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Allogeneic heart transplantation following xenogeneic bridging

A. Schütz[1], J. Pratschke[2], M. Breuer[1], C. Hammer[2], M. Engelhardt[1], U. Brandl[1], R. Babic[3], B. Reichart[1], and B. M. Kemkes[1]

[1] Department of Cardiac Surgery, [2] Institute for Surgical Research, and
[3] Institute for Pathology, Ludwig-Maximilians-University, Klinikum Großhadern, Munich, FRG

Abstract. Xenografting seems to be a solution to bridge the time intervals when an essential allograft cannot be obtained. A subsequent allograft was never tried. Eight dogs (20–24 kg) of 2 years of age underwent right cervical heart transplantation. Donors were silver foxes (3–4 kg). The animals were treated by triple drug therapy consisting of cyclosporin A, methylprednisolone and azathioprine in clinical dosages. For control, six recipients received allogeneic heart transplantation (AHTP) and the identical immunosuppression. After rejection of the xenograft, a second allogeneic heart was anastomosed to the same right cervical vessels. Routine histology and immunohistology were performed. Thromboxane B2 and 6-keto-prostaglandin F1a were determined daily in peripheral blood. After final rejection sensitization of the recipient was controlled by haemagglutination tests. Survival times of the xenografts were 9.6 ± 1.2. The subsequent hearts under the same therapy beat for 4.5 ± 5.0 days. The average survival time of control hearts was 18 ± 1.9 days. The five hyperacute second allografts showed signs of humoral rejection by absence of inflammation. The release of thromboxane B2 was different in hyperacute, accelerated or cellular rejection. In contrast to the long-functioning grafts, thromboxane B2 persisted during hyperacute rejection at a high level. However 6-keto-prostaglandin F1a showed no significant differences between long-time survivors and hyperacute rejecting hearts. After xenogeneic transplantation all recipients showed haemagglutinating titres between 1:4 and 1:16. Allogeneic grafts have different kinetics of rejection following xenogeneic heart transplantation (XHTP) compared with control hearts. Thromboxane B2 seems to be an important mediator in hyperacute rejection. This type of rejection is not associated with a change in 6-keto-prostaglandin F1a levels. These results indicate, that xenogeneic bridging under a common immunosuppressive regimen could lead to accelerated rejection of the following allograft. Under this condition clinical bridging is not advisable.

Key words: Allogeneic heart transplantation – Xenogeneic bridging

Offprint requests to: A. Schütz, M. D., Department of Cardiac Surgery, University of Munich, Klinikum Großhadern, Marchioninistr. 15, 8000 Munich 70, FRG

The continuing shortage of human donor hearts has led to the suggestion that hearts from appropriate animal species could be used for bridging a time interval in which a desperately needed heart allograft is not available. The question raised was whether such an intermediate XHTP would jeopardize the success and survival time (SVT) of the following AHTP, due to the sensitization with xenogeneic heart antigen. The aim of this study was to determine the SVT of allografts after XHTP and the rejection type occurring after xenogeneic bridging uder standardized immunosuppression (IS) using a fox/dog model. In addition a potential correlation between thromboxane B2 (TxB2), 6-keto-PGF_{1a} liberation and the type of rejection was investigated.

Materials and methods

XHTP was performed on nine mongrel dogs of 20 to 25 kg aged under 2 years. Xenogeneic donors were silver foxes of 3–4 kg. After rejection of the sensitizing xenografts, the second allogeneic heart was anastomosed onto the same cervical vessels. Adult beagles of 10–12 kg served as donors for the AHTP. As control group, six AHTP were performed under the same conditions. The operation technique has previously been described, as has the anaesthesia [16]. The sensitization to the xenograft was monitored by haemagglutination of fox red blood cells. All transplanted grafts were under continuous observation for the first 3 h. Blood samples were drawn every 15 min during the first postoperative hours, and subsequently daily. The samples for TxB2 and 6-keto-PGF_{1a} were centrifuged at 4 °C and stored at − 70° until use.

