

## RAPID COMMUNICATION

# Impact of topically-applied lpd-glucose on tracheal mucociliary clearance after warm and cold ischemia: short communication

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## INTRODUCTION

Tracheal transplantation remains a challenge for General Thoracic Surgery.<sup>1</sup> A large number of surgical techniques and implantable devices has been tested with poor results.<sup>1,2</sup> In spite of that, some recent findings on tracheal revascularization and graft epithelial replacement have renewed the interest on tracheal transplantation, with special attention to graft bioengineering.<sup>2-5</sup> However, in order to succeed in tracheal transplantation, the effects of tracheal ischemic injury secondary to harvesting need to be better understood.

Functional preservation of solid organs is usually achieved by the use of intravascular preservation solutions.<sup>6</sup> However, because of the particular segmental pattern of the tracheal vascularization, with no major tracheal vessels,<sup>7</sup> intravascular administration of preservation solutions into tracheal grafts is technically complex,<sup>8</sup> and far from being useful in a clinical scenario. The trachea is composed of thin cell layers and maintains an open lumen after harvesting. Based on those anatomical characteristics, we hypothesized if a topically-applied preservation solution could penetrate into tracheal layers and maintain mucociliary function of tracheal grafts submitted to either warm or cold ischemia.

Our objectives were: 1) to evaluate if topically-applied LPD-glucose, a solution largely used for lung preservation, could ameliorate the effects of warm (room temperature) ischemia on tracheal mucociliary clearance; and 2) to evaluate if topically-applied LPD-glucose could ameliorate the effects of cold ischemia on tracheal mucociliary clearance.

## METHODS AND MATERIALS

This research was approved by the Ethical Committee of our Institution. All animals were treated in agreement with the Brazilian regulation for the use of animals for scientific research.

In order to address the first objective, we obtained 31 tracheal segments from 16 Wistar male rats (weight,

300 ± 50g). The rats were anesthetized with intraperitoneal pentobarbital (50mg/kg), and euthanized by exsanguination. A median cervicosternotomy was performed, followed by tracheal harvesting. The trachea was sectioned at the middle length providing two tracheal segments from each rat. Right after harvesting, the tracheal segments were submerged in saline solution or LPD-glucose (Vitrolife AB, Gothenburg, Sweden), and stored at room temperature during the established ischemic time. The tracheal segments were allocated to one of six groups as follows: Group 1A (Saline solution, 6h of ischemia); Group 1B (Saline solution, 16h of ischemia); Group 1C (Saline solution, 24h of ischemia); Group 2A (LPD-glucose, 6h of ischemia); Group 2B (LPD-glucose, 16h of ischemia); Group 2C (LPD-glucose, 24h of ischemia).

In order to address the second objective, we obtained 34 tracheal segments from 17 Wistar male rats (weight, 300 ± 50g). The anesthetic procedure, the surgical technique, and the tracheal harvesting were the same as described above. Right after harvesting, the tracheal segments were submerged in saline solution or LPD-glucose, and stored at 4°C during the established ischemic time. The tracheal segments were allocated to one of six groups as follows: Group 3A (Saline solution, 6h of ischemia); Group 3B (Saline solution, 16h of ischemia); Group 3C (Saline solution, 24h of ischemia); Group 4A (LPD-glucose, 6h of ischemia); Group 4B (LPD-glucose, 16h of ischemia); Group 4C (LPD-glucose, 24h of ischemia).

In order to achieve a baseline measure, 05 tracheal segments were obtained from 03 Wistar male rats as described above, and analyzed just after harvesting (Control Group).

## Mucociliary clearance analysis

The mucociliary clearance was analyzed by the ciliary beating frequency (CBF) and the *in situ* mucociliary transport (MT), as described previously.<sup>9-12</sup> Briefly, after the established ischemic period, the ventral wall of each tracheal segment was opened to expose the epithelium. The tracheal segment was placed under a light microscope (Olympus BX50, Tokyo, Japan), that was connected to a video camera (Sony Triniton 3CCD, Tokyo, Japan), with a 100x magnification. MT was measured by timing the movement of mucous particles across the tracheal surface with the aid of a reticulated eyepiece. MT was considered

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present when there was any measurable transportation, and was considered absent when there was no measurable transportation.

Afterwards, under the same microscope, a stroboscope (Machine Vision Strobe, Cedar-Hurst, USA) was placed in front of the tracheal segment, and CBF was measured by synchronization between the cilia movement and the stroboscope flashlight. CBF was considered present when there was any measurable ciliary beating, and was considered absent when there was no measurable ciliary beating. When present, the CBF was expressed in Hertz (Hz).

