

The Efficacy of PAF Antagonist TCV-309 in a Canine Single Lung Allotransplantation after 24 hour Preservation

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To evaluate the efficacy of TCV-309, platelet activating factor (PAF) antagonist, we investigated the use of this agent when added to the Euro-Collins solution or administered to the donor and the recipient as well as the Euro-Collins solution in the preservation of a canine lung for 24 hours. The function of the graft was assessed before harvesting and one hour after reperfusion by a unilateral pulmonary artery occlusion test. In the ECS group, donor lungs were flushed with 500 ml of the Euro-Collins solution containing 375 μg TCV-309. In the TCV group, donor and recipient dogs were pretreated with TCV-309, and donor lungs were flushed as the ECS group. Donor dogs received TCV-309 100 $\mu\text{g}/\text{kg}$ bolus followed by continuous infusion of 500 $\mu\text{g}/\text{kg}/\text{hr}$ for one hour. Recipient dogs received TCV-309 100 $\mu\text{g}/\text{kg}$ bolus followed by continuous infusion of 500 $\mu\text{g}/\text{kg}/\text{hr}$ for two hours, encompassing two hours of reperfusion.

Better posttransplantation pulmonary function was obtained in the TCV group. In conclusion the PAF antagonist TCV-309 is a beneficial agent when it is administered to the donor and the recipient and added to the Euro-Collins solution in a canine model of a single lung transplantation after 24 hr preservation.

Introduction

In organ transplantation, reperfusion following prolonged ischemia results in organ injury which is mediated by activated neutrophils. Preserved organs are injured from sequela during ischemia and subsequent reperfusion. Ischemia reperfusion injury is integrated by various mechanisms, including leukocyte, endothelial activation, platelet activation, oxygen free radicals, complement activation, the generation of inflammatory mediators and arachidonic acid metabolites.¹⁾ Since PAF was first reported in 1972 by Benveniste considerable attention has been directed toward the pathophysiology of PAF as a mediator of acute inflammatory and vasoactive reaction, including the responses of cells related to ischemia-reperfusion injury. PAF is known to have a potent platelet activation and aggregation released by neutrophils, monocytes, eosinophils, macrophages, lymphocytes, endo-

thelial cells, platelets, as well as endothelial cells.²⁾³⁾⁴⁾ Moreover, PAF activates neutrophils leading to their aggregation, adherence, chemotaxis and degranulation; consequently, inflammatory mediators including leukotrienes, superoxide anions, and eicosanoids are released.¹⁾ Conte⁵⁾ and coworkers clarified that pulmonary dysfunctions manifested by low oxygen tension, high pulmonary vascular resistance, decreased pulmonary compliance and lung edema following transplantation are similar to the pathophysiological effects of PAF. They proved that the PAF antagonist BN-52021 significantly improved the posttransplant lung function after 20-24 hr preservation.

Since Terashita⁶⁾ developed CV-3988, several kinds of PAF antagonists have been reported. CV-3988, a specific PAF antagonist which is classified as a PAF-related compound and has a beneficial effect on endotoxin shock and anaphylactic shock.⁷⁾⁸⁾ TCV-309, also developed by Terashita⁹⁾, is more selective and potent than CV-3988 *in vitro* and *in vivo*.

Tagawa⁹⁾ et al. demonstrated the amelioration of a 24-hour preserved canine lung with the PAF antagonist (TCV-309) in an isolated lung perfusion model. Following the results of previous studies in which TCV-309 attenuated reperfusion injury when added to preservation solution (Euro-Collins solution) or perfusion medium (fresh allogeneous blood) by Tagawa, the present study was designed to validate the efficacy of TCV-309 *in vivo* and to investigate the use of this agent when added to the Euro-Collins solution and administered to the donor and the recipient as well as the Euro-Collins solution in the preservation of a canine lung for 24 hr.

Materials and methods

TCV-309

TCV-309 was provided by Takeda Chemical Industries, Ltd. (Osaka, Japan), and was dissolved in normal saline at a dose of 0.1 mg/ml.

