Eur Respir J
1991, 4, 5–9

Effect of heart-lung transplantation on airway potential difference in patients with and without cystic fibrosis

E.W.F.W. Alton*, A. Khagani**, R.F.H. Taylor†, R. Logan-Sinclair††, M. Yacoub, D.M. Geddes

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ABSTRACT: Measurement of the potential difference (PD) across the airways provides an indication of the viability and integrity of the lining epithelium. PD was recorded from the lower airways in "diseased controls" and in patients following heart-lung transplantation. Diseased controls showed a high PD centrally which fell (became less negative) peripherally (trachea -15.8 mV (SEM 1.0), lobar bronchi -12.6 mV (1.2), segmental bronchi -9.8 mV (1.2)). Following heart-lung transplantation (HLT) the profile of PD with airway size was altered in comparison to non-transplanted patients with reduced values in the large airways. Host tracheal values above the anastomosis were similarly reduced. Two episodes of rejection were associated with a lower mean airway PD; no significant changes were found with infection. In patients with cystic fibrosis (CF), values in the donor lung did not differ from those in non-CF transplanted patients up to one year following transplantation, although nasal PD in the host remained elevated. HLT selectively alters the PD profile only of larger airways, which may relate to the interruption of the bronchial arterial supply to these sites.

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Keywords: Ion transport; potential difference; transplantation.

Received: December 20, 1989; accepted after revision July 23, 1990.

E.W.F.W. Alton was supported by the National Heart and Chest Hospitals Clinical Research Committee and by an MRC Training Fellowship.

In 1981, KNOWLES and co-workers [1] first described the *in vivo* measurement of potential difference (PD) across the epithelial lining of the human respiratory tract. As PD is dependent upon both active ion transport and the resistance of the epithelium to passive ion fluxes [2], this parameter provides an indirect measure of the activity of these processes and the integrity of the mucosal surface. The PD is highest in the central airways and falls peripherally probably reflecting regional variations in transport of electrolytes (and water) across the airways. Airway PD is reduced by squamous metaplasia [3] and viral infection [4], and may be influenced by cigarette smoking [2, 5].

With the increasing use of heart-lung transplantation (HLT) as treatment for a variety of pulmonary disorders, *in vivo* recordings of lower airway PD provides a technique to study the bioelectrical physiology of the donor lungs. These lungs have been subjected to ischaemic injury, lack normal innervation, lymphatic drainage and bronchial blood supply, and are prone to immunological injury and repeated infection. The PD of these donor lungs could also be influenced by the host disease. Thus, patients with CF demonstrate a markedly more negative PD across both upper and lower respiratory epithelia [1]. We have, therefore, recorded the lower airway PD profile in patients following HLT and compared these values to unoperated "diseased controls".

Methods

Equipment

PD was measured using a fine polyethylene tube (outer diameter 1.5 mm) (Jencons, Leighton Buzzard, UK) passed through the biopsy channel of a fibreoptic bronchoscope. Contact with the mucosal surface was maintained through Ringers solution at room temperature (flow rate 0.3 ml·min^{-1}) (Travenol, Thetford, UK) using a syringe pump (Model 355, Sage Instruments, Massachusetts, USA). The Ringers solution was connected to a calomel half cell (Kent Industrial Measurements, Stonehouse, UK) using a three way tap. Values were recorded with reference to an intravenous cannula (gauge 20) (Venflon, Viggo, Helsingborg, Sweden) similarly perfused with Ringers solution and connected to a second calomel electrode. Both calomel electrodes were connected to a high impedance (10^{11} ohms) voltmeter (WPI Instruments). Prior to measurement the offset of the electrodes was noted, ±5 mV being acceptable. Suitable adjustments

were made to recorded values. Only stable potentials (±10% over 10 s) were noted and values rounded to the nearest whole number. Measurements took approximately 5 min to perform.

In cases where topical anaesthesia was applied to the vocal cords (4% lignocaine) values in the upper trachea regularly showed a reduction by the end of the procedure. Therefore, only the initial readings, taken immediately after the bronchoscope was passed through the vocal cords, were used for analysis. No such effect was seen in the distal trachea or bronchi.

Patients

PD was recorded in 23 subjects (mean age 57.4 yrs, range 31–75 yrs, 15 male) undergoing bronchoscopy for diagnostic purposes under local anaesthesia (non-CF non-HLT). Diagnoses in these patients included carcinoma of the lung, sarcoidosis, tuberculosis, cryptogenic fibrosing alveolitis and asbestos-related lung disease. Twelve were current smokers (mean age 57.4 yrs) and six ex-smokers (mean age 57.0 yrs) with a mean time from stopping smoking of 7 yrs (range 3–11 yrs). Two patients with CF, aged 20 and 31 yrs both female, were similarly studied (CF non-HLT). Medication at the time of bronchoscopy included gentamicin, azlocillin, prednisolone, salbutamol, ipratropium bromide, pancreatic supplements, cimetidine, vitamin E and multivitamins.

