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Tc-99m-HMPAO uptake by bronchoalveolar cells

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Lung uptake of intravenously injected Tc-99m-HMPAO is observed in smokers and in lung toxicity due to various agents. We investigated the Tc-99m-HMPAO uptake of bronchoalveolar lavage (BAL) cells in the lungs after incubation in *in vitro* conditions (6 patients), intravenous injection (IV) (7 patients) and inhalation (INH) (6 patients) of Tc-99m-HMPAO in order to show whether BAL cells are also responsible for Tc-99m-HMPAO uptake in the lungs. Cell/supernatant (C/S) count ratio was 7.0 ± 3.5, 29.3 ± 40.8 and 8.4 ± 4.5 for *in vitro*, IV and INH groups, respectively. $C/S_{in\ vitro}$ showed a positive correlation with % alveolar macrophages ($r = 0.943$, $p = 0.0048$) and a negative correlation with % neutrophils ($r = -0.945$, $p = 0.0045$). Cells/whole BAL fluid ratio correlated with the amount of daily cigarette consumption in INH group ($r = 0.95$, $p = 0.0037$). Tc-99m-HMPAO showed adherence to mucus after inhalation. Tc-99m-HMPAO diffuses into alveolar spaces after injection and is present in BAL fluid and BAL cells both after injection and inhalation. Glutathione concentration and oxido-reductive state of the epithelial lining fluid and BAL cells may influence the lung uptake of Tc-99m-HMPAO.

Key words: Tc-99m-HMPAO, bronchoalveolar lavage, radioaerosols, alveolar macrophages, smoking

INTRODUCTION

LUNG UPTAKE of intravenously injected Tc-99m-hexamethylene propyleneamine oxime (HMPAO) is observed in smokers and in lung toxicity due to various agents.[1–6] There is no clear definition of which cells are responsible for this uptake though some authors have proposed that endothelial cells responsible from this retention.[6–8] Tc-99m-HMPAO is also administered as a radioaerosol and its clearance rate from the lungs was found to be slower than that of Tc-99m-DTPA.[9–11] Though lipophilic agents are supposed to use the whole alveolar surface for diffusion and are expected to have fast disappearance from the lungs, this slow clearance of Tc-99m-HMPAO has not been fully explained.

To our knowledge, there are no reported data in the English literature about the presence of Tc-99m-HMPAO in the bronchoalveolar lavage fluid (BALF) and bronchoalveolar lavage cells (BAL cells). Since Tc-99m-HMPAO is a lipophilic agent used for cell labeling, it is also quite likely that it diffuses into the alveolar spaces after intravenous injection and penetrates into the cells within the alveolar space. Furthermore, if it is administered as a radioaerosol by inhalation, it comes into close contact with the alveolar cells. Its' cellular uptake depends mainly on the lipophilic properties, and its intracellular retention is caused by conversion to the hydrophilic nondiffusable form in the presence of glutathione (GSH) or other reducing agents.[12–14]

We designed this study to investigate the presence of Tc-99m-HMPAO in BALF and BAL cells in the lungs after incubation with Tc-99m-HMPAO in *in vitro* conditions, after intravenous injection of Tc-99m-HMPAO and after the inhalation of Tc-99m-HMPAO radioaerosol. Using these data, we attempt to explain whether BAL cells are also responsible for Tc-99m-HMPAO uptake in the lungs.

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MATERIALS AND METHODS

Patients about to undergo bronchoscopy as a part of their clinical evaluation for diagnostic purposes were informed about the study. Nineteen patients who provided consent were enrolled. A full history of smoking and drug treatment was taken from the patients. Bronchoscopy was performed under topical anesthesia with lignocaine. Bronchoalveolar lavage (BAL) was performed from the right middle lobe or left lingula using a total of 120 m*l* normal saline in 20 m*l* aliquots. None of the patients had a significantly disturbed ventilation or perfusion at the region where BALF was obtained. BALF was filtered through sterile gauze swabs. Thirty milliliters of the recovered fluid was placed in ice and brought to the Nuclear Medicine Department for the experiments. The total and differential cell counts of the BALF were also determined.