Experimental design

Twenty-two size- and weight-matched adult mongrel dogs weighing 10-12 kg were randomly divided into the two groups. The ECS group consisted of 5 donors and 5 recipients; the donor lungs were flushed with 500 ml of the Euro-Collins solution containing 375 μg TCV-309. The TCV group consisted of 6 donors and 6 recipients; donor and recipient dogs were pretreated with TCV-309 and donor lungs were flushed as in the ECS group. Donor dogs were administered with TCV-309 100 $\mu\text{g}/\text{kg}$ bolus followed by continuous infusion of 500 $\mu\text{g}/\text{kg}/\text{hr}$ for one hour. Recipient dogs were administered with TCV-309 100 $\mu\text{g}/\text{kg}$ bolus followed by continuous infusion of 500 $\mu\text{g}/\text{kg}/\text{hr}$ for two hours, encompassing two hours of reperfusion.

Donor operation

The donor dogs were anesthetized with pentobarbital 25 mg/kg intravenously, then endotracheally intubated, and were mechanically ventilated with a tidal volume of 35 ml/kg at a rate of 14 breaths/min while monitoring the airway pressure. An FiO_2 of 1.0 was maintained throughout the experiment. A 5 Fr. thermodilution catheter (CO-S 5861, TERUMO, Tokyo) was placed in the main pulmonary artery (PA) through the left jugular vein, and cardiac output was measured by cardiac output computer (MTC-6100, NIHON KOHDEN, Tokyo). A 5 Fr. arterial line (ELECATH-2900, ELECTRO-CATHETER CORP., RATHWAY N.J.) was established through left femoral artery to monitor systemic pressure and extravascular thermal volume by lung water computer (MTV-1100, NIHON KOHDEN, Tokyo). A 1.9 mm diameter cut-down tube was placed in the left atrium (LA) as a pressure line. Airway pressure, arterial pressure, PA pressure and LA pressure were measured by using polygraph (RMP-6004 MS, NIHON KOHDEN, Tokyo). A median sternotomy was done, and an accessory lobe lobectomy was performed in order to facilitate light microscopic examination, assay as a control lipid peroxide level (LPO) and myeloperoxidase activity (MPO). After ligating and dividing the azygos vein, the right bronchus, main PA and right PA were isolated and encircled. Prior to donor organ procurement, a unilateral PA occlusion test was performed occluding the right PA and the right mainstem bronchus for ten minutes with a tidal volume of 25 ml/kg. Thus cardiac output, main PA pressure, LA pressure, airway pressure and arterial blood gas analysis were obtained.

Organ harvest and preservation

After systemic heparinization (500 U/kg), a 24 Fr. perfusion cannula was inserted into the proximal PA through the right ventricle. 100 μg of PGE₁ in 20 mL of normal saline was given as a bolus via a PA pressure line.

The venae cavae were ligated and the left atrial appendage was amputated to allow egress of the pulmonary perfusate. The PA was flushed with 500 ml of 4°C Euro-Collins solution containing 375 μg TCV-309. PA flush pressure was continuously monitored and maintained below 10 mm Hg by adjusting the height of the drip chamber. During flushing, ventilation was continued. Heart-lung block removal was performed with trachea clamped at a point of end-inflation and placed in a sterile plastic bag with 500 ml of 4°C Euro-Collins solution. Great care was taken not to injure any bronchus or lung parenchyma during this procedure. The block was stored at a temperature of 4-6°C for 24 hours.

Recipient operation

On the following day, weight and size matched recipient animals were anesthetized and ventilated in an identical fashion to the donor. A left pneumonectomy was performed through a left thoracotomy. In the TCV group the six recipients received 100 $\mu\text{g}/\text{kg}$ of TCV-309 as a bolus followed by continuous infusion of TCV-309 500 $\mu\text{g}/\text{kg}/\text{hr}$ for two hours. (e.g., TCV-309 was administered for 60 minutes before reperfusion and 60 min after reperfusion.)

