In vitro effect of multiple hydrogen peroxide gas plasma sterilizations on the rate of closure of ameroid constrictors

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Objective—To evaluate the effect of multiple hydrogen peroxide gas plasma (HPGP) sterilizations on the rate of closure of ameroid constrictors.

Sample—Thirty-six 5.0-mm ameroid constrictors.

Procedures—Ameroid constrictors were randomly allocated to 6 groups. Each group underwent 1, 2, 3, 4, 5, or 6 HPGP sterilizations. Ameroid constrictors were then incubated for 35 days in canine plasma and digitally imaged at predetermined times during incubation. One individual, who was unaware of the group to which each ameroid constrictor was assigned, measured the lumen area of the constrictor on each digital image. Mean lumen area was compared among groups.

Results—No ameroid constrictors were completely closed after 35 days of incubation in canine plasma. Mean lumen area after incubation did not differ among constrictors that underwent 1, 2, and 3 sterilizations. Constrictors that underwent 4 sterilizations were closed significantly more than were those that underwent 1, 2, or 3 sterilizations. Mean lumen area after incubation did not differ significantly between constrictors that underwent 5 and 6 sterilizations, although the final lumen areas for those constrictors were significantly smaller than those for constrictors that underwent 1, 2, 3, and 4 sterilizations.

Conclusions and Clinical Relevance—Ameroid constrictors that underwent 5 and 6 HPGP sterilizations had a 9% to 12% decrease in lumen area, compared with that of constrictors that underwent ≤ 4 plasma sterilizations, and the use of such constrictors could increase the risk of portal hypertension and secondary acquired shunting or decrease the risk of persistent shunting. (*Am J Vet Res* 2014;75:924–928)

Portosystemic shunts are common hepatic vascular anomalies in small animal patients.[1–3] Medical management of PSSs has been associated with chronic hepatic fibrosis and portal hypertension, which can result in progressive hepatic failure, as well as clinical signs of persistent or progressive neurologic and urinary tract abnormalities.[4–7] The long-term prognosis is generally more favorable following treatment with gradual surgical attenuation of a PSS, compared with that following medical management, provided the patient survives the immediate postoperative period.[6–10]

Various methods have been used for PSS attenuation including silk ligatures,[11] intravascular thrombogenic coils,[11,12] hydraulic occluders,[4,13] cellophane banding,[8,11,14] and ameroid constrictors.[2,15,16] Among those devices, ameroid constrictors have gained popularity because they gradually occlude the PSS, provide predictable closure, and are associated with low surgical and postoperative complications.[1,15,16] Ameroid is a hygroscopic, compressed casein that undergoes expansion when immersed in fluid, which is characterized by rapid expansion initially followed by a period of slower expansion.[1,15,17]

Abbreviations

HPGP	Hydrogen peroxide gas plasma
PSS	Portosystemic shunt

Investigators of a retrospective study[14] that involved 106 dogs and 5 cats with congenital PSSs in which the shunt was surgically attenuated by use of a cellophane band suggest that the initial constriction of the PSS should not be < 2.0 to 3.0 mm. Because scientifically sound recommendations regarding the appropriate size of ameroid constrictor for use in attenuation of PSSs with particular diameters are lacking, the decision of which size of constrictor to use for attenuation of each PSS is subjective. Surgeons may handle ameroid constrictors of multiple sizes before choosing which size of constrictor to use during a PSS attenuation procedure. Consequently, for the purpose of frugality and because the manufacturer does not advise against multiple sterilizations, unused ameroid constrictors are frequently

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resterilized after surgical procedures. The casein material in ameroid constrictors is a type of protein. In 1 study,[18] the use of heat to sterilize micellar casein concentrates caused proteolysis and protein dissociation. Thus, resterilization of ameroid constrictors could cause denaturation of the casein material and increase its permeability, which could affect the rate of closure of the constrictors when used for PSS attenuation.

The objective of the study reported here was to evaluate the lumen area of ameroid constrictors after ≥ 1 HPGP sterilization procedure. Our hypothesis was that the number of times that an ameroid constrictor undergoes HPGP sterilization would have no effect on its final lumen area.

