Bronchial hyper-responsiveness after human cardiopulmonary transplantation

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SUMMARY

1. Bronchial responsiveness to inhaled methacholine was studied in ten heart-lung transplant (HLT) recipients a mean 10.6 months (range 1.5–28 months) post-HLT and in ten normal subjects.

2. The mean provocation dose of methacholine producing a 20% fall in FEV₁ (PD20 FEV₁) was significantly lower in the HLT recipients (1.70 ± 3.96 mg vs 11.55 ± 3.79 mg; \( P < 0.001 \)), as was the mean provocation dose of methacholine producing a 50% fall in specific airway conductance (PD50 sGAW) (0.08 ± 0.08 mg vs 5.13 ± 5.47 mg; \( P < 0.005 \)).

3. These results establish the presence of significant bronchial hyper-responsiveness to inhaled cholinergic agonists in the transplanted lung.

Key words: autonomic regulation, bronchial hyper-responsiveness, heart-lung transplantation, methacholine, smooth muscle.

Abbreviations: BHR, bronchial hyper-responsiveness; HLT, heart-lung transplant; ITGV, intrathoracic gas volume.

INTRODUCTION

Bronchial hyper-responsiveness (BHR), resulting in bronchoconstriction after a variety of physical, chemical or pharmacological stimuli, is a characteristic feature of asthma. A similar phenomenon has been demonstrated in a small number of apparently normal subjects and in a variable proportion of patients with chronic airflow limitation, allergic rhinitis, and after some viral infections [1].

Despite intensive investigation, the mechanism of bronchial hyper-responsiveness is not currently understood, but factors which may be important include reduced basal airway calibre, increased contractility of bronchial muscle, altered mucosal permeability, circulating humoral or cellular agents, and disorders of autonomic regulation [1]. The demonstration that in vivo BHR is not reproducible in vitro provides indirect evidence that airway innervation may play a role in the genesis of BHR [2].

The clinical cardiopulmonary transplantation programme in this institution provided a unique opportunity to evaluate patients with denervated lungs. We investigated airway response characteristics after methacholine inhalation in ten HLT recipients and ten normal subjects. To avoid any possible discordance between the effect of volumetric changes on airway resistance and alteration in flow rates, we assessed the airway response to methacholine by measuring changes in both specific airway conductance (sGAW) and forced expiratory volume in 1 s (FEV₁). The purpose of this study was to gain insight into bronchial reactivity in the human transplanted lung and into the role of the autonomic nervous system in the genesis of airway hyper-responsiveness.

PATIENTS AND METHODS

Permission to perform this study was obtained from the Stanford Medical Center Committee for the Protection of Human Subjects. In addition, informed consent was obtained individually from each participating subject.

Methacholine challenge

The experimental group comprised ten cardiopulmonary transplant recipients who had survived a mean 10.6 months (range 1.5–28 months) after surgery. Before transplantation, all patients had advanced pulmonary
hypothesis as a result of primary pulmonary hypertension (n = 5) or Eisenmenger syndrome (n = 5). No clinical or functional evidence of asthma was evident before operation in the recipients but precise historical details regarding the donors' previous pulmonary status were not always available. Serial pulmonary function tests were obtained in our laboratory after transplantation; at the time of this study, eight had normal gas exchange with no evidence of airflow limitation [3]. The remaining two patients each had demonstrated an obstructive ventilatory defect in keeping with a diagnosis of obliterative bronchiolitis, although histopathological confirmation had not been obtained at the time of this study [4]. Patient no. 9, who has been the subject of a separate report [5], had responded to augmented immunosuppression and had had a stable lung function for 12 months before this study. The post-transplant immunosuppressant regimen consisted of prednisone (0.2 mg day⁻¹ kg⁻¹), cyclosporin in doses sufficient to maintain trough serum levels (measured by radioimmunoassay) between 75 and 100 ng/ml and azathioprine (1–1.5 mg day⁻¹ kg⁻¹). None of the patients had symptoms or signs of viral infection for at least 4 weeks before the study.

