

# Technetium-99m-HMPAO as a Marker of Chemical and Irradiation Lung Injury: Experimental and Clinical Investigations

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We evaluated the ability of  $^{99m}\text{Tc}$ -hexamethylpropyleneamine oxime ( $^{99m}\text{Tc}$ -HMPAO) to serve as a sensitive marker of lung injury. **Methods:** Two experimental rabbit models of minimal lung injury were designed using injections of a low dose (0.05 ml/kg) of oleic acid or 50 Gy of irradiation. In addition, we clinically investigated whether patients who received chemotherapy ( $n = 14$ ) or radiotherapy ( $n = 13$ ) for lung cancer showed high uptake of  $^{99m}\text{Tc}$ -HMPAO in the lungs. **Results:** Despite the minimal endothelial lesions visualized by electron microscopy (edematous changes and blebbing), in both animal models, the lungs showed high uptake of  $^{99m}\text{Tc}$ -HMPAO, which occurred rapidly within 1 min after injection. Clinically, the mean lung-to-liver ratio of  $^{99m}\text{Tc}$ -HMPAO activity in the patients who received chemotherapy ( $0.649 \pm 0.185$ ,  $p < 0.01$ ) was significantly higher than that of the controls ( $n = 16$ ;  $0.387 \pm 0.108$ ), and all 12 patients who received more than 30 Gy of irradiation showed abnormal uptake in the irradiated lungs, despite the lack of abnormal opacities on chest CT. **Conclusion:** These findings suggest that  $^{99m}\text{Tc}$ -HMPAO has the potential to be a sensitive marker of chemical and irradiation lung injury.

**Key Words:** technetium-99m-HMPAO; lung; oleic acid; irradiation; endothelium

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The pulmonary microvascular endothelium manifests structural and functional alterations following exposure to various toxic agents and irradiation. This endothelial damage is considered to be an early event in the development of severe lung injury (1-7). However, this injury is often difficult to detect due to the lack of a readily available marker. The availability of such a marker may aid in preventing subsequent lung injury.

In the past two decades, numerous studies established that the endothelium of many species actively takes up and metabolizes circulating biogenic amines (8-11). Technetium-99m-hexamethylpropyleneamine oxime ( $^{99m}\text{Tc}$ -

HMPAO), which has a molecular mass of 380 daltons, is a lipophilic, basic, cyclic amine as are *N*-isopropyl- $p$ - $^{123}\text{I}$ -iodoamphetamine ( $^{123}\text{I}$ -IMP) and  $^{123}\text{I}$ -*N,N,N'*, trimethyl-*N*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propanediamine (I-123-HIPDM) (12-14). In the lung,  $^{99m}\text{Tc}$ -HMPAO presumably is localized in the endothelium (12,15). This agent may have potential for showing altered kinetics or extraction due to endothelial damage and therefore may be useful to evaluate endothelial function.

Technetium-99m-HMPAO also has been used as an imaging agent reflecting tumor blood flow (16-19). We applied this agent to thoracic tumors and frequently observed an intense uptake in the lungs of patients who had undergone chemotherapy or radiotherapy, despite the absence of abnormal opacities on chest CT (18). Therefore, we concluded that this agent might be a sensitive detector for early and subclinical pulmonary injuries.

The purpose of the present two animal studies was to investigate the potential of  $^{99m}\text{Tc}$ -HMPAO as a sensitive marker for minimal lung injuries produced by a low dose of oleic acid and irradiation. In the past, oleic acid was used extensively in an animal model of pulmonary fat embolism and diffuse alveolar damage, and its initial effects on the endothelium have been demonstrated pathologically (20-24). The initial effects of irradiation on the endothelium also have been well documented in many pathologic studies (2-4, 25-29).

Although a portion of our  $^{99m}\text{Tc}$ -HMPAO study in irradiated animals was published earlier (30), in this report we expand the study by using more animals. In addition, to evaluate clinical application of this agent, we performed  $^{99m}\text{Tc}$ -HMPAO scintigraphy in patients with lung cancer who had received various cytotoxic anticancer drugs or irradiation.

## MATERIALS AND METHODS

### Animal Studies

**Anesthesia.** Japanese white rabbits weighing 2.3 to 3.5 kg were anesthetized with 50 mg/kg of sodium pentobarbital (Nembutal) administered intravenously via the ear vein.

**Oleic Acid Study.** Oleic acid was intravenously administered to 20 anesthetized rabbits. Eight rabbits received 0.05 ml/kg; six

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rabbits, 0.10 ml/kg; and six rabbits, 0.20 ml/kg. Ten other rabbits served as controls. Although 0.10 ml/kg of oleic acid had been used as a standard dose in past experimental animal models (20,23,24), we used a lower dose (0.05 ml/kg) to minimize lung injury. A  $^{99m}\text{Tc}$ -HMPAO scan was obtained 30 min following injection of oleic acid.

**X-ray Irradiation Study.** A total of 38 anesthetized rabbits were irradiated through a right chest field,  $5 \times 2.5$  cm, with a single dose of 50 Gy using 200 keV, 20 mA x-ray, with a half value layer of 1.28-mm copper and 1-mm aluminum filters. The irradiation area included the right lung and right hemimediastinum. A relatively high dose of 50 Gy was given since previous investigators demonstrated difficulty in producing so-called exudative radiation pneumonitis in rabbits compared to other animals such as rats and mice (25,28,29). Between 24 hr and 28 days following irradiation, the  $^{99m}\text{Tc}$ -HMPAO scan was obtained. Twelve rabbits were examined at 24 hr after irradiation; six rabbits, on Day 2; six rabbits, on Day 14; six rabbits, on Day 21; and the remaining eight rabbits were examined on Day 28 following irradiation.