PD was recorded on 18 occasions from 16 patients undergoing bronchoscopy following HLT. Indications included suspected infection (9 cases), rejection (6) and regular review (3). Five had CF (mean age 23.4 yrs, range 15–32 yrs, 4 female) and 11 had other disorders including bronchiectasis, primary pulmonary hypertension, double-inlet left ventricle, univentricular heart, ventral septal defect and cardiomyopathy (mean age 22.0 yrs, range 4–48 yrs, 7 female). No patient was a current smoker. Measurements were made under general anaesthesia: no lignocaine gel or spray was used. Treatment included cyclosporin A, prednisolone, azathioprine, disopyramide, aspirin, frusemide, ranitidine, antibiotics and antifungal agents, vitamins and iron replacements. Measurements were made from 13–990 days after transplantation.

Sites of measurement

In the non-transplant patients values were recorded from upper, mid and lower thirds of the trachea both anteriorly and posteriorly. Amongst the transplant patients, readings were taken above and below the anastomosis both from the anterior and posterior trachea. In both groups of patients further readings were taken from main stem, lobar, segmental and subsegmental bronchi as well as a "wedged" value, being the furthest peripheral airway accomodating the exploring electrode. Values from the floor of the nasal cavity were measured in the HLT patients following transplantation, using our previously described method [6]. "Mean airway PD" was obtained by summing values (one per airway level) from all airway levels in a particular group.

Other investigations

Bronchoalveolar lavage was performed by advancing the bronchoscope into a lower lobe basal segment. One hundred and sixty ml of sodium bicarbonate-buffered normal saline (pH 7.2) were injected and approximately 60–80 ml recovered. Samples were transported on ice in sterile siliconized glass bottles and routinely assayed for cytomegalovirus (CMV), pneumocystis and bacterial growth.

Transbronchial biopsies were taken from basal segments of the lower lobe. Specimens were placed in formol saline and processed in the routine manner for haematoxylin and eosin (H+E) sections. Thus, the entire specimen was sectioned and one in every three ribbons stained with H+E. One section of one of the larger cuts was stained with elastic van Gieson's and 1–2 with Grocott's to look for evidence of pneumocystis infection.

Statistics

The Mann Whitney U test was used to compare PD at different airway levels within each group, at comparable levels between two groups and for combined values for all airway levels within a group of patients. For convenience data are expressed as mean±SEM. Because nasal PD is normally distributed [7] Student's t-test was used to compare nasal PD between groups. Correlation was calculated using the Pearson product-moment coefficient. The Null hypothesis was rejected at $p<0.05$.

Results

The PD profile in non CF HLT patients showed no change in values with decreasing airway size (fig. 1). This was related to a significant reduction in large airway values when compared with non-CF non-HLT subjects, amongst whom all airway levels showed values significantly greater than the wedge recordings (fig. 2). In neither group was there a significant difference between values in the anterior and posterior trachea (non-HLT -14.6 mV (SEM ±1.1) and -16.4 mV (1.3), n=59, respectively; HLT -8.8 mV (1.0) and -9.4 mV (1.0), n=34, respectively) and therefore the results were combined for further analysis. Combined mean airway PD of the transplanted patients (-8.6 mV (SEM ±0.55), n=88) was significantly lower than for the non-CF non-HLT group (-12.7 mV (0.55), n=170; $p<0.001$). There was no correlation of PD at any airway level in the HLT group with the sex or age of the donor, nor with pre-transplantation use of inotropic agents: no

