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Bronchial reactivity to methacholine after combined heart-lung transplantation

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ABSTRACT The operation of combined heart-lung transplantation results in acute denervation of the heart, lungs, and airways below the level of the trachea. The bronchoconstrictor response to inhaled methacholine of 12 recipients of heart-lung transplants was compared with that of 12 recipients of heart transplants having similar medication and 12 normal subjects. The median dose of methacholine that produced a reduction of at least 20% in the FEV_1 (PC_{20}) for the recipients of heart-lung transplants (8 mg/ml) was significantly lower than that for the recipients of heart transplants (64 mg/ml) and normal subjects (>64 mg/ml). The increased airway reactivity may be related to the effects of chronic pulmonary denervation or subclinical inflammation in the airways. The effect of denervation on the response to full inspiration during bronchoconstriction was studied in six patients with heart-lung transplants by means of partial and maximal forced expiratory manoeuvres. Four showed bronchodilatation after a deep breath, indicating that this response can occur after extrinsic pulmonary denervation in man. The patients with heart-lung transplants described a "tight" sensation in the anterior chest during bronchoconstriction, indicating that this sensation is not dependent on pulmonary innervation.

Introduction

The human bronchial tree responds to varied physical, chemical, and pharmacological stimuli by bronchoconstriction. The magnitude of the response to any stimulus varies widely between individuals, asthmatic subjects characteristically showing substantial bronchial hyperresponsiveness.[1] The factors responsible for this variation are unknown.[2] Airway responsiveness in vivo correlates poorly with the reactivity of isolated bronchial smooth muscle strips from the same individual,[3,4] suggesting that increased airway responsiveness is not a property of bronchial smooth muscle but requires an intact lung or whole organism. The role of airway innervation in influencing bronchial reactivity is not clear. Vagal innervation is a determinant of resting airways tone.[5] Patients with diabetic autonomic neuropathy show a reduced airways response to cold air inhalation,[6] although their response to inhaled histamine is increased.[7]

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The operation of combined heart and lung transplantation provides an opportunity to study the effects of airway denervation below the level of the trachea on airway responsiveness in man. We have compared the airway resonse to methacholine in recipients of heart-lung transplants and of heart transplants having similar medication and in normal subjects. Increased bronchial responsiveness has been documented in recipients of heart-lung transplants but whether this is related to the transplant procedure or other factors, such as drug treatment, has not been established.[8]

In normal subjects and, more variably, in asthmatic subjects a deep inhalation to total lung capacity during pharmacologically induced bronchoconstriction leads to transient bronchodilatation lasting from 15 to 45 seconds.[9,10] Animal studies suggest that the bronchodilatation depends on intact airways innervation[11] but this is less certain in man as the response is not influenced by cholinergic blockade.[12] A recent study failed to show bronchodilatation after a deep inhalation in recipients of heart-lung transplants, suggesting that the response might depend on extrinsic pulmonary innervation.[13] We studied the effect of inhaled methacholine on flow rates measured during partial and maximal

forced expiration in recipients of heart-lung transplants to determine the effect of deep inspiration on airways calibre after bronchoconstriction.

Methods

SUBJECTS

Twelve heart-lung transplant recipients were compared with 12 orthotopic heart transplant recipients and 12 normal subjects (study 1). The characteristics of the groups are shown in table 1; the predicted lung function values were estimates based on the subjects' anthropometric data.[14] The indications for transplantation in the heart-lung transplant group were: Eisenmenger's syndrome in four, primary pulmonary hypertension in four, emphysema in two, complex pulmonary atresia in one, and lymphangioleiomyomatosis in one. Nine of the heart transplants were performed for dilated cardiomyopathy, two for ischaemic heart disease, and one for congenital heart disease. The recipients of transplants were all clinically well at the time of study—patients with heart-lung transplants who had airways obstruction as a complication of the transplant procedure were excluded. All patients with transplants were receiving cyclosporin and most were taking azathioprine, low dose aspirin, and dipyridamole. Control subjects had no history of chest disease or allergy, were free of respiratory symptoms, and were non-smokers. All subjects gave informed consent to the study.