Materials and Methods

Ameroid constrictors—Thirty-six standard 5.0-mm ameroid constrictors[a] with a metal key were randomly allocated to 6 treatment groups with 6 constrictors allocated to each group. Constrictors in treatment groups 1, 2, 3, 4, 5, and 6 underwent 1, 2, 3, 4, 5, and 6 HPGP sterilizations, respectively. To mimic a clinical situation, each ameroid constrictor was removed from its package and handled in an aseptic manner for 30 minutes in an operating room before each sterilization. Within each group, each ameroid constrictor was identified with 1 of 6 different colors of surgical identification tape (ie, a 2- to 3-mm piece of surgical identification tape was attached to each constrictor). Each group of constrictors and a sterilization indicator strip were packaged within 2 layers of protective covering and sterilized with an HPGP sterilization unit.[b]

Plasma preparation and incubation—Three units of fresh frozen plasma that was obtained from the same donor dog at different times was thawed in a 37°C water bath and mixed on a rocking platform for 10 minutes. All 3 units of plasma was then combined in a sterile manner, and the protein and albumin concentrations in the combined plasma were determined by means of a standard biochemical analyzer.[c] The protein concentration was 5.3 g/dL, and the albumin concentration was 3.2 g/dL. A sample of the combined plasma was streaked onto a chocolate agar plate[d] for bacteriologic culture, the results of which were negative. Streptomycin (5 µg/mL) and amphotericin B (2 mg/mL) were added to the combined plasma to protect against bacterial and fungal contamination. The plasma was then divided into 85-mL aliquots in a class 2 biosafety cabinet,[e] placed in sterile glass bottles, and stored frozen at –20°C until used.

Following completion of the designated number of HPGP sterilizations, each group of 6 ameroid constrictors was placed into a sterile glass Petri dish within a class 2 biosafety cabinet.[e] One Petri dish was used for each group, and each Petri dish was identified with a different color of surgical identification tape. The Petri dishes containing the ameroid constrictors were then submerged in plasma (day 0) and incubated in 5% CO_2 at 37°C in a standard microbiological incubator[f] for 35 days. The CO_2 concentration and temperature within the incubator were monitored, verified, and recorded daily. The plasma in which the Petri dishes were submerged was changed every 96 hours.

Data collection—Data were recorded and maintained in a commercially available software program.[g] Each ameroid constrictor was weighed on a gram scale immediately prior to (day 0) and after (day 35) incubation in plasma and digitally imaged on days 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 21, 28, and 35 at approximately the same time each day. The digital camera[h] was mounted on a miniature tripod positioned 8.9 cm above and oriented perpendicular to the constrictors. Each petri dish was set on a place card that depicted the group number, date, and time that the image was obtained. Prior to an image being obtained, a label was placed adjacent to each constrictor that depicted the color of the surgical identification tape that was applied to it. A ruler was also placed in the image field to provide a scale for dimensional analysis. A computer software image program[i] was used to measure the lumen area of each ameroid constrictor as described.[4] For each constrictor on each image, the lumen area was measured 5 times and the mean was calculated. One investigator (HATM), who was unaware of the group to which each constrictor was allocated, performed all measurements.

Statistical analysis—A Shapiro-Wilk test was used to assess the distribution of the data for normality. Outcomes of interest were constrictor weight and lumen area. A 2-way ANOVA for repeated measures was used to evaluate the mean for each outcome among the treatment groups. Independent variables included in the model were treatment group, day that measurement was obtained, and the interaction between treatment group and day. Within each day, comparisons between treatment groups were performed with the Student Newman-Keuls method to adjust for type I error inflation caused by multiple comparisons. Linear regression was used to determine the rate of reduction of lumen area over time. Results were reported as the mean ± SD. All analyses were performed with commercially available software,[j] and values of $P < 0.05$ were considered significant.

Results

For ameroid constrictors in groups 1 through 6 after the designated number of HPGP sterilizations, the mean ± SD weight was 1.40 ± 0.01 g on day 0 (immediately before incubation in plasma), and the mean ± SD lumen area was 0.22 ± 0.01 cm^2 on day 0 and 0.12 ± 0.01 cm^2 on day 35 (immediately after incubation in plasma). The mean ± SD constrictor weight on day 35; lumen area on days 0, 7, and 35; and percentage decrease in lumen area between days 0 and 35 for each group were summarized (**Table 1**). None of the ameroid constrictors had closed completely on day 35.

