Eur J Vasc Endovasc Surg (2010) **39**, 620–626

Cryoplasty of the Venous Anastomosis for Prevention of Intimal Hyperplasia in a Validated Porcine Arteriovenous Graft Model

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Submitted 6 October 2009; accepted 25 December 2009
Available online 12 February 2010

KEYWORDS
Animal model;
Arteriovenous graft;
Cryoplasty;
Intimal hyperplasia;
Percutaneous transluminal angioplasty;
Stenosis

Abstract *Objectives:* Cryoplasty combines conventional angioplasty — percutaneous transluminal angioplasty (PTA) — with cold thermal energy. In this animal study, we investigated if preventive cryoplasty could reduce intimal hyperplasia (IH) at the venous anastomosis.
Design: We investigated cryoplasty versus PTA of the venous anastomosis in a validated porcine, bilateral, arteriovenous graft model.
Animals and methods: In 12 pigs, 24 expanded polytetrafluoroethylene (ePTFE) grafts were bilaterally inserted between the common carotid artery and internal jugular vein. Directly after surgery, one venous anastomosis was treated with cryoplasty at −10 °C, the contralateral anastomosis with conventional PTA. At 4 weeks, graft flow was measured, quantitative angiography was performed and grafts with adjacent vessels were excised for histological analysis.
Results: Due to a number of thromboses, data for paired analysis were available from eight pigs. Angiographic outflow vein diameter and graft blood flow were not different between treatment groups. Compared with the control group, IH at the venous anastomosis was reduced by 47% ($P = 0.21$) and intima/media ratio was reduced by 45% ($P = 0.07$) by cryoplasty. Effects were most profound in those animals that tended to develop most IH.
Conclusion: Our results suggest that preventive cryoplasty of the venous anastomosis might help to reduce IH in those cases that develop most profound IH.

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1078-5884/$36
doi:10.1016/j.ejvs.2009.12.030

Introduction

Arteriovenous vascular access grafts are currently used by 42% of haemodialysis patients in the United States.[1] One-year primary and secondary patency rates of these grafts are 40% and 60%, respectively.[2] Haemodialysis graft failure is predominantly caused by progressive intimal hyperplasia (IH) in the venous outflow tract,[3,4] which is characterised by vascular smooth muscle cell proliferation, extracellular matrix deposition and angiogenesis within the neointima and adventitia.[5,6] According to the current guidelines, pre-emptive treatment of the stenosis is mandatory to prevent graft thrombosis.[7,8] Percutaneous transluminal angioplasty (PTA) is the preferred method for graft salvaging.

In search for treatment alternatives, most clinical studies aimed at secondary IH prevention while a validated animal *in vivo* restenosis model is not available. Cryoplasty is a novel endovascular technique that starts with nucleation of small ice crystals in the extracellular fluid in tissue adjacent to the balloon. Since ice does not incorporate solutes, a hypertonic environment is created, leading to osmotic dehydration of cells.[9] When the balloon is removed, the tissue thaws, isotonic conditions are regained and smooth muscle cells rehydrate,[9] inducing apoptosis. First short-term clinical studies of cryoplasty in femoropopliteal arterial disease appear favourable compared with conventional angioplasty.[10–13] In haemodialysis accesses outcome after cryoplasty is still under debate.[14,15] So far, clinical studies have not provided enough evidence for beneficial effects of cryoplasty in the reduction nor the treatment of AV graft stenosis. The only cryotherapy experimental animal *in vivo* study has been conducted in rabbit iliac arteries after balloon angioplasty.[16]

In the present study, we tested the hypothesis that primary cryoplasty at the venous anastomosis immediately following surgery reduces IH in a porcine, bilateral, arteriovenous graft model.

Animals and methods

Graft implantation

In 12 female Landrace pigs weighing 52.3 ± 0.8 kg expanded polytetrafluoroethylene (ePTFE) grafts (W.L. Gore and Associates, Flagstaff, AZ, USA) were inserted bilaterally between the common carotid artery (CCA) and the internal jugular vein as described previously.[17–19] In each pig, one graft was randomly assigned to undergo cryoplasty immediately after surgery. The contralateral graft served as control and underwent conventional PTA treatment. The study protocol was approved by the Institutional Review Board for animal experimentation of the University Medical Center Utrecht and conforms to the 'Guidelines for the Care and Use of Laboratory Animals', published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Anaesthesia

Before graft implantation and termination, the animals were fasted overnight. On day −1, a fentanyl plaster (25 $\mu g\,h^{-1}$) was applied to the skin. Animals were premedicated with an intramuscular injection containing ketamine 10 $mg\,kg^{-1}$, midazolam 0.4 $mg\,kg^{-1}$ and atropine 0.5 mg. After cannulation of an ear vein, an intravenous injection of thiopental sodium 4 $mg\,kg^{-1}$ was administered. Then, they were intubated and ventilated with a mixture of O_2 and air (1:2). During the operation, the ear vein was used for continuous administration of 0.3 $mg\,kg^{-1}\,h^{-1}$ midazolam, 2.5 $\mu g\,kg^{-1}\,h^{-1}$ sufentanil and 50 $\mu g\,kg^{-1}\,h^{-1}$ pancuronium. The pigs were monitored by electrocardiogram and capnography.

Oral anti-platelet therapy

Starting 6 days preoperatively, the pigs received acetylsalicylic acid 80 $mg\,day^{-1}$. Clopidogrel (Sanofi-aventis, Gouda, the Netherlands) 225 mg was added 1 day preoperatively and continued at a dose of 75 $mg\,day^{-1}$ until termination. Before graft puncturing for cryoplasty and conventional angioplasty, an intravenous bolus of 10 mg abciximab was administered.[18]

Antibiotics

Before operation, the animals received intravenous amoxicilline/clavulanic acid 500/125 mg. Oral amoxicilline/clavulanic acid 600/157.5 mg was administered on day 1 postoperatively.

Operative procedure

Operational procedure was performed as described earlier.[17] In short, through a longitudinal incision in the midline of the neck, the CCA and the internal jugular vein were dissected bilaterally. Baseline flow through the CCA was measured using a 4-mm perivascular flow probe (Transonic Systems, Maastricht, the Netherlands). Intravenous heparin 100 $IU\,kg^{-1}$ was administered before vessel manipulation.

Two vascular surgeons (G.J.d.B. and H.J.M.V.) created the end-to-side anastomoses at a 45° angle using a continuous 8/0 polypropylene suture. Sterile, reinforced, thin-walled, ringed, ePTFE grafts (W.L. Gore & Associates, Flagstaff, AZ, USA) were implanted (diameter 5 mm, length 7 cm). Then, blood flow through the artery and vein was measured and graft flow was calculated as caudal CCA flow minus cranial artery flow. After administration of abciximab, each graft was punctured separately to perform the interventional radiological procedure. After treatment, the puncture hole was sutured using 6/0 polypropylene.

Cryoplasty

The PolarCath™ Peripheral Dilatation System (Boston Scientific, Maastricht, the Netherlands) was used for cryoplasty. This system consists of a nitrous oxide-expanded dual balloon catheter, a microprocessor-based inflation unit and a nitrous oxide cylinder. A 6F sheath is placed in the graft and the PolarCath™ balloon is advanced to the venous anastomosis over a 0.035-inch guidewire. Upon entering the balloon, the pressurised liquid nitrous oxide changes phase, resulting in balloon expansion. The 4-mm diameter balloon[17] is inflated in 2-atmosphere (atm) increments until