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Preservation of Canine Platelets with Iloprost (ZK 36374) during Extracorporeal Circulation

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Abstract

(J. Extra-Corp. Technol. 19 [3] p. 258–264 Fall 1987, 23 ref.) Contact between blood and synthetic/air interfaces during extracorporeal circulation (ECC) results in adverse platelet alterations. We tested the efficacy of a new reversible potent prostacyclin analogue, iloprost (ZK 36374), in preventing these untoward consequences in fifteen mongrel dogs undergoing extracorporeal circulation using either a membrane or bubble oxygenator. In treated groups, infusion of iloprost was incrementally increased to 150 ng/kg/min at least 15 minutes prior to incision. Equal infusion volumes of saline were administered to the control group. Platelet counts in control dogs (n = 6, membrane/control) decreased to 57 ± 10% (mean ± standard error of the mean) of initial levels by 30 minutes of ECC; in contrast, dogs infused with iloprost demonstrated consistently stable platelet counts despite perfusion with either membrane (107 ± 10%, n = 6) or bubble (81 ± 13%, n = 3) oxygenators. At 30 minutes of ECC, the iloprost infusion was discontinued. Although the platelet counts in the membrane iloprost-treated group continued to remain stable (99 ± 7%), platelet counts in the bubble iloprost-treated group dropped precipitously to 49 ± 10% within 60 minutes of discontinuance of infusion. Furthermore, by 90 minutes of ECC, platelets continued to demonstrate reduced sensitivity to ADP as measured by percent light transmission in both control (53 ± 15%) and bubble iloprost-treated (20 ± 16%) groups. In contrast, the membrane iloprost-treated group regained normal reactivity (80 ± 15%). We conclude that iloprost effectively prevents adverse platelet alterations during ECC. Furthermore, its salutary effects in membrane oxygenator systems persist long after platelet functional inhibition is reversed.

Introduction

Extracorporeal circulation (ECC) with bubble and membrane oxygenators results in extensive contact between blood, air and the synthetic surface.1 Resultant decreases in platelet number and function are associated with prolonged post-operative bleeding times and increased blood loss.2,3 Use of prostanoids, such as prostaglandin E₁ (PGE₁) and prostacyclin (PGL₂) in in vitro simulated bypass circuits, has prevented these untoward platelet changes.4 Their clinical use, however, has been precluded largely due to their vasoactive properties and, in the case of prostacyclin, its instability at neutral pH.5,6 Recently, a new stable prostacyclin analog, iloprost (ZK 36374), has proven to be a more potent antplatelet agent than either PGE₁ or PGL₂ and reversible in its action.7,10 We therefore tested the efficacy of iloprost in providing temporary control of platelet reactivity in canines undergoing partial extracorporeal circulation using either membrane or bubble oxygenators.
Materials and Methods

Experimental Design

All animal studies were approved by the University of Pennsylvania Animal Use Committee and conform with the "Guiding Principles in the Care and Use of Animals."

Fifteen mongrel dogs weighing from 23.5-30 kg were premedicated with 1.0 mg/kg morphine sulphate and 0.04 mg/kg atropine. Anesthesia was induced with 3.0 mg/kg morphine sulphate, 0.4 mg/kg succinylcholine and 0.2 mg/kg diazepam. All dogs received 20 mg/kg cefazolin. After endotracheal intubation, anesthesia was maintained with 1.75 mg/kg/hr morphine sulphate. Additionally, 0.25 mg/kg morphine sulphate, 0.25 mg/kg succinylcholine and 0.2 mg/kg diazepam were administered as needed. Mean arterial blood pressure measurements and blood samples were obtained through the indwelling catheters in the carotid artery and central venous pressure through the internal jugular vein. All animals were anticoagulated with porcine heparin at 600 U/kg and maintained at an activated clotting time greater than 500 seconds. Vascular access was obtained through the femoral artery and vein and internal jugular vein using 16F, 18F, and 16F USCI catheters, respectively. Animals were placed on partial extracorporeal circulation using either a hollow-fiber membrane or a hard-shell infant-sized bubble oxygenator in a circuit assembled with a centrifugal vortex pump and standard medical grade polyvinylchloride tubing. Extracorporeal circuits were flushed with 100% carbon dioxide for 15 minutes prior to priming directly from the animal. Animals received 1500 ± 90 ml Ringer's lactate for 15 minutes prior to incision for vascular access and received an additional 820 ± 92 ml during the bypass period. Extracorporeal circulation at a rate of 1000 ml/min in bubble and 1500 ml/min in membrane oxygenators was maintained for 3 hours in each animal. All oxygenators were ventilated with 100% oxygen at a rate of 1.0 L/min. Extracorporeal circulation was carried out at normothermic temperatures for all groups.

