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Behavior of plastic and metal ameroid constrictors during *in vitro* incubation in physiologic solutions of varying glucose concentration

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ABSTRACT

The objective of this study was to evaluate the influence of ameroid constrictor (AC) composition as well as glucose concentration in the surrounding fluid on the rate and completeness of AC closure. In a pilot study, four ACs (two metal, two plastic) were incubated in a solution containing 100 mg/dL glucose, and in a follow-up study, two additional ACs (one metal, one plastic) were incubated in a solution of 100 mg/dL glucose and six ACs (three metal, three plastic) were incubated in a solution of 50 mg/dL glucose. Dimensions of the ACs were analyzed weekly for 57 days. No significant difference was found in the rate or overall proportionate closure for either metal *versus* plastic ACs or ACs incubated in 50 mg/dL *versus* 100 mg/dL glucose. As there was no statistically significant difference in the proportionate closure of metal and plastic ACs, both types are clinically suitable for gradual attenuation of portosystemic shunts in animal patients. The lack of a significant difference in rate and completeness of closure of ACs incubated in different concentrations of glucose provides evidence that the glucose concentration of the surrounding fluid likely does not have a significant effect on AC closure. However, a significant difference in the proportionate closure of ACs occurred within the first week of the study between constrictors incubated in 50 mg/dL glucose and those incubated in 100 mg/dL glucose, and additional studies are indicated to determine the significance of this early difference *in vivo*.

1. Introduction

Portosystemic shunts are congenital vascular abnormalities that link the portal circulation to the systemic circulation, allowing portal blood to bypass the liver and directly enter the systemic circulation (Johnson et al., 1987; Martin, 1993; Winkler et al., 2003; Hunt, 2004). Gradual ligation of the shunt is desired in order to allow the liver time for vascular remodeling and to thereby avoid potentially life threatening portal hypertension following complete shunt attenuation. Devices such as the ameroid constrictor (AC) have thus become standard of practice for gradual shunt occlusion (Falls et al., 2013). Ameroid constrictors contain hydrophilic casein that absorbs fluid in the postoperative period, thereby resulting in a gradual closure of the vessel (Lange et al., 1985). According to Poiseuille's law, the resistance to flow through a vessel is inversely proportional to the radius raised to the fourth power (Pfitzner, 1976). A small change in radius of a blood vessel results in a dramatic change in resistance to flow, and ACs that close rapidly can have a substantial early effect on diminishing blood flow through the shunt vessel. This may be detrimental, as it allows less time for hepatic remodeling prior to the establishment of normal blood flow to the liver and may resultantly increase the risk of portal hypertension. Therefore, the rate of closure of ACs holds great clinical significance. Numerous experimental studies have evaluated the behavior of ACs *in vivo* and clinical outcomes in patients have been reported, but until recently it was not possible to accurately measure the degree of closure of the AC *in vivo* as the metal sheath created a metallic streak artifact on computed tomography (Barrett and Keat, 2004). A recent study using ACs encased in plastic rather than metal showed variable degrees of closure in clinical patients (Hunt et al., 2014). Patients with congenital portosystemic shunts (CPS) show a variety of biochemical perturbations associated with poor liver function (Center and Magne, 1990). Some of these products of hepatic metabolism (namely glucose and albumin) affect the osmolality and oncotic pressure of plasma and therefore might be expected to alter the rapidity and effectiveness with which ACs draw water from the body fluids and constrict.

Previous studies have evaluated some factors suspected to affect the rate and completeness of AC closure. Monnet and Rosenberg studied the effect of varying protein concentrations and found that high protein concentrations were associated with more rapid closure of ACs *in vitro*, although glucose and osmolality were not held constant (Monnet and Rosenberg, 2005). Adin et al. postulated that coating ACs

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with petrolatum might prolong the time to closure after implantation in the peritoneal cavity of rats, but no significant difference was found between treated and untreated ACs and the authors speculated that petrolatum coating was rapidly removed by peritoneal macrophages (Adin et al., 2004). Youmans et al. demonstrated that ACs tended to attenuate the femoral veins more rapidly than cellophane banding in experimental dogs (Youmans and Hunt, 1999). Thompson et al. studied the effect of the number of sterilization procedures (1–6) with hydrogen peroxide gas plasma on the rate of closure of ACs and found that ACs that underwent four, five, or six sterilizations constricted faster and to a greater proportionate amount than those that underwent three or fewer sterilizations (Thompson et al., 2014). Previous studies have reported that ACs should completely close within 65 days given an albumin concentration of 2.6 g/dL (Monnet and Rosenberg, 2005). However, the initial two weeks of constriction appear to be very important with rapid changes in constrictor ID, and complete occlusion of vessels can occur within this time frame (Monnet and Rosenberg, 2005; Youmans and Hunt, 1999). An *in vitro* study further demonstrated that flow through the AC is most rapidly decreased within the first three days, and flow through the AC is reduced by approximately 90% within the first week of attenuation (Elzinga, 1969).

The goal of the present study was to evaluate the behavior of plastic *versus* metal encased ACs that were incubated in solutions with similar oncotic pressure but different glucose concentrations and osmolality.

Our hypotheses for this study were as follows:

Metal ACs incubated in a given concentration of albumin and glucose will close at the same rate and to the same proportionate amount as plastic ACs.