Tc-99m-d,l HMPAO (Ceretec, Amersham, UK) was labeled according to the manufacturer's recommendations. Quality control was performed using instant thin layer chromatography and used if the labeling efficiency was >90%. Tc-99m-HMPAO was used within 10 minutes after labeling.

In Vitro *Studies (*In Vitro*):*
BALF of 6 patients was used. Thirty milliliters of BALF was centrifuged in 4,250 rpm for 10 minutes to separate the cells from the fluid. The cell sediment was incubated with 74 MBq Tc-99m-HMPAO for 10 minutes at room temperature. After incubation, the cells were washed with the previously separated fluid and centrifuged as before. One milliliter of the supernatant and one milliliter of the cell sediment were counted in a multi-crystal gamma counter for 30 seconds (LB 2111-LBIS, EG&G Berthold) using 20% window at 140 keV. Cell count/supernatant counts ($C/S_{in\ vitro}$), and cell count/total counts ($C/T_{in\ vitro}$) ratios (total counts = supernatant counts + cell counts) and percentage of activity washout from the BAL cells at 1 hour (% WO) were calculated.

Intravenous Injection (IV):
Before the BAL procedure 185 MBq Tc-99m-HMPAO was injected intravenously to 7 patients. The time between Tc-99m-HMPAO injection and BAL was 27 ± 15 minutes. Five milliliters of venous blood was drawn simultaneously with BAL. One milliliter of the BALF was counted in the gamma counter as described previously, and the whole BALF count is obtained ($WBALF_{iv}$). The remaining fluid was centrifuged as described. One milliliter of the supernatant and the cell sediment were counted in the gamma counter. Whole blood was also centrifuged to separate the cells from the plasma. One milliliter of the plasma and blood cells were counted in the gamma counter. Cell count/supernatant count (C/S_{iv}), cell count/whole BALF count ($C/WBALF_{iv}$), supernatant count/whole BALF count ($S/WBALF_{iv}$), whole BALF count/plasma count ($WBALF/P_{iv}$), cell count/plasma count

Table 1 Patient characteristics of *In Vitro*, IV and INH groups (COPD: Chronic Obstructive Pulmonary Disease)

	In Vitro	IV	INH
Number of patients	6	7	6
Gender	3 F, 3 M	1 F, 6 M	3 F, 3 M
Age	55 ± 16	61 ± 13	51 ± 14
Diagnosis	3 COPD	3 COPD	1 COPD
	2 lung cancer	4 lung cancer	3 lung cancer
	1 tuberculosis		1 tuberculosis
			1 radiation pneumonitis
Smoking history	2 smokers	4 smokers	4 smokers
	(20 ± 0 cigarettes/day	(33 ± 10 cigarettes/day	(33 ± 22 cigarettes/day
	for 42 ± 12 years)	for 38 ± 10 years)	for 22 ± 8 years)
	1 ex-smoker	3 ex-smokers	2 nonsmokers
	3 nonsmokers		

Table 2 Total number of cells and differential cell counts in BAL fluid of patients in three patient groups

	In Vitro	IV	INH
Total BAL cells × 10^3/m*l*	412 ± 580	437 ± 352	444 ± 305
% Alveolar macrophages	69 ± 31	83 ± 25	92 ± 3
% Lymphocytes	4 ± 6	4 ± 7	5 ± 3
% Neutrophils	26 ± 27	13 ± 25	3 ± 0
% Monocytes	6 (one patient)		
% Eosinophils			2 (one patient)

(C/P_{iv}), supernatant count/plasma count (S/P_{iv}), blood cell count/total blood cell + plasma count (BC/T_{iv}) ratios were calculated.

