Pre-treatment of donor with 1-deamino-8-arginine vasopressin could alleviate early failure of porcine xenograft in a cobra venom factor treated canine recipient

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Abstract

Objective: Unlike cardiac or renal xenotransplants, the depletion of complement using cobra venom factor (CVF) does not improve pulmonary xenograft survival. Several cases suggest that the swine von Willebrand factor (vWF) may play a major role in presenting a different pathogenesis of pulmonary xenograft dysfunction from other organs. To evaluate the role of vWF and the complement system in mediating hyperacute vascular injury of pulmonary xenografts and elucidate pathogenesis of the injury, we performed swine-to-canine orthotropic single lung xenotransplantation after pre-treatment of 1-deamino-8-arginine vasopressin (DDAVP) and CVF. Methods: We set up three groups for lung xenotransplantation: group I served as the control group; group II, recipients pre-treated with CVF; group III, donors pre-treated with DDAVP (9 mg/kg, 3 days)/recipients pre-treated with CVF (60 u/kg). Hemodynamic data, coagulation and complement system parameters, and grafted lung pathologies were examined serially for 3 h after transplantation. Results: DDAVP infusion reduced the vWF content in swine lung tissue in vivo (7.7 ± 2.4 AU/mg vs 16.0 ± 5.6 AU/mg, P < 0.0001). Infusion of CVF 24 h prior to transplantation effectively depleted the recipient’s serum C3 and complement hemolytic activity below the detectable range. Regardless of the use of CVF, both groups I and II transplanted with unmodified grafts showed an immediate drop in leukocytes and platelet counts after transplantation. However, in group III, in recipients transplanted with DDAVP pre-treated swine lung, the platelet count did not decrease after transplantation (P = 0.0295). The decrease of plasma antithrombin and fibrinogen tended to be attenuated in group III. Light microscopic examination revealed extensive vascular thromboses in both capillary and larger vessels, as well as early pulmonary parenchymal damage in groups I and II, but were rarely observed in group III. Conclusions: Complement inhibition alone was not enough to alleviate intravascular thrombosis, the main pathology in pulmonary xenotransplantation. Pre-infusion of DDAVP to the donor animal was effective in preventing platelet sequestration and attenuated intravascular thrombosis. It is suggested that the strategies targeting vWF would be promising for successful pulmonary xenotransplantation.

Keywords: Hyperacute rejection; Pulmonary xenotransplantation; Platelet; Desmopressin; Thrombosis

1. Introduction

The hyperacute rejection of an organ transplant between discordant species is generally thought to be mediated by the binding of natural antibodies to endothelial cells of the xenograft and subsequent activation of the complement cascade. Deposition of xenoreactive antibodies, as well as complement fragments in the rejected xenograft, is evidence of this concept. In addition, it has been well documented that swine heart or a kidney xenograft functions well for several months in a primate recipient under the condition of antibody depletion and anti-complement treatment [1]. However, in the context of pulmonary xenotransplantation, the pathogenesis of hyperacute injury seems to be different from that in renal and cardiac xenografts. Pulmonary xenografts rapidly fail following exposure to human or primate blood, showing a marked increase of pulmonary vascular resistance and fulminant pulmonary edema associated with micro- or macrovascular thrombosis. Strategies employed to deplete natural antibodies and/or to make an anti-complement condition were only partially effective in prolonging pulmonary xenograft survival [2–4]. Despite this strategy, histologic examinations
of xenografts revealed the persistent presence of microvascular thrombosis with fibrin plugs [4]. Therefore, the mechanism for hyperacute injury in pulmonary xenograft is described as hyperacute pulmonary xenograft dysfunction (HPXD) rather than hyperacute rejection [5].

