A Comparison of ^{99m}Tc-DTPA and ^{113m}In-DTPA Aerosol Clearances in Humans

Effects of Smoking, Hyperinflation, and *in vitro* Oxidation[1-3]

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SUMMARY As an index of permeability of the alveolar epithelium, the clearance of an inhaled aerosol of ^{99m}Tc-DTPA is increased in several disease states. However, the usefulness of the test to assess the severity of disease is limited because healthy smokers also have abnormally rapid rates of clearance. Because the stability of the ^{99m}Tc-DTPA bond might be a contributory factor, we tested the affinity of ^{99m}Tc for DTPA *in vitro*, and in groups of healthy smokers (n = 13) and nonsmokers (n = 7) we measured the clearances of ^{99m}Tc-DTPA and ^{113m}In-DTPA, which have a similar molecular shape and charge. *In vitro*, sodium hypochlorite or hydrogen peroxide released as much as 98% of free ^{99m}Tc from the ^{99m}Tc-DTPA complex. When incubated with human neutrophils stimulated with phorbol myristate acetate, between 4 and 7% of free ^{99m}Tc-DTPA was released after 30 min, and 12% was released after 60 min. *In vivo*, the clearances of both ^{99m}Tc-DTPA and ^{113m}In-DTPA in the smokers (n = 13) were faster than in the nonsmokers (n = 7) ($p < 0.05$). Within the smokers, the mean ^{99m}Tc-DTPA clearance ($T_{½}$ 25 ± 4 min) was faster than the mean ^{113m}In-DTPA clearance (34 ± 6 min), ($p < 0.05$). For nonsmokers, the difference was smaller ($T_{½}$ ^{99m}Tc-DTPA, 56 ± 6; $T_{½}$ ^{113m}In-DTPA, 62 ± 6) and not significant. During hyperinflation, smokers (n = 8) and nonsmokers (n = 8) both demonstrated an increase in ^{113m}In-DTPA clearance. Although some oxidative dissociation of the ^{99m}Tc-DTPA complex may occur in a smoker's lungs, it is not sufficient to explain the difference in ^{99m}Tc-DTPA clearance between healthy smokers and nonsmokers.

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Introduction

Aerosols of radiolabeled solutes are commonly used to measure the permeability of the alveolar epithelium. The most popular solute is diethylene triamine penta-acetate (DTPA), a low molecular weight chelating agent (MW 393 daltons). After labeling with ^{99m}Tc, DTPA is aerosolized, passed through a particle separator, and inhaled by the subject. The rate of clearance of ^{99m}Tc-DTPA from lung to blood is increased in a variety of situations, including chronic interstitial lung diseases (1), systemic sclerosis (2), hyaline membrane disease (3), and most patients with the adult respiratory distress syndrome (4). However, the clinical value of ^{99m}Tc-DTPA clearance is limited by the fast clearances observed in otherwise healthy smokers (5) and normal volunteers who breathe at high lung volumes (6). Furthermore, smokers fail to show a further increase in their clearance of ^{99m}Tc-DTPA when breathing at high lung volumes (7).

Explanation for these findings is hampered by the lack of definitive information concerning the pathways by which solutes such as ^{99m}Tc-DTPA travel across the alveolar surface and out of the field of the scintillation detector. One potential problem is the stability of the ^{99m}Tc-DTPA complex. With its short half-life and sharp energy peak, ^{99m}Tc is a widely used radionuclide, but its chemistry is complex and poorly understood (8). Most compounds using ^{99m}Tc as a radiolabel require its reduction from the stable $^{99m}TcO_4$ state before it can be complexed successfully with a molecule such as DTPA. However, the stability of the ^{99m}Tc-DTPA complex *in vivo* during exposure to oxidizing agents is unknown. Preliminary data from Huchon and coworkers (9) in dogs suggest that ^{99m}Tc-DTPA has a faster clearance than DTPA complexed with other radiolabels. It is possible that part of the increase in ^{99m}Tc-DTPA clearance seen in patients with lung inflammation and smoke exposure may be due to the clearance of $^{99m}TcO_4$ formed *in vivo*, which has a clearance $T_{½}$ in normal subjects of 10 min compared to 60 min for ^{99m}Tc-DTPA (2).