Histopathology

The rejected hearts were explanted with the recipients still alive. Sections of standardized areas were stained with HE, methylgreen pyronin and PAS. Ischaemic necroses within the first 6 h were demonstrated by succinate dehydrogenase (SDH) staining, modified according to previously described methods [3, 14]. AR was graded ac-

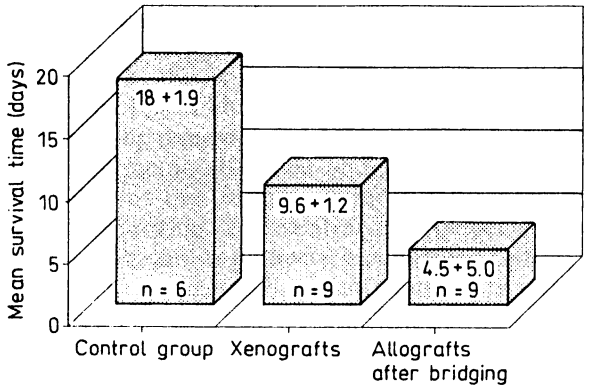

Fig. 1. Average graft survival time of xenogeneic, control and subsequent allogeneic hearts

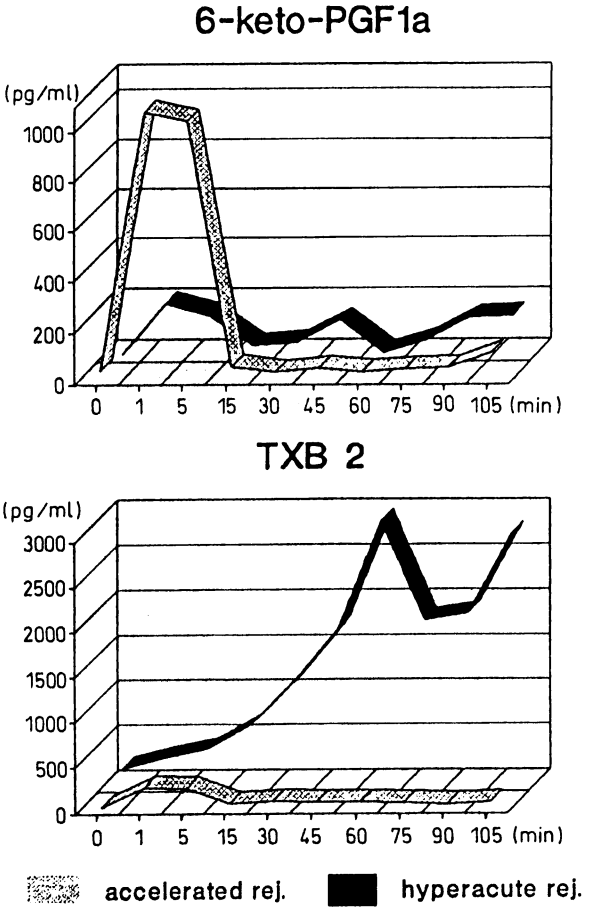

Fig. 2. Liberation of TxB2 and 6-keto-PGF_{1a} plotted against time after opening the anastomoses. During hyperacute rejection a sharp increase in TxB2 could be observed, while 6-keto-PGF_{1a} remained at the orginal level. In contrast the accelerated rejection shows an increase in 6-keto-PGF_{1a} and unchanged levels of TxB2

cording to Billingham et al. [4]. Monoclonal antibodies directed against canine B cells, CD3 positive cells, monocytes and IgM were visualized with peroxidase conjugated antibodies [11].

Immunosuppression

Allogeneic and xenogeneic recipients were immunosuppressed in the same fashion using a triple drug therapy. Prior to perfusion of the graft the recipients received MP i. v. 250 mg and AZA i. v. 50 mg. The trough blood levels of CyA ranged between 500 and 700 ng/ml. AZA 1.25 mg/kg and MP 0.25 mg/kg were given daily. Imipenem/cilastatin (Zienam) was injected i. v. for the first 5 days followed by benzylpenicillin i. m. for another 7 days in order to prevent infections.

