Influence of Hypothermia and Cardioplegic Solutions on Expression of α-Gal Epitope on Porcine Aortic Endothelial Cells

Miriam Keller, Andres Beiras-Fernandez, Michael Schmoeckel, Bruno Reichart, Paolo Brenner

Abstract

Objectives: **The Galα1-3Galβ1-4GlcNAc-R is the major antigen on pig tissue bound by human xenoreactive natural antibodies in xenotransplant. We have investigated in vitro the influence of hypothermic storage with cardioplegic solutions on expression of Galα1-3Galβ1-4GlcNAc-Rs and hyperacute xenograft rejection.**

Materials and Methods: **To analyze effects of hypothermia on the Galα1-3Galβ1-4GlcNAc-Rs, cultured porcine aortic endothelial cells were exposed to a temperature of 4°C for 1 hour, 4 hours, and 6 hours. Cell cultures of the control groups were incubated at the same time at 38°C. To investigate the influence of cardioplegic solutions on the Galα1-3Galβ1-4GlcNAc-Rs, porcine aortic endothelial cells were exposed to 4°C for 4 hours in the presence of University of Wisconsin solution or histidine-tryptophan-ketoglutarate solution. Cells of the control groups were cooled at 4°C for 4 hours without cardioplegic solution. After treatment, porcine aortic endothelial cells were submitted to fluorescence-activated cell sorter.**

Results: **Hypothermia of 4°C showed no significant effect on the quantity of Galα1-3Galβ1-4GlcNAc-Rs. However, the treatment of porcine aortic endothelial cells with University of Wisconsin solution resulted in a highly significant reduction of Galα1-3Galβ1-4GlcNAc-Rs by 50% (*P* = .006). Treatment of porcine aortic endothelial cells with histidine-tryptophan-ketoglutarate solution decreased α-Gal quantity significantly by 32% (*P* = .011).**

Conclusions: **Our data offer new perspectives in the prevention of hyperacute, humoral xenograft rejection by reducing the Galα1-3Galβ1-4GlcNAc-Rs after exposure to different cardioplegic solutions.**

Key words: *Hyperacute xenograft rejection, Xenotransplant, University of Wisconsin solution, Histidine-tryptophan-ketoglutarate solution*

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Introduction

Xenotransplant offers a potential solution to the worldwide organ shortage crisis in transplant medicine. In case of a discordant xenotransplant (like pig-to-[non]human primate), a vascularized organ is rejected within minutes to a few hours (1-3). The responsible antigen for this so-called hyperacute xenograft rejection is the Galα1-3Galβ1-4GlcNAc-R, a carbohydrate structure expressed in great quantities (1×10^6 to 30×10^6 per cell) on the surface of porcine endothelial cells (4-7).

In a previous study with a working heart perfusion model, we could not show aggravation of hyperacute xenograft rejection by a long ischemic time, but we could show the beneficial effects of prolonged ischemic time regarding hyperacute xenograft rejection, contrary to expectations. Hearts exposed to a long ischemic time of 4 hours showed no signs of a hyperacute xenograft rejection, such as macroscopic and microscopic tissue damage, immunoglobulin and complement deposition, or a decreased coronary flow. Perfusion time of these hearts was significantly prolonged when compared with a control group with a short ischemic time, and hemodynamic function of these hearts was stable until 6 hours of perfusion, whereas hemodynamic

parameters of hearts with short ischemic time decreased significantly after 2 hours (8, 9).

In this current in vitro study, we wanted to answer the following question: Do hypothermia or cardioplegic solutions, both components of ischemic time, have an influence on the Galα1-3Galβ1-4GlcNAc-Rs of porcine aortic endothelial cells?

