The efficacy of inhaled nanoparticle tacrolimus in preventing rejection in an orthotopic rat lung transplant model

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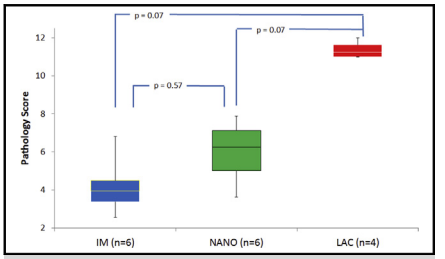
ABSTRACT

Objective: The immunosuppressive efficacy of inhaled nanoparticle tacrolimus was compared with systemic tacrolimus in a rodent allogeneic lung transplant model.

Methods: Sixteen rats underwent allogeneic left orthotopic lung transplantation and were divided into 3 treatment groups: (1) inhaled nanoparticle tacrolimus: 6.4 mg tacrolimus/6.4 mg lactose twice per day; (2) intramuscular tacrolimus: 1 mg/kg tacrolimus once per day; and (3) inhaled lactose: 6.4 mg of lactose twice per day. Five days after transplant, the rats were necropsied and underwent histologic rejection grading and cytokine analysis. Trough levels of tacrolimus were measured in allograft, blood, and kidney.

Results: Both intramuscular (n = 6) and nanoparticle tacrolimus (n = 6) rats displayed lower histologic grades of rejection (mean scores 3.4 ± 0.6 and 4.6 ± 0.9, respectively) when compared with lactose rats (n = 4) (mean score 11.38 ± 0.5, $P = .07$). Systemic tacrolimus trough levels (median) were lower in nanoparticle tacrolimus–treated rats versus intramuscular-treated rats (29.2 vs 118.6 ng/g; $P < .001$ in kidney, and 1.5 vs 4.8 ng/mL; $P = .01$ in blood).

Conclusions: Inhaled nanoparticle tacrolimus provided similar efficacy in preventing acute rejection when compared with systemic tacrolimus while maintaining lower systemic levels. (J Thorac Cardiovasc Surg 2017;154:2144-51)



Box-whisker plot of pathology score in the treatment (IM and NANO) and control (LAC) groups.

Central Message

nTAC administered via inhalation shows similar attenuation of acute rejection in a rodent lung allograft as systemic therapy.

Perspective

Inhaled therapy of antirejection medication may provide adequate immunosuppression while maintaining low systemic levels and therefore less adverse toxic effects.

See Editorial Commentary page 2152.

According to the 2016 Registry of the International Society for Heart and Lung Transplantation, more than 3900 lung transplants were performed in 2014 and the overall median survival for lung transplant recipients is 5.8 years, with approximately 30% of patients experiencing an episode of acute rejection within the first year of transplant.[1] These episodes of acute rejection are known risk factors for the development of chronic rejection and bronchiolitis obliterans syndrome within a few years after lung transplantation.[2,3] Although the strategies vary among transplant centers and continue to evolve, the mainstay for prevention of acute and chronic rejection remains systemic immunosuppression, with initial induction by a 3-drug regimen (calcineurin inhibitor or other interleukin [IL]-2 antagonists, in addition to corticosteroids and an antimetabolite), followed by maintenance therapy.[4]

Current therapy with tacrolimus (oral and intravenous) can cause systemic toxicity (especially renal) and increase the risk of opportunistic infections. Lungs offer a direct route for administering therapy by inhalation, which may circumvent some of the associated systemic toxicity and potentially achieve higher concentrations at the target site

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Abbreviations and Acronyms

IL = interleukin
IM = intramuscular
LAC = lactose
NANO = nanoparticle
nTAC = nanoparticle tacrolimus
Q1, Q3 = first and third quartiles of the median value
SD = standard deviation

in lung transplant recipients. Prior attempts using aerosolized cyclosporine had limited success because of airway reactivity to the polyethylene glycol solvent in these formulations.[5,6]

A novel formulation of tacrolimus has been developed by the College of Pharmacy, University of Texas in Austin, prepared from anhydrous tacrolimus using a thin film freezing process and stabilized with lactose molecules.[7] This brittle matrix formulation of nanoparticle tacrolimus (nTAC) provides greater aqueous solubility, a greater surface area relative to conventional tacrolimus, and a dispersion size less than 5 μm, theoretically enabling it to reach the lung's smallest and most distal airways and therefore behaving like a nanoparticle.[7,8]

Previous work using this nTAC in a single-dose nonrejection (syngeneic) orthotopic rat lung transplantation model confirmed satisfactory lung tissue levels with negligible systemic levels.[8]

The objective of this study was to compare the antirejection efficacy of this novel inhaled nTAC with that of systemic (standard) tacrolimus in an allogeneic orthotopic rat lung transplantation model, to measure blood and tissue tacrolimus levels, and to perform cytokine analysis.

