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PEG-hirudin/iloprost Coating of Small Diameter ePTFE Grafts Effectively Prevents Pseudointima and Intimal Hyperplasia Development

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Objectives. *Small diameter PTFE grafts are prone to thrombosis and intimal hyperplasia development. Heparin graft coating has beneficial effects but also potential drawbacks. The purpose of this study was to evaluate the experimental efficacy of PEG-hirudin/iloprost coated small caliber PTFE grafts.*
Methods. *Thirty-six femoro-popliteal ePTFE grafts (expanded polytetrafluoroethylene, diameter 4 mm) were inserted into 18 pigs. Grafts were randomised individually for each leg and grouped for 3 groups. Group I consisted of native ePTFE grafts, group II were grafts coated with a polylactide polymer (PLA) without drugs and group III grafts were coated with PLA containing a polyethylene glycol (PEG)-hirudin/iloprost combination. The follow-up period was 6 weeks. Patency rates were calculated and development of pseudointima inside the grafts was noted. Thickness of intimal hyperplasia at the distal anastomoses was measured using light microscopy.*
Results. *Patency rates for group I were 6/9 (67%), for group II 9/10 (90%) and 12/12 (100%) for group III. In groups I and II there was a significant reduction of blood flow proximal to the graft at graft harvest, to* 29 ± 12 *and* 28 ± 20 *ml/min respectively (both* $p < 0.01$ *versus preoperative value), whilst in group III blood flow,* 99 ± 21 *ml/min, remained at the preoperative level. Subtotal stenosis due to development of pseudointima was noted in each of the native and PLA coated grafts but not in group III grafts. Intimal hyperplasia at the distal anastomosis was lowest in group III.*
Conclusions. *The PEG-hirudin/iloprost coating of ePTFE prostheses effectively reduced pseudointima and intimal hyperplasia development and led to superior graft patency.*

Keywords: Intimal hyperplasia; Peripheral grafts; Antithrombotic coating; Hirudin; Iloprost.

Introduction

It is generally accepted, that the primary choice for infrainguinal reconstructions is the autologous vein.[1,2] However, in the absence of a suitable vein, a prosthetic graft has to be inserted. Small calibre vascular prostheses are associated with high rates of graft failure due to thrombosis and development of intimal hyperplasia.[3,4] Particularly the prognosis of crural PTFE grafts remains poor. In order to prevent early graft failure, systemic anticoagulation using vitamin K antagonists is commonly administered.[5,6] Since the PTFE surface is highly thrombogenic, especially small prostheses providing marginal flow rates are prone to early graft thrombosis.[7,8] Several approaches have been developed in order to reduce the thrombogenicity of graft surfaces.[9–11] In the past, various efforts were undertaken to facilitate endothel cell seeding to enhance the thromboresistance.[11] However the endothel cell seeding procedure is cumbersome and time consuming since the cells have to be harvested from a patient's vein, several weeks ahead of the definite peripheral reconstruction.[11,12] Alternatively prosthetic grafts can be bonded with antithrombotic drugs like heparin or dipyridamole.[10,13] However the biggest drawback of the use of heparin remains the risk of heparin-induced thrombocytopenia.[14–16]

A promising and safe alternative to heparin with even higher anticoagulative properties represents the combination of hirudin and iloprost.[17] Hirudin is

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a direct thrombin antagonist and inhibits both free and clot-bound thrombin. It is not dependent, unlike heparin, on antihrombin III. Iloprost acts as a strong inhibitor of thrombocyte activation and aggregation.[17,18] In addition it has a strong vasodilator effect which could be particularly important for peripheral grafts with poor runoff.[19,20] The use of a polylactide polymer coating as a drug carrier allows a safe delivery of the anticoagulant drugs from the graft surface to reduce the thrombogenicitiy of the prosthesis during several weeks and months.

