Effect of protein concentration on rate of closure of ameroid constrictors in vitro

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Objective—To evaluate the effect of protein concentration on rate of closure of ameroid constrictors in vitro.

Sample Population—Twenty-four 3.5-mm ameroid constrictors.

Procedure—Ameroid constrictors were equally allocated into 4 treatment groups; constrictors were placed in saline (0.9% NaCl) solution (control) or plasma diluted with saline solution to obtain protein concentrations of 1.5, 3, or 6 g/dL. Ameroid constrictors were incubated for 27 days. A digital camera was used to image ameroid constrictors in culture at 1, 6, 11, 16, 21, and 27 days, and the lumen diameter of each constrictor was measured.

Results—None of the rings were completely closed at 27 days. Mean final lumen diameter was 0.205 ± 0.22 mm. Mean final lumen diameter of constrictors in the control group was significantly larger than that of constrictors in the 1.5, 3, and 6 g/dL groups. Constrictors in the 1.5 g/dL group closed to a larger diameter than that of constrictors in the 6 g/dL group. Constrictors in the control group had the smallest overall change in lumen diameter, compared with constrictors in the 3 and 6 g/dL groups. Constrictors in the 1.5 g/dL group had a significant decrease in overall lumen diameter, compared with constrictors in the 3 and 6 g/dL groups. The diameter of the ameroid lumen was a function of time and protein concentration.


Congenital portosystemic anomalies resulting in shunting of portal blood into the systemic circulation are described in dogs and, less frequently, in cats. Surgical correction of the shunt is the recommended treatment to improve long-term outcome.

The goal of surgical treatment is to redirect portal blood through the liver to palliate the clinical signs of hepatic encephalopathy. Multiple techniques for surgical occlusion of intrahepatic portosystemic shunts and extrahepatic portosystemic shunts have been described. Because complete occlusion of the shunt has been associated with a better long-term prognosis, different techniques have been used to gradually and completely occlude the shunt without increasing the risk of acute portal hypertension.

The ameroid constrictor has been described as providing reliable and predictable gradual shunt occlusion. The device consists of a slotted stainless-steel ring surrounding an inner compressed casein band with a casein key mechanism, permitting the constrictor to be placed around a vessel. Ameroid is a hygroscopic casein derivative that undergoes rapid volumetric expansion that plateaus in 60 days. As the casein gradually absorbs fluid and expands, the inner diameter closes and constricts the shunt vessel. The ameroid also triggers an inflammatory reaction around the shunt that contributes to the closure of the shunt. Ameroid may also contribute to thrombosis of the shunt.

Even with slow occlusion, some dogs have persistent or recurrent signs of hepatic encephalopathy. It is possible that constriction of the implant over a duration that is too short may result in blood being redirected through the hepatic portal system before the vasculature has adequate time to undergo additional development. This would result in the generation of portal hypertension, which would favor the development of acquired shunting. Because the casein absorbs moisture from the surrounding tissue, the rate of closure of the ameroid constrictor may be influenced by oncotic pressure. Albumin and other proteins are the major factors determining oncotic pressure. Therefore, the rate of closure of the ameroid constrictor may be influenced by the overall plasma protein or albumin concentration of the surrounding fluid. Animals with portosystemic shunts have wide variability in plasma protein and albumin concentrations.

The rate of closure of the ameroid constrictor has only been examined in vitro by use of saline (0.9% NaCl) solution and in vivo with various animal models. The effect of plasma protein concentration, which should influence the rate of diffusion of fluid into the constrictor casein, has yet to be examined.

The purpose of the study reported here was to evaluate the effect of protein concentration on the rate of closure of ameroid constrictors in vitro. We hypothesized that the rate of closure of the ameroid constrictor would not be influenced by the protein concentration in vitro.

Materials and Methods

Twenty-four 3.5-mm ameroid constrictors were equally allocated into 4 treatment groups; constrictors were placed in saline solution (control group) or plasma diluted with saline solution to obtain protein concentrations of 1.5, 3, or 6 g/dL.
Plasma protein was aseptically obtained from a single dog donor, and plasma was serially diluted to obtain protein concentrations of 1.5, 3, and 6 g/dL. All protein concentrations were confirmed with a blood chemistry analyzer. One milliliter of streptomycin (0.1% solution) and 1 mL of amphotericin B (0.05% solution) were added to the plasma to provide protection against bacterial and fungal contamination, respectively. Sterile 35-mL aliquots of all dilutions were stored frozen in 50-mL conical vials at −20°C until needed. The albumin concentration was 0.80, 1.10, and 2.90 g/dL for the control group and the 1.5, 3, and 6 g/dL treatment groups, respectively. The sodium concentration was 151, 157, 162, and 169 mEq/L for the control group and the 1.5, 3, and 6 g/dL treatment groups, respectively. Osmolality was 282, 316, 338, and 358 mOsm/kg for the control group and the 1.5, 3, and 6 g/dL treatment groups, respectively.