Transplantation procedure

The transplantation was carried out in a fashion similar to that described by Veith.¹⁰⁾ Prior to transplantation, the right PA and the right main bronchus were isolated and encircled to prepare for an immediate postoperative unilateral PA occlusion test. After completing recipient's pneumonectomy with long stumps being left on artery, veins, and bronchus, the heart-lung blocks were removed from storage and the donor's left lung was isolated and trimmed for immediate transplantation. The atrial anastomosis was made with a 5-0 Prolene suture (Ethicon, Inc, Somerville, N.J.) in a horizontal mattress pattern. The pulmonary artery anastomosis was made with a running 6-0 Prolene suture. The bronchial anastomosis was made with a running 4-0 Prolene suture using a telescoping technique. The total ischemic time was defined as the time from PA flushing until reperfusion was begun. The implantation time was defined as the time from the beginning of the atrial anastomosis until blood flow was established to the organ.

Data collection, assessment

Posttransplant assessment

One hour after reperfusion, a unilateral pulmonary artery occlusion test was performed occluding the right PA and right mainstem bronchus for ten minutes to determine posttransplant graft function.

Histological assessment

Three sections of the lower lobe segment from the transplanted lung were stained with hematoxylin and eosin and examined with a light microscope and assayed for MPO activity and LPO. Two of the samples were frozen in liquid nitrogen and kept at -70°C for MPO and LPO measurements.

LPO, MPO assay

LPO in lung tissue was measured according to Ohkawa's¹¹⁾ method. Protein content was measured by Lowry's¹²⁾ method. LPO levels were corrected by protein content, and expressed as n mol malonaldehyde (MDA) /mg protein. MPO activity was assayed as a marker of pulmonary neutrophil sequestration as described by Rubin¹³⁾.

Immunosuppression

The chest was closed in a standard fashion after inserting a 20 Fr. chest tube. Each animal was treated with cyclosporin (10 mg/kg intramuscularly) and penicillin potassium (200,000 unit/body intramuscularly) postoperatively. No inotropic drugs or diuretics were given through the experiment and the chest tube was removed as soon as the animal awoke from anesthesia.

Animal care

Animals in this study were maintained in accordance with the guidelines of the Committee on Animals of the Nagasaki University School of Medicine. All animals received humane care in compliance 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 Institute of Laboratory Animal Resources, National Academy of Sciences, and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Statistical analysis

Statistical analysis was performed by Mann-Whitney U test. A P value less than 0.05 was considered significant. Results were presented as mean \pm standard deviation.

Results

Transplant results

All animals survived the procedure. All animals underwent successful lung transplantation after 24 hours of

graft ischemia. In the ECS group, three recipients died within 10.5 ± 6.7 hours postoperatively because of pulmonary edema. One recipient died on the 5th postoperative day from bronchial anastomotic dehiscence. The remaining recipient was sacrificed on the 4th postoperative day. In the TCV group, one recipient died of pulmonary edema 6 hours after implantation. The other recipients were sacrificed accordingly on either the 4th or the 14th postoperative day after the unilateral pulmonary artery occlusion test. (Data are not shown.) Total ischemic times were not different between the groups. (ECS group, 25 hour 18 min \pm 19 min; TCV group, 24 hour 38 min \pm 49 min) Groups were similar with regard to animal weights, harvest time, preservation time.

Arterial blood gas analysis

There was no significant difference in the preharvesting PaO₂ between the two groups, (396.7 ± 100 mm Hg for the ECS group and 457.5 ± 129 mm Hg for the TCV group.) One hour after reperfusion PaO₂ was significantly higher in the TCV group. The mean PaO₂ was 386.8 ± 186 mm Hg for the TCV group and 141.3 ± 102 mm Hg for the ECS group. (Fig. 1) There was no significant difference in arterial PaO₂ during the study.