Intravascular thrombosis and systemic coagulopathy are other barriers for xenotransplantation [6]. Quiescent endothelium normally maintains an anticoagulant profile to the adjacent blood. However, any failure of endothelial regulators of coagulation can lead to serious procoagulant consequences. The von Willebrand factor (vWF) has been proposed as a major contributor of coagulopathy associated with xenotransplantation [7]. vWF is a protein stored in platelets and endothelial cells that is released upon endothelial cell activation [8]. vWF binds to glycoprotein Ib (GPIb) on platelets in high shear stress leading to platelet adhesion and activation. Swine vWF has a supranormal capacity to bind to human GPIb, resulting in platelet aggregation even in the absence of shear stress [9]. This behavior contributes to the immediate sequestration of circulating platelets in the lung [2,10]. The interaction between swine vWF and human platelet involves the platelet mediated thrombus formation, which results in disseminated coagulopathy [7]. Therefore, blocking the vWF-platelet interaction could be a promising strategy to reduce initial aggregation even in the absence of sheer stress [9]. DDAVP, an analog of vasopressin, has been used to treat patients with a deficiency of vWF [11]. It raises the plasma levels of vWF and FVIII by releasing Weibel-Palade (WP) bodies in endothelial cells. The release of vWF from WP bodies has been demonstrated by the binding of DDAVP to a vasopressin type 2 receptor located on the surface of endothelial cells without up-regulation of endothelial vWF mRNA synthesis.

In order to deplete or reduce the amount of vWF in endothelial cells of pulmonary xenografts, we designed a novel strategy—the administration of DDAVP to donor swine before transplantation. The effects of DDAVP were evaluated to the extent of vWF depletion and inhibitory effect on pulmonary microvascular thrombosis and platelet sequestration in the swine-to-canine pulmonary xenograft model.

2. Materials and methods

2.1. Treatment of donor with DDAVP

All of the animals in the study were treated humanely in compliance with the ‘Guide for the Care and Use of Laboratory Animals’ published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985). Three adult swine (about 30 kg) were anesthetized with intramuscular administration of ketamine hydrochloride (20 mg/kg) and were intubated and ventilated with 100% oxygen at a rate of 10 breaths per minute with a tidal volume of 12 ml/kg. A 9 Fr. Hickman catheter (Bard, USA) was inserted through the neck incision and the tip was placed in the back. Three small incisions were placed in the right chest and a lung biopsy was taken with video-assisted technique. Three micrograms per kilogram of DDAVP (Ferring Pharmaceuticals, USA) was injected every 12 h intravenously through a Hickman catheter for 3 days before transplantation.

2.2. Measurement of vWF Ag content in swine lung tissue

To determine if DDAVP could deplete vWF from pulmonary endothelial cells, we obtained lung samples at the time of lung retrieval from four controls and two DDAVP-treated pigs, and measured the content of vWF Ag in the lung tissue. The tissue was immediately frozen in liquid nitrogen. Two pieces from different sites of each specimen were selected and each tissue piece was ground and treated with protein extraction buffer (50 mM Tris-Cl, 150 mM NaCl, 5 mM EDTA, 1% triton-X 100, 1 mM PMSF, pH 8.0). From each extract, the vWF Ag and total protein levels were determined. The vWF Ag level was determined by a sandwich enzyme-linked immunosorbent assay (ELISA), consisting of a capture antibody, rabbit anti-human vWF antibody (A0082, Dako Corp., Carpinteria, CA) and peroxidase-conjugated detecting antibody. The pooled normal swine plasma in serial dilutions was tested in parallel and used as a calibrator standard. The vWF Ag content expressed as the ratio of tissue vWF Ag level to total amount of protein was compared between the DDAVP treatment and control groups.

2.3. Assessment of expression of vWF mRNA in lung tissue

To determine if DDAVP induced the expression of vWF mRNA in vivo, the level of vWF mRNA expression was compared in both lung tissues obtained before and after the administration of DDAVP in two donor pigs. Swine endothelial cells were studied in parallel as a positive control. vWF and GAPDH mRNA were analyzed by reverse transcriptase and polymerase chain reaction (RT-PCR). The primer sequence was designed from the swine vWF CDNA sequence (sense 5′-gga ggc ctg tct act caa cg-3′, antisense 5′-tga ccc gac tct act cca cag-3′). The change of the vWF mRNA amount was determined by a visual comparison of the band density of PCR product.

