Safety profile of repeated infusion of platelet-like nanoparticles in healthy dogs

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OBJECTIVE
To assess the safety and efficacy of the platelet-like nanoparticle (PLN), and to assess its safety in repeated administration.

ANIMALS
6 purpose-bred dogs.

PROCEDURES
The PLN was administered IV at 3 different doses using a randomized crossover design. Each dog received a full dose of 8 X 10^10 particles/10 kg, half dose, and 10 times the dose, with a 14-day washout period between doses. Biochemical, prothrombin time, partial thromboplastin time, and fibrinogen analyses were performed at baseline and 96 hours postinfusion. A CBC, kaolin-activated thromboelastography, platelet function assay closure time, and buccal mucosal bleeding time were performed at baseline and 1, 6, 24, 48, 72, and 96 hours postinfusion.

RESULTS
No significant changes were observed over time in the thromboelastography parameters, closure time, and buccal mucosal bleeding time. After the administration of the half dose, hematocrit levels decreased significantly at 1, 6, 24, 48, and 96 hours, with all values within the reference range. The platelet count was decreased significantly at hours 1, 6, 24, 48, and 72 after administration of the half dose, with values less than the reference range at all hours but hour 72. No significant changes in serum biochemistry, coagulation panel, and fibrinogen were observed for all doses. No adverse events were noted during the first infusion. Three dogs experienced transient sedation and nausea after repeat infusion.

CLINICAL RELEVANCE
The PLN resulted in a dilution of hematocrit and platelets, and did not significantly alter hemostasis negatively. The safety of repeated doses should be investigated further in dogs.
and development of storage lesions.\textsuperscript{9,10} Moreover, although platelet transfusions in people account for about 10% of blood product transfusions, they account for 25% to 35% of all transfusion-associated reactions.\textsuperscript{10,11} Such information is unavailable in veterinary medicine. Despite efforts to enhance safety, storage, portability, efficacy, and availability of natural platelet products, a suitable natural product has not been identified in veterinary medicine.\textsuperscript{12–14}

In light of these challenges, significant research has been focused on developing an alternative to fresh stored platelets, including nanoparticles designed to mimic the physical characteristics and biological functionality of natural platelets.\textsuperscript{15–16} SynthoPlate (Haima Therapeutics) is one such platelet-like nanoparticle (PLN) that has shown promising results in various animal models.\textsuperscript{17–20} It is a biocompatible and biodegradable PLN engineered on a unilamellar liposomal platform approximately 170 nm in diameter, surface-decorated with a combination of von Willebrand factor (vWF)-binding peptides (VBPs), collagen-binding peptide (CBP), and active platelet glycoprotein (GP) Iib–IIa-binding fibrinogen mimetic peptide (FMP).\textsuperscript{16,20,21} These surface motifs mimic the adhesion and aggregation properties of natural platelets, without activation of the host’s natural platelets.\textsuperscript{16,17}

Using the tested PLN, a clinically significant reduction in bleeding times has been observed in thrombocytopenic mice, as well as in a porcine model of arterial hemorrhage.\textsuperscript{17–20} Although other liposomal therapeutics have been investigated in dogs, particularly in the context of chemotherapeutic agents, no studies of synthetic platelets using a liposomal platform have been conducted in canine models.\textsuperscript{22–24}

The primary objective of our study was to assess safety in repeated IV administration of an initial version of the PLN in dogs. Secondary objectives included characterizing the impact of PLNs on organ function and hemostatic testing. We hypothesized that PLNs would be safe in dogs at 3 administered doses. We also hypothesized that no difference would be observed in organ function or hemostatic testing in healthy dogs.

