



Effects of positive expiratory pressure on pulmonary clearance of aerosolized technetium-99m-labeled diethylenetriaminepentaacetic acid in healthy individuals

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INTRODUCTION

The alveolar-capillary barrier, also known as the blood-gas barrier, is excellent at maintaining the separation between alveolar air and pulmonary capillary blood, allowing rapid, efficient exchange of respiratory gases while preventing the diffusion of water-soluble particles suspended in alveolar air.^(1,2) The integrity of the blood-gas barrier is extremely important to the maintenance of pulmonary homeostasis. In 1953, Frank Low published the first high-resolution electron micrographs of the human pulmonary blood-gas barrier, showing that a structure only 0.3 μm thick separates capillary blood from alveolar gas, which suggested that the barrier

might be vulnerable to mechanical failure if the capillary pressure increased⁽³⁾ or in states of high lung inflation, in which the capillary wall is under tension because of longitudinal stress in the alveolar walls.⁽⁴⁾

Technetium-99m-labeled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) radioaerosol inhalation lung scintigraphy is a rapid, easily performed, extremely sensitive, noninvasive technique for assessing lung epithelial permeability.^(5,6) When inhaled, ^{99m}Tc-DTPA particles arrive at the alveolar epithelial surface and then diffuse from the air space into the vascular space. The clearance rate of ^{99m}Tc-DTPA is a reliable index of alveolar epithelial permeability. Therefore, ^{99m}Tc-DTPA aerosol-inhaled scintigraphy has been used in numerous

ABSTRACT

Objective: To evaluate the effects of positive expiratory pressure (PEP) on pulmonary epithelial membrane permeability in healthy subjects. **Methods:** We evaluated a cohort of 30 healthy subjects (15 males and 15 females) with a mean age of 28.3 ± 5.4 years, a mean FEV₁/FVC ratio of 0.89 ± 0.14 , and a mean FEV₁ of $98.5 \pm 13.1\%$ of predicted. Subjects underwent technetium-99m-labeled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) radioaerosol inhalation lung scintigraphy in two stages: during spontaneous breathing; and while breathing through a PEP mask at one of three PEP levels—10 cmH₂O (n = 10), 15 cmH₂O (n = 10), and 20 cmH₂O (n = 10). The ^{99m}Tc-DTPA was nebulized for 3 min, and its clearance was recorded by scintigraphy over a 30-min period during spontaneous breathing and over a 30-min period during breathing through a PEP mask. **Results:** The pulmonary clearance of ^{99m}Tc-DTPA was significantly shorter when PEP was applied—at 10 cmH₂O (p = 0.044), 15 cmH₂O (p = 0.044), and 20 cmH₂O (p = 0.004)—in comparison with that observed during spontaneous breathing. **Conclusions:** Our findings indicate that PEP, at the levels tested, is able to induce an increase in pulmonary epithelial membrane permeability and lung volume in healthy subjects.

Keywords: Lung/metabolism; Technetium Tc 99m pentetate/pharmacokinetics; Radiopharmaceuticals; Positive-pressure respiration.

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experiments and clinical investigations designed to assess the integrity of the respiratory epithelium.^(7,8)

Positive expiratory pressure (PEP) therapy involves breathing with slightly active expiration against mild expiratory resistance (typically 10-20 cmH₂O).⁽⁹⁾ Its application via a PEP mask represents a reliable, safe, low-cost intervention that can increase lung volumes and intrathoracic pressure.^(10,11) It has been shown to improve lung volume, promote dilation of the airways, and decrease pulmonary resistance. The purpose of PEP therapy is to increase the transpulmonary pressure gradient and improve pulmonary expansion, which consequently improves oxygenation and the response to inhaled bronchodilators.^(12,13)

The effects of different intensities of PEP on the pulmonary clearance of ^{99m}Tc-DTPA in healthy subjects have been largely unexplored in the literature and require further elucidation. Therefore, the aim of the present study was to evaluate the effects of PEP on pulmonary epithelial membrane permeability in healthy subjects.

METHODS

We studied 30 healthy subjects (15 men and 15 women). The mean age was 28.3 ± 5.4 years. Subjects with cardiovascular or neuromuscular disease were excluded, as were those with a history of smoking or respiratory disease, as well as those who were pregnant. All participants were recruited from a tertiary care hospital in the city of Porto Alegre, Brazil, and were evaluated between January and July of 2012. The study was approved by the Research Ethics Committee of the Porto Alegre *Hospital de Clínicas* (Protocol no. 04-418), which is accredited by the Brazilian National Commission on Research Ethics, and all subjects gave written informed consent.