Infusion of iloprost was begun after induction of anesthesia at a rate of 75 ng/kg/min and increased to 150 ng/kg/min for at least 15 minutes prior to incision for vascular access. Fifteen minutes after initiation of extracorporeal circulation, the drug was decreased to 75 ng/kg/min and finally stopped at 30 minutes of bypass. Equal infusion volumes of normal saline were administered to control animals.

Sample Acquisition

Whole blood samples for hematocrit, platelet count and aggregation studies were withdrawn from the arterial lines immediately after induction of anesthesia, 15 minutes prior to incision for vascular access and 15 minutes after administration of heparin. In addition, samples were obtained at 5, 15, and 30 minutes and every subsequent half-hour interval during extracorporeal circulation. Recorded at each sample period were the following hemodynamic data: mean arterial pressure, central venous pressure, heart rate and bypass flow rates. Arterial blood gases were measured periodically to maintain normal metabolic status.

Sample Preparation

Whole blood samples for hematocrit and platelet count were drawn into plastic syringes containing one volume of 5.3% sodium citrate for every nine volumes of blood. In addition, 10⁻³ M PGE₁ was added to prevent formation of microaggregates. Whole blood platelet counts were performed by phase microscopy and corrected for hemodilution with the following equation: control hematocrit divided by sample hematocrit multiplied by sample whole blood platelet count.

Whole blood samples for platelet aggregation studies were drawn into additional plastic syringes containing one volume of 5.3% sodium citrate for every 9 volumes of blood. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by previously described method with the following modifications. Prior to centrifugation of PRP, the whole blood was maintained at room temperature for 15 minutes. Calcium chloride to achieve a final concentration of 6 meq/L was added back to each PRP aggregation sample prior to the addition of the agonist. Following a one minute preincubation at 37°C, the threshold concentration of adenosine diphosphate (ADP) was determined for the post-induction of anesthesia sample (control) and tested in each subsequent sample. ADP thresholds were determined as previously described by Carvalho and modified by Clemmons. Briefly, the threshold concentration of ADP added to PRP was defined as the lowest logarithmic concentration of aggregating agent necessary to achieve at least 65% light transmittance within five minutes. Threshold concentrations ranged from 5 to 20 uM ADP.

Statistical Analysis

Mean, standard deviation and standard error of the mean were calculated for all groups. Statistical differ-

References:

a. C.R. Bard, Inc., Billerica, MA 01821
b. Maxima, J & J Cardiovascular, King of Prussia, PA 19406
c. S 070, Shiley, Inc., Irvine, CA 92714
d. BF 50, Bio-Medicus, Inc., Minneapolis, MN 55344
e. American Bentley Laboratories, Inc., Irvine, CA 92714
f. Berlex Laboratories, Inc., Cedar Knolls, NJ 07927
ences within each group were compared utilizing a one-way analysis of variance. Comparison of the control group with the experimental groups were compared using a student's t test. A p value <.05 was considered to be statistically significant.

Results

General Parameters
All groups prior to initiation of the study were identical with respect to weight, hematocrit, platelet count and mean arterial pressure (Table 1). Heart rates and central venous pressures were well within normal limits for canines undergoing extracorporeal circulation.

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<th>Table 1</th>
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<td><strong>Initial Study Characteristics</strong></td>
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<td>Mean Arterial Weight</td>
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*Mean ± standard error of the mean

Mean arterial blood pressures in both membrane-treated and bubble-treated iloprost groups were significantly lower than in the control membrane group at all points recorded during the infusion. Following discontinuation of iloprost, the drug-treated groups showed a gradual return of mean arterial pressures with no significant difference noted with the control group (Figure 1).

Hematocrits for all groups showed no significant differences at any point except for the sampling period at 15 minutes after heparinization (Figure 2).

Platelet Counts

In the control membrane group (n=6) mean platelet counts, expressed as a percentage of the initial platelet count, decreased to 76 ± 11% (mean ± standard error of the mean) within 5 minutes of extracorporeal circulation and continued to decline to 57 ± 10% by 30 minutes of bypass. By 3 hours of extracorporeal circulation, the platelet counts in the control group gradually rose to 87 ± 7% (Figure 3). For the iloprost-treated bubble group (n=3), mean platelet counts declined slightly to 88 ± 9% and 81 ± 13% at 5 and 30 minutes of bypass, respectively. However, once the infusion was terminated in the bubble group, platelet counts fell within 60 minutes to 49 ± 10% (Figure 3). No recovery of platelet count was noted throughout the remainder of the 3 hours of extracorporeal circulation. In contrast, in the iloprost-treated
membrane group (n = 6), mean platelet counts remained completely preserved throughout the period of infusion as well as during the entire 3 hour bypass (100 ± 5%) period despite the termination of iloprost at 30 minutes of extracorporeal circulation (Figure 3).