The control group consisted of ten age and sex matched normal subjects with normal spirometrically derived flow rates who had no risk factors for airflow hyper-responsiveness, as determined by the American Thoracic Society Epidemiological Questionnaire [6].

The Jaeger Body Test (constant volume) Plethysmograph was used to measure airway resistance and intrathoracic gas volume (ITGV). Pressure changes in the box expiratory flow rates derived from a pneumotachograph were measured by a transducer linked to a compensation chamber to eliminate ambient pressure variations. To allow for slow pressure changes, a calibrated leak with a half-time of 7 s was incorporated into the box. Resistance measurements were made during tidal breathing from an insulated bag with a water reservoir and temperature regulator; this system ensured constant humidity and temperature conditions throughout the breathing cycle and so avoided the necessity to measure resistance during non-physiological panting manoeuvres. Measurements of ITGV were made in standard fashion and, in addition, expiratory flow rates derived from a pneumotachograph in line with the mouthpiece were calculated at each stage.

A dosimeter (Jaeger Asthma Provocation System) in line with the mouthpiece inside the closed box was used to deliver aerosolized methacholine. Aerosol delivery was triggered by the inspiratory phase of quiet tidal respiration and lasted 1.0 s/breath. Since the volume and concentration of nebulized solution were known, the exact dose of methacholine delivered in milligrams could be calculated, obviating the necessity to use imprecise inhalation units. The aerosol particle size generated by this system varies from 0.5 to 0.7 μm. No studies using this equipment are available in HLT recipients to comment on the degree of variability of deposition of the aerosol throughout the tracheobronchial tree. Measurements of inspiratory airway resistance (RAW), ITGV (and hence sGAW was calculated) and FEV₁ were made in that order at baseline, 3 min after inhalation of 154 mmol/l NaCl solution (saline) and 3 and 6 min after successive methacholine inhalations. The initial dose of methacholine was 0.05 mg and this was doubled at successive stages until a cumulative dose of 12.75 mg was reached, or until sGAW and FEV₁ fell by 50% and 20% respectively.

The methacholine dose–response relationships were analysed by constructing a semilog dose–response curve by plotting the log dose of methacholine (mg) on the abscissa against the fall from baseline (%) of FEV₁ and sGAW on the ordinate. The PD20 FEV₁ and the PD50 sGAW were calculated by linear interpolation from the graph. Since the study was continued in each subject until both FEV₁ and sGAW fell by at least 20% and 50% respectively (or until the maximum dose of methacholine was given), extrapolation of the curve to calculate these endpoints in reactive patients was not necessary. The maximum dose of methacholine given (12.75 mg) was used for statistical purposes in subjects whose sGAW and FEV₁ values remained greater than 50% and 80% of baseline respectively, throughout the study period. Conversely, the lowest dose of methacholine given (0.05 mg) was used for statistical purposes in subjects whose sGAW and FEV₁ fell by at least 50% and 20% respectively, after this dose.

Statistical analysis was performed by using an unpaired Student's t-test. Predicted values for lung function were derived from the following sources: FEV₁, Morris et al. [7] and sGAW, Zarin & Clausen [8]. In the HLT patients, parameters of the recipient were utilized.

RESULTS

Baseline individual and mean values of FEV₁, FEV₁ as percentage predicted, FEV₁/FVC ratio and sGAW plus sGAW as percentage predicted are shown in Table 1, together with PD20 FEV₁ and PD50 sGAW for the normal subjects and the HLT patients. Baseline FEV₁ was significantly lower in the HLT group than in the controls (2.90 ± 0.76 litres vs 3.96 ± 0.81 litres; P < 0.05), principally reflecting the postoperative restrictive ventilatory defect in the HLT group. Conversely, baseline sGAW was significantly higher in the HLT group (0.29 ± 0.14 s⁻¹·cm⁻² water vs 0.19 ± 0.10 s⁻¹·cm⁻² water; P < 0.05). No significant difference was found whether patients with obliterative bronchiolitis were included or excluded.