**Technetium-99m-HMPAO Scintigraphy.** Technetium-99m-HMPAO was prepared by adding 5 ml of  $^{99m}\text{TcO}_4^-$  at 222 MBq/ml to a freeze-dried HMPAO and  $\text{Sn}^{II}$  (Ceretek, Amersham, England) used within 5 min of preparation since it becomes unstable after 30 min (13–18). The anesthetized rabbits were fixed symmetrically in the supine position over a collimator and detector. Immediately after the bolus injection of 37 MBq of  $^{99m}\text{Tc}$ -HMPAO via the ear vein, posterior sequential images were collected at 1-sec intervals for 60 sec and subsequent images were obtained at one frame/min over a 30-min period, using a gamma camera (Toshiba, GCA 901-A/W2) fitted with a low-energy, parallel-hole, high-resolution collimator. Pulse-height analysis was performed at 140 keV with a 20% window, and the images were acquired on a  $64 \times 64$  matrix. Simultaneously, a static image was obtained 30 min after the  $^{99m}\text{Tc}$ -HMPAO injection.

Time-activity curves (TACs) were acquired by setting square regions of interest (ROIs) of  $4 \times 4$  pixels in the lower lung fields. In the oleic acid-treated rabbits, the degree of lung uptake was assessed by the ratio of  $^{99m}\text{Tc}$ -HMPAO activity at 60 sec ( $A_{60}$ ) to the initial highest peak activity ( $A_{\text{peak}}$ ) following injection ( $A_{60}/A_{\text{peak}}$  ratio). In the irradiated rabbits, symmetrical ROIs were set in both the irradiated and the contralateral nonirradiated lung, and the degree of  $^{99m}\text{Tc}$ -HMPAO uptake in the irradiated lung was expressed as the mean count-per-voxel of the irradiated lung over that of the contralateral nonirradiated lung. The data were analyzed with a data processor (Toshiba, GMS-550 U).

**Technetium-99m-Macroaggregated Albumin Scintigraphy.** To compare the results of  $^{99m}\text{Tc}$ -HMPAO scans with changes of pulmonary arterial perfusion, pulmonary perfusion scans with 37 MBq of  $^{99m}\text{Tc}$ -macroaggregated human serum albumin ( $^{99m}\text{Tc}$ -MAA), containing  $5 \times 10^4$   $^{99m}\text{Tc}$ -MAA particles 10 to 60  $\mu\text{m}$  in size, were obtained for the six rabbits given 0.05 ml/kg of oleic acid, the six rabbits given 0.10 ml/kg, and the six rabbits given 0.20 ml/kg. Scans were obtained 30 min after the oleic acid was injected. Also, pulmonary perfusion scans were obtained in the 15 irradiated rabbits, all of which had shown uptake of  $^{99m}\text{Tc}$ -HMPAO in the irradiated lung on the  $^{99m}\text{Tc}$ -HMPAO scan obtained 3 days earlier (see Table 2). In these rabbits, the ROIs were set in the same manner as for the  $^{99m}\text{Tc}$ -HMPAO scans, and we judged pulmonary perfusion as being decreased when the mean count-per-voxel of the irradiated lung was more than 20% lower than that of the contralateral nonirradiated lung, and as being unchanged when the difference was within 20%.

**Technetium-99m-Oxygen-4 and  $^{99m}\text{Tc}$ -DTPA Scintigraphy.** Technetium-99m-oxygen-4 scans were performed to exclude non-specific accumulation due to the free  $^{99m}\text{TcO}_4^-$  released from  $^{99m}\text{Tc}$ -HMPAO, and  $^{99m}\text{Tc}$ -DTPA scans were obtained to investigate whether increases in vascular permeability and extravascular space occurred (6,31).

Each scan was obtained 30 min after oleic acid injection in the six rabbits given 0.05 ml/kg, in six rabbits given 0.10 ml/kg, and in six rabbits given 0.20 ml/kg. Each scan was also obtained in six irradiated rabbits at 24 hr after irradiation and in six control rabbits. The rabbits were injected with 37 MBq of  $^{99m}\text{TcO}_4^-$  or  $^{99m}\text{Tc}$ -DTPA intravenously, and the images were obtained in the same manner as the  $^{99m}\text{Tc}$ -HMPAO scans.

**Chest Radiographic and Histologic Studies.** Chest radiographs were obtained for all rabbits immediately after  $^{99m}\text{Tc}$ -HMPAO examination to observe whether there was an abnormal infiltrative opacity.

Light microscopic study was performed on hematoxylin and eosin-stained specimens of the resected lung in the 14 rabbits given oleic acid that showed uptake on the  $^{99m}\text{Tc}$ -HMPAO scans. Six rabbits had been administered 0.05 ml/kg of oleic acid; four rabbits, 0.10 ml/kg; and four rabbits, 0.20 ml/kg. All of these rabbits were killed immediately after chest radiographs were obtained. Also, a light microscopic study was performed on the 14 irradiated rabbits with  $^{99m}\text{Tc}$ -HMPAO uptake (five rabbits at 24 hr, five rabbits at 2 days and four rabbits at 28 days after irradiation).