**Materials and methods**

**Animals**

Six purpose-bred intact male hound dogs between 1 and 2 years of age with a mean weight of 27.76 kg (SD, ± 1.9 kg) were purchased (Marshall BioResources). Dogs were acclimated for a minimum of 1 week prior to baseline health assessments. Daily enrichment was provided, and group housing was performed, if possible, according to The Ohio State University Laboratory Animal Resources protocols. Free access to water was allowed, and a commercial dry kibble was fed twice daily. Food was withheld for 12 hours prior to PLN infusion. Dogs were deemed healthy prior to the study based on physical examination, CBC, biochemical profile, prothrombin time (PT), partial thromboplastin time, fibrinogen concentration, platelet function assay (PFA), kaolin-activated thromboelastography (TEG), and buccal mucosal bleeding time (BMBT). All animal experiments were approved and conducted in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee (IACUC) of The Ohio State University (IACUC 2017A00000007).

**PLN Manufacture**

Manufacture of the tested PLN (SynthoPlate, Haima Therapeutics), a heteromultivalent ligand decorated poly-(ethylene glycol) liposomal construct, was performed as previously described.\textsuperscript{19,20} 1,2-Distearoyl-sn-glycero-3-phosphocholine, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(methoxy[polylethylene glycol]-1000) (DSPE-mPEG1000), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(maleimide[polylethylene glycol]-2000) (DSPE-PEG2000-Mal), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-(azido[polylethylene glycol]-2000) (DSPE-PEG2000-azide) were used (Avanti Polar Lipids), as well as rhodamine B-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (DHPERhB; Invitrogen). The peptides VBP (sequence CTRYLRIHPQSWVHQI), CBP (sequence CGP17), and FMP (sequence cyclo(Pra)CNPRGD(Tyr[OEt])RC) were custom-synthesized (GenScript). Cholesterol was purchased (Sigma-Aldrich). vWF-binding peptide and CBP were conjugated to DSPEPEG2000-Mal using thiol–maleimide coupling, and FMP was conjugated to DSPE-PEG2000-azole via copper-catalyzed alkyne–azole cycloaddition.\textsuperscript{25,26} The conjugates were purified by dialysis and characterized by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry. A standard film rehydration and extrusion process was used for the manufacture of the tested PLN.\textsuperscript{21,27} Dynamic light scattering and electron microscopy characterization confirmed an approximate 200-nm diameter.

**Experimental model**

The tested PLN was administered at 3 different doses using a randomized crossover design, with each dog serving as its own control. Dogs were first randomized into 2 groups of 3 (to accommodate all experiments to be performed by 2 people in a timely manner) using an electronic randomization software (Excel, Microsoft Corp). Each dog was then assigned randomly to a series of 3 treatment doses—full dose, half dose, and 10 times the dose—using the same randomization software. Dose was defined as 8 X 10\textsuperscript{10} PLNs per 10 kg of body weight, based on recommended platelet dosing for dogs.\textsuperscript{28} Dogs were given at least 14 days between treatment doses as a washout period, corresponding to greater than 5 half-lives of the PLN in mice—time that would allow for more than 97% of the PLNs to be excreted, providing that pharmacokinetics in dogs are the same as in mice.\textsuperscript{29} Each dog received each dose once, with 1 dog requiring a repeat dosing because of an adverse event. A total of 19 experiments were conducted over a 7-week period. Authors administering the infusion were blinded to the dose given at each experiment.
Dogs received a full physical examination, including an accurate body weight and blood pressure measurement, prior to each experiment (Cardell 9402, Midmark). The PLN solutions (1 mL/kg) were diluted to a standard volume of 100 mL in saline (0.9% sodium chloride) solution. A 20-gauge cephalic catheter was placed for each experiment, and doses were infused over a 30-minute period using an infusion pump. Dogs were monitored during the entirety of the infusion by at least one of the investigators. The following data were recorded prior to administration of the solution, at 15 minutes, and at the end of infusion (30 minutes): temperature, heart rate, respiratory rate, and blood pressure.

