First experience with heterotopic thoracic pig-to-baboon cardiac xenotransplantation


Abstract: Background: Heterotopic thoracic heart transplantation may be an alternative to the established heterotopic abdominal or orthotopic cardiac xenotransplantation model as it combines the safety of heterotopic transplantation with the benefit of a working heart model.

Methods: In a first series of two animals, we tested the surgical feasibility of this procedure with non-transgenic pig hearts transplanted into pre-sensitized baboons (killed after weaning from cardiopulmonary bypass). In a second group (n = 2), double-transgenic α(1,3)galactosyl-transferase knock out/hCD46 pig hearts were transplanted into naïve baboons after immunoadsorption. The immunosuppressive regimen consisted of anti-CD20-mAb, tacrolimus, sirolimus, MMF and steroids.

Results: The first baboon was successfully transplanted, but died of an air embolism, while in the second animal graft survival was 50 days with the recipient in good physical condition. One rejection reaction was successfully treated by immunoadsorption, ATG and the proteasome inhibitor bortezomib. Post-mortem histopathology showed no evidence for humoral or cellular rejection of the cardiac xenograft.

Conclusions: This is the first description of a heterotopic thoracic pig-to-baboon heart transplantation. The procedure combines the advantages of a working heart model with the safety of heterotopic transplantation. In contrast to orthotopic transplantation, the recipient heart can assist the donor heart during episodes of rejection. We believe that the heterotopic thoracic model may accelerate the progress into the clinical application of cardiac xenotransplantation. However, successful combination of this heterotopic transplantation with an experimental model of cardiac failure may be needed before this technique can be promoted to clinical trials.

Introduction

Heterotopic abdominal and orthotopic cardiac xenotransplantation are the two established animal models for immunological studies on acute and delayed xenograft rejection and graft survival. Non-life-supporting heterotopic abdominal hearts from transgenic pigs have survived for 6 months in non-human primates [1]; however, the survival time after life-supporting orthotopic pig-to-baboon heart transplantations exceeds 1 month only in a limited number of experiments [2,3]. So far, orthotopic pig-to-baboon heart transplantation is the only accepted preclinical model for cardiac xenotransplantation in humans. A survival time of 3 months in at least 60% in a series of consecutive life-supporting experiments, with a minimum number of 10 non-human primates surviving for this period of time has been suggested as a prerequisite before clinical transplantation may be started [4].

Barnard and Losman [5] performed the first heterotopic thoracic heart transplantation as a biologic left ventricular assist in 1974. This technique was further modified to enable biventricular assistance [5,6]. The main advantages of this technique are the use of donor hearts even if they are smaller than the recipient’s native heart, its usefulness in patients with high pulmonary artery...
pressure and the possible assistance of the recipient’s native heart during episodes of rejection [7].

As heterotopic thoracic pig-to-baboon heart transplantation combines the safety of a non-life-supporting graft with the benefits of a real working heart model, we tested the feasibility of this procedure.

**Materials and methods**

**Animals and anaesthesia**

Non-transgenic landrace (n = 2; Department of Veterinary Medicine, Ludwig Maximilian University, Munich, Germany) and double-transgenic α(1,3)galactosyl-transferase knock out (GalT-KO)/hCD46 (n = 2; Revivicor Inc., Blacksburg, VA, USA and Institute for Molecular Animal Breeding and Biotechnology, Gene Centre, Ludwig Maximilian University, Munich, Germany) pigs were used as heart donors. Donor anaesthesia was conducted with fentanyl and propofol. Two presensitized and two naïve captive-bred baboons served as recipients (German Primate Centre, Göttingen, Germany). After intramuscular premedication with midazolam and ketamine, the baboons were anesthetized with propofol and fentanyl. This study was approved by the Local Bavarian Government and the Institutional Ethical Committee. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health.

**Surgical procedure**

The inferior vena cava in the donor heart was ligated and the full length of the SVC was harvested for anastomosis of the right atria. The right pulmonary veins were closed and the muscle bridge between the left pulmonary veins was divided to create an opening for the anastomosis of the left atria.

A right-sided pericardial flap was created in the recipient, laid over the hilum of the right lung serving as a cradle for the donor heart. The donor heart was placed into the right chest, the opening within the left atrium facing anteriorly. The two left atria and thereafter the right atria were connected in a diamond-shaped manner. The implantation continued with the aortic end-to-side anastomosis. Finally, the two main pulmonary arteries were joined end-to-side. This anastomosis was either performed directly (n = 2), or by interposition of a vascular graft (n = 2, see Fig. 1).