Platelet Function

Reactivity of platelets to ADP in the control membrane group prior to heparinization was 74 ± 17% of the initial aggregation at threshold dose achieved in the post-induction of anesthesia sample (Figure 4). Platelet responsiveness gradually declined from 68 ± 10% following initiation of extracorporeal circulation to 54 ± 9% after three hours of bypass. In both drug-treated oxygenator groups platelets became insensitive to ADP during the inception of iloprost infusion, initially showing 27 ± 15% in the iloprost-treated membrane group and 8 ± 4% in the iloprost-treated bubble group (Figure 4). Transient increase of platelet reactivity to 51 ± 22% in membrane and 57 ± 29% in bubble iloprost-treated groups was noted following administration of heparin (Figure 4). By 5 minutes of extracorporeal circulation, platelet reactivity again declined to 11 ± 8% and 7 ± 4% of initial values in membrane and bubble iloprost-treated groups, respectively (Figure 4). With discontinuance of the iloprost, platelets from the bubble-treated group continued to remain unresponsive to ADP (Figure 4). In contrast, despite termination of the iloprost, platelet reactivity to ADP from the membrane iloprost-treated group returned to 62 ± 18% of initial aggregation levels within 30 minutes of iloprost, and continued to show improved platelet responsiveness by 3 hours of bypass (71 ± 15%) (Figure 4).

Discussion

Several investigators have demonstrated that extensive contact between blood and the extracorporeal circuit results in both qualitative and quantitative alterations to platelets. This occurs not only during routine cardiopulmonary bypass but also with the institution of long-term support systems such as extracorporeal membrane oxygenation and ventricular assist devices. These platelet defects result in both prolongation of bleeding times and increased postoperative blood loss.

This observed decrease in platelet number and function is thought to result from fibrinogen deposition on the artificial surface. This fibrinogen layer provides a milieu for platelet adhesion followed by aggregation and release of platelet granule contents. Activated platelets can combine with circulating proteins to activate blood coagulation.

Attempts to find a truly biocompatible surface have been unsuccessful. Consequently, attention has been focused on altering or concealing the thrombogenic properties of the artificial surface. Adsorption of albumin or the binding of heparin to the surface has been successful in certain settings but clearly not in the clinical panacea. Alternatively, investigators have concentrated on providing pharmacologic inhibition of formed blood elements in an attempt to limit the blood-artificial surface interaction.

Extensive research has been conducted in the field of prostaglandins and their ability to effectively preserve platelet number and functional integrity. Recent studies utilizing PGE and PGI, although successful
in simulated in vitro bypass circuits,\textsuperscript{5,6} have been unsuccessful in clinical trials.\textsuperscript{7,8} Both of these endoperoxide metabolites exhibit unacceptable vasoactivity; additionally, PG\textsubscript{I\textsubscript{2}} is chemically unstable. Recently a new potent prostacyclin analogue, iloprost, has been synthesized which maintains the desired antiplatelet properties of prostacyclin but is less vasoactive and chemically stable at neutral pH. In vitro testing of this analogue against the platelet agonist ADP has shown iloprost to be 100 times more potent than PGE\textsubscript{1} and 10 times more potent than PG\textsubscript{I\textsubscript{2}}.\textsuperscript{9} Iloprost is also more effective than either PGE\textsubscript{1} or PG\textsubscript{I\textsubscript{2}} at raising intracellular levels of cAMP,\textsuperscript{10} the proposed mechanism for prostanoid-induced inhibition of platelets.

Our present study indicates that iloprost is highly effective as an inhibitor of platelet reactivity to ADP in both membrane and bubble oxygenator systems. Despite the introduction of a direct gas interface from the bubble oxygenator, platelet number was preserved with platelet functional inhibition noted during the drug infusion. However, following termination of iloprost in this group, irreversible changes in both platelet number and function were evident. In contrast, the iloprost-treated membrane group, with a closed-system circulation, regained normal platelet reactivity following termination of iloprost, together with continued preservation of platelet count, despite a 3-hour bypass period.

The need for iloprost only for the initial blood-surface contact in a closed system presumably results from stereo-chemical changes of fibrinogen previously adherent to the synthetic surface, creating a diminished affinity for platelets.\textsuperscript{20,21} However, the direct gas-to-blood interface occurring in the bubble oxygenator causes a mechanical disruption of blood components and denaturation of plasma proteins, which results in progressive damage and a need for continual protection of formed blood elements.\textsuperscript{3,22,23}

It is worthwhile noting that while a transient sensitivity of platelet reactivity to ADP was observed in both iloprost-treated groups following the initial administration of heparin, no loss of platelet number occurred. Maximal inhibition of platelet reactivity did return prior to initiation of bypass. Interestingly, iloprost has been used successfully in patients with heparin-induced thrombocytopenia requiring open-cardiac surgery.\textsuperscript{23}

In summary, iloprost effectively prevents surface-mediated platelet alterations during extracorporeal circulation utilizing either a membrane or bubble oxygenator system. If, as in the case of a bubble oxygenator system, a direct gas interface is introduced, continued platelet protection may be required until the gas-to-blood interface can be eliminated. Furthermore, although iloprost is reversible in its inhibition of platelets, its salutary antithrombotic effects outlast its presence in plasma. These findings have important therapeutic implication for critically ill patients requiring prolonged blood contact with an artificial surface, including those present in long-term membrane oxygenators and the total artificial heart.

