

## Development of a new method for simultaneously evaluating mucociliary clearance and pulmonary epithelial permeability in rabbit experiments by means of $^{18}\text{F}$ FDG, three-dimensional positron emission tomography and rectilinear scan

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We tried to simultaneously obtain the elimination constant of mucociliary clearance and the pulmonary epithelial permeability constant after inhalation of 2- $^{18}\text{F}$ fluoro-2-deoxy-D-glucose ( $^{18}\text{F}$ FDG) solution by carrying out whole lung positron emission tomography and a rectilinear scan in rabbit experiments. The elimination constant of pulmonary epithelial permeability was obtained from the decrease in the amount of the radioactivity with time in the region of interest (ROI) confined to the lungs, trachea and tracheal cannula in the rectilinear scan. The total elimination constant of the radioactivity in the lungs was obtained from the ROI confined to the lungs in the tomography. The mucociliary clearance rate constant in the lungs was then obtained after subtracting the elimination constant of the pulmonary epithelial permeability from the total elimination constant of the  $^{18}\text{F}$ FDG in the lungs. The mucociliary clearance constant in the trachea was calculated from the residual radioactivity in the trachea and the mucociliary clearance constant in the lungs. The mean pulmonary epithelial permeability constant was  $0.0020\% \text{ min}^{-1}$  obtained from the rectilinear scan. The mean mucociliary clearance constants of the lungs and the trachea were  $0.0006$  and  $0.025\% \text{ min}^{-1}$ , respectively. These results indicated that the pulmonary epithelial permeability and mucociliary clearance could be evaluated simultaneously with  $^{18}\text{F}$ FDG by using three-dimensional positron emission tomography and a rectilinear scan.

**Key words:**  $^{18}\text{F}$ FDG, mucociliary clearance, PET, pulmonary epithelial permeability, rabbit

### INTRODUCTION

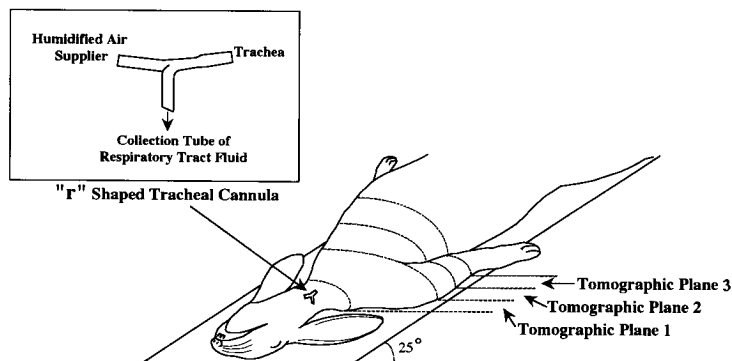
VENTILATION, pulmonary epithelial permeability, mucociliary clearance and other lung functions can be assessed in nuclear medicine.<sup>1</sup> The clearance of inhaled materials from the lungs is caused by mucociliary transport and

permeation through the alveolar epithelial surface of the capillary into the pulmonary capillary. Large molecular weight materials cannot pass through the epithelial junction pores and are transferred to the upper bronchial airways by the mucociliary clearance, although small molecular weight materials can pass through the epithelial membrane into the capillary blood stream and are also transported by the mucociliary clearance.<sup>2</sup> In nuclear medicine, both lung functions were examined separately in humans<sup>3</sup> or individually in animal studies,<sup>4,5</sup> because it is hard to distinguish exactly the contribution ratio of pulmonary permeation from the total lung clearance when