Pulmonary vascular resistance

Before harvesting, the pulmonary vascular resistance was similar in the two groups. (1383.6 ± 527 dynes \cdot sec \cdot cm⁻⁵ for the ECS group and 1456.9 ± 854 dynes \cdot sec \cdot cm⁻⁵ for the TCV group) After reperfusion the TCV group showed less elevation in PVR than the ECS group (1806.6 ± 881 dynes \cdot sec \cdot cm⁻⁵ for the TCV group and 2261.4 ± 1285 dynes \cdot sec \cdot cm⁻⁵ for the ECS group); however, there was no significant difference in PVR between the two groups. (Fig. 2)

Lung compliance

Dynamic lung compliance was similar in both groups throughout this study. Before harvesting, the dynamic lung compliance was 16.8 ± 3.3 mL/cm H₂O for the ECS group, 16.2 ± 4.4 mL/cm H₂O for the TCV group. After implantation the dynamic lung compliance decreased slightly. (15.6 ± 3.1 mL/cm H₂O for the ECS group and 15.4 ± 4.4 mL/cm H₂O for the TCV group) (Table 1)

ETV

Extravascular thermal volume (ETV) were comparable. ETV was 3.98 ± 0.97 mL/kg for the ECS group and 2.78 ± 0.91 mL/kg for the TCV group. ETV increased after reperfusion, in the TCV group ETV was 7.58 ± 4.48 mL/kg as compared with 10.80 ± 4.06 mL/kg for the ECS group.

Table 1. Pulmonary compliance, ETV and MPO before harvesting and One Hour after Reperfusion

Group	Pulmonary compliance mL/cmH ₂ O		ETV mL/kg		MPO unit	
	before harvesting	one hour after Tx	before harvesting	one hour after Tx	before harvesting	one hour after Tx
ECS group	16.8±3.3	15.6±3.1	3.98±0.97	10.80±4.06	9.7±1.6	16.2±3.2
TCV group	16.2±4.4	15.4±4.4	2.78±0.91	7.58±4.48	7.7±2.7	12.1±6.9

Definition of abbreviation : Tx = Transplantation

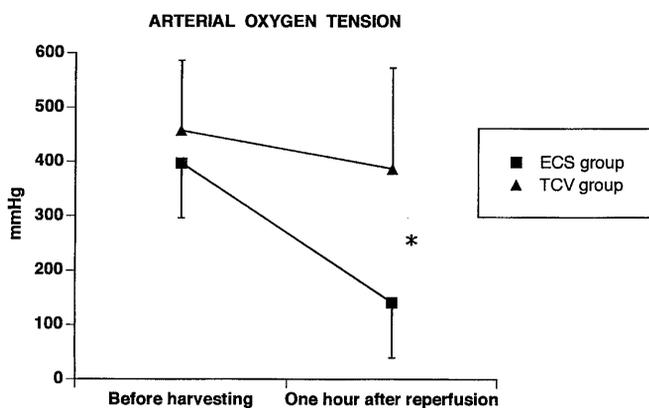


Fig. 1 arterial oxygen tension before harvesting and one hour after reperfusion

* $p < 0.05$ ECS group VS TCV group

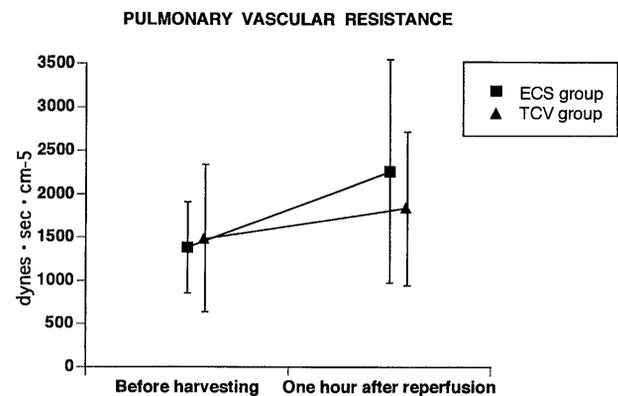


Fig. 2 Pulmonary vascular resistance before harvesting and one hour after reperfusion

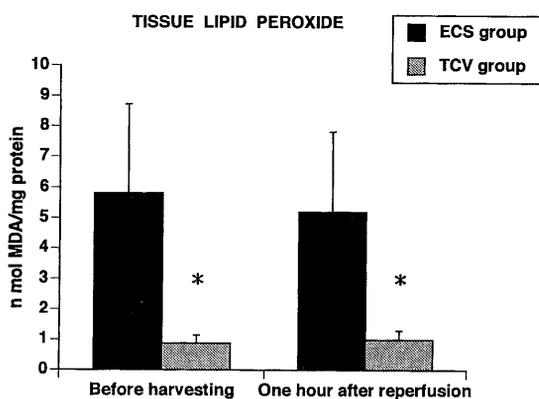


Fig. 3 Lipid peroxide concentration in lung tissue before harvesting and one hour after reperfusion