Materials and Methods

Cell culture

Fresh aortas of German landrace pigs were obtained in a sterile manner from a local private slaughterhouse and transported in sodium chloride 0.9% containing 1% amphotericin B, penicillin, streptomycin (APS; Sigma-Aldrich, St. Louis, USA). Aortic endothelial cells were isolated using standard procedures (10). Briefly, aortas were washed 3 times in sodium chloride 0.9% and 1% amphotericin B, penicillin, streptomycin, then the fat and connective tissue were trimmed from the aortas with sterile scissors, and the vessel was cut longitudinally. Next, aortas were put into a frame with the endothelial side facing up and washed once more. Then they were incubated with dispase (Sigma-Aldrich; 2.5 units/mL phosphate-buffered saline) for 1 hour at 37°C in a humidified incubator. Endothelial cells were dislodged with sterile L-15 medium Leibovitz (Sigma-Aldrich), collected, and afterwards, seeded onto 100 × 20 mm plastic culture dishes in L-15 medium Leibovitz containing 10% fetal calf serum (Biochrom, Berlin, Germany) and 1% amphotericin B, penicillin, streptomycin (Sigma-Aldrich). The work with L-15 medium Leibovitz permitted incubation of the endothelial cells in carbon-dioxide–free systems (11). Cell culture medium was replaced every day.

Selection of cell cultures for the experiments

To avoid influence of subculturing methods on the porcine aortic endothelial cells and the Galα1-3Galβ1-4GlcNAc-Rs, only endothelial cells of primary cultures were used for experiments. To avoid manipulation of long cultivation time on the expression rate of the Galα1-3Galβ1-4GlcNAc-R, all experiments were realized on day 6 ± 1. Cell cultures not ready for analysis at day 7 because of low cells growth rate were thrown away. To eliminate individual differences in the expression rate of the Galα1-3Galβ1-4GlcNAc-R, we worked with paired, random samples, using cell cultures from the same individual for the test and the control group.

Cell viability

To test the cell viability after treatment, living cells were analyzed by trypan blue exclusion test, as follows: After detachment of the endothelial cells from the culture dishes with Acutase (PAA Laboratories, Pasching, Austria), cells were resuspended in 3 mL phosphate-buffered saline. Next, 50 μL of this cell suspension was mixed with 50 μL of 0.4% trypan blue (Sigma-Aldrich). After incubation of approximately 3 minutes, stained and unstained cells (always 100 cells) were counted under light microscopy, and the percentage of viable cells was calculated.

Characterization of endothelial cells

To identify endothelial cells, we combined morphologic studies and determined the characteristic endothelial marker platelet endothelial cell adhesion molecule 1 (CD31). Cell cultures accepted for experiments showed the typical cobblestone morphology. Platelet endothelial cell adhesion molecule 1 expression rate was determined by flow cytometry analysis: endothelial cells were stained with mouse anti-pig CD31 antibody (AbD Serotec; Oxford; UK) (Figure 1). Only pure cell Serotec; Oxford; UK) (Figure 1). Only pure cell cultures were included in the experiments.

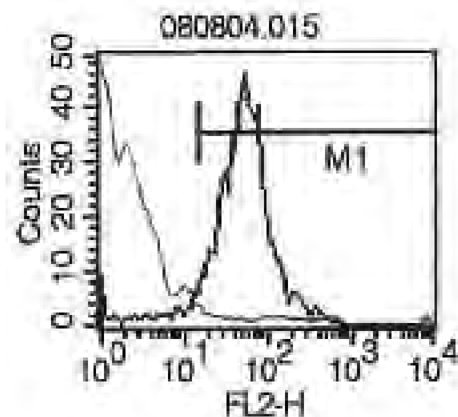

Figure 1. Identification of endothelial cells plateleted endothelial cell adhesion molecule 1 expression rate was determined by flow cytometry analysis. Only pure cell cultures were included into experiments.

Detection of Galα1-3Galβ1-4GlcNAc-Rs on porcine aortic endothelial cells

α-Gal expression was determined by flow cytometry analysis: After removing L15 medium from the

culture dishes, and after washing the cells with phosphate-buffered saline, endothelial cells were detached from the culture dishes by 1 mL Acutase (PAA Laboratories, Pasching, Austria). To establish methods for this study, we compared cell detachment with Acutase, trypsin, and nonenzymatic citrate-buffer solution (2 mM EDTA, 135 mM potassium chloride, 15 mM sodium citrate). Acutase was the gentlest way of cell detachment regarding Galα1-3Galβ1-4GlcNAc-Rs.