Radioaerosol Inhalation (INH):
Before the BAL procedure, patients inhaled 1,110 MBq Tc-99m-HMPAO for 4–5 minutes in the sitting position from a nebulizer (UltraVent Aerosol Delivery System, Mallinckrodt Medical, Petten, Holland) which produces submicronic aerosols (MMAD 0.89 μm, GSD 1.85) with 10–12 liters/min O_2 flow rate. The time between inhalation and BAL was 23 ± 8 minutes. Five milliliters of venous blood was drawn simultaneously with BAL. Two milliliters of the mucus obtained from the fluid was placed in a separate tube. One milliliter of the whole BALF was counted in the gamma counter. BALF and whole blood were centrifugated and counted as described before. Cell count/supernatant count (C/S_{inh}), cell count/whole BALF count ($C/WBALF_{inh}$), supernatant count/whole BALF count ($S/WBALF_{inh}$), whole BALF count/plasma count ($WBALF/P_{inh}$), cell count/plasma count (C/P_{inh}), supernatant count/plasma count (S/P_{inh}), blood cell count/total blood cell + plasma counts (BC/T_{inh}) ratios were calculated. One milliliter of the mucus was also counted and mucus/plasma (M/P) and mucus/whole BALF count (M/WBALF) ratios were obtained.

The data were analyzed using Pearson correlation coefficient to search for a linear relationship with a confidence interval of 90–95%. $p < 0.05$ is accepted as significant.

RESULTS

Patient characteristics of *In Vitro*, IV and INH groups are summarized in Table 1. Total number of cells and differential cell counts in BALF of patients are shown in Table 2. The ratios for *In Vitro* group are in Table 3 and the ratios for IV and INH groups are in Table 4. Forty-two percent of Tc-99m-HMPAO in the blood was in the blood cells at about half an hour after i.v. injection (Range 35%–52%)

Table 3 Cell count/supernatant count (C/S), cell count/total count (counts in the total volume of supernatant + cells) (C/T) ratios and percentage of activity washout from the BAL cells at 1 hour (% WO) of the *In Vitro* group

	In Vitro
C/S	7.0 ± 3.5
C/T	0.19 ± 0.08
% WO	9.3 ± 4.2

Table 4 Cell count/supernatant count (C/S), cell count/whole BALF count (C/WBALF), supernatant count/whole BALF count (S/WBALF), whole BALF count/plasma count (WBALF/P), cell count/plasma count (C/P), supernatant count/plasma count (S/P_{inh}), blood cell count/total blood cell + plasma counts (BC/T), mucus/plasma (M/P) and mucus/whole BALF count (M/WBALF) ratios of IV and INH groups

	IV	INH
C/S	29.3 ± 40.8	8.4 ± 4.5
C/WBALF	9.4 ± 10.1	5.7 ± 3.07
S/WBALF	0.65 ± 0.51	0.70 ± 0.16
WBALF/P*	0.010 ± 0.015	4.0 ± 4.2
C/P	0.16 ± 0.30	19.8 ± 27.0
S/P	0.003 ± 0.003	3.1 ± 3.7
BC/T	0.42 ± 0.06	0.43 ± 0.14
M/P	—	19.3 ± 16.9
M/WBALF	—	5.0 ± 2.8

