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Platelet Activation in Ovines Undergoing Sham Surgery or Implant of the Second Generation PediaFlow™ Pediatric Ventricular Assist Device

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Abstract

The PediaFlow™ pediatric ventricular assist device (VAD) is a magnetically levitated turbodynamic pump under development for circulatory support of small children with a targeted flow rate range of 0.3 - 1.5 L/min. As the design of this device is refined, ensuring high levels of blood biocompatibility is essential. In this study we characterized platelet activation during the implantation and operation of a second generation prototype of the PediaFlow VAD (PF2) and also performed a series of surgical sham studies to examine purely surgical effects on platelet activation. In addition, a newly available monoclonal antibody was characterized and shown to be capable of quantifying ovine platelet activation. The PF2 was implanted in 3 chronic ovine experiments of 16, 30, and 70 days, while surgical sham procedures were performed in 5 ovines with 30 d monitoring. Blood biocompatibility in terms of circulating activated platelets was measured by flow cytometric assays with and without exogenous agonist stimulation. Platelet activation following sham surgery returned to baseline in approximately 2 weeks. Platelets in PF2 implanted ovines returned to baseline activation levels in all three animals, and showed an ability to respond to agonist stimulation. Late term platelet activation was observed in one animal corresponding with unexpected pump stoppages related to a manufacturing defect in the percutaneous cable. The results demonstrated encouraging platelet biocompatibility for the PF2 in that basal platelet activation was achieved early in the pump implant period. Furthermore, this first characterization of the effect of a major cardiothoracic procedure on temporal ovine platelet activation provides comparative data for future cardiovascular device evaluation in the ovine model.

Keywords

platelet activation; pediatric ventricular assist devices; surgical sham studies; ovines; biocompatibility assessment; magnetic levitation

Introduction

Congenital heart disease (CHD) affects an estimated 36,000 infants per year and more than 9,200 children born with CHD require invasive surgery to prevent death in their first year of life. In addition CHD has a total mention mortality of more than 6,800 [1]. A number of these patients could benefit from mechanical circulatory support to provide the necessary cardiac perfusion to bridge them to a heart transplant [2-5]. Ventricular assist devices (VADs) have demonstrated a significant survival benefit, reduction in morbidity, and enhanced quality of life in adults with end stage heart failure [6-8]. The success of VADs in adults make these devices an attractive treatment modality for children suffering with congenital and/or acquired cardiac disease, however there is a dearth of such therapy options for infants and young children [2-5, 9]. This situation provided the impetus for the implementation of the United States National Heart, Lung, and Blood Institute's Pediatric Circulatory Support Program [2]. As part of this program the PediaFlow™ device, a maglev turbodynamic VAD, is currently under development with an objective of providing bridge to transplant circulatory support for newborns and small children at a flow rate range of 0.3 to 1.5 L/min [5, 9-10]. The second generation prototype of the PediaFlow VAD (PF2; shown in Figure 1) was able to achieve higher flow rates than our first generation PediaFlow VAD through supercritical operation by utilizing a more efficient 4-pole motor. Although the fluid path remained the same, the outer housing has a significantly reduced device volume of 35.3 cc (40% decrease) and improved cannula connections when compared to the first generation PediaFlow design [10].

Blood biocompatibility is an important design goal in the development of blood contacting artificial organs. Together with infection, thromboembolic complications remain significant sources of morbidity and mortality in adult heart failure patients [8, 11-14]. Coupled with the risk of thromboembolism is the risk of bleeding associated with anticoagulant and anti-platelet therapy required to prevent thromboembolism. Improved device design might mitigate these risks, but sub-clinical markers indicating cellular biocompatibility are needed. Numerous studies have evaluated direct and indirect markers of platelet activation as well as other hemostatic indices in relation to thrombosis concerns in VAD patients [15-20]. Flow cytometric assays are particularly attractive in that they provide a snapshot of the level of circulating activated platelets [21-24].

The use of large animal models to assess VAD performance and basic biocompatibility is essential before proceeding to clinical trials [24-27]. In the past decade circulating activated platelets have been quantified in bovines and ovines implanted with VADs under development [10, 22-23, 28-30]. As a result, assessment of platelet activation has demonstrated utility in evaluating pump design and candidate surface coatings to improve device hemocompatibility [10, 23, 30]. Several of these studies further demonstrated potential relationships between elevated platelet activation and partial thrombotic occlusion of the device flow path, increased number and size of renal infarcts and other pump-related complications [10, 23]. Preclinical assessment of platelet activation can further be enhanced with surgical sham studies as such studies enable characterization of purely surgical effects on temporal platelet activation. As a result, in a healthy animal the effects of surgery can be discounted, and platelet activation sustained or elevated above that for surgical sham controls can be directly attributed to the device under investigation [23].