To investigate ^{99m}Tc-DTPA stability and its effect on alveolar clearance rates, we labeled DTPA with an alternative radiolabel, ^{113m}Indium, which has a higher energy peak than ^{99m}Tc but a shorter radioactive half-life (100 min for ^{113m}In versus 360 min for ^{99m}Tc). The ^{113m}In-DTPA complex has a low dissociation constant (10) and has been used previously for liver scanning (11), brain scanning (12) and the estimation of glomerular filtration rate (13).

In vitro assessments of ^{99m}Tc-DTPA and ^{113m}In-DTPA affinty were performed after challenge with either oxidants or stimulated neutrophils. Then in groups of nonsmokers and healthy smokers, we compared in a randomized order the clearances of ^{113m}In-DTPA and ^{99m}Tc-DTPA. Finally, we measured the effects of hyperinflation on ^{113m}In-DTPA clearance.

Methods

Subjects

Healthy smoking and nonsmoking volunteers from the Royal Postgraduate Medical School were recruited. For the comparison of ^{99m}Tc-DTPA and ^{113m}In-DTPA clearances, 7 nonsmokers (mean age, 33 yr; range, 31 to 37 yr) and 13 smokers (mean age, 38 yr; range, 29 to 50 yr) were studied. For the hyperinflation study, 8 nonsmokers (mean age, 34 yr; range, 26 to 48 yr) and 8 smokers (mean age, 37 yr;

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range, 29 to 50 yr) were studied. None of the subjects had had a recent upper respiratory tract infection. The study was approved by the Hospital Ethics Committee, and all subjects gave informed consent.

Preparation of Radiolabeled DTPA

Sterile bottles containing 9.10 mg of DTPA and 0.45 mg of $SnCl_2$ in a nitrogen atmosphere (Cis (UK) Limited, London, UK) were used for all studies. ^{99m}Tc was eluted from a ^{99}Mo generator with saline and added to the DTPA kit in an oxygen-free atmosphere to form ^{99m}Tc-DTPA with a concentration of 5 mCi/ml. ^{113m}In was eluted from a ^{113}Sn generator with 0.05 M hydrochloric acid and added to the DTPA kit to form ^{113m}In-DTPA at a concentration of 15 mCi/ml. This was then neutralized to pH 7.0 to 7.5 with sodium bicarbonate.

Measurement of Radiolabel-DTPA Affinity

The stability of the radiolabeled-DTPA complex was measured using silica gel thin-layer chromatography as previously described (14). Five μl of a solution of radiolabeled DTPA was placed at the same base of each strip, which was then eluted with a solvent. Using acetone as a solvent, free $^{99m}TcO_4$ traveled with the solvent front and ^{99m}Tc-DTPA remained at the origin. Using water as a solvent, free ^{113m}In stayed at the origin, whereas ^{113m}In-DTPA traveled with the solvent front. After migration of the solvent, the strips were dried, cut into thirds, and counted in a well counter.

Neutrophil Separation and Activation

Samples of heparinized blood were obtained from healthy volunteers. The neutrophils were separated by centrifugation in a dextran gradient (15) and resuspended in albumin. Aliquots of neutrophils were then incubated with radiolabeled DTPA in the presence of phorbol myristate acetate (20 μg/ml) or its diluent. After various times of incubation, percentages of free $^{99m}TcO_4$ and ^{113m}In were determined.

Measurement of Pulmonary DTPA Clearance

We measured the pulmonary clearance of inhaled radiolabeled DTPA as described previously (5), using a particle separator (Venticis; Cis (UK) Limited) to produce an aerosol of particles with a mass median aerodynamic diameter of 0.5 μ. With the nose occluded, each subject inhaled the aerosol through a mouthpiece during tidal breathing for 3 min. Initial count rates were approximately 20,000 cpm for ^{99m}Tc-DTPA and 6,000 cpm for ^{113m}In-DTPA. Radioactivity was then counted for 10 min with a scintillation counter placed over the right anterior chest. After background subtraction (150 to 200 cpm for ^{99m}Tc and 1 to 2 cpm for ^{113m}In), the decreases in corrected lung counts were plotted on a logarithmic scale against time. From a computer-fitted regression line, the half-time clearance ($T_{½}$) of radiolabeled DTPA from lung to blood was calculated and corrected for radioisotopic decay.

Counting Technique

The same scintillation detectors were used for ^{99m}Tc and ^{113m}In, but they were shielded with more than 25 mm of lead. Single-bore collimators (6.5-cm internal diameter), placed up against the chest wall, restricted the counting field to the lung parenchyma. There was negligible contribution from extrapulmonary tissues, assessed by a counter placed over the thigh.

