Comparison of Lung Vascular and Epithelial Permeability Indices in the Adult Respiratory Distress Syndrome[1-3]

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SUMMARY Measurements of pulmonary clearance of inhaled ^{99m}Tc-DTPA and transvascular ^{113m}In-transferrin flux were made in 12 patients with established ARDS and 14 volunteer control subjects (7 smokers and 7 nonsmokers). Smokers had significantly increased ^{99m}Tc-DTPA clearance (clearance rate constant, 3.6 ± 0.8; mean ± SEM) compared with nonsmokers (1.2 ± 0.1). All patients with ARDS had increased clearance of ^{99m}Tc-DTPA (5.2 ± 0.9), but the finding was nonspecific in that increased clearance overlapped with the findings in normal smokers. Protein flux in smokers (protein flux units, 0.0 ± 0.2) was similar to that in nonsmokers (0.3 ± 0.2). In 9 of the 12 patients with ARDS, protein flux was increased, and as a group (3.2 ±1.0) they differed significantly ($p < 0.01$) from the combined smoking and nonsmoking control subjects (0.2 ± 0.1, n=14). The parameters of DTPA clearance and transvascular protein flux correlated well in the patients with ARDS (Spearman's rank correlation = 0.71, $p < 0.01$). Although ^{99m}Tc-DTPA clearance is a sensitive technique in ARDS, a single study in this context does not allow a diagnostic conclusion because of its nonspecificity. Abnormal protein flux appears to be more specific for ARDS but was not a universal finding in the patients studied.

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Introduction

The adult respiratory distress syndrome (ARDS) is a form of acute lung injury defined by fulfillment of radiologic and physiologic criteria. The pathologic correlate of this clinical syndrome is severe and usually diffuse damage to alveolar-capillary units, with accumulation of protein-rich pulmonary edema fluid. The immediate result of damage to these units is increased transvascular fluid and solute flux, which, if lymphatic clearance is overcome, leads to pulmonary edema. Thus, in mild or early pulmonary injury, lung water itself may not be increased (1). Likewise, early in the development of lung injury, conventional measurements of gas exchange or lung mechanics are poor predictors of the specific abnormalities that will produce edema (2).

Despite modern intensive care management, mortality in ARDS has remained high, exceeding 60% in a recent series (3). The development of relatively noninvasive methods of examining alveolar capillary barrier function may improve prognosis because of earlier diagnosis and more accurate monitoring of the effects of treatment. We have compared indices of permeability of both components of the alveolar-capillary barrier in order that the specificity and sensitivity of the 2 techniques could be assessed. Previous studies of lung permeability in ARDS have examined either transvascular protein flux (4) or the clearance of an inhaled, radiolabeled, low molecular weight solute, ^{99m}Tc diethylene triamine pentacetate (DTPA) (5) as indices of endothelial and epithelial injury, respectively. DTPA clearance is increased in this syndrome, but this finding is not specific to ARDS as increased clearance has been reported in otherwise normal smokers (6), patients with interstitial lung disease (7), and in normal subjects while breathing with 10 cm H_2O of positive end expiratory pressure (PEEP) (8). No previous study has, however, compared the extent of increased DTPA clearance in ARDS with that found in normal smokers. In view of this and in an attempt to define the relationship of DTPA clearance to macromolecular vascular leakage, we studied both indices in a group of patients with ARDS as well as in normal smokers and nonsmokers.

In this study, endothelial injury was assessed by measuring the transvascular flux of ^{113m}In-transferrin, corrected for changes in pulmonary blood volume using ^{99m}Tc-labeled red blood cells. Previous studies in patients with ARDS using a similar approach had suggested that the determination of protein flux was both sensitive and specific. These studies, however, had possible sources of error introduced by either not monitoring changes in pulmonary blood volume (4) or relying on only 1 scintillation detector to study 2 regions of interest (9).

Methods

Subjects

Twelve consecutive nonsmoking patients admitted to the Intensive Care Unit were studied as soon as they fulfilled the following criteria for the diagnosis of ARDS: (*1*) sudden onset of acute respiratory failure requiring mechanical ventilation; (*2*) diffuse airspace infiltrates on chest radiograph; (*3*) ratio of arterial to alveolar partial pressure of oxygen less than 0.25; (*4*) total static thoracic compliance less than 30 ml/cm H_2O; (*5*) pulmonary capillary wedge pressure less than 16 mmHg when measured. Clinical details are summarized in table 1.

