Improving the Results of Small-Bore Vascular Graft Testing in Rabbits: A Technical Primer

FILIP PEKAR, MD, ALBERT C. GROBE, MS, PATRICIA A. WEBER, DRPH, DAVID T. CHEUNG, PHD, AND CARLOS M. G. DURAN, MD, PHD*

Appropriate models to evaluate the in vivo behavior of small-diameter grafts are varied. To evaluate the behavior of small-diameter, bovine-derived grafts in the arterial circulation, we chose the rabbit abdominal aorta model. In the development of our procedure, we evaluated several models published in the literature, with unsatisfactory results. The high incidence of postoperative mortality and morbidity led us to modify published methods to incorporate cautious surgical technique and mild systemic hypothermia with cross-clamp times shorter than 30 min, as well as perioperative administration of agents with metabolic, rheologic, and neuroprotective properties. These modifications enabled us to achieve 100% operative survival with a very low incidence of postoperative paralysis. The presented model will be used for further evaluation of small-diameter grafts in our laboratory.

(As requested by an anonymous reviewer, and accepted by the authors, this manuscript is published while noting that the method of anesthesia is considered to be less than optimal for current standards for research animal anesthesia. Specifically, 1) the intramuscular route of administration of ketamine poses a higher risk of tissue inflammation than do the subcutaneous or intravenous routes; 2) endotracheal intubation and adequate ventilation are preferred to spontaneous ventilation without intubation during major abdominal surgery; 3) additional monitoring of rabbits, such as the use of pulse oximetry and other methods is desirable; and 4) the current report utilized an analgesic dose of 2.3 mg/kg BID which is more than twice the recommended does of 1.1 mg/kg SID or BID. Routine use of a high dose of this analgesic may increase the risk of nephrotoxicity. –Editor).

Vascular grafts represent a large component of all medical device implantations performed worldwide (1), with clinical applications that include coronary artery revascularization, peripheral vascular surgery, and arteriovenous shunt construction. The rabbit model has been used quite extensively for the in vivo evaluation of small-bore (< 6 mm) vascular grafts (2-6). The thrombogenic properties of rabbit blood are similar to those of humans (5, 7), as are the thromboplastic and fibrinolytic properties of rabbit arteries (7, 8), making the rabbit an ideal model for experimental small-diameter vascular graft studies. The rabbit also develops intimal hyperplasia as a result of vascular injury, which is a recognized mode of failure for small-bore grafts implanted in humans (9). Furthermore, the ease of animal handling, size, and cost effectiveness make this model attractive. To establish this model in our laboratory, we adapted existing models to minimize potential perioperative risks.

Materials and Methods

Animal source and care. Female New Zealand White rabbits (*Oryctolagus cuniculus*) were obtained from Western Oregon Rabbit Company (Philomath, Oreg.) and housed two per cage. Rabbits were allowed to acclimate for at least 14 days before undergoing any procedures and had normal rabbit chow (Laboratory Rabbit Diet HF, #5326, Lab Diet, Richmond, Idaho) and water ad libitum throughout the duration of the study. Average room temperature was 17 to 18°C, with a 12:12-h day:night cycle and 10 to 12 air changes/h. All animals appeared clinically normal throughout the 14-day quarantine period, and no other pathogen testing was done. Animals were cared for in accordance with the *Principles of Laboratory Animal Care* formulated by the National Society of Medical Research and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The use of the animals for this project was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Montana. The submitted protocol also included criteria for analgesia and euthanasia.

Preoperative preparation. Rabbits 4.3 ± 0.3 kg (mean ± SD, range 3.8-4.8 kg) at the time of surgery were sedated with an intramuscular dose of ketamine (50 mg/kg), the abdominal region shaved, and the rabbit placed in dorsal recumbency on a heating pad. The heating pad was not switched on during the initial phase of the surgery. Anesthesia was induced and maintained with 1.5-2% isoflurane in oxygen mixture administered via a ventilator and anesthesia delivery system (North American Drager, Telford, Pa.); the level of isoflurane was adjusted as needed to keep the animal sufficiently anesthetized, as evaluated by ear pinch or any evidence of discomfort. Because we did not open the chest cavity, we chose to allow for passive, spontaneous ventilation by the animal (10, 11). The temperature was monitored with a rectal temperature probe, and electrocardiographic leads were attached so the heart rate could be monitored. Ice bags were placed around and between the hind legs and along the lower flanks. An intravenous cannula (22-gauge) was introduced into the marginal ear vein, and cold (4°C) supplemented Lactated Ringers solution was given. The supplemented Lactated Ringers solution contained per liter: 20 ml sodium bicarbonate (84 mg/ml and 50 mEq/50ml, American Pharmaceutical Partners, Inc. Los Angeles, Calif.), 50 ml of 20% mannitol, and 0.5 ml dexamethasone sodium phosphate (4 mg/ml, American Regent Laboratories, Shirley, N.Y.).