Blood samples (10 mL) were collected atraumatically by direct jugular venipuncture using a 10-mL syringe and an 18-gauge needle. Alternatively, a 19-gauge butterfly catheter and 10-mL syringe were used to obtain samples from a lateral saphenous or dorsal pedal vessel when jugular venipuncture could not be performed with minimal restraint (n = 10). Blood samples were collected at baseline and 1, 6, 24, 48, 72, and 96 hours after each dose infusion. Venipuncture of the same vessel was not repeated within 24 hours. Samples were collected in the following order: 1 serum tube, 3 1.8-mL 3.2% sodium citrate tubes, and 1 2-mL EDTA tube. All tubes were gently inverted 5 to 7 times. Biochemical analysis was performed on serum samples at baseline and 96 hours (Hitachi 501, Roche). A CBC was performed at all time points using EDTA blood on an automated analyzer, with differential counts confirmed by manual blood smear review (ADVIA 2120i, Siemens Healthcare Diagnostics). One sodium citrate tube was used for PT, activated partial thromboplastin time, and fibrinogen analysis, conducted on an automated hemostasis analyzer at baseline and 96 hours (STA Compact Max, Diagnostica Stago). The remaining 2 sodium citrate samples were used for TEG (TEG model 5000, Haemonetics Corp) and PFA analysis at all time points (PFA-100, Siemens Healthcare Diagnostics).

Kaolin-activated TEG was performed using recalcified citrated whole blood according to current guidelines. Samples were rested at room temperature (20 °C) at least 30 minutes after collection. Mean time to sample analysis was 47 ± 15 minutes. Briefly, 1 mL of citrated whole blood was added to a kaolin vial and inverted gently 5 times. Calcium chloride (20 μL) was added to a standard prewarmed cup (37°C), followed by the kaolin-activated citrated whole blood (340 μL). TEG analysis was run for as long as 60 minutes. The following values were determined by proprietary software and recorded: reaction time, clot kinetic time (defined as the time to reach a clot strength of 20 mm), α angle, maximum amplitude, and global clot strength (TEG Manager software, Haemonetics Corp).

Platelet function was assessed using a commercially available point-of-care PFA that has been evaluated in dogs. Cartridges coated with the agonists collagen and adenosine diphosphate were used for all measurements. Closure time (CT) was measured in duplicate from the same sodium citrate tube. In the event of a flow obstruction, a third measurement was obtained. Mean CT for each sample was used for statistical analysis. The coefficient of variance (CV) was calculated as an SD divided by the mean multiplied by 100, and samples with a CV greater than 20% were excluded from analysis, as recommended. Cartridges were stored at 4°C and warmed to room temperature at least 15 minutes prior to running the test. All samples were run within 90 minutes after collection.

BMBTs were acquired using the same technique by 1 of 2 operators (PWS, supervised by JG, or PEY) on the buccal mucosa at each time point. A standard commercial device was used and the presence of hemostasis was agreed upon by the operators (Surgicutt Vet, Jorgensen Labs). Gentle restraint was used to maintain dogs in a stable position during testing.

Statistical analysis

Data were tested for normality using a D’Agostino-Pearson test, and results are presented as mean ± SD because the majority of the data were normally distributed. The primary outcome was change of recorded variables compared to baseline. Given the large number of outcomes, a gatekeeping approach was used to control the false discover rate. A 2-stage approach was applied to each group of outcomes (CBC, chemistry, TEG, PFA, BMBT, coagulation, and vitals). In the first stage, we tested for changes over time under each dose and controlled false discover rates across tests using the Benjamini-Hochberg method. If the first-stage analysis revealed temporal changes in an outcome at a particular dosage (P < .05), we compared each time point to the baseline and applied the Benjamini-Hochberg method to these paired comparisons. If data were missing, a linear mixed model with Kenward-Roger degrees of freedom was used to analyze the data; otherwise, repeated-measures ANOVA with Greenhouse-Geisser degrees of freedom was used. All analyses were performed using statistical software packages (MedCalc Statistical Software version 15.4, MedCalc Software bvba; SAS version 9.4, SAS Inc).

