Efficacy of a Platelet-Activating Factor Antagonist TCV-309 for Lung Preservation

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To evaluate the efficacy of a platelet-activating factor (PAF) antagonist, TCV-309, for the prevention of lung reperfusion injury, an experimental study was performed using a canine isolated lung reperfusion model. The canine lungs were flushed for 24 hours at 4°C. The animals were classified into 4 groups: Group A, donor lungs were flushed with a Euro-Collins (EC) solution only and preserved for 24 hours at 4°C as controls (n=7); Group B, donor dogs received intravenous TCV-309 at a dose of 30 µg/kg 15 minutes before heparin sodium administration, which was given prior to isolating the lungs and donor lungs were preserved for 24 hours at 4°C (n=8); Group C, donor lungs were flushed with an EC solution containing TCV-309 (375 µg/500ml) and preserved for 24 hours at 4°C (n=6). Group D, donor lungs were flushed and stored with an EC solution and then reperfused with allogeneous blood containing TCV-309, 0.75 µg/ml of blood volume (n=6). The pulmonary vascular resistance (mmHg/l/min) was significantly lower for Groups B, C and D than for Group A (A vs. B or C or D: 270.8±111.3 vs. 96.2±78.3 (p <0.01) or 122.6±75.4 (p <0.05) or 120.3±89.7 (p <0.05), respectively) (120 min). The airway pressure (AWP, cmH2O) was significantly lower for Groups B and C than for Group A (A vs. B or C or D: 21.0±3.3 vs. 16.0±2.2 (p <0.01) or 15.7±2.2 (p <0.01) or 18.3±1.4 (N.S.), respectively) (0 min). The AWP was significantly lower for Group D than for Group A (A vs. B or C or D: 30.4±7.0 vs. 23.4±2.1 (N.S.) or 21.5±1.8 (N.S.) or 19.1±2.0 (p <0.01), respectively) (120 min). The static lung compliance (ml/cm H2O) was significantly higher for Groups B, C and D than for Group A throughout the reperfusion. The blood oxygen tension (ΔPO2, mmHg) and lung water (%) were significantly improved for Groups C and D, but not for Group A (ΔPO2. A vs. B or C or D: 161.1±66.2 vs. 246.7±82.4 (N.S.) or 309.9±62.2 (p <0.05) or 307.2±79.0 (p <0.05), lung water; 89.6±0.8 vs. 87.3±2.6 (N.S.) or 86.0±1.7 (p <0.001) or 85.2±2.3 (p <0.005). At the end of the harvest, the tissue level of lipid peroxidation (nmolMDA/mg protein) was significantly lower for Group B than for the others (B vs. others: 1.17±0.2 3 vs. 1.51±0.14 (p <0.05)). At the end of storage, the tissue level of myeloperoxidase (Units/g wet tissue) was significantly lower for Group C than for Groups A and D (C vs. A or B or D: 13.5±18.4 vs. 42.2±14.3 (p <0.05) or 40.8±52.2 (N.S.) or 42.6±18.8 (p <0.05)). In conclusion, the PAF antagonist, TCV-309, is effective for the prevention of ischemic reperfusion injury in 24-hour cold preserved canine lungs.

Introduction
The platelet-activating factor (PAF) was originally found to be released by antigen-sensitized basophils and is a phospholipid mediator defined as 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine. It has been shown to be produced by a variety of inflammatory cells including neutrophils, monocytes, eosinophils, macrophages, lymphocytes, and platelets, as well as endothelial cells. The PAF is suspected to be involved in a variety of physiopathological conditions, including arterial thrombosis, acute inflammation, endotoxic shock, acute allergic disease, and transplant rejection. Systemic effects of the PAF are hypotension, pulmonary hypertension, increased resistance of airways and increased vascular permeability. The PAF activates platelets and neutrophils which produce inflammatory mediators and free radical generations. The PAF is also produced by the stimulation of arachidonic acid metabolites and free radical generations. During the period preservation, the donor organ is injured by mechanical trauma, cold ischemia and hypoxia, and ischemic reperfusion injuries which occur after transplantation. Generally, ischemic reperfusion injuries are related to free radical generations originating from neutrophils. It is also related to the PAF receptor. The PAF antagonist inhibits ischemic reperfusion injury of the organ. Conte et al. showed that the use of a PAF antagonist, BN 52021, at donor harvest, flushing solution and recipient improved the post-transplant pulmonary function in dogs following a period of preservation of 20 hours. Therefore, we studied the effect of a novel PAF antagonist TCV-309 (Takeda Chemical Industries Ltd, Osaka, Japan) (Fig.1) for the prevention of reperfusion injuries in 24-hour cold preserved lungs of canines and evaluated when and how the PAF affects the lung preservation.