ACs incubated in a solution with higher glucose concentration will constrict at the same rate and to the same proportionate amount as those incubated in a solution with lower glucose concentration.

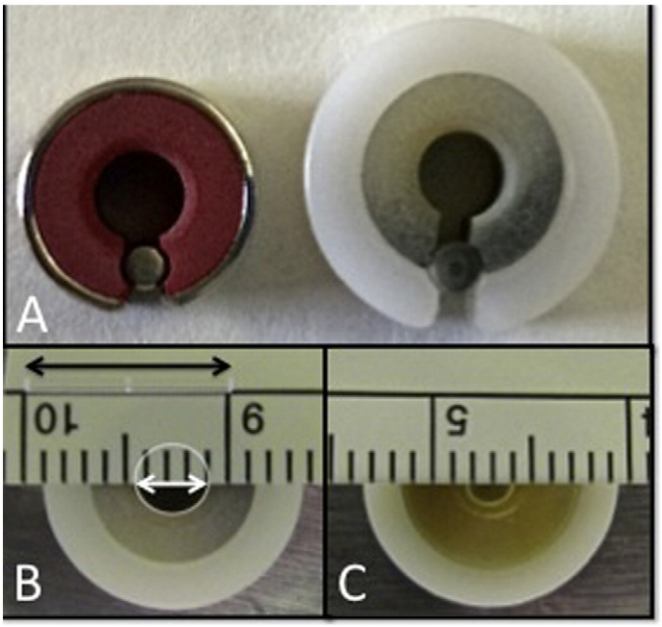

Fig. 1. ACs. (A) Images of one metal and one plastic AC (B) Image of plastic AC with ruler edge centered above AC to approximate diameter of the AC; circle has been fitted to internal ring of AC, and arrows depict measurements made for ID and 1 cm as comparison (C) Image of plastic AC with ruler edge centered above AC to approximate diameter of the AC; no circles fitted or measurement arrows depicted.

2. Materials and methods

2.1. Study design

This study consisted of two phases: a pilot phase (four ACs) and a follow-up phase (eight ACs). Preliminary results from the pilot study have been reported by Hunt et al. (2014).

2.2. Ameroid constrictors

Two forms of AC were used (Fig. 1A). Both forms contained a casein cylinder with a reported internal diameter (ID) of 3.5 mm (actual measurements varied from 3.26 mm to 4.35 mm). One type (metal) had an external casing of surgical stainless steel; the other (plastic) had an external casing of polyacetal homopolymer (Fig. 1A).[a] Although the ACs were standardized with reference to their ID, the clay core of the metal ACs was red or white whereas the clay core of the plastic ACs was gray, indicating that they came from different batches of clay. Nevertheless, they represented ACs that would normally be used to attenuate CPS in clinical patients. The ACs were sterilized using hydrogen peroxide (H_2O_2) according to normal protocols.

2.3. Study groups

ACs were divided into four distinct groups: plastic *versus* metal, and 100 mg/dL glucose (Group 100G) *versus* 50 mg/dL glucose (Group 50G). Six plastic and six metal ACs were used such that a total of six ACs (three plastic and three metal) were evaluated following incubation in 50 mg/dL glucose solution and another six ACs (three plastic and three metal) were evaluated following incubation in 100 mg/dL glucose solution. A pilot study of four ACs (two metal and two plastic) incubated in 100 mg/dL glucose solution was followed by a second phase in which two ACs (one metal and one plastic) were incubated in 100 mg/dL glucose and six ACs (three metal and three plastic) were incubated in 50 mg/dL glucose.

2.4. Perfusate

The perfusates were mixed to include 3.0 g/dL of albumin[b] in 0.9%NaCl[c] with either 100 mg/dL (pilot study and 100G group) or 50 mg/dL (50G group) glucose[d] concentration with a final volume of 334 mL. To protect against bacterial and fungal contamination, 0.53 mL of Tobramycin[e] and 0.67 mL of Amphotericin B[f] were added. Albumin concentration was verified with a blood chemistry analyzer[g] and electrolyte and glucose measurements were made with a blood gas analyzer.[h] Osmolality[i] was measured for the pilot study perfusate solution.

After making the perfusate solution, it was stored sterilely as 10 mL aliquots at −20 °C until its use. The ACs were incubated in individual wells containing 10 mL aliquots of the solution, such that the whole of each AC was covered with solution.

During each trial, approximately 0.5 mL of perfusate from each of the wells was collected every seven days (days 1, 8, 15, 22, 29, 36, 43, 50, and 57), combined in a red top plastic vacutainer tube respective of study group (pilot, Group 100G, and Group 50G), and stored at −20 °C.

At the same time points (starting on day 8), each AC was transferred using sterile technique to a new well and fresh solution was added to the wells.

After transferring the ACs to their new wells on day 8 of the study, the initial perfusate solutions were submitted for aerobic and anaerobic culture as well as collected and stored for the perfusate assay. The remainder of the initial perfusates was maintained in each well within the original covered chamber until day 64, at which time they were submitted for microbial culture.

2.5. Incubation

Ameroid constrictors were incubated in covered plastic chambers at 38 °C in an atmosphere containing 5% CO_2 for 57 days.