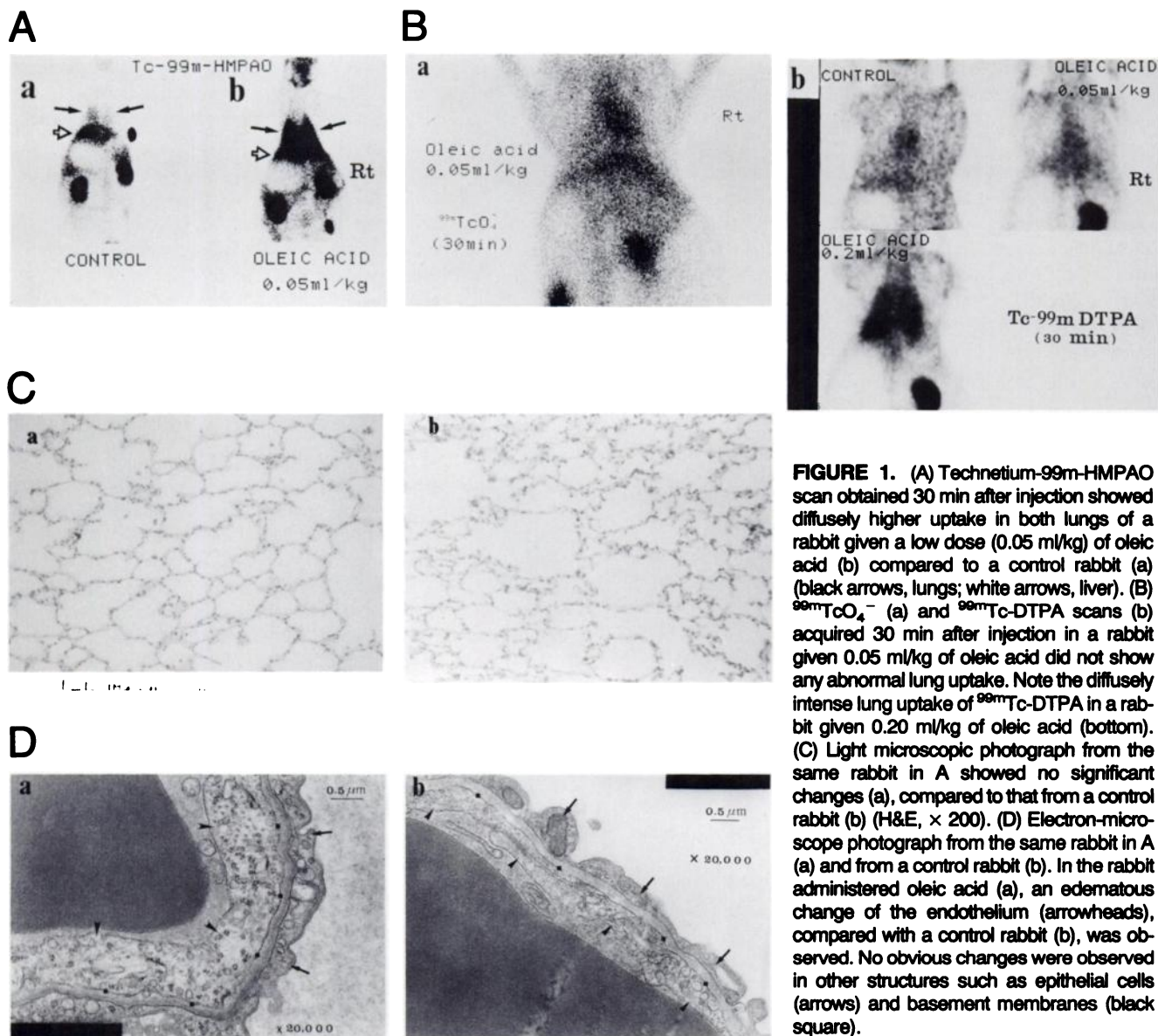
Electron microscopic study was performed to clarify the injured site of the lungs in five rabbits given 0.05 ml/kg of oleic acid and the 11 irradiated rabbits (five rabbits were at 24 hr, three rabbits at 2 days, and three rabbits at 28 days after irradiation) that showed  $^{99m}\text{Tc}$ -HMPAO uptake. The light and electron microscopic findings in these rabbits were compared with those of the three control rabbits.

## Clinical Studies

**Chemotherapy.** To evaluate the clinical applicability of  $^{99m}\text{Tc}$ -HMPAO, we investigated whether patients who had been administered anticancer drugs showed high uptake of  $^{99m}\text{Tc}$ -HMPAO in the lung. The possibility that the lung in these patients may be affected by cytotoxic drugs is likely since several previous pathologic studies on pneumonitis caused by anticancer drugs demonstrated that the endothelial cell was the primary and main affected site (7,8,32–34).

Patients included 11 men and three women with lung cancer (mean age,  $59 \pm 18$  yr) who had received chemotherapy with various anticancer drugs (cisplatin plus vindesine or vinblastine—8 patients; cisplatin plus cyclophosphamide plus doxorubicin and/or methotrexate—5 patients; cyclophosphamide plus bleomycin—1 patient). Of the 14 patients, eight were smokers (Brinkmann index range, 400–1000) and six were nonsmokers.

As a control group, we selected 16 patients (10 men and 6 women; mean age,  $57 \pm 14$  yr) with no pulmonary disease from the patients who underwent a brain perfusion study with  $^{99m}\text{Tc}$ -HMPAO for evaluation of cerebral disease. Of the 16 control patients, nine were smokers (Brinkmann index range, 450–900) and seven were nonsmokers. Each patient fasted prior to the examination to prevent changes in liver  $^{99m}\text{Tc}$ -HMPAO uptake. An anterior static image was acquired 30 min after the injection of 740 MBq of  $^{99m}\text{Tc}$ -HMPAO, using a gamma camera (Toshiba, GCA 901-A/W2), and the lung-to-liver activity ratios were evaluated.