Surgical protocol. After sterile draping, a midline laparotomy was performed. The skin was retracted with traction sutures, and the intestines were displaced to the right side of the animal and wrapped in lap sponges moistened with cold sterile saline. Isolation of the abdominal aorta from the surrounding tissues was meticulously performed to avoid accidental injury to the inferior vena cava and its branches. Care was taken to identify all aortic branches along the entire circumference. A 5-cm-long segment of the abdominal aorta was isolated below the left renal artery and proximal to the iliac bifurcation. If it was feasible, anastomotic sites were planned to avoid cutting of the large dorsal aortic branches. One ml of heparin (1000 U/ml, American Pharmaceutical Partners, Inc., Los Angeles, Calif.) was administered intravenously, and further cooling of the animal was achieved by using sterile saline slush applied directly into the abdominal cavity and to the lap sponge covering the intestines.

The International Heart Institute of Montana Foundation at St. Patrick Hospital and Health Sciences Center and the University of Montana

**Corresponding author*

When the rectal temperature of the animal reached 34.5°C, the ice packs and the saline slush were removed, as the temperature continued to drop without additional cooling. Dorsal aortic branches within the previously prepared aortic segment were clipped and cut. The aorta was occluded proximally and distally with microsurgical vascular clamps (MORIA 200/A, angled, 30 mm, Fine Science Tools, Inc., Foster City, Calif.) placed below the left renal artery and above the inferior mesenteric artery, and because of the elastic recoil of the aorta, it was cut approximately 1 cm from each clamp to avoid space issues.

All animals had a 3- to 4-mm internal diameter bovine-derived conduit implanted into the infrarenal abdominal aortic position. An end-to-end distal anastomosis was performed with a continuous, single 8-0 Prolene suture (Polypropylene, Ethicon, Inc., Somerville, N.J.). On completion of the distal anastomosis, the cold supplemented Lactated Ringers solution was exchanged for warm (37°C), warm saline packs were placed next to the rabbit under the sterile draping, and the heating pad under the rabbit was switched on to start the rewarming phase of the procedure. The distal microvascular clamp was removed, and a 2.5- to 3-mm Cooley Dilator (Pilling Surgical Instruments, Fort Washington, Pa.) was inserted through the graft to check the patency of the distal anastomosis, the clamp was reapplied, and the proximal anastomosis was performed in the same manner. Before tying of the suture, the distal vascular clamp was removed to flush air from the graft by filling the graft with blood. After the suture was tied, the proximal vascular clamp was removed, and additional 8-0 Prolene suture used as needed to control anastomotic or graft bleeding.

After checking for bleeding, the presence of a pulse was verified by direct observation and palpation. The intestines were returned to the abdominal cavity and repeatedly lavaged with warm sterile saline to achieve appropriate rewarming of the animal. When the rectal temperature of the rabbit reached 36.5°C, the saline was removed and the laparotomy closed in two layers with 3-0 Vicryl (Polygalactin 910, Ethicon, Inc.). The supplemented Lactated Ringers solution was discontinued, with a total amount of 100 to 150 ml having been administered for both the cold and warm solutions. All rabbits received 0.2 ml flunixin meglumine (50 mg/ml, Equileve, Phoenix Scientific, Inc., St. Joseph, Mo.) intramuscularly as an analgesic immediately after surgery.

The animal was covered with towels and kept between two heating pads with warm packs to rewarm it to physiological temperature (38 to 39°C) before being transferred to a cage. The rabbits were encouraged to eat and to move their hind limbs throughout the rewarming period. The rabbits received 0.2 ml flunixin meglumine intramuscularly twice daily for 4 days postoperatively and were monitored for neurologic or ischemic complications of the hind limbs throughout the study. No antibiotic prophylaxis, platelet aggregation inhibitors, or anticoagulants were used postoperatively.

Results

Twelve female New Zealand White rabbits underwent surgery, and a small-diameter bovine conduit (internal diameter, 3 to 4 mm) was implanted into the infrarenal abdominal aorta. The average length of the implanted graft was 4.4 ± 0.3 cm (Mean ± SD, Range 3.6-4.7 cm) with an average cross-clamping time of 20.6 ± 2 min (mean ± SD, range 17-29 min). All grafts were patent immediately after implantation, as demonstrated by a visible and palpable pulse, and at euthanasia at 2, 4, or 6 months.