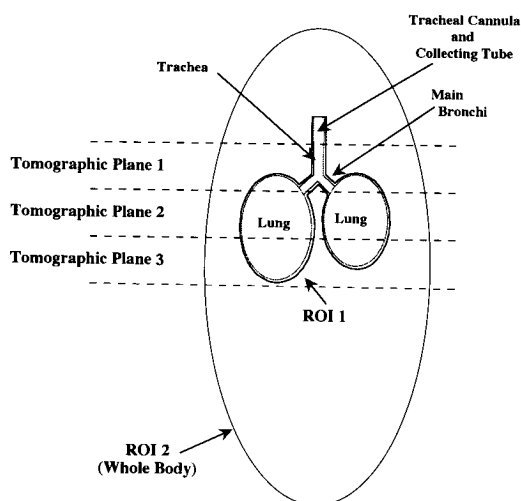
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**Fig. 1** Schema of tomographic scan and "r" shaped tracheal cannula. The rabbit was restrained in a supine position with its head downwards on a 25°-inclined board to avoid the respiratory tract fluids being retained in the airway. The trachea, main bronchi and lungs were completely included in 3 consecutive tomographic scans.



**Fig. 2** Region of interest (ROI) 1 set in the rectilinear scan. The radioactivity transported by mucociliary clearance remained in the ROI 1. The elimination of the radioactivity from ROI 1 indicated the clearance by permeation through the pulmonary epithelial surface.

using small molecular weight materials alone, such as  $^{99m}\text{Tc}$ -diethylenetriaminepentaacetic acid (DTPA).<sup>3</sup> We attempted to simultaneously evaluate the mucociliary clearance and pulmonary epithelial permeability after inhalation of 2-[ $^{18}\text{F}$ ]fluoro-2-deoxy-D-glucose ( $^{18}\text{FDG}$ ) solution by using three-dimensional positron emission tomography and rectilinear scan in a rabbit experiment.

## MATERIALS AND METHODS

### Animal

Male normal New Zealand white rabbits (Funabashi Farm) weighing about 2.5 kg were used in these experiments. After anesthetizing with urethane (1.1 g/kg), the rabbit was fixed on its back and an "r" shaped tracheal cannula (Fig. 1) was inserted into the trachea. One of its three openings was connected to an air outlet of a humidifier to supply air of approximately 100% humidity maintained at  $39 \pm 1^\circ\text{C}$  to provide air the same as that passing through the nasal cavity<sup>6-8</sup> (Fig. 1). The rabbit was then restrained

in a supine position with its head downwards on a 25°-inclined board to avoid respiratory tract fluids being retained in the airway<sup>6-8</sup> (Fig. 1). A test tube was connected to the last opening of the "r" shaped tracheal cannula to collect the respiratory tract fluid including the  $^{18}\text{FDG}$  and that transported by the mucociliary clearance.

$^{18}\text{FDG}$  was automatically synthesized and dissolved in normal saline (74 MBq/mL) before inhalation. After a transmission scan, measured with an external ring source of  $^{68}\text{Ge}$  to correct for body mass, the  $^{18}\text{FDG}$  solution was inhaled via the tracheal cannula for 5 min with an ultrasonic nebulizer (NEU-06, Omron, mass median aerodynamic diameter:  $4.6 \mu\text{m}$ ) in normal respiration. Just after inhalation, the remaining  $^{18}\text{FDG}$  solution deposited inside the tracheal cannula was wiped away, and the rabbit was again placed on the bed for positron emission tomography (PT931/04, CTI, Knoxville, TN) on the 25°-inclined board (Fig. 1).

### PET scan

Three serial tomographic scans of the neck and thorax including the trachea and lungs were performed every 30 min for 120 min, and a rectilinear scan of the whole body was performed after each tomographic scan. The venous blood was sampled to measure the blood radioactivity with a crosscalibrated well counter at 0, 5, 10, 20, 30, 45, 60, 90 and 120 min. The venous blood radioactivity was determined after normalizing with the body weight and the inhalation radioactivity.