Ventilatory Monitoring

For the hyperinflation study, ventilation was monitored with inductance plethysmography (Respitrace®; Ambulatory Monitoring Systems, Ardsley, NY) calibrated with a single-posture technique as previously described (16). The inductance plethysmograph was operated in the DC mode so that the signals from the bands could be processed to produce a display of lung volume on an oscilloscope. In this way, the subject could monitor his functional residual capacity. At the beginning and end of each study period, the expiratory reserve volume was measured with a spirograph to obtain the difference in lung volumes between the 2 sets of treatment periods. In all subjects, FEV_1, VC, and TLC were obtained by body plethysmography. Supine expiratory reserve volume and VC were obtained from a Collins survey spirometer (Warren E. Collins, Braintree, MA) and corrected to body temperature and pressure.

Protocol

Each subject was studied while supine. For the comparison of ^{99m}Tc-DTPA and ^{113m}In-DTPA clearances, each subject was studied during 2 separate 10-min periods of relaxed breathing. On one day, ^{113m}In-DTPA clearance was measured, and on another day ^{99m}Tc-DTPA clearance was measured. The order was randomized. In the study assessing the effect of hyperinflation on ^{113m}In-DTPA clearance, scintillation counts were recorded for two 10-min periods. During one period, the subject was resting quietly. During the other 10-min period, the subject voluntarily breathed at the highest lung volume he could comfortably maintain for 10 min. The study periods were formally randomized. All subjects who smoked more than 10 cigarettes/day smoked their last cigarette within 1 h of both measurements of radiolabeled DTPA clearance. Those who smoked less than 10 cigarettes/day had smoked within the 24 h preceding the measurement. Mixed expired CO concentrations were measured in the final 8 smokers with an electrochemical sensor (Ecolyser, Energetics Science Inc., Elmsford, NY).

Statistical Analysis

Analysis of variance for unbalanced design (General Linear Interactive Modeling [GLIM], Royal Statistical Society) was used, and a value of $p < 0.05$ was accepted as being statistically significant. Values are presented as means ± standard error.

Results

In Vitro Analysis

The percentages of ^{99m}Tc-DTPA dissociation after challenge with sodium hypochlorite or hydrogen peroxide are listed in table 1. With either agent, free ^{99m}Tc was dissociated from DTPA in a dose-dependent manner to a maximum of 98% at the highest concentrations. There was no detectable dissociation of

TABLE 1

DISSOCIATION OF ^{99m}Tc-DTPA SOLUTIONS BY OXIDANTS *IN VITRO*

Experiment No.	Substance	Concentration (*mM*)	Free Tc (%)
1	Diluent	–	0.9
	Sodium hypochlorite	270*	96.3
2	Diluent	–	0.6
	Hydrogen peroxide	0.15	0.6
		15	3.6
		150	88.4
3	Diluent	–	0.1
	Sodium hypochlorite	1.4	89.6
		14	89.8
		140	90.4
	Hydrogen peroxide	15	94.3
		150	97.7
4	Diluent	–	0.1
	Sodium hypochlorite	0.14	0.9
		1.4	0.4
		14	76.9
		140	85.1
	Hydrogen peroxide	0.15	13.1
		1.5	46.6
		15	97.1
		150	97.7

* All sodium hypochlorite concentrations are expressed as mM free chlorine.

TABLE 2

DISSOCIATION OF ^{99m}Tc-DTPA SOLUTIONS BY NEUTROPHILS STIMULATED WITH PHORBOL MYRISTATE ACETATE (PMA) COMPARED WITH CONTROLS

Experiment No.	Neutrophils/ml	Content	Incubation Time (*min*)	Free Tc (%)
1	16.6×10^6	Diluent	30	0.3
		Cells alone	30	0.1
		Cells + PMA	30	4.2
2	13.3×10^6	PMA alone	60	0.1
		Cells alone	30	0.1
		Cells alone	60	0.1
		Cells + PMA	0	0.2
		Cells + PMA	30	7.4
		Cells + PMA	60	12.1
3	10×10^6	PMA alone	60	0.1
		Cells alone	60	1.6
		Cells + PMA	0	1.4
		Cells + PMA	15	6.1
		Cells + PMA	30	4.7
		Cells + PMA	60	6.1

^{113m}In-DTPA when subjected to the same concentrations of oxidizing agents.