* $p < 0.05$ ECS group VS TCV group

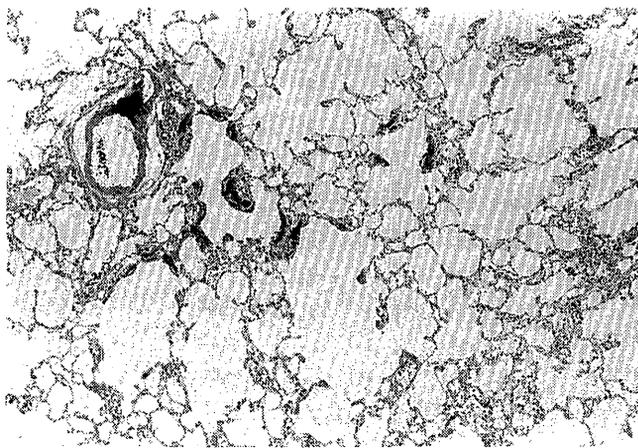
The TCV group showed less extravascular water content than the ECS group. (Table 1)

LPO

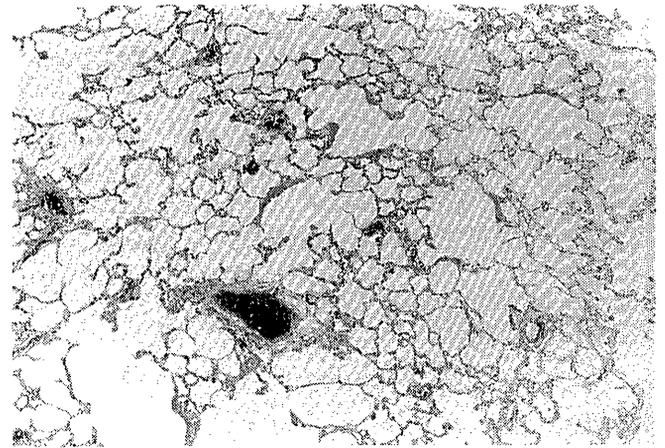
LPO, measured as thiobarbituric acid reactive material, was significantly lower in the TCV group than in the ECS group before harvesting and post implantation. ($p < 0.05$) (Fig. 3) Before harvesting lipid peroxide concentration was 5.81 ± 2.91 nmol MDA/mg protein in the ECS group and 0.88 ± 0.26 nmol MDA/mg protein in the TCV group. After reperfusion LPO was 5.20 ± 2.62 nmol MDA/mg protein and 1.00 ± 0.30 nmol MDA/mg protein for ECS and TCV groups respectively.

MPO

In both groups MPO activity increased following reper-



A : ECS group



B : TCV group

Acute vascular congestion, mild alveolar edema and intraalveolar hemorrhage are seen in both groups. In the TCV group pulmonary architecture was better preserved than the ECS group.

Fig. 4 Light micrograph of lungs biopsied one hour after reperfusion.

fusion. In the ECS group MPO activity was 9.7 ± 1.6 units, which increased to 16.2 ± 3.2 units. In the TCV group MPO activity was 7.7 ± 2.7 units, which increased to 12.1 ± 6.8 units after reperfusion. However, statistical significance was not seen. (Table 1)

Histopathology following lung transplantation

Light microscopic examination of sections of the transplanted lung specimens demonstrated acute vascular congestion, mild alveolar edema and intraalveolar hemorrhage. In the TCV group the pulmonary architecture was better than ECS group, although significant difference between the groups was not seen. (Fig. 4)

Discussion

Pulmonary dysfunction following transplantation is defined as reimplantation response¹⁵⁾ which is related to ischemia, reperfusion injury, denervation and disruption of the bronchial arteries and lymphatics of the grafted lung. Posttransplant lung dysfunction is characterized by pulmonary edema, increased pulmonary vascular resistance, decreased compliance and hypoxia.

