Effects of Bronchial Transection and Reanastomosis on Mucociliary System*

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Study objectives: The mechanisms involved in the impairment of mucociliary function after lung transplantation are not completely understood. The purpose of the present study was to isolate the effects of unilateral bronchial transection and reanastomosis in a rat model.

Design: In situ bronchial mucociliary transport (MCT) was determined proximal and distal to the bronchial anastomosis, as well as in the right bronchus, in 48 rats classified into six groups: intact rats, and rats at 1 day, 2 days, 7 days, 15 days, and 30 days after bronchial transection and reanastomosis of the left main stem bronchus.

Results: Distal to the anastomosis site, left bronchus in situ MCT (mean ± SD) was 0.26 ± 0.19 mm/min for the intact group, and 0.11 ± 0.13 mm/min, 0.07 ± 0.04 mm/min, 0.03 ± 0.04 mm/min, 0.07 ± 0.12 mm/min, and 0.05 ± 0.06 mm/min for 1 day, 2 days, 7 days, 15 days, and 30 days after surgery, respectively (all significantly reduced, p < 0.05). No intergroup differences were found proximal to the anastomosis (p = 0.30). When comparing the left and right bronchi, differences were detected in both distal (p < 0.0001) and proximal sides (p = 0.0001). No significant differences in mucus transportability in vitro were found (p = 0.15). Mucus contact angle of the left bronchus (52.8 ± 20.5°) was significantly greater than that of the mucus from the right bronchus (34.4 ± 12.9°; p < 0.05).

Conclusions: We conclude that bronchial transection and reanastomosis lead to a marked impairment of MCT in distal airways, which can in part be explained by alterations in the surface properties of mucus.

Key words: bronchial transection; mucociliary clearance; rat

Abbreviation: MCT = mucociliary transport

Mucociliary transport (MCT) of the airways is often regarded as a primary defense mechanism, because inhaled particles and bacteria entrapped in the mucus layer covering the epithelial surfaces are continuously removed from the airways by the active beating of cilia.1–3 This mucociliary function in the respiratory tract is necessary for health and for the normal functioning of the tissue, particularly in resisting respiratory infection,4 and has been reported to be impaired after lung transplantation.5–9 Such alteration, if present, may place the immunocompromised patients at an ever-greater risk for respiratory-tract infection, one of the limiting factors in their survival.10–13

The mechanisms involved in the impairment of mucociliary function after lung transplantation have not yet been well established and may be related to a number of factors, such as surgical trauma from bronchial transection, denervation, and devascularization.14–17 Furthermore, immunosuppression or

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possible subclinical rejection may also have an effect on mucociliary function. MCT is dependent on factors related to the mucus, to the cilia, and to the interaction between them, and an understanding of its dysfunction could conceivably lead to treatments aimed at reducing the incidence of pulmonary infections in recipients of lung and heart-lung transplants.

The purpose of the present study was to isolate a single factor or factors that might be involved in the mucociliary impairment in transplant recipients. The effect of bronchial transection and reanastomosis was studied using bronchial MCT in a rat model, where factors such as rejection and immunosuppression are not present. In order to better understand the impact of this procedure on MCT, the acute effects in short-term operated animals from the first to 30th day after surgery were studied. In this rat model, it was possible to determine, by direct visualization of carbon particle suspension, the MCT both proximal and distal to the bronchial anastomosis, as well as that in the contralateral bronchus. In addition to determining in situ MCT, in vitro mucus transportability was investigated using the frog palate method and the contact angle, a mucus surface property.

