BASIC RESEARCH

The effects on mucociliary clearance of prednisone associated with bronchial section

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OBJECTIVE: Infections have been and remain the major cause of morbidity and mortality after lung transplantation. Because mucociliary clearance plays an important role in human defense mechanisms, the influence of drugs on the mucociliary epithelium of patients undergoing lung transplantation must be examined. Prednisone is the most important corticosteroid used after lung transplantation. The aim of this study was to evaluate the effects of bronchial transection and prednisone therapy on mucociliary clearance.

METHODS: A total of 120 rats were assigned to 4 groups according to surgical procedure or drug therapy: prednisone therapy (1.25 mg/kg/day); bronchial section and anastomosis + prednisone therapy (1.25 mg/kg/day); bronchial section + saline solution (2 ml/day); and saline solution (2 ml/day). After 7, 15, or 30 days, the animals were sacrificed, and the lungs were removed from the thoracic cavity. The *in situ* mucociliary transport velocity, ciliary beat frequency and *in vitro* mucus transportability were evaluated.

RESULTS: Animals undergoing bronchial section surgery and anastomosis had a significant decrease in the ciliary beat frequency and mucociliary transport velocity 7 and 15 days after surgery ($p<0.001$). These parameters were normalized 30 days after the surgical procedure. Prednisone improved mucous transportability in the animals undergoing bronchial section and anastomosis at 15 and 30 days ($p<0.05$).

CONCLUSION: Bronchial section and anastomosis decrease mucociliary clearance in the early postoperative period. Prednisone therapy improves mucus transportability in animals undergoing bronchial section and anastomosis.

KEYWORDS: Prednisone; Mucociliary clearance; Bronchi; Lung transplantation; Immunosuppression.

Braga KAO, Nepomuceno NA, Correia AT, Jatene FB, Pêgo-Fernandes PM. The effects on mucociliary clearance of prednisone associated with bronchial section. Clinics. 2012;67(6):647-651.

Received for publication on February 15, 2012; **First review completed on** February 27, 2012; **Accepted for publication on** February 27, 2012

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INTRODUCTION

Lung transplantation exposes the bronchial tree to a series of events, such as anesthesia, mechanical ventilation, and immunosuppression, which may alter the mucociliary epithelium (1).

Among the factors involved in lung transplantation surgery, bronchial section may play an important role in general post-operative complications. A major problem after transplantation seems to be related to deficient bronchial scarring, leading to stenosis and dehiscence (2). Studies have reported that, after transplantation, *in situ* mucociliary transport velocity (MCTV) and ciliary beat frequency (CBF) are reduced and that there is an increase in mucus stiffness (3-5). This reduction leads to mucus retention, causing pulmonary infection. This condition, associated with immunosuppression protocols and with the lung being a transplanted organ exposed to environmental air, explains why infection is frequent in these patients.

Another extremely relevant factor associated with the increase in infections in such patients is related to immunosuppression. A few years ago, immunosuppression protocols after organ transplantation were restricted and quite often limited to the empirical and unmonitored administration of azathioprine and prednisone. The introduction of multiple drugs in the 1990s increased the success rates of most solid organ transplantations with more specific and better controlled results, as is the case with prednisone (6,7).

Studies have demonstrated that both bronchial injury and immunosuppressive agents, such as cyclosporine, mycophenolate sodium and azathioprine, impair MCTV (8-12). Initially, mucociliary clearance was observed in bronchi undergoing bronchial section and unilateral bronchial anastomosis (8). This impairment was attributed to alterations in mucus viscoelastic properties in the affected bronchial region. Another study demonstrated that azathioprine reduces the

No potential conflict of interest was reported.

mucociliary transport velocity in animals evaluated 7 days after the surgical procedure (bronchial section and unilateral bronchial anastomosis), with animal recovery evaluated 30 days after surgery (11). Despite MCTV impairment, this study showed the improvement of mucus viscoelastic properties in animals undergoing bronchial section and bronchial anastomosis treated with azathioprine. Three studies evaluated the effects of cyclosporine on mucociliary clearance (5,9,10). Using the bronchial section and bronchial anastomosis model, it was demonstrated that cyclosporine decreases MCTV as a result of impaired CBF and *in vitro* mucus transportability (MT), and it also decreases mucus secretion (5,10). It was observed that cyclosporine exerts a synergistic effect when associated with bronchial section injury and mucociliary clearance.