Materials and Methods

Experimental model
In this study, the isolated lung perfusion model was used
as previously reported by Kawahara et al.24) (Fig.2). Twenty-seven adult mongrel dogs weighing 8-12 kg were anesthetized with sodium pentobarbital (0.5 mg/kg of body weight) administered intravenously and intubated for mechanical ventilation. After median sternotomy, heparin sodium (five hundred units/kg of body weight) was administered intravenously, and five minutes later both lungs and the heart were removed. A polyethylene cannula was secured in the truncus of the pulmonary artery. An endobronchial tube (9.0 mm in diameter) was placed in the trachea. Initially the lung was flushed with approximately 500 ml of a 4-6°C Euro-Collins solution through the pulmonary artery at a pressure of 3-5 mmHg. During the period of flushing, the lung was ventilated with room air using a Harvard ventilator at a tidal volume of 35 ml/kg and a rate of 10 cycles/min. At the end of the flushing, the lung was inflated to 40% of the end-tidal volume and the trachea was clamped. Both lungs and the heart were placed in a plastic box filled with the same flush solution and stored in a refrigerator at 4°C for 24 hours. After a hypothermic preservation period, both right pulmonary artery and bronchus were ligated and divided. As a result, the lung was isolated. After this procedure, the left lung was reperfused for 120 minutes. An arterial cannula was connected to a circuit containing a reservoir primed with 400 ml of allogeneous blood. The left atrial cuff was left open allowing the lung perfusate to drain freely into the reservoir. The left lung was ventilated with room air at a tidal volume of 20 ml/kg and a rate of 10 cycles/min. The perfusate was pumped continuously with a roller pump and maintained at 37°C in a small temperature-controlled warm bath. Small catheters were inserted into the cannulas of the pulmonary artery and trachea, respectively. They were connected to pressure transducers and were continuously monitored. The preserved lung was ventilated with a tidal volume of 20 ml/kg. The pulmonary arterial flow rate was maintained at 10 ml/kg/min.

Measured parameters
The number of white blood cells (WBC) in the perfusate was counted before reperfusion, and 30, 60, 90 and 120 minutes after the commencement of the period of reperfusion. The pulmonary vascular resistance (PVR) was defined as the mean pulmonary arterial pressure/flow rate (mmHg/l/min). The airway pressure (AWP, cmH2O) was obtained from pressure monitoring. The static lung compliance (effective lung compliance, Cat) was defined as flow rate/pressure (ml/cmH2O) at 1.2 sec of the end-inspiratory plateau. The blood oxygen tension (\(\Delta P_{O_2}\)) at the end of the reperfusion of isolated lungs was measured as follows. The 100ml of allogeneous venous blood was perfused at a rate of 5ml/kg/min after the left lower pulmonary artery was ligated. The FiO2 was set at 1.0. The final perfusate of 100ml of allogeneous venous blood was sampled from the left atrial cuff. The PO2 of the perfusate were measured before and after perfusion and \(\Delta P_{O_2}\) was determined using the formula: \(P_{V_{O_2}} - P_{A_{O_2}}\) (mmHg), where \(P_{V_{O_2}}\) is the oxygen tension of venous blood after perfusion and \(P_{A_{O_2}}\) that of venous blood before perfusion. After the period of reperfusion, the lung water in the left lower lobe was measured as follows. The reperfusion lungs were weighed and placed in a desiccator at 160°C for 48 hours to obtain the dry lung weight. The ratio of lung water weight to reperfused lobe weight was determined using the formula: \((W_r - W_d)/W_r\) (%), where \(W_r\) is the weight of the reperfused lobe and \(W_d\) is the blood-free dry weight of the lobe. After finishing the measurement of parameters during the period of reperfusion, the lipid peroxidation (LPO) and the myeloperoxidase (MPO) of the tissue were