**FIGURE 1.** (A) Technetium-99m-HMPAO scan obtained 30 min after injection showed diffusely higher uptake in both lungs of a rabbit given a low dose (0.05 ml/kg) of oleic acid (b) compared to a control rabbit (a) (black arrows, lungs; white arrows, liver). (B)  $^{99m}\text{TcO}_4^-$  (a) and  $^{99m}\text{Tc}$ -DTPA scans (b) acquired 30 min after injection in a rabbit given 0.05 ml/kg of oleic acid did not show any abnormal lung uptake. Note the diffusely intense lung uptake of  $^{99m}\text{Tc}$ -DTPA in a rabbit given 0.20 ml/kg of oleic acid (bottom). (C) Light microscopic photograph from the same rabbit in A showed no significant changes (a), compared to that from a control rabbit (b) (H&E,  $\times 200$ ). (D) Electron-microscope photograph from the same rabbit in A (a) and from a control rabbit (b). In the rabbit administered oleic acid (a), an edematous change of the endothelium (arrowheads), compared with a control rabbit (b), was observed. No obvious changes were observed in other structures such as epithelial cells (arrows) and basement membranes (black square).

**Radiotherapy.** In addition, we used SPECT images to investigate whether the irradiated lung showed high  $^{99m}\text{Tc}$ -HMPAO uptake in the 13 patients with lung cancer. The patients had received a total dose of 16 to 50 Gy during the course of radiotherapy, with a single dose of 2 Gy. Of the 13 patients, two had an abnormal opacity on chest CT due to radiation-related pneumonitis in the irradiated lung, but the remaining 11 patients did not have any significantly abnormal opacity due to radiation pneumonitis. Results were judged positive when abnormal  $^{99m}\text{Tc}$ -HMPAO uptake was seen in a pulmonary portion distant from the lung tumor to separate from its tumor uptake.

SPECT images were acquired 30 min after  $^{99m}\text{Tc}$ -HMPAO injection using the rotating SPECT apparatus with a single-head detector (Toshiba, GCA 901-A). Same-sized ROIs ( $4 \times 4$  voxels) were set in both the abnormal uptake site in the irradiated lung and the contralateral nonirradiated lung, and the degree of  $^{99m}\text{Tc}$ -HMPAO uptake was assessed by irradiated lung-to-contralateral lung activity ratios using mean counts per voxel.

### Statistics

Results were expressed as mean  $\pm$  s.d., and statistical analysis was performed using Student's t-test. A probability value of less than 0.05 indicated a significant difference.

## RESULTS

### Animal Studies

**Oleic Acid Study.** For all 20 rabbits administered oleic acid, static  $^{99m}\text{Tc}$ -HMPAO images made 30 min after the injection showed diffusely higher uptake in both lungs compared to that in the 10 controls (Fig. 1A). The mean  $A_{60}/A_{\text{peak}}$  ratio for the rabbits given a dose of 0.05 ml/kg was significantly higher than that for the controls (Table 1). These ratios tended to increase with the dose of oleic acid. The results also indicated that high pulmonary extraction of  $^{99m}\text{Tc}$ -HMPAO manifested rapidly within 1 min following the first pulmonary transit of this agent.

**TABLE 1**  
Mean  $A_{60}/A_{peak}$  Ratios in the Rabbits Administered Oleic Acid

Rabbits	$A_{60}/A_{peak}$ ratios
Controls (n = 10)	0.129 ± 0.023**
0.05 ml/kg of oleic acid (N = 8)	0.214 ± 0.031**
0.10 ml/kg of oleic acid (N = 6)	0.264 ± 0.024**
0.20 ml/kg of oleic acid (N = 6)	0.325 ± 0.027**

\*\*Significant differences were found (Student's t-test, \*p < 0.05; †p < 0.01).

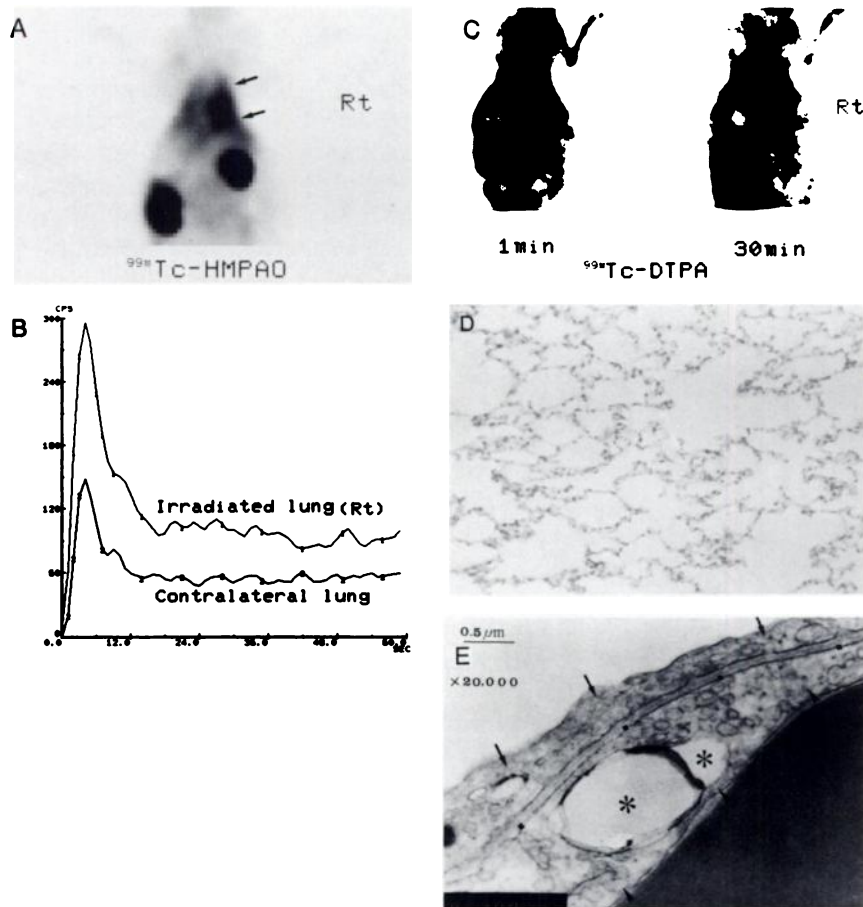
$A_{60}/A_{peak}$  ratio is the ratio of  $^{99m}\text{Tc}$ -HMPAO activity at 60 sec ( $A_{60}$ ) over the initial highest peak activity ( $A_{peak}$ ) following the injection.