Transplanted subjects demonstrated a striking and significant reduction in PD20 FEV₁ (1.70 ± 3.97 mg vs 11.55 ± 3.79 mg; P < 0.001). PD50 sGAW was also significantly lower in the transplanted group (0.08 ± 0.08 mg vs 5.13 ± 5.47 mg; P < 0.005).

These data provide evidence of bronchial hyper-responsiveness to methacholine in the transplanted lung. No relationship was found between either PD20 FEV₁ or PD50 sGAW and length of time post-HLT nor between baseline FEV₁ (as percentage predicted) or sGAW, and PD20 FEV₁ or PD50 sGAW respectively.

Individual log dose–response curves are displayed in Fig. 1, which graphically demonstrates the magnitude of the difference in airway responsiveness to methacholine between HLT recipients and normal controls.
DISCUSSION

The present data document the presence of BHR to methacholine after cardiopulmonary transplantation in nine of the ten patients available for study, one patient having a 'normal' PD20 FEV1 of 12.75 mg but low PD50 sGAW of 0.30 mg. Although we found a wide variation in individual PD20 FEV1 and PD50 sGAW values, this is consistent with previous studies of bronchial responsiveness in both normal subjects and patients with rhinitis, chronic airflow limitation and asthma [6, 9-11]. Notwithstanding this variability, mean PD20 FEV1 and PD50 sGAW values in the transplant group were significantly less than those found in normal subjects.

There are several possible mechanisms to account for post-transplant BHR. Parasympathetic denervation hypersensitivity is one such mechanism. Evidence for the central role of the vagus nerve in the control of bronchial smooth muscle tone has been provided by experimental work in animals and clinical observation in man [12]. Electrical stimulation of vagal motor fibres produces marked bronchoconstriction in animals, which is potentiated by acetylcholine and attenuated by atropine. In addition, chemical or mechanical airway irritation causes bronchoconstriction, which is prevented by prior administration of atropine or section of the vagus nerve. These experiments suggest that central or reflex stimulation of vagal motor activity causes airway narrowing by the action of acetylcholine on muscarinic receptors. Although similar experiments cannot be performed in man, general agreement exists that bronchial smooth muscle is maintained in a tonic state by the release of acetylcholine from vagal efferents, and that reflex bronchoconstriction after airway irritation is mediated by both afferent and efferent vagal fibres [12].

It seems reasonable to speculate that post-transplant BHR could result from hypersensitive muscarinic receptors deprived of tonic vagal stimulation.

A second possible mechanism for post-transplant BHR is a modification of the third nervous system in the lung [13, 14]. It is possible that post-transplant BHR may be related to modification of this non-cholinergic non-adrenergic system, which normally inhibits bronchial smooth muscle tone.

A third possible mechanism involves damage to the respiratory epithelium, which might cause BHR [15]. In this respect two of the transplanted patients are considered to have obliterative bronchiolitis. Subclinical bronchiolitis may be difficult to detect and a failure to establish a clinical diagnosis or even a biopsy diagnosis would not be surprising. As well, possible abnormalities in mucociliary clearance or even a failure to establish a normal clearance could have been due to the failure of the systemic immune system to control airway inflammation. To date, no knowledge regarding the extent of airway alterations in mucociliary clearance or even a failure to establish a normal clearance has been performed in HLT recipients, so this is a possibility that should be considered.

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Fig. 1. Individual dose–response curves to methacholine in normal control subjects (●) and heart–lung transplant patients (○).

possibility remains unsubstantiated. Finally, although few data are available on the influence of immunosuppressant regimens on bronchial responsiveness [16], it seems improbable that low-dose prednisone, azathioprine and cyclosporine therapy was responsible for the marked BHR observed. Although the effect of cyclosporine on airway responsiveness is unknown, the former two agents might reasonably be expected to reduce BHR by suppressing any inflammatory component.