Six recipients of heart-lung transplants fulfilling the same criteria took part in study 2; their characteristics are shown in table 2.

METHACHOLINE CHALLENGE TESTS

Three baseline measurements of FEV_1 were performed and the highest value obtained was used for subsequent analysis. Normal saline (9 g/l) was administered from a Wright's nebuliser (driven with oxygen at a flow rate of 7 l min^{-1}: output 0·15 ml/min) while the subject, wearing a nose clip, inhaled from a mouthpiece during tidal breathing for one minute. Forced expiratory volume in one second (FEV_1) was measured immediately after nebulisation and again after 60 and 150 seconds. The procedure was repeated at five minute intervals with doubling concentrations of methacholine from 2 to 64 mg/ml or until the concentration of methacholine producing a fall in the FEV_1 of at least 20% (PC_{20}) was reached.

Table 1 *Study 1: characteristics of the subjects (means with SD in parentheses)*

	HLT	HT	N
Number	12	12	12
Sex	3M:9F	9M:3F	3M:9F
Age (years)	27·9 (9·1)	29·3 (9·6)	27·5 (8·0)
Months after operation	8·2 (5·5)	10·8 (4·4)	—
FEV_1 (litres)	3·0 (0·7)	3·4 (0·7)	3·7 (0·6)
FEV_1 (% pred)	90 (14)*	87 (13)*	107 (13)
FVC (litres)	3·2 (0·6)	3·8 (0·9)	4·0 (0·6)
FVC (% pred)	86 (10)*	85 (12)*	103 (10)

HLT—heart-lung transplant recipients; HT—heart transplant recipients; N—normal subjects; % pred—percentage of predicted value.[14]
*$p < 0·05$ in the comparison with normal subjects (see text).

Table 2 *Study 2: characteristics of the subjects and results*

	Subject No					
	1	2	3	4	5	6
Age (years)	39	17	20	23	31	34
Sex	F	F	M	M	F	M
Months after transplantation	12	36	24	12	3	24
FEV_1 (litres)	2·74	3·43	4·22	4·61	2·90	5·18
(% pred)	87	99	94	108	90	132
FVC (litres)	2·85	3·70	4·69	5·12	3·01	5·90
(% pred)	79	97	89	103	82	127
PC_{20} (mg/ml)	2	8	2	16	4	64
$\dot{V}_{40}$max (1 sec^{-1})	1·30	1·53	1·5	1·60	1·00	1·50
$\dot{V}_{40}$p (1 sec^{-1})	1·6	0·35	0·50	0·60	1·25	0·70

PC_{20}—concentration of methacholine producing a reduction of at least 20% in FEV_1; $\dot{V}_{40}$max—flow rate 60% of FVC below TLC during forced expiration after inspiration to TLC; $\dot{V}_{40}$p—flow rate at this lung volume during forced expiration after inspiration to a point 40% of FVC below TLC.

EFFECT OF DEEP INSPIRATION

Baseline measurements of partial and maximal flow-volume loops were performed by using a computerised spirometer system incorporating a visual display screen that allowed real time display of the patient's flow-volume loop (Autospiror Discom-14, Chest Corporation, Tokyo). The forced vital capacity (FVC) was determined. The volume at 40% of FVC below total lung capacity (TLC) was marked on the screen. The subject (wearing a nose clip) was asked to go on to the mouthpiece at the end of a normal tidal expiration, inspire to the target volume, and then perform a maximal forced expiration to residual volume (RV) followed by a full inspiration to TLC and a further forced expiration to RV—thus producing superimposed partial and complete maximum expiratory flow-volume loops.[15] This enabled flow rates to be compared at equivalent lung volumes relative to TLC (which has been shown to remain constant during methacholine induced bronchoconstriction[16]). The procedure was repeated several times until the subject was familiar with the technique and consistent results were obtained.