In table 2, 3 studies are summarized of ^{99m}Tc-DTPA affinity after incubation at 37°C for varying periods of time with neutrophils and phorbol myristate acetate (PMA). When activated by PMA, the neutrophils dissociated between 4 and 7% of the ^{99m}Tc-DTPA after 30 min, and as much as 12% after 60 min. No dissociation of ^{113m}In-DTPA was produced under similar conditions of incubation.

In Vivo *Results*

For the comparison of ^{99m}Tc-DTPA and ^{113m}In-DTPA clearances, table 3 lists lung volumes, cigarette consumption, and $T_{1/2}$ values for each subject. Mixed expired carbon monoxide concentrations were 12.6 ± 3 ppm prior to the measurement of ^{99m}Tc-DTPA clearance, and 12.6 ± 2 ppm prior to ^{113m}In-DTPA clearance. Individual half-times of clearance are shown graphically in figure 1. The mean ^{113m}In-DTPA clearance for the smokers was significantly faster than that of the nonsmokers ($p < 0.05$). Within the smoking group, but not the nonsmoking group, the mean ^{113m}In-DTPA clearance was significantly slower than the ^{99m}Tc-DTPA clearance ($p < 0.05$). This difference remained significant even when the data from the smoker with the slowest clearance of ^{113m}In-DTPA (Subject 12) was excluded from the analysis. Also, subjects who were younger and smoked less heavily tended to have slower clearances of both forms of DTPA.

In the hyperinflation study, the mean values of FEV_1, VC, TLC, and FRC for the nonsmokers (3.7 ± 0.4, 4.5 ± 0.5, 6.5 ± 0.7, and 2.7 ± 0.3 L, respectively) tended to be higher than the corresponding values for the smokers (3.0 ± 0.3, 4.0 ± 0.3, 5.8 ± 0.4, and 2.2 ± 0.2 L, respectively), although the values were not statistically different. Likewise, the mean difference in FRC between the 2 study periods for the nonsmokers (1.7 ± 0.3 L) was not statistically different from the 1.2 ± 0.3 L obtained by the smokers. Individual data points for ^{113m}In-DTPA clearance are shown in Figure 2. There was a marked overlap in baseline clear-

TABLE 3

AGE, FEV_1, VC, RATIO OF FEV_1 TO VC, CIGARETTE USE, AND CLEARANCE HALF-TIMES FOR ^{113m}In-DTPA AND ^{99m}Tc-DTPA

	Age (*yr*)	FEV_1 (*L*)	VC (*L*)	FEV_1/VC	Cig/day	^{113m}In-DTPA ($T_{1/2}$ *min*)	^{99m}Tc-DTPA ($T_{1/2}$ *min*)
Nonsmokers							
1	32	3.7	4.7	0.79	None	60	48
2	31	3.6	4.6	0.77	None	86	85
3	32	2.7	3.8	0.72	None	61	56
4	36	3.6	5.1	0.70	None	32	42
5	37	4.4	5.4	0.82	None	54	59
6	32	3.5	4.2	0.83	None	74	62
7	34	5.9	7.4	0.80	None	64	31
Mean	33.4	3.92	5.02	0.78		61.8	54.6
SE	0.9	0.38	0.44	0.02		6.3	6.5
Smokers							
1	55	2.6	4.0	0.64	35	14	14
2	49	2.6	4.2	0.62	20	21	15
3	19	3.5	5.0	0.70	15–20	59	54
4	60	1.6	2.7	0.56	10–20	23	23
5	20	4.2	5.6	0.75	25–30	39	25
6	29	4.1	5.0	0.82	20	33	19
7	46	3.7	4.9	0.76	30	8	8
8	55	1.8	3.7	0.47	20–30	15	17
9	17	4.1	5.2	0.80	10–15	29	32
10	32	3.9	4.2	0.90	20	13	8
11	31	3.8	4.5	0.84	10	57	29
12	50	2.9	3.4	0.85	10–15	83	49
13	29	2.4	3.0	0.80	5–10	52	32
Mean	37.8	3.16	4.27	0.73		34.4	25.0
SE	4.2	0.25	0.24	0.03		6.3	4.0