Each treatment group of ameroid constrictors was placed in standard 10 × 100-mm sterile polystyrene Petri dishes for 27 days at 37°C and 5% CO2. All 6 constrictors of a given treatment group were cultured in the same Petri dish, with labels identifying each constrictor to ensure serial measurements of individual constrictors. Plasma was changed every 48 hours to prevent contamination and provide a consistent protein concentration. A digital camera was used to image the ameroid constrictors in culture at 1, 6, 11, 16, 21, and 27 days. The camera was held perpendicular to each Petri dish from a consistent height, and the same zoom and image-quality settings were used for each photograph. A commercial computer software image program was used to measure the lumen diameter of the ameroid constrictors in each time. Three measurements of each constrictor lumen were obtained, and the mean value was used for statistical analyses. The stainless-steel band of the constrictor was measured with calipers to establish a reference, which was used to calibrate measurements and correct for the effect of focal length and magnification between digital images.

Statistical analyses—Data were determined to be normally distributed by use of the Kolmogorov-Smirnov test for normality. A test for sphericity by use of the Mauchly criterion was performed to evaluate homoscedasticity. Because results of the sphericity test indicated that homoscedasticity (Mauchly criterion, P = 0.02) was not respected, we used an epsilon-adjusted test (Geisser-Greenhouse) to perform an ANOVA. A 2-way ANOVA with repeated measures evaluated the effect of treatment (protein concentration), time, and treatment-time interaction on the lumen diameter of the ameroid constrictor. The Fisher least significant difference test was used to further describe the difference between treatment groups. A 1-way ANOVA evaluated the percentage decrease in the diameter of the constrictors within each group. A multiple regression analysis was performed to determine which factors influenced the rate of closure of the ameroid constrictors. Values of P < 0.05 were considered significant. Data are expressed as mean ± SD.

Table 1—Mean ± SD values for initial and final lumen diameters of ameroid constrictors cultured in saline (0.9% NaCl) solution (control) or solution containing plasma protein concentrations of 1.5, 3, or 6 g/dL for 27 days.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Initial lumen diameter (mm)</th>
<th>Final lumen diameter (mm)</th>
<th>Percentage change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled data</td>
<td>3.43 ± 0.11</td>
<td>2.05 ± 0.22</td>
<td>40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>6.0 g/dL</td>
<td>3.41 ± 0.08</td>
<td>1.85 ± 0.14</td>
<td>46</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3.0 g/dL</td>
<td>3.50 ± 0.11</td>
<td>1.87 ± 0.12</td>
<td>47</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1.5 g/dL</td>
<td>3.40 ± 0.16</td>
<td>2.29 ± 0.14</td>
<td>33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Control</td>
<td>3.40 ± 0.08</td>
<td>2.19 ± 0.08</td>
<td>36</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Results

Initially, mean lumen diameter of constrictors was similar in each group (P = 0.334; Table 1). Mean final lumen diameter was 2.05 ± 0.22 mm, which was a change of 40% (P < 0.001). None of the constrictors were completely closed at 27 days (Figure 1). Treatment (P = 0.001), time (P < 0.001), and treatment-time interaction (P < 0.001) had a significant effect on the lumen diameter of constrictors during the study. The final lumen diameter for constrictors in the saline solution control group was significantly larger than that of constrictors in the 1.5 (P = 0.046), 3 (P = 0.004), and 6 g/dL (P < 0.001) treatment groups. In addition, the lumen of constrictors in the 1.5 g/dL group closed to a larger diameter than constrictors in the 6 g/dL group (P = 0.019). There was no significant difference in final lumen diameter between constrictors in the 1.5 and 3 g/dL groups (P = 0.267) or between constrictors in the 3 and 6 g/dL groups (P = 0.173).

Constrictors in the 3 and 6 g/dL groups had the largest change in lumen diameter (Figure 1). Constrictors in the control group had a smaller overall change in lumen diameter, compared with those in the 3 (P = 0.013) and 6 g/dL groups (P < 0.001). Constrictors in the 1.5 g/dL group had a significant decrease in overall lumen diameter, compared with those in the 3 (P < 0.001) and 6 g/dL groups (P < 0.001). However, there was no difference in the overall change in lumen diameter between constrictors in the control group and 1.5 g/dL group (P = 0.214) or in the 3 and 6 g/dL groups (P = 0.153).