Detached cells were collected with phosphate-buffered saline, and this cell suspension was centrifuged (4 minutes, 1500 *U*, at room temperature). Cell pellets were now diluted with 1 mL phosphate-buffered saline, and the cell concentration was determined by the Coulter (Beckman Coulter, Fullerton, USA). Next, single-cell suspension was incubated with fluorescein isothiocyanate-conjugated BS-I Isolectin B4 (Sigma-Aldrich; 50 μL/100 000 cells) according to results of realized saturation tests, for 1 hour at room temperature, washed and submitted to fluorescence-activated cell sorter (FACSort, Becton Dickinson).

Of each cell culture, 2 samples were submitted to fluorescence-activated cell sorter analysis. Finally, the mean of the 2 results was used for statistics. In each case, porcine aortic endothelial cells of the same cell culture not stained with BS-I Isolectin B4 were submitted to fluorescence-activated cell sorter and consulted as control sample. BS-I Isolectin B4 of Bandeiraea simplicifolia has been shown to interact specifically with Galα1-3Galβ1-4GlcNAc-Rs (12).

Experimental procedure: influence of hypothermia (4°C) on the Galα1-3Galβ1-4GlcNAc-Rs

To investigate the influence of hypothermia on the Galα1-3Galβ1-4GlcNAc-Rs, we compared the α-Gal quantity of different groups: the cell cultures of the test groups were cooled at 4°C (standard preservation temperature in organ transplant) for 1 hour (n=6), 4 hours (n=6), or 6 hours (n=6), and the cell cultures of the control groups were incubated for the same time at 38°C (corporal temperature of pigs). We chose 1 hour to examine the influence of hypothermia after a short period of time, 4 hours, because present ischemic tolerance limit in cardiac transplant is 4 hours and 6 hours with respect to improved techniques of organ preservation. Longer periods of hypothermia, such as 24 or 48 hours, were not tested because of the impracticality in cardiac transplant.

For cooling at 4°C, cell cultures were put in a compressor cooler (Waeco Cool Freeze; Emsdetten; Germany) with electronic temperature controller. In addition, temperature was controlled regularly with a manual mercury thermometer. The cell cultures of the control group were put in an incubator at 38°C. To generate comparable atmospheric conditions in the cooler and the incubator, no carbon dioxide was added in the incubator.

Experimental procedure: influence of cardioplegic solutions on the Galα1-3Galβ1-4GlcNAc-Rs

To investigate the influence of cardioplegic solutions on the Galα1-3Galβ1-4GlcNAc-Rs, we compared 2 groups: the endothelial cells of the test group were coated with University of Wisconsin solution (n=4), or histidine-tryptophan-ketoglutarate solution (n=4) at 4°C for 4 hours. The endothelial cells of each control group were cooled at 4°C for 4 hours without any cardioplegic solution. Cell cultures of the control group were put in the compressor cooler for 4 hours, as described above. From the culture dishes of the test group, the medium was removed, and replaced by 10 mL University of Wisconsin solution (ViaSpan; Bristol-Myers Squibb; NY, USA) or histidine-tryptophan-ketoglutarate solution (Custodiol; Dr. Franz Köhler Chemie GmbH; Alsbach-Hähnlein; Germany). Then, cell cultures of the test group, as well, were put in the cooler at 4°C for 4 hours.

Statistical analyses

The influence of hypothermia on the Galα1-3Galβ1-4GlcNAc-Rs was investigated by a 2-way repeated measure ANOVA. The control and test groups about the influence of cardioplegic solutions on the Galα1-3Galβ1-4GlcNAc-Rs were compared by paired *t* test. $P < .05$ was considered to be significant. Results are presented as means ± SEM.

