Improved Microsurgical Creation of Venous Pouch Arterial Bifurcation Aneurysms in Rabbits

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BACKGROUND AND PURPOSE: The choice of the experimental aneurysm model is essential for valid embolization-device evaluations. So far, the use of the rabbit venous pouch arterial bifurcation aneurysm model has been limited by demanding microsurgery, low aneurysm patency rates, and high mortality. This study aimed to facilitate microsurgery and to reduce mortality by optimized peri-/postoperative management.

MATERIALS AND METHODS: Aneurysms were created in 16 New Zealand white rabbits under general intravenous anesthesia. Using modified microsurgical techniques, we sutured a jugular vein pouch into a bifurcation created between both CCAs. Aggressive anticoagulation (intraoperative intravenous: 1000-IU heparin, 10-mg acetylsalicylic acid/kg; postoperative subcutaneous: 14 days, 250-IU/kg/day heparin) and prolonged postoperative anesthesia (fentanyl patches: 12.5 μg/h for 72 hours) were applied. Angiographic characteristics of created experimental aneurysms were assessed.

RESULTS: The reduced number of interrupted sutures and aggressive anticoagulation caused no intra-/postoperative bleeding, resulting in 0% mortality. Four weeks postoperation, angiography showed patency in 14 of 16 aneurysms (87.5%) and Ohshima type B bifurcation geometry. Mean values of parent-artery diameters (2.3 mm), aneurysm lengths (7.9 mm), and neck widths (4.1 mm) resulted in a mean 1.9 aspect ratio.

CONCLUSIONS: Compared with historical controls, the use of modified microsurgical techniques, aggressive anticoagulation, and anesthesia resulted in higher aneurysm patency rates and lower mortality rates in the venous pouch arterial bifurcation aneurysm model. Gross morphologic features of these aneurysms were similar to those of most human intracranial aneurysms.

ABBREVIATIONS: CCA = common carotid artery; DSA = digital subtraction angiography
that this chronic inflammatory reaction to nonresorbable sutures is locally confined to the outer parts of the respective vessel walls. What is more, the aneurysm wall in the venous pouch arterial bifurcation model consists of an unaltered vein transplant with just a few elastic membranes. These features can also be seen in human intracranial aneurysm walls. The major shortcomings, resulting in less frequent use of this aneurysm model in recent years, are the high demands of the microsurgical technique, low aneurysm patency rates, and high morbidity rates. The present study aimed to show that these shortcomings could be minimized by facilitating microsurgical procedures and improved peri-/postoperative management.

Materials and Methods
The aneurysms were created in 16 female New Zealand white rabbits (body weight, 3.1–4.7 kg). All surgical procedures were performed at the Department of Clinical Research at the University of Berne. The study was approved by the responsible veterinarian agency of the Canton of Berne, Switzerland.

Anesthesia
All rabbits were premedicated with subcutaneous injection of 65-mg/kg xylazine and 4-mg/kg ketamine and received, preoperatively, a single dose of antibiotics (50-mg penicillin) intravenously. For general anesthesia, a solution of 130-mg/kg xylazine and 8-mg/kg ketamine in 100-mL 0.9% saline was intravenously administered via a perfusor with a rate of 1 mL/s. Animals received oxygen (1 L/min) via ventilation mask.

Microsurgical Aneurysm Creation
The animals were fixed in a supine position on a body warming plate. All operative procedures were performed under sterile conditions. A midline incision was made from the manubrium sterni up to the jaw. The following microsurgical procedures were performed by using an operation microscope: First, a 1.3-cm-long segment of the left external jugular vein without venous branches was prepared, ligated proximally and distally with 4–0 silk (Ethilon 4–0; Ethicon, West Somerville, New Jersey), resected, and kept in heparinized saline (a mixture of 1000-IU heparin in 20-mL 0.9% saline and 1-mL papaverin HCl 4%). The left CCA was then prepared over a distance of approximately 5 cm, starting from the aortic arch to the carotid bifurcation. Tiny arterial branches running medially and supplying laryngeal and tracheal structures and neural structures were preserved. The right CCA was then also isolated and mobilized up to the carotid bifurcation and proximally down to the brachiocephalic branching. Then the animals received 1000-IU heparin intravenously. To obtain a long donor artery for a tensionless anastomosis, we temporarily clipped the right CCA distally just below the carotid bifurcation and then proximally ligated it above the brachiocephalic branching and cut it just above this ligature. This stump was extensively irrigated with heparinized saline as described above, and the adventitia at the cut free end was carefully resected.