Treatment group ($P < 0.013$), day ($P < 0.001$), and the interaction between treatment group and day ($P < 0.001$) were significantly associated with the lumen area of the constrictors. The mean lumen area did not differ significantly among the 6 groups on day 0. For all groups, the mean lumen area was decreased by 20% on day 7, by 25% on day 10, and by 28.8% on day 14 (**Figure 1**). The reduction in mean lumen area during

Table 1—Mean ± SD final weights and lumen areas for 5.0-mm ameroid constrictors (n = 36) that were randomly allocated into 6 groups of equal size (6) and were sterilized 1 to 6 times with HPGP; following the final sterilization (day 0), constrictors were incubated in canine plasma for 35 days.

Treatment group	Weight on day 35 (g)	Lumen area (cm^2)			Percentage decrease in lumen area between days 0 and 35
		Day 0	Day 7	Day 35	
1	1.623 ± 0.021[a]	0.227 ± 0.007[a]	0.185 ± 0.005[a]	0.134 ± 0.005[a]	41.0
2	1.624 ± 0.012[a]	0.229 ± 0.009[a]	0.178 ± 0.002[a]	0.131 ± 0.006[a]	42.8
3	1.605 ± 0.009[a]	0.227 ± 0.007[a]	0.176 ± 0.001[a]	0.131 ± 0.006[a]	42.3
4	1.655 ± 0.026[b]	0.215 ± 0.007[a]	0.173 ± 0.006[a,b]	0.121 ± 0.006[b]	43.7
5	1.657 ± 0.011[b]	0.217 ± 0.007[a]	0.164 ± 0.005[b]	0.103 ± 0.006[c]	52.5
6	1.650 ± 0.152[b]	0.217 ± 0.006[a]	0.163 ± 0.004[b]	0.102 ± 0.010[c]	53.0

[a–c]Within a column, values with different superscript letters differ significantly ($P < 0.05$).

Groups 1, 2, 3, 4, 5, and 6 were subjected to 1, 2, 3, 4, 5, and 6 HPGP sterilizations, respectively. For all groups, the mean weight of the ameroid constrictors was 1.40 ± 0.01 g on day 0, and the percentage decrease in lumen area between days 0 and 35 was significant ($P < 0.001$).

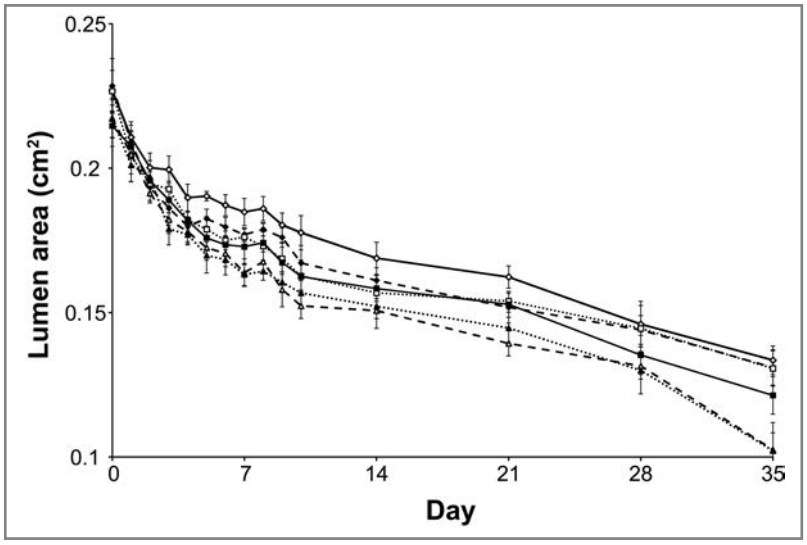

Figure 1—Mean ± SD lumen area for 5-mm ameroid constrictors (n = 36) that were randomly allocated into 6 groups of equal size (6) and were sterilized 1 to 6 times with HPGP; following the final sterilization (day 0), constrictors were incubated in canine plasma for 35 days. Groups 1 (solid line with open diamonds), 2 (dashed line with black diamonds), 3 (dotted line with open squares), 4 (solid line with black squares), 5 (dashed line with open triangles), and 6 (dotted line with black triangles) were subjected to 1, 2, 3, 4, 5, and 6 HPGP sterilizations, respectively. For constrictors in all groups, the mean lumen area was decreased by 20% on day 7, 25% on day 10, and 28.8% on day 14, compared with that on day 0. On day 35, the mean lumen areas for constrictors in groups 1, 2, and 3 differed significantly ($P < 0.05$) from those of constrictors in groups 4, 5, and 6, and the mean lumen area for constrictors in group 4 differed significantly from the mean lumen areas for constrictors in groups 5 and 6.

the first 7 days of incubation (slope coefficient, –0.0058 cm^2/d) was 3.7 times that during the period between days 8 and 35 of incubation (slope coefficient, –0.0018 cm^2/d; $P < 0.001$). For each group, the percentage decrease in the mean constrictor lumen area between days 0 and 35 was significantly ($P < 0.001$)different from 0.

