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.1 A large number of surgical techniques and implantable devices has been tested with poor results.1,2 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.3-5 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.6 However, because of the particular segmental pattern of the tracheal vascularization, with no major tracheal vessels,7 intravascular administration of preservation solutions into tracheal grafts is technically complex,8 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 that 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.9-12 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
Therefore, the maintenance of tracheal mucociliary clearance is essential; 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. 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. 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 absent 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 the mucus and impair the mucociliary clearance. 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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