Our objective in this study was to characterize platelet activation during the implant and operation of the second generation design of the PediaFlow VAD (PF2) and also perform a series of surgical sham studies to examine purely surgical effects from the implantation procedure. Both circulating platelet activation status and the ability of circulating platelets to respond to agonists delivered in vitro were quantified. In addition a newly available

antibody marker to quantify ovine platelet activation was characterized as was the effect of obtaining pre-operative blood from venipuncture versus an indwelling venous catheter. The results provide future guidance for ovine cardiovascular device biocompatibility assessment and demonstrate promising blood biocompatibility for the PF2 device.

MATERIALS & METHODS

Blood collection

All animal studies were performed in compliance with the University of Pittsburgh Institutional Animal Care and Use Committee, and National Institutes of Health (NIH) guidelines for the care and the use of laboratory animals. An indwelling catheter was inserted in the jugular vein of Dorset-cross, Cheviot, and Suffolk sheep for pre-operative blood collection for each of the PF2 implants and surgical sham studies. Samples were collected by withdrawing 20 mL of blood from the catheter, then the sample volume (2.7 mL), and then re-infusing the initial 20 mL of blood. The blood (2.7 mL) was added to monovettes containing 0.3 mL of 0.106M trisodium citrate (Sarstedt, Newton, NC). At least three pre-operative samples were collected from the indwelling catheter and averaged to obtain the pre-operative data point (post-operative day zero).

Pre-operative blood samples were also used to assess the ability of MCA2418 (an anti-human CD62P antibody-clone Psel.KO.2.5; AbD Serotec; Raleigh, NC, USA) to preferentially bind to activated ovine platelets in nine ovines. To assess the effect of the jugular vein catheter versus jugular venipuncture on pre-operative platelet activation, blood was also drawn slowly (to avoid mechanical stress) via jugular venipuncture using an 18-gauge 1.5-in needle with syringe and stopcock, discarding the first 3 mL for twelve ovines and 2.7 mL was added to sodium citrate monovettes. Post-operative samples were collected from the carotid arterial line, inserted during surgery. Samples were obtained on post-operative days 1, 2, and 3, and then twice weekly for the duration of each surgical study (sham study and implanted animal).

In vitro biocompatibility flow loop setup

A mock circulatory test loop with a blood bag reservoir and including the PF2 pediatric VAD was prepared as previously described [10]. Ovine whole blood (360 mL) was collected by jugular venipuncture into a blood bag containing 40 mL of 0.106M sodium citrate solution. Ovine blood (240 mL) was added to the reservoir and circulated for 2 hr each at 0.5, 1.0, and 1.5 L/min. Resistance of the circuit (a tubing clamp downstream of the pump) was used to set the afterload pressure. A bag with the same ovine blood (~100 mL) served as the control and was used to replace blood volume taken from the test loop. Blood was collected hourly for assessment of platelet activation. A similar test loop was constructed with another prototype pediatric VAD device which utilized an axial thrust bearing for comparison and evaluated for one hr at 1.8 L/min.

Assessment of platelet activation

Platelet activation was quantified during the 6 hr in vitro study before and after exogenous stimulation with platelet activating factor (PAF; Calbiochem, San Diego, CA, USA). Platelet activation was assessed pre and post surgery with samples from each PF2 implant and sham surgery animal. In addition, platelet activation was assessed following stimulation using adenosine diphosphate (ADP; Calbiochem, San Diego, CA, USA) or PAF in samples from three of the sham animals and from all of the PF2 implants.

Blood (5 μ L) was transferred from monovette tubes into 12 $\times$ 75 mm polystyrene tubes with 5 μ L of CAPP2A (7.5 μ g/mL; Veterinary Medical Research and Development; VMRD,

Pullman, WA, USA), 5 μ L of goat anti-mouse IgG-phycoerythrin (60 μ g/mL; Invitrogen, Carlsbad, CA, USA), and 35 μ L of Tyrode's buffer (Electron Microscopy Services, Hatfield, PA, USA) with 1% bovine serum albumin (BSA) and 0.106M sodium citrate and incubated for 20 min. Each unstimulated sample was performed in duplicate.