2.4. Swine-to-canine left single lung xenotransplantation

A swine, weighing about 30 kg, was anesthetized with intramuscular administration of ketamine hydrochloride (20 mg/kg) and an intravenous line was placed in a vein of its ear. Thiopental sodium of 200-250 mg and 0.2 mg of atropine were administered. The swine was then intubated and ventilated with 100% oxygen at a rate of 12 breaths per minute with a tidal volume of 15 ml/kg. Arterial pressure and blood gases were monitored through a cannula placed in the femoral artery. A median sternotomy and thymectomy were performed and the pericardium was opened. The left side azygos vein was ligated and divided. The inferior and superior vena cavae were loosely encircled with heavy silk. After injection of 20,000 IU of heparin intravenously, a 7 Fr. cannula was placed in the main pulmonary artery. Five micrograms per kilogram of alprostadil (PGE1, Eglandin®, Welfide, Korea) was injected directly into the pulmonary artery 10 min before harvest. The superior vena cava was ligated and the inferior vena cava and left atrial appendage.
were incised. Euro-Collins solution (100 ml/kg) was administered into the pulmonary artery through the pulmonary arterial cannula at a height of 40 cm. The inferior pulmonary ligaments were sharply divided and the trachea was divided with a stapling device. The heart and lungs were then removed en bloc and immersed in cold (4 °C) saline solution. The cold ischemic time in all the animals was within 3 h.

Swine-to-canine orthotopic single lung transplantsations were performed in three groups: the control group, a mongrel dog transplanted with unmodified swine lung (n = 3); the CVF group, CVF-pretreated dog transplanted with unmodified swine lung (n = 3); the CVF + DDAVP group, CVF-pretreated dog transplanted with DDAVP treated swine lung (n = 3). Nine adult male mongrel dogs weighing 26 to 42 kg were used as recipients. The recipients of the CVF and CVF + DDAVP groups were given 60 units/kg of CVF intravenously 24 h prior to transplantation in order to deplete complement components. The recipient dogs were anesthetized with intramuscular administration of ketamine hydrochloride (20 mg/kg) and were then intubated and ventilated with 100% oxygen at a rate of 12 breaths per minute with a minute ventilation of 180 ml/kg. The right femoral artery was cannulated and the left atrial pressure was monitored. The inferior pulmonary vein, jugular vein and continuous pressure monitoring of the pulmonary artery and right atrium were performed. Cardiac outputs were measured using the thermodilution method. The dog was positioned at right lateral decubitus and the left thoracotomy was placed through the fourth intercostal space. A left pneumonectomy was performed and the left lung of the swine was transplanted. The left atrium was sutured with 5-0 Prolene, and the pulmonary artery was anastomosed with 6-0 Prolene. The bronchus was sutured with a 4-0 Prolene suture. The left atrial auricle was sutured and the left atrial pressure was monitored. The animal was given 2,500 IU of heparin and the pulmonary artery was unclamped at the time of reperfusion. A 12-mm ultrasonic flow meter (Transonic, USA) was placed around the left main pulmonary artery and the flow to the transplanted left lung was measured. The hemodynamic data was measured repeatedly at 10, 20, 30, 60, 90, 120, and 180 min after reperfusion. Based on this data, we calculated physiological variables such as pulmonary vascular resistance and systemic vascular resistance. Serial lung biopsies, as well as blood samples, were collected during each time. After 3 h of reperfusion were completed, the animal was sacrificed by giving it 20 mequiv. of KCl and an autopsy was performed. The total warm ischemic time was consistently less than 60 min.