Results

Cardiorespiratory parameters

Mean ± SD baseline heart rate, respiratory rate, rectal temperature, and mean blood pressure values were 97 ± 12 beats per minute, 25 ± 2 breaths per minute, 101.4 ± 0.4 °F, and 116 ± 34 mm Hg, respectively. Repeated measures at 15 and 30 minutes from the start of infusion were not significantly different for each dose (P = .678 to .817).

TEG

Mean TEG parameters for baseline were within institutional reference intervals (Table 1). No significant changes were observed over time (P = .628 to .655). All mean TEG parameters were within
institutional reference ranges for each dose and each time (Table 1).

### PFA

Baseline CT for all dogs was within the institutional reference interval (Table 1). The mean CT value for the half dose at 1 hour, full dose at 2 hours and 10 times the dose at 1 to 2 hours were more than the institutional reference interval. However, mean CT at 1, 6, 24, 48, 72, and 96 hours postinfusion was not significantly different for any of the administered doses (P = .216). CTs at each dose and time point are summarized in Table 1. Flow obstruction occurred in 7 samples (3% of all samples run), and samples were rerun. The mean CV for all samples was 9.9 ± 6.8%, and 7 samples were excluded from analysis because of a CV greater than 20%, consistent with data in people. 34 None of the rerun samples resulting from flow obstruction had a CV greater than 20%.

### BMBT

Baseline BMBT for all dogs was within the published reference interval (Table 1). 38 When compared to baseline, no significant difference was found over time for all doses (P = .568). All mean BMBTs were greater than 20%.

### CBC

At baseline, all values were within the institutional reference interval except for the platelet count for dog A, which was 135 X 10^9/L (Table 1). After administration of the half dose, Hct decreased significantly at hours 1, 6, 24, 48, and 96 (Table 1). All values remained within the institutional reference
interval. When compared to baseline, plasma protein level decreased significantly at multiple doses and time points (Table 1). However, mean plasma protein level values were all within the reference interval at each dose and time point (Table 1).

Platelet count was decreased significantly at hours 1, 6, 24, 48, and 72 after administration of the half dose, with values less than the reference interval at hours 1, 6, 24, and 48 (Table 1). Platelet clumps were identified on microscopic review of 9 samples, 5 of which had absolute counts that were less than the reference interval (mean, 110 ± 15 X 10⁹/L for those 5 samples).

Biochemistry and coagulation variables
Serum biochemistry, coagulation panel, and fibrinogen values for each dog were within institutional reference intervals at baseline except for PT, when only 1 dog had a value within institutional reference intervals (Table 2). No significant difference was observed 96 hours postinfusion for all doses (P = .111 to 1.000). All mean chemistry and coagulation values were within institutional reference intervals for each dose, except for PT (Table 2).

Adverse events
No dogs showed evidence of clinically relevant thromboembolic events during the 9-week duration of the study. No dog experienced an adverse event during the first infusion. Three dogs (50%) experienced adverse events during 4 infusions (Table 3). Sedation (n = 3), nausea (n = 3), vomiting (n = 1), and hypotension (n = 1) were noted, occurring 10 to 15 minutes after the start of infusion. Mild events (sedation and nausea), experienced by dogs A and F, resolved within 2 to 3 minutes after completion of the infusion. Dog D experienced an adverse event during the second and fourth infusions (both of which were the half dose). During the second infusion, dog D experienced sedation, nausea, and hypotension.