Venous blood radioactivity (cps/mL/MBq  $\times$  kg)

$$= \frac{\text{Time corrected blood radioactivity (cps/mL)}}{\text{Inhalation radioactivity (MBq)}} \times \text{Body weight (kg)}$$

### Analyzing the pulmonary epithelial permeability

In the rectilinear scan, the region of interest (ROI) 1 was confined to the area of the collection tube for respiratory tract fluid, the tracheal cannula, the trachea and lungs, and ROI 2 involved the whole body (Fig. 2). The  $^{18}\text{FDG}$  deposited and transferred by the mucociliary clearance remained in ROI 1 in the rectilinear scan. Therefore, the  $^{18}\text{FDG}$  eliminated from ROI 1 in the rectilinear scan means that the radioactivity was transferred to the circu-

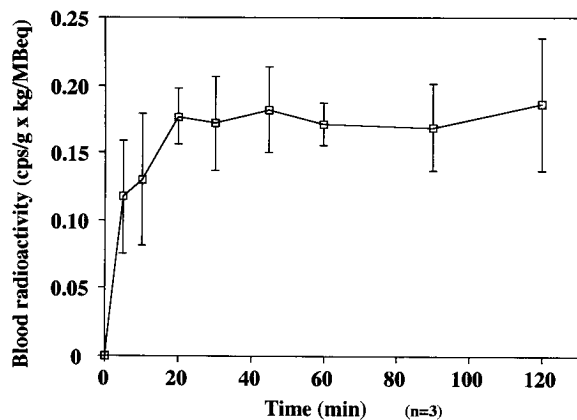


Fig. 3 Changes in the decay corrected whole blood radioactivity normalized with inhalation volume and body weight.

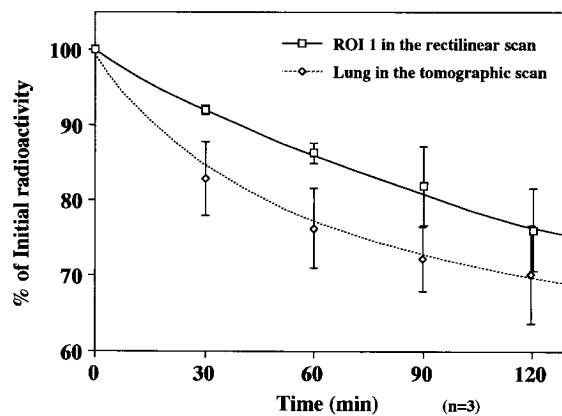


Fig. 4 Decline in the amount of the radioactivity in the ROI 1 in the rectilinear scan and the lungs in the tomographic scan.

Table 1 Elimination rate constant calculated with the declining rate of radioactivity in the tomographic scan and rectilinear scan after inhalation of  $^{18}\text{F}$ FDG in the normal rabbits

Rabbit No.	$K_{p\text{Lung}}$	( $r^2$ )	$K_{e\text{Lung}}$	( $r^2$ )	$K_{m\text{Lung}}$	$K_{m\text{Trach}}$
1	0.0019	(0.992)	0.0028	(0.855)	0.0009	0.029
2	0.0019	(0.992)	0.0024	(0.893)	0.0005	0.027
3	0.0022	(0.953)	0.0025	(0.959)	0.0003	0.018
MEAN (SD)	0.0020 (0.0002)		0.0026 (0.0002)		0.0006 (0.0003)	0.025 (0.006)

$K_{p\text{Lung}}$ : Pulmonary epithelial permeability constant,  $K_{e\text{Lung}}$ : Total elimination rate constant of the lung,  $K_{m\text{Lung}}$ : Mucociliary clearance rate constant of the lung,  $K_{m\text{Trach}}$ : Mucociliary clearance rate constant of the trachea,  $r^2$ : Square of coefficient of correlation

lation. The elimination constant obtained from ROI 1 of the rectilinear scan represents the pulmonary epithelial permeability ( $K_{p\text{Lung}}$ ).

#### Evaluation of the mucociliary clearance rate of the lung and trachea

The PT-931/04 with four detector rings (512 BGO detector/ring) provides seven 47 mm thickness tomographic scans acquired simultaneously, so that a part of the trachea and all parts of the lungs of the rabbit could be imaged with 3 tomographic scannings. The total elimination rate constant of the lungs ( $K_{e\text{Lung}}$ ) was obtained from the decline in the amount of radioactivity in the ROI confined to the lungs in the tomographic scans. The  $K_{e\text{Lung}}$  was the total elimination constant for mucociliary clearance and permeation of the lungs. The mucociliary clearance rate constant for the lungs ( $K_{m\text{Lung}}$ ) was obtained by subtracting  $K_{p\text{Lung}}$  from  $K_{e\text{Lung}}$  ( $K_{m\text{Lung}} = K_{e\text{Lung}} - K_{p\text{Lung}}$ ). The tracheal mucociliary clearance constant ( $K_{m\text{Trach}}$ ) was calculated with the residual radioactivity in the trachea obtained from the tomographic scans and the amount of the mucociliary clearance from the lung that was calculated with the  $K_{m\text{Lung}}$ .