2.5. Complement hemolytic activity

To see the effect of CVF on the complement system, the recipients’ complement hemolytic activity on rabbit erythrocytes was evaluated as previously described. Briefly, 0.1 ml of 10^8 rabbit erythrocytes in gelatin veronal buffer containing dextrose (57 mM NaCl, 5 mM sodium diethyl barbiturate, 3% dextrose, 0.1% gelatin, 0.06 mM CaCl₂, 0.4 mM MgCl₂) were incubated with 0.1 ml dog serum diluted in the same buffer and incubated for 60 min at 37 °C. After adding 1.5 mL of ice-cold PBS and centrifugation for 10 min at 1500g, hemolysis was determined in the supernatant by measuring the absorbance of released hemoglobin at 412 nm.

2.6. Measurement of blood cell counts and coagulation parameters

We monitored the changes of the following parameters up to 3 h after transplantation: blood cell counts, prothrombin time and levels of fibrinogen, antithrombin and D-dimer. The blood cell count was performed by ABCvet CBC analyzer (ABX hematology, France) and coagulation parameters by STA Compact analyzer (Diagnostica Stago, France). Each parameter was expressed as a percentage of baseline value prior to reperfusion.

2.7. Histologic evaluation

A series of lung biopsies of a xenograft were performed prior to and at 10, 20, 30, 60, 120, and 180 min after reperfusion. The tissue samples were fixed in 10% buffered formalin, washed, dehydrated and embedded in paraffin. The embedded samples were cut into 4-µm thick sections, re-hydrated and stained with hematoxylin and eosin.

2.8. Statistical analysis

Data was expressed as the mean±SEM. Comparisons between groups were analyzed using a mixed model. Significance was accepted at P<0.05.

3. Results

3.1. DDAVP depleted vWF Ag from swine lung tissue

The content of vWF Ag in lung tissue was significantly decreased in the DDAVP-treated group compared to that in the control group (7.7±2.4 vs 16.0±5.6 AU/mg, P<0.0001, Fig. 1). Based on RT-PCR, the band density of PCR product for vWF mRNA seemed to be equal. We confirmed that the treatment of DDAVP depleted the amount of vWF and did not increase synthesis of mRNA for vWF (Fig. 1).

3.2. DDAVP treatment abrogated the increase of pulmonary vascular resistance (PVR) on the native canine lung caused by CVF administration

In all three groups, graft flows were low and decreased gradually with time (Fig. 2). Pulmonary vascular resistance of the CVF group increased after reperfusion and stayed high throughout the measurement. However, the CVF + DDAVP group, as well as the unmodified group, did not show such changes and remained stable (P<0.001, Fig. 2). This phenomenon suggested that PVR of the native canine right
lung increased with CVF administration and was prevented by the pre-treatment of the donor lung with DDAVP.

3.3. DDAVP treatment abrogated platelet sequestration

Serum complement hemolytic activity in the recipient dogs was abrogated at 6 h after CVF infusion and depletion of complement was sustained through transplantation. The blood counts prior to reperfusion in recipients were not different between groups. In the unmodified control group, platelet counts dropped immediately after reperfusion, and the decreased count of platelets after reperfusion in the CVF group was more prominent than in the unmodified group \( (P Z 0.0628) \). Pre-treatment of donor with DDAVP significantly blunted the decrease of the platelet count after reperfusion in CVF-treated recipients \( (P Z 0.0295, \text{Fig. 3}) \).

Leukocyte counts in recipients of the CVF group initially decreased immediately after reperfusion and began to increase shortly thereafter \( (P < 0.0001) \). Additive DDAVP treatment of the donor significantly reduced the reactive leukocytosis after reperfusion \( (P = 0.0452, \text{Fig. 3}) \). There was no significant difference in hemoglobin level according to time and group.