The segment of the left CCA planned for the anastomosis was also meticulously freed of the adventitia and then temporarily clipped distally and proximally. Between the clips, an elliptic arteriotomy was performed to accommodate the circumferences of the right CCA and the venous pouch, and then this portion between the clips was also extensively irrigated with heparinized saline to remove intraluminal clots. The posterior circumference of the right CCA stump was now sutured into the arteriotomy in the left CCA, by using 4–5 nonresorbable monofilament sutures (Ethilon10–0; Ethicon). Then a longitudinal cut was made in the stump of the right CCA to accommodate half the circumference of the venous pouch. The back side of the venous pouch wall was now first anastomosed with the arteriotomy in the left CCA by again using 4–5 sutures, and then it was anastomosed with the cut in the right CCA with 3–4 sutures (Fig 1A). The same procedures were performed in the same order at the anterior side of the anastomosis (Fig 1B). Then the distal end of the venous pouch was clipped, and the distal clips on the right CCA were removed. After prompt filling of the aneurysm, trapped air and debris were washed out by opening the clip at the dome of the venous pouch, which was then tightly sealed with a 4–0 polyfilament suture (Vicryl, Ethicon). The suture lines around the anastomosis and the aneurysm neck were then covered with small pieces of adipose tissue for additional hemostasis (Fig 2A). During the operation, 4% papaverin HCl solution and antibiotic solution (neomycin sulfate, 5 mg/mL) was frequently ap-
plied topically on the anastomoses to prevent vasospasm and local infections.

For deep and superficial wound closure, 4–0 resorbable sutures (Monocryl, Ethicon) were used.

**Postoperative Management**

Immediately after we finished the operation, all animals received 10-mg/kg acetylsalicylic acid intravenously and 60-mL 5% glucose subcutaneously to compensate for dehydration during surgery. For prolonged anesthesia, transdermal fentanyl matrix patches releasing 12.5 μg/h were applied for 72 hours in the shaved neck region of the animals. All animals received daily 250-IU/kg low-molecular heparin subcutaneously for 2 weeks.

**Angiographic Follow-Up**

Four weeks postoperatively, all animals underwent DSA, performed with the animal under general anesthesia, by using standard techniques via a transfemoral approach.21 To assess the gross morphologic characteristics, we measured the aneurysm parameters, total length, neck width, and parent-vessel diameters (at the level of the aneurysm orifice) on DSA images and calibrated them by paravertebraally placed skin clamps. The aneurysm aspect ratio (length/neck width) was calculated.25 Additionally, the distance of the neck plane center from the contour of the parent artery was measured (Fig 2) to determine the Ohshima-type bifurcation aneurysm.26

All animals showing a patent aneurysm at follow-up were not killed but were included in ongoing experiments at the Department of Clinical Research of the University of Berne.

**Results**

With the modified microsurgery, on average, 150 minutes were needed for the operations, decreasing from 225 to 115 minutes in the course of the experimental series. The duration of the clamping time of both CCAs was, on average, 65 minutes, decreasing from approximately 120 to 45 minutes. Altogether, on average, 22 interrupted sutures (range, 20–29) were needed to create the anastomoses and aneurysms.

Perioperative and postoperative mortality was 0%. Despite intra- and postoperative high-dose heparinization, no spontaneous bleeding occurred at the site of the anastomoses. No postoperative wound infections and no gastric stress ulcer were observed in the present series; however, 1 animal (the heaviest at 4.7 kg) was impaired by slight hemiparesis but survived until follow-up.

**Angiographic Follow-Up**

Four weeks postoperatively, DSA showed patency of both CCAs and the aneurysms in 14 of 16 animals (87.5%), while aneurysm occlusion was observed in the hemiparetic animal (4.7 kg) and another animal (4.5 kg). Both aneurysms were created, however, at the beginning of the series (aneurysms 1 and 8). As measured on the calibrated DSAs, parent-artery diameters ranged between 1.6 and 2.7 mm (mean, 2.3 mm), the mean aneurysm length was 7.9 mm (range, 7.4–8.6 mm), and the mean aneurysm neck width was 4.1 mm (range, 3.7–4.8 mm). These measurements resulted in a mean aspect ratio of 1.9 (range, 1.67–2.08). The neck orifice positioning in relation to the parent-artery axis (Fig 2B) showed a mean distance of 1.7 mm (range, 1.3–2.1 mm), thus corresponding to Ohshima type B aneurysms (Table). In human aneurysms, this type B geometry is statistically significantly correlated with a high risk of aneurysm rupture.26

**Discussion**

The results of the present study favor the venous pouch arterial bifurcation aneurysm model in rabbits. Unfortunately, in the available literature on this model, only general descriptions of the operative technique can be found, without special emphasis on surgical details or the importance of peri- and postoperative management strategies.4,21,24,27 In the authors’ opinion, the following factors play key roles in facilitating microsurgery and improving the outcome of this experimental aneurysm creation:

1) Careful preparation of a long segment of the left CCA, but without destruction of the superior laryngeal nerves and small vessels.

