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Experimental Arteriovenous Fistulas: Treatment with Porous Metallic Stents

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PURPOSE: To determine the efficacy of porous metallic stents in the treatment of experimentally created carotid-jugular fistulas. METHODS: Carotid-jugular fistulas were constructed surgically in five mongrel dogs. Porous metallic stents were placed endovascularly across the fistula holes within the carotid artery; carotid angiography was performed before, immediately after, and 1 and 2 months after stent placement. The fistula specimens were resected 2 months after stent placement; gross and light microscopic analyses were performed. RESULTS: Angiography revealed complete closure of three of the five fistulas 1 month after stent placement; two of the five fistulas remained patent but demonstrated diminished flow rate. All carotid arteries were widely patent throughout the study. Gross pathology of the carotid-jugular specimens revealed fibrous connective tissue and collagen within the fistula hole. A thin layer of endothelium covered the stent wires and the fibrous connective tissue overlying the fistula hole. CONCLUSIONS: The stents were effective in closing three of the five fistulas and reducing flow through the fistulas in the remaining animals. With further refinements and variations in technique, porous metallic stents may prove a viable alternative to current endovascular devices for treatment of certain arteriovenous fistulas.

Index terms: Fistula, arteriovenous; Interventional instruments, stents; Animal studies


Intravascular stents are used currently for intraluminal dilatation of atherosclerotic plaque and are designed to compress plaque and dilate tightly stenotic vessels (2–7) (Richter G, Noldege G, Troeren T, et al, “First Long-term Results of a Randomized Multicenter Trial: Iliac Balloon-Expandable Stent Placement versus Regular Percutaneous Transluminal Angioplasty” (abstr), Radiology 1990;177(P):152). Unlike balloons, navigation of those devices is not dependent on the size of the fistula hole or the effects of blood flow. The stent is attached to a catheter that tracks over a guide wire and is deployed on the arterial side of the fistula. Previous experiments with porous arterial grafts and stents have demonstrated filling of the pores with fibrous connective tissue and endothelium over time (8, 12) (Verdon S, Gould K, Termin P, et al, “Scanning Electron Microscopy of a Self-expanding Vascular Stent in a Canine Animal Model,” presented at the XIth International Congress for Electron Microscopy, San Francisco, Calif, 1990). We sought to determine whether overgrowth of fibrous connective tissue...
through the stent pores would be adequate to close experimentally constructed fistulas.

Materials and Methods

Experimental arteriovenous fistulas were created in five mongrel dogs weighing approximately 25 kg. The surgical procedure and handling of these dogs complied with our hospital’s Institutional Animal Care and Use Committee.

Surgical Technique for Creation of an Arteriovenous Fistula in the Necks of Dogs

The anterolateral cervical portion of the neck is shaved and surgically prepped with povidone-iodine (Betadine) antiseptic solution. Surgical draping is arranged to isolate the incisional area. An 8- to 10-cm anterior midline incision is made over the cervical trachea and extended down through the subcutaneous tissue and underlying superficial fascia. Hemostasis is maintained by use of a Bovie electrocoagulator. Using Metzenbaum dissecting scissors and DeBakey tissue forceps, sharp dissection is continued by separating the plane between the sternocephalicus and sternohyoideus strap musculature. A Weitlaner retractor is inserted into the dissected space, exposing the encapsulated common carotid artery and vagus nerve. The common carotid artery sheath is invaded and the vagus nerve separated. The mobilization of the common artery does not involve the cranial or caudal thyroid arteries or veins. Suture ligatures, size 2-0 silk, are placed proximally and distally around the common carotid artery and the Weitlaner retractor is removed. The external jugular vein then is mobilized from its subcutaneous bed by sharp dissection with Metzenbaum dissecting scissors. Suture ligatures, size 2-0 silk, are placed proximally and distally around the common carotid artery and the external jugular vein. The wire has a diameter of 0.0035 in and the stent measures 42 mm in length and 5 mm in diameter. The wire is tensioned. To prevent stent migration, the stent diameter should be slightly larger than the intraluminal diameter of the native vessel. Thus, stent diameters of 5 mm were chosen, because arterial diameters measured 3.5 mm to 4.0 mm in diameter by ultrasound.

Gross and microhistopathologic studies were performed on the specimens.