3.4. DDAVP administration attenuated coagulation abnormalities

Prothrombin time tended to be prolonged in the unmodified control group but there was no significant difference between groups. In contrast, fibrinogen and antithrombin levels decreased after reperfusion \( (P = 0.0879 \text{ for fibrinogen, } P = 0.0034 \text{ for antithrombin, Fig. 4}) \). The decrease of fibrinogen and antithrombin levels tended to be attenuated in the CVF + DDAVP group. The D-dimer level was pitched occasionally during transplantation but there was no significant difference between elapsed time and group.

3.5. Intravascular thrombosis and alveolar injury was less severe in the CVF + DDAVP group.

Light microscopic examination revealed early extensive capillary congestion and intravascular thrombosis in both the unmodified and CVF groups after reperfusion. The graft injury progressed with time resulting in alveolar edema and hemorrhage at the later period of time. In the CVF + DDAVP group, the extent of the injury was far less (Fig. 5).

3.6. Discussion

The binding of xenoreactive antibodies and consequent complement activation is believed to be the primary
mechanism of hyperacute rejection of xenografts. The beneficial effects of expressing human complement regulatory protein, such as CD45, CD55 and CD59, or depleting the recipient’s complement protein with CVF in both the heart and kidney xenotransplantation models, prove that complement activation is a major player in the tissue damage of xenografts. However, the mechanism of acute pulmonary xenograft injury has not yet been elucidated. HPXD is characterized by elevated PVR, microvascular thrombosis and fibrin deposition, which differ from the typical hyperacute rejection findings in heart or kidney xenotransplantations. Indeed, the expression of hDAF/hCD59 in swine lung is relatively resistant to acute xenograft injury but cannot abolish microscopic intravascular thrombosis and intra-alveolar hemorrhaging [12].

In this study, CVF administration depleted complement proteins and inhibited the activation of the recipient’s complement system against xenografts. Serum complement hemolytic activity was maintained below a detectable range throughout the experiment and only scarce depictions of C3 fragments were seen in xenografts. However, it failed to improve the recipients’ hematologic, hemodynamic and histopathologic findings after xenotransplantation. On the contrary, the pulmonary vascular resistance increased and systemic vascular resistance decreased; the sequestration of platelets was more profound in CVF-treated recipients compared to the unmodified control group. This suggests that the hemodynamic deteriorations shown in the CVF-treated group may not be dependent on the formation of membrane attack complex on xenograft but rather relate to other factors. Theoretically, CVF administration does not completely inhibit the complement system. A small amount of activation of complement can release anaphylatoxins such as C3a and C5a, which can activate pulmonary endothelial cells. Moreover, a preformed large amount of anaphylatoxins in circulation, which have been continuously produced and released by CVF, may lead to the strong activation of pulmonary endothelial cells. It is conceivable that activation of endothelial cells is a more important cause of HPXD than complement activation itself. This would explain why the CVF group showed a more prominent increase of pulmonary vascular resistance, as well as the severer platelet sequestration than those seen in the unmodified control group. The phenomenon observed in the CVF-treated group was similar to the typical features seen in the complete
discordant pulmonary xenotransplantation model. Such observations agree with the results of other researchers [4]. In terms of the xenotransplantation model, the CVF treated canine model should be considered more suitable than the unmodified model as a control.

Intravascular thrombosis and disseminated coagulopathy are two of the main pathologies of pulmonary hyperacute rejection in xenotransplantation. Depletion of xenoreactive antibodies or inhibition of complement activation only partially improves xenograft functions [3] and both cannot abolish coagulopathy. Although the mechanism of coagulopathy in pulmonary xenotransplantation is not still fully understood, it has been proposed that the activation of endothelial cells secondary only to the binding of antibodies, including anti α Gal or anti-non α Gal antibodies, or a sublytic amount of complement activation play an important role [13,14]. Endothelial activation enhances intravascular thrombosis and platelet sequestration by expressing adhesion molecules, producing vasoconstrictors and releasing secretory granules containing platelet activators. Swine vWF which is released from endothelial cells binds to human platelets, resulting in platelet activation and aggregation. It was reported that approximately 82% of the platelets disappeared from the perfusate during the first minute of perfusion of swine lung with human blood [2]. Therapy with anti-GPlb monoclonal antibody inhibited platelet deposition on the xenograft [15] and inhibits for the interaction between GPlb or GPIIa and vWF attenuated the increase of pulmonary vascular resistance and release of thrombin and histamine [15]. However, an anti-platelet monoclonal antibody can lead to sequestration of platelets to the reticuloendothelial system [16] and thus, non-selective inhibition of the recipient’s platelet function can cause possible hemorrhagic complications or systemic shock. Therefore, it is necessary to develop strategies to modulate vWF on the part of the donor.

