Differential, time-dependent effects of perivenous application of fibrin glue on medial thickening in porcine saphenous vein grafts

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Abstract

Objective: Neointimal and medial thickening play a critical role in late vein graft failure following CABG. Previous ex vivo experiment suggested that perivenous application of fibrin glue may reduce the damage in the circular smooth muscle cell layer of the media of the vein graft shortly after exposing to arterial pressure. However, the in vivo as well as the longer term impact of this intervention remain unknown. Methods: Bilateral saphenous vein-carotid artery interposition grafting was performed in eight large white pigs (35—45 kg). In each pig, one of the grafts was randomly selected to receive perivenous fibrin glue support while the contralateral graft served as control. At 1 and 4 months following surgery (n = 4 pigs in each group), all 16 patent vein grafts were removed and pressure-fixed. Multiple histological sections from each graft were prepared. Proliferating cell nuclear antigen (PCNA) was detected by immunocytochemistry. Vein graft morphology was assessed using computer-aided planimetry. Results: Although perivenous application of fibrin glue had little effects either on medial thickness 1 month after implantation or on PCNA index, it significantly increased medial thickness (control: 0.37 ± 0.02 mm; treated: 0.55 ± 0.02 mm, p < 0.001) and total wall thickness (control: 0.75 ± 0.04 mm; treated: 0.92 ± 0.04 mm, p = 0.008) at 4 months (mean ± SEM; n = 4 in each group). Conclusions: Our data indicated that perivenous application of fibrin glue enhances graft thickening and as such does not constitute a strategy for preventing late vein graft failure after CABG.

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1. Introduction

Coronary artery bypass grafting (CABG) is associated with better survival and fewer repeat revascularizations as compared to percutaneous stent implantation in patients with multivessel ischemic heart disease [1,2]. Autologous saphenous vein continues to be the most commonly used conduit for CABG, owing to the fact that complete graft failure that include the placement of a porous, non-restrictive, polyester stent [8—10] , or a bioabsorbable sheath [11]. Strategies have been proposed recently for preventing vein graft failure that include the placement of a porous, non-restrictive, polyester stent [8—10] , or a bioabsorbable sheath [11].

Another extravascular support strategy has been that of perivenous application of fibrin glue [12]. Commercially available fibrin glue is a two-component fibrin sealant...
2. Materials and methods

2.1. Surgical procedure

The study was approved by the ethics committee of the Chinese University of Hong Kong. Experimental interventions were performed in 12 large white pigs (initially weighing 35–45 kg), which received humane care according to the European Convention on Animals Care.

All animals underwent bilateral saphenous vein into carotid artery interposition grafting as previously described [13]. The pigs were fasted for 12 h before operation. Anesthesia was induced with ketamine (30 mg) and atropine (0.6 mg), administered intramuscularly. Following endotracheal intubation, the animals received 4% isoflurane in 1:1 oxygen and nitrous oxide gas. Amoxicillin (Merck & Co., USA) was given intramuscularly for antimicrobial prophylaxis at a dose of 20 mg/kg prior to skin incision. The pigs also received intravenous normal saline throughout the operation, and they were monitored with ECG and pulse oxymeter. Approximately 12 cm of the saphenous vein from each pig’s right leg was dissected free of surrounding tissue and all side branches were secured with a 3–0 silk ligature. The vein was removed from the animal, rinsed in isotonic sodium chloride solution (0.9 g/L) containing 2 IU/mL heparin and 50 μg/mL glyceryl trinitrate, and stored in the same solution at room temperature (24°C) until needed.

Two para-sternomastoid muscle longitudinal neck incisions were made and the common carotid arteries carefully dissected from the internal jugular vein and vagus nerve within the carotid sheath. Following systemic heparinization (200 IU/kg), a segment of the common carotid artery was isolated and divided between vascular clamps, bevelling the cut ends obliquely to 45°. The saphenous vein was reversed and similarly beveled. The end-to-end anastomoses of the vein to common carotid artery were performed using continuous 7–0 Polypropylene (Prolene, Ethicon, San Angelo, TX, USA) sutures. The graft was de-aired through the suture line before the suture of the second anastomosis was tied, then the vascular clamps were removed and the graft perfused at arterial pressure (Fig. 1a). In each pig, one of the grafts was randomly selected to receive 2 mL perivenous fibrin glue (Tisseel®, Hyland Immuno, Baxter AG, Vienna, Austria) support (Fig. 1b) while the contralateral graft served as control. Hemostasis was checked, neck and leg wounds were closed with 2–0 polyglycolic acid sutures (Dexon, Davis & Geck, Hampshire, UK) and inhalational anesthetic agents were discontinued. Animals were extubated and, when in a satisfactory condition, returned to their pens and fed a normal chow diet.

The pigs were randomly divided into two groups. The vein grafts were removed at the end of the first month after operation in group I, and at the end of the 4th month in group II. Two pigs in group I died on the same day (postoperative day 17 and day 23, respectively) due to accidental trauma. Two other pigs in the group II died 9 and 11 weeks after surgery, respectively, likely due to pneumonia. Eventually, four pigs in each group, with total 16 patent vein grafts, were included in the final study.