The subject carried out a methacholine challenge test to obtain a PC_{20} value. Flow rates at 60% of FVC below TLC ($\dot{V}_{40}$) for the partial and complete flow-volume loops were compared. Subjects were

questioned about the respiratory sensation associated with bronchoconstriction.

ANALYSIS

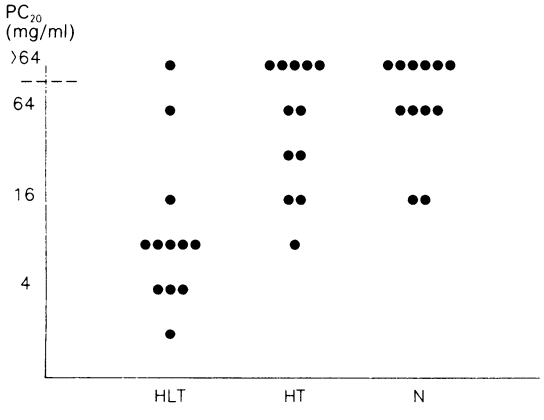
Baseline lung function for the three groups in study 1 was compared by analysis of variance and comparisons between pairs of groups by the Newman-Keuls multiple comparisons technique. Comparison of PC_{20} values in the three groups in study 1 was performed by means of the non-parametric Kruskal-Wallis analysis of variance.

Results

Baseline lung function for the three groups in study 1 is given in table 1. The difference in FEV_1 between groups did not quite reach conventional statistical significance ($p = 0{\cdot}08$) and there was no significant difference between any two groups. FEV_1, expressed as a percentage of the predicted normal value, was significantly different in the three groups ($p < 0{\cdot}01$) and was lower in both transplant groups than in the control subjects ($p < 0{\cdot}05$). There was no significant difference, however, between the two transplant groups. The overall difference in FVC was significant ($p = 0{\cdot}05$) but the differences between pairs of groups did not reach significance. FVC, expressed as a percentage of the predicted value, was significantly different in the three groups ($p < 0{\cdot}01$) and was lower in both transplant groups than in the normal subjects ($p < 0{\cdot}05$). There was again no significant difference between the transplant groups.

METHACHOLINE CHALLENGE

The PC_{20} values of the three groups of subjects in study 1 are shown in the figure. The recipients of heart-lung transplants had a significantly lower median PC_{20} (8 mg/ml) than the recipients of heart transplants (64 mg/ml) or the normal controls (>64 mg/ml; $p < 0{\cdot}001$).



Concentration of methacholine (mg/ml) producing a fall in FEV_1 of at least 20% (PC_{20}) in each subject (study 1). HLT—heart-lung transplant recipients; HT—heart transplant recipients; N—normal subjects.

EFFECT OF DEEP INHALATION

Four of the six recipients of heart-lung transplants developed bronchodilatation after a deep breath and two did not (table 2). All six subjects described a "tight" sensation in the anterior chest associated with the feeling that it was difficult to take a deep breath. The sensation appeared to be similar to that experienced by normal subjects undergoing bronchial challenge testing.

Discussion

We have found greater bronchial responsiveness to methacholine in recipients of heart-lung transplants than in normal subjects and recipients of cardiac transplants. Glanville and colleagues also found greater bronchial reactivity to methacholine in patients with heart-lung transplants than in normal subjects,[8] though their study did not include heart transplant recipients as controls. There was no significant difference in bronchial reactivity between the patients with heart transplants and the normal subjects in our study. This indicates that factors such as previous surgery and immunosuppressive drug treatment are not the cause of the bronchial hyperresponsiveness of the patients with heart-lung transplants. We were not able to match the heart and heart-lung recipients for sex but, as the heart transplant group behaved similarly to the normal subjects, this does not seriously affect the interpretation of the results.