*p = 0.045

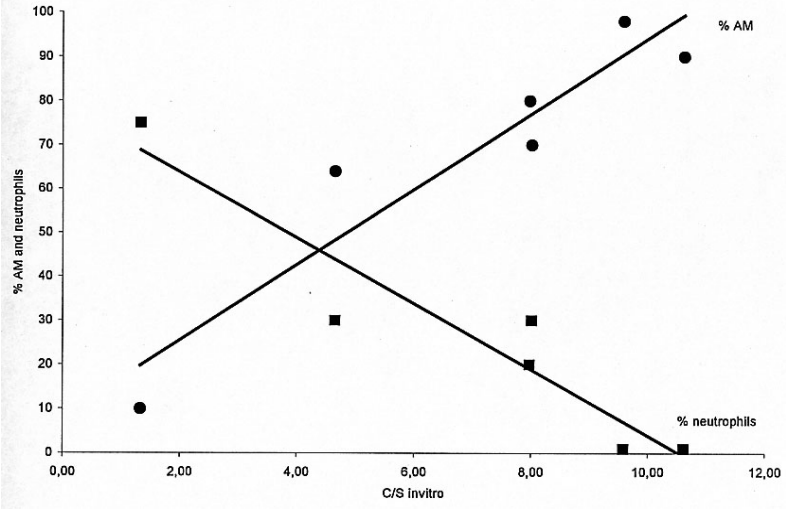

Fig. 1 The ratio of cell counts/supernatant counts in the *in vitro* study (C/S in vitro) showed a positive correlation with % AM and a negative correlation with % neutrophils (r = 0.943, p = 0.0048 and r = –0.945, p = 0.0045).

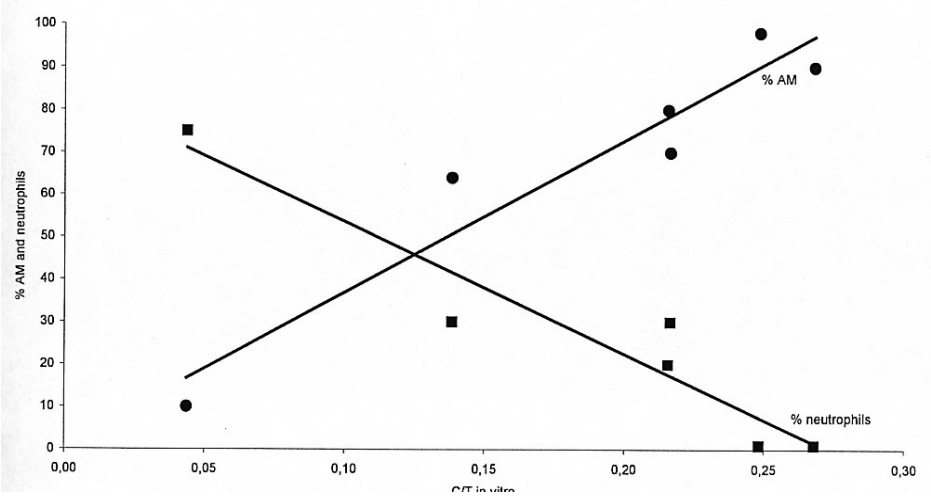

Fig. 2 The ratio of cell counts/total counts in the *in vitro* study (C/T in vitro) showed a positive correlation with % AM and a negative correlation with % neutrophils (r = 0.957, p = 0.0026 and r = –0.95, p = 0.0037).

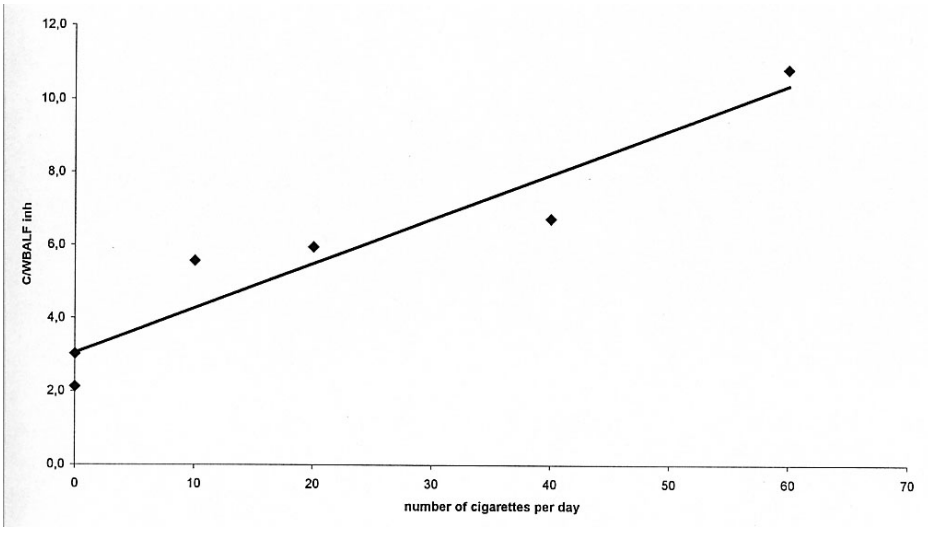

Fig. 3 The amount of daily cigarette consumption of patients in the INH group was positively correlated with $C/WBALF_{inh}$ (r = 0.95, p = 0.0037).