Results

Influence of hypothermia (4°C) on the Galα1-3Galβ1-4GlcNAc-Rs

To examine if hypothermic storage at 4°C shows influence on the quantity of Galα1-3Galβ1-4GlcNAc-Rs we looked at 2 parameters. First, we investigated the percentage of porcine aortic endothelial cells showing Galα1-3Galβ1-4GlcNAc-Rs on their surface (Figure 2). There were no significant differences of the percentage of α-Gal–positive cells between the

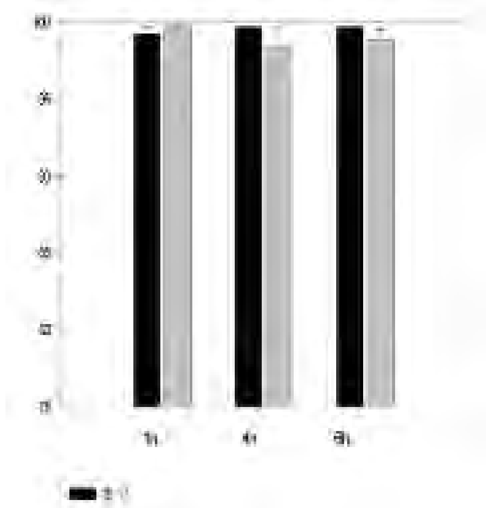

Figure 2. Hypothermia of 4°C had no significant influence on the percentage of porcine aortic endothelial cells stained positively with BS-I Isolectin B4. Not even a long storage time of 6 hours at 4°C resulted in a decrease of α-Gal positive cells. Nearly all porcine aortic endothelial cells cooled at 4°C, as well as those incubated at 38°C showed Galα1-3Galβ1-4GlcNAc-Rs on their surface.

porcine aortic endothelial cells cooled at 4°C and those incubated at 38°C. Nearly all analyzed porcine aortic endothelial cells carried Galα1-3Galβ1-4GlcNAc-Rs on their surface.

The second parameter we looked at was the mean quantity of Galα1-3Galβ1-4GlcNAc-Rs per cell (Figure 3). Therefore, we referred to the mean (mean fluorescence intensity of 1 cell) in fluorescence-activated cell sorter analysis. There also were no significant differences in the mean between porcine aortic endothelial cells exposed to 4°C and those exposed to 38°C. Expression of epitopes depending on the ischemic time showed following results:

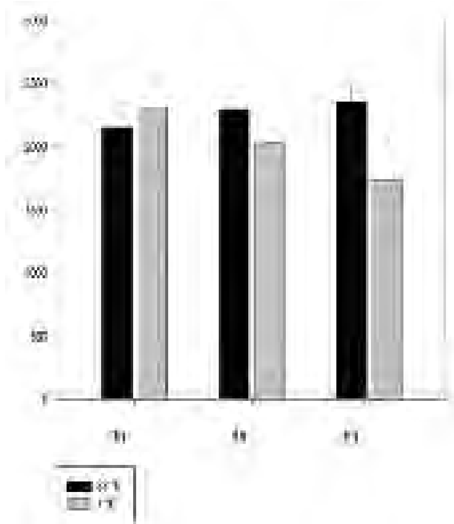

Figure 3. Hypothermia of 4°C did not result in a significant reduction of the mean in flow cytometry. Also, a long hypothermic time of 6 hours did not significantly reduce the quantity of Galα1-3Galβ1-4GlcNAc-Rs per cell.

1-hour group at 4°C, 2310.36 ± 191.03 (at 38°C, 2142.04 ± 193.48), 4-hour group at 4°C, 2024.44 ± 127.88 (at 38°C, 2287.06 ± 90.92), and the 6-hour group 1731.45 ± 311.07 (at 38°C, 2355.93 ± 159.41). No significant differences were observed.

Influence of cardioplegic solutions on the Galα1-3Galβ1-4GlcNAc-Rs

The first series of tests was made with the University of Wisconsin solution. The treatment of porcine aortic endothelial cells with University of Wisconsin solution for 4 hours at 4°C resulted in a highly significant reduction of Galα1-3Galβ1-4GlcNAc-Rs by 50% (P = .006) (Figures 4 and 6a). Whereas the mean of the control group was 1861.17 ± 116.29, the mean of porcine aortic endothelial cells treated with University of Wisconsin solution was only 931.65 ± 20.44. The percentage of cells stained positively with BS-I Isolectin B4 was 99.95% ± 0.01% in the group treated with University of Wisconsin solution and 99.69% ± 0.17% in the group without University of Wisconsin solution (Figure 5). Thus, there was no significant difference in the number of cells showing Galα1-3Galβ1-4GlcNAc-Rs on their surfaces, but there was a highly significant reduction of Galα1-3Galβ1-4GlcNAc-Rs, per cell, by treatment with University of Wisconsin solution.

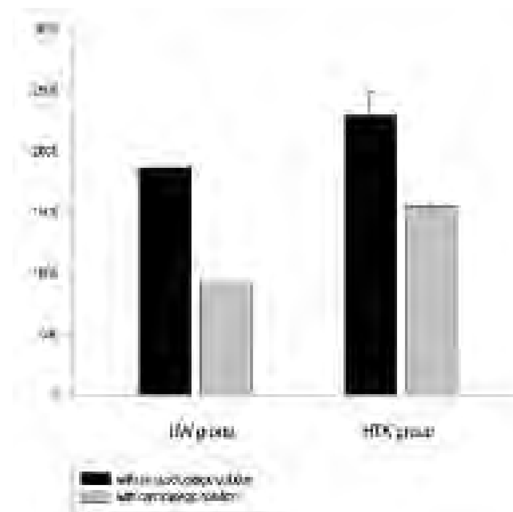

Figure 4. Treatment of porcine aortic endothelial cells with cardioplegic solutions resulted in a significant reduction of Galα1-3Galβ1-4GlcNAc-Rs; 50% less Galα1-3Galβ1-4GlcNAc-Rs were detectable on porcine aortic endothelial cells treated with UW solution at 4°C for 4 hours if compared with the control group cooled at 4°C without any cardioplegic solution (P = .006). In the histidine-tryptophan-ketoglutarate series, 32% less Galα1-3Galβ1-4GlcNAc-Rs were detectable on porcine aortic endothelial cells treated with histidine-tryptophan-ketoglutarate solution at 4°C for 4 hours if compared to the control group cooled at 4°C without any cardioplegic solution (P = .011).

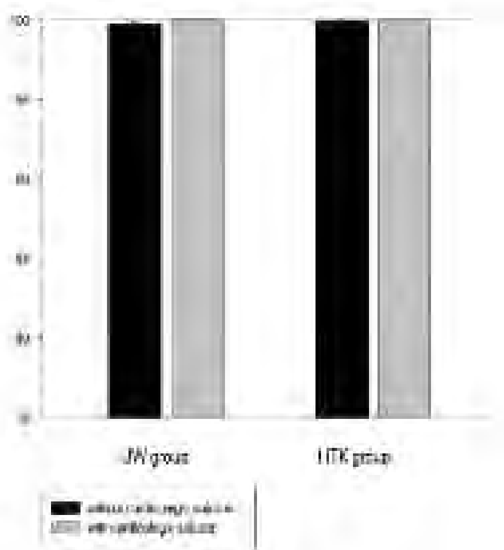

Figure 5. Treatment of porcine aortic endothelial cells with cardioplegic solutions showed no significant influence on the percentage of α-Gal positive cells. Neither the treatment with UW solution nor treatment with histidine-tryptophan-ketoglutarate solution resulted in a significant decrease of cells positively stained with BS-I Isolectin B4. Nearly 100% of the cells treated with UW or histidine-tryptophan-ketoglutarate solution, as well as those without any contact to cardioplegic solutions, expressed Galα1-3Galβ1-4GlcNAc-Rs on their surface.

The second series of tests was made with histidine-tryptophan-ketoglutarate solution. The results were similar to the observations of the University of Wisconsin series. There could not be demonstrated significant differences in the percentage of cells stained positively with BS-I Isolectin B4 (with histidine-tryptophan-ketoglutarate solution: 99.92% ± 0.04%; without histidine-tryptophan-ketoglutarate solution: 99.87% ± 0.06%) (Figure 5). But the treatment of porcine aortic endothelial cells with histidine-tryptophan-ketoglutarate solution showed a significant reduction of Galα1-3Galβ1-4GlcNAc-Rs per cell by 32% (P = .011) (Figures 4 and 6b). Porcine aortic endothelial cells exposed to histidine-tryptophan-ketoglutarate solution at 4°C for 4 hours showed a mean of 1541.27 ± 57.77. Porcine aortic endothelial cells exposed to 4°C for 4 hours without histidine-tryptophan-ketoglutarate solution showed a mean of 2281.51 ± 187.97.

Discussion

Our in vitro study about the influence of hypothermia and cardioplegic solutions on Galα1-3Galβ1-4GlcNAc-Rs demonstrates a significant reduction of the major xenoantigen in flow cytometry analysis after incubation of porcine aortic endothelial cells with special cardioplegic solutions at 4°C for 4 hours. Hypothermia at 4°C alone does not show any Galα1-3Galβ1-4GlcNAc-R reducing effects.

The Galα1-3Galβ1-4GlcNAc-R reducing effect of cardioplegic solutions may clarify the observation previously made in the working heart perfusion model: hearts exposed to a long ischemic time of 4 hours showed no histologic signs of a hyperacute xenograft rejection, and were impressed with longer perfusion times than control hearts with short ischemic time (1 hour) and with a stable hemodynamic function until 6 hours of perfusion (8, 9).

In this study, we compared 2 intracellular standard cardioplegic solutions and their effect on the Galα1-3Galβ1-4GlcNAc-Rs: University of

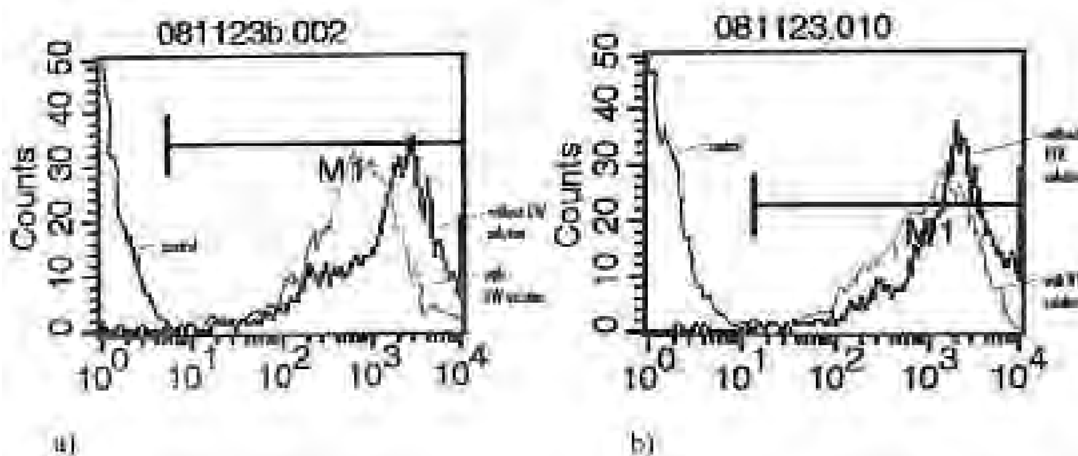

Figure 6. Galα1-3Galβ1-4GlcNAc-R detection with BS-I Isolectin B4 in flow cytometry. a) Fluorescence intensity decreased high significantly by 50% by the treatment of porcine aortic endothelial cells with UW solution. b) Fluorescence intensity decreased significantly by 32% by treatment of porcine aortic endothelial cells with histidine-tryptophan-ketoglutarate solution.

Wisconsin and histidine-tryptophan-ketoglutarate solution. Table 1 shows the ingredients of each solution. We have shown the Galα1-3Galβ1-4GlcNAc-R reducing effect of both solutions, with superiority of the University of Wisconsin solution.

Table 1. Ingredients of UW solution and histidine-tryptophan-ketoglutarate solution.