**Immunosuppressive regimen**

The two initial experiments using non-transgenic donor hearts were proof of principle and the baboons were killed after weaning from cardiopulmonary bypass. Thus, no immunosuppressant was given. In the second baboon, we used TPC (50 mg/kg IV; Nextran, Princeton, NJ, USA) to overcome hyperacute xenograft rejection. In the two consecutive experiments with transgenic GalT-KO/hCD46 grafts, the immunosuppressive regimen consisted of anti-CD20 and was started 2 weeks before transplantation, tacrolimus, sirolimus, MMF and tapered steroids. Immunoabsorption (TheraSorb; Miltenyi Biotec, Bergisch Gladbach, Germany) was done immediately before transplantation thus reducing the total plasma concentration of antibody by approximately 80% in the recipients. Human anti-CMV-IgG (150 mg/kg Cytotect; Bio-test Pharma GmbH, Dreieich, Germany) was given immediately after immunoabsorption to normalize IgG levels.

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**Fig. 1.** (A) Intraoperative situs during pulmonary artery anastomosis in a baboon undergoing heterotopic thoracic cardiac xenotransplantation. (B) Schematic artist’s view of the same stage during operation.
Monitoring of graft function

Perioperatively, heart rate, rhythm and ST-segment were analysed continuously in ECG-leads II and V5 (Sirecust 960; Siemens, Erlangen, Germany). Arterial blood pressure and cardiac function were monitored continuously via a catheter in the femoral artery (Pulsion, Munich, Germany) and a central venous catheter (Arrow, Erding, Germany) introduced via the cephalic vein. Cardiac output was measured with the femoral arterial thermodilution technique (PiCCO; Pulsion). On each postoperative day (POD), we assessed heart rate of the recipient and the graft by external ECG positioned over the right chest wall.

We conducted echocardiographic examinations of the graft in regular intervals using an ultrasonographic scanner and a 10-MHz phased-array transducer (Sonos 5500; Hewlett Packard, Andover, MA, USA).

Before termination of the experiment, a CT angiogram was done (Somatom Definition; Siemens Medical Solutions, Forchheim, Germany). Complete necropsy of the recipient was carried out after the graft had failed and histopathological examination and immunohistochemistry was performed.

Results

In the first heterotopic xenotransplantation, the cardiac graft from a non-transgenic donor pig started to be rejected within minutes after reperfusion. The rejected xenograft dilated and substantially compromised right ventricular filling of the recipient’s heart. Weaning from cardiopulmonary bypass (CPB) was only possible with massive volume loading and high dose inotropic and vasopressive pharmacological support.

Hence, the α-galactosylpolyethylene-glycol-conjugate TPC (Nextran) was given before reperfusion in the second transplantation of a non-transgenic porcine donor heart. No clinical signs of hyperacute xenograft rejection were seen. The function of the recipient’s heart was also severely compromised with reduced right ventricular filling but the graft was not dilated. In both operations, no vascular graft for the end-to-side anastomosis of the pulmonary arteries was used.

In the following two operations, we choose an interposition of a graft for the pulmonary arteries anastomosis. In one baboon, a 10-mm spiral supported Gore Tex vascular graft (Gore, Putzbrunn, Germany) was implanted. Weaning from CPB was possible with moderate vasopressive and positive inotropic pharmacological support and with no signs of dilatation of the ventricles. Due to an unfortunate intraoperative dislocation of the venous cannula, air was sucked into the closed-circuit CPB. Despite all efforts to fully de-air the extracorporeal circuit, an air embolism occurred resulting in consecutive brain damage and leading to the death of the baboon 10 h after termination of CPB. Transthoracic echocardiography, however, revealed good function of the porcine graft within the first hours after reperfusion.

In the fourth heterotopic thoracic pig-to-baboon heart transplantation of this series, a 10-mm polytetrafluoroethylene graft (Vascutek, Hamburg, Germany) was used for the pulmonary artery anastomosis. Moderate doses of vasoactive and inotropic drugs were needed during the weaning phase from CPB. The animal could be weaned from the ventilator 5 h after the end of CPB.