Lung volumes were lower in both transplant groups than in the normal subjects. This might affect bronchial reactivity by virtue of an initial reduction in airway calibre. There was, however, no significant difference in baseline lung function between the two transplant groups, suggesting that this was not the main factor producing increased airway reactivity in the heart-lung transplant group.

The mechanism responsible for the increased bronchial responsiveness to methacholine in recipients of heart-lung transplants has not been established. One possibility is that it is due to pulmonary denervation following the transplant procedure. The donor heart and lungs are placed en bloc in the recipient in the normal anatomical position with an anastomosis between the donor's and the recipient's trachea just above the carina.[17] The recipient retains tracheal innervation down to the level of the anastomosis. Although pulmonary innervation may be re-established in dogs within six months of lung trans-

plantation,[18] there appear to be important species differences in reinnervation. After orthotopic cardiac transplantation dogs have shown evidence of subsequent reinnervation,[19] whereas this has not been found in man.[20] The presence of an innervated trachea above the anastomosis makes it difficult to detect pulmonary denervation non-invasively. Although we do not have direct evidence of continuing pulmonary denervation in these patients, we might reasonably assume that their lungs were partly, if not completely, denervated at the time of study. The increased bronchial reactivity might represent a denervation hypersensitivity of muscarinic receptors in the airways. A similar phenomenon has been found in recipients of heart transplants, who show an increased response to beta adrenergic agonists,[21] presumably owing to up regulation of adrenergic receptors. Alternatively, the increased responsiveness might result from loss of a bronchodilator effect of non-adrenergic, non-cholinergic nerves in the airways.[22]

Another possible mechanism is the effect of previous episodes of rejection or inflammation in the airways. The subjects studied were clinically well, with no airways obstruction or pulmonary infiltrates on their chest radiographs. The development of late onset chronic airflow obstruction in some recipients of heart-lung transplants, associated with histological evidence of bronchiolitis, indicates that these patients are at risk of developing an inflammatory process in the airways.[23] The hyperresponsiveness might result from subclinical inflammation. In asthmatic patients an inflammatory infiltrate in the airway wall or loss of epithelial cells from the lining of the bronchi is thought to play a part in the pathogenesis of increased reactivity[2]—a theory that is supported by human[24] and animal[25,26] experimental data.

It is uncertain whether the transient bronchodilatation seen in normal subjects after a full inspiration depends on pulmonary innervation.[9,10] Animal experiments have suggested that it may be due to length-tension hysteresis of airways smooth muscle[27] or a reflex response.[11] A recent study found that recipients of heart-lung transplants did not develop bronchodilatation after a deep breath during methacholine induced bronchoconstriction,[13] whereas in the present study four of the six subjects did show bronchodilatation. A possible explanation for the different results is that some of our patients were developing pulmonary reinnervation. As, however, the two studies were conducted at a similar time after transplantation (mean interval 18·5 months in our study and 25·1 months in the study by Glanville[13]) this is unlikely. Although a small number of patients has been studied and further investigations are required, our results indicate that the increased bronchial reactivity in the recipients of heart-lung transplants is not always associated with loss of bronchodilatation after deep inspiration. This is consistent with the results of studies that have shown that the bronchodilator response is not abolished by cholinergic blockade,[12,28] adrenergic blockade,[29] or ganglion blockade[29] in man. In asthmatic subjects absence of bronchodilatation during pharmacologically induced bronchoconstriction appears to be related to the degree of hyperreactivity.[30,31]

The sensations described by the recipients of heart-lung transplants were similar to those described by normal subjects during pharmacologically induced bronchoconstriction. This suggests that pulmonary innervation is not essential for the sensation associated with bronchoconstriction, but it does not exclude a role for tracheal stretch receptors.

In conclusion, recipients of heart-lung transplants show increased bronchial reactivity to methacholine, which may be related to pulmonary denervation or to a subclinical inflammatory process in the airways of the transplanted lung. Further studies are required to define the mechanisms. Bronchodilatation can still follow full inspiration in some of these patients, suggesting that this response does not depend on pulmonary innervation.

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