Small-caliber Mesothelial Cell–layered Polytetrafluoroethylene Vascular Grafts in New Zealand White Rabbits

Steven R. Sparks, MD, Uttam Tripathy, MD, Abraham Broudy, MD, John J. Bergan, MD, Norman H. Kumins, MD, and Erik L. Owens, MD, San Diego, California

Reduction in the thrombogenicity of small-caliber synthetic vascular grafts by lining them with mesothelial cell has been suggested as a method to reduce thrombosis. The purpose of this research is to determine whether creation of a mesothelial lining on the inner surface of a synthetic vascular graft would improve the patency rate of a small-caliber vascular grafts. Carotid interposition grafting was performed using mesothelial-lined grafts (MLG) in 30 New Zealand rabbits and compared with similar carotid interposition grafts using non-mesothelial-lined grafts (NLG) on the contralateral side. The mesothelial lining was created by suturing a piece of harvested peritoneum with the visceral surface toward the lumen onto a 2-mm polytetrafluoroethylene (PTFE) graft. Graft patency was studied by in vivo Dopler. In vitro evaluations were done with hematoxylin–eosin stains, broadband cytokeratin staining, and monoclonal antibodies for macrophages. Explanation of the grafts was done in terminal operation at 7, 14, and 21 days. The MLG showed progressive fibroblastic proliferation in direct proportion to the age of the graft, but this did not lead to graft occlusion. However, a significant number of NLG were not patent at each time period studied. We concluded that mesothelial cell lining of small-caliber PTFE grafts could enhance the short-term patency more than using the PTFE without the mesothelial lining. The use of such hybrid small-caliber grafts has a potential for improving the patency of these artificial vascular graft substitutes.

INTRODUCTION

The successful development of a small-diameter artificial vascular prosthesis continues to be a challenge because of poor durability and shortened patency due to its enhanced thrombogenicity. This is further perpetuated by a lack of contact inhibition exhibited by endothelial cells at this interface that causes endothelial cell hyperplasia. Failure of the synthetic graft to completely endothelize in humans leads to continued platelet deposition and thrombotic events on the surface of the graft that ultimately contribute to the development of myointimal hyperplasia, which is a common cause of failure of arterial reconstructions.

Endothelial seeding has been suggested to reduce the thrombogenicity of synthetic vascular grafts, however, this method has had limited success. Furthermore, the limited availability of endothelial cells and the tedious process of seeding and implantation with the failure to achieve a
predictable confluent monolayer of endothelial cells on the surface of the synthetic graft has been an overwhelming problem. Endothelial cells are known to have a biologic behavior similar to that of endothelial cells. The use of endothelial cells to cover vascular prosthesis is an alternative that may solve the problem of having to obtain autologous endothelial cells from the venous territory. Mesothelial cells have been known to produce prostacyclin, have fibrinolytic activity, and have been shown to support blood flow. Our objectives in this study were fourfold: to show that (1) acute lining of small-caliber grafts is possible; (2) mesothelial cell lining will enhance 2-mm graft patency; (3) the mesothelial cells will adhere to the polytetrafluoroethylene (PTFE); and (4) mesothelial cells should inhibit endothelial cell ingrowth.

MATERIALS AND METHODS

The Ethics Committee at the University of California Medical Center approved this project. Thirty male New Zealand white rabbits (NZWR) weighing 3.5 to 4.0 kgs were divided into three groups of 10. Each animal received a nonlined graft (NLG) as an interposition in the left common carotid artery as well as a mesothelial-lined graft (MLG) as an interposition in the right carotid. Creation of peritoneal mesothelial-lined grafts is described below. The groups consisted of 10 animals in each time period (7, 14, and 21 days). After the proximal and distal anastomoses were completed, the grafts were examined for pulse and Doppler signal. After the defined time period, the grafts were then removed in a terminal procedure using induction medications. Subsequently, both of the carotid grafts in each animal were examined for gross luminal patency with the Doppler. The luminal diameter was measured in longitudinal segments with the specimen stained with hematoxylin-eosin (H-E) staining. Mesothelial cells were stained for using monoclonal broadband. Cytokeratin staining (Dako-Cytokeratin, 34BE12, Dako Corp. Carpentry, CA), and macrophages were identified by using monoclonal antibody staining. (RAM 11, Dako). Internal diameter measurement of these lengthwise-cut segments of grafts, as taken at the center at the grafts, as endothelial ingrowth and luminal compromise are common at the anastomotic ends. The macrophages were counted randomly at the most pronounced areas of intimal hyperplasia using monoclonal antibody stain. Statistical comparison between groups was achieved by using a paired t-test.

The animals were anesthetized by means of ketamine induction (20 mg/kg) and atropine (0.05 mg/kg), followed by endotracheal halothane inhalation anesthetic. The NZWR were given one preoperative dose of antibiotic (gentamicin). The abdomen and neck were shaved, prepped, and draped in a sterile fashion. Using a 4-cm suprumbilical incision to the left of the midline, the peritoneal cavity was entered and a 20 x 20 mm piece of peritoneum was removed. The abdomen was then closed. This piece of peritoneum was used to construct a peritoneal-lined PTFE graft in the following manner (see Fig. 1). The sheet of peritoneum was placed over an approximately 2 x 2 cm piece of PTFE, making sure that the visceral side of