In vitro effects of lipid emulsion on platelet function and thromboelastography in canine blood samples

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Objective—To determine whether soybean oil emulsion has an in vitro effect on platelet aggregation and thromboelastography in blood samples obtained from healthy dogs.

Animals—12 healthy adult dogs.

Procedures—Blood samples were collected from each dog into tubes containing EDTA, hirudin, or sodium citrate for a CBC, collagen- and ADP-induced impedance aggregometry, or thromboelastography, respectively. Whole blood platelet aggregation, determined with ADP or collagen agonists, was measured in blood samples containing hirudin and final lipid concentrations of 0, 1, 10, and 30 mg/mL. The thromboelastographic variables R (reaction time), K (clotting time), α angle, and maximum amplitude were evaluated in blood samples containing sodium citrate and final lipid concentrations equivalent to those used for assessment of platelet aggregation.

Results—Median maximum ADP- and collagen-induced platelet aggregation in blood samples containing 1, 10, or 30 mg of lipid/mL did not differ significantly from the value for the respective lipid-free blood sample. Maximum amplitude determined via thromboelastography was significantly reduced in blood samples containing 10 and 30 mg of lipid/mL, compared with findings for lipid-free blood samples. Values of other thromboelastographic variables did not differ, regardless of lipid concentrations.

Conclusions and Clinical Relevance—Maximum amplitude determined via thromboelastography in canine blood samples was significantly affected by the addition of lipid to final concentrations that are several orders of magnitude higher than clinically relevant lipid concentrations in dogs. Lipid treatment appears to have no significant effect on hemostatic variables in dogs, although clinical studies should be performed to confirm these in vitro findings. (Am J Vet Res 2013;74:567–571)

Parenteral nutrition has an important role in the support of critically ill hospitalized veterinary patients that cannot have their nutritional needs met through enteral-assisted feeding alone. The administration of parenteral nutrition formulations with a moderate to high lipid content to critically ill animals has several advantages. Lipid is a concentrated source of energy and provides more calories per unit volume than does dextrose or amino acids, allowing adequate energy to be more easily delivered to patients without the development of volume overload. Lipid emulsions also decrease the overall osmotic load of the parenteral nutrition solution. Finally, lipids are an efficient source of energy for critically ill animals in which increased protein catabolism and peripheral insulin resistance result in an adaptation to use lipids efficiently for energy in an effort to spare endogenous proteins and to preserve glucose for glucose-dependent tissues. This is particularly true after several days of inadequate caloric intake, which is common in veterinary patients in intensive care.

Although uncommon, there are isolated case reports in the human medical literature documenting the occurrence of bleeding disorders associated with the administration of IVLEs as a component of parenteral nutrition. Although the causes for development of bleeding disorders are varied and not always definitively identified, reduction in platelet numbers and altered platelet function have been cited. Clinical reports of IVLE administration–associated bleeding disorders in humans have mostly involved immature or low–birth weight neonates who have been found to have a diminished ability to metabolize lipids. In addition, some adverse effects have been related to the administration of IVLEs at a high rate. However, despite these concerns, IVLEs continue to be a component of parenteral nutrition in infants and adults and are clinically well tolerated in most cases.
In veterinary medicine, there is a lack of consensus regarding the use of the lipid component of parenteral nutrition partly because of the hypothesized possibility of bleeding complications. Although there are no reports of thrombocytopenia or platelet dysfunction resulting from administration of currently manufactured IVLEs in dogs, to the authors’ knowledge, the risk of bleeding in humans has resulted in a preference of some veterinary clinicians to choose low-lipid or lipid-free parenteral nutrition formulations for administration to patients with thrombocytopenia or other perceived risk factors for bleeding.

In the human medical literature, reports on the effect of IVLEs on platelets have centered on assessment of platelet function determined via platelet aggregometry methods. Thromboelastography is a method of measuring coagulation from initiation of clot formation through fibrinolysis in whole blood samples.\(^1\,2\) It has recently gained popularity in veterinary medicine and can be used as another means to evaluate coagulation status in dogs.\(^3\)

The objective of the study reported here was to determine whether a soybean oil emulsion has an in vitro effect on platelet aggregation and thromboelastography in blood samples obtained from healthy dogs. We hypothesized that there would be a lipid concentration–dependent reduction in canine platelet function.

**Materials and Methods**

**Dogs**—Healthy client-owned dogs were recruited for participation in the study. Dogs were considered for inclusion if they were between 2 and 6 years of age, had a body weight > 5 kg, and had no recent history of systemic illness or major surgery; a physical examination was performed to ensure that the dogs were clinically normal. Dogs were excluded if they had been administered any of the following within 1 month prior to blood sample collection: medications known to affect platelet function, general anesthetic agents, fatty acid supplements, or diets enriched with omega-3 fatty acids. Dogs were also excluded if a platelet count performed on the day of blood sample collection for platelet aggregometry and thromboelastography indicated that they were thrombocytopenic. The study was approved by the Purdue Animal Care and Use Committee, and informed owner consent for each dog’s participation was obtained.