Seven smoking and 7 nonsmoking volunteers (respective age ranges, 23 to 33 and 22 to 41 yr) were studied as a control group. All were healthy and none had a recent history of upper respiratory tract infection or medication use. The study was approved by the Hospital Ethical Committee, and subjects or their next of kin gave informed consent.

Pulmonary DTPA Clearance

Clearance of inhaled ^{99m}Tc-DTPA was measured as previously described (10). Briefly, an aerosol of DTPA was generated with a jet nebulizer equipped with a particle separator that produces particles with a mass median aerodynamic diameter of 0.5 μm (Cis Limited, London, UK). In normal subjects, the nose was occluded, and each subject inhaled the aerosol for 2 to 3 min. In the ARDS group, radioactivity was introduced by manual ventilation using a nonrebreathing system. Radioactivity was counted with 2 scintillation de-

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TABLE 1

CLINICAL DETAILS FROM THE PHYSIOLOGIC AND PERMEABILITY STUDIES OF PATIENTS WITH ARDS

Patient No.	Age (yr)	Sex	Diagnosis	Physiologic Status at Time of Study: Arterial/Alveolar P_{O_2} Ratio	Physiologic Status at Time of Study: Total Thoracic Compliance (ml/cm H_2O)	Physiologic Status at Time of Study: PCWP (mmHg)	Permeability Indices: Rate Constant K (DTPA)	Permeability Indices: Flux Units (protein)
1	28	M	Post-BMT (no pulmonary pathogen)	0.14	14	10	6.9	9.5
2	53	F	Laporotomy x 3; transfusion > 100 units	0.18	20	CVP, 3 cm	1.9	-0.9
3	22	F	Post-BMT (cytomegalovirus)	0.15	17	12	3.8	3.2
4	72	F	Postcardiopulmonary bypass	0.23	22	15	2.6	1.6
5	26	F	Post-BMT (Herpes simplex)	0.19	24	11	3.7	0.2
6	27	M	Post-BMT (no pathogen)	0.18	17	CVP, 4 cm	3.3	-0.3
7	48	M	Inf. endocarditis; ARDS postcardiopulmonary bypass for valve replacement	0.15	20	4	4.6	4.1
8	19	M	Postoperative aspiration	0.18	23	10	5.8	7.6
9	28	M	Post-BMT (no pathogen)	0.15	14	CVP, 3 cm	7.9	1.6
10	74	M	Acute fibrosing alveolitis (Hamman–Rich)	0.10	18	CVP, 2 cm	4.9	2.1
11	70	M	Acute myocardial infarction with renal failure and sepsis	0.09	29	10	3.9	1.6
12	23	M	Post-BMT (no pathogen)	0.10	20	CVP, 6 cm	13.3	8.5

Definition of abbreviations: PCWP = pulmonary capillary wedge pressure; BMT = bone marrow transplant; CVP = central venous pressure (measured from anterior axillary line).

tectors placed over the right anterior chest and right thigh, and an intravenously administered dose of ^{99m}Tc-DTPA (100 μCi) was used for correction of blood and tissue background as previously described (10). The decrease in corrected lung counts was plotted semilogarithmically against time. A regression line was fitted by computer, from which the clearance rate constant *K* (percent decline in activity per minute) was derived.

Transvascular Protein Flux

This technique modified from Gorin and coworkers (11), and more recently described by Dauber and colleagues (12), examines dynamic flux of transferrin from the intravascular pool (monitored by the scintillation detector over the heart) into the lung. False positive or negative protein flux caused by progressive changes in pulmonary blood volume were prevented by correcting for these alterations by simultaneously measuring ^{99m}Tc-labeled red cell flux.

Red blood cell labeling. Twenty minutes after intravenous injection of 20 μg/kg of stannous medronate (Amersham International, Amersham, UK) injected intravenously, a 10-ml sample of heparinized blood was drawn from a central venous line in patients and from a 21-gauge forearm butterfly needle in control subjects. After separating and washing the red cells in saline, they were labeled *in vitro* with 1 mCi of ^{99m}Tc. Labeled cells were subsequently reinjected and used as the blood pool marker. Binding efficiency was consistently greater than 95%.

Labeling of protein with ^{113m}Indium. Approximately 1 mCi of ^{113m}In (half-life, 100 min) was injected intravenously (along with the labeled autologous red cells); ^{113m}In binds to plasma transferrin *in vivo*. Thirty minutes thereafter, ultrafiltration of plasma (Micropartition System; Amicon, Gloucester, UK) showed less than 3% of ^{113m}In to be nonprotein bound. Acrylamide gel electrophoresis and subsequent counting of sliced gel in a scintillation well counter showed binding to a protein with electrophoretic mobility consistent with transferrin (molecular weight, 76,000).