There are no studies in the literature reporting the effect of systemic corticosteroids on the mucociliary tract. *In vitro* studies evaluating the effect of steroids on MCTV demonstrated that prednisolone increases mucus production, and topical steroids, such as budesonide, decrease CBF in the respiratory mucosa (13-15). Furthermore, postoperative death may be related to complications, such as low scarring and steroid-induced infections (16).

The objective of this study was to evaluate the effects of prednisone on mucociliary clearance in rats undergoing bronchial section and bronchial anastomosis.

MATERIALS AND METHODS

Study Design

A total of 120 male Wistar rats were distributed into four groups: prednisone therapy (Pd); prednisone therapy + surgical procedure (PdSc); saline solution (Sal); and saline solution + surgical procedure (SalSc). The animals in the Pd group received 1.25 mg/kg/day of prednisone. The PdSc group rats underwent bronchial section and bronchial anastomosis and were treated with 1.25 mg/kg/day of prednisone. SalSc group animals underwent bronchial section and bronchial anastomosis and a daily gavage of saline solution. After treatment (7, 15, or 30 days), the animals were sacrificed, and CBF, MCTV and MT were assessed. The animals were cared for according to the Guide for the Care and Use of Laboratory Animals (17). Our institution's Ethical Committee approved the protocol.

Gavage

Animals were sedated in a closed chamber containing inhaled isoflurane anesthetic (Isoforine, Cristália, Itapira, SP, Brazil). The solution was prepared by dissolving and homogenizing a 5 mg prednisone tablet (Meticorten, Mantercop, Rio de Janeiro, RJ, Brazil) into 10 ml of 0.9% saline solution, which resulted in a dose volume of 2.5 ml/kg/day for the Pd and ScPd groups. The Sal and ScSal group animals received a volume corresponding to 2.5 ml/kg/day of 0.9% saline solution. The gavage was performed daily using the orogastric route and a No. 7 polyethylene catheter.

Surgical Procedure

Anesthesia was induced by inhaled isoflurane in a closed chamber, followed by orotracheal intubation with a 6.5 cm long 14-gauge catheter. General anesthesia was maintained by inhalation isoflurane (2%) from a nebulizer (Model 1223, Takaoka, São Paulo, SP, Brazil). Ventilation was achieved using a volume-cycled ventilator (Harvard Apparatus 683, Holliston, MA, USA), with a respiratory rate of 70 breaths/min and a tidal volume of 10 ml/kg.

A left thoracotomy was performed. The left main stem bronchus was dissected, clamped, and completely transected, followed by an end-to-end anastomosis with a running 8-0 polypropylene suture.

Euthanasia

After 7, 15, or 30 days of treatment, 10 animals from each group were anesthetized with intraperitoneal pentobarbital (50 mg/kg, Syntec do Brasil, Cotia, SP, Brazil) and euthanized by exsanguination, according to the Report of the American Veterinary Medicine Association Panel on Euthanasia (18).

Data Collection and Analysis

The lungs were removed from the thoracic cavity, an incision was performed in the left main stem bronchus, and mucus samples were collected and stored at -80°C (5,8). *In vitro* MT was evaluated with a bullfrog (*Rana catesbeiana*) palate model as follows: the transport velocity of the mucus sample placed on a frog's excised palate was determined with the aid of a stereomicroscope equipped with a reticulated eyepiece. The velocity of the rat mucus samples was compared with the transport speed of autologous frog mucus, and the results were expressed as relative speed (rat/frog).

After mucus sample collection, the ventral wall of the left main stem bronchus was opened to expose the ciliated epithelium. The bronchus was placed under a light microscope (Olympus, BX50, Tokyo, Japan) that was connected to a camera (Sony Trinitron, 3CCD, Tokyo, Japan). A stroboscope (Machine Vision Strobe, Cedarhurst, NY, USA) was placed in front of the bronchus, and CBF was measured by synchronization between cilia movement and the stroboscope flashlight. CBF was measured in relation to the section of the bronchus. Subsequently, under the same microscope, *in situ* MCTV was timed by direct observation of talc particle movement across the bronchus.

Statistical Analysis

A triple-factor variance analysis (drug, time, and surgery) was used to evaluate the interference of the analyzed factors on dependent variables as well as a possible interaction among them, followed by a post-hoc Bonferroni test for multiple analyses among groups. Normality and homogeneity were evaluated in the groups using the Kolmogorov-Smirnov test with the Lilliefors correction ($p>0.05$ for all groups) and Levene's test ($p>0.05$). Descriptive and inferential statistical analyses were performed with SPSS software version 13 (IBM, Armonk, NY, USA).