and one percent of plasma activity was in the BALF (Range 0.01%–3.6%). After inhalation, 25% ± 24% of BALF activity was in the plasma and 43% of Tc-99m-HMPAO in the blood was in the blood cells (Range 19%–59%). There was no statistically significant difference between the ratios of IV and INH groups except the WBALF/P ratio (p = 0.045), due to the wide standard deviation.

$C/S_{in\ vitro}$ and $C/T_{in\ vitro}$ showed a significant positive correlation with % alveolar macrophages (AM) (r = 0.943, p = 0.0048 and r = 0.95, p = 0.0026, respectively) and a negative correlation with % neutrophils (r = –0.945, p = 0.0045 and r = –0.95, p = 0.0037, respectively) (Fig. 1 and Fig. 2). We were unable to show a correlation with the count ratios and the fraction of BAL cells in IV and INH groups.

$C/WBALF_{inh}$ correlated with the amount of daily cigarette consumption (r = 0.95, p = 0.0037) (Fig. 3). $C/WBALF_{iv}$ and the amount of daily cigarette consumption had a correlation of borderline significance (r = 0.725, p = 0.065) (Fig. 4). Three currently nonsmokers (exsmokers) in IV group and two nonsmokers in INH group tended to have lower $C/WBALF_{iv}$ (1.1 ± 0.6 versus 15.5 ± 9.3) and $C/WBALF_{inh}$ ratios (2.6 ± 0.6 versus 7.3 ± 2.4) compared to smokers.

DISCUSSION

Lungs can take up and metabolize biogenic amines such as amphetamines, which are taken up by the endothelial

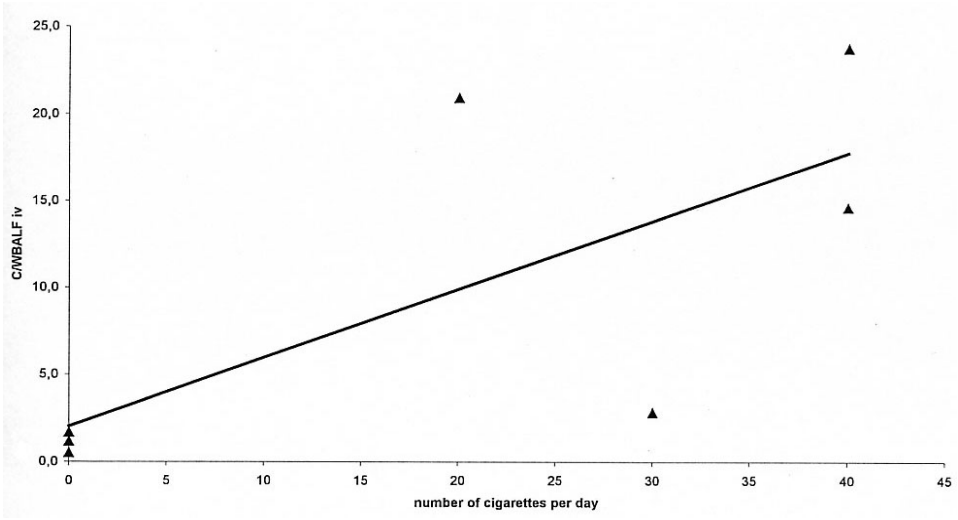

Fig. 4 $C/WBALF_{iv}$ and the amount of daily cigarette consumption of patients in the IV group had a correlation of borderline significance ($r = 0.725$, $p = 0.065$).