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**Fig. 3 Study and experimental groups**

- No PAF antagonist (Group A) (Control)
- PAF antagonist (Group B) → HARVEST
- PAF antagonist (Group C) → STORAGE
- PAF antagonist (Group D) → REPERFUSION
measured at the end of the harvest, at the end of storage
and at the end of reperfusion in the biochemical labora-
tory. The LPO assay was the modified method previously
yepted by Ohkawa et al. and the MPO assay, that of
Bradley et al. Experimental groups (Fig.3)
The perserved lungs were classified into four groups;
Group A, donor lungs were flushed with a Euro-Collins
(EC) solution only and perserved for 24 hours at 4°C (n =
7) as controls; Group B, donor dogs received intravenous
TCV-309 at a dose of 30 μg/kg 15 minutes before heparin
sodium administration, which was given prior to isolating
the lungs and donor lung were preserved for 24 hours at 4°C
(n = 8); Group C, donor lungs were flushed with a EC
solution containing TCV-309 (375 μg/500ml) and pre-
served for 24 hours at 4°C (n = 6); Group D, donor lungs
were flushed and stored with a EC solution and then
reperfused with allogeneous blood containing TCV-309,
0.75 μg/ml of blood volume (n = 6).
All animals in the study were maintained in accordance
with the guidelines of the Committee of Animal Care of
the Nagasaki University School of Medicine, Nagasaki,
Japan.
Statistical analysis.
Each value is shown as mean ± SD. The data from four
groups were compared using the Mann-Whitney’s U
nonparametric test. A probability value of less than 0.05
was considered significant.

Results

The number of white blood cells decreased to 3000/mm³
after 30 min of reperfusion in four groups and remained at
the same level until the end of reperfusion.
The pulmonary vascular resistance (PVR) increased for
Group A gradually, while it decreased for Groups B, C and
D (Fig.4). At 0 min, the PVR was lower for Groups B and
C which received TCV-309 before reperfusion than for
Groups A and D but statistically not significantly lower.
After 120 min of reperfusion, the PVR (mmHg/l/min) was
significantly lower for Groups B, C and D than for Group
A (A vs. B or C or D: 270.8±111.3 vs. 96.2±78.3 (p <0.01)
or 122.6±75.4 (p <0.05) or 120.3±89.7 (p <0.05)).
The air way pressure (AWP) increased for Group A; in
the same way, it increased for Groups B, C and D but
gradually (Fig.5). At 0 min, the AWP (cmH2O) was sig-
nificantly lower for Groups B and C which received TCV-
309 before reperfusion than for Group A (A vs. B or C or
D: 21.0±2.2 vs. 16.0±2.2 (p <0.01) or 15.7±2.2 (p <0.01)
or 18.3±1.4 (N.S)). After 120 min of reperfusion, the
AWP was significantly lower for Group D than for Group
A (A vs. B or C or D: 30.4±8.6 vs. 23.4±7.0 (N.S.) or 21.
5±7.8 (N.S.) or 19.1±2.0 (p <0.01)).
The static lung compliance (Cst) decreased gradually for all groups, while more quickly for Group A (Fig. 6). Especially, throughout the period of reperfusion, the Cst, (ml/cmH2O) was significantly higher for Groups B, C and D than for Group A (120 min, A vs. B or C or D: 9.7 ± 2.5 vs. 16.7 ± 3.9 or 18.0 ± 3.8 or 17.4 ± 4.3 (p < 0.05)).

The blood oxygen tension (mmHg) was 161.1 ± 66.2 for Group A, 246.7 ± 82.4 for Group B, 309.9 ± 62.2 for Group C and 307.2 ± 79.0 for Group D (Fig. 7). There was a significant difference between Group A, and Groups C and D (p < 0.05).