2.2. Morphometric analysis

Following harvesting, all patent vein grafts were immediately pressure-fixed ex vivo at 100 mmHg for 20 min using 10% formaldehyde. The grafts were then kept in the same solution for 12–18 h after the pressure fixation before storage in phosphate buffer solution at 4°C. For wax embedding, three segments (approximately 5 mm apart) were removed from each vein graft, dehydrated and embedded in paraffin wax with their axis perpendicular to the cutting plane. Transverse (5 μm thick) sections were cut at four different levels approximately 0.6 mm apart and mounted on glass slides. Sections were stained with Hematoxylin and Eosin, and Elastic van Gieson for examination under light microscopy. Vessel wall dimensions were measured by computer-aided planimetry with use of a Nikon microscope and camera. The area enclosed by the endothelium defined the lumen. The area enclosed between the

derived from human plasma. In short, fibrinogen is reconstituted in aprotinin solution to give the first component of the sealant, while thrombin is reconstituted in the calcium chloride solution to give the second component. During or immediately before its application, the two components are mixed and quickly form an elastic mass, which firmly adhere to the tissue. The product is therefore used in the clinical setting to achieve hemostasis, to seal or glue tissue. In fact, the solidified fibrin sealant is completely absorbable, permeable, and does not impede ingrowth of vasa vasorum.

In an ex vivo model, Stooker et al. [12] found that perivenous application of fibrin glue may reduce the damage in the circular smooth muscle cell layer of the media of the saphenous vein segments within the first hour of exposing to arterial pressure.

The present study was therefore designed to evaluate the in vivo as well as the longer term impact of perivenous application of fibrin glue on vein graft medial thickening and neointima formation in a porcine model of saphenous vein—carotid artery interposition grafting.
internal elastic lamina and the lumen defined the intima. The area enclosed between the inner aspect of the external elastic lamina and the internal elastic lamina defined the media. Measurements of the perimeters of the lumen and internal and external elastic lamina were also obtained. Mean values of intimal and medial circumferences, as well as intimal and medial thickness, were then calculated for all sections from the same graft.

2.3. Cell proliferation

Proliferating cell nuclear antigen (PCNA) was detected in the sections by immunocytochemistry using a primary monoclonal antibody (PC10, Dako Ltd., High Wycombe, Bucks, UK) at a 1/100 dilution. This was followed by a 1/50 dilution of biotinylated anti-rabbit IgG (Dako) and avidin—biotin—peroxidase conjugate (Dako) according to the manufacturer’s instructions. Sections were counterstained with Harris’ hematoxylin. Total number of cells and number of strongly biotinylated anti-rabbit IgG (Dako) and avidin—biotin—peroxidase conjugate (Dako) at a 1/100 dilution. This was followed by a 1/50 dilution of monoclonal antibody (PC10, Dako Ltd., High Wycombe, Bucks, UK) at a 1/100 dilution. This was followed by a 1/50 dilution of biotinylated anti-rabbit IgG (Dako) and avidin—biotin—peroxidase conjugate (Dako) according to the manufacturer’s instructions. Sections were counterstained with Harris’ hematoxylin. Total number of cells and number of strongly positive nuclei were counted in both intima and media in five microscopic fields per section using a ×40 objective.

2.4. Statistical analysis

Values are presented as mean ± standard error of the mean with the number of observations in parentheses. All data were stored and analyzed using a standard computer statistical software program (Statistical Package for the Social Sciences 11.0; SPSS Inc., Chicago, IL, USA). Inter-group comparisons (i.e., 1 month vs 4 months) were established using ANOVA with paired values. For comparisons between paired control and treated grafts in each group (with each pig serving as its own control), a Wilcoxon signed-rank test was used. Statistical significance was inferred at two-sided p values less than 0.05.

3. Results

3.1. Impact of time (group I vs group II)

All 16 vein grafts were nicely patent. For those fibrin glue supported grafts, no residual perivenous glue could be identified in either group upon harvesting. In fact, we observed that the fibrin glue could be absorbed as early as 1—2 weeks after operation and barely lead to remarkable local tissue adherence thereafter (data not shown). As expected, the intimal and medial areas, and the total wall thickness, were all significantly greater in the group II than those in the group I (Table 1, p = 0.009, 0.013, 0.02, respectively, for the control grafts; and p = 0.014, <0.001, 0.018, respectively, for the glue-treated grafts). However, no inter-group difference was detected in the PCNA index (Table 1), although the percentage of fibrosis was much higher in group II than in group I (p = 0.03 and 0.04 for the control and glue-treated grafts, respectively).

3.2. Impact of perivenous application of fibrin glue (control vs treated grafts)

In both groups, no significant differences between the control and treated grafts were shown on the PCNA index and the percentage of fibrosis. Perivenous application of fibrin glue appeared to limit intimal thickening in group I, although it was not statistically significant (Table 1). On the contrary, glue treatment had little effects on grafts’ medial thickness 1 month after implantation (Fig. 2a and b). Moreover, external support with fibrin glue even significantly increased vein grafts’ medial thickness and total wall thickness at 4 months (Table 1 and Fig. 2c and d).