ADP and PAF stimulated samples were prepared as above using 25 μ L of Tyrode's buffer with BSA, and 5 μ L of either 200 μ M ADP or 100 μ M PAF, and 5 μ L of 20-mM glycine-proline-arginine-proline (GPRP for inhibition of fibrin polymerization, Anaspec, San Jose, CA, USA). Samples were then washed with 1 mL of Tyrode's buffer with 1% BSA and 0.106 M sodium citrate (washing buffer) and mixed. Samples were then centrifuged for 10 min at $132 \times g$. The supernatant was removed and the pellet resuspended. IgG1-Alexa Fluor 488 (isotype control; 5 μ L of 25 μ g/mL; MCA928A488, AbD Serotec), MCA2418-Alexa Fluor 488 (5 μ L of 25 μ g/mL; MCA2418-A488), or MCA2419-Alexa Fluor 488 (5 μ L of 25 μ g/mL; anti-human CD62P-clone Psel.KO.2.7, AbD Serotec) were then added to tubes and incubated for twenty min. Samples were then mixed with 1 mL of washing buffer and centrifuged at $132 \times g$. Following removal of the supernatant, samples were fixed with 1% paraformaldehyde. Flow cytometric analysis was performed as previously described [24]. MCA2419 was utilized as the platelet activation marker to quantify the effect of the jugular vein catheter on pre-operative platelet activation. Blood was sent out to a veterinary diagnostic laboratory (ANTECH Diagnostics; Lake Success, NY, USA) weekly to quantify platelet counts for each in vivo study.

In vitro characterization of MCA2418 binding to activated ovine platelets

MCA928-A488 was used as the isotype control antibody for the evaluation of MCA2418-A488. Unstimulated samples were prepared as above replacing the 35 μ L of citrated Tyrode's buffer with 30 μ L of Tyrode's buffer with (BSA) and 5 μ L of GPRP. ADP and PAF stimulated samples were prepared as above. Fluorescence associated with MCA2418-A488 for control and activated samples was compared to evaluate whether MCA2418 preferentially bound to activated ovine platelets. Flow cytometric analysis was performed as previously described [24].

Implant procedure

Three sheep were implanted with two PF2 prototypes (PF2.1, PF2.2- Table 1). A problematic sensor wire (discussed below) was replaced between the first and second PF2.1 implants. Anesthesia was induced with ketamine and maintained with inhalation isoflurane. An arterial line for pressure measurement was placed in the left carotid artery along with a venous line for pharmacologic and fluid administration into the jugular vein. The PF2 pump was placed into the fourth intercostal space via a left thoracotomy. The inflow cannula was an 18F cardiopulmonary bypass cannula (DLP; Medtronic, Minneapolis, MN, USA) trimmed to 7 cm and communicated with the left ventricle through a stab incision to the heart and secured with a felt-coated sewing ring fixed on the apex of the heart with pledgeted sutures. After bolus administration of heparin (150 U/kg), the outflow cannula (20F EOPA cannula trimmed to 9 cm, Medtronic) was inserted by an introducer through a snare incision into the descending aorta and the cannula was then fixed directly onto the pump outflow connector. The pump was started and operated at the desired RPM (Table 1). The blood contacting surface of the PediaFlow is TiAl_6V_4 . The blood contacting surface of the cannulae is Carmeda® BioActive Surface (heparin bonded surface coating) on polyurethane.

After closure of the chest, each animal was allowed to recover from anesthesia, and spontaneously ventilate. Bupivacaine hydrochloride (Hospira Inc, Lake Forest, IL, USA) was used in the intercostal muscles and flunixin meglumine (Phoenix Pharmaceutical, Inc;

St. Joseph, MO, USA; 25 mg intravenously) was used for analgesia for the first 5 days and then as needed. No heparin was administered for 24–48 hrs after surgery. Heparin was then administered to maintain the activated clotting time between 180–200 secs. Warfarin (intravenous coumadin; Bristol-Myers Squibb; Princeton, NJ, USA) was administered concurrently in an effort to maintain the INR in the range of 2.0–3.0. Kefzol (Cefazolin) was given prophylactically for the first 50 days of the 70 day implant and for the duration of the other implants. One unit of blood was transfused on the day of surgery into the animal from the 30 day PF2.1 study.

Sham surgeries were performed on five animals as described for the implant with the following alterations: No inflow cannula was inserted into the sewing ring. After the stab incision into the apex of the left ventricle the sewing ring site was stapled shut. The descending aorta was partially clamped with a vascular clamp and an outflow graft was anastomosed onto the aorta and tied off at the anastomotic site. Coumadin was not used in the sham studies, although heparin was administered to maintain the activated clotting time between 180–200 secs for the duration of the study.

Statistical Analyses

All data are presented as mean with standard deviation. Statistical analyses were performed using SPSS 12.0.1 (SPSS, Chicago, IL, USA). One-way analysis of variance with repeated measures (Bonferroni post hoc test) was used to compare the means of MCA2418 binding to unstimulated and stimulated ovine platelets. A paired samples t-test was used to compare means of pre-operative platelet activation from jugular venipuncture and jugular venous catheter samples. Significance was considered to exist for $p < 0.05$.

RESULTS

Effect of venous line placement and in vitro assessment of platelet activation

In Figure 2 the MCA2418 antibody bound at significantly higher levels to ADP and PAF-stimulated ovine platelets when compared to unstimulated ovine platelets. Pre-operative platelet activation after jugular venipuncture and following placement of a jugular venous catheter is shown in Figure 3. The level of platelet activation measured in samples obtained from the venous catheter was significantly reduced from those drawn through jugular vein venipuncture. There was also less variance observed in these activation levels when the catheter was utilized.

