Safety Evaluation of Lyophilized Canine Platelets in a Model of CABG
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Background
Cellphire has completed a Phase 1 micro-dose clinical safety trial in normal healthy subjects using lyophilized human platelets. In anticipation of future clinical studies Cellphire evaluated the safety of Lyophilized Canine Platelets (LCP) in comparison to Liquid Stored Canine Platelets, following intravenous administration in a model of on-pump coronary artery bypass graft (CABG) in the canine. This safety study was in support of a future Phase II human clinical trial in cardiac patients.

Surgical Procedures
A splenectomy was performed to reduce the influence of reserve endogenous platelets on experimental outcome, and to avoid immediate sequestration of infused materials. The left external jugular vein was exposed, and a triple lumen catheter was inserted. This was used to monitor central venous pressure and for drug administration. A left lateral thoracotomy was performed, and the pericardium was opened. The ascending aorta was dissected and looped with umbilical tape or a 12F catheter tourniquet for traction purposes. Heparin (250 IU/kg) was administered to increase the Activated Coagulation Time (ACT) to a target range of 400 to 660 seconds. Cardiopulmonary Bypass was then initiated using a system consisting of a pediatric-sized integrated membrane oxygenator, venous reservoir, arterial filter, and tubing treated with phosphocholine surface treatment, thus representing a typical clinical perfusion system. ECG and heart rate, blood pressure (SAP, DAP, MAP, and CVP), oxygen saturation, end-tidal carbon dioxide (ETCO2), pO2, pCO2, base excess (BE), HCO3–, pH, spun hematocrit (HCT), Na+, K+, Ca++, ACT, rectal and esophageal body temperatures, and urine output were all measured. The coronary artery bypass grafting was then performed using the femoral vein. After the anastomosis was complete the cardiopulmonary bypass was terminated and protamine reversal initiated. Stored whole donor blood was added through the cardiopulmonary circuit to increase the hematocrit to above 20%, if necessary. Once stable, the chest cavity was packed with gauze and the test article administered. Additional blood loss was determined at hourly intervals up to 4hrs post-dosing.

Experimental Design
Splenectomy
Heparinized
Triple Lumen Catheter Placed
Cardiopulmonary Bypass Initiated
Coronary Artery Bypass Termination
Protamine Reversal
Hourly Blood Loss Measured (up to 4hrs)
Dosing of LCP

Anastomosis Grafting

Table 1. Group Assignments

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose Particles/Kg</th>
<th>Number of Animals</th>
<th>Recovery Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>10 mL</td>
<td>4</td>
<td>4 hours*</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>Liquid Stored Platelets STD Dose*</td>
<td>4</td>
<td>4 hours*</td>
</tr>
<tr>
<td>3</td>
<td>LCP – 33.3% TCP</td>
<td>5.11 x 10^5</td>
<td>4</td>
<td>4 hours*</td>
</tr>
<tr>
<td>4</td>
<td>LCP – 10% TCP</td>
<td>1.57 x 10^6</td>
<td>4</td>
<td>4 hours*</td>
</tr>
<tr>
<td>5</td>
<td>LCP – 3.3% TCP</td>
<td>5.24 x 10^6</td>
<td>4</td>
<td>4 hours*</td>
</tr>
</tbody>
</table>

LCP – Lyophilized Canine Platelets
TCP – Total Circulating Platelets
* Post the start of dosing
*Dose Equivalent to 10% TCP

Figure 2. Blood flow through the anastomosis site was monitored for 4 hours post-infusion. No significant differences were observed between groups.

Figure 3. After coming off of pump, the chest cavity was packed with gauze and the test article was administered. The gauze packing was exchanged each hour up to 4 hours post-infusion. The total blood loss was measured and calculated per kg

Lyophilized Canine Platelets Help Mitigate Blood Loss
(Total Blood Loss (gm/kg) 4 Hour Post Infusion)

No Change in Blood Flow Through the Anastomosis Site

Figure 4. Representative histological images of the anastomosis site. No evidence of thrombosis was observed in the lumen of the anastomosis regardless of test article or dosing.

Conclusion
• Administration of LCP up to 5.11 x 10^9 particles/kg was safe in a canine CABG model
• Dosing of 1.57 x 10^9 particles/kg and 5.11 x 10^9 particles/kg reduced overall blood loss and was comparable to 2 day old liquid stored platelets
• No thrombus formation was noted following administration of LCP or liquid stored platelets
• The starting effective dose may vary depending on the clinical indication, further studies are required

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