Technetium-99m-MAA scans did not show any reduced pulmonary perfusion sites in the six rabbits given a dose of 0.05 ml/kg, whereas in the six rabbits given a dose of 0.10 or 0.20 ml/kg, reduced or defective perfusion sites were demonstrated in both lungs.

Technetium-99m-oxygen-4 and  $^{99m}\text{Tc}$ -DTPA scans performed in the six rabbits given 0.05 ml/kg of oleic acid did not show any abnormal uptake in the lung (Fig. 1B). The TACs also did not show any significant difference compared to those of the controls. However, both scans performed in the 24 rabbits given 0.10 or 0.20 ml/kg of oleic acid showed diffusely abnormal uptake in both lungs.

The chest radiographs of all eight rabbits given a dose of 0.05 ml/kg did not show any abnormal opacity in the lung; however, the remaining 12 rabbits given a dose of 0.10 or 0.20 ml/kg showed infiltrates predominantly in both lower lung fields or throughout the whole lung. Light microscopy revealed no clear changes in the lungs of the six rabbits given a dose of 0.05 ml/kg compared to the three controls (Fig. 1C), while the lungs of the 12 rabbits given 0.10 or 0.20 ml/kg had an extensive exudate within the alveolar space and thickening of the interalveolar septa. In contrast, electron microscopy showed edematous changes in the endothelial cytoplasm with or without blebs in all five rabbits given 0.05 ml/kg compared to the three controls. Neither destruction of the basement membrane nor significant degeneration of the alveolar epithelium were found (Fig. 1D).

**X-Ray Irradiation Study.** The  $^{99m}\text{Tc}$ -HMPAO scans acquired 30 min after injection showed higher uptake within the irradiated lung than in the contralateral nonirradiated lung in 33 (86.8%) of the 38 irradiated rabbits. The irradiated-to-nonirradiated lung ratio of  $^{99m}\text{Tc}$ -HMPAO activity ranged from 1.05 to 1.72 in the 33 rabbits with positive results, and the mean ratio was  $1.38 \pm 0.17$ . Of the 12 rabbits examined at 24 hr after irradiation, 11 (91.6%) showed abnormal uptake (Fig. 2A). Negative results were found in one of the 12 rabbits at 24 hr after irradiation, one of the six rabbits at 2 days, one of the six rabbits at 14 days,



**FIGURE 2.** (A) Technetium-99m-HMPAO scan obtained 30 min after injection in a rabbit that received 50 Gy of irradiation 24 hr before (right lung) demonstrated higher uptake in the irradiated lung (arrows) than in the contralateral nonirradiated lung. (B) The time-activity curves during the first 60 sec in the same rabbit in A revealed that a higher uptake of  $^{99m}\text{Tc}$ -HMPAO in the irradiated lung occurred rapidly after the injection. (C)  $^{99m}\text{Tc}$ -DTPA scans did not demonstrate significantly high uptake in the irradiated lung (right) of the rabbit. (D) In the  $^{99m}\text{Tc}$ -HMPAO-positive rabbit that received irradiation 2 days before, light microscopy did not reveal any clear alterations in the irradiated lung (H&E, × 200). (E) Electron microscopy in the rabbit presented in D reveals an edematous endothelial cytoplasm (arrowheads) with blebs (asterisks) in the irradiated lung. However, no significant changes were noted in the basement membrane (black boxes) and epithelial cells (arrows).

and two of the six rabbits at 21 days following irradiation. In all 33 rabbits with positive results, the TACs revealed that the high uptake of  $^{99m}\text{Tc}$ -HMPAO occurred during the first minute immediately after the injection (Fig. 2B).

The results of  $^{99m}\text{Tc}$ -MAA scans performed in the 15 irradiated rabbits showing  $^{99m}\text{Tc}$ -HMPAO uptake are presented in Table 2. Significantly reduced perfusion was noted in only eight rabbits (63.3%). The pulmonary perfusion tended to decrease as the time following irradiation increased; however,  $^{99m}\text{Tc}$ -HMPAO uptake tended to increase conversely. Neither the  $^{99m}\text{TcO}_4^-$  nor the  $^{99m}\text{Tc}$ -DTPA scans performed in the six irradiated rabbits demonstrated abnormal uptake in the irradiated lung (Fig. 2C). The TACs of both scans did not show any significant differences between the irradiated and the contralateral non-irradiated lung.

Chest radiographs of all 33 rabbits showing  $^{99m}\text{Tc}$ -HMPAO uptake did not show any abnormal opacity in the irradiated lung. Light microscopy in all 14 irradiated rabbits did not reveal any significant changes in the pulmonary peripheral structures compared to the contralateral non-irradiated lung (Fig. 2D), and there was no obvious thickening of interalveolar septa or exudative changes within the alveolar spaces. Cellular infiltrations were not observed.