**Sample collection**—Samples of blood (9 mL total) were collected from a jugular vein of each dog by atraumatic venipuncture with a 23-gauge winged infusion catheter directly into 3 tubes containing EDTA, hirudin, and sodium citrate in sequential order for a CBC (including platelet count), platelet aggregometry, and thromboelastography, respectively. All blood samples were analyzed within 2 hours after collection.

**IVLE**—A commercially available lipid emulsion was used for all assays. The IVLE was a 20% soybean oil emulsion that contained soybean oil (200 g/L), egg yolk phospholipids (12 g/L), and glycerin in water (22.5 g/L).

**Platelet aggregation**—Platelet aggregation was measured with an impedance-based whole blood platelet aggregometer.\(^4\,5\) This aggregometer has been described\(^14\,15\) and the assay was performed on the basis of the manufacturer’s recommendations. Blood samples containing hirudin were added to the test channels in 300-µL aliquots. The undiluted lipid emulsion was added to the test channels in increasing volumes (0, 1.5, 15.8, and 53 µL) to obtain final concentrations of 0 (control), 1, 10, and 30 mg of soybean oil/mL, respectively. Preheated (37°C) irrigation saline (0.9% NaCl) solution was added to each test channel at a volume of 300 µL. Each diluted sample was incubated and stirred at 37°C for 5 minutes in the single-use test channels, followed by the addition of 20 µL of agonist. The resultant total reaction volume for aggregation was 620 µL for the control samples and 620 µL plus the additional lipid volumes for the treated samples. Platelet aggregation was assessed in response to both collagen (3.2 µg/mL) and ADP (6.5µM) agonists. Platelet aggregation was measured by recording the change in electrical impedance for 15 minutes following the addition of the agonist. The magnitude of electrical impedance was expressed as aggregation units, and the AUC was recorded as an expression of the platelet aggregation response over the
measured period. The AUC values were used for statistical comparison among groups because AUC reflects both the magnitude of aggregation (in aggregation units) and the velocity of aggregation.

**Thromboelastography**—The lipid emulsion was added in increasing volumes (0, 2.5, 26.3, and 88 µL) to 0.5 mL of the blood sample containing sodium citrate from each dog to obtain final soybean oil concentrations of 0, 1, 10, and 30 mg/mL; lipid-treated samples remained in plastic vials for 3 minutes at room temperature (approx 20°C). Thromboelastographic assays were performed by adding 20 µL of calcium chloride (0.2M) to the reaction cup, followed by 340 µL of the citrated blood or blood and lipid sample to result in a final reaction volume of 360 µL. Calcium chloride was added to recalcify the sample and initiate clotting; additional activators to speed clotting were not used. The standard thromboelastographic variables R (reaction time), K (clotting time), α angle, and MA were recorded for each assay.

**Statistical analysis**—Data were assessed for normality via the Shapiro-Wilk test. Parametric data (platelet aggregation values) were analyzed via a paired t test. Nonparametric data (thromboelastographic variables) were analyzed via a Wilcoxon signed rank test. A Bonferroni correction was performed for multiple comparisons. A value of \( P = 0.05 \) was adjusted for the number of tests performed; as a result, values of \( P \leq 0.0025 \) were considered significant.

**Results**

**Dogs**—Twelve dogs met all inclusion requirements and were enrolled in the study. The median age was 4 years (range, 2 to 6 years), and the median body weight was 23 kg (range, 7 to 28 kg). Dogs included 1 Golden Retriever, 1 Border Collie, 1 Greyhound, 1 Boston Terrier, 1 Cairn Terrier, 1 Beagle, and 6 mixed-breed dogs.

**Platelet aggregation**—The maximum ADP-induced platelet aggregation measured in blood samples with lipid concentrations of 1, 10, and 30 mg/mL did not differ significantly from the maximum ADP-induced platelet aggregation measured in the blood samples without added lipid (Figure 1). When ADP was used as an agonist, the AUC obtained for blood samples with 30 mg of lipid/mL was increased relative to the AUC for the lipid-free blood samples, but the difference was not significant (\( P = 0.027 \)).

**Discussion**

In the present study, addition of a commonly used lipid emulsion at a lipid concentration of 1 mg/mL to canine blood samples had no effect on platelet aggregation or thromboelastographic variables. Although the maximal blood concentration of lipid attained in veterinary patients receiving parenteral nutrition is not known, the concentration of 1 mg/mL was extrapolated from the human medical literature. The lipid concentrations of 10 and 30 mg/mL had no effect on the other thromboelastographic variables, increasing lipid concentration did not result in a significant difference from values for the lipid-free control blood samples (Table 2).