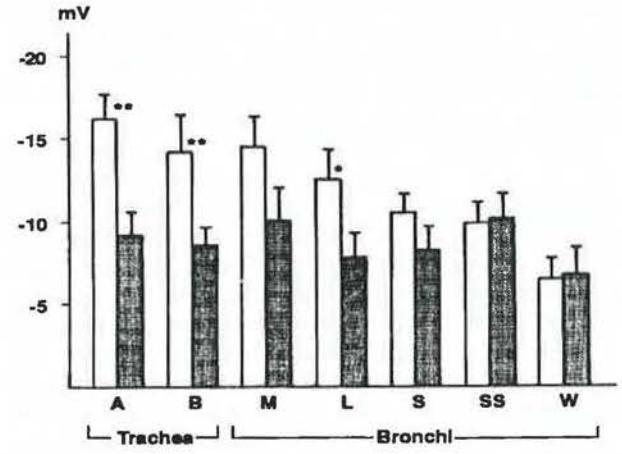

Fig. 1. – Comparison of lower airway PD of non-CF non-HLT (open bars, n=23) and HLT patients (shaded bars, n=13). For the former group, values in the upper and mid thirds of the trachea have been combined as an "above" value to allow for comparison (Trachea: above (A), below (B); Bronchi: main (M), lobar (L), segmental (S), subsegmental (SS), wedge (W)). PD was significantly lower in the host (**: p<0.01) and donor trachea (**: p<0.01) and lobar bronchi (*: p<0.05) of the HLT subjects, and for a combined value of all airway levels (***: p<0.001). Error bars indicate SEM. PD: potential difference; CF: cystic fibrosis; HLT: heart-lung transplant.

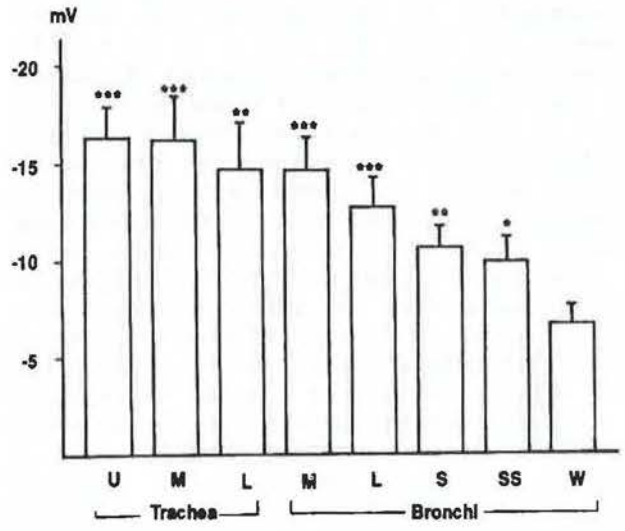

Fig. 2. – Lower airway PD in the non-CF non-HLT group showing a fall in PD with decreasing airway size (Trachea: upper (U), mid (M), lower (L); Bronchi: main (M), lobar (L), segmental (S) subsegmental (SS), wedge (W)). Values for all airway levels are compared to those of the wedge recordings (*: p<0.05; **: p<0.01; ***: p<0.001). Error bars indicate SEM. For further abbreviations see legend to figure 1.

donor was a smoker. Similarly, neither the ischaemia time for the donor lungs nor the time of PD measurement following transplantation (up to 990 days) was correlated with PD.

Two of the HLT patients (1 CF, 1 non-CF) showed histological evidence of rejection at the time of measurement, in the form of perivascular large lymphoid cell cuffing. Their mean combined airway PD (-6.65 mV (SEM ±1.3), n=13) was significantly (p<0.05) lower than 9 patients with normal bronchial histology (-9.3 mV, (0.6), n=62). Evidence of infection was obtained from bronchial lavage or histology at the time of PD recordings in 8 patients (*H. influenza* (n=3), *P. aeruginosa* (n=2), *S. aureus* (n=1), cytomegalovirus (n=1), *Pneumocystis carinii* (n=1)). Of these 8 patients, 7 had symptoms of infection including an increase in dyspnoea, pyrexia, reduction in exercise tolerance and suggestive chest X-ray changes. In one case, *H. influenza* was isolated during routine follow-up in an asymptomatic patient. In comparison to subjects without evidence of infection, there was no significant difference either in mean airway PD (-8.5 mV (SEM ±0.7), n=52 *vs* -8.4 mV, (0.7), n=55) or at individual airway levels. The two asymptomatic patients undergoing routine follow-up, who showed no evidence of infection or rejection, had a combined mean PD of -7.7 mV (SEM ±1.4), n=14 significantly (p<0.01) lower than the non-CF non-HLT group.

Amongst CF HLT patients, PD did not differ from non-CF HLT readings at any airway level (fig. 3) or as a combined mean value. Again no reduction in values with decreasing airway size was seen. Tracheal PD above the anastomosis was no different from non-CF HLT values (-10.5 mV (SEM ±2.4) *vs* -9.2 mV (1.5); p=NS), but nasal PD was significantly higher (-35.5 mV (3.0) *vs* -18.0 mV, (2.3); p<0.01). No correlation of lower airway PD with time following HLT was seen up to a maximum of 370 days.