Platelet activation during a 6 hr in vitro study with the PF2.2 VAD is illustrated in Figure 4. CD62P-positive platelets remained low ($< 11\%$) during this entire study; and the percentage of CD62P-positive platelets increased markedly following stimulation with 10^{-6} M PAF throughout the study. In contrast platelet activation following one hr of contact with the other pediatric pump employing the axial thrust bearing rose to 18% in one hr and platelet activation following PAF stimulation rose only to 22%.

Sham surgery studies

In Figure 5A platelet activation quantified by MCA2419 rose following sham surgery; reaching a peak on day 7 and then steadily declined, returning to baseline levels within the first 17 days. P-selectin 2418 was a new antibody that was not available for the initial two surgical sham studies. Therefore only MCA2419 was plotted in Figure 5A because it had the larger sample size ($N = 5$). In Figure 5B platelet activation quantified by MCA2418 with and without agonist stimulation of the drawn sample is presented. Platelet activation increased following stimulation with ADP and PAF but the difference between stimulated and unstimulated activation levels was diminished early in the post-operative period

compared to the pre-operative difference. The difference in platelet activation before and after stimulation increased as surgery-associated platelet activation dissipated. In Figure 5C platelet count rises on day one after surgery and then follows a biphasic pattern, before stabilizing to baseline levels on day 16, similar to the platelet activation results. No kidney infarcts were observed at necropsy in any of these sham surgery animals.

Ovine implants of the PF2 VAD

Figure 6 shows platelet activation results and necropsy images from the 17-day PF2.1 VAD implantation. Platelet activation rose following surgery reaching a maximum on day 3 and returned to baseline levels on day 6. Platelet activation remained low before sharply rising on day 16 subsequent to numerous pump stoppages (rotor de-levitation) that occurred prior to termination of the study. These events caused rotor touchdown and were found to be the result of a manufacturing defect in a sensor wire in the percutaneous cable. In Figure 6B platelet activation following agonist stimulation was elevated above platelet activation found in unstimulated blood over the course of the implant. As with the sham surgery animals, this difference in activation increased as unstimulated platelet activation returned to baseline levels. However, in the final samples taken after the pump stoppages the difference between unstimulated and stimulated samples again decreased. In Figure 6C platelet count follows a similar pattern as platelet activation rising after surgery in the first week before returning to baseline. As seen in Figure 6D, no obvious kidney infarcts were observed at necropsy and the outlet region of the impeller had a large number of scratches covering the surface that were attributable to the numerous pump stoppages. Histopathology of kidney sections did not reveal clear evidence of micro-infarcts, although some chronic cortical nephritis was observed that lacked the typical wedge shaped appearance one would expect to see with an infarct.

Platelet activation data and necropsy images for the 30-day PF2.1 VAD implant are seen in Figure 7. Platelet activation rose slightly following surgery reaching a maximum on day 1 and returned to baseline on day 2. The low platelet activation observed in this study was accompanied by an ability of platelets to become further activated following incubation with ADP and PAF. Platelet count decreases after surgery and then rises to a level slightly above baseline for the duration of the end of study. There were two small surface infarcts on the left kidney which did not penetrate past the cortex and a relatively low number of scratches on the impeller at necropsy. Histopathology indicated a healing area around the focal infarct.

Platelet activation and necropsy images for the 70-day PF2.2 VAD implant are displayed in Figure 8. Platelet activation in the 70 day study sharply rose following surgery reaching a maximum on day 3. On day 6 platelet activation began to decline, however between days 9 and 23 platelet activation measurements fluctuated with relatively high levels of activation detected by both antibodies on days 13 and 20. When platelet activation was high in the unstimulated samples the ability to respond further to ADP and PAF was limited. From day 23 until study termination on day 70, circulating activated platelet levels were relatively low, approaching baseline, and the platelets were highly responsive to agonist stimulation. Platelet count follows a similar trend as the platelet activation data; sharply rising following surgery before returning to baseline by the end of the third week. At the time of device explantation several surface infarcts were observed on the left kidney; these infarcts did not penetrate into the medulla. Histopathology indicated healed areas around the focal infarcts. There were also a moderate number of scratches found on the impeller of the device.