Because a reduction in the radius of a narrow airway causes a greater increase in resistance than a similar change in the radius of a wider airway, it has been suggested that BHR may be a function of reduced baseline airway calibre [1]. However, many studies have shown a wide variation in airway responsiveness among subjects with similar baseline pulmonary function [1]. Our data are consistent with these studies in demonstrating no correlation between PD20 FEV₁ or PD50 sGAW and baseline FEV₁ as percentage predicted and sGAW as percentage predicted in either the transplant group or normal controls. Thus, post-transplant BHR does not appear to be a function of resting airway calibre. While hypertrophy and hyperplasia of bronchial smooth muscle may play a minor role in asthmatic BHR, no evidence of increased bronchial muscle mass was seen in two other transplant recipients who subsequently came to autopsy [17]. Furthermore, baseline pulmonary function showed no evidence of airflow limitation in eight of the ten transplant recipients studied. It appears that hypertrophy of airway muscle is unlikely to explain post-transplant BHR.

Although some evidence suggests that pulmonary innervation may be re-established 6 months after lung reimplantation in dogs [18], the present data, by demonstrating no concordance between BHR and duration of post-transplant survival, are more consistent with the possibility that human heart–lung transplantation results in longstanding pulmonary denervation. This same lack of concordance also attests to the fact that BHR is not a simple post-surgical artifact. The extent of the surgery associated with combined heart–lung transplantation and the demonstration that cardiac reinnervation does not occur after human heart transplantation [19] also argue against the possibility of pulmonary reinnervation in our patients. Indeed, it is possible that BHR may prove to be an index of pulmonary denervation, although the evidence to support such a speculation is not yet available and the wide discordance of PD20 FEV₁ and PD50 sGAW in one HLT recipient serves to underline the importance of utilizing volume corrected parameters of flow. At the present time, independent confirmation of pulmonary denervation in human subjects is not possible; no precise method of evaluation of denervation is feasible in human heart–lung transplantation recipients. Inhalation of citric acid to stimulate irritant receptors and provoke cough and bronchoconstriction cannot be used as an index of innervation of the transplanted lung in these subjects, since receptors above the tracheal anastomosis would be stimulated in this fashion. Similarly, selective stimulation of the upper respiratory tract with sulphur dioxide, a method used in animals to provoke reflex bronchoconstriction and thereby demonstrate intact innervation [20], is clearly not desirable in human subjects as it requires tracheostomy. Finally, although some of these patients have undergone bronchoscopy after transplantation, passage of the bronchoscope through the larynx obviously necessitates both sedation and local anaesthesia...
delivered directly through the bronchoscope channel from above. Lidocaine used in this fashion invariably gravitates distal to the tracheal anastomosis. Thus attenuated cough reflex observed during bronchoscopy in these subjects cannot be used as an index of denervation, and such observations are in any case difficult to interpret objectively.

We have demonstrated the presence of marked BHR in nine of ten cardiopulmonary transplant recipients, none of whose donors had evidence of lung disease. Post-transplant BHR differs from asthmatic BHR in a number of important respects: transplant recipients have no clinical or physiological evidence of episodic bronchospasm and do not demonstrate eosinophilia. It therefore seems that the documentation of post-transplant BHR furnishes unique physiological data but does not provide a clinical model of the asthmatic state, although the possibility that these patients may develop asthma in the future cannot be excluded. The clinical implications of BHR in the management of lung transplant recipients remain to be elucidated. However, chronic alterations of airway function are a major cause of long-term mortality and morbidity in these patients [3], and the current data provide some insight into the nature of airway regulation in the denervated lung post cardiopulmonary transplantation.

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REFERENCES