DEVELOPING UNIVERSAL BLOOD TYPE DONOR ORGANS USING EX VIVO ABO ENZYMATIC TREATMENT

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Background:

Lung transplantation (LTx) is a life-saving therapy for end-stage lung diseases. Successful organ Tx depends on finding donor organs that display on their surfaces antigens that are immunologically compatible with those of the recipient. Among the more critical antigens requiring matching are the A- and B-antigens, which are part of the ABO histo-blood group system. The need to match multiple antigens reduces the probability of finding a compatible donor, thereby increasing times on waitlists. This situation not only results in increased waitlist mortality for some patients but also in wastage of donor organs that cannot be matched. For instance, blood group O candidates have a nearly 2-fold higher risk of dying than do those of type A or B while waiting for an acceptable lung allograft.1 Any means to decrease matching requirements would effectively expand the donor pool for a given recipient and dramatically improve organ allocation.

A small subset of transplants, mostly kidneys, are performed in adults using ABO-incompatible (ABOi) organs, if an ABO-compatible donor cannot be found. The procedure is performed primarily with sub-group A2 donors (low antigen expression) into group O/B recipients (with low anti-A antibodies titers) along with recipient desensitization protocols to minimize rejection.2 While these patients may begin to produce ABO antibodies again soon after ABOi transplantation, graft injury often does not occur. This is a phenomenon called “accommodation”, caused by mechanisms that remain incompletely understood.3 Although 5-year outcomes yielded equivalent between ABOi vs ABO compatible kidney Tx, higher early mortality has been observed in ABOi patients.4 This is likely due to the recipient-centric desensitization protocols: for instance, the over-suppressed immune system leads to higher risk of infectious complications, and changes in the coagulation system from plasmapheresis lead to higher rates of bleeding events.4 Therefore, a donor-centric antigen removal approach can be envisioned to relieve the burden of augmented immunosuppression on recipient as well as to greatly expand the practice of ABOi Tx to all mismatch combinations (instead of the current small subset).

Our collaborators from University of British Columbia recently identified enzymes that carry out the conversion of whole units of A blood to O very efficiently (Figure 1-A, S. Withers, Nature Microbiology 2019).5 In collaboration with this group, we explored the feasibility and safety of using the enzymes (FpGalNAc deacetylase and FpGalNase) to remove histo-blood type antigens from donor lungs during ex vivo lung perfusion (EVLP). EVLP is fairly mature technique used clinically for organ preservation, assessment, and restoration. With EVLP, donor lungs are maintained, perfused and ventilated at physiological conditions for several hours before transplantation.6 EVLP has been used as a platform to deliver treatment to donor lungs – treating donor bacterial and virus infection, decreasing donor lung inflammation associated with brain death, and treating donor lungs affected with thromboembolic events.7-10 We predict that converting organs to O type (universal blood type organs) during EVLP prior to LTx will reduce
initial immune responses, especially hyperacute rejection (HAR) and allow survival of grafts from ABOI donors. In this study, the enzymes’ efficacy was examined sequentially from in vitro cell-based and tissue-based studies to ex vivo whole organ studies (Figure 1-B).

Methods and Results

Compatibility of enzymes with organ perfusion/preservation solution. To ensure compatibility of the enzymes with the EVLP system, 1% human type A red blood cells (RBCs, n=5/group) were treated with enzyme-containing EVLP solution (37°C) and lung preservation solution (4°C) for 1 hour. Phosphate buffered saline (PBS) at 37°C, the standard solutions used for blood treatment, was used as a comparison group. The temperatures chosen for EVLP solution and lung preservation solution were based on their working temperature in clinical practice. After enzyme treatment, RBCs were incubated with mouse anti-human blood type A (BTA) monoclonal antibody followed by FITC labeled goat anti-mouse IgM. The level of antigen removal was analyzed by flow cytometry. Dose escalation study was carried out to help predict the appropriate dose to be used in organs. The unit of dose used throughout the study is defined as weight of enzymes (µg) over volume of solution (mL).