Tissue injury occurs upon reperfusion and is mediated by activated neutrophils¹⁵⁾¹⁶⁾. Neutrophils release PAF and PAF activates neutrophils which results in endothelial dysfunction which is significant event causing ischemia and reperfusion. Experimental study demonstrates that PAF itself causes bronchoconstriction, increases vascular permeability, decreases cardiac output, hypotension, cellular edema, and increases tracheal secretion, which is similar to posttransplant pulmonary dysfunction. The present study was designated to evaluate the effect of PAF antagonist (TCV-309) in a canine model of a single lung

allograft transplantation following 24-hour preservation. TCV-309⁹⁾, first reported in 1990, is as potent as WEB 2086 and more potent and specific than CV-6209, where as CV-6209 is about 80 times more potent than CV-3988.⁸⁾ Our previous study²⁾ using an isolated lung perfusion model validated a decrease in pulmonary vascular resistance and lung water content, and an increase in pulmonary compliance when TCV-309 was added at a dose of $0.075 \mu\text{g/mL}$ to perfusion medium (fresh allogeneous blood). In our left lung allotransplantation model, we assessed the function of the graft before harvesting and one hour after reperfusion by occluding the right pulmonary artery and bronchus. The TCV group had significant higher arterial oxygen tension and lower extravascular water content than the ECS group. Terashita⁹⁾⁸⁾ showed that CV-3988 protected against congestion of the lung and infiltration of leukocytes in its blood vessels in mice with anaphylactic shock. We speculate that the improvement of microcirculation in the pulmonary vasculature resulted in better oxygenation and protected against vasoconstriction and pulmonary edema. The TCV group showed lower MPO activity after reperfusion, however, it was not statistically significant and light microscopic examination showed no difference between the groups. The fact that PAF induced leukocyte activation caused the infiltration of leukocytes in the lung, and the release of diverse chemical mediators including leukotrienes were investigated by Schlondorff and Neuwirth¹⁷⁾. TCV-309 might have prevented the leukocyte adherence to the endothelial cell, consequently resulting in a decreased release of immediate chemical mediators. In the TCV group lipid peroxide concentration was significantly lower than the ECS group throughout the experiment, suggesting less oxygen free radical formation in the TCV-309 treated group. However, the reason for the significant lower lipid peroxide level before harvesting in the TCV group without ischemia nor

reperfusion remains to be elucidated.

There is increasing evidence that PAF antagonists can lessen reperfusion injury. Conte¹⁸⁾ and coworkers validated that PAF or factors associated with PAF might mediate preservation and reperfusion injury in the canine lung allotransplantation model following 20-24-hour preservation using BN 52021 that is classified as a natural product and was administered to donors and recipients and added to modified Eurocollins solution. Another study by Corcoran¹⁹⁾ and coworkers demonstrated that BN 52021 enhanced 6 hour pulmonary preservation compared with iron chelator deferoxamine. They also proved oxygen-derived free radicals were likely mediators of PAF-induced pulmonary dysfunction, and posttransplantation pulmonary dysfunction was reversed by a variety of effects of BN 52021. In a swine model of heart-lung transplantation after 5 hours of preservation CV-3988 reduced the production of superoxide anions.²⁰⁾ Those authors demonstrated better results by using several kinds of PAF antagonists in heart-lung or lung transplantation models. The results fully support the significant importance of PAF as a important mediator in reperfusion injury in heart-lung or lung transplantation. Our results clearly support the conclusions of these authors that PAF antagonist ameliorates ischemia-reperfusion injury and improves post-transplant lung function.

In conclusion better posttransplantation pulmonary function was obtained when the PAF antagonist TCV-309 was administered to the donor and the recipient and added to the Euro-Collins solution in a canine model of a single lung allotransplantation. The PAF antagonist, TCV-309, by attenuating ischemia reperfusion injury is a beneficial agent in a canine lung transplantation following 24 hour preservation with the Euro-Collins solution.

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