**Materials and Methods**

**Experimental Design**

Two sets of experiments were performed. The first set, based on experiments on 48 Wistar male rats, weighing 350 to 400 g and classified into six groups of eight animals according the time of study after bronchial transection, focused on bronchial MCT. The animals came from the vivarium of the Medical School of the University of São Paulo, where they received food and water ad libitum. The groups of animals were designated by numbers: group 1, intact rats (animals not operated on and submitted to libitum terms of time elapsed between bronchial transection and MCT study); and groups 2 to 6, rats submitted to transection and reanastomosis of the left bronchus. Groups 2 to 6 differ in MCT study after bronchial transection, focused on bronchial MCT. Groups 2 to 6, rats submitted to transection and reanastomosis of the left bronchus. Groups 2 to 6 differ in terms of time elapsed between bronchial transection and MCT study: day 1, day 2, days 7, 15 days, and 30 days for group 2, group 3, group 4, group 5, and group 6, respectively. In situ MCT in the animals operated on was measured in the operated left bronchi at sites proximal and distal to bronchial anastomosis as well as in the nonoperated right bronchi. The in situ MCT of the intact animals was also measured at both locations in both the left and right main bronchi. The right lung, which was not operated on, also served as an internal control for these studies. The second set of experiments was based on another group of eight rats that were operated on and allowed to recover for 1 week; these rats were used for the collection of mucus samples to study in vitro mucus transportability (frog palate preparation) and contact angle measurements.

**Bronchial Transection Procedure**

The rats were preanesthetized by inhalation of ether, which was sufficient to permit oral intubation with a 6.5-cm long, 14-gauge catheter. The animals were then connected to a rodent ventilator (model 683; Harvard Apparatus; Holliston, MA) that was adjusted to maintain normal ventilation (respiratory rate, 70 breaths/min; tidal volume, 10 mL/kg of body weight). Anesthetic for the surgical procedure was a 2% halothane mixture delivered by 100% oxygen at a flow rate of 0.5 L/min. A thoracotomy was performed at the fifth intercostal space; the main stem bronchus was dissected, and all visible nerve bundles around the left bronchus region were severed. The main stem bronchus was also cut in order to interrupt nerve bundles within the wall of the bronchus, and then reanastomosed with 5.0 polypropylene using continuous sutures. The lungs were inflated to remove atelectasis, a chest tube was inserted, and the thoracic cavity was closed. The endotracheal and chest tubes were removed when spontaneous breathing resumed. An operating stereoscopic microscope with 8 × magnification was used for the procedure. All the operations were performed by the same surgeon using clean, yet nonsterile technique.

**In Situ Bronchial MCT**

Based on MCT in the rat trachea, a new approach to its study in the rat bronchus was developed. In situ bronchial MCT was observed by means of tracer displacement, using a 100 × microscope. The rats were anesthetized with pentobarbital sodium (30 mg/kg intraperitoneal) and killed by sectioning the abdominal aorta. The lung was removed and carefully dissected in order to obtain total visibility of the trachea and the main bronchi. The cartilaginous fraction of each bronchus was also removed, thus exposing the membranous fraction. After dissection, the surface of the bronchus was placed under the microscope (Olympus BX50; Olympus; Tokyo, Japan) connected to a camera (Sony Triniton 3CCD; Sony; Tokyo Japan), and a stream of nebulized saline solution was passed over it to provide humidification. In situ mucus clearance was monitored by direct observation of charcoal particles in saline solution mixed with common talc particles, in order to increase solution viscosity. This charcoal solution (1 μL) was placed on the surface of each main bronchus (both distal and proximal to the anastomosis). The progression of the leading edge of the charcoal drop toward the bronchus was timed and expressed as a velocity (millimeters per minute of distance traveled/time elapsed).

**Mucus Collection**

Mucus samples were collected from the bronchial passages by inserting a small brush in each bronchial cavity for 30 min. The brush was then withdrawn and immediately immersed in mineral oil to prevent mucus dehydration, and the samples were stored at −2°C to 0°C until analyzed.