cells and released into the circulation with a half time of 3 minutes.[15] I-123 iodoamphetamine (IMP) is expected to follow the same fate, but it remains in the lungs longer, and so an additional site of accumulation is considered necessary. Ikeda et al. have reported I-123-IMP uptakes in BAL cells after intravenous injection.[16] Though the authors did not calculate a ratio and their calculation method was different from ours, the division of BAL cell counts to total BALF activity is 0.40 in their study. If their data are calculated with our method, BAL cells/blood serum and total BALF activity/blood serum ratios are found to be 2.1 and 5.3, respectively. Itasaka et al. have also performed a similar study, in which they have found BAL cells/blood serum and total BALF activity/blood serum ratios to be 2.71 and 6.86, respectively.[17] Ikeda et al. claimed that the prolonged retention of I-123-IMP in the diseased lungs is due to the diffusion of I-123-IMP from the endothelial cells to the pulmonary interstitium and alveolar space and absorption by the increased alveolar cells.[16]

Tc-99m-HMPAO is a lipophilic radiopharmaceutical like I-123-IMP. In numerous studies, the lung retention of Tc-99m-HMPAO was related to endothelial cell uptake, endothelial cell damage or microvascular injury.[6–8] In this study, we showed that Tc-99m-HMPAO is present in BAL fluid and BAL cells. If we recalculate our IV data using Ikeda et al's method, the ratio of BAL cell counts to total BALF activity happens to be 0.54 ± 0.27 in our study. BALF/serum ratios of I-123-IMP in the aforementioned studies are much more higher than BALF/plasma ratios of Tc-99m-HMPAO found in our study, which might be due to higher blood activity of Tc-99m-HMPAO.

In vitro incubation of BAL cells with Tc-99m-HMPAO resulted in the incorporation of 19% of the radiopharmaceutical added to the medium. Because the extracellular medium may be free of reducing or oxidizing agents in *in vitro* cell suspension, intracellular diffusion and retention of Tc-99m-HMPAO are mainly effected by the lipophilicity of the prepared compound, intracellular conversion to the hydrophilic form by means of intracellular glutathione or other thiols and backdiffusion. 9.3% of the initial radioactivity has washed out of the cells after one hour. The negative correlation with % neutrophils was an interesting finding and Tc-99m-HMPAO seems to prefer alveolar macrophages. Our number of experiments may be small to draw definite conclusions but a study on guinea pigs showed that the GSH content was 17.93 nmol/mg in AM and 11.67 nmol/mg in polymorphonuclear leukocytes (PNL), which, after oxidant injury is increased to 51.22 nmol/mg in AM with no change in the GSH content of PNL.[18] These findings need further confirmation.

In IV group, we found the highest BAL cells to supernatant ratio, but with great variability (range 0.5–89.5). The great variation in C/S ratio in our study may be related to the presence of multiple factors affecting the uptake. The maintenance of the lipophilicity of Tc-99m-HMPAO in the plasma and presence of a uniform and adequate pulmonary perfusion are prerequisites for Tc-99m-HMPAO diffusion into the endothelial cells of the pulmonary capillaries. Just like I-123-IMP, Tc-99m-HMPAO may diffuse through the endothelial cells to the pulmonary interstitium, alveolar epithelium and alveolar space. During its passage from the capillary endothelium or alveolar epithelial cells, Tc-99m-HMPAO may be converted to its nondiffusable hydrophilic form in the presence of intracellular reducing agents. Epithelial lining fluid (ELF) coats the alveolar surface and contains GSH[19] that can reduce Tc-99m-HMPAO to its hydrophilic form, and thus the converted amount of Tc-99m-HMPAO will be trapped in the alveolar space. Any

remaining lipophilic Tc-99m-HMPAO can penetrate into the BAL cells. Briefly, once Tc-99m-HMPAO reaches the alveolar spaces after i.v. injection, it might be trapped either in the ELF or in the BAL cells. Since the BAL cells are encountered with Tc-99m-HMPAO directly before reaching the blood, the C/P ratio of INH group is higher compared to IV group.