## RESULTS

### Blood radioactivity

The changes in the blood radioactivities with time are shown in Fig. 3. The blood radioactivity reached a plateau within 20 min and the same level continued to 120 min.

### Pulmonary epithelial permeability constant ( $K_{p\text{Lung}}$ ) and the mucociliary clearance rate constant of the lungs ( $K_{m\text{Lung}}$ ) and the trachea ( $K_{m\text{Trach}}$ )

The decline in the amount of radioactivity in the ROI 1 in the rectilinear scan is shown in Fig. 4. The mean  $K_{p\text{Lung}}$  was 0.0020%  $\text{min}^{-1}$  and the squares of the coefficients of correlation were 0.953–0.992 (Table 1). The decline in the amount of  $^{18}\text{F}$ FDG radioactivity in the lungs obtained from the ROI confined to the lungs in the tomographic scan is shown in Fig. 4. The mean  $K_{e\text{Lung}}$  was 0.0026%  $\text{min}^{-1}$  and the squares of the coefficient of correlation were 0.855–0.959 (Table 1). The mean  $K_{m\text{Lung}}$  which was obtained from  $K_{e\text{Lung}}$  and  $K_{p\text{Lung}}$  was 0.0006%  $\text{min}^{-1}$  (Table 1). The mean  $K_{m\text{Trach}}$  which was obtained from residual counts in the trachea and  $K_{m\text{Lung}}$  was 0.025%  $\text{min}^{-1}$  (Table 1).

## DISCUSSION

In animal experiments, the mucociliary clearance of the lungs and the expectorant activity of drugs have been investigated with radioactive tracers binding large molecular weight materials,<sup>9,10</sup> carbon particles,<sup>11</sup> latex particles,<sup>12</sup> cork particles<sup>13</sup> or measurement of ciliary beat frequency.<sup>14</sup> Because small molecular weight materials are eliminated by the mucociliary clearance and permeation through the alveolar epithelial surface, it is difficult to determine the exact percentage of mucociliary clearance in total elimination in the lungs. In human studies, the lung permeation and the mucociliary clearance activity could be evaluated on separate days in the same subjects with <sup>99m</sup>Tc-human albumin or <sup>99m</sup>Tc-DTPA, respectively.<sup>3</sup> But because a surgical operation on the trachea was necessary for tracheal cannulation, it is difficult to evaluate both lung functions in the same animal on separate days.<sup>10-14</sup>

Because the PT931/04 can obtain 47 mm thickness tomographic scans in 7 slices during one scanning procedure, three-dimensional analysis can be performed with it. All of the lungs and a part of the trachea where the tracheal cannula was not inserted were able to be measured in three PT931/04 scannings in the rabbit experiment. The total elimination ratio of the radioactivity in the lung and the residual radioactivity in the trachea could be obtained separately. On the other hand, in ROI 1 including the collecting tube for the respiratory tract fluid, the tracheal cannula, trachea and lungs in the rectilinear scan, the radioactivity initially deposited there and/or transported by mucociliary clearance could not leave the ROI 1. The alveolar macrophage plays an important role in scavenging the inhaled materials from the lungs,<sup>15</sup> but because <sup>18</sup>FDG is an analog of glucose and a substrate of hexokinase, the <sup>18</sup>FDG entrapped in the myocardia or other cells remains there for more than 2 hours.<sup>16-18</sup> The <sup>18</sup>FDG incorporated in the alveolar macrophage might be restrained from elimination from ROI 1. Coughing also plays an important role in the clearance of inhaled materials. Because the rabbits used in these experiments did not cough during the PET scannings, elimination from ROI 1 would occur only by permeation through the pulmonary epithelial membranes to the systemic circulation. The  $K_{mLung}$  could be obtained after subtraction of the  $K_{pLung}$  from  $K_{eLung}$  measured with tomographic scans.  $K_{mTrach}$  could be calculated from the residual radioactivities of the trachea and lung, and the  $K_{mLung}$  was obtained as previously described.