Results

All 9 XHTP and the following AHTP were performed without surgical problems and became perfused uniformly after connection with the blood circulation. Four xenogeneic and three allogeneic hearts showed spontaneous contractions, and two xenogeneic and six allogeneic grafts needed defibrillation. The controls functioned as described previously [15]. SVT of the control group (Fig. 1) was 18 ± 1.9 days. Xenogeneic fox hearts for 9.6 ± 1.2 days (Fig. 1). In contrast the subsequent allografts survived only 4.5 ± 5.0 days (Fig. 1), with a range between 105 min and 14 days. The observed immunoreactions were classified as hyperacute (n = 5), accelerated (n = 3) and acute rejection (n = 1). The haemagglutination titre after sensitization by XHTP varied between 1:4 and 1:16. There was no correlation between the level of the haemagglutinating titre and the type of rejection which occurred after the second transplantation.

Hyperacute rejected grafts

Hyperacute rejection (HXR) was defined as rejection within less than 48 h. According to this definition, five hearts (55.5%) were rejected hyperacutely. Visible evidence of AR was present after about 30 min of haemoperfusion in three cases. During the course of AR the hearts became progressively enlarged, livid and finally cyanotic. Routine histological examination of the rejected hearts revealed no signs of cellular rejection in terms of infiltration. In this type of rejection focal ischaemic necroses were observed in two cases. In three cases even with SDH no significant morphological changes could be detected. Congestion of arterioles with platelets and RBC was a rare and inconstant feature. Discrete extravascular haemorrhage and perivascular oedema could be detected in all cases. Immunohistology showed IgM-producing plasma cells, but no infiltration by monocytes or CD3-positive cells. Traces of IgM were found around muscle cells and predominantly on the endothelium of vessels. Levels of TxB2 increased continuously during the first hour in all cases (Figs. 2 and 3). After a moderate reduction it reached a second peak, lasting to the final stage of rejection in three cases. In the other recipients with HXR, TxB2 concentration increased immediately after reperfusion and remained at a high level until final rejection. In contrast the levels of 6-keto-PGF_{1a} did not change in this group.

Accelerated rejection

The three accelerated rejected hearts (33.3%) showed a cellular infiltrate corresponding to grade 2–4. Only distinct focal ischaemic necroses could be found, in contrast extravascular haemorrhage with large infiltrates of B cells and monocytes was characteristic. Thrombi consisting of

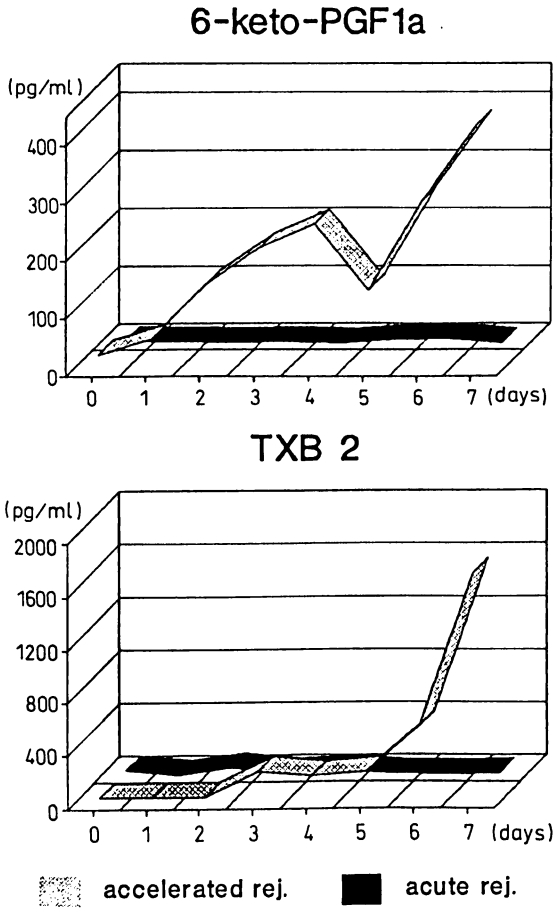

Fig. 3. Time course of daily TxB2 and 6-keto-PGF_{1a} levels during accelerated and acute rejection. During the final stage of accelerated rejection TxB2 increased and 6-keto-PGF_{1a} showed only slight alteration. In the case of acute rejection neither 6-keto-PGF_{1a} nor TxB2 changed