We demonstrated that the enzymes are fully compatible with organ perfusion and preservation solutions in the dose range of 0.2–4 µg/mL (Figure 2-A). The enzyme was able to remove over 95% of antigen of RBCs in EVLP and lung preservation solutions at the dose of 1µg/mL, while the same effect in PBS was achieved at the dose of 4µg/mL.

Static treatment of human artery with the enzymes. To test the enzymes’ efficacy on the tissue level, an in vitro model of human artery was used. From each human donor (type A1), aorta (n=3/group) were split into a control group (EVLP solution) and treatment group (enzyme-containing EVLP solution), and incubated statically at 37°C for 4 hours respectively. Biopsies were taken at the end of incubation for both groups. The change in expression of blood type antigen was analyzed by immunohistochemistry. Consecutive sections of the biopsies were double-stained with CD31 antibody (a marker of endothelial cells) to show the location of inner surface of blood vessel and blood type A (BTA) antibody to show the expression of blood type antigen.

The histo-blood type antigens in human arteries were observed primarily on endothelial cells – and as expected, BTA antigens co-localized with endothelial marker CD31 (Figure 2-B, Pretreatment). Static treatment of aorta using 1 µg/mL of enzymes in EVLP solution demonstrated 92±1% of BTA antigen removal (Figure 2-BC). The dose effect amongst the tested range were similar, implying the lowest dose tested could be the effective dose in EVLP. Static cold treatment of aorta in lung preservation solution also demonstrated antigen removal, despite less consistent results, highlighting that normothermic temperature may lead to higher efficacy of the enzymes.

Ex vivo treatment of human lungs. The efficacy of enzyme-containing EVLP solution in removing histo-blood type antigens were studied in human lungs under the Toronto EVLP settings. Donor human lungs assessed with clinical EVLP and determined as unsuitable for transplantation were used here. After lungs were declined for transplant, enzymes were added to the perfusion liquid to initiate treatment. Biopsies were taken before and after treatment. The expression of BTA antigen was analyzed by immunohistochemistry. As described in previous section, consecutive sections of the biopsies were double-stained with CD31 and BTA antibodies. The function of lungs were monitored for potential acute side effects throughout treatment.

Comparison of sections of the pre-treatment biopsy reveals that the BTA antigens in lungs are located both on endothelial and epithelial cells (Figure 2-D, solid arrow for endothelial and dashed
arrow for epithelial). After treating the lungs (n=3) with enzymes ex vivo under different conditions, biopsies were taken from different areas of the lungs to examine changes in BTA antigens. Within 1h of perfusion, remarkable intravascular clearance of BTA antigens was observed (Figure 2-E). Similar results were observed after 3h treatment of low (1µg/mL) and high dose (5µg/mL) of the enzyme (Figure 2-F). Importantly, no acute physiological side effects were observed from the enzymatic treatment. These studies demonstrate that within the time span of normal clinical EVLP (4 hours), the enzymes are able to effectively remove BTA antigens along the intravascular endothelium of perfused human lungs.

**Ex vivo hyperacute rejection (HAR) model to evaluate the effects of ABO removal using enzymatic treatment.** In this pre-clinical stage, in order to study post-transplant efficacy of the enzyme treatment, an ex vivo HAR model was established by adding mis-matched plasma to the EVLP system to trigger antibody-mediated rejection. Type O plasma along with necessary anti-A antibodies and complement components was used as the surrogate for “recipient blood”. The potential effect of antigen removal on preventing or alleviating HAR was examined.

Type A1 human lungs declined for transplantation were used. Upon arrival, lungs were split into two blocks, placed on two independent EVLP circuits, and randomized into control (EVLP) and treatment group (EVLP + enzyme) (Figure 3-A). The enzyme was added to the treatment lung after 1 hour of EVLP when full flow and ventilation have been achieved. Type O plasma was added to both groups to induce “post-transplant” immune responses after 4 hours of perfusion/treatment. Lungs were subsequently observed for 4 hours (blood mismatch reperfusion period). Physiologic parameters such as airway pressures, pulmonary compliance, and oxygenation capacity were evaluated hourly. Biopsies were taken before EVLP, before adding O plasma, and at the end of mis-match perfusion. Expression of BTA was analyzed by IHC as described previously. Hallmarks for HAR such as formation of fibrin thrombi, neutrophil infiltration, diffuse alveolar damage, and complement (C4d) deposition were examined.