Hind limb paraparesis developed in one rabbit within the first postoperative day, and the animal died on the third postoperative day. Postmortem examination did not show graft occlusion, and the paraplegia most probably was a result of spinal cord ischemia. A second animal developed bilateral dry ulcerations of the footpad. A third animal died suddenly at 5 months, and post-mortem examination revealed intestinal ischemia and graft thrombosis.

Discussion

The implantation of small-diameter vascular grafts, either of human or synthetic origin, is performed daily in current human medical practice, and the number of vascular grafts implanted annually exceeds one million throughout the world. In the United States alone, 571,000 coronary artery bypass grafts were performed in 1999 (12). Autologous arteries and veins still represent the best treatment option, however pre-existing vascular diseases or previous cardiovascular procedures emphasize the need for a safe, durable, and long-term alternative graft of an acceptable diameter. Advances in tissue engineering may ultimately provide material meeting most of these optimal graft characteristics.

The rabbit model has been used quite extensively to study the properties of small-diameter (< 6 mm) graft implants under various conditions. Postoperative ischemic spinal cord injury resulting in paraplegia represents a serious complication after surgical procedures on the abdominal aorta and limits the use of small-diameter graft implantations at this site in rabbit models. Early failure of the procedure may also be the result of technical problems such as hemorrhage, anastomotic tearing or leaks, prosthetic graft thrombosis, and infection.

During the development phase of the presented model, we explored the methodology from previously published models. The pilot group consisted of 11 rabbits (data not shown), and the basic surgical protocol put forth by Nordestgaard et al. (6) was used. Further modification by the addition of hypothermia (5) and supplemented Lactated Ringers solution (13) brought us to our final surgical technique, which was used for the remainder of the graft implantations (the 12 animals presented in this paper).

Initially we used mechanical retractors to expose the field, which was discontinued after post-mortem examination of several animals that died early revealed the presence of unilateral subcapsular hematomas of the right kidney. We speculated the hematoma was a result of direct pressure on the kidney by the retractor, and we substituted retracting sutures with satisfactory results.

The surgeries initially were performed under normothermic conditions. After several cases of postoperative paraplegia and death thought to be related to normothermic ischemic injury of the spinal chord, we decided to apply controlled mild hypothermia (32-34°C). Mild hypothermia previously had been shown to offer pronounced protection from ischemic spinal chord injury with increased recovery of motor function (5, 11, 13) and reduced metabolic demands during the ischemic period (13). The mild hypothermia was achieved by external and internal cooling of the animal by the use of ice bags, cold intravenous Lactated Ringers, and saline slush. The Lactated Ringers solution contained sodium bicarbonate, mannitol, and dexamethasone sodium phosphate to aid in controlling metabolic acidosis, ischemic cellular swelling, and inflammation, respectively. This solution is similar to cardioplegia formulations used during bypass surgery to protect the heart from ischemic damage, and we also took into account the considerable neuroprotective effects of these agents.

We initially performed an end-to-side type of anastomosis, with the intention of sparing the spinal aortic branches from the abdominal aorta by ligating the aorta between the anastomoses. The decreased technical performance of an end-to-side anastomosis, prolonged cross-clamping time of the aorta, and the possibility of anastomotic occlusion by thrombus extension from the point of the tied native aorta eliminated this option. Rather, end-to-end anastomoses were performed at both ends, in combination with previously

mentioned changes. The continuous suture pattern was chosen to minimize the cross-clamp time and is routinely used in human patients. An interrupted suture pattern is more commonly used with smaller grafts under conditions where there is a possibility of producing a stenosis at the anastomosis.

A concern has been raised that flunixin might have an antiplatelet activity and could therefore be serving as an anticoagulant. Information available in the literature suggests that Flunixin (an NSAID and COX 1 & 2 inhibitor) may affect platelet aggregation via the nonspecific inhibition of COX, which results in the inhibition of prostaglandin and thromboxane production from arachidonic acid (14). Prostaglandin I2 is inhibitory for platelet aggregation, whereas thromboxane A2 is involved in the aggregation of platelets (15), and this combined action may exert an anticoagulant effect. However, NSAIDs do not affect the production of platelet activating factor, which is also involved in platelet aggregation.

We achieved a 100% immediate patency rate and low incidence of postoperative complications in the study group of animals with no incidents of early graft thrombosis by using a 3- to 4-mm-diameter graft with a 4-cm length. By comparison, Nordestgaard et al. implanted polytetrafluoroethylene conduits (2 or 3 mm × 1 cm) into the abdominal position of rabbits, with a patency rate at 3 months of 24% for 2-mm grafts and 72% for 3-mm grafts (6). Of the 17 rabbits with 2-mm grafts, 12 were killed or died within the first 7 days because of graft thrombosis, whereas four of the five survivors had patent grafts at the end of 3 months. Of the 25 rabbits they grafted with 3-mm grafts, 19 grafts were patent at 1 week, and 18 were patent at 3 months. Of the 42 rabbits implanted with 2- or 3-mm grafts, 18 developed paralysis during their study. The paralysis may have been related to the cross-clamp time, which varied from 20 to 50 min under normothermic conditions.