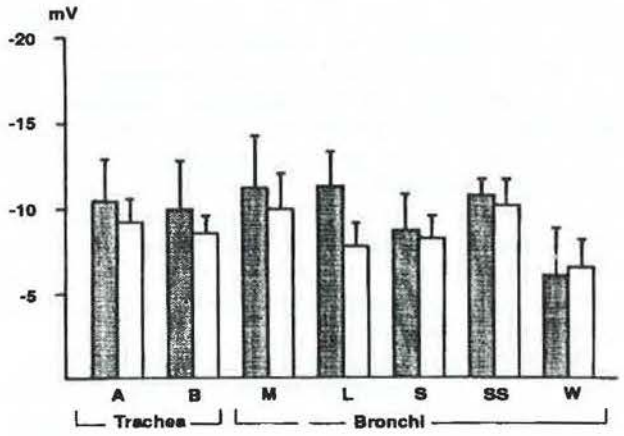

Fig. 3. – Lower airways PD of CF (shaded bars, n=5) and non-CF HLT (open bars, n=13) patients. Error bars indicate SEM. For further abbreviations see legend to figure 1.

The combined mean airway PD of the two non-CF non-HLT patients (-21.6 mV (SEM ±3.0), n=14) was significantly higher (p<0.01) than for the 23 non-CF non-HLT patients (-12.7 mV (0.55), n=170). Values for each airway level in the two CF patients were: upper trachea -22, -13, mid-trachea -22, -13, lower trachea -28, -12, main bronchi -37, -17, lobar bronchi -27, -, segmental bronchi -18, -49, subsegmental bronchi -20, -, wedge -3, -21.

Discussion

Up to 3 yrs following HLT the lower airways of non-CF subjects have an abnormal bioelectrical profile. The PD is reduced in the large airways, with normal values at the segmental level and more peripherally. Interestingly, PD in the upper trachea above the anastomosis was also significantly lower than in non-HLT controls. There was no relationship of PD at any airway level with any of the donor-related factors studied, or with time following HLT.

The effect of topical lignocaine in reducing airways PD is well documented [2] and we were careful to minimize these effects in the non HLT patients. The small quantities that undoubtedly did pass the vocal cords will have been primarily deposited in the upper trachea and indeed a time-dependent reduction of PD in this region was noted. Therefore, for purposes of analysis only, the values obtained from the upper trachea immediately upon entry of the bronchoscope were noted. However, these values may still have been reduced by the effect of this drug, and readings from the mid and lower thirds of the trachea may be a more accurate representation. In comparing the measurement of the transplanted patients recorded without using such topical anaesthesia, to those of the non-transplanted group, the effects of lignocaine would serve only to diminish the separation between the groups, making any differences obtained more significant.

Previous studies have shown no effect of general anaesthesia on airway PD [8]. However, to further assess this possibility we measured the PD profile in one patient under local and general anaesthesia [9]. The very similar results do not support the contention of a depressant effect of general anaesthesia. Finally, nasal PD recorded under general anaesthesia showed a significantly elevated value in the CF HLT patients. with mean values in both this group and the non-CF HLT subjects very similar to those noted previously [6]. It is unlikely that general anaesthesia has a selective effect on reducing lower airways PD, without altering upper tract values.

Similar reasoning suggests that the many drugs being taken by the HLT patients did not artefactually lower large airways PD. Furthermore, apart from the topical application of the sodium-channel blocker amiloride [3], few pharmacological agents are known to alter the net sodium-absorption which is likely to be principally responsible for the generation of PD in these airway regions.

To assess the possibility that denervation of the donor lung may lower PD we have recorded values in a patient following single lung transplantation [10]. In this case, the subject acted as her own control, having one normally innervated lung and one lacking nervous control. PD was similar on both sides and no different to values from the non-CF non-HLT patients.

Two patients were subsequently shown to have histological evidence of rejection at the time of PD measurement. Their combined mean value for all airway levels was significantly lower than for HLT patients with normal bronchial histology. Because rejection may be patchy, and because of the small numbers involved, further studies will be required to validate this finding, although interestingly similar findings have been noted following small-bowel transplantation [11].

Eight patients were found to have concurrent infection as judged by bronchial lavage and transbronchial biopsy, of whom seven showed typical clinical evidence of infection. No significant change in PD was seen in this group as a whole. This is perhaps not surprising, in view of the heterogeneity of causative organisms, degrees of colonization and phase of clinical illness. Thus, we have previously shown that the changes in PD caused by upper respiratory tract infections may vary with viral type as well as with the stage of illness [4]. However, because of the limitations of lavage and a restricted number of transbronchial biopsies it is clear that we cannot fully assess the extent of infection in a given patient, nor could we objectively assess disease severity in this small group.