After 4 hours of EVLP, the normalized expression of BTA antigen in the control lung remained at 99.2% as compared to baseline. The overall tissue expression of BTA antigen in the treated lung reduced to 50.3% and near complete removal was observed in the endothelium, similar to that described in the previous section. After adding the “recipient” (300 mL type O plasma), the control lung showed worsen physiological parameters represented by increases in airway pressure and decreases in pulmonary compliance (Figure 3-B-blue). On the other hand, physiology of the treatment lung remained stable throughout the whole course (Figure 3-B-red). H&E staining of the control lung shows a fairly normal appearance at 0th and 4th hour of EVLP. At the end of mismatched blood reperfusion, the three lobes of the control lung (RUL, RML, RLL) ubiquitously shows infiltration of leukocytes, and presence of red cells in the alveoli (Figure 3-C). Histology of the treatment lung shows minimal lung injury (Figure 3-D). Although the physiological deterioration in the control group was rather mild than expected for HAR, this could be a dilution effect of the antibodies and complement factors, since the type O plasma added in this case was sub-physiological (~15% vs physiological 55%). Future studies with near physiological level of O plasma are planned to confirm these results.

**Conclusions:**

Ex vivo enzymatic treatment can effectively remove blood type antigen in donor lungs without acute side effects. Preliminary results of an ex vivo rejection model shed light on the enzymes’ ability to alleviate ABO incompatible antibody-mediated rejection in lungs. This treatment has potential to develop universal blood type organs, resulting in great expansion of safe ABOi organ transplantation, and leading to a much fairer allocation of organs for transplantation.
Figure 1: The concept of enzymatic removal of ABO antigen and study design. A. Basic ABH antigens on red blood cells. Enzymatic reactions marked by black arrows remove the terminal α-N-acetyl-galactosamine or α-galactose monosaccharide and turn A/B red cells to an O type. B. Schematic overview of the study design. In Aim 1, the enzymatic effects was examined on the cellular level with human red blood cells (Phase I), on the tissue level with human artery (Phase II), and on the whole organ level with ex vivo perfused human lungs (Phase III). In Aim 2, the impact of treatment on post-transplant outcome was studied with an ex vivo hyperacute rejection (HAR) model.
Figure 2: Effect of enzyme on human red blood cells (A) and artery (B-C) and human lungs (D-F). A. Flow cytometry of RBC treated with enzymes at different doses in different media. C. Representative image of immunohistochemical staining of artery treated without (Control) and with enzymes (Treatment). Positive staining in brown color. BTA colocalize with endothelial cells (CD31 positives) in untreated artery. BTA is absent in treated artery. D. Dose effects of enzymes on type A human aorta in different media/condition. D-E. Representative immunohistochemical staining images of lung before (D) and after (E) being treated with enzymes. Positive staining in red color. Before treatment, BTA locates both inside the blood vessels (solid arrow) and air ways (dashed arrow). After treatment, vascular BTA disappears. F. Expression of BTA based on area quantification of positive immune staining and normalized against the pre biopsy using the formula: \[% Normalized Antigen = \frac{Area_{BTA}(Post) + Area_{total}(Post)}{Area_{total}(Pre)} \]
Figure 3: Ex vivo HAR model for ABOi lung transplantation and effects of enzymatic treatment.

A. Paired study design: lungs from same donor were separated to two distinct EVLP circuits and randomized into control (standard EVLP) and treatment (EVLP + enzyme) group. Type O plasma was added to initiate antibody mediated rejection. B. Lung physiology before and after addition of O plasma (dashed line) on peak airway pressure (Peak awP), dynamic compliance.
(Cdyn), plateau airway pressure (Plat awP), static compliance (Cstat), and functional oxygenation (deltaPO2). C-D. Low power H&E images of the control lung (C) and treatment lung (D) from baseline, before addition of O plasma and at the end of perfusion. Control lung at the end shows patchy inflammatory infiltrates and thickened interstitium.

References: