Efficacy of Double Filtration Plasmapheresis in Removing Xenoantibodies and Prolonging Xenograft Survival in an Ex Vivo Swine Perfusion Model


ABSTRACT

Introduction. In xenotransplantation, antibodies mediate humoral rejection, resulting in organ dysfunction. Removal of xenoantibodies is likely a first step for successful transplantation. Double filtration plasmapheresis (DFPP) selectively removes large molecular weight pathogenic substances, such as immunoglobulins (Ig), without other plasma proteins. The antibodies fractions removed and the changes in blood biochemistry are unanswered questions after DFPP in addressed this ex vivo swine heart perfusion model.

Materials and Methods. Twelve swine hearts were perfused with human blood in a modified Langendorff’s apparatus. The perfusate containing human blood was divided into 2 groups: controls (N = 6) and DFPP-treated group (N = 4). Blood counts, biochemistry data, and immunological profiles were compared at 3 time points: before and after DFPP and after heart perfusion.

Result. Perfusion times of control and DFPP groups were 5.43 ± 1.81 vs 9.25 ± 3.00 hours, respectively. Only the values of albumin and total protein showed difference. The immunologic profile revealed complete removal of IgM and most IgG, IgA, C3, and C4, namely, 79.95%, 88.58%, 83.15%, and 87.97%, respectively.

Conclusion. DFPP showed excellent efficacy to remove xenoantibodies and prolong xenograft survival in an ex vivo perfusion model.

In xenotransplantation, antibodies mediate hyperacute humoral rejection. Removal of these antibodies significantly prolongs xenograft survival. There are several approaches to remove these antibodies: double filtration plasmapheresis (DFPP), immunoabsorption (IA), and intravenous immunoglobulin (IVIG). Only DFPP shows consistent benefits. The DFPP uses 2 of artificial membranes with different pore sizes. The first separates blood cells from plasma and the second excretes unwanted pathogenic proteins, such as Ig or lipoproteins, recycling useful low to middle molecular weight proteins, such as albumin. Currently, DFPP has been applied to removal of xenoantibodies, alloantibodies is highly sensitized transplant recipients and treatment of acute humoral rejection. There was unanswered questions about the antibodies fractions that are removed and the changes in blood biochemistry values, which were the purpose of this study.

MATERIALS AND METHODS

This study received approval of an institutional board review and followed the guidelines for our “Guide for the Care and Use of Laboratory Animals”.

Animal

We purchased 12 Landrace adult pigs of 60–90 kg and age 8–12 months from The Animal Technology Institute Taiwan. Hearts (Miaoli, Taiwan) recovered under anesthesia were perfused with human blood in a modified Langendorff’s apparatus, specially designed for large animal hearts. We regularly evaluated electrocardiography, echocardiography, blood counts, biochemistry values, and blood gas analyses as well as constantly monitored pH.
Electrolytes, and temperature of the perfusate. We recorded survival time, which was defined by cessation of the heart beat or ventricular tachycardia.

Human Blood

Human blood obtained from a volunteer donor just before each study, blood was heparinized (300 μg/kg pig weight). The pig hearts were divided into 2 groups: controls (N = 6) and perfused hearts treated with DFPP (DFPP group N = 6). The DFPP or control blood was diluted with lactates Ringer’s solution to achieve a hematocrit level of 28–32%. An extra 50 mL of 25% human albumin was added to 4 of 6 donated bloods in the DFPP group to correct for its hypo-osmolarity. The DFPP used 2 artificial membranes: the first was Infomed Plasmatfilters LF-030 polyethersulfone (pore size 0.2 μm) and the second was plasma fractionator Evaflux 4A (pre size 30 nm). In total 10 sessions were performed in each DFPP group. Blood count, biochemistry values, and immunologic profiles were examined at 3 times: before and after DFPP and after perfusion of the swine heart. IgG, IgA, IgM, C3, and C4 were measured using enzyme-linked immunosorbent assay (ELISA) in our central laboratory.

RESULTS

The survival times of the controls and the DFPP group were 5.43 ± 1.81 versus 9.25 ± 3.00 hours, respectively (P = 0.0423). Blood counts, biochemistry values, and immunologic profiles are summarized in Table 1. The blood count and biochemistry values, except albumin and total protein, did not show any difference between the 2 groups at various times. Although the purpose of DFPP was to retain albumin in the body, a huge proportion of this protein (63%) was removed.

Changes in IgM and IgG are shown in Figure 1 in different times DFPP completely removed IgM, where as only 22.48% of IgM was absorbed in the control group. The reduction in IgG, IgA, IgM, C3, and C4 were as 79.95%, 88.58%, 80.83%, 30.3, and 87.97%, respectively.

DISCUSSION

In xenotransplantation, nonhuman primates are is used as organ recipients. Xenograft rejection often leads to animal death. Ethical considerations are important in performing work with nonhuman primate. We maintained heart survival up to 48 hours without compromising contractility by using syngeneic blood. The method provided an opportunity to examine xenograft function. In the present study, the survival time was better among the DFPP group (5.43 ± 1.81 vs 9.25 ± 3.00 hours; P = 0.0423), which showed that antibody mediates the early rejection event.

In the present study, IgM was removed completely by an Evaflux 4A membrane (pore size 30 nm). The reduction in IgG and IgA were about 75%–85%. Only a few articles have discussed the efficiency of antibody removal by DFPP. Most reports only demonstrated changes in affinity and titer. In our study we also noted 80% reductions in complement components C3 and C4. In clinical conditions of treating highly sensitized transplant recipients, it has been recommended to use Evaflux 2A (pore size 10 mm) to remove more IgG. However this suggestion could lead to complications such as interstitial edema and coagulation dysfunction. In the first 2 pig hearts in the DFPP group, albumin and total protein were depleted profoundly: total

<table>
<thead>
<tr>
<th>Table 1. Blood Count, Biochemistry Values, and Immunologic Profiles at Different Time Points</th>
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<tbody>
<tr>
<td>WBC (1/μL)</td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>4850 ± 1372</td>
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<tr>
<td>5810 ± 7189</td>
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<tr>
<td>TP (mg/dL)</td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>5.64 ± 1.91</td>
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<tr>
<td>3.52</td>
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<tr>
<td>2.3 ± 1.08</td>
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<tr>
<td>IgG (mg/dL)</td>
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<tr>
<td>Baseline</td>
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<tr>
<td>1995 ± 85.83</td>
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<tr>
<td>220.8 ± 6.64</td>
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<td>18.27</td>
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Note: The value was expressed as mean ± SD.

Abbreviations: WBC, white blood cell count; Hb, hemoglobin; PLT, platelet; Neu, neutrophil percentage; Lymp, lymphocyte percentage; TP, total protein; Alb, albumin.

†The data in 4 samples after DFPP supplement with the albumin to correct the osmolarity.

§The blood did not pass through DFPP, and only checked after perfusion with heart.
72.80% and albumin 62.53%. The survival times were less than 3 hours due to severe hemolysis and hyperkalemia. If a smaller pore size membrane was used, more useful proteins are removed; adding albumin back into blood can correct the hemolysis.1,11

In conclusion the efficacy of DFPP to remove xenoantibodies, especially IgM, achieved enhanced survival of xenografts. By expanding these results into daily practice, DFPP can efficiently remove autoantibodies or alloantibodies. We suggest that using larger pore size membranes in DFPP provides equal clinical benefit and avoids complications.

REFERENCES