The initial mean lumen diameter of constrictors in the 3 g/dL group was 3.50 ± 0.11 mm and closed to a final mean lumen diameter of 1.87 ± 0.12 mm, which was a change of 47% (P < 0.001; Table 1). Constrictors in the 6 g/dL group had a similarly large change in lumen diameter; the initial mean lumen diameter of 3.41 ± 0.08 mm closed to a final lumen diameter of 1.85 ± 0.14 mm, which was a change of 46% (P < 0.001). Constrictors in the 1.5 g/dL group had a decrease in lumen diameter from an initial mean diameter of 3.40 ± 0.16 mm, which closed to a final mean diameter of 2.28 ± 0.14 mm, representing a change of 33% (P < 0.001). Constrictors in the control group had a 36% decrease in lumen diameter, with an initial mean lumen diameter of 3.40 ± 0.08 mm and a final mean lumen diameter of 2.19 ± 0.08 mm.

Protein concentration and osmolality of the medium were significantly correlated (r² = 0.96; P = 0.001). Protein concentration and colloid osmotic pressures of the medium were significantly correlated (r² = 0.99;
In dogs, the normal colloid osmotic pressure is reported to be around 18 to 20 mm Hg. In our study, the colloid osmotic pressure was lower than the normal colloid osmotic pressure because the protein concentrations were adjusted by dilution of plasma and streptomycin and amphotericin B were added. Colloid osmotic pressure is a better reflection of the osmotic pressure exerted by plasma protein and associated electrolytes because it takes into consideration the electrostatic attraction of different proteins. Colloid osmotic pressure is controlled primarily by albumin and globulin, and, to a lesser degree, by fibrinogen and other protein concentrations. In our study, it would have been preferable to establish treatment groups as a function of albumin concentration rather than total protein concentration. However, we chose to examine the total protein concentration in plasma to better reproduce the most common protein concentrations reported for dogs with portosystemic shunts.

Fluid exchange is influenced by osmolality of the surrounding fluid. Plasma osmolality in dogs is approximately 300 mOsm/kg. Constrictors in the medium containing 1.5 g of plasma protein/dL were in medium that was isotonic to plasma. Groups in which medium contained 3 and 6 g of plasma protein/dL were hypertonic to plasma, whereas the saline solution group was hypotonic to plasma. Because serial dilutions of plasma protein from 1 dog were used, there was a significant correlation between the protein and albumin concentrations.

Animals with portosystemic shunts have wide variability in plasma protein and albumin concentrations. Initial studies that characterized ameroid dynamics did not account for the solute concentration of the surrounding fluid. When constrictors in animals with high plasma protein concentrations close to a small diameter in a short duration, the effect on blood flow through the shunt vessel will be greater than constrictors with slower closure. Poiseuille’s law predicts that conductance of blood through a vessel is directly proportional to the fourth power of the radius of the vessel. Thus, small changes in vessel diameter are magnified and have a large effect on conductance through the vessel. Rapid vessel closure directly translates into less time available for hepatic vascular remodeling.

In the study reported here, the ameroid constrictors did not close completely after 27 days of treatment in vitro. Greater than 50% of the lumen diameter of the constrictors remained open 27 days after treatment. This has been previously reported. Results of a study by Vogt et al indicate that most of the portosystemic shunts treated with an ameroid constrictor had a shunt fraction during transrectal scintigraphy < 20% and were considered closed within 30 days after placement around a shunt. In our study, results of regression analysis indicated that an ameroid constrictor in a dog with an albumin concentration of 2.6 g/dL, which was the mean concentration of albumin in a study by Center et al, should completely close in 65 days.
would tend to suggest that closure of the shunt is caused by events other than swelling of the casein within the constrictor. Results of a study by Adin et al. indicate that a severe inflammatory reaction occurs around the ameroid constrictor, which should contribute to the rate of closure of the shunt. Results of a study in dogs in which ameroid constrictors were placed around the caudal mesenteric artery indicate that the wall of the artery included in the constrictor underwent necrosis and calcification. In a study by Besancon et al., thrombosis contributed to premature closure 10 days after placement around a shunt.

Various attempts have been made to slow the rate of closure of ameroid constrictors. Results of a study performed in vivo comparing closure rates in ameroid constrictors coated in petrolatum indicate that the coating had no effect on closure of the constrictor or local inflammatory reaction. Coating the ameroid constrictor with silicone grease slowed the rate of closure. Nine days after implantation, flow through a simulated vessel was decreased by approximately 90% in noncoated ameroid constrictors, whereas ameroid constrictors covered with silicone had an approximately 50% decrease in flow. In that study, histologic examination to evaluate the degree of inflammatory reaction around the ameroid constrictor with and without silicone coating was not performed. Silicone may be superior to petrolatum in isolating the ameroid constrictor from the surrounding tissue and fluid.

By permitting greater time for hepatic remodeling, slowing shunt occlusion may decrease the rate of complications in animals undergoing partial shunt occlusion surgery with an ameroid constrictor. Additional studies are required to better characterize the local reaction to ameroid constrictors and the ways in which closure of ameroid constrictors can be attenuated to permit additional time for vascular remodeling.

References

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g. JMP, SAS Institute Inc, Cary, NC.

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