Histologic Processing and Photography

Before trimming, the blood vessel containing the stent was photographed. Under the supervision of a pathologist, an experienced histotechnician trimmed the tissues as follows: (a) Segments of the blood vessel proximal and distal to the stent were trimmed (transverse sections, 3 to 4 mm in thickness) using a razor blade. The portion of the vessel containing the stent was trimmed gently through the vessel wall to the outer surface of the stent wires and completely...
around the stent using a razor blade (3- to 4-mm-thick sections); the stent wires were then individually cut using small wire cutters. (b) The stent wire segments were removed from the ends of the transverse sections using fine forceps under a dissecting microscope (stereoscope) and were photographed. (c) The segments of the blood vessel (with stent wires removed) were processed using an automated tissue processor and embedded in paraffin such that cross (transverse) sections could be seen. (d) After processing and embedding, the paraffin blocks were cut at approximately 5 μm. Three slides from each of the sections were cut. One slide from each section was stained with hematoxylin and eosin; one with Masson’s trichrome stain and one with Verhoeff’s elastin stain.

Results

Three of the five arteriovenous fistulas were closed 4 weeks after stent placement (Figs 1 and 2), and remained closed and unchanged on the 8-week follow-up study. The remaining two dogs revealed patent fistulas at both 4- and 8-week intervals, but relative decrease in flow through the fistula. The carotid arteries remained widely patent in all dogs throughout the study.

The arteriovenous fistulas specimens were resected 8 weeks after stent placement.

Longitudinal sectioning with removal of half the arterial wall revealed fibrous connective tissue filling the pores of the stent (Fig 3). Cross sections through the fistula (section 3) also demonstrated dense (white) connective tissue within the fistula hole, causing complete separation of the arterial and venous lumens (Fig 4). There was a small organized thrombus that was attached to the fistula site by a small stalk.

The neointima was comparatively uniform in thickness around the arterial lumen (Fig 5). All specimens through the stent revealed fragmentation (disruption) of the internal elastic lamina caused by outward pressure of the stent wires (Fig 6). The area between the artery and vein consisted of fibrous connective tissue with fibro-
blasts, macrophages, and giant cells (Fig 7). Vascular spaces of various sizes were present in this area (Fig 7). The increased amount of mature collagen and the decreased cellularity along the fistula margins suggested that the margins of the fistula were thrombosed first. The connective tissue within the fistula on the arterial side was organized in such a fashion that the spindloid cells (fibroblasts) were oriented parallel to the endothelial surface of the artery, whereas the cells within the thrombus of the vein were much more haphazard. The significance of this lies in the body’s apparent attempt to form a more organized, properly oriented border around the artery.

Discussion

The dog was chosen for our experiment because it is one of the best experimental animals for studying the ingrowth of fibrous tissue through the wall of the porous graft. Sauvage et al implanted 1084 porous Dacron arterial grafts in experimental animals (dogs, pigs, and calves) (13). Six-centimeter grafts were used to bridge defects created by excision of 5- to 6-cm lengths of descending thoracic aorta of the dog, growing pig, mini pig, and calf. Transinterstices ingrowth refers to the movement of fibrous tissue through the wall of the porous graft. Transinterstices ingrowth occurred quickly in the pigs and calves, but more slowly in the dogs. In general, they postulate that the longer it takes for a prosthesis to heal in an experimental animal, the more probable it is that the data from that animal will be relevant in humans. Arterial prostheses heal much slower in humans than in any experimental animal thus far tested. For this reason, they prefer to use mongrel dogs.
as models because of their slower healing rate, which approximates the healing rate in humans more closely than that of other animals.

Hemorrhage within and adjacent to the stent was seen in our experiment (Fig 3). Based on information from coagulation and fibrinolytic assays, the incidents of delayed hemorrhage in the interstices of an arterial prosthesis are much higher in dogs than in humans (13). This is thought to be caused by a highly active fibrinolytic system associated with insufficient reserves of fibrinogen. Plasmin, the active fibrinolytic enzyme, often is detectable in dog blood, but seldom in human blood. Fibrinogen levels in humans do not change substantially after placement of an intraarterial graft, as opposed to in dogs. Fibrinogen levels were tested in dogs receiving micronet arterial grafts: a dramatic increase in fibrinogen level was seen in the first 4 to 6 postoperative days, followed by a large decrease (13). This decrease was followed shortly by evidence of major transinterstices hemorrhage in many cases.

Treatment of arteriovenous fistulas with coated (nonporous) stents has been attempted. Wakhloo et al used stents coated with autologous vein grafts for treatment of arteriovenous fistulas created in Labrador dogs, but his study yielded mixed results (Wakhloo A, Schumacker M, de Vries J, et al, “Coated and Noncoated Stents in Treatment of Carotid A-V Fistulas and Aneurysms: An Experimental Study,” presented at the 30th Annual Meeting of the American Society of Neuroradiology, St Louis, Mo, May 30–June 6, 1992). In stents coated with autologous vein fixed to the stent with prolene sutures, the stent was eventually encased in fibrocellular tissue without significant narrowing.
of the vessel lumen; however, lyophilized sutured vein graft and autologous vein attached with cyanoacrylate led to an early and complete thrombosis of the stented vessel 1 week after implantation because of immunologic rejection and distinct foreign-body reaction, respectively.