Calculation of protein flux. A period of 15 min was allowed for equilibration after isotope injection. Scintillation detectors were placed over the lung (right upper zone) and heart (as a measure of blood pool activity). The lung detector was placed regardless of distribution of radiologic abnormalities in the patients with ARDS, whereas the heart detector was placed where counts from labeled red cells were maximal 10 min after injection. The detectors used in both studies comprised 5.1 cm by 5.1 cm collimated NaI crystals. The spectrometer (NE 4697; Nuclear Enterprises, Edinburgh, UK) was fitted with 2 pulse height analyzers enabling simultaneous recording of counts caused by both isotopes from each probe detector. This was interfaced with a dedicated minicomputer (Dragon 64; Compusense Ltd., London, UK) that facilitated display and recording of data. A correction factor for influence of ^{113m}In on ^{99m}Tc was derived initially and applied to subsequent ^{99m}Tc counts. In all studies, counts were serially recorded for at least 30 min. To correct for any dynamic changes in pulmonary blood volume, the ^{113m}In-protein lung/heart ratio for each minute was divided by the lung/heart ratio for labeled red cells at that time. The rate of change of this corrected ^{113m}In lung/heart ratio was quantitated by plotting the individual points versus time and drawing a computer-fitted regression line. The regression-equation-derived slope was divided by the Y intercept at time zero to correct for differences in physical factors between studies (12). This quotient was used as the index of vascular permeability and expressed as protein flux units (× 10^{-3}/min). A representative protein flux study from Patient 1 is shown in figure 1.

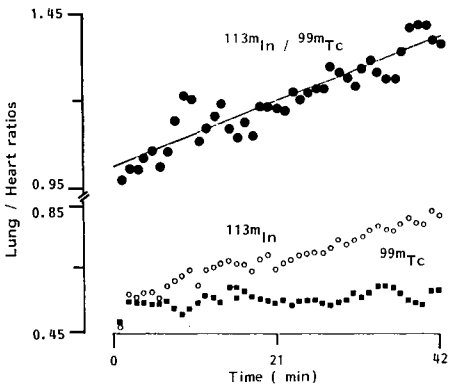

Fig. 1. Protein flux study in Patient 1. Linear regression equation for uppermost slope; y = 1.01 + 0.0096 × 1 (r = 0.94). Therefore, calculated protein flux index allowing for correction for physical factors (see text) = 9.5 (× 10^{-3}).

Study Protocol

All patients with ARDS were studied in the supine position while mechanically ventilated, and in 4 of them, greater than 6 cm H_2O of PEEP was being applied (maximum, 10 cm). Volunteer control subjects were studied on one day while resting comfortably in a semisupine position. In both groups, DTPA clearance was measured initially, and the transferrin flux study was started within 30 min of its completion. The counts from the ^{99m}Tc-labeled red cells were approximately 10 times the residual counts from the prior ^{99m}Tc-DTPA study and counts from this source were ignored in the transferrin flux study.

Statistical Analysis

For the DTPA clearance study, the 3 groups were analyzed by one-way analysis of variance and unpaired *t* tests on logarithmically transformed data. When appropriate, possible Type 2 errors (false acceptance of the null hypothesis) were investigated by calculating, the power and beta value (13). Transferrin flux was compared using the Mann-Whitney U test

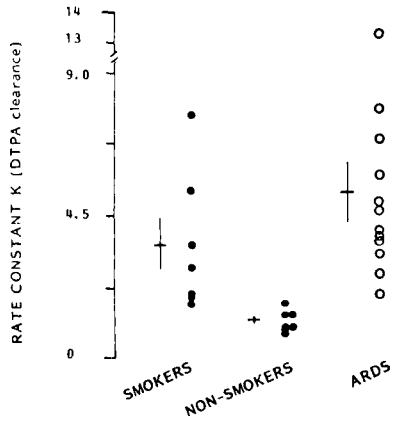

Fig. 2. Clearance of DTPA expressed as rate constant K (%/min) in the 3 study groups. Nonsmokers versus both smokers and patients with ARDS: $p < 0.001$.

because of the larger variances. A p value less than 0.05 was deemed significant. All results are expressed as mean ± standard error of the mean.