Discussion

Results of the present in vitro study indicated that the number of times ameroid constrictors underwent HPGP sterilization significantly affected their rate of closure. Ameroid constrictors that underwent ≥ 4 HPGP sterilizations constricted faster and had a smaller mean final lumen area than did ameroid constrictors that underwent ≤ 3 HPGP sterilizations. Constrictors that underwent 5 or 6 HPGP sterilizations had the quickest rate of closure on days 7 and 35 after initiation of incubation in canine plasma. Thus, the hypothesis that multiple HPGP sterilizations would have no effect on the lumen area of ameroid constrictors was rejected.

Investigators of another study[18] hypothesized that HPGP sterilization might denature casein proteins, of which ameroid is composed. Denaturation of casein might make it more absorptive, resulting in rapid and complete expansion of the ameroid constrictor. This phenomenon was partially validated by the results of the present study because, although the mean final weight of the constrictors did not differ significantly among some of the treatment groups, the final weight of the constrictors tended to increase as the number of HPGP sterilizations that the constrictors underwent increased. Given that the magnitude of increase in mean weight was greatest for constrictors that underwent 5 or 6 HPGP sterilizations, it is possible that a similar proportionate increase in weight would be observed for constrictors that undergo > 6 HPGP sterilizations; however, additional in vitro studies would need to be conducted to determine whether constrictor weight continues to increase or reaches a plateau (ie, ceiling) as the number of sterilizations constrictors are subjected to increases.

Results of other studies[17,19,20] indicate that ameroid constrictors undergo rapid expansion during the first 4 to 14 days after implantation, followed by gradual, slow expansion during the next 60 to 80 days. In the present study, the mean lumen area of the constrictors during the initial 7 days of incubation in plasma was reduced 3.7 times, compared with that during days 7 to 35 of incubation. The percentages of reduction in the constrictor lumen area on days 7 (20%), 10 (25%), and 14 (28.8%) of incubation in the present study were similar to those reported by investigators of other studies.[19,21,22] Moreover, the results of the present study suggested that the percentage of reduction in constrictor lumen area during the initial 7 days of incubation in plasma

was positively associated with the number of HPGP sterilizations that the constrictor underwent prior to incubation (ie, ameroid constrictors that underwent 5 and 6 HPGP sterilizations had a greater percentage reduction in lumen area during the first 7 days of incubation than did constrictors that underwent < 5 HPGP sterilizations). The clinical implication of this finding is that ameroid constrictors that undergo > 4 HPGP sterilizations may not provide the gradual occlusion desired for attenuation of PSSs.

Consistent with results of other studies,[18,20] none of the ameroid constrictors in the present study had closed completely after 35 days of incubation in plasma. It has been suggested that occlusion of PSSs following implantation of ameroid constrictors likely results from the centripetal swelling of the ameroid as well as from a fibrovascular response within the vascular wall,[11,17,20,21] and the formation of an intraluminal thrombus that arises concentrically from the intima.[2,15,17,19,20,23] When correctly implanted, most ameroid constrictors achieve complete occlusion of the shunt by swelling of the ameroid and a fibrovascular response.[10,15] However, in 1 study,[1] 8 of 14 cats had persistent shunting 8 to 10 weeks after surgical implantation of ameroid constrictors, and the development of multiple acquired shunts or incomplete shunt attenuation could not be ruled out in those cats. In another study,[16] 21 of 99 (21%) dogs with extrahepatic PSSs had a persistent shunt after implantation of an ameroid constrictor. In the present study, after 35 days of incubation in plasma, the mean lumen areas for constrictors in groups 5 and 6 were 9% to 12% smaller, compared with the mean lumen areas for constrictors in groups 1 through 4. It is probable that the likelihood of persistent shunting will decrease as the percentage of constriction of an ameroid constrictor increases.

The performance of in vivo studies are necessary to determine whether the rapid expansion of ameroid constrictors during the first 7 days after implantation could predispose patients to portal hypertension and secondary acquired shunting and whether the extent of shunt occlusion 35 days after ameroid constrictor implantation is associated with persistent shunting. The rate of gradual occlusion necessary to prevent the formation of multiple acquired shunts, the clinical impact of persistent shunting, and the percentage of persistent shunting required for manifestation of clinical signs associated with PSSs are unknown. Thus, recommendations regarding the number of times ameroid constrictors can undergo HPGP sterilization without adversely affecting their performance cannot be made until prospective in vivo studies are conducted.