Proliferation of neointima and endothelium over the stent wires is a well-documented phenomenon. This neointima/endothelial layer provides a smooth, nonthrombogenic surface. In our experience, this proliferative tissue response may be sufficient to cover partially or completely the pores of a stent (14).

Schatz et al studied the acute and chronic biological changes caused by balloon-expandable intracoronary stents in the adult mongrel dog (11). They placed 20 stainless steel stents in the coronary arteries of 20 dogs. Angiography was performed at 1, 3, 6, and 12 months. The animals were killed in groups of three at 1, 3, 8, and 32 weeks for gross, light, and electronmicroscopic analysis. All stents were patent, and there was no evidence of myocardial infarction. The stent was covered initially by a thin layer of thrombus that was later replaced by neointimal muscular proliferation that reached maximal thickness by 8 weeks. Histopathologic analysis at 1 week revealed that the struts of the stent had compressed the arterial wall; and the indentations were filled with thrombus material largely composed of red cells and fibrin covered by immature endothel-

Fig 5. A, Cross section through carotid artery (approximately 4 mm from the fistula) after removal of the stent wires. The neointima lies deep to the stent wires (holes) and encircles the inner lumen of the artery (Verhoeff's van Gieson's elastic stain, magnification × 3.3). B, The stent wires (holes) depressed the elastic lamina. Note the fragmentation (disruption) of the internal elastic lamina (arrows) caused by the outward pressure of the stents wires (Verhoeff's van Gieson's elastic stain, magnification × 33).

Fig 6. Cross section through the fistula. The area between the artery and vein consists of fibrous connective tissue with fibroblasts and macrophages. Vascular spaces (arrows) of various sizes lie within the fibrous connective tissue filling the fistula hole (Verhoeff's van Gieson's elastic stain, magnification × 6.6).

Fig 7. Note that the margin of the fistula (large arrows) contains blue material (collagen) that is darker (more mature and less cellular) than the less mature cellular fibroblasts in the central area (arrowheads). This probably indicates that the margins of the fistula were thrombosed (and subsequently organized) first. The connective tissue around the artery is organized in such a fashion that the spindloid cells (fibroblasts) are oriented parallel to the endothelial surface of the artery, whereas the cells within the thrombus of the vein are much more haphazard. This may represent an attempt by the body to form a more natural, organized, properly oriented border around the artery (Masson's trichrome stain).
lium. In the 3-week specimen, the neointima covering the stent was more cellular, and hemosiderin-laden macrophages partially replaced the underlying thrombus. At 8 weeks, the degree of intimal hyperplasia peaked because of fibroblast proliferation, so that intimal thickness was greatest; the amount of underlying residual thrombus was minimal. By 32 weeks, the neointima was thinnest and appeared less cellular. Schatz's results indicated that the early thrombus covering the stent wires is replaced ultimately by a thin layer of neointima that is grossly indistinguishable from that of adjacent nonstented artery.

Palmaz et al demonstrated total early endothelialization of intravascular stents in 1 to 3 weeks in the peripheral vessels (15–17). Excessive intimal hyperplasia was a rare finding. The biological effects of vascular implants are related to phenomena that occur at the interface between the host tissue and the foreign implant material (10, 18). The series of events after stent placement facilitates the growth of endothelial cells over the surface of the stent (19). When observed on cross sections of the stented arterial wall, replacement of thrombus by fibromuscular intima occurred first around the stent struts and then expanded eccentrically. Fibroblasts in direct contact with the metal surface develop physical attachment. It has been suggested that the attachment occurs between the cell membrane and the metal oxide layer through an intervening proteinaceous film. Eight weeks after stent placement, when thrombus has been replaced by fibromuscular tissue, neovascularization is present and is more dense around stent struts than near the pores between the struts.