**Mucus Transportability**

Mucus transportability by ciliary beating was evaluated using an in vitro frog palate preparation. The frog (Rana catesbiana) was selected, because its palate is lined with a pseudostratified epithelium, similar to that found in human conductive airways. Briefly, the transport velocity of the mucus sample placed on a mucous depleted frog palate was determined with the aid of a stereoscopic microscope equipped with a reticulated eyepiece. The mucus samples were rinsed with petroleum ether to remove the oil before placement on the surface of the palate. The velocity of the rat mucus samples was compared to the transport speed of autologous frog mucus, and the results were expressed in terms of relative speed (rat/frog). The experiments were carried out at room temperature (20°C to 25°C) and 100% humidity maintained by using an ultrasonic spray of 2/3 normal saline solution (0.9% NaCl).
Contact Angle Measurement

The contact angle indicates the ability of a fluid to spread when deposited on a solid planar surface. The contact angle of all mucus samples were measured on microscope glass slide, which was treated with sulfochromic acid to remove any electrical charge. It is the angle existing between the tangent to the liquid-air interface and the solid surface at the point where the three phases meet.22 This spread occurs because a finite interaction exists between the solid surface and the molecules present in the liquid. Physically speaking, the contact angle is dependent on the nature of the solid, that of the liquid, and that of the interaction between them. The contact angle was observed using eyepiece with 25× magnification, with two movable arms (right/left and forward/backward). The eyepiece has a goniometer with an angular scale of 0° to 180°, which measures the angle between the mucus drop and the surface of the glass. A perforated tempered-iron support is placed under the glass allowing humidification by the vapor from a water bath kept at 3°C to 7°C.

Statistical Analysis

The significance of the results for in situ bronchial MCT was assessed by profile analysis.23 Briefly, profile analysis consists of a multivariate method in that a single statistical model is applied. This model considers the groups and one other factor (in this study, bronchial side); basic hypotheses can thus be tested to identify significant differences. The basic hypotheses were as follows: hypothesis 1, there is no interaction between the factors of group (group 1 to group 6) and side (left or right bronchus); hypothesis 2, no differences because of groups are observed; and hypothesis 3, the bronchi on the two sides present similar results. The analysis thus investigates the effects of parallelism (hypothesis 1), coincidence (hypothesis 2), and effect of side (left or right bronchus; hypothesis 3). When hypothesis 1 was accepted, hypotheses 2 and 3 were then tested. Values of p < 0.05 were considered statistically significant.

In vitro mucus transportability and contact angle measure were assessed using a Student paired t test with a value of p, 0.05 taken as significant. Linear correlation evaluated the relationship between in vitro mucus transportability and contact angle.

RESULTS

The results obtained showed that in situ MCT was significantly impaired distal to anastomosis (groups 2 to 6), in comparison to group 1 (intact rats; p < 0.05). All five groups of rats operated on were found to provide similar results (p = 0.39). When comparing the left vs right bronchus in group 1 (intact animals), no difference of MCT was observed (p = 0.54). In contrast, MCT of the left bronchus was significantly lower in all groups operated on (groups 2 to 6), in comparison to the right bronchi (p < 0.0001; Fig 1). The proximal site to the anastomosis showed a different pattern of mucociliary impairment. No significant intergroup differences of MCT were observed (p = 0.30). A significant decrease in MCT was only detected when operated left bronchus was compared to right bronchus in all groups operated on (groups 2 to 6; p = 0.0001). No such difference was observed in group 1 (intact animals; Fig 2).

Figure 3 shows the results of in vitro mucus transportability of the operated left bronchi and intact right bronchi. No significant differences were observed (p = 0.15). The results of contact angle measurement (Fig 4), however, showed that the mucus collected from the operated bronchi had a significantly greater contact angle (p < 0.05) than mucus collected from intact right bronchi. There was a significant inverse correlation between in vitro mucus transportability and contact angle (r = −0.575; p = 0.02; n = 16).