Electron microscopy in all 11 irradiated rabbits with abnormal uptake of  $^{99m}\text{Tc}$ -HMPAO revealed degeneration of the endothelium which manifested as intracytoplasmic edema with blebs (blebbing). In the five rabbits at 24 hr and the three rabbits at 2 days after irradiation, these changes were not widespread, and no significant changes were noted in the other structures such as epithelial cells, interstitium,

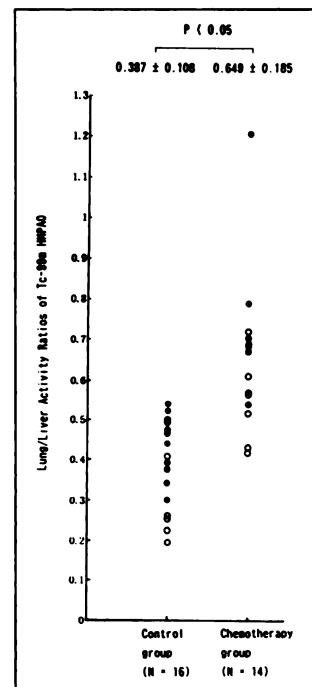
**TABLE 2**  
The results of  $^{99m}\text{Tc}$ -MAA Scans Obtained in 15 Irradiated Rabbits Showing  $^{99m}\text{Tc}$ -HMPAO Uptake in the Irradiated Lung on the  $^{99m}\text{Tc}$ -HMPAO Scans Performed 3 Days Earlier

Rabbit no.	Time interval after irradiation (day)	Irradiated lung-to-contralateral lung activity ratios	
		( $^{99m}\text{Tc}$ -HMPAO)	( $^{99m}\text{Tc}$ -MAA)
1	1	1.33	0.87
2	1	1.21	0.91
3	1	1.31	0.97
4	1	1.34	0.86
5	1	1.18	0.78*
6	1	1.21	0.96
7	14	1.05	0.73*
8	14	1.11	0.68*
9	14	1.12	1.00
10	14	1.61	0.75*
11	14	1.40	0.93
12	21	1.36	0.69*
13	21	1.54	0.73*
14	21	1.57	0.64*
15	21	1.68	0.68*

\*The pulmonary perfusion decreased more than 20% lower than that of the contralateral nonirradiated lung.

No. 4 is the same rabbit presented in Figure 2A.

**FIGURE 3.** Comparison of the lung-to-liver ratios of  $^{99m}\text{Tc}$ -HMPAO activity in the patients who received chemotherapy and the control subjects. The mean activity ratio in the patients who received chemotherapy was significantly higher than that in the controls ( $p < 0.01$ , Student's t-test). The mean value for the smokers who received chemotherapy ( $0.730 \pm 0.207$ ) was significantly higher than that for smokers ( $0.429 \pm 0.080$ ) in the control group ( $p < 0.01$ ), and the mean ratio for nonsmokers in the chemotherapy group ( $0.544 \pm 0.114$ ) was significantly higher than that for nonsmokers ( $0.333 \pm 0.130$ ) in the control group ( $p < 0.05$ ) (black circles, smokers; white circles, nonsmokers).



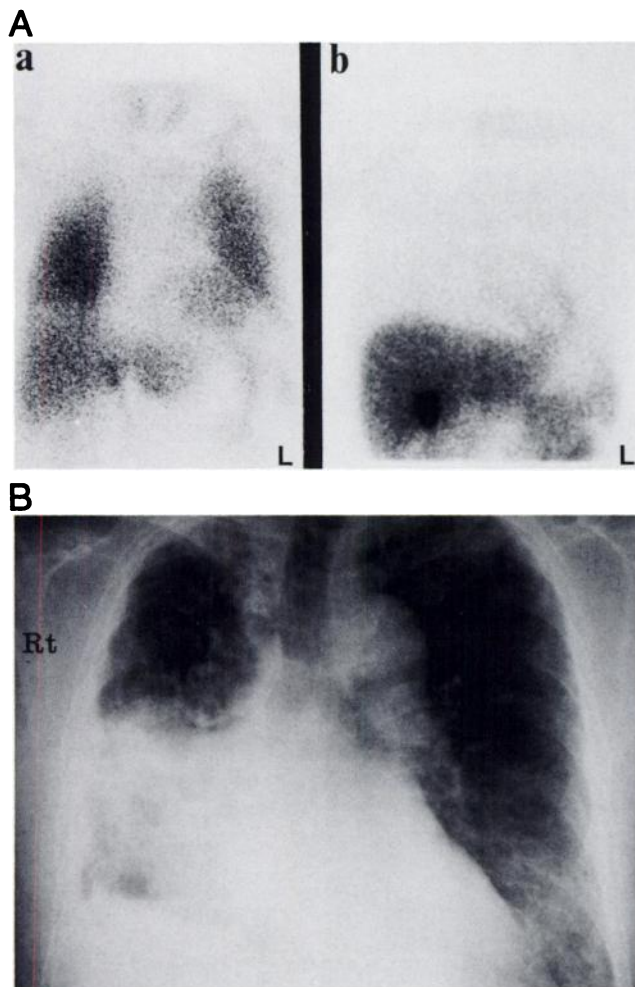
and basement membrane (Fig. 2E). In the three rabbits at 28 days after irradiation, the number of endothelia manifesting these changes increased, and scattered stripping off the basement membrane of the endothelium was observed.