The PEG-hirudin/iloprost coating technology has previously been shown to effectively reduce thrombogenicity of clinically used vascular prostheses *in vitro*.[21] We therefore intended to compare a PEG-hirudin/iloprost coated prosthesis against an uncoated and a graft with polylactide sealing in a randomized fashion in an animal experimental setting. We used a porcine model, since pigs produce a reliable intimal hyperplastic response and because the coagulation system of the pig resembles the human system.[22–24]

Material and Methods

Graft coating

Eight centimeter long segments of 4 mm diameter ePTFE grafts were coated under sterile conditions with a polylactide polymer (PLA, MW 30 kDa, Resomer R203, Boehringer Ingelheim, Ingelheim, Germany) as described previously.[17] An 8% PLA solution was used, containing 5% polyethylene glycol (PEG) hirudin (lepirudin, Aventis Pharma GmbH, Germany) and 1% iloprost (Ilomedin®, Schering AG, Berlin, Germany). The grafts were dip-coated twice into the solution to achieve a homogenous coating and then dried and sterile packed. The detailed release characteristics of PEG-hirudin and iloprost from the coating have been described elsewhere.[21] After an initial accelerated release of both drugs during the first 48 h, a slower and continuous release follows over a period greater than 3 months. After 90 days approximately 60% of the PEG-hirudin and 10% of the iloprost were released.

Animals, surgical and randomization procedures

Thirty-six 4-mm ePTFE grafts were implanted in eighteen female domestic pigs (25-30 kg, age 10 to 16 weeks, German Landrace). Since each animal received two grafts, each side was randomised using closed envelopes. Two animals died during the course of the experiments, one due to an infected port system and one due to malignant hyperthermia (each ePTFE/PLA combination). One infected graft (ePTFE) was excluded from the study. Eventually, group I consisted of native ePTFE grafts (n = 9), group II comprised PLA coated ePTFE grafts (n = 10) and group III PLA containing PEG-hirudin/iloprost (n = 12).

After intramuscular sedation with 4 mg/kg azapiron (Stresnil, Janssen, Germany), 10 mg/kg ketamine (Ursotamin, Serumwerk Bernburg, Germany) and 0.05 mg/kg atropine (B.Braun AG Melsungen, Germany), a 20-gauge needle was placed in an ear vein. General anaesthesia was maintained after endotracheal intubation with isoflurane (Baxter AG, München, Germany) and oxygen. Antibiotic prophylaxis was provided with 1.2 g amoxicillin (Augmentan, GlaxoSmithKline GmbH, München, Germany) intravenously during surgery and for two doses after surgery. A port system for intravenous administration of drugs and blood sampling was placed into the first 5 animals. This procedure was discontinued after one animal died due to an infection of the port system.

Longitudinal incisions (12 cm) were made in both hind limb groins. The femoral artery was dissected free from the inguinal ligament to the first segment of the popliteal artery. The deep femoral artery was ligated and dissected out. The baseline flow was measured by means of an ultrasonic flow meter (T206, Transonic Systems, Ithaca, USA). Following systemic heparinisation with 300 IE/kg heparin the common femoral artery was clamped. The proximal anastomosis was created end-to-end using a 7-0 polyproylene (Prolene, Ethicon, Germany) running suture. The distal anastomosis was sutured to the distal superficial femoral artery in an end-to-side fashion using a 7-0 Prolene running suture. After restoring blood flow, the flow rate was measured again to confirm patency. The wounds were closed subcutaneously with a running 4-0 polyglactin 910 (Vicryl, Ethicon, Germany) suture and a 4-0 poliglecaprone 25 (Monocryl, Ethicon, Norderstedt, Germany) suture for the skin. All animals received 100 mg aspirin/day (ASS 100, Ratiopharm, Germany). The study protocol was approved by the local ethical committee. Animal care complied with the ''Principles of Laboratory Animal Care'' and the Guide for the Care and Use of Laboratory Animals'' (NIH Publication No. 80-23, revised 1985).

Follow-up and termination

Graft patency was confirmed once a week and on the day of the harvest using color-coded duplex sonography under sedation, as described previously.