DISCUSSION

The recently available MCA2418 antibody was shown to cross-react and to differentiate agonist-stimulated from unstimulated ovine platelets, demonstrating its potential utility in

quantifying platelet activation associated with blood contacting artificial organs. These results are similar to results previously reported with MCA2419 and MCA2420 [24]. Each of these antibodies recognize human p-selectin on the surface of activated platelets, but being distinct clones they will recognize different epitopes on exposed p-selectin and may have differential binding to a given level of platelet activation. In assessing all of the reported data on these three antibodies with ovine platelets, MCA2420 appears to have the lowest affinity for p-selectin relative to MCA2418 and MCA2419, and thus would be expected to be the least sensitive of the three platelet activation markers [10, 24]. Recently we have observed binding of MCA2420 to activated ovine platelets that was reduced compared to published data [24]. This reduced antibody binding persisted even with increased antibody concentrations. As a result, in future studies it is recommended to utilize two distinct antibody clones for control purposes and our recommendation would be to utilize MCA2418 and MCA2419 rather than MCA2420.

The in vitro study of the PF2 VAD in a blood perfusion circuit showed promising results with low platelet activation over the entire six hr study. The relatively low levels of platelet activation were accompanied by an ability to respond to PAF stimulus. The platelet response to PAF decreased over time and at the higher flow rates, but even at six hrs there was still a four-fold increase in the level of p-selectin positive platelets following stimulation. The PF2 results observed in vitro were similar to the low platelet activation observed in vivo, demonstrating the potential utility of the described platelet activation assays for in vitro testing. Measurements of platelet activation with an axial bearing pump in vitro showed elevations in the first hr to 18%. After stimulation however, platelet activation only rose to 22%. This minimal response to PAF suggested marked platelet dysfunction. The rise to 18% in the first hr of pumping by itself would not necessarily have been considered evidence of lack of platelet biocompatibility; however, the inability of platelets to respond to stimulation suggests that substantial levels of activation and platelet damage have occurred.

This report is the first to apply these monoclonal antibodies for biocompatibility assessment in an in vitro mock circulatory loop using ovine blood. While limited to a single study, the contrast between the two pump types was dramatic and demonstrates the potential utility for more sophisticated in vitro studies. For example, this evaluation modality might be able to discern differences between similar pumps with different design parameters i.e. gap width, number of impeller blades, stator vanes; that would enable more focused research on the promising pumps in the more costly chronic in vivo studies.

Elevated pre-operative platelet activation has been reported previously in ovines [10, 29]. In these reports stress was speculated to be a significant factor in this elevation and the stress was attributed to the necessity of animal restraint and jugular venipuncture to obtain blood samples. The time period for the sheep to acclimate to its new surroundings was also considered to be a potential factor. The use of an indwelling jugular vein catheter was applied in one animal in the previous study as a means to reduce stress-induced artifact [10]. In this report we performed a substantive comparison of platelet activation before and after placement of a jugular venous catheter. Figure 3 shows pre-operative platelet activation was significantly decreased following placement of the jugular venous catheter, demonstrating the utility of the catheter in obtaining pre-operative platelet activation values. It is interesting to note though, that jugular venipuncture does not altogether preclude the acquisition of low pre-operative platelet activation as the spread in the data for venipuncture in Figure 3 indicates. However, this large variability in platelet activation from venipuncture samples provides further argument for the placement and use of a venous catheter. The use of a venous catheter is further merited because all post-operative samples are collected via an indwelling catheter thus ensuring a more appropriate comparison between pre-operative and

post-operative values, in addition to avoiding comparison of post-operative data to artificially elevated baseline measurements.

VAD implantation generally requires invasive surgery (thoracotomy) that causes significant tissue damage, inflammation, and platelet activation. The effect of surgery on ovine platelet activation had not been previously quantified. Further, determining the extent to which surgery causes ovine platelet activation theoretically allows one to distinguish platelet activation that can be attributed to the VAD post-operatively. In this report platelet activation rose following the sham surgical procedure peaking on day 7 and then steadily dissipated over time back to baseline in approximately two and a half weeks. The ability of the platelets to respond to agonist similarly returned to baseline levels in this period. If temporal platelet activation after VAD implantation followed a trend similar to the temporal sham surgery it would indicate that minimal platelet activation was attributable to the device, a promising biocompatibility result.

Platelet activation in each of the PF2 VAD implants rose following surgery. In each case platelet activation returned to a baseline level. In the 17-day implant there was a large rise in activation on day 16 (Figure 6) which coincided with numerous pump stoppages due to a manufacturing defect in the axial position sensor cable in the percutaneous lead. With each pump stoppage there was rotor touchdown accompanied by regurgitant flow through the device creating a flow field that may have promoted platelet activation. The numerous scratches found on the outflow section of the impeller (Figure 6D) may also have created a nidus for platelet activation and locally disturbed flow. At explant, however, there was no thrombotic deposition seen in association with these scratches. This large rise in platelet activation following pump stoppage was also observed in implants with an earlier generation of the PF2 VAD [10]. In the 30-day implant of the PF2 VAD (Figure 7) platelet activation only rose to a modest degree ($< 20\%$). There were several impeller scratches observed in this study, but the number of scratches appeared to be the least of the three implants. In the 70-day implant (Figure 8) platelet activation had the largest rise following surgery of the three implants but returned to baseline levels on day 9. However, in this study there were two subsequent sharp rises in platelet activation on days 13 and 20 although there were no observed pump stoppages or other pump complications on these days. At necropsy impeller scratches were identified and the cause of these scratches may have been related to the two spikes in platelet activation. Platelet activation did eventually moderate towards baseline on day 23 of this study.