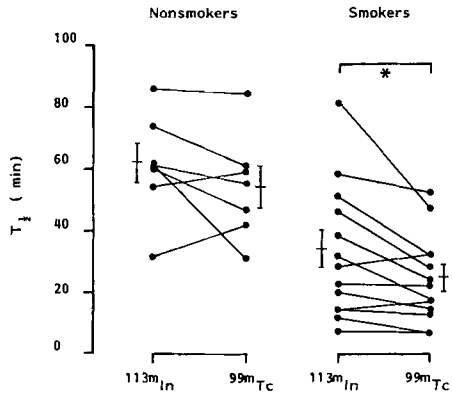

Fig. 1. Clearance half-times in minutes for nonsmokers and smokers. Each pair of data points represents the clearance for ^{113m}In-DTPA (*left*) and ^{99m}Tc-DTPA (*right*). Means ± SE. Asterisk indicates $p < 0.05$.

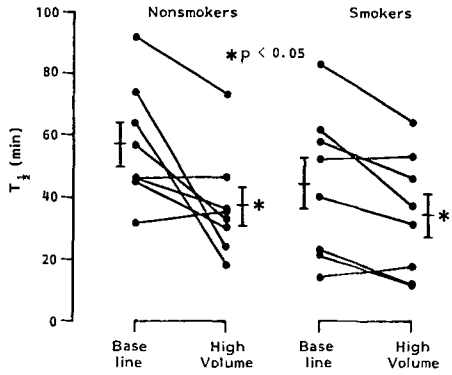

Fig. 2. Clearance half-times for ^{113m}In-DTPA for nonsmokers and smokers at baseline (*left*) and while breathing at a high lung volume (*right*). Means ± SE. Asterisk indicates $p < 0.05$ compared to baseline.

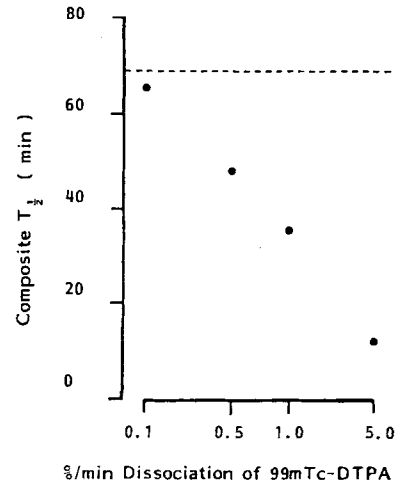

Fig. 3. The composite half-times of clearance ($T_{½}$) in minutes for ^{99m}Tc after inhalation of ^{99m}Tc-DTPA, assuming various hypothetical rates of dissociation of $^{99m}TcO_4$ from ^{99m}Tc-DTPA. $T_{½}$ in absence of dissociation (*interrupted line*) is 72 min.

ances between the 2 groups. Both groups also showed a significant decrease in $T_{½}$ when breathing at a high lung volume ($p < 0.05$). For the nonsmokers, the mean $T_{½}$ decreased from 57.1 ± 6.9 min to 36.8 ± 6.0 min. For the smokers, the mean $T_{½}$ decreased from 44.1 ± 8.3 min to 34.2 ± 7.0 min.

Discussion

The increased clearance of inhaled ^{99m}Tc-DTPA in smokers compared with that in nonsmokers has not been adequately explained. It is surprising that the increased clearance values seen in asymptomatic smokers approach those found in acutely ill patients with ARDS (17). On the other hand, in light to moderate cigarette smokers, the clearance returns to virtually normal values within 3 wk of stopping smoking (18). The findings in the current study suggest that instability of the Tc-DTPA complex plays only a minor role in the increased clearance seen in smokers. As shown in table 3, the clearance of the much more stable In-DTPA complex in smokers ($T_{½}$, 34 ± 6 min) was still much faster than in the nonsmokers ($T_{½}$, 62 ± 6). Nevertheless, there was a relatively greater difference between the Tc-DTPA and In-DTPA clearances in smokers (9.4 min, 27%) than in nonsmokers (7.2 min, 12%). This difference was significant only in the smoking group. The nonsmoking group was smaller in number (7 against 13), and a statistically significant difference (though smaller than that seen in smokers) between In-DTPA and Tc-DTPA might exist in a larger group.