UW solution		Histidine-tryptophan-ketoglutarate solution	
Poly(O-2-hydroxyethyl) starch	50.000 g/L	Sodium chloride	0.8766 g/L
Lactobionic acid	35.830 g/L	Potassium chloride	0.6710 g/L
Potassium hydroxide 56%	14.500 g/L	Magnesium chloride × 6 H_2O	0.8132 g/L
Sodium hydroxide 40%	3.769 g/L	Histidine HCl × H_2O	3.7733 g/L
Adenosine	1.340 g/L	Histidine	27.9289 g/L
Allopurinol	0.136 g/L	Tryptophan	0.4085 g/L
Potassium dihydrogen phosphate	3.400 g/L	Mannitol	5.4651 g/L
Magnesium sulphate × 7 H_2O	1.230 g/L	Calcium chloride × 2 H_2O	0.0022 g/L
Raffinose × 5 H_2O	17.830 g/L	Potassium hydrogene-2-ketoglutarate	0.1842 g/L
Glutathione	0.922 g/L		

With respect to our results and literature research, so far, we cannot answer in detail, which component of each solution is responsible for the Galα1-3Galβ1-4GlcNAc-R reducing effect. In further experiments, every component of the cardioplegic solutions must be analyzed separately. There are 3 possible mechanisms that could cause the reduction of Galα1-3Galβ1-4GlcNAc-Rs shown in the flow-cytometry analysis (Figure 7). First, components of cardioplegic solutions could cause changes in the chemical structure of the Galα1-3Galβ1-4GlcNAc-Rs and, in consequence, xenoreactive antibodies cannot bind to these epitopes. Second, detachment of the Galα1-3Galβ1-4GlcNAc-R can be hypothesized. Electrolytes, such as the high-potassium concentration of intracellular solutions generally, and the University of Wisconsin solution especially, could be responsible for these mechanisms. Third, a coating effect of the cardioplegic solutions is imaginable. Here, above all, sugar molecules are probably the decisive components that cover the Galα1-3Galβ1-4GlcNAc-Rs.

The superiority of University of Wisconsin solution if compared to histidine-tryptophan-ketoglutarate solution in this case could be attributed to the poly (O-2-hydroxyethyl) starch or to the osmotic active and high molecular sugar raffinose. On the other hand, the waterproofed molecule lactobionic acid, the antioxidant glutathione, or the

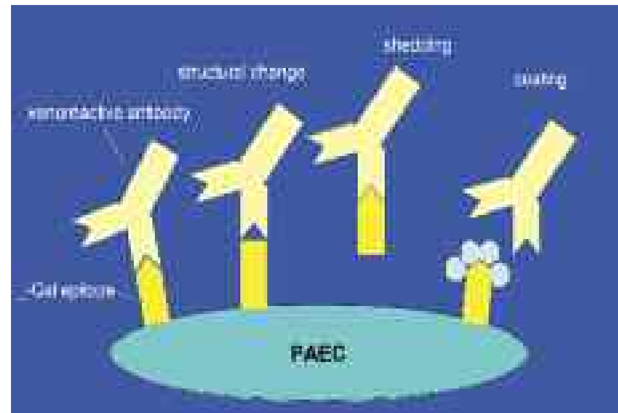

Figure 7. Influence of special cardioplegic solutions on xenograft endothelial cell surface. We suggest 3 theories explaining the reduced antigen-antibody interaction on porcine aortic endothelial cells: It is possible that cardioplegic solutions (1) cause structural changes on the endothelial cell surface, (2) result in a detachment (shedding) of Galα1-3Galβ1-4GlcNAc-Rs, or (3) coat the endothelial surface.

ATP-synthesis important adenosine could contribute to the superiority of University of Wisconsin solution.

To identify the exact mechanism and the responsible components of the cardioplegic solutions further experiments must be realized: for example, analysis of the supernatant with enzyme-linked immunosorbent assay tests regarding detached Galα1-3Galβ1-4GlcNAc-Rs and analyses of every component separately. If antigen-coating would confirm the results of the flow cytometry, other preservation solutions should be tested. Also, further preservation solutions like Celsior and Perfadex (for lung preservation) should be tested with regard to these effects. This cell studies are not whole organ studies and must be reproduced in a working heart perfusion and primate model.

The most important question surely is: Do we actually have to make use of the beneficial effects by cardioplegic solutions in the era of α1,3-galactosyltransferase gene-knockout (GT-KO) pigs? Since 2003, homozygous α1,3-galactosyltransferase gene-knockout pigs have been available for preclinical trials (13, 14). The production of homozygous pigs, with a disruption in the α1,3GT gene, represents a critical step forward in the field of xenotransplantation. Transplant of α1,3-galactosyltransferase gene-knockout organs prevents hyperacute rejection and reduces acute vascular rejection, but finally, xenografts fail due to a type of chronic rejection (15, 16).