fibrinogen or platelets and RBC were found occasionally. Immunohistology showed a massive deposit of IgM predominantly around muscle cells, endothelium of vessels and perivascular tissue, suggesting that a mixed rejection type with strong humoral participation occurred. TxB2 and 6-keto-PGF_{1a} showed no significant alterations during the first postoperative hours and days (Figs. 2 and 3). While TxB2 increased rapidly 1 day prior to final rejection, 6-keto-PGF_{1a} levels altered little.

Acute rejection

The AHTP of the control group showed typical severe cellular rejection. One juvenile AHTP after bridging reacted in the same way as described for the control group. Interstitial haemorrhage and widespread myocytolysis were found. Only slight B-cell infiltrates and accumulations of IgM could be detected.

Discussion

Organ availability is a worsening problem in clinical transplantation. The shortage of human organs has prompted trials for the use of organs from other species. New, potent immunosuppressive drugs have pushed xenotransplantation into a new era [8, 9]. Preformed natural antibodies [7] and cross-species antibodies, generated in response to transplantation, blood transfusion or pregnancy, interfere with this type of organ transplantation. It was, however, not clear whether these antibodies also cross-react with allografts. Investigations into bridging time in the clinical situation have been published using artificial organs [5]. Allogeneic transplantation following xenografting could not be attempted up to now due to previously described unsolved problems [2]. Experiments in rodents have been described. However, the size of the recipients made transplantation of two consecutive organs of the same type impossible [10]. The fox-to-dog model represents an adequate model to examine a bridging procedure. The animals are large enough to receive two consecutive transplants at the same site. Fox and dog are closely related species and according to their evolutionary and genetic background comparable with the baboon – man system [8]. Antigenic similarity induces the formation of anti-fox antibodies which have partial identity with antigens of the canine relative, the dog [17]. This is postulated to be the explanation for the histological findings revealing a purely humoral rejection mechanism in five cases, comparable with the mechanism found after sensitization with an allograft [6, 13]. In the case of HXR immunohistology showed that antibodies are reacting predominantly with the endothelium of the donor graft's vessels. Some of the allografts were rejected by cellular and humoral mechanisms in an accelerated fashion. As described in previous experiments, single allogeneic transplanted hearts follow a mainly cellular rejection of the acute type. One juvenile allograft in our study resulted in a SVT close to that of unsensitized recipients. The rejection mechanism was of the cellular type with confluent myocytolyses and haemorrhage.

These findings were confirmed by the liberation of TxB2, which was only found during HXR. High levels of TxB2 were reached after reperfusion of the allograft, when HXR occurred, but not during cellular events. The failure to detect an increase in 6-keto-PGF_{1a} levels in HXR may be attributed to damage to the endothelium caused by antibodies and complement activation. On the other hand, the damage of the endothelium activates thrombocytes and leads to liberation of TxB2. The mediation of cardiac ischaemia by TxB2 released from platelets is well described [12]. We suggest that cardiac ischaemia, caused by such potent vasoconstrictors as TXB2 and possibly endothelin [1], is an important factor in HXR cases. These findings would lead to the suggestion that organs from closely related species are of clinical value because of the relatively long survival time. This procedure, however, might be dangerous due to the close antigenicity. The antibodies directed against the xenogeneic species recognize similar targets on the subsequent allogeneic graft and are thus able to jeopardize the prospective permanent allograft. Without more information about possible immunosuppression or modulation of both allogeneic and xenogeneic graft rejection, this bridging should not be ventured in a clinical situation at the present time under conventional triple drug therapy.

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