At POD 12 an increase in troponin I levels, accompanied by a 2-fold increase in the titre of anti-pig antibodies as assessed by hemagglutination assay, was interpreted as a rejection reaction. We started an anti-rejection therapy consisting of immunoadsorption and IV IgG infusion, high dose corticosteroids and ATG. Cardiac markers increase in the following 2 days, hence immunosuppressive rescue therapy with a course of the proteasome inhibitor bortezomib was started on POD 15 [8]. Both troponin I and CK-MB levels decreased after that anti-rejection therapy, but remained elevated throughout the observation period. Good graft function was confirmed by transthoracic echocardiography until POD 45; however, marked concentric hypertrophy of the left ventricle was detected from POD 15 onwards (see Fig. 2 and Supporting Information).

On POD 46, ventricular fibrillation of the graft was detected by ECG, but could be successfully terminated by defibrillation. A stable heart rhythm of the graft could only be achieved for 48 h before another defibrillation was needed. Echocardiography now revealed continuous poor cardiac function resulting in a third episode of vetricular fibrillation and defibrillation on POD 49. We terminated the study on POD 50.

A CT scan on POD 49 showed patency of all anastomoses of the graft and the recipient’s heart. Good perfusion of the coronary arteries of the xenograft could be shown; however, myocardial perfusion was poor due to vetricular fibrillation during the scanning period (Fig. 3 and Supporting Information).

Throughout the whole study period, we found the baboon in an otherwise good physical conditions with no signs of infection.
Necropsy revealed a hypertrophic left ventricular myocardium of the xenograft. The weight of the porcine heart was 230 g at POD 50, a nearly 2-fold increase compared to the weight before transplantation (118 g). All anastomoses were patent and no outflow obstructions were found in the series of echocardiographic investigations and the CT scan. No fibrosis of the anastomoses was seen during necropsy.

Histologic analysis of myocardial morphology demonstrated subendocardial patchy necrosis without evidence of active rejection (Fig. 4). Immunohistochemical staining exhibited only minimal evidence for endothelial staining of IgM but no deposition of complement components (C4d) and fibrin. No intramyocardial haemorrhages or cellular infiltrations (CD3) were seen.

Discussion
This is to our knowledge the first description of a heterotopic thoracic pig-to-baboon heart transplantation. Losman and Barnard have reported on a series of heterotopic thoracic allogeneic heart transplantation in baboons [9,10]. In 1977, the same group transplanted a baboon and a chimpanzee cardiac xenograft in the heterotopic position into two patients in cardiogenic shock after cardiopulmonary bypass [11]. These were the first heterotopic thoracic cardiac xenotransplantations; however, the feasibility of a pig-to-primate heterotopic thoracic heart transplantation has not yet been tested. Losman and Barnard already stated that for heterotopic allotransplantation in man and baboons a vascular graft for the pulmonary artery anastomosis is essential to enable an unobstructed filling of both the recipient’s native and the donor’s heart. Our series in the pig-to-baboon setting confirmed that such an interposition is mandatory. In all other aspects, our experience suggests that the use of a porcine donor organ in the non-human primate does not add any technical problems to this operation. It has to be noted however that a baboon’s chest is narrower and longer in comparison to the human anatomy, thus special care has to be taken that the donor hearts are smaller than the recipient native hearts. Choosing donor pigs with 20% lower body weight than the recipient baboons seems to be acceptable.

We believe that there are several advantages of the thoracic compared to the abdominal heterotopic heart transplantation technique in the xenogeneic pig-to-baboon model. Firstly, the parallel position of the graft enables a more physiological blood flow pattern in both the right and the left ventricle of the donor heart. Secondly, abdominal complications are avoided like adhesions or mechanical bowel...
obstruction resulting in an ileus and contributing to morbidity in the primates. Finally, the heterotopic thoracic cardiac xenotransplantation may be a possible procedure for the first cardiac xenotransplantation in man.

In the pre-cyclosporine era, it was suggested that the main benefit of heterotopic thoracic heart transplantation resulted from the possibility that the recipient’s diseased, however still functioning heart could maintain a minimal life-saving function during episodes of rejection. Even with acceptable long-term survival of non-human primates after cardiac xenotransplantation with pig hearts, similar results are not guaranteed in men. Rejection episodes are likely to occur in the first patients receiving cardiac xenotransplantation. The heterotopic thoracic approach may increase the level of safety for this procedure. It was shown in the past that even during a severe rejection crisis with temporary failure of the transplant, the residual function of the recipient’s native hearts was able to bridge to a successful treatment of rejection [12]. However, this possible advantage needs to be confirmed in a study combining the heterotopic thoracic pig-to-baboon heart transplantation with a cardiac failure model [10].