We previously had determined that a cross-clamp time exceeding 30 min under normothermic or mild hypothermic conditions contributed to a high rate of postoperative paralysis. This observation is supported by the study of Tolwani et al. (5), where mild hypothermia and a mean cross-clamp time of 30 min or less resulted in a 3% incidence of paraplegia, whereas without hypothermia, the incidence of paraplegia was 100%.

We obtained our favorable results because of improvements in surgical technique, use of mild hypothermia, and limiting of ischemic periods to < 30 min as well as to the perioperative administration of agents with metabolic, rheologic, and neuroprotective properties. The presented model will be used for further small-bore vascular graft implantation studies in our laboratory.

Acknowledgments

We thank Leslie Trail for assistance during surgery and postoperative care.

References

1. **Zdrahala, R. J.** 1996. Small caliber vascular grafts. Part I: state of the art. J. Biomater Appl. **10:**309-329.
2. **Baumann, D. S., M. Doblas, A. Daugherty, G. Sicard, and G. Schonfeld.** 1994. The role of cholesterol accumulation in prosthetic vascular graft anastomotic intimal hyperplasia. J. Vasc. Surg. **19:**435-445.
3. **Greisler, H. P., J. J. Klosak, E. D. Endean, J. F. McGurrin, J. D. Garfield, and D. U. Kim.** 1991. Effects of hypercholesterolemia on healing of vascular grafts. J. Invest. Surg. **4:**299-312.
4. **Lee, E. S., M. P. Caldwell, A. S. Tretinyak, and S. M. Santilli.** 2001. Supplemental oxygen controls cellular proliferation and anastomotic intimal hyperplasia at a vascular graft-to-artery anastomosis in the rabbit. J. Vasc. Surg. **33:**608-613.
5. **Tolwani, R. J., K. Yamamoto, K. Waggie, S. Green, G. Otto, K. Takahashi, Y. D. Rubinstein, and R. E. Pratt.** 1998. Hypothermia reduces neurologic deficits associated with placement of a vascular prosthesis in the abdominal aorta of rabbits. Lab. Anim. Sci. **48:**282-287.
6. **Nordestgaard, A. G., J. A. Buckels, and S. E. Wilson.** 1986. A laboratory model for the evaluation of thromboembolic complications of small diameter vascular prostheses. Br. J. Exp. Pathol. **67:**839-849.
7. **van der Lei, B., P. H. Robinson, H. L. Bartels, and C. R. Wildevuur.** 1989. Microarterial grafting into the carotid artery of the rabbit: some considerations concerning species-dependent thrombogenicity. Br. J. Plast. Surg. **42:**59-64.
8. **Astrup, T. and K. Buluk.** 1963. Thromboplastic and fibrinolytic activities in vessels of animals. Circulation Research **13:**253-260.
9. **Cassel, W. S., R. A. Mason, R. Campbell, G. B. Newton, J. C. Hui, and F. Giron.** 1989. An animal model for small-diameter arterial grafts. J. Invest. Surg. **2:**181-186.
10. **Kreitmann, B., A. Riberi, M. Zeranska, G. Novakovitch, and D. Metras.** 1997. Growth potential of aortic autografts and allografts: effects of cryopreservation and immunosuppression in an experimental model. Eur. J. Cardiothorac Surg. **11:**943-952.
11. **Kazama, S., Y. Miyoshi, M. Nie, H. Imai, Z. B. Lin, A. Kurata, and M. Machii.** 2001. Protection of the spinal cord with pentobarbital and hypothermia. Ann. Thorac. Surg. **71:**1591-1595.
12. **American Heart Association.** 2001. 2002 heart and stroke statistical update. American Heart Association, Dallas.
13. **Ueno, T., K. Furukawa, Y. Katayama, H. Suda, and T. Itoh.** 1994. Spinal cord protection: development of a paraplegia-preventive solution. Ann. Thorac. Surg. **58:**116-120.
14. **Babu, K. S. and S. S. Salvi.** 2000. Aspirin and asthma. Chest **118:**1470-1476.
15. **Boothe, D. M.** http://www.cvm.tamu.edu/vcpl/CE/NSAIDS_COX.htm. Non-steroidal anti-inflammatories and cyclo-oxygenase: is there a basis for selection? Texas A&M University, College Station, Tex. Site visited in 2003 [Online.]