RESULTS

Animals undergoing bronchial section and anastomosis had a significant CBF reduction 7 and 15 days after surgery ($p<0.001$). CBF recovery was observed after 30 days (Figure 1).

The SalSc and PdSc groups did not show significant differences in CBF at any of the study periods ($p = 0.084$), indicating that there is no interaction between prednisone and the surgical procedure.

The SalSc and PdSc animals showed significant reductions in MCTV at 7 and 15 days ($p<0.001$) compared to the Sal and Pd animals, respectively. However, no significant differences were evident between the groups after 30 days ($p>0.1$). The MCTV was slower in the SalSc and PdSc

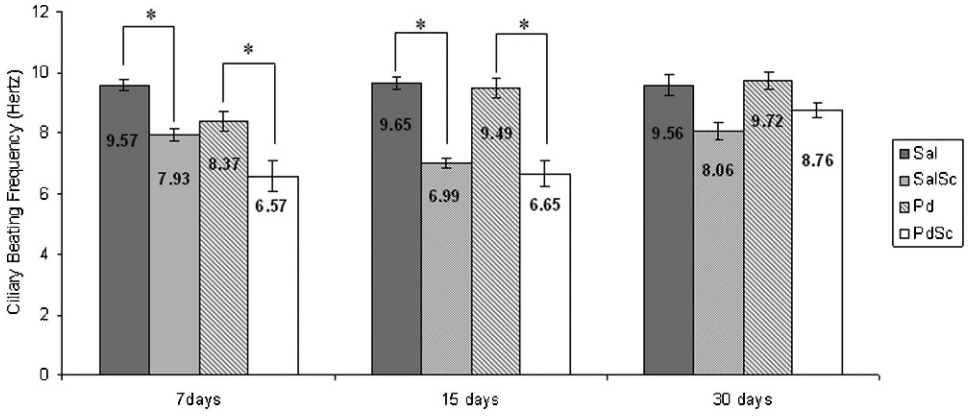

Figure 1 - The ciliary beat frequency (mean ± standard deviation) of animals undergoing or not undergoing bronchial section and anastomosis and treated with prednisone or saline solution for 7, 15, or 30 days. Animals undergoing the surgical procedure had a significant CBF decrease at 7 and 15 days after the surgical procedure ($p<0.001$).

groups at 7 and 15 days after surgery ($p<0.05$) compared to 30 days after surgery (Figure 2).

MCTV did not significantly differ between the SalSc and PdSc groups in any of the study periods ($p=0.139$).

The Pd group had a lower MT than the Sal group in all of the study periods ($p<0.03$). Mucus transportability was lower in the SalSc group compared to the Sal group in all of the study periods, indicating that the surgical procedure worsens this variable ($p<0.001$). Group PdSc showed better mucus transportability at 15 and 30 days compared with the SalSc group ($p<0.05$), indicating that prednisone improves mucus transportability in animals undergoing bronchial section and anastomosis (Figure 3).

DISCUSSION

Mucus transportability is directly related to its chemical composition (19). In this study, we observed that prednisone interferes with mucus quality by reducing its transportability. However, when prednisone therapy was associated with bronchial section and anastomosis, there was an improvement in mucus transportability, possibly because this drug modulates local inflammatory conditions. Studies have reported a reduction in mucin production and improvement in mucociliary transportability in asthmatic patients after treatment with corticosteroids. Such results are justified because inflammation in animal models causes mucus hypersecretion and therefore reduces mucus transportability (20). Although the mechanisms are not completely understood, IgG increases mucus viscoelasticity. Therefore, the control of inflammatory processes reduces viscoelasticity in response to a decrease in mucosal IgG (19). In these cases, improvement in this variable is easily understood because prednisone improves the general inflammatory conditions of patients. Our study used animals free of diseases whose only condition was daily treatment with steroids, and in this case, the drug impaired mucus transportability.