### Data presentation and Statistical analysis

Data was presented as mean  $\pm$  standard deviation for numerical variables. ANOVA with Bonferroni post-test was used for numerical variables when appropriate. Fisher's exact test was used for categorical variables when appropriate. Statistical significance was considered when  $p < 0.05$ .

## RESULTS

### Groups submitted to warm ischemia

When the tracheal segments were submitted to warm ischemia, the mucociliary clearance was severely impaired, chiefly in the groups with more than 6h of ischemia. Two of three tracheal segments in Group 1A, and three of four tracheal segments in Group 2A had present CBF. However, the CBF was absent in all tracheal segments in Groups 1B, 1C, 2B and 2C. The MT was absent in all groups (Group 1A to 2C), but for one tracheal segment in Group 2B.

### Groups submitted to cold ischemia

The CBF values (in Hz) found  $10.94 \pm 1.13$  in Group 3A;  $12.70 \pm 3.10$  in Group 3B;  $9.32 \pm 4.46$  in Group 3C;  $8.90 \pm 6.18$  in Group 4A;  $14.38 \pm 1.80$  in Group 4B; and  $11.59 \pm 1.89$  in Group 4C. The CBF could be measured in all but two segments (Group 4A and Group 3C, each). There was no statistical difference among Groups 3A to 4C ( $p = 0.11$ ). Even when the Control Group (CBF =  $15.21 \pm 4.39$ ) was included in the analysis, there was no significant difference on CBF among the groups ( $p = 0.054$ ).

The MT was present in 26% of tracheal segments submerged in saline solution (Groups 3A, 3B, and 3C), and in 46% of tracheal segments submerged in LPD-glucose (Groups 4A, 4B, and 4C). However, this difference was not statistically significant ( $p = 0.28$ ).

## DISCUSSION

Mucociliary clearance is the primary defense mechanism of the respiratory system.<sup>13</sup> The trachea has an essential role in mucociliary clearance by impelling the mucus from the lower to the upper airways to be expelled by cough or swallowed.<sup>14</sup> Therefore, the maintenance of tracheal mucociliary clearance seems to be an important aim for tracheal graft preservation.

Based on tracheal anatomy, we proposed a novel approach to tracheal graft preservation. Because of the semi-rigid tracheal structure,<sup>7</sup> the tracheal lumen remains open after harvesting. This could allow continuous contact of the preservation solution with the respiratory epithelium during the ischemic period, what could enhance epithelium preservation. Preservation solution could also penetrate into

the other tracheal layers through the highly water-permeable respiratory epithelium.<sup>15</sup>

Because of the simpler anatomic and physiologic structure of the trachea, its metabolic requirements are supposed to be lower than those of other solid organs.<sup>16</sup> This could allow at least a short period of warm ischemia after tracheal harvesting. However, based on the showed data, warm ischemia cannot be allowed after tracheal harvesting, even during short periods.

When the tracheal segments where stored at 4°C, the CBF could be measured on almost all of them. The ischemic time up to 24h at 4°C did not seem to severely impair the tracheal CBF in our study. Interestingly, even when the Control Group is included in the analysis, the difference among groups did not reach statistical significance. Although this finding might be related to the small sample size of the groups, it suggests that tracheal mucociliary clearance could benefit from cold storage. Besides that, MT was present in several tracheal segments after cold storage. Although MT was absent in the majority of tracheal grafts submitted to cold ischemia, more tracheal segments submerged in LPD-glucose had present MT compared to those submerged to saline solution. However, the difference did not reach statistical significance. Thereafter, the major factor implicated on tracheal preservation, as assessed by mucociliary clearance, seems to be the storage temperature, and not the preservation solution.

However, the mucociliary clearance is directly affected by the physical properties of the mucus. Topically-applied solutions, including saline solution, can alter the rheological properties of mucus and impair the mucociliary clearance.<sup>17,18</sup> So, the not significant difference on mucociliary clearance of the tracheal segments submerged to LPD-glucose or saline solution could be related solely to the modification of rheological properties of the mucus.

Our study has some limitations. We did not perform histological evaluation of the tracheal segments. Therefore, in spite of the better mucociliary function of tracheal segments submitted to cold ischemia, there are no data about the cellular integrity of some important tracheal tissues, namely respiratory epithelium and cartilage. Other limitation is the small sample size in some groups, which could lead to statistical misinterpretation.

## CONCLUSIONS

We conclude that topically applied LPD-glucose did not ameliorate the effects of both warm and cold ischemia on tracheal mucociliary clearance in rats. However, studies with larger sample size, including evaluations about the effects of preservation solutions on cellular morphology and mucous rheology are necessary in order to discard or not the use of topically-applied preservation solutions for tracheal preservation.

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