4. Discussion

Although CABG is highly effective in improving quality of life and prolonging survival in patients with multivessel coronary artery disease, the long-term success of this therapy is limited by vein graft failure. Vein grafts fail due to their greatly increased susceptibility to atherosclerosis which progresses in a rapidly accelerated form leading to premature occlusion. The substrate for accelerated atherosclerosis in vein grafts is abnormal remodeling in the early period after implantation of the vein into the arterial circulation, characterized by formation of a neointima, composed principally of VSMC and extracellular matrix, in association with endothelial dysfunction. Several novel strategies to limit vein graft occlusion have been evaluated experimentally in recent years, including pharmacological interventions [13,14], gene therapy [15], and extravascular support [8—12]. In particular, Stooker et al. [12] demonstrated that perivenous application of fibrin glue inhibits endothelial and VSMC damage in an isolated human saphenous vein ex vivo model (using heart—lung machine with non-pulsatile flow) during 1-h exposure to arterial pressure. Nevertheless, the longer term results of the present study demonstrate that placement of fibrin glue initially (within 1 month) exerts no inhibitory effect on saphenous vein graft thickening in a porcine model, in vivo. By contrast, when assessed at 4 months, fibrin glue even elicited an increase in graft thickening.
Previous observations proposed potential beneficial effects of fibrin glue early after its application. Perivascular delivery of losartan (an angiotensin II receptor antagonist) with fibrin glue prevents neointimal hyperplasia after balloon angioplasty-induced injury in the pig saphenous artery [16]. Fibrin and fibrin fragments are potent chemotactic substances for VSMC [17]. Fibrin glue induces the migration of VSMCs from rabbit aortic explants [18]. It is reasonable to suggest, therefore, that the perivenous placement of the fibrin glue (at least initially) promotes the migration of medial VSMCs preferentially toward the adventitial region of the graft rather than toward the intima, thereby preventing neointima formation and medial thickening.

Fibrin has also been implicated in other facets of vein graft disease and prevention. Bioabsorbable (vicryl) and non-absorbable (polyester) loose fitting stents markedly inhibit medial and neointimal thickening of porcine saphenous vein grafts when assessed at both 1 and 6 months after implantation [8,11,19]. It has been firmly established that these stents have to be non-restrictive, i.e., loose fitting, in order for them to exert an inhibitory effect on vein graft thickening [9,20]. The degree of the non-restriction is considerable since the optimal diameter of the stents was 8 mm compared to 5 mm fully distended saphenous vein graft. Tight fitting, restrictive stents were found to actually promote vein graft thickening [20]. It was discovered that the...
space between the graft and the stent is rapidly filled with an exudate that is likely to be derived from the anastomoses, which was covered by the external stents and sheaths in these studies [11,19,20]. What was notable about the exudates was that it was fibrin-rich and promoted new microvessel growth [11,19,20]. It was reasoned that fibrin, being one of the most potent angiogenic factors known [17], promotes angiogenesis thereby creating a new vasa vasorum which in turn prevents graft hypoxia. Whilst hypoxia promotes VSMC proliferation, this mechanism was proposed as being central to the considerable impact of the external stent [11,19,20]. As mentioned above, this intra-stent fibrin would also augment migration of VSMCs toward the adventitia rather than toward the intima. Such effects of fibrin would readily explain the initial (i.e., within 1 month) observations in the current study.

In complete contrast, at 4 months after implantation, perivascular application of the fibrin glue actually increased vein graft thickening compared to controls. It is likely that the fibrin glue would have been rapidly biodegraded by proteases and therefore not have had a long-term action that would explain the present effects. However, fibrin also possesses a number of potent biological effects that may promote graft thickening. Remarkably little is known about the effects of fibrin or fibrin glues on VSMC proliferation but one study has shown that fibrin glue promotes fibroblast cell proliferation in the gut [21]. Fibrin is also classically associated with inflammation, in particular, the promotion of macrophage accumulation in vascular tissues [22]. Macrophages release a plethora of factors and mitogens that would augment VSMC proliferation and therefore graft thickening. Although inflammation may not exert an effect on vein graft thickening at the early phase, over the longer term perivascular inflammation may be highly deleterious to the vein graft.

We conclude that the perivascular application of fibrin glue does not inhibit vein graft thickening. On the contrary, it may even promote the process over time, possibly through differential, time-dependent effects on VSMC proliferation and inflammation. Although perivascular application of fibrin has been advocated as a means of preventing vein graft thickening, the present study indicates that this strategy could potentially jeopardize long-term venous graft patency in patients undergoing CABG. Further investigation is warranted to elucidate the possible side effect of fibrin glue as a hemostatic agent in vascular reconstructive surgery.

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References


Appendix A. Conference discussion

Dr J. Roquette (Lisbon, Portugal): So you think we should not use fibrin glue when we are doing some distal anastomosis venous, it might jeopardize the patency in the long term? Dr Wan: Yes, based on our findings, we propose not to use fibrin glue (at least not for the purpose of preventing late vein graft failure).