of the normal volunteer deposition in the trachea and the primary bronchi was measured mainly. Since the radioactivity was recognized in peripheral area of the lung, $^{18}$FDG powder was delivered to the alveoli or respiratory bronchioles. And an alteration of the radioactivity could be measured.

Further investigations must be required about the $^{18}$FDG powder inhalation aerosol. The $^{18}$FDG powder inhalation will be used for diagnosis of the lung diseases, and then reveal the conditions of mucus in deposition sites of air tract since $^{18}$FDG is soluble in water.

References

### Table 1. Effective cut-off diameter for 50% collection efficiency (ECD50) of multistage liquid impinger.

<table>
<thead>
<tr>
<th>Location</th>
<th>Throat</th>
<th>Stage 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Filter</th>
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</thead>
<tbody>
<tr>
<td>ECD50(µm)</td>
<td>27.7</td>
<td>21.2</td>
<td>7.1</td>
<td>5.2</td>
<td>3.3</td>
<td>1.9</td>
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### Table 2. Relationship of radioactivity incorporation yield and methylalcohol volume.

<table>
<thead>
<tr>
<th>Methylalcohol(ml)</th>
<th>2.0</th>
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<th>0.1</th>
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<tr>
<td><strong>Yield (%)</strong></td>
<td>30.7</td>
<td>38.6</td>
<td>45.0</td>
<td>55.1</td>
<td>62.6</td>
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### Table 3. Dispersion test of $^{18}$FDG powder inhalation.

<table>
<thead>
<tr>
<th>Location</th>
<th>Throat</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>Filter</th>
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<tbody>
<tr>
<td>Rate(%)</td>
<td>12.1</td>
<td>34.5</td>
<td>13.1</td>
<td>11.1</td>
<td>8.5</td>
<td>1.3</td>
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### Table 4. Dispersion test of $^{18}$FDG powder inhalation in normal volunteer.

<table>
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<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Rate(%)</td>
<td>7.9</td>
<td>37.7</td>
<td>13.2</td>
<td>10.7</td>
<td>9.4</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Fig. 1. Diagram showing multistage liquid impinger.

Fig. 2. Particle size distribution of crystals of $^{18}_{\text{FDG}}$ and Neu5Ac-Na.
*FDG Solution
↓ Evaporation
↓ - EtOH, 1.5ml, EtO, 0.7ml, US
↓ 3000rpm×5min
Supernatant
↓ Evaporation
↓ - MeOH, 0.1ml, US
↓ - Neu5Ac-Na, 20mg, US
↓ - EtO, 0.10ml, US
↓ 2500rpm×5min
Precipitation
↓ - EtO, 0.4ml, US
↓ 2500rpm×5min, US
Precipitation
↓ - EtO, 0.4ml, US
↓ 2500rpm×5min, US
Precipitation
↓ - EtO, 0.4ml, US
↓ - Lactose, 180mg, US
↓ Evaporation
↓ Dry with Vacuum, 60°, 10min
Capsule

Fig. 3. Preparation procedure of \(^{18}\)FDG powder inhalation. Abbreviations: EtOH = ethylalcohol; MeOH = methyl-alcohol; Et\(_2\)O = diethylether; Neu5Ac-Na = sodium N-acetylneuraminic acid; US = ultrasonication.

Fig. 4. Elimination and uptake of \(^{18}\)FDG in trachea, lung, myocardium and blood.
Fig. 5. Positron emission tomography of lung including the trachea (left) and mid-heart level (right).

Fig. 6. Rectilinea scan of the lung after inhalation of 18FDG powder aerosol.
Fig. 7. Alteration of $^{18}$FDG radioactivity in lung and blood level after inhalation in human normal volunteer.