References

Questions from the Audience

Question: Aaron Hill, Falls Church, VA: I was wondering about thromboxine B2 levels, and if those were measured, I'm especially interested in the bubble group. Did you measure thromboxine B2?
Response: No, we did not measure it, although we did take samples and save them for future testing.
Question: I would think there would be a significant difference in light of some of your results already. Another question I have is: Since this is a very vasoactive substance, were you able to maintain pressures well? And did you have to use any extraordinary means or pharmacologic manipulations in order to maintain pressures?
Response: We did not use any pressors in any of the drug-treated animals, although the dosage in a canine model is, specifically in this case, set at an extremely high level. We were able to tolerate the hypotension with minimal fluid infusion. Clinically, this drug is being tested in multi-center trials and it is fairly well controlled with either fluid administration or pressors: Much more so than with either prostaglandin E1 or prostacyclin.
Comment: Right, with using alpha receptor manipulators in the clinical trials, I assume.
Response: I'm not sure.
Question: Steve Murphy, Southern California: Tell us more about the drug itself and its potential use in humans.
Response: Our particular hope is to find the means to provide some protection to platelets during prolonged exposure of blood to the artificial surface. In the past there have been many studies trying to find the truly biocompatible surface, either with the material or with manipulation through coating with albumin or perhaps heparin binding. All of these, although they had limited success in the clinical panacea have a long way to go. So our choice is to look for pharmacologic intervention in the hope that by inhibiting the reactivity of the platelets prior to exposure to an artificial surface, the time necessary with the protein layer to occur on an artificial surface and thereby decrease the affinity of platelets to that surface. If we can protect the platelets until that time and provide a pharmacological vehicle that is reversible to allow the reactivity of the platelets to return and maintain the platelet number as well, that's our goal.
Question: Richard Berryessa, Denver, CO: Nice study, nicely presented. Do you think this provides additional evidence for our being able to drive another nail into the coffin of that primitive device the "bubble generator"?
Response: The intent of the study was not to compare membrane and bubble oxygenators. It was to investigate the efficacy of the drug and its ability to preserve and protect platelets at various points with blood contact to an artificial surface. I will tell you that our methods were somewhat unorthodox in the way we primed our dogs. Basically, it entails exsanguinating them into a dry circuit and it was to maximize the initial exposure of blood to the artificial surface. In the case of a bubble oxygenator, and particularly with that priming technique, there was probably no opportunity whatsoever for the normal protein layer to occur. And through three hours of bypass, any protein laydown was denatured and accounts for the rather dramatic effect of the bubble oxygenator in this study. Clinically the protocol currently used requires that the drug be run through the duration of bypass with both membrane and bubble oxygenators. Our system was specifically set up to mimic an ECMO or LVAD type system. But as most of us know, those are rarely instituted without the preliminary adventure of cardiopulmonary bypass in which you have not only air to blood interface with the suction system, but you probably have
arterial filters. You have a number of factors that were not in our system. In the normal clinical protocol at this time, the drug is run until protamine is given. And it has, to date, been quite promising.

**Question:** Bill Pelley, Portage, MI: Very nice presentation. Have you all done any work at all on long-term use—greater than 12 to 14 hours on bypass? And have you noticed your ability to decrease the amount of heparin that is being required?

**Response:** From the multi-study trials I’m not aware as to whether or not there’s a decrease in the need for heparin. As you saw on the slide, there was a transient response to heparin when it was given as a bolus at heparinization in the animals. But the reactivity diminished and we did preserve complete inhibition prior to the exposure of the blood to the artificial surface. We have not in our institution used the drug in the long term. You perhaps might wish to elaborate on that.

**Comment:** We had occasion two months ago to use Iloprost on a patient that was antithrombin 3 deficient. He was two days postoperative of an aortic valve replacement when he went into failure. And we pumped him for 22 hours on a ECMO circuit with Iloprost. We noticed an ability to decrease our heparin to extremely low levels and we were able to maintain our ACTs at about 150 to 180 seconds with a hollow fiber membrane. It seems very effective. We were also flying by the seat of our pants. Thank you, very nice presentation.