DDAVP has been used to treat vWF-deficient patients because it induces the release of vWF from endothelial cells without up-regulation of vWF synthesis in endothelial cells [17]. The data showed that pre-treatment of DDAVP prevented platelet sequestration, reduced reactive leukocytosis, and attenuated subsequent coagulopathy demonstrated by the fall of both fibrinogen and antithrombin levels. The effect of DDAVP administration on preventing platelet activation was surprisingly successful considering that canine platelets have not been reported to have a strong interaction with swine vWF, unlike a primate’s platelets. We believe that the effect of DDAVP would be more prominent if we were to use a discordant primate as a recipient.

We speculated that the beneficial effect of DDAVP might be caused by reducing the release of vWF from the xenograft endothelial cells because DDAVP administration reduced the amount of vWF in swine lung tissue to 50% in our experiment and also the association between vWF release and platelet activation had been already proven [16]. However, there is a possibility that vWF may not be the only player for the favorable effect of DDAVP. The alteration of P-selectin expression after DDAVP treatment might have played a certain role because P-selectin and vWF are contained in WP bodies of endothelial cells together. To clarify this, the relationships between the amount of vWF depletion and extent of platelet activation should be investigated further and the effect of DDAVP on P-selectin expression of endothelial cells also should be studied.

There is evidence that a xenoreactive natural antibody, predominately IgM, binds to the xenograft and activates the complement. Subsequent deposition of IgM, IgG, C1q, C3, C4, C9 will lead to the rapid failure of xenograft [3,18]. However, whether xenoreactive antibodies actually remain bound to swine endothelium is not certain [19]. It was demonstrated that, although xenoreactive antibodies can bind to pulmonary xenografts, many of these antibodies are shed as a form of antibody-antigen complex [20]. On the other hand, an acute pulmonary xenograft injury was characterized by a rise of pulmonary vascular resistance, which is associated with the profuse release of thromboxane A2 [21]. Thromboxane A2 is produced from arachidonate in platelets by the aspirin sensitive cyclooxygenase pathway [22]. Collins et al. demonstrated that elimination of a pulmonary intravascular macrophage with liposome chlorodronate also reduces the synthesis of thromboxane [21]. An activated macrophage in the lung may trigger endothelial cells to release vWF, which sequentially induces binding, aggregation of platelets, and the synthesis and release of thromboxane into circulation from the platelets. Thus, strategies to reduce interaction between endothelial cells and platelets would be beneficial in preventing the rapid increase of pulmonary vascular resistance, maintaining barrier function of pulmonary endothelial cells, as well as ameliorating platelet sequestration.