At times this proliferative tissue reaction can lead to significant narrowing of the intraluminal diameter of the vessel; however, stent-related stenosis was not present in our specimens. Stenosis of the stented vessel is related to cellular proliferation within the vessel wall, stimulated by interactions between platelets adherent to damaged intima, endothelial cells, and smooth muscle cells (20, 21). Endovascular placements of stents may induce this process either by outward pressure of the stent, as seen with the self-expandable stents (Wallstent and Gianturco Z stent), or as a result of inflation of a delivery balloon (Palmaz, Streecker, Gianturco-Robin). Intimal fibrocellular tissue growth is a known phenomenon of implantable stents. Waxhloo et al compared balloon-expandable tantalum stents and self-expanding nitinol stents for the treatment of carotid aneurysms in Labrador dogs (22). The stents consisted of elastic, loosely connected woven loops made of biocompatible tantalum or nitinol filaments, 100 μm thick. Nine-month angiographic follow-up revealed maximal stenosis of the stented vessel segment of up to 40% after placement of the tantalum stent; however, no more than 15% stenosis followed placement of the nitinol stents. Significantly greater intimal fibrocellular tissue growth surrounded a tantalum filament when compared with the nitinol filaments. The stent-induced stenosis was usually located at the distal rather than the proximal portion of the stented segment. This was appreciated 3 weeks after implantation of the stent and persisted over 6 months. Histologic examination revealed thick intimal fibrocellular proliferative tissue surrounding the tantalum filaments (300 to 350 μm). The nitinol filaments were covered with a smooth and regular neointimal layer of approximately 150 μm. Few hemosiderin-laden macrophages were present in the fibrotically thickened intimal wall underlying either type of stent, and the media of the stented vessels revealed minor signs of atrophy. The proposed theory is that intimal-media injury caused by dilating the stent toward the arterial wall may induce this fibroproliferative reaction. The greater proliferative reaction in the vessels containing the balloon-expandable tantalum stent versus the self-expanding nitinol prosthesis may be caused by the intimal-medial injury induced by the dilating balloon.

Duprat et al assessed vessel patency in stented arteries with their computed stent:artery ratio (8). The stent:artery ratio was computed using the diameter of the stent when fully expanded and the angiographic diameter of the artery to be stented. Arterial diameter was determined by measuring the vessel on a selective arteriogram made immediately before the stent was inserted. Good vascular patency rates were observed when the stent:artery ratio was less than or equal to 1.2. A stent:artery ratio greater than 1.2 was associated with spasm, immediate or delayed thrombosis, or excessive intimal proliferation. In another experiment by Duprat et al small stents (2.5-mm diameter unconstrained) were placed in small vessels with similar diameters in four mongrel dogs (9). A fibrocellular proliferation of the intima and media was re-
sponsible for a 20% to 25% luminal narrowing in all vessels. This proliferative response was associated with multiple disruptions of the internal elastic membrane and occasional intimal splits with associated intimal hemorrhage.

Plugging of the stent pores with neointimal tissue seems more likely if the density of the woven wire network is greater and the pores between the wire filaments are smaller. For this reason, we chose the Wallstent for our experiment. It is constructed with 20 wires, 0.0035-in diameter; the pore dimension is 1.17 mm in length by 1.53 mm in width; the surface area covered by the stent wire is 17.67%, with the surface free area (open space) being 82.33%. This results in a pore density of 97.6 pores per square centimeter at 5-mm diameter. In comparison, the balloon-expandable tantalum stent has a pore density of 34.7 pores per square centimeter at 5-mm diameter; the self-expanding nitinol stent, made of heat-treated nickel-titanium alloy, has a density of 62.4 pores per square centimeter at 5-mm diameter (22). The greater pore density of the Wallstent results in smaller pores, with a greater number of wire segments per given length, and thus provides a more favorable substructure upon which organized fibrous tissue can proliferate.

The purpose of this study was not to determine whether stents may act as a source of emboli. The study of thromboembolic disease caused by stent implantation was beyond the scope of this work. The possibility of stents acting as a potential source of emboli exists. Endothelialization of stent wires several weeks after implantation has been documented (15, 16). This endothelium provides a smooth, protective, nonthrombogenic surface. Previous studies have suggested that stent-induced thrombus formation can be avoided successfully with systemic anticoagulation (23, 24).

An endovascular stent constructed for neurorinterventional applications has not yet been developed. The 7F introducer catheter with affixed stent used in our study would be much too stiff to negotiate the curvature of the cervical/intracranial carotid artery of humans. A minified version, perhaps 4F, with great flexibility and trackability, would need to be developed before stents could be considered seriously for the treatment of intracranial vascular disease.

In conclusion, the proliferation of fibrous tissue, collagen, and endothelium is a known phenomenon after intravascular implantation of a vascular graft or stent. Placement of a tubular woven wire mesh (stent) across a fistula hole provides the foundation for this fibrous tissue process to occur. The result was either complete or near-complete closure of the fistulous communication between the carotid artery and jugular vein. Although preliminary, these results may stimulate interest in further development in stent technology, with particular focus on neurorinterventional applications.

References


Please see the commentary on page 1974 in this issue.