Results

Values for both permeability indices in the patients with ARDS are described in table 1. Individual data for pulmonary clearance of ^{99m}Tc-DTPA for the 3 study groups are represented in figure 2. Linear correlation coefficients for all DTPA clearance slopes were at least −0.96; K values for ^{99m}Tc-DTPA clearance did not differ significantly between normal smokers and patients with ARDS (3.6 ± 0.8 and 5.2 ± 0.9, respectively), but both groups differed significantly from normal nonsmokers (1.2 ± 0.1, $p < 0.001$). The possibility of a beta error (1- power) calculated for the comparison between smokers and patients with ARDS was 0.64. Transvascular protein flux data (figure 3) revealed no significant differences between smokers and nonsmokers (protein flux units of 0.0 ± 0.2 and 0.3 ± 0.2, respectively), and therefore their data were combined to form 1 control group (0.2 ± 0.1, n=14). Results from this control group differed significantly from that of the 12 patients in the ARDS group (3.2 ± 1.0, $p < 0.01$).

Within the ARDS group there was significant Spearman's rank correlation ($r_s = 0.71$, $p < 0.01$) between the index of protein flux and K value for ^{99m}Tc-DTPA clearance in each individual patient (figure 4). Although all 12 patients with ARDS had K values for ^{99m}Tc-DTPA clearance greater than 95% confidence limits derived from 7 normal nonsmokers, only 9 had indices of protein flux greater than the 95% confidence limits established in the combined control group. The linear correlation coefficient for the plots of corrected ^{113m}In-transferrin lung/heart ratios versus time in those patients with positive slopes outside the control values ranged from 0.58 to 0.94 (mean r = 0.74).

Only 2 of the 12 patients studied (Patients 7 and 8) recovered from respiratory failure, and 1 of these died during the same admission from an unrelated cause (Patient 7). Autopsies were performed in 7 patients. Six showed diffuse alveolar damage but in 1 (Patient 10), the predominant feature was diffuse fibrosis.

Discussion

These studies demonstrate similarly increased ^{99m}Tc-DTPA clearance in healthy smokers and patients with ARDS. Although increased transvascular protein flux was not found in 3 of the 12 patients studied, the group as a whole differed significantly from control subjects. Thus far, this finding appears specific to ARDS as normal values were found in the smokers in this study and in patients with cardiogenic pulmonary edema (4). Close rank correlation was shown between the 2 indices of lung injury in the patients with ARDS.

^{113m}In-Transferrin Flux

An experimental study using a similar technique suggested that abnormal protein flux could be detected in mild injury despite unchanged gravimetric extravascular lung water (12). In addition, the investigators showed that corrections for regional blood volume and physical factors (geometry of the chest, chest wall thickness, lung tissue density, etc.) increased the specificity of the protein flux data. Patients with ARDS, however, unlike laboratory animals after a discrete noxious insult, are probably in a steady state with regard to vascular derecruitment and permeability changes. An explanation for the failure to detect abnormal protein flux in 3 patients in this study is the possible transient nature of protein leakage. This is supported by experimental evidence that pulmonary edema formation (and presumably increased vascular permeability) usually reaches a maximum within 24 h of acute lung injury (14). Thereafter, the edema fluid is gradually resorbed, and hyaline membranes and interstitial inflammation become more prominent histologically. All 3 patients were studied within 24 h of fulfillment of ARDS criteria, but abnormal protein flux may have been too transient for detection with this technique. Alternatively, because blood flow to the more injured areas is reduced (15), normal flux data may represent more active derecruitment of these regions compared with that in the other patients. Gas exchange was, however, severely impaired in all 3, and none survived.

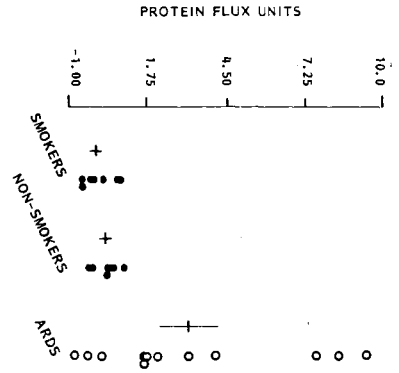

Fig. 3. Protein flux expressed as protein flux units (see text) in the 3 study groups. Patients with ARDS versus combined smokers and nonsmokers: $p < 0.01$.