Pulmonary scintigraphy with ^{99m}Tc-DTPA radioaerosol was conducted over two 30-min periods: during spontaneous breathing; and while subjects were breathing via a PEP mask (Vital Signs/GE Healthcare, Totowa, NJ, USA), which we adapted to fit each subject by using headgear, coupled to a PEP therapy device (Resistex®; Mercury Medical, Clearwater, FL, USA). Using computer-generated randomization codes, we divided the subjects into three groups: those receiving PEP at 10 cmH₂O (PEP₁₀); those receiving PEP at 15 cmH₂O (PEP₁₅); and those receiving PEP at 20 cmH₂O (PEP₂₀). During data acquisition, the subjects were in a sitting position with their hands on their thighs and their arms held away from their body. For each subject, the spontaneous breathing period served as the control.

A spirometer (MasterScreen v4.31; Jaeger, Würzburg, Germany) was used in order to measure FEV₁ and FVC. The technical procedures, acceptability criteria, and reproducibility criteria, as well as standardization of measures, were in accordance with the guidelines established by the Brazilian Thoracic Association.⁽¹⁴⁾ Subjects received instruction regarding the procedures

to be performed during the spirometry assessment. The spirometer was routinely calibrated (on a daily basis) with a 3-L syringe in order to compensate for ambient temperature conditions. A minimum of three and a maximum of eight tests were conducted with a 1-min interval between them. Three reproducible maneuvers were performed, and the best curve was considered for the study. Results are expressed as absolute and percent-of-predicted values.⁽¹⁵⁾

The ^{99m}Tc-DTPA was chelated by adding ^{99m}Tc-pertechnetate (^{99m}Tc-O₄⁻, IPEN-TEC; Institute for Energy Research and Nuclear Science, São Paulo, Brazil) to 740 MBq (20 mCi) of DTPA (Institute for Energy Research and Nuclear Science) in 5 mL of normal saline. Using instant thin layer chromatography, we determined the labeling efficiency to be above 98%. The solution was placed in the reservoir of the nebulizer (Aerogama Medical, Porto Alegre, Brazil) and was inhaled by the volunteer during 3 min of normal tidal breathing, with an oxygen flow rate of 9 L/min. During nebulization, the subjects remained under supervision, which allowed us to verify the appropriate performance of the inhalation maneuvers, as well as to correct any errors in inhalation techniques. The subjects were placed in a sitting position in front of a gamma camera (Starcam 4000i; GE Medical Systems, Milwaukee, WI, USA), and images were obtained every 20 s over a 30-min period. Two regions of interest were defined—the left lung and the right lung—and were drawn manually, a time-activity curve being constructed by the same investigator. For each lung, we employed the negative slope of the curve to define clearance, using the minimum and maximum clearance values. The ^{99m}Tc-DTPA clearance rate was expressed as its half-time (T_{1/2})—i.e., the time for its activity to decrease to 50% of the peak value.

All statistical analyses were performed with the SPSS Statistics software package, version 20.0 (IBM Corp., Armonk, NY, USA). The normality of the variables was assessed with the Shapiro-Wilk test. Categorical data are presented as absolute and relative frequencies. Continuous data with normal distribution are expressed as means and standard deviations. Anthropometric variables and lung function parameters were compared among groups by one-way ANOVA (with the exception of gender, which was compared by the chi-square test). The difference in T_{1/2} among groups was determined with one-way analysis of covariance, with body weight, height, and body mass index (BMI) as the dependent variables. The influence of the pressure levels on T_{1/2} was compared among the groups by two-way ANOVA. The level of statistical significance was set at 5% (p<0.05).