### Table 2—Biochemistry and coagulation variables at baseline and 96 hours after IV infusion of the platelet-like nanoparticle (SynthoPlate, Haima Therapeutics) in 6 dogs at various doses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference range</th>
<th>Baseline</th>
<th>Dose</th>
<th>96 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemistry profile</td>
<td></td>
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<tr>
<td>BUN (mg/dL)</td>
<td>5–20</td>
<td>14.3 ± 1.8</td>
<td>H</td>
<td>18.3 ± 3.3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>14.0 ± 2.6</td>
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<td></td>
<td></td>
<td></td>
<td>T</td>
<td>16.0 ± 1.8</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.6–1.6</td>
<td>0.9 ± 0.2</td>
<td>H</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Alanine transaminase (IU/L)</td>
<td>10–55</td>
<td>33.3 ± 4.7</td>
<td>H</td>
<td>41.0 ± 10.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>51.7 ± 39.6</td>
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<td></td>
<td></td>
<td></td>
<td>T</td>
<td>40.8 ± 5.9</td>
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<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>15–120</td>
<td>34.5 ± 12.8</td>
<td>H</td>
<td>35.5 ± 13.4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>34.5 ± 12.8</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>33.8 ± 13.6</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>0.1–0.4</td>
<td>0.1 ± 0.0</td>
<td>H</td>
<td>0.1 ± 0.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>0.1 ± 0.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.9–4.2</td>
<td>3.6 ± 0.2</td>
<td>H</td>
<td>3.6 ± 0.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>3.6 ± 0.2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>2.2–2.9</td>
<td>2.4 ± 0.5</td>
<td>H</td>
<td>2.4 ± 0.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>2.5 ± 0.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>77–126</td>
<td>93.8 ± 4.4</td>
<td>H</td>
<td>95.8 ± 5.6</td>
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<td></td>
<td></td>
<td></td>
<td>F</td>
<td>100.7 ± 8.3</td>
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<td></td>
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<td>T</td>
<td>95.8 ± 8.6</td>
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<tr>
<td>Coagulation</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Prothrombin time (s)</td>
<td>6–7.5</td>
<td>7.7 ± 0.3</td>
<td>H</td>
<td>7.7 ± 0.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>7.9 ± 0.5</td>
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<td></td>
<td></td>
<td>T</td>
<td>7.6 ± 0.3</td>
</tr>
<tr>
<td>Activated partial</td>
<td>9–21</td>
<td>10.6 ± 0.4</td>
<td>H</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>thromboplastin time (s)</td>
<td></td>
<td></td>
<td>F</td>
<td>10.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>T</td>
<td>10.7 ± 0.3</td>
</tr>
<tr>
<td>Fibrinogen (g/L)</td>
<td>100–384</td>
<td>149.7 ± 17.7</td>
<td>H</td>
<td>175.0 ± 15.4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>F</td>
<td>176.2 ± 36.1</td>
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<td></td>
<td></td>
<td></td>
<td>T</td>
<td>186.2 ± 42.0</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD and reflect pooled data from 6 dogs at each dose and time point. Baseline data were collected once at the start of the study. F = Full dose; H = Half dose; T = 10 Times the dose. A dose was defined as 8 X 10¹⁰ platelet-like nanoparticles per 10 kg of body weight. CHEM = chemistry profile.
Table 3—Order of dose administration of the platelet-like nanoparticle (SynthoPlate, Haima Therapeutics) for each dog and adverse event noted.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dog</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>F</td>
<td>T*</td>
<td>H</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>F</td>
<td>F^H</td>
<td>T</td>
<td>H^H</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>T</td>
<td>F</td>
<td>H^H</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>F</td>
<td>T</td>
<td>H</td>
<td>N/A</td>
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<tr>
<td></td>
<td>C</td>
<td>T</td>
<td>F</td>
<td>H</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>H</td>
<td>T</td>
<td>F</td>
<td>N/A</td>
</tr>
</tbody>
</table>

F = Full dose; H = Half dose; N/A = not applicable; T = Times the dose.

*Adverse event included a mild reaction. **Adverse event included a severe reaction, resulting in discontinuation of the infusion.

There was a washout period of 14 days between doses. A dose was defined as 8 X 10^13 platelet-like nanoparticles per 10 kg of body weight. Only 1 dog received a fourth dose, as a result of prior adverse events resulting in discontinuation of the infusion. The dogs were divided into 2 groups to facilitate the experiments carefully and ensure appropriate handling and processing of all samples in a timely manner.