Mucus transportability reduction was observed at 7, 15, and 30 days after bronchial section and bronchial anastomosis. Rivero et al. (8) evaluated the effects of bronchial section and unilateral anastomosis on mucus transportability

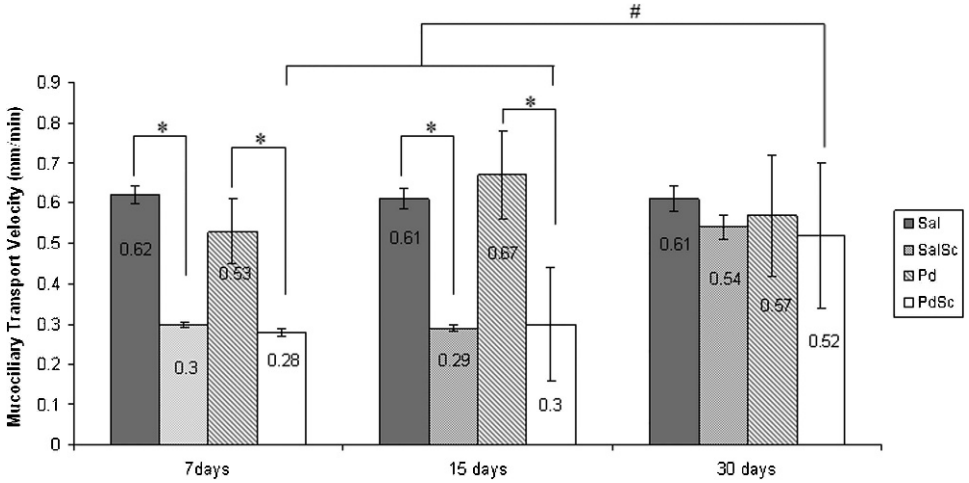

Figure 2 - The mucociliary transport velocity (mean ± standard deviation) of animals undergoing or not undergoing bronchial section and anastomosis and treated with prednisone or saline solution for 7, 15, or 30 days. Animals assessed 7 and 15 days after the surgical procedure had a lower mucociliary transport velocity compared with the non-surgical groups (*$p<0.001$). Groups assessed after 30 days did not present a significant difference in this variable. Groups undergoing surgery had a lower mucociliary transport velocity 7 and 15 days after the surgery compared to animals assessed after 30 days (# $p<0.05$).

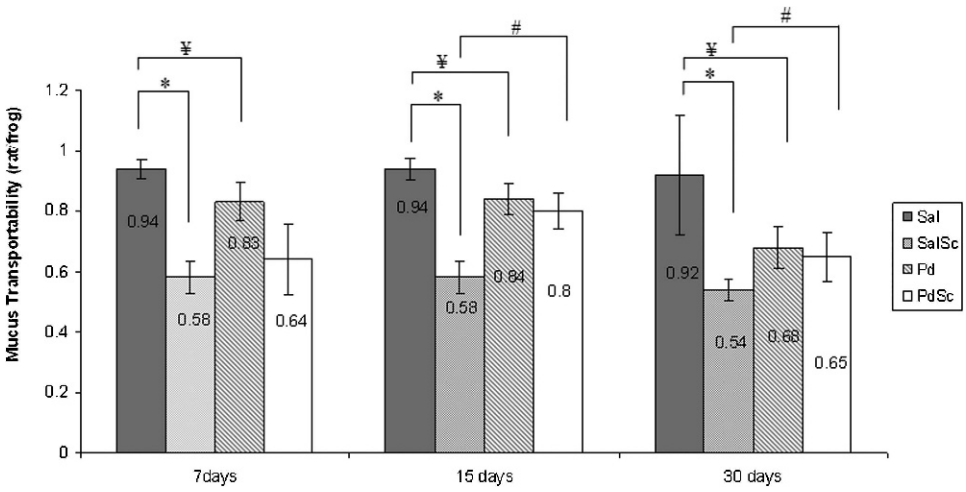

Figure 3 - The in vitro mucus transportability (mean ± standard deviation) of animals undergoing or not undergoing bronchial section and anastomosis treated with prednisone or saline solution for 7, 15, or 30 days. Animals treated with prednisone showed a significant reduction of mucus transportability in all of the study periods (Sal vs. Pd group, ¥$p<0.03$). Animals undergoing the surgical procedure and treated with saline solution had lower transportability compared to the control group (SalSc vs. Sal, *$p< 0.001$). Animals assessed 15 and 30 days after the surgical procedure had better transportability when they received prednisone therapy (# $p<0.05$).

and did not observe changes in this parameter. However, this study used the contralateral bronchus as a control, i.e., surgery was carried out on the left bronchus, and the right bronchus was used as a control. In the present study, one animal without surgical intervention was used as the control because we considered that, although the injury was induced in the left bronchus, a diffuse inflammatory process may affect the production and consequently contralateral mucus transportability. We believe a methodological difference could justify the difference in the final results.