RESULTS

We evaluated 30 healthy subjects (15 males and 15 females), with a mean age of 28.26 ± 5.40 years, a mean FEV₁/FVC ratio of 0.89 ± 0.14, and a mean FEV₁ of 98.5 ± 13.1% of the predicted value. The anthropometric and lung function parameters are presented in Table 1. The spirometric parameters were

similar among the three study groups. Body weight, height, and BMI were higher in the PEP₂₀ group than in the PEP₁₅ group ($p = 0.038$, $p = 0.027$, and $p = 0.015$, respectively). The ^{99m}Tc-DTPA pulmonary clearance rate was not found to correlate significantly with age ($r = -0.120$; $p = 0.951$), body weight ($r = 0.115$; $p = 0.545$), height ($r = 0.085$; $p = 0.655$), or BMI ($r = 0.120$; $p = 0.528$). In the analysis of the results related to the ^{99m}Tc-DTPA pulmonary clearance rate, we considered the mean values for the left and right lungs together, given that the difference between the two lungs was not found to be statistically significant in the PEP₁₀ group ($p = 0.258$), the PEP₁₅ group ($p = 0.908$), or the PEP₂₀ group ($p = 0.570$).

In comparison with the value obtained during spontaneous breathing, the mean $T_{1/2}$ was significantly lower in the PEP₁₀ group— 90.3 ± 25.4 min versus 73.3 ± 30.6 min ($p = 0.044$), as can be seen in Figure 1. That difference was also significant in the PEP₁₅ group— 89.8 ± 28.9 min versus 63.1 ± 22.1 min ($p = 0.044$)—and in the PEP₂₀ group— 99.3 ± 49.6 min versus 64.5 ± 29.6 min ($p = 0.004$). Regarding the delta variation in $T_{1/2}$ values, no statistically significant difference was found among the groups ($p = 0.322$).

DISCUSSION

To our knowledge, this was the first study to investigate the effects that different levels of PEP (10, 15, and 20 cmH₂O) have on the rate of pulmonary clearance of ^{99m}Tc-DTPA. We demonstrated that PEP was able to induce increases in epithelial permeability, at all of the levels tested. Another important finding is that no significant variation in pulmonary clearance rate was observed among the three groups under the effects of any of the three PEP levels.

The effect of positive end-expiratory pressure (PEEP) on solute clearance has been described as a sigmoidal dose-response relationship dependent on the pressure level applied (5-15 cmH₂O); in other words, the rate of pulmonary clearance of ^{99m}Tc-DTPA accelerates exponentially due to the increase in lung volume caused by the administration of different PEEP

levels.⁽¹⁶⁾ Contrary to our hypothesis, we did not find any differences among the three levels of PEP. In accordance with our results, the study conducted by Bishai et al.⁽¹⁷⁾ showed an increase in the pulmonary epithelial permeability in mice at a PEEP level of 10 cmH₂O. This difference in pulmonary clearance might be because mice have smaller alveoli and are probably more sensitive to the distension of inter-epithelial junctions of the alveolar epithelium induced by the application of lower pressure levels.

Our findings differ from those reported by Paiva et al.,⁽¹⁸⁾ who assessed the permeability of the alveolar

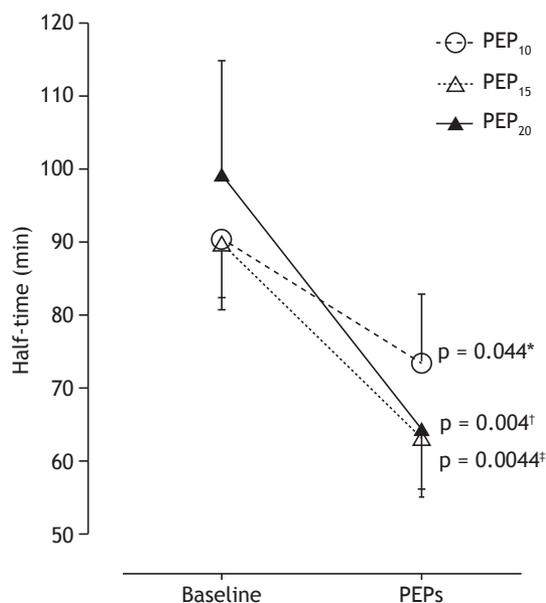


Figure 1. Effects of positive expiratory pressure (PEP) on the pulmonary clearance of technetium-99m-labeled diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA). At all PEP levels, there was a significant decrease in the ^{99m}Tc-DTPA half-time (the time for its activity to decrease to 50% of the peak value) in comparison with the baseline (spontaneous breathing) values (analysis of covariance, $p < 0.05$ for all). PEP₁₀: PEP at 10 cmH₂O for 30 min; PEP₁₅: PEP at 15 cmH₂O for 30 min; and PEP₂₀: and PEP at 20 cmH₂O for 30 min. *PEP₁₀. †PEP₂₀. ‡PEP₁₅.