The infusion was discontinued and the dog excluded from data collection for that experiment. These signs resolved within 45 minutes after the infusion was stopped. Dog D received diphenhydramine 2 mg/kg IM and a 15-mL/kg crystalloid bolus as treatments during the event. The same dog (dog D) showed no adverse effect during the third infusion (10 times the dose). Dog D completed the study 4 weeks later, with the fourth infusion (the half dose again) resulting in vomiting and mild sedation, resolving 2 to 3 minutes after completion of the infusion.

Discussion

In our study investigating the use of a PLN in dogs, we showed no changes in organ function and hemostatic parameters after infusion. However, 3/6 dogs (50%) experienced some side effects, ranging from nausea to hypotension, during 4/13 repeated infusions.

This is the first time the tested PLN, an early version of SynthoPlate, has been evaluated in dogs. As reviewed comprehensively elsewhere, several synthetic platelet-like designs have been reported that primarily mimic platelets’ aggregatory capability only via surface decoration of polymeric, lipidic, or albumin particles with fibrinogen or fibrinogen-relevant peptides. The tested PLN is a clinically relevant biocompatible liposomal platform that is surface-decorated hetero-multivalently with 3 types of peptides. The peptides VBP and CBP mimic platelets’ bleeding-site-selective adhesion mechanisms to vWF and collagen, whereas the active platelet integrin GPIIb-IIIa-binding FMP augments the activated platelet aggregation mechanism.

The lack of changes in platelet function assessed by TEG tracings, PFA, CT, and BMBT was expected. Because of the nature of the surface decoration, the tested PLN should not activate native patient’s platelets independently. We showed that the presence of the tested PLN did not create a detectable procoagulant state in the studied dogs based on our in vitro testing. It has been documented that PLN adhesion to a control bovine serum albumin surface is minimal under various shear stresses, whereas the PLN will adhere to a vWF-collagen-coated surface under similar shear stresses. Similarly, in a murine model, PLN did not activate and aggregate resting platelets in vitro, but the addition of PLNs to a platelet-poor plasma improved platelet aggregation significantly. Although we demonstrated a lack of activation of the hemostatic system in vitro within the constraints of our study design, there is a concern that PLN infusion would increase the risk of thromboembolic events in vivo. However, none were detected during the 9-week study period, while the dogs received daily monitoring and care.

When injected in a mouse with thrombocytopenia, PLNs were able to achieve a reduction of more than 60% in tail bleed time. Similarly, injection of PLNs 1 minute after liver laceration in a murine model or 1 minute after traumatic arterial hemorrhage in a porcine model showed a reduction in blood loss in both models. Based on these findings, PLNs have been shown to demonstrate a hemostatic impact when a bleeding defect is present (either because of reduced platelet count, hemodilution, or severe injury).

The changes in CBC variables such as Hct, platelet count, and plasma protein may be related to hemodilution resulting from the volume of 100 mL infused rapidly to the patient. It is unlikely that it is a result of an impact of the tested PLN, because the statistically significant difference in Hct and platelets was only present for the half dose of PLNs, with a decrease averaging approximately 4 Hct percentage points and 30 X 10^12/L, respectively. Although the full dose and 10-times dose PLN saw a decrease in hematocrit and platelet levels, it was not statistically different. Furthermore, the changes to the CBC variables are unlikely to be clinically significant. Even if the platelet count is decreased to less than the reference intervals, the lowest platelet count was still more than 95 X 10^12/L, which should be enough to maintain primary hemostasis.

The coagulation variables were remarkably unchanged after infusion of PLNs at each dose and each time point, which was expected because of the lack of the known impact of PLNs on secondary hemostasis. However, the baseline values for PT being mildly out of the institutional reference interval was unexpected. Our hospital routinely ran a control dog alongside the patient’s coagulation variables on clinical patients, for the sake of comparison, and seldomly used reference intervals. That control part was not done during our experiment, and therefore institutional reference intervals were used. It is possible that our institutional reference interval needs to be updated.