Prednisone therapy after bronchial section and bronchial anastomosis improved mucus transportability when we compared these animals to those submitted to surgery alone. This result reinforces the idea that, under conditions in which inflammation is activated, prednisone alters mucus quality, improving its transportability. In addition, prednisone improves bronchial circulation after transplantation. Inui et al. (21) studied the bronchial circulation of transplanted animals undergoing an immunosuppression protocol (azathioprine and cyclosporine) with or without the addition of prednisolone and observed an improvement in blood flow and a reduction of bronchial ischemia in the group receiving corticosteroids.

Regarding the frequency of ciliary beats, we did not observe differences among the groups treated with prednisone during the study time points, suggesting that prednisone does not change the ciliary beat. We did not find studies in the literature evaluating the systemic use of prednisone on ciliary beat frequency.

In vitro investigations evaluating the effect of topical corticosteroids, such as budesonide, showed a reduction of ciliary beat frequency in the respiratory mucosa of treated animals (14,15). However, these studies suggest that the damage caused to the mucociliary system is more closely associated with medication preservation solutions, such as benzalconium chloride, than to the corticosteroids themselves (22,23).

Regarding bronchial section and anastomosis, ciliary beat frequency reduction was observed in animals evaluated 7 and 15 days after the surgical procedure. Such complications are due to autonomic denervation and blood supply discontinuation after bronchial section without reestablishing bronchial circulation after surgery. Moreover, the inflammatory stimulus in this region after surgery may lead to mucus hypersecretion, as previously discussed. According to Allegra et al. (24), mucus hypersecretion may decrease the ciliary beat frequency during bronchial inflammation and cause functional and structural damage to the mucociliary apparatus.

Pazetti et al. (5) observed an impairment of the ciliary beat frequency 30 days after animals underwent bronchial section and anastomosis surgery. The same result was found by Veale et al. [30] while studying the anastomosed bronchial region of lung transplant patients compared to their non-transplanted (native) contralateral bronchi.

We observed that the group of animals sacrificed after 30 days presented improvement in the parameters evaluated 7 and 15 days after surgery, suggesting that the bronchial ciliated epithelium and the function of the ciliated cells in these animals had regenerated. Although this parameter was recovered 30 days after surgery, there is controversy in the literature regarding the period over which the regenerative process should be evaluated. A study of lung transplantation in dogs observed the recovery of mucociliary transport within the first weeks after surgery (25). Pazetti et al. (5) observed a decrease in ciliary beat frequency 30 days after the procedure, with a trend toward recovery observed in animals evaluated later, which in this case was 90 days after surgery.

The interaction between the prednisone and surgery had no effect on ciliary beat frequency. Auteri et al. (26) did not observe bronchial scarring impairment in animals treated with prednisone within 28 days after surgery. Nevertheless, other studies have shown scarring process impairment when animals are treated with corticosteroids (16).

The efficiency of mucociliary clearance depends on three factors: the density of the cilia and their beat frequency, the amount of mucus, and the viscoelastic properties of the mucus. In this study, we observed that prednisone improves mucus transportability after surgery; therefore,

we believe that the mucus composition was altered. However, this improvement was not enough to impact the ciliary beat frequency or mucociliary transport velocity. In general, we observed that prednisone does not affect mucociliary clearance even though it impairs mucus transportability.

All of the groups undergoing surgery showed impairment of the three study variables. Therefore, we suggest that mucociliary clearance was impaired by surgery. This result corroborates literature data (5,8).

Despite the effective improvement in mucus quality in animals that underwent surgery and were treated with prednisone, we cannot state that the association of drugs and surgical intervention affects mucociliary clearance because no changes were observed in the mucociliary transport velocity in these animals.

Bronchial section and anastomosis decrease mucociliary clearance in the early postoperative period, affecting the mucociliary transport velocity, ciliary beat frequency and mucus transportability.

Prednisone therapy improves mucus transportability in animals undergoing bronchial section and anastomosis.

ACKNOWLEDGMENTS

This study was performed at the Thoracic and Cardiovascular Surgery Graduation Program, Heart Institute, Hospital das Clínicas, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil. The study was supported by grants by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, SP, Brazil.

AUTHOR CONTRIBUTIONS

Braga KAO executed the experimental protocol, performed the data analysis and wrote the paper. Nepomuceno NA executed the experimental protocol and performed the data analysis. Correia AT performed the statistical analysis. Jatene FB supervised all of the stages. Pêgo-Fernandes PM supervised all of the stages and wrote the paper.

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