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<table>
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Blood samples were collected from each dog into tubes containing hirudin; lipid emulsion was added before sample aliquots underwent ADP-induced and collagen-induced impedance aggregometry.
interactions. Given that, for each dog, platelet concentration of lipid/mL was significantly decreased. The MA represented the final clot strength and is mainly dependent on specific platelet-to-platelet binding induced by the high concentration of lipid.

Although there were no significant differences in platelet aggregation with either agonist at any lipid concentration, compared with findings for the lipid-free blood samples, a nonsignificant increase in AUC was detected when ADP was used as the agonist. This result was unexpected and was discordant with the thromboelastographic findings, which indicated that there was a significant reduction in the MA of blood samples as a result of the addition of lipid to a concentration of 30 mg/mL. It was suspected that the increase in platelet aggregation in those blood samples was because of non-specific platelet-to-platelet binding induced by the high concentration of lipid.

Compared with the MA of lipid-free blood samples, the MA in blood samples containing either 10 or 30 mg of lipid/mL was significantly decreased. The MA represents the final clot strength and is mainly dependent on platelet number and function, including platelet-fibrin interactions. Given that, for each dog, platelet numbers were verified to be within the reference interval prior to its inclusion in the study and evaluated blood samples were collected during 1 venipuncture procedure, the data suggest that the decrease in MA may have been due to decreased platelet function, lipid-induced interference with fibrin-platelet interactions, or another qualitative defect in fibrin or fibrinogen. However, it is also known that soybean oil increases viscosity of porcine whole blood samples in a concentration-dependent manner in vitro. A study in clinically normal Greyhounds revealed that the whole blood MA in that breed is typically lower than values in other breeds; those researchers suggested that because of the relatively high whole blood viscosity of this breed, the pin movement of the instrument could be restricted. It is also possible that the reduced MA in the present study was caused by an in vitro phenomenon, such as interference between fibrin and the wall of the reaction cup, which resulted in reduced torque on the torsion pin and a reduced magnitude of the measured MA. Because the platelet aggregation and thromboelastographic results were divergent at these supraclinical lipid concentrations in the present study, the most likely conclusion is that there was an in vitro effect on the assay and not a true change in platelet function.

Several mechanisms have been proposed to account for the platelet dysfunction in humans with bleeding-related adverse effects following the administration of lipid-containing parenteral nutrition. Lipid-laden platelets with accompanying morphological changes and impaired platelet aggregation were detected in a human who developed a bleeding disorder following the administration of an IVLE for 6 weeks. Phagocytosis of lipid by platelets after short-term administration of IVLEs has also been detected. In those studies, the IVLEs were administered over periods of hours to days to either healthy adults or adults with noncritical illnesses and were not associated with the development of any adverse effects. Another possible mechanism is a change in the fatty acid composition of the platelet membrane phospholipids. Some studies have revealed changes in the platelet membrane fatty acid profile that mirrors the fatty acid profile of the IVLE. In such study, a reduction in platelet function paralleled a transient increase in the proportion of linoleic acid (C18:n2-6) in the platelet membrane following a single 6-hour infusion of a soybean oil emulsion in healthy adult volunteers. Results of the present in vitro study are in contradistinction to findings of those studies because canine platelet function was not reduced even at excessive supraclinical lipid concentrations.

There were limitations associated with the present study. First, it was conducted in vitro, which precludes potentially confounding physiologic interactions such as platelet-endothelial signaling. Additionally, the contact time between the lipid emulsion and the blood components was limited to 5 minutes. Some proposed mechanisms behind lipid-induced platelet dysfunction.

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<td>3.4 (1.8–6.8)</td>
<td>3.8 (2.6–6.5)</td>
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<td>K (min)</td>
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<td>3.4 (1.2–6.2)</td>
<td>2.8 (1.6–9.3)</td>
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<td>Angle (°)</td>
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<td>65.4 (57.3–73.9)</td>
<td>61 (57.2–73)</td>
<td>58.1 (56.7–68)</td>
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<td>MA (mm)</td>
<td>56.9 (40.9–60.8)</td>
<td>53 (24.6–62.1)</td>
<td>43.8 (31.1–52.3)</td>
<td>44.4 (26.1–57.3)</td>
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Blood samples were collected from each dog into tubes containing sodium citrate; lipid emulsion was added before sample aliquots underwent thromboelastography.
involve a longer exposure time, so it is possible that in vivo effects of lipid on platelet function may not be identified via this experimental method.

Results of the present study have suggested that, although there may be potential for the development of hypocoagulability following administration of IVLEs at excessive doses in dogs, there appears to be no effect on platelets at lipid concentrations likely to be attained in clinical settings. Whereas platelet effects in humans appear to be most relevant with prolonged lipid administration, which is not a commonly performed procedure in veterinary medicine, alterations in platelet structure and function in humans have been demonstrated with short-term infusion of IVLEs, which is a more typical procedure in veterinary medicine. Follow-up ex vivo studies are needed to determine whether lipid administration at a clinically relevant dose and duration has the potential to result in changes to canine platelet number or function.

References