Inhaled ^{99m}Tc-DTPA Clearance

In contrast to the data for protein flux, clearance of inhaled ^{99m}Tc-DTPA was increased in every patient with ARDS studied, but this finding was not specific to the syndrome. These data show that as far as the extent of increased clearance is concerned, the technique cannot distinguish between patients with ARDS and normal smokers, although the former group did tend towards higher rate constants for tracer solute clearance. Although the number of subjects studied was relatively small for this comparison, thus reducing the power of the statistic, it is unlikely that the failure to define a

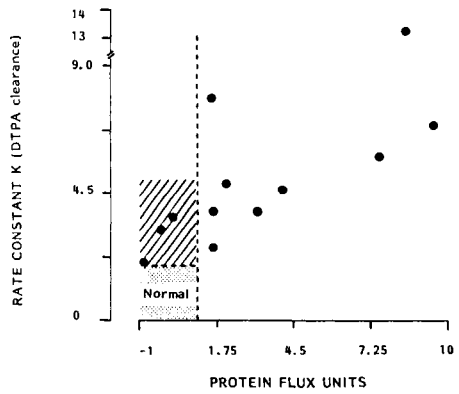

Fig. 4. Protein flux units versus rate constant K (%/min) for DTPA clearance in each patient with ARDS. Spearman's rank correlation coefficient (r_S) = 0.71; $p < 0.01$. Normal (*dotted area*): area bound by 95% confidence intervals in control subjects (see text). Hatched area represents significantly increased DTPA clearance, normal protein flux.

difference between these groups represents a statistical Type 2 error. Other larger studies in smokers have demonstrated similar means and standard error for the DTPA clearance rate constant (16, 17) to that in this study. The healthy smoking subjects must have near normal lung structure in contrast to the severe diffuse lung injury of the patients with ARDS. The finding of similarly increased clearance in both groups may support previous suggestions that the site of increased solute flux in smokers is in respiratory bronchioles rather than in alveoli (18). The alveoli, unlike the bronchioles, do not show inflammatory changes in young smokers (19). Implicit in these inferences from the ^{99m}Tc-DTPA clearance data is the actual deposition of aerosolized tracer solute on peripheral nonciliated epithelium including respiratory bronchioles and alveoli. Undoubtedly there must be some deposition of aerosol on more proximal airways; the relative surface areas of air spaces and airways together with the DTPA particle size must, however, imply that the contribution from alveoli is orders of magnitude greater than from airways. It seems reasonable, therefore, to regard this technique as an index of alveolar epithelial permeability.

This study suggests that measurement of DTPA clearance in patients with a recent smoking history will not provide diagnostically useful information. In nonsmoking patients, a single study showing increased solute clearance implies an active inflammatory process, and serial studies may provide useful information of recovery of alveolar-capillary barrier integrity (20). The use of PEEP in many of the patients with ARDS introduces a possible source of error for the clearance data because PEEP increases the clearance of DTPA in normal nonsmokers (8). However, preliminary experimental evidence (21) has demonstrated that in the presence of acute lung injury, clearance is unaffected by PEEP. It seems unlikely, therefore, that this factor influenced DTPA clearance in the patient group.

Correlation between ^{113m}In-Transferrin Flux and ^{99m}Tc-DTPA Clearance

The significant rank correlation in the patients with ARDS between DTPA clearance and transvascular flux was an unexpected finding. The alveolar epithelium and the pulmonary capillary endothelium have very different reflection coefficients for a substance of known molecular radius (22). This is particularly marked for small molecules like DTPA (molecular radius, 0.6 nm) where the epithelium is virtually 10 times less permeable than the endothelium. Thus, most of the resistance to diffusion across the barrier of a hydrophilic solute like DTPA is in the epithelium rather than in the endothelium. These relationships apply, however, to normal rather than to injured lung. The strong relationship found between the 2 indices of barrier integrity, despite the heterogeneity of predisposing conditions to lung injury, would suggest that both epithelium and endothelium were damaged proportionately. This is in accord with a recent study of patients with acute alveolitis, where a significant correlation was shown between DTPA clearance and lung lavage albumin concentrations (23).

In summary, although DTPA clearance is a sensitive technique in ARDS, a single finding of increased clearance in a patient who smokes is nonspecific. Its use, therefore, in smokers with clinical acute lung injury cannot be advocated. Transvascular protein flux, although not as sensitive as DTPA clearance, is not affected by smoking and appears at present specific for the syndrome. The particular advantage of these solute flux techniques lies in their sensitivity in early or mild injury when extravascular lung water and the chest radiograph may be normal (12, 20). In addition, serial studies should provide more dynamic information about alveolar-capillary recovery. The use of these techniques will, hopefully, focus attention on the early phase of acute lung injury, when the potential benefit of appropriate therapy is greatest.

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