Table 1. Baseline characteristics of the study groups.^a

Variable	PEP group			p*
	PEP10 (n = 10)	PEP15 (n = 10)	PEP20 (n = 10)	
Male gender, n (%)	6 (60)	2 (20)	7 (70)	0.061
Age, years	27.7 ± 5.1	30.4 ± 5.9	26.6 ± 5.1	0.286
Weight, kg	70.7 ± 13.6	60.4 ± 5.2	76.5 ± 11.5	0.009†
Height, cm	173 ± 7.7	165.5 ± 5.5	176.1 ± 8.5	0.017‡
BMI, kg/m ²	23.4 ± 2.8	22.1 ± 1.5	24.5 ± 2.1	0.004§
FVC, % of predicted	99.5 ± 15.9	97.1 ± 17.8	99.4 ± 18.5	0.347
FEV1, % of predicted	97.8 ± 12.3	99.3 ± 12.6	98.6 ± 14.4	0.356

^aValues are expressed in mean ± SD, except where otherwise indicated. PEP: positive expiratory pressure; PEP₁₀: PEP at 10 cmH₂O for 30 min; PEP₁₅: PEP at 15 cmH₂O for 30 min; PEP₂₀: PEP at 20 cmH₂O for 30 min; and BMI: body mass index. *Between-group comparisons. †Significant difference between the PEP₁₅ group and the PEP₂₀ group ($p = 0.038$). ‡Significant difference between the PEP₁₅ group and the PEP₂₀ group ($p = 0.027$).

§Significant difference between the PEP₁₅ group and the PEP₂₀ group ($p = 0.015$).

epithelial membrane in 36 healthy individuals submitted to 10 cmH₂O and 20 cmH₂O under continuous positive airway pressure (CPAP). The authors showed that 20 cmH₂O of CPAP induced an increase in epithelial permeability, whereas 10 cmH₂O of CPAP did not.

Previous studies in humans,⁽¹⁹⁾ sheep,^(20,21) and dogs^(22,23) showed that the application of PEEP increases lung volume and accelerates the rate of pulmonary clearance of ^{99m}Tc-DTPA. Therefore, the increased end-expiratory lung volume produced by PEP reduces airway resistance and promotes an increase in functional residual capacity.⁽²⁴⁾ However, other factors, such as airway/alveolar distension, prevention of alveolar collapse during expiration, and recruitment of collapsed alveoli, can also increase functional residual capacity.⁽²⁵⁾

In keeping with the findings of the present study, Suzuki et al.⁽²⁶⁾ observed that, during the application of 20 cmH₂O of PEEP, pulmonary clearance of ^{99m}Tc-DTPA increased but returned to baseline values when PEEP was discontinued. That suggests that the increase in the ^{99m}Tc-DTPA pulmonary clearance rate achieved through the application of PEEP is reversible after breathing returns to atmospheric pressure levels.

The mechanisms by which pulmonary insufflation accelerates the pulmonary clearance of ^{99m}Tc-DTPA

remain unclear. Some authors attribute this effect to an increase in the alveolar surface area available for diffusion,⁽²²⁾ due to either the increase in epithelial permeability,⁽²⁷⁾ functional alterations in the surfactant layer,⁽²⁸⁾ or distension of the intercellular junctions of the alveolar epithelium.⁽²⁹⁾

The present study has some limitations. First, we evaluated subjects with healthy lungs. Therefore, any conclusions drawn from this study cannot be applied to patients with lung disease. This needs to be investigated in different, clearly specified disease groups, especially because PEP is not usually applied in patients with healthy lungs. Second, the upper airway critical closing pressure was not measured. Nevertheless, our findings offer new perspectives on the role of noninvasive ventilation, particularly in relation to PEP and its influence on the alveolar epithelium.

On the basis of the findings of the present study, we conclude that the application of 10, 15, or 20 cmH₂O of PEP for 30 min can induce an increase in epithelial permeability and lung volume in healthy subjects. Given that the application of PEP represents a reliable, safe, and low-cost intervention in a variety of clinical situations, future investigations should be conducted with a focus on expanding upon these findings.

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