### Clinical Studies

**Chemotherapy.** Figure 3 compares the lung-to-liver ratios of  $^{99m}\text{Tc}$ -HMPAO activity in the chemotherapy and control groups. The mean activity ratio in the chemotherapy group (mean  $\pm$  s.d.,  $0.649 \pm 0.185$ ) was significantly higher than that in the control group ( $0.387 \pm 0.108$ ) ( $p < 0.01$ ). The ratios for smokers tended to be higher than for nonsmokers, although there were no statistically significant differences in either chemotherapy (smokers:  $0.730 \pm 0.207$ ; nonsmokers:  $0.544 \pm 0.114$ ;  $p < 0.10$ ) and control (smokers:  $0.429 \pm 0.080$ ; nonsmokers:  $0.333 \pm 0.130$ ;  $p < 0.10$ ) groups. However, chemotherapy resulted in higher lung uptake in both smokers and nonsmokers, as the ratio for smokers who received chemotherapy was significantly higher than that for smokers in the control group ( $p < 0.01$ ), and the ratio for nonsmokers who received chemotherapy was also significantly higher than that for nonsmokers in the control group ( $p < 0.05$ ).

Scans of a patient showing diffuse  $^{99m}\text{Tc}$ -HMPAO uptake in both lungs and with the highest lung-to-liver activity ratio are presented in Figure 4A. Chest radiographs of this patient 3 mo later showed a diffuse abnormal opacity in both lungs (Fig. 4B), which improved with steroid therapy and was clinically diagnosed as anticancer, drug-induced interstitial pneumonia.

**Radiotherapy.** All 12 patients who received more than 30 Gy of irradiation showed a higher uptake of  $^{99m}\text{Tc}$ -HMPAO in the corresponding irradiated lung than the surrounding and



**FIGURE 4.** (A) Patient with the highest lung-to-liver ratio of  $^{99m}\text{Tc}$ -HMPAO activity (1.204). The patient had been administered CDDP (total dose, 900 mg) and VDS (total dose, 36 mg). Diffusely higher uptake of  $^{99m}\text{Tc}$ -HMPAO (a) compared to a control subject (b) was demonstrated. (B) A chest radiograph obtained 3 mo later showed a diffusely abnormal infiltrate in both lungs. Drug-induced pneumonitis was clinically suspected. The abnormal opacity in the right lower lung was due to lung cancer.

contralateral nonirradiated lung (Fig. 5). Table 3 shows the irradiated lung-to-contralateral lung ratios of  $^{99m}\text{Tc}$ -HMPAO activity. The patients who received a 50-Gy dose tended to have a higher activity ratio than did those who received lower doses, and the highest activity ratios were noted in the patients with an infiltrate on their chest CT.

## DISCUSSION

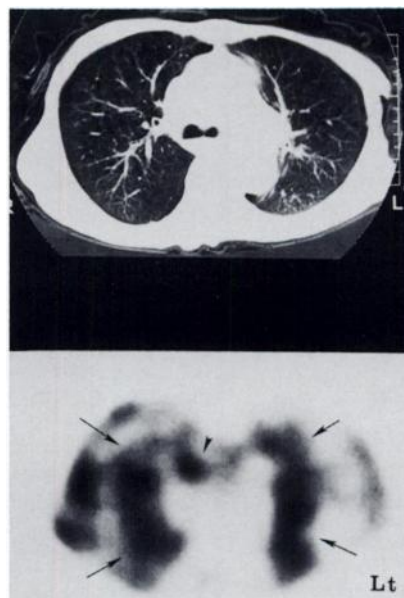
In both animal models given a low dose of oleic acid (0.05 ml/kg) or 50 Gy of irradiation, the lungs showed high uptake of  $^{99m}\text{Tc}$ -HMPAO with a high incidence, although the morphologic change was localized in the microvascular endothelium by the electron microscopy. Furthermore, the irradiated rabbits showed uptake as early as 24 hr following irradiation. Although early radiation-induced lung injury has been detected by various radiopharmaceuticals such as  $^{67}\text{Ga}$  (35,36),  $^{99m}\text{Tc}$ -DTPA (31), and  $^{123}\text{I}$ -IMP (37,38), in

our survey these agents did not detect it so early. Previous electron microscopic studies also demonstrated endothelial damage to be an initial event in more advanced lung injuries induced by oleic acid and irradiation (2–4, 20–24). Thus, our results indicate that  $^{99m}\text{Tc}$ -HMPAO has potential as a highly sensitive indicator for detecting early and minimal lung injuries induced by oleic acid and irradiation.

Technetium-99m-HMPAO is a cyclic amine and its localization in the lung is presumably in the endothelium (12,39). Therefore, the high lung uptake of  $^{99m}\text{Tc}$ -HMPAO in both experimental models is most likely related to dysfunction of amine metabolism in the injured endothelium. On the other hand, as a lipophilic, nonparticle substance,  $^{99m}\text{Tc}$ -HMPAO characteristically penetrates easily into endothelial cytoplasm through pores in the cell membrane (40–45). Therefore, the rapid manifestation of abnormal uptake of this agent following injection, as seen in both models, may be partially caused by enhanced transport across the injured cell membrane of endothelium.

Unchanged  $^{99m}\text{Tc}$ -MAA lung images in the  $^{99m}\text{Tc}$ -HMPAO-positive rabbits given a dose of 0.05 ml/kg of oleic acid and a lower incidence of significantly reduced  $^{99m}\text{Tc}$ -MAA uptake in the  $^{99m}\text{Tc}$ -HMPAO-positive, irradiated rabbits (Table 2) indicate that  $^{99m}\text{Tc}$ -HMPAO is more sensitive in the detection of lung injury than is  $^{99m}\text{Tc}$ -MAA. That  $^{99m}\text{Tc}$ -HMPAO accumulates despite the reduced  $^{99m}\text{Tc}$ -MAA uptake in the lungs of rabbits given 0.10 or 0.20 ml/kg of oleic acid or irradiation suggests that  $^{99m}\text{Tc}$ -HMPAO reaches the peripheral lung areas where  $^{99m}\text{Tc}$ -MAA can not reach. It is possible that nonparticle  $^{99m}\text{Tc}$ -HMPAO can reach the sites where the large particles of  $^{99m}\text{Tc}$ -MAA (10–60  $\mu\text{m}$ ) cannot reach (37,46).