Temporal platelet activation in ovines implanted with the PF2 VAD, particularly in the 17 and 30-day studies, showed similar trends to that of the sham studies in that there was a return to baseline values in the first two weeks. In fact, in these two studies platelet activation was lower than that observed in the sham surgery animals and platelet activation resolved more quickly. This is an encouraging biocompatibility result for the PF2 device, but merits some consideration as to how the VAD implanted animals would achieve such a result. In the sham studies the aorta had to be partially clamped in order to sew the outflow graft onto the aorta whereas with PF2 VAD placement the aortic cannula was inserted by a snare incision with the use of an introducer that required a much shorter aortic clamp time. In addition, coumadin was not used in the sham surgery studies and while coumadin does not have a direct effect on platelets, the use of coumadin in the sham studies might have reduced thrombin generation and thus had an effect on the resolution of platelet activation after surgery. Platelet activation in the 70-day study was higher in the immediate post-operative period in comparison to the sham surgery control animals, which suggests that there was some ongoing activation directly attributable to the device. However, pump effects did resolve as platelet activation returned to a sustained baseline level on day 23.

In this report we also show platelet activation following stimulation with platelet agonists in PF2 VAD-implanted ovines. Low platelet activation levels would suggest good platelet biocompatibility however low platelet activation accompanied with an inability to respond to exogenous stimulation would suggest blunted platelet function as a result of irreversible pump-related damage or perhaps due to heavy anti-platelet medication. Assessing platelet responsiveness may be particularly useful in preclinical studies since a healthy animal would be expected to fully respond to platelet stimulus given that other pre-existing conditions (e.g. vascular disease, coagulopathies), prior surgery or device blood contact would not be present. The mostly low platelet activation measurements observed in the PF2 VAD-implanted ovines was accompanied by an ability of the platelets to respond strongly to exogenous ADP and PAF stimulation. At the end of the 17-day implant study and early in the 70-day study when platelet activation was high, there was a limited amount of further activation that was measured following stimulation.

Previous studies have related evidence of thromboembolism, as measured by kidney infarcts observed at necropsy, to levels of platelet activation measured to VAD implantation [10, 23]. The initial level of platelet activation in the 70-day implant was higher than what was observed in the other PF2 studies and at necropsy there were more infarcts that were larger in size relative to the other two VAD-implanted ovines, although there was no evidence of kidney dysfunction during the implant. One of the reasons for the higher platelet activation and increased kidney infarcts may have been that the pump was operated at a much lower flow rate (0.5 L/min) than the first two implants. In the 17-day PF2.1 study (Figure 6) there was a large rise in activation at the end of the study without the presence of kidney infarcts. In this study the animal was terminated within 48 hrs of an increased frequency of pump stoppages and there may not have been enough time for a large thrombus to form or for a kidney infarct to develop grossly from a thromboembolus shed late in this period. A utility of flow cytometric assessment of platelet activation versus necropsy evaluation alone is demonstrated here in that it can provide an ongoing assessment of how the device is performing whereas it can be difficult to determine when an infarct has developed and how it might relate to pump or animal complications observed over the course of the study.

In comparing the results of these studies with the PF2 VAD to a previous report on ovine implants with the first generation of the PediaFlow VAD, some comments can be made. For that earlier design platelet activation became elevated at the end of the study or was sustained throughout the study for all three animals [10]. In the PF2 series of implants all three had periods of sustained low platelet activation and only the initial 17-day study ended with elevated levels of platelet activation. Many of the complications encountered with the first generation PediaFlow implants were related to peripheral system failures (namely cannula connections). This pump issue was eliminated in the PF2 VAD and the device underwent a motor redesign to achieve higher flow rates. Given the higher flow rates and higher associated shear the improved platelet biocompatibility observed in the PF2 VAD implants is encouraging. The PF2 VAD represents a promising next step in the PediaFlow development strategy to develop a pediatric VAD for infants and small children with high blood biocompatibility.

CONCLUSIONS

This report summarizes our efforts to enhance blood biocompatibility assessment of cardiovascular devices in the ovine model. A new monoclonal antibody was characterized and demonstrated an ability to quantify circulating activated ovine platelets. The placement of a jugular venous catheter for blood collection was shown to significantly reduce pre-operative platelet activation artifact due to venipuncture. The effect of VAD placement surgery on ovine platelets was characterized and platelet activation attributable to the

surgery was found to return to baseline levels in approximately two weeks. Platelet activation following implant of the PF2 VAD also returned to baseline levels during the implant periods and platelets in contact with the PF2 VAD had preserved platelet function. The PF2 VAD demonstrated promising platelet biocompatibility and represents a critical next step in the development of a highly biocompatible PediaFlow pediatric VAD.