DISCUSSION

Respiratory infection is a significant cause of morbidity and mortality in lung and heart-lung transplant...
The immunosuppressive effect of antirejection agents such as cyclosporine and prednisone is easily identified as a risk factor for pulmonary infection. However, drug-induced immunosuppression alone may not be the only cause of the significantly higher prevalence of pulmonary infection in comparison to that of other organs that undergo comparable immunosuppression. Considering the consequences of the mechanical manipulation of the bronchial blood supply and of autonomic innervation when the new lung is transplanted to the recipient, it is possible to speculate that changes in MCT may contribute to the greater susceptibility to respiratory infection presented by patients in the postoperative period of lung transplantation.

Bronchial denervation may be the major factor responsible for the impairment of mucociliary transport in operated airways, although it is beyond the scope of the present article to deal with the mechanisms responsible for the neural control of mucociliary transport. Here, the emphasis is on the changes of mucus clearance as a function of time after surgically induced bronchial transection. However, the effects of autonomic innervation on mucociliary properties are well known in the clinical setting. For instance, anesthesiologists are quite aware of the adverse effects of atropine on mucus rigidity, an effect that makes patients (especially those with hypersecretion) more prone to airway obstruction by mucus plugs. The techniques involved in transplantation result in total extrinsic denervation of the transplanted organs, because pulmonary nerves, as well as various other structures, are severed and not reconnected. Springall and coworkers, for example, have demonstrated the disappearance of subepithelial and intraepithelial nerves in the trachea below the anastomosis, as well as in the stem bronchi in the transplanted respiratory tract. The mucociliary apparatus is innervated predominantly by the parasympathetic nervous system, and cholinergic agonists and β-adrenergic agonists seem to increase the frequency of ciliary beat as well as tracheal mucus and water secretion.

No clinical data seem to be available involving the acute measurement of MCT in patients undergoing lung transplantation. The present study is the first study to employ a rat bronchial transection and reanastomosis model for the investigation of MCT. In this investigation, the effects of bronchial transection on the functioning of the mucociliary system in short-term operated animals could be considered while avoiding the effects of severance of other paths of nerve supply to the lungs, such as pulmonary artery and vein. It was also possible to determine MCT both proximal and distal to the bronchial anastomosis as of the first day up to the 30th day after surgery. The acute mucociliary impairment of the distal bronchus supports the hypothesis that bronchial denervation and devascularization are probably responsible for the decrease in MCT. The MCT proximal to anastomosis, however, present a different pattern of mucociliary impairment. No difference of mucociliary transport was observed when comparing operated to intact animals. Mucociliary impairment was only detected when operated bronchi were compared to right bronchi (nonoperated). Because the right bronchus showed no mucociliary alteration during the same period, it seems to have constituted an adequate internal control of mucociliary function, thus reinforcing the results observed in the left distal bronchus.

**Figure 3.** Values of in vitro mucus transportability of mucus samples collected from a separate group of rats on postoperative day 7 in transected and intact bronchi, showing no statistical difference (values are means and SDs).

**Figure 4.** Values of contact angle measurements of mucus samples collected from a separate group of rats on postoperative day 7 in transected and intact bronchi, showing greater mucus contact angle for operated left bronchi (*p < 0.05; values are means and SDs).
Some of the properties of the mucus collected from the operated left bronchus were also compared to those of the right bronchus after 7 days of transection. This time period was selected because the analysis of the first experiment indicated that the maximal effects on mucus transport were readily evident at this time. In vitro mucus transportability showed no significant differences, although a significantly greater contact angle of the mucus collected from the operated bronchus was noted, indicating that surface forces are altered in airways submitted to bronchial transection and subsequent anastomosis.

This type of study has some limitations. Immersing the specimens in mineral oil and subsequently washing them with petroleum ether could conceivably have affected the surface properties of the secretions (ie, contact angle). Nevertheless, this did not seem to affect the correlation between contact angle and in vitro mucus transportability. In our study, it was not possible to collect mucus from only below the anastomosis site, and it is conceivable that the left bronchus mucus represents a mixed sample (distal-proximal). It is possible that the differences observed in mucus contact angle and mucus transportability by frog palate could be even bigger if samples collected exclusively from the distal segment were studied.