As opposed to positive  $^{99m}\text{Tc}$ -HMPAO results in the rabbits given a dose of 0.05 ml/kg of oleic acid or irradiation,  $^{99m}\text{Tc}$ -DTPA did not show any abnormal uptake



**FIGURE 5.** A patient with lung cancer who received 30 Gy of irradiation in the mediastinal and both hilar regions for lung cancer showed an abnormal uptake of  $^{99m}\text{Tc}$ -HMPAO in the corresponding irradiated lung on the SPECT image (bottom: arrows), despite the absence of clear abnormal opacity on the chest CT (upper). Intense mediastinal uptake was caused by metastatic lymphadenopathy (arrow-head) (bottom).

**TABLE 3**  
Results of the  $^{99m}\text{Tc}$ -HMPAO SPECT in the Patients Who Received Irradiation

Patient no.	Age	Sex	Radiation dose (Gy)	Chest CT findings in the irradiated lung	Irradiated lung-to-contralateral lung ratios
1	70	M	16	No opacity	No abnormal uptake
2	77	M	30	No opacity	1.21
3	64	M	30	No opacity	1.19
4	51	M	30	No opacity	1.24
5	58	F	36	No opacity	1.23
6	69	M	40	No opacity	1.27
7	58	F	40	No opacity	1.35
8	72	F	50	No opacity	1.29
9	64	M	50	No opacity	1.47
10	81	M	50	No opacity	1.58
11	70	M	50	No opacity	1.49
12	67	M	50	Infiltrate	1.98
13	73	M	50	Infiltrate	2.17

All 12 patients who received more than 30 Gy showed abnormal  $^{99m}\text{Tc}$ -HMPAO uptake in the irradiated lung. The patients who received 50 Gy tended to show a higher uptake than those who received lower doses.

(Figs. 1B and 2C). As a small hydrophilic substance,  $^{99m}\text{Tc}$ -DTPA (molecular mass, 492 daltons) easily transfers across capillaries through interendothelial gaps into the extravascular space (6, 31, 40, 47), manifesting high lung uptake as a result of enhanced microvascular permeability or increased extravascular space or both. Therefore, the negative  $^{99m}\text{Tc}$ -DTPA results indicate that lipophilic  $^{99m}\text{Tc}$ -HMPAO did not show high uptake by these abnormalities in the rabbits with minimal injuries. This is in accordance with the light and electron microscopic findings revealing no evidence of increased extravascular space (Figs. 1C, 1D, 2D and 2E). On the other hand, the negative  $^{99m}\text{TcO}_4^-$  results in these rabbits indicate that abnormal  $^{99m}\text{Tc}$ -HMPAO uptake was not simply caused by nonspecific uptake of free  $^{99m}\text{TcO}_4^-$  released from  $^{99m}\text{Tc}$ -HMPAO.

Our clinical studies revealed high  $^{99m}\text{Tc}$ -HMPAO uptake in the lungs of patients treated with chemotherapy or irradiation (Fig. 3, Table 3) who did not have either symptoms related to the drug-induced pneumonitis or abnormal opacity within the irradiated field (Table 3). High uptake of  $^{99m}\text{Tc}$ -HMPAO has also been demonstrated in the lungs of smokers (12, 39, 48) and patients with a history of lung edema (49), and may be related to endothelial damage due to substances contained in cigarette smoke and edema. These and our clinical findings suggest clinical applicability of  $^{99m}\text{Tc}$ -HMPAO for identifying early or subclinical lung injuries, even in less critical situations.

In our series, chemotherapy resulted in a higher lung uptake of  $^{99m}\text{Tc}$ -HMPAO in both smokers and nonsmokers compared to the controls, possibly indicating that chemotherapy's influence on the lung is more dominant than the influence of cigarette smoke. As suggested by Figure 4, the degree of  $^{99m}\text{Tc}$ -HMPAO uptake might be used to predict later developing lung injury induced by chemotherapy. However, further investigations are necessary.

Currently, as potential agents protective of endothelium

from free radicals and lipid peroxides activated by various chemical substances and irradiation, nitric monoxide, superoxide dismutase, and catalase have been candidates in animal models (50-52). When such agents become available for clinical use,  $^{99m}\text{Tc}$ -HMPAO may be useful in assessing the effect of treatment.

A biogenic amine,  $^{123}\text{I}$ -metaiodobenzylguanidine ( $^{123}\text{I}$ -MIBG), was recently proposed as an endothelial metabolic imaging agent, and its uptake decreases with endothelial injury in animal studies (8-10). In contrast to this negatively depicting agent of lung injury,  $^{99m}\text{Tc}$ -HMPAO may have the advantage of depicting it positively. Moreover,  $^{99m}\text{Tc}$ -HMPAO scanning has the advantage of simplicity compared to a dual-isotope technique detecting endothelial damage (20). Inhaled  $^{99m}\text{Tc}$ -DTPA also can be used to detect early lung injury, but it is used for assessing alveolar epithelial damage (53).

## CONCLUSION

Although further studies including microautoradiography are necessary to clarify the exact mechanism of an abnormal uptake of  $^{99m}\text{Tc}$ -HMPAO in an injured lung, our animal and clinical studies indicate that this agent is available as a sensitive marker of early and minimal chemical and irradiation lung injuries.

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