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REFERENCES

1. Lloyd-Jones D, Adams RJ, Brown TM, et al. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation*. 2010; 121(7):e46–e215. [PubMed: 20019324]
2. Baldwin JT, Borovetz HS, Duncan BW, et al. The National Heart, Lung, and Blood Institute Pediatric Circulatory Support Program. *Circulation*. 2006; 113(1):147–55. [PubMed: 16391168]
3. Baldwin JT, Duncan BW. Ventricular assist devices for children. *Prog Pediatr Cardiol*. 2006; 21(2): 173–184.
4. Blume ED, Naftel DC, Bastardi HJ, Duncan BW, Kirklin JK, Webber SA. Outcomes of children bridged to heart transplantation with ventricular assist devices: a multi-institutional study. *Circulation*. 2006; 113(19):2313–9. [PubMed: 16702487]
5. Borovetz HS, Badylak S, Boston JR, et al. Towards the development of a pediatric ventricular assist device. *Cell Transplant*. 2006; 15(Suppl 1):S69–74. [PubMed: 16826798]
6. Pagani FD, Miller LW, Russell SD, et al. Extended mechanical circulatory support with a continuous-flow rotary left ventricular assist device. *J Am Coll Cardiol*. 2009; 54(4):312–21. [PubMed: 19608028]
7. Slaughter MS, Rogers JG, Milano CA, et al. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med*. 2009; 361(23):2241–51. [PubMed: 19920051]
8. Rose EA, Gelijns AC, Moskowitz AJ, et al. Long-term mechanical left ventricular assistance for end-stage heart failure. *N Engl J Med*. 2001; 345(20):1435–43. [PubMed: 11794191]
9. Wearden PD, Morell VO, Keller BB, et al. The PediaFlow pediatric ventricular assist device. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2006; 9:92–8. [PubMed: 16638553]
10. Johnson CA Jr. Biocompatibility Assessment of the First Generation PediaFlow™ Pediatric Ventricular Assist Device. *Artif Organs*. 2011 (in press).
11. Simon MA, Watson J, Baldwin JT, Wagner WR, Borovetz HS. Current and future considerations in the use of mechanical circulatory support devices. *Annu Rev Biomed Eng*. 2008; 10:59–84. [PubMed: 18647112]
12. Mussivand T. Mechanical circulatory support devices: is it time to focus on the complications, instead of building another new pump? *Artif Organs*. 2008; 32(1):1–4. [PubMed: 18181797]
13. Deng MC, Edwards LB, Hertz MI, et al. Mechanical circulatory support device database of the International Society for Heart and Lung Transplantation: third annual report--2005. *J Heart Lung Transplant*. 2005; 24(9):1182–7. [PubMed: 16143231]
14. Kirklin JK, Naftel DC, Kormos RL, et al. Second INTERMACS annual report: more than 1,000 primary left ventricular assist device implants. *J Heart Lung Transplant*. 2010; 29(1):1–10. [PubMed: 20123242]