To our knowledge, this is the first study to assess repeat PLN administration in dogs, some of which experienced reactions such as vomiting, sedation, and hypotension. Reactions have been described...
with other lipid-based nanoparticles and are usually grouped under the term complement activation-related pseudoldergy (CARPA). CARPA is a nonimmunoglobulin E-mediated hypersensitivity reaction similar to Cremophor or Polysorbate 80 reactions, which have been described in dogs after the injection of IV tacrolimus, cyclosporine, vitamin K, and amiodarone. The frequency of liposome-induced CARPA can be as much as 45% in people and pigs, and is characterized by cardiovascular (eg, tachycardia, bradycardia, arrhythmia, hypotension, hypertension, chest pain, back pain), respiratory (eg, tachypnea, urticaria, erythema, pruritus) reactions depending on species, liposome composition, and dose. Cutaneous signs are less common in dogs compared to people or pigs. Dogs may also exhibit leukopenia, leukocytosis, and thrombocytopenia (salivation, urination, and defecation). However, CARPA usually occurs during the first injection, and its frequency and severity decrease during subsequent injections. In our experiment, none of the dogs experienced a reaction during the first infusion. One dog who received the injection 4 times had no reaction from the first and third injections. Indeed, that dog exhibited a more severe reaction to the second infusion, but did not show any adverse effects during the third infusion. The fourth infusion did result in a mild reaction. It is therefore unclear whether the reaction seen is consistent with CARPA or another unknown factor, such as an idiosyncratic reaction to the lipid base used in the PLN.

There are several limitations to our study. Because of the use of healthy dogs, the efficacy of the PLNs to halt bleeding secondary to thrombocytopenia or thrombocytopenia cannot be established. Also, because of our crossover design, and although our statistical analysis was designed to reduce false significance, the clinical relevance of the changes in the CBC is unclear, because they did not follow a specific pattern. Last, the dose, injection volume, and injection rate of the product represent a scenario that may have to be optimized in future studies. In our study, we elected considered 1 PLN equivalent to 1 platelet, and the dose of 8 × 10^{10} PLNs per 10 kg to be a full dose, with 8 × 10^{11} PLNs per 10 kg considered to be 10 times the appropriate dose. However, the appropriate dose of platelets in people and animals is controversial. In people, various doses have been described and tested, from 1.5 to 2.9 × 10^{11} per patient up to 4.4 × 10^{11} platelets/m^{2}. Assuming a “typical” person weighs 70 kg (or 2 m^{2}), this represents 3 to 10 × 10^{10} platelets per 10 kg, and the dose of PLNs used in our study thus represents the mid-high end of the range. However, because an actual platelet is approximately 10 times larger compared to the PLN, the dose of PLNs used in a porcine model of traumatic arterial hemorrhage that showed decreased blood loss and improved survival was 1.7 × 10^{11}. As such, different volumes of infusion, dilutions of the PLN, and speeds of infusion may have a different impact on hemostatic variables, side effects, or both.

In conclusion, we demonstrated that the tested PLN is safe as a single infusion, and does not lead to platelet activation in healthy dogs. Given the species-agnostic nature of fully synthetic PLNs, and the potential translation to people, the clinical efficacy and understanding of side effects during repeated infusion warrants continued investigations and refinement of the PLN.

Acknowledgment

This project was funded internally through the Ohio State University Canine Fund. Dr. Sen Gupta is supported by an NIH research award (HL121212) for the development of the tested PLN.

Dr. Buckman is the chief executive officer for Haima Therapeutics, which manufactures SynthoPlate. Dr. Sen Gupta is the inventor of SynthoPlate, and co-founder and chief scientific advisor for Haima Therapeutics. The other authors declare no conflicts of interest relevant to the manuscript.

The authors thank Dr. Michael Pennel from the Ohio State University Department of Biostatistics for his statistical expertise.

Data were presented in abstract format at the European Veterinary Emergency and Critical Care Society meeting, June 2020, Ghent, Belgium.

The authors declare no off-label use of antimicrobials.

References