The difference between ^{113m}In-DTPA and ^{99m}Tc-DTPA clearance could be explained in several ways. With isoelectric focusing, no differences in charge have been found *in vitro*, nor have any differences in molecular shape been revealed by viscometry (J.S. Fleming and D. Royston, personal communication). Ultrasonic nebulization can disrupt the ^{99m}Tc-DTPA complex (19), but this effect should occur equally in nonsmokers and smokers. The *in vitro* experiments (tables 1 and 2) and the relatively greater difference in the smoking group suggested that oxidative dissociation of ^{99m}Tc-DTPA might be occurring *in vivo*. From the mean $T_{½}$ values in table 3, the percentage of ^{99m}Tc present as $^{99m}TcO_4$ and $^{99m}TcO_2$ which is required in theory to explain the differences between the $T_{½}$ for ^{113m}In-DTPA and ^{99m}Tc-DTPA, can be calculated, assuming a $T_{½}$ for $^{99m}TcO_4$ of 10 min (2). The effect on the overall clearance of increasing the fraction of TcO_4 is shown in figure 3 for a lung with a ^{99m}Tc-DTPA clearance half-time of 72 min. The calculated combined clearance of TcO_4 and Tc-DTPA remained linear ($r = 1.0$) for at least 10 min. The increase in $T_{½}$ clearance for the ^{99m}Tc-labeled DTPA compared to ^{113m}In in smokers ($T_{½}$, 34.4, and 25 min, respectively) can be explained in theory by a constant fractional dissociation of TcO_4 from Tc-DTPA of 1.8% min^{-1}; for nonsmokers the value is 0.4% min^{-1}. Nevertheless, this difference is insufficient to fully explain the faster ^{99m}Tc-DTPA clearance in the smoking group.

Sampson and associates (20) have shown that in air, but not in a lower oxygen environment, ^{99m}Tc is gradually oxidized and released as TcO_4 from its complex with DTPA. In the current study, the addition of oxidizing agents released as much as 98% as free $^{99m}TcO_4$. *In vivo*, ^{99m}Tc-DTPA injected intravenously is relatively immune to oxidation because of the presence of antioxidants in the blood (21). Nevertheless, the alveolar surface and interstitium of the lung is likely to be a more fertile environment for the generation of oxidant species, especially when infiltrated with activated cells (22). For example, Johnson and coworkers (23) have estimated that as much as 100 nmol of hydrogen peroxide and 500 nmol of superoxide anion may be released from activated neutrophils into lung tissue over a 4-h period. More recently, Winterbourn and colleagues (24) have calculated that intracellular hydrogen peroxide within neutrophils may reach concentrations as high as 100 mM, which is within the range of concentrations used in our *in vitro* studies (table 1). Although the PMA-activated neutrophils (table 2) only caused ^{99m}Tc to dissociate at a rate of approximately 0.2% min^{-1}, i.e., less than that required in theory to explain the *in vivo* differences (0.4 to 1.8% min^{-1}), the local tissue concentration of activated neutrophils in the lungs of smokers is still a matter of conjecture.

There is some suggestive evidence that compared to nonsmokers, smokers have increased oxidant activity in their lungs. For example, neutrophil counts are elevated in lung lavage fluid (25) and in macerated lung tissue (26). Smokers also have increased numbers of macrophages in lavage fluid and histologic sections (27). *In vitro*, phagocytic cells obtained from smokers by bronchoalveolar lavage produce a greater amount of oxidant species (28–30).

Increases in lung volume accelerate the clearance of ^{99m}Tc-DTPA in nonsmokers possibly because of pore-stretching (6). Nevertheless, smokers do not increase their ^{99m}Tc-DTPA clearance with hyperinflation (7). This study (figure 2) shows

that DTPA transfer is accelerated by hyperinflation in both smokers and nonsmokers if DTPA is labeled with ^{113m}In. Thus, the clearance of ^{99m}Tc-DTPA in smokers differs qualitatively as well as quantitatively from that observed in nonsmokers.

In conclusion, ^{99m}Tc-DTPA is highly susceptible to oxidative dissociation *in vitro*. The difference between ^{99m}Tc-DTPA and ^{113m}In-DTPA clearances in smokers suggests, albeit indirectly, that dissociation might occur *in vivo* also. If direct proof for this contention were gained, the difference in clearance might in itself serve as an indicator of the lung's oxidant burden; further investigation of ^{99m}Tc-DTPA and ^{113m}In-DTPA differences in the adult respiratory distress syndrome and chronic inflammatory disorders (alveolitis) would be of interest.

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