The tracheobronchial mucociliary clearance rate of the rabbits was measured by a method of counting residual fluorescent latex particles previously inhaled<sup>12</sup> or the rate of transportation of carbon particles on the tracheal mucous membrane.<sup>11</sup> Few studies have been reported on the mucociliary clearance rate of rabbits by means of a radionuclide. Schlesinger et al. examined the effects of

ambient pollution on the mucociliary clearance of rabbits after inhalation of <sup>99m</sup>Tc-tagged ferric oxide; but they reported the mean residence time of the inhaled tracer in the irritant-exposed rabbit versus the normal baseline value.<sup>19-21</sup> Saano et al. reported that the tracheobronchial mucociliary clearance was 6.82% h<sup>-1</sup> with a <sup>99m</sup>Tc-labeled homologous blood cell inhalation technique in the rabbits.<sup>10</sup> Therefore, there were no comparative data on the mucociliary clearance constant of the rabbits obtained in this study.

The pulmonary permeability was affected by the indicator's molecular weight, lipid solubility and other factors due to the subjects.<sup>22,23</sup> Few experiments have been reported on pulmonary permeability when using rabbits and sugars such as <sup>18</sup>FDG, but when cyanocobalamin was used as a tracer, the lung permeation constant was reported to be 0.0013 min<sup>-1</sup> in rabbit experiments.<sup>22</sup> It was considered that the  $K_{pLung}$  obtained in this experiment would not be discrepant to it.

The radioactivity in the blood was kept at a constant level from 20 min to 120 min after <sup>18</sup>FDG inhalation. No absorption could have occurred in the digestive tract because <sup>18</sup>FDG was only administered via the tracheal cannula and all of the <sup>18</sup>FDG transported in the respiratory tract fluid was recovered in the collection tube placed with the tracheal cannula. Therefore, the radioactivity observed in the blood was due to the penetration from the lung through the alveolar epithelium. Absorption of a drug from the respiratory tract was examined for the administration system,<sup>24,25</sup> because a substance absorbed from the lung would be transported to the circulation and not be inactivated by the first-pass effect in the liver on oral administration. Although the inhalation maneuvers of a metered-dose inhaler were very variable within individual subjects<sup>26,27</sup> and the absorption of deposited drugs in the oral cavity and oropharynx would make the blood level complicated, inhalation might be considered as a unique administration route, because the blood radioactivity was kept at a constant level to 120 min.

The experimental technique used in this study was originally described by Perry and Boyd<sup>6</sup> and developed by Miyata et al.<sup>7,8,13</sup> for evaluating the mucociliary transport activity and the expectorant activity of drugs. The collection of respiratory tract fluids including inhaled <sup>18</sup>FDG into the collecting tube attached to the tracheal cannula during the experiment was important for evaluating pulmonary epithelial permeability. All of the <sup>18</sup>FDG transported by the mucociliary clearance remained in the tracheal cannula and the collection tube. Only the  $K_{pLung}$  could be consequently estimated with the declining rate of the radioactivity in ROI 1 in the rectilinear scan. The total decline in the amount of radioactivity in the lung was obtained from the tomographic scans with PT-931. Therefore, the  $K_{pLung}$ ,  $K_{eLung}$  and  $K_{mLung}$  could be obtained simultaneously during one experimental procedure in the rabbit with <sup>18</sup>FDG, three-dimensional positron emission

tomography and the rectilinear scan. Nevertheless, further evaluations of mucociliary clearance and pulmonary epithelial permeability in a diseased animal model and/or the efficacy of a drug as an expectorant or anti-inflammatory drug and comparison with those of normal rabbits would be necessary to ascertain the utility of this technique.

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