MATERIALS AND METHODS

All of the animals received humane treatment in accordance with the ''Principles of Laboratory Animal Care'' formulated by the National Society for Medical Research and the ''Guide for the Care and Use of Laboratory Animals'' prepared by the National Academy of Sciences and published by the National Institutes of Health, under a protocol approved by the Institutional Animal Care and Use Committee of The University of Texas Health Science Center at San Antonio (protocol number 10084x).

Tacrolimus monohydrate (Batch #090703) was purchased from CQ International Co Inc (Cambridge, Mass). nTAC powder was produced as a 1:1 mixture with lactose by the College of Pharmacy, University of Texas at Austin, using a thin film freezing process described previously.[7]

Allogeneic orthotopic left lung transplants were performed using inbred adult male Brown Norway rats as donors and inbred Lewis rats as recipients (Charles River, Wilmington, Mass) using the modified cuff technique previously described by Mizuta and associates.[9]

Donor rats were anesthetized using 3% to 5% inhaled isoflurane (Halocarbon, River Edge, NJ) admixed with oxygen and were ventilated via tracheostomy using a Harvard Model 683 Small Animal Ventilator (Harvard Apparatus, Holliston, Mass) with a tidal volume of 10 mL/kg at a rate of 60 to 80 breaths/min with positive end-expiratory pressure of 2 cm of water. Animals were dosed with heparin (APP Pharmaceuticals, Schaumburg, Ill) 1000 units/kg intravenously through the penile vein (alternatively into the hepatic bed) to achieve systemic heparinization over 5 minutes. The thoracic cavity was exposed via a generous laparotomy incision, and the lungs were flushed in anterograde fashion using cooled low potassium dextrose preservative solution, Perfadex (XVIVO, Goteborg, Sweden), to achieve uniform pallor of the lung graft. The entire heart-lung block was then excised, and the left hilum was dissected on a cold operating stage. The pulmonary vein, pulmonary artery, and main bronchus of the left lung were mounted with cuffs fashioned out of 14-gauge Angiocath (Becton Dickinson, Sandy, Utah). Lungs were stored at 4°C in a partially inflated state (end-tidal volume, ~3-4 cm water pressure), immersed in the preservative solution.

After the explant, the recipient procedure was performed. Recipient rats were anesthetized, orally intubated using a 14G cannula, and ventilated with similar settings as the donor. Body temperature was maintained at approximately 36.5°C using a water-bath warmer. The left pulmonary hilum was exposed via a third intercostal thoracotomy, and the pulmonary artery, vein, and bronchus were individually clamped using microaneurysm clips (Yasargil; Aesculap, Center Valley, Pa). The donor pulmonary artery, vein, and bronchus were then aligned and telescoped into the respective structures of the recipient and anastomotic cuffs secured using 6-0 silk ties. After completion of the transplant, the recipient was allowed to recover from the effects of anesthesia in a warm chamber with 100% oxygen and given a single dose of 5 mg/kg enrofloxacin (Baytril; Bayer, Shawnee Mission, Kans) intramuscularly (IM) for infection prophylaxis.

Grouping

After transplantation, the recipients were divided into 1 of 3 groups, the lactose (LAC) group, IM group, and nanoparticle (NANO) group, as depicted in the flow diagram of experimental design (Figure 1).

The NANO group received nebulized nTAC administered every 12 hours by inhalation in a 4-port nose-only dosing chamber using the AeroNeb Pro nebulizer (Aerogen, Galway, Ireland). An amount of 12.8 mg of nTAC powder (6.4 mg tacrolimus + 6.4 mg lactose) was dispersed in 6 mL of normal saline by sonication for 3 minutes for nebulization.

The LAC group was treated with nebulized lactose every 12 hours in a similar manner as nTAC in the NANO group. Lactose solution was prepared by mixing 6.4 mg of lactose powder with 6 mL of normal saline by agitation for 3 minutes. The IM group received anhydrous tacrolimus suspended in normal saline and administered at a concentration of 1 mg/mL/kg of recipient body weight into the thigh once every 24 hours.[10] The injected side was alternated each day. The first dose of the each medication was administered after the animals had recovered fully from anesthesia and breathing spontaneously.

Experimental End Point

The recipient animals were electively euthanized at postoperative day 5 by exsanguination through a cardiac puncture under deep anesthesia. The time of euthanasia was adjusted to coincide with the approximate time for their next treatment (the IM group was euthanized 24 hours after the final dose, whereas animals in the NANO and LAC groups were killed 12 hours after the final dose). Thus, the drug concentration levels in the blood and tissue would reflect trough levels of tacrolimus. At necropsy, allografts were examined for aeration and then divided into 3 coronal segments. The superior and middle segments of the allograft, and both kidneys from the recipient were snap-frozen in liquid nitrogen and stored at −80°C for later analysis. The inferior segments were preserved in 10% buffered formalin for histologic examination. A portion of blood collected in ethylenediaminetetraacetic acid from cardiac puncture at the time of euthanasia was centrifuged, and the supernatant plasma was saved at −80°C for tacrolimus level analysis. The remaining sample was saved as whole blood at −80°C for later analysis. A brief video has been compiled to illustrate this methodology (Video 1).