2) Careful removal of the highly thrombogenic adventitial connective and fatty tissue from both parent arteries at the planned junction before the creation of the anastomosis.

3) Creation of a tensionless anastomosis between both CCAs. Apparently, it makes no difference that in the present series, the right CCA was anastomosed to the left CCA, in contrast to the originally described and later modified procedure.21
4) Reduction of the number of sutures and adjustment of the sequence of suturing around the anastomosis. The number of necessary sutures could be reduced to an average of 22, compared with the ≥32 sutures reported in the literature.21,24 For the sequence of suturing, the proposed beginning at the posterior aspect of the anastomosis (Fig 1) gives better visual control of these most difficult sutures, in contrast to previously proposed procedures21,24,27

5) The intimal layer around the anastomosis may not be penetrated by sutures, to avoid intimal damage causing thrombosis in the parent arteries. A reduced number of sutures also causes less injury to the vessel walls, further minimizing the risk of aneurysm thrombosis or embolism.

6) Sealing of the outside of the suture lines with a thin layer of fatty tissue proved important to avoid bleeding from the anastomoses, despite the reduced number of sutures and the aggressive anticoagulation regimen, as discussed below.

7) A reduced number of sutures results in shorter clamping times of both CCAs and shorter operation times, reducing the risk of neurologic deficits and complications from anesthesia.

**Improved Anticoagulation Management**

With the prolonged and more aggressive anticoagulation regimen, a high 87.5% aneurysm and parent-vessel patency could be achieved, but without the incidence of spontaneous aneurysm hemorrhage. These findings go along with Grunwald et al29 during elastase aneurysm creation in an extremely large model.6 Gross morphologic features of the created aneurysms are similar to those of most human intracranial aneurysms.