Sasaki et al.,[13] reported that mouse lung contains 1.880 ± 0.067 mM GSH and 1.880 ± 0.087 mM nonprotein thiols, which is approximately 92.6% of levels in the brain. In their study, diethyl maleate treatment caused a significant reduction of both Tc-99m meso HMPAO and Tc-99m-d,l HMPAO (to 68.3% and 79.2%, respectively), in the lungs of the control mice, respectively. Lung was the only organ to show a significant reduction in uptakes of both of the isomers in parallel with GSH depletion, indicating that Tc-99m-HMPAO retention in the lung is related to GSH concentration. El-Shirbiny et al.[20] reported that rat lung contains 1.756 ± 0.189 μM GSH, which is approximately 88.6% of the brain. Though they could not find a significant relation, diethyl maleate treatment caused a reduction in % injected dose per gram of Tc-99m-d,l HMPAO in the lungs, to 63.8% of the control mice, but the control group had a very wide standard deviation.

Cigarette smoking in humans is associated with augmentation of antioxidant enzyme activities[21] and causes oxidant/antioxidant balance alterations in the airspaces.[19] Thus, the air spaces of smokers are exposed to oxidants both from cigarette smoke and those released from inflammatory leukocytes.[19] In normal conditions, we do not observe a substantial amount of Tc-99m-HMPAO accumulation in the lungs. In the presence of oxidants in the alveolar spaces due to smoking or other chemical and radiation hazards, GSH is converted to its oxidized form[22] and the amount of reduced GSH needed to convert Tc-99m-HMPAO into its hydrophilic form will be decreased. Thus, in such an environment, Tc-99m-HMPAO will be more stable and its' lipophilicity will be prolonged,[9] facilitating its diffusion into cells. The increased number of BAL cells in lung inflammation can facilitate cellular incorporation as well. In the study of Suga et al., considerable Tc-99m-HMPAO uptake occurred rapidly in the lungs although the damage to the endothelium was minimal by electron microscopy.[6] We think that trapping of Tc-99m-HMPAO in the ELF or BAL cells depending on the intracellular or extracellular GSH or oxidoreductive state are needed to be considered while explaining the Tc-99m-HMPAO uptake mechanisms in such studies. There is no proposed mechanism in the literature explaining how and where Tc-99m-HMPAO is localized in the endothelial cells after injury. It is not clear in the literature whether the injured endothelial cells themselves are trapping Tc-99m-HMPAO, or the conditions that cause endothelial cell injury or the chemical results of this injury are responsible for the retention of Tc-99m-HMPAO in the lungs. On the contrary, we expect Tc-99m-HMPAO to be more stable in its lipophilic diffusible form following endothelial injury due to the increase of oxidized GSH in the endothelium, and so trapping of Tc-99m-HMPAO in endothelial cells seems less likely.

After inhalation, the C/S_{inh} ratio is similar to the *in vitro* ratio, but the $WBALF/P_{inh}$ ratio is 400-fold higher than in the IV group. In this model, Tc-99m-HMPAO is directly encountered with alveolar cells, ELF, alveolar and bronchial epithelium and mucus. There is a high adhesion of Tc-99m-HMPAO to mucus, which is found to be five times more than the BALF, and the M/P ratio is very similar to C/P_{inh} ratio. We do not have any data on the Tc-99m-HMPAO uptake of alveolar and bronchial epithelium, but it seems that there is considerable uptake in BAL cells. If the inhaled Tc-99m-HMPAO is converted into the nondiffusable hydrophilic form, it will have a long clearance time from the alveolocapillary membrane because of its molecular weight.[23]

In conclusion, our data showed that Tc-99m-HMPAO diffuses into the alveolar spaces after intravenous injection and is present in BALF and cells both after intravenous injection and inhalation. GSH concentration in ELF and BAL cells and the oxido-reductive state of the environment may influence the lipophilicity of the compound and can facilitate its intracellular diffusion or result in Tc-99m-HMPAO trapping in alveolar spaces. Intravenously injected Tc-99m-HMPAO may be the method of choice for detecting lung injury, due to the high adhesion of Tc-99m-HMPAO to the mucus, which can effect the lung clearance rate of Tc-99m-HMPAO.

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