Nevertheless, heterotopic thoracic heart transplantation has some disadvantages in comparison with the orthotopic technique. Perioperatively, it is difficult to estimate how much of the cardiac output will be pumped by the donor heart. Untypical turbulent flow and or stasis may be present and long-term anticoagulation is recommended. The transplanted heart will partially compress the right middle and lower lobe of the lung. This will result in a ventilation–perfusion mismatch with a pulmonary shunt fraction. Furthermore, the chronic atelectasis may be a focus for infection being even more significant under immunosuppression. In our baboon surviving more than 7 weeks, we however neither experienced postoperative oxygenation problems nor detected any pulmonary infection.

The elevation of both, troponin I and anti-pig antibody titres were taken as supporting evidence for a rejection reaction, which was subsequently treated aggressively. All markers for myocardial damage decreased thereafter. In this experiment, however, rejection was not confirmed by biopsies thus we cannot prove reversibility of a xenograft rejection episode—a prerequisite for future clinical studies [4].

The immunosuppressive regimen used in this study appeared to protect the porcine graft from humoral and cellular rejection, as no histological signs for an immunologically mediated injury could be shown at day 50 after transplantation. The recipient was in good physical condition throughout the experiment and tolerated the immunosuppression well. The complete, gross

Fig. 4. Immunohistochemistry of the xenograft revealed only minimal endothelial deposition of IgM (A), but no C4d staining along the vascular endothelium (B). No intravascular fibrin deposition (C) could be shown and no infiltration of the myocardium with CD3 positive cells was seen (D).
and microscopic post-mortem examination did not reveal opportunistic infections. However, atrophy of lymphatic tissues and signs of a pancytopenia were seen and therefore the immunosuppressive regimen may be reduced in future studies, particularly on the background of the absence of any histological signs of rejection.

Taking the absence of any histological signs of a xenograft rejection into consideration, the most likely reason for the elevated myocardial markers during the second half of the postoperative period and the recurrent ventricular fibrillation in the last week of graft survival was the myocardial hypertrophy. The normal weight of a porcine heart at that time would have been approximately 150 g (heart weight at explantation: 230 g) [13]. Biomechanical stress as a major trigger for cardiac hypertrophy needs to be discussed as a possible cause in this context. We found however no evidence for an outflow obstruction of the xenograft. Nevertheless, the uncompromised function of the recipient’s native heart may have increased the afterload of the xenograft. Marked hypertrophy of the transplanted hearts was not seen in patients after heterotopic heart transplantation. One has to consider however that in these instances the recipients’ hearts were severely insufficient, thus the haemodynamic situation may not be comparable. The influence of primate hormones and growth factors on the juvenile porcine heart may also contribute to the hypertrophy but this possible mechanism needs to be further investigated.

In conclusion, heterotopic pig-to-baboon cardiac xenotransplantation is feasible and may combine the safety during episodes of rejection with the benefits of a real working heart model. Graft survival and the effectiveness of treatment strategies for rejection episodes can be investigated. Only future studies with experimental recipient heart failure, however, will enable a stepwise approach to the clinical situation of future cardiac xenotransplantation in man. The possibility of the recipient’s heart to be lifesaving during an episode of rejection still has to be proven in the setting of xenotransplantation and the reversibility of rejection reactions has do be shown. If successful, this procedure may increase the safety of clinical xenogeneic heart transplantation and thus be an important step towards clinical trials.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1 and S2. Transthoracic echocardiography of the donor heart 2 h after weaning from CPB (POD 0) and at day 30 after heterotopic thoracic cardiac xenotransplantation. Concentric hypertrophy of xenograft can be seen at POD 30.

Video S3. 3D reconstruction of a CT angiogram (Somatom Definition; Siemens Medical Solutions, Forchheim, Germany) at POD 50. Starting from a
lateral right view on the porcine xenograft the reconstruction is rotated into a lateral view of the recipient’s heart. The final close-up of the transplanted xenograft shows the pulmonary trunk and the left coronary artery.

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