The present study had several limitations, with the most obvious being that it was conducted in vitro instead of in vivo. Although ameroid constrictors were available in a variety of sizes (3.5, 5, 6.4, 7, 8, and 9 mm), only 5.0-mm ameroid constrictors were used in this study because a search of medical records from the Purdue University Veterinary Teaching Hospital between 2003 and 2013 indicated that was a commonly used size of constrictor for treatment of PSSs. Of the 76 patients with PSSs that were treated with surgical implantation of an ameroid constrictor during that period, 31 (40.7%) had a 3.5-constrictor implanted, 30 (39.5%) had a 5.0-mm constrictor implanted, 9 (12%) had a 6.0-mm constrictor implanted, 3 (4%) had a 9.0-mm constrictor implanted, 2 (3%) had a 11.0-mm constrictor implanted, and 1 (1%) had a 7.5-mm constrictor implanted. We chose to use 5.0-mm constrictors for this study because that size of constrictor was commonly used in clinical practice and was easier to image and measure, compared with 3.5-mm constrictors. Also, we only used standard ameroid constrictors with metal keys. Additional studies need to be conducted to determine whether the percentage of reduction of lumen area is constant regardless of ameroid constrictor size or whether ameroid constrictors with a slim design or a casein key react in a similar manner as standard ameroid constrictors to multiple HPGP sterilizations.

In the present study, the protein concentration of the plasma in which the ameroid constrictors were incubated was held constant for all groups throughout the 35-day incubation period. Results of another study[22] indicate that a high plasma protein concentration increases the rate of casein constriction in ameroid constrictors. Additional changes in constrictor lumen area might have been observed in this study had the protein concentration of the plasma in which the constrictors were incubated been varied. An important point of clarification is that 5-mm ameroid constrictors should have an initial area of 0.19 cm^2 prior to sterilization. The initial measurements of the ameroid constrictors evaluated in the present study were obtained on day 0 after they underwent the designated number of HPGP sterilizations and immediately prior to incubation in plasma. Although the mean lumen area of the constrictors on day 0 did not differ significantly among the 6 treatment groups, the actual differences in mean lumen area on day 0 reflect the effect and variation caused by multiple HPGP sterilizations.

Results of the present study indicated that the number of times that an ameroid constrictor underwent HPGP sterilization was positively associated with its extent of constriction when incubated in canine plasma. Therefore, it would be beneficial for clinician awareness to label sterilization packages with the number of times that the enclosed ameroid constrictor has been sterilized. The performance of prospective in vivo studies is necessary to determine whether the initial rapid rate of expansion for ameroid constrictors that have undergone multiple HPCP sterilizations will adversely affect the gradual occlusive function of the constrictors sufficiently to induce portal hypertension and secondary shunting or whether the proportionately smaller final lumen area for constrictors that have undergone 5 or 6 HPGP sterilizations, compared with the final lumen area for constrictors that have undergone < 5 sterilizations, will reduce the likelihood of persistent shunting. Once those in vivo studies are conducted, recommendations regarding the number of times that an ameroid constrictor can undergo HPGP sterilization without adversely affecting its function can be made.

a. Ameroid constrictor, Research Instruments NW, Lebanon, Ore.
b. VHP MD140X biodecontamination unit, STERIS Corp, Mentor, Ohio.
c. VITROS 5,1 FS, Ortho Clinical Diagnostics Inc, Rochester, NY.
d. Chocolate agar plate, Remel Inc, Lenexa, Kan.

e. Class II Type A/B3 biological safety cabinet, NuAire, Plymouth, Minn.
f. IR Autoflow CO_2 water jacketed incubator, NuAire, Plymouth, Minn.
g. Microsoft Excel, version 14.06129.5000, Microsoft Corp, Redmond, Wash.
h. Olympus Tough TG-820 12-megapixel digital camera, Olympus Corporation of the Americas, Tokyo, Japan.
i. NIH Image J, National Institutes of Health, Bethesda, Md. Available at: rsb.info.nih.gov/nih-image. Accessed Nov 1, 2013.
j. STATA SE, version 12.1, StataCorp, College Station, Tex.

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