As we measured pulmonary vascular resistance while the right lung was perfused, the increase of pulmonary vascular resistance observed in the CVF group was primarily caused by the canine native right lung. The starting point of the PVR measurement was when the left pulmonary artery was being clamped just before the reperfusion, and thus represented right pulmonary vascular resistance. After reperfusion, the PVR in both the unmodified and CVF+DDAVP groups decreased to a small degree and maintained its level thereafter. This result suggested that DDAVP administration, which achieved the depletion of vWF, played an important role in preventing a rise of PVR in the native recipient’s right lung which was presented in the CVF group. It has been well studied in the swine-to-baboon model that the immune complex between the swine vWF and the xenoreactive natural antibody from the baboon is shed into the blood from the transplanted xenograft and causes a disseminated intravascular coagulopathy (DIC) [7]. We hypothesized that the vWF immune complex secreted from the transplanted xenograft could cause DIC. These immune complexes could further activate the complement system, generate anaphylatoxins and cause systemic shock. As another explanation, increased amounts of anaphylatoxins in CVF treated recipients could have led to the release of vWF from endothelial cells and subsequent activation of platelets, which induced profuse release of thromboxane A2. An elevated level of thromboxane A2 in circulation could be responsible for the increase of PVR in the native recipient’s right lung. In the CVF+DDAVP group, the depletion of vWF secretion might have ameliorated such release of thromboxane A2 and its consequent systemic effect. A decrease of platelet count, increase of leukocyte count and decrease of fibrinogen
and antithrombin levels were observed in both the unmodified and CVF groups while those findings were not observed in the CVF + DDVAP group. Those results can also be explained in the same context. Even though the beneficial effect of DDVAP was transitory, it may prove to be worthwhile as the reduction of the initial inflammation would be beneficial to prolong overall graft survival.

The swine vWF molecule can be a target for gene alteration in a genetically modified swine production project for xenotransplantation. However, considering its role in maintaining physiological hemostasis, complete ablation of the vWF gene would not be desirable. Transient inhibition of gene expression might be an alternative way to control vWF. How to achieve timely and well-tuned control on the extent of vWF expression is the next hurdle to overcome.

Our data suggested that swine vWF plays an important role in HPXD and the depletion of donor vWF would be a safe and effective strategy to prevent microvascular thrombosis in xenografts. However, the number of each experiment group was rather small. Moreover, our experimental model was a swine-to-canine transplantation which is not completely discordant and might not be appropriate for evaluating the agents or strategies to prevent HPXD in regard to natural antibodies. Before reaching a final conclusion, we need to confirm our postulation through ex-vivo perfusion using human blood or the swine-to-baboon xenotransplantation model. An experiment to define a quantitative relationship between the beneficial effect of DDVAP and extent of vWF depletion is also necessary in order to be clarified. In this study, we confirmed that DDVAP pre-treatment is a very effective and safe preconditioning regimen for pulmonary xenotransplantation experiments.

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References


Appendix A. Conference discussion

Dr D. van Raemdonck (Leuven, Belgium): I did not find in your abstract how many animals were in each group, and I am not sure that you have presented this.

Dr Kim: Each group has 3 animals.

Dr van Raemdonck: Is that not too small to show any differences?

Dr Kim: Even though we did not use the baboon, this study took a lot of our time, effort, and expense. I think for a large animal model, 3 in each group are reasonable.
If we had used baboons, for example, 1 or 2 for each group would have been enough. As our model is not a discordant xenotransplantation model, but a concordant model, we want to know what will happen if we apply the same model using a baboon or human. For this finding, we are now working on the ex-vivo perfusion model. Once we have baboon facilities in my country in the future, we are planning to do that type of research.

**Dr Van Raemdonck:** Can you repeat what this drug DVAP is doing. What is the working mechanism?

**Dr Kim:** That is the drug which has been used for Type I von Willebrand’s disease. For Type I von Willebrand’s disease, the patients have a smaller amount of von Willebrand factor in their blood, and if we use the injection of vasopressin, it causes the endothelial cells, especially from the lung, to secrete the von Willebrand’s factor from the WP body. And what became apparent is that although several treatments work fine at the beginning, it did not increase serum von Willebrand factor after repeated injections, which means the von Willebrand factor had been depleted from the WP body. We tested this with several lung biopsies, treating a pig with DDAVP for 10 days. What we found is 2 days are enough to deplete pulmonary endothelial von Willebrand factor.

**Dr Van Raemdonck:** Did you look at the effect of this drug in an allotransplant model?

**Dr Kim:** Actually, we did not.