There is evidence that α1,3-galactosyltransferase gene-knockout cells express low levels of Galα1-

3Galβ1-4GlcNAc-Rs (17). These low, but immunologically significant levels of Galα1-3Galβ1-4GlcNAc-Rs expressed on tissue of α1,3-galactosyltransferase gene-knockout animals, are synthesized by an alternative galactosyltransferase called iGb3 synthase (18, 19). Prolonged organ storage time with reduced presence of α-Gal owing to these preservation solutions may contribute to an extended xenograft survival. Furthermore, if antigen detachment explicates the α-Gal reducing effect, we could profit from an additional therapeutic way: the Galα1-3Galβ1-4GlcNAc-Rs could be isolated from the supernatant and be injected in the organ recipient before organ reperfusion. The injected Galα1-3Galβ1-4GlcNAc-Rs could have a kind of hapten effect and bind xenoreactive natural antibodies immediately after the start of reperfusion.

It is not unlikely that injected Galα1-3Galβ1-4GlcNAc-Rs will have greater potential in reducing xenograft rejection than synthetic soluble oligosaccharides, like GAS or BSA-Gal, which have been tested in xenotransplant. The results of this study also implicate practical and economic aspects: if long ischemic time actually does not trigger xenograft rejection but diminishes hyperacute xenograft rejection, it is possible to transport xenografts over longer distances in clinical practice similar to allotransplant. Therefore, the practical realization of xenotransplant could be simplified because the number of expensive breeding centers for donator pigs could be kept to a minimum.

Our study shows a reduction of Galα1-3Galβ1-4GlcNAc-Rs by 50% after treatment of porcine aortic endothelial cells with University of Wisconsin solution and a reduction by 32% after treatment with histidine-tryptophan-ketoglutarate solution.

Treatment with these solutions does not result in a complete elimination of Galα1-3Galβ1-4GlcNAc-Rs, but nevertheless, as we have seen in the working heart perfusion experiments, the reduction caused by cardioplegic solution seems to be sufficient for diminishing hyperacute xenograft rejection. There is evidence that nonvascularized α-Gal expressing grafts could become resistant to antigen-antibody rejection through a reduction in α-Gal expression below a threshold (20). It is possible that the same also applies to vascularized organs. In this case, reduction of Galα1-3Galβ1-4GlcNAc-Rs by cardioplegic solutions seems to be sufficient to press α-Gal expression below this threshold.

We elucidated the influence of cardioplegic solution after an ischemic time of 4 hours. So far, we do not have information on the influence of cardioplegic solutions upon the Galα1-3Galβ1-4GlcNAc-Rs after 2 or 3 hours' of ischemic time. It is necessary to investigate these times in further experiments, at least for detecting the role of preservation time in the preventing hyperacute xenograft rejection. Moreover, if treatment of porcine aortic endothelial cells PAEC with cardioplegic solutions is just as effective after 2 or 3 hours, the α-Gal reducing effect could be used without the disadvantage of an increased ischemic-reperfusion injury.

We have demonstrated beneficial effects of cardioplegic solutions on xenograft rejection. In flow cytometry, the quantity of Galα1-3Galβ1-4GlcNAc-Rs on the surface of porcine aortic endothelial cells is reduced significantly after treatment with University of Wisconsin or histidine-tryptophan-ketoglutarate solution. If the results of this in vitro study can be reproduced in an organ transplant study, the influence of cardioplegic solutions on Galα1-3Galβ1-4GlcNAc-Rs in the future can offer a strategy itself in diminishing hyperacute xenograft rejection, and above all, in eliminating residues of Galα1-3Galβ1-4GlcNAc-Rs on α1,3-galactosyltransferase gene-knockout organs.

In our next experiments, we want to answer the outstanding questions discussed in this paper, as well as examine the α-Gal reducing effect and its benefits in primate trials. Owing to the results of our study, we use University of Wisconsin solution in our current heterotopic thoracic pig heart to baboon xenotransplants series.

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