15. Radovancevic R, Matijevic N, Bracey AW, et al. Increased leukocyte-platelet interactions during circulatory support with left ventricular assist devices. *ASAIO J.* 2009; 55(5):459–64. [PubMed: 19730003]
16. Dewald O, Schmitz C, Diem H, et al. Platelet activation markers in patients with heart assist device. *Artif Organs.* 2005; 29(4):292–9. [PubMed: 15787623]
17. Houel R, Mazoyer E, Boval B, et al. Platelet activation and aggregation profile in prolonged external ventricular support. *J Thorac Cardiovasc Surg.* 2004; 128(2):197–202. [PubMed: 15282455]
18. Matsubayashi H, Fastenau DR, McIntyre JA. Changes in platelet activation associated with left ventricular assist system placement. *J Heart Lung Transplant.* 2000; 19(5):462–8. [PubMed: 10808153]
19. Wilhelm CR, Ristich J, Kormos RL, Wagner WR. Monocyte tissue factor expression and ongoing complement generation in ventricular assist device patients. *Ann Thorac Surg.* 1998; 65(4):1071–6. [PubMed: 9564930]
20. Slaughter MS, Sobieski MA, Gallagher C, Graham J, Brandise J, Stein R. Fibrinolytic activation during long-term support with the HeartMate II left ventricular assist device. *ASAIO J.* 2008; 54(1):115–9. [PubMed: 18204326]
21. Michelson AD, Barnard MR, Krueger LA, Frelinger AL 3rd, Furman MI. Evaluation of platelet function by flow cytometry. *Methods.* 2000; 21(3):259–70. [PubMed: 10873480]
22. Baker LC, Davis WC, Autieri J, et al. Flow cytometric assays to detect platelet activation and aggregation in device-implanted calves. *J Biomed Mater Res.* 1998; 41(2):312–21. [PubMed: 9638537]
23. Snyder TA, Watach MJ, Litwak KN, Wagner WR. Platelet activation, aggregation, and life span in calves implanted with axial flow ventricular assist devices. *Ann Thorac Surg.* 2002; 73(6):1933–8. [PubMed: 12078793]
24. Johnson CA Jr, Snyder TA, Woolley JR, Wagner WR. Flow cytometric assays for quantifying activated ovine platelets. *Artif Organs.* 2008; 32(2):136–45. [PubMed: 18005275]
25. Carney EL, Clark JB, Myers JL, Peterson R, Wilson RP, Weiss WJ. Animal model development for the Penn State pediatric ventricular assist device. *Artif Organs.* 2009; 33(11):953–7. [PubMed: 19849686]
26. Litwak KN, Unger LS, Fukamachi K, Saeed D. Retrospective analysis of adverse events in preclinical ventricular assist device experiments. *ASAIO J.* 2008; 54(4):347–50. [PubMed: 18645350]
27. Saeed D, Fukamachi K. In vivo preclinical anticoagulation regimens after implantation of ventricular assist devices. *Artif Organs.* 2009; 33(7):491–503. [PubMed: 19566726]
28. Kilic A, Nolan TD, Li T, et al. Early in vivo experience with the pediatric Jarvik 2000 heart. *ASAIO J.* 2007; 53(3):374–8. [PubMed: 17515732]
29. Dasse KA, Gellman B, Kamenewa MV, et al. Assessment of hydraulic performance and biocompatibility of a MagLev centrifugal pump system designed for pediatric cardiac or cardiopulmonary support. *ASAIO J.* 2007; 53(6):771–7. [PubMed: 18043164]
30. Snyder TA, Tsukui H, Kihara S, et al. Preclinical biocompatibility assessment of the EVAHEART ventricular assist device: coating comparison and platelet activation. *J Biomed Mater Res A.* 2007; 81(1):85–92. [PubMed: 17109415]

Figure 1.
The PediaFlow PF2 ventricular assist device.

Figure 2. CD62P positive platelets quantified by MCA2418 antibody binding to control and 20 μ M ADP and 10 μ M PAF-stimulated platelets (N = 9). MCA2418 binding to ADP and PAF stimulated platelets was significantly higher ($p < 0.001$) than its binding to unstimulated platelets.

Figure 3.

Boxplot comparison of pre-operative platelet activation from blood taken by jugular venipuncture and after insertion of a jugular venous catheter (N = 12 each). Platelet activation following placement of the jugular vein catheter was significantly lower ($p < 0.05$) than platelet activation from jugular venipuncture samples.

Figure 4.

A) Platelet activation during a six hr in vitro circulatory loop with the PF2.2 pump before and following stimulation with 10 μ M PAF.

Figure 5.

A) Platelet activation in five sham surgical studies ($n = 5$), B) platelet activation in sham surgical studies following stimulation of blood with $20 \mu\text{M}$ ADP and $10 \mu\text{M}$ PAF ($N = 3$), and C) platelet count ($\times 10^3/\mu\text{L}$).

Figure 6.

A) Platelet activation in the first PF2 VAD chronic implant, B) platelet activation following stimulation of blood with 20 μM ADP and 10 μM PAF, C) platelet count ($\times 10^3/\mu\text{L}$), and D) image of a kidney and the outflow region of the PediaFlow impeller at necropsy. Note: There is light reflection artifact on the left border of the kidney.

Figure 7.

A) Platelet activation in the second PF2 VAD chronic implant, B) platelet activation following stimulation of blood with 20 μM ADP and 10 μM PAF, C) platelet count ($\times 10^3/\mu\text{L}$), and D) image of the left kidney and the outflow region of the PediaFlow impeller at necropsy. The arrow denotes the presence of a kidney infarct. Note: There is light reflection artifact on the left portion of the kidney.

Figure 8. A) Platelet activation in the PF2 VAD chronic implant, B) platelet activation following stimulation of blood with 20 μM ADP and 10 μM PAF, C) platelet count ($\times 10^3/\mu\text{L}$), and D) image of the left kidney and the outflow region of the PediaFlow impeller at necropsy. Arrows denote the presence of kidney infarcts.

Table 1

Ovine PF2 Implants

Study #	Pump Prototype	Length of Study (Days)	Average Pump Speed (krpm)	Estimated Average Pump Flow (L/min)
1	2.1	17	13.5	1.2
2	2.1	30	15.5	1.5
3	2.2	70	8.5	0.5