Both contact angle and adhesiveness are surface properties dependent on tensioactive properties present in mucus and influence the interface interaction between mucus and the epithelium. Surface tension has previously been shown to play a significant role in the mechanics of the coupling between mucus and cilia.34,35 Surfactant is probably responsible for the displacement of particles from the air to the aqueous phase of the mucus layer, in a magnitude inversely proportional to surface tension: the lower the surface tension, the greater the immersion of the particles in the aqueous subphase.36

A high contact angle should correspond to a mucus that sticks more strongly. Contact angle (θ) is directly correlated to the work of adhesion (Wad) by the equation:

\[ Wad = YLV(1 + \cos \theta), \]

in that YLV is the surface tension of the liquid with its saturated vapors. The work of adhesion is related both to the surface tension and to the contact angle and is a measure of adhesivity, which could be defined as the energy per square centimeter to separate two phases that initially have a common interface. Therefore, the increase in the contact angle should contribute to the increase in the work of adhesion of bronchial secretions.37 Mucus that sticks more strongly corresponds to mucus with an increased work of adhesion, and the contact angle is a surrogate measure of the work of adhesion. Adhesive forces are involved in the mechanical coupling between cilia and the mucus layer, as well as cough transport,38,39 probably by interfering with mucus surface mechanical impedance (frictional adhesiveness). According to this concept, tensioactive liquids in the sol phase increase cough clearance of mucus simulants40 and MCT34 when applied directly to the frog palate. In addition, cough clearance was shown to be inversely correlated with mucus contact angle in patients with cystic fibrosis37,41 and patients receiving mechanical ventilation.42 In this study, a negative correlation was found between contact angle and in vitro mucus transportability. This data are consistent with the theory that surface properties contribute not only to determine cough clearance, but also MCT.

This study suggests that the impairment of the in situ bronchial MCT after bronchial transection may be attributable, at least in part, to changes in surface properties of the mucus layer and its interface with the underlying cilia. Although the difference between in vitro transportability of the mucus samples from the left and right bronchus was not statistically significant, we cannot discount some contribution of mucus rheologic properties in altering in situ MCT proximal to the anastomosis. In situ MCT can also be influenced by ciliary-beat frequency, which was not measured in the present study.

The results found here are consistent with those reported in dogs. Brody and coworkers5 showed that bronchial MCT from large airways was impaired during in the first 3 days after lung denervation and transection of the bronchus. Marelli and coworkers6 also showed that the movement of carbon particles could not be detected after 3 weeks when they were deposited distal to bronchial anastomosis. Tomkiewicz and coworkers15 evaluated mucus rheology and transport properties after autotransplantation and found the presence of a less rigid mucus and more clearable just at 2 months after surgery. Most studies in the literature, however, involve the inhalation of radiolabeled particles, and overall lung MCT is measured in long-term transplanted animals. The present method allowed the measurement of MCT in specific regions, specifically just above and below bronchial anastomosis, in short-term operated animals. There are no studies in the literature that allow a direct comparison of the obtained results. One of the strengths of our studies lies in the direct measurement of in situ MCT velocity, discriminating precisely between regions that were proximal and distal to the anastomosis. Such topographic information is of paramount importance to support the concept that bronchial transection and anastomosis impairs MCT distally to the anastomosis.

In conclusion, the present data show that bronchial MCT distal to anastomosis is acutely impaired after bronchial transection, with no recovery apparent up to...
1 month after surgery. In contrast, proximal to anastomosis, a different pattern of mucociliary impairment is involved. The data presented here suggest that acute mucociliary impairment is because of bronchial denervation, although bronchial devascularization may contribute by altering mucosal structures in the early postoperative period after lung transplantation. An increased contact angle of the mucus collected from an operated bronchus 1 week after surgery may explain the reduced MCT. Further studies are necessary to investigate mucus and ciliary function in order to elucidate the mechanisms of this alteration.

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