Following heart-lung transplantation the values from all airway regions in five CF patients were no different from other transplant patients. However, nasal PD in the CF group was raised as expected. These findings suggest that the electrochemical defect typical of CF epithelia is not found in the donor lungs up to one year following HLT. The values above the suture line in CF patients were lower than those in the CF non-HLT controls but similar to those in the non-CF HLT patients as noted in our previous report [9] and by Wood *et al.* [12]. HLT thus appears to lower tracheal PD both above and below the suture line in both groups of patients. We cannot disprove that donor cells have crossed the anastomosis to repopulate the CF portion of the trachea, but this has not been reported from animal studies.

Following HLT, patients are prone to infection and immunological injury, and undergo repeated invasive investigations, all of which predispose to changes in the normal epithelial lining of the airways and in turn PD. Whilst we have tried to assess the individual contributions of these factors, it is possible that various combinations underlie the altered PD profile. However, the two asymptomatic patients with no evidence of infection or rejection still showed the same abnormal values in the upper airways. During HLT the bronchial arterial supply to the trachea and larger bronchi is cut, revascularization of this region occurring *via* collaterals from the pulmonary circulation. There is, therefore, a prolonged period of ischaemia in the regions above and below the anastomosis, down to the segmental bronchi. We suggest that this may contribute to the abnormal PD profile in these regions. The possible role of ion transport in airway defences and the consequences of such changes are at present unclear.

Few patients with CF undergo bronchoscopy, and amongst those doing so there is little opportunity to perform anything other than the clinically indicated procedures. We have only been able to study two such patients, adding to PD profiles for three patients reported previously [1]. Whilst markedly more negative values were found at all airway levels except the trachea,

these changes were "patchy". It is well recognized that infection or inflammation can produce squamous metaplasia and that in turn this type of epithelium is associated with a lower PD. A similar picture is often demonstrated within the nasal cavity [3].

In conclusion, in patients following HLT the normal airway PD profile is altered with a reduction in values in the large airways, including those of the host trachea. HLT patients with CF show no difference in PD in the donor lung from values found in non-CF subjects, suggesting that the donor lungs do not acquire the CF electrochemical defect.

Acknowledgements: The authors would like to thank the staff of the bronchoscopy units at the London Chest and Brompton Hospitals, and the theatre staff at Harefield Hospital for their help and courtesy. Also L.S. Nakhla and P. Vuddamalay (Microbiology) and A. Pomeroy (Histopathology), Harefield Hospital for access to their records, P. Baldock and H. Blair for help with collection of data and M. Delaney for typing the manuscript.

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Effet de la transplantation cardio-pulmonaire sur la différence de potentiel des voies aériennes chez les patients avec ou sans fibrose kystique. E.W.F.W. Alton, A. Khagani, R.F.H. Taylor, R. Logan-Sinclair, M. Yacoub, D.M. Geddes.

RÉSUMÉ: La mesure de la différence de potentiel (PD) au travers de la voie aérienne fournit une indication de la viabilité et de l'intégrité de l'epithélium de recouvrement. PD a été enregistré au niveau des voies aériennes inférieures chez des "contrôles-malades" et chez des patients après une transplantation cardio-pulmonaire. Les contrôles malades montraient une différence de potentiel élevée dans la région centrale, avec diminution (valeur moins négative) vers la périphérie (trachée -15.8 mV (SEM ±1.0), bronche lobaire -12.6 mV (1.2), bronche segmentaire -9.8 mV (1.2)). Après transplantation cardio-pulmonaire, le profil de PD en rapport avec la dimension de la voies aériennes est modifié par comparason avec les patients non transplantés, les valeurs dans les voies aériennes de grand calibre étant réduites. Les valeurs trachéales de l'hôte au-dessus de l'anastomose sont également diminuées. Deux épisodes de rejet ont été associés à une PD des voies aériennes moyennes abaissée; aucune modification significative n'ai été observée en cas d'infection. Chez les patients atteints de fibrose kystique, les valeurs dans le poumon du donneur ne diffèrent pas de celles observées chez les patients transplantés sans fibrose kystique, jusqu'à un an après la transplantation, quoique la différence de potentiel nasal de l'hôte reste élevée. La transplantation cardio-pulmonaire modifie de façon sélective le profil des différences de potentiel uniquement au niveau des grandes voies aériennes, ce qui peut être en relation avec l'interruption de la circulation artérielle bronchique à ce niveau.
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