Conclusions

The improved venous pouch arterial bifurcation aneurysm model using modified microsurgical techniques, aggressive anticoagulation, and anesthesia resulted in aneurysm patency rates and mortality rates comparable with those of the rabbit elastase model. Gross morphologic features of the created aneurysms are similar to those of most human intracranial aneurysms.
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References
1. Molyneux AJ, Kerr RS, Yu LM, et al. International Subarachnoid Aneurysm Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: a randomised comparison of effects on survival, dependency, seizures, rebleeding, subgroups, and aneurysm occlusion. Lancet 2005;366:809–17
2. Mitchell P, Kerr R, Mendelow A, et al. Could late rebleeding overturn the superiority of cranial aneurysm coil embolization over clip ligation seen in the International Subarachnoid Aneurysm Trial? J Neurosurg 2008;108:437–42
3. Bavinzski G, Richling B, Binder BR, et al. Histopathological findings in experimental aneurysms embolized with conventional and thrombogenic/anti-thrombolytic Guglielmi coils. Minim Invasive Neurosurg 1999;42:167–74
4. Bocher-Schwarz HG, Ringel K, Bohl J, et al. Histological findings in coil packed experimental aneurysms 3 months after embolization. Neurosurgery 2002;50:379–84, discussion 384–85
5. Sherif C, Plenk H Jr, Grossschmidt K, et al. Computer-assisted quantification of occlusion and coil densities on angiographic and histological images of experimental aneurysms. Neurosurgery 2006;59:539–66, discussion 559–66
6. Bouzeghrane F, Naggara O, Kallmes D, et al. Creation of four experimental aneurysms: a systematic review. AJNR Am J Neuroradiol 2010;31:418–23. Epub 2009 Oct 29
7. Massoud T, Guglielmi G, Ji C. Experimental saccular aneurysms. 1. Review of surgically constructed models and their laboratory applications. Neuroradiology 1994;36:537–46
8. Anidjar S, Salzmann J, Gentic D. Elastase-induced experimental aneurysms in rats. Circulation 1990;82:973–81
9. Wakhloo A, Schellhammer F, de Vries J. Histopathologic and immunohistochemical comparison of human, rabbit, and swine aneurysms embolized with platinum coils. AJNR Am J Neuroradiol 2005;26:2560–68
10. Shin YS, Niihi Y, Yoshino Y, et al. Creation of four experimental aneurysms with different hemodynamics in one dog. AJNR Am J Neuroradiol 2005;26:1764–67
11. Abruzzo T, Shengelaia GG, Dawson RC 3rd, et al. Histologic and morphologic comparison of experimental aneurysms with human intracranial aneurysms. AJNR Am J Neuroradiol 1998;19:1309–14
12. Dai D, Ding Y, Danielson M, et al. Endovascular treatment of experimental aneurysms with use of fibroblast transfected with replication-deficient adenovirus containing bone morphogenic protein-13 gene. AJNR Am J Neuroradiol 2008;29:739–44. Epub 2008 Jan 9
13. Grunwald IJ, Romeike BF, Roth G, et al. Anticoagulation regimes and their influence on the occlusion rate of aneurysms: an experimental study in rabbits. Neurosurgery 2005;57:1048–55, discussion 48–55
14. Cavley CM, Dawson RC, Shengelaia G, et al. Arterial saccular aneurysm model in the rabbit. AJNR Am J Neuroradiol 1996;17:1761–66
15. Krings T, Moeller-Hartmann W, Hans PJ, et al. A refined method for creating saccular aneurysms in the rabbit. Neuroradiology 2003;45:423–29
16. Cruise G, Shum J, Plenk H. Hydrogel-coated and platinum coils for intracranial aneurysm embolization compared in three experimental models using computerized angiographic and histologic morphometry. J Mater Chem 2007;17:5965–73
17. Yoneyama Y, Fandino J, Taub E. Surgical therapy. In: Fisher M, Borggrefe M, eds. Current Review of Cerebrovascular Disease. Philadelphia: Current Medicine; 2001:254
18. Grunwald IQ, Romeike BF, Roth C, et al. Anticoagulation regimes and their influence on the occlusion rate of aneurysms: an experimental study in rabbits. Neurosurgery 2005;57:1048–55, discussion 48–55
19. Yoneyama Y, Fandino J, Taub E. Surgical therapy. In: Fisher M, Borggrefe M, eds. Current Review of Cerebrovascular Disease. Philadelphia: Current Medicine; 2001:254
20. Sherif C, Marbacher S, Fandino J. High-resolution three-dimensional MT magnetic resonance angiography for the evaluation of experimental aneurysms in the rabbit. Neuroradiology 2009;51:217–23. Epub 2009 Feb 12
21. Bavinzski G, Al-Schameri A, Kilner M, et al. Experimental bifurcation aneurysm: a model for in vivo evaluation of endovascular techniques. Minim Invasive Neurosurg 1998;41:129–32.
22. Plenk H, Shum J, Cruise G, et al. Cartilage and bone neof ormation in rabbit carotid bifurcation aneurysms after endovascular coil embolization. Eur Cell Mater 2008;16:69–79
23. Sherif C, Bavinzski G, Dorfer C, et al. Computerized assessment of angiographic occlusion rate and coil density in embolized human cerebral aneurysms. AJNR Am J Neuroradiol 2009;30:1046–53
24. Spetzger U, Reul J, Weis J, et al. Microsurgically produced bifurcation aneurysms in a rabbit model for endovascular coil embolization. J Neurosurg 1996;85:488–95
25. Lall R, Edidumen C, Bendok B, et al. Unruptured intracranial aneurysms and the assessment of rupture risk based on anatomical and morphologic factors: sifting through the sands of data. Neurosurg Focus 2009;26:E2:1–5
26. Ohshima T, Miyachi S, Hattori K, et al. Risk of aneurysm rupture: the importance of neck orifice positioning—assessment using computational flow simulation. Neurosurgery 2008;62:767–75
27. Forrest MD, O’Reilly GV. Production of experimental aneurysms at a surgically created arterial bifurcation. AJNR Am J Neuroradiol 1989;10:400–02
28. Grunwald I, Romeike B, Roth C, et al. Anticoagulation regimes and their influence on the occlusion rate of aneurysms: an experimental study in rabbits. Neurosurgery 2005;57:1048–54
29. Lewis D, Ding Y, Dai D, et al. Morbidity and mortality associated with creation of elastase-induced saccular aneurysms in a rabbit model. AJNR Am J Neuroradiol 2009;30:91–94
30. Hoh BL, Rabinov JD, Pryor JC, et al. A modified technique for using elastase to create saccular aneurysms in animals that histologically and hemodynamically resemble aneurysms in humans. Acta Neurochir (Wien) 2004;146:705–11
31. Cloft HJ, Altes TA, Marx WF, et al. Endovascular creation of an in vivo bifurcation aneurysm model in rabbits. Radiology 1999;213:223–28