The lung water (%) of the perfused lung was 89.6 ± 0.8 for Group A, 87.3 ± 2.6 for Group B, 86.0 ± 1.7 for Group C and 85.2 ± 2.3% for Group D (Fig. 8). There was a significant difference between Group A, and Groups C (p < 0.0001) and D (p < 0.005).

The lipid peroxidation (LPO, nmolMDA/mg protein) was 1.17 ± 0.23 for Group B and 1.51 ± 0.14 for Groups A, C and D (p < 0.05) (Fig. 9). There was a trend toward a significant difference at the end of storage; the LPO was 1.84 ± 0.31 for Group C, 2.35 ± 0.53 for Group A, 2.38 ± 0.59 for Group B and 2.36 ± 0.80 for Group D. No significant difference existed in the value of the LPO after reperfusion: 1.13 ± 0.28 for Group A, 1.10 ±
The significant difference of the myeloperoxidase (MPO) existed at the end of storage; the MPO (Units/g wet tissue) was 13.5 ± 18.4 for Group C, 42.2 ± 14.2 for Group A (p < 0.05), 40.8 ± 52.2 for Group B (N.S.) and 42.6 ± 18.8 for Group D (p < 0.05) (Fig.10).

Discussion

The results demonstrated that the value of PVR, AWP and lung water were lower, Cst maintained higher, and oxygenation function better in the lungs which were treated with TCV-309 than in the lungs which were not treated with TCV-309. This suggests that TCV-309 prevented 24-hour cold preserved lungs form ischemic reperfusion injury. TCV-309 is a white to pale yellow powder or masses at room temperature and soluble in water and saline. The chemical name of TCV-309 is 3-bromo-5-[N-phenyl-N-[2-[1,2,3,4-tetrahydro-2-isouinolycarbonyloxy]ethyl] carbamoyl]ethyl] carbamoyl]-1-propylpyridinium nitrate. The molecular formula is C₃₀H₃₂BrN₅O₇ and the molecular weight, 656.5. TCV-309 was as potent as WEB 2086 and more potent than CV-6209 and CV-3988, and did not cause hemolysis or vascular tissue damage due to a detergent-like action seen with other PAF-related compounding antagonists at higher concentrations or doses. In our study, there was no difference in the PVR, AWP, Cst, lung water or oxygenation function in the lungs whenever TCV-309 was administered, before donor lung harvesting, during storage, or just before and after reperfusion. This suggests that TCV-309 can be administered to donors intravenously or added to flushing solution, or administered to recipients intravenously just before and after reperfusion.

The number of WBC did not change significantly for either group. We supposed that it decreased significantly for the group which did not receive TCV-309, because TCV-309 blocked the PAF effect and the WBC were prevented from adhesion and migration to the pulmonary vascular endothelium. It is unknown whether the number of WBC in the perfusate were reduced after reperfusion. The majority of WBC in the perfusate may be trapped in the circuit or reperfused lung. But the isolated lung perfusion model has been established as a useful tool for evaluating the lung oxygenation function, the isolated lungs were finally flushed with allogeneous venous blood which was taken from another dog.

The LPO and MPO of the tissue were measured. Generally, the LPO of the tissue reflects the consequence of the free radical generation of the preserved lung and the lung content of MPO was measured to quantitate the degree of pulmonary neutrophil sequestration. During storage, the tissue LPO level was maintained at a standard level and the tissue MPO was decreased in the lungs which were flushed with the EC solution containing TCV-309. However, the LPO level was reduced and the MPO increased in the lungs after reperfusion, whether lungs were treated with TCV-309 or not. The tissue LPO might be quickly flushed out into the perfusate during reperfusion, and TCV-309 might not be able to inhibit the trapping of neutrophils in the lungs due to reperfusion.

In conclusion, TCV-309, the PAF antagonist, is useful for the prevention of ischemic reperfusion injury in the 24-hour cold preserved lungs of canines, and this suggests that the agent can be administered to the donor before and during harvesting, flushing solution during storage, or recipients just before and after reperfusion.

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