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Does mild heat combined with external stenting prevent from intimal hyperplasia and medial thickening in the venous grafts?

Experimental study

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Abstract

Introduction. *Intimal hyperplasia and medial thickening of the venous grafts used in coronary artery bypass grafting (CABG) often leads to wall thickening and ultimately to conduit occlusion.*

The purpose was to investigate the effects of mild heat (85°C) followed by utilization of restrictive sleeve on histological changes of the venous grafts implanted into an arterial system.

Material and methods. *Reversed external jugular vein interposition grafting of the carotid artery on the mongrel dogs (n = 18) was performed. The experimental animals were split into three groups: H (n = 6) — grafts were exposed to mild heat and an external sleeve was placed around, S (n = 6) — grafts only with the sleeve and C (n = 6) — control group. The grafts were explanted after 3 months. Prior to explantation the grafts' patency was checked using flowmeter. Afterwards harvested veins were examined in light (LM), scanning (SEM) and transmission electron microscope (TEM). Cross-sectional intima (IA), media (MA) and relative intima area (RIA) for all grafts were calculated. Tissue samples from all grafts before implantation (harvested veins and veins after exposition to mild heat) were also examined.*

Results. *Mild heat destroyed endothelial cells (ECs) and, to a lesser degree, basement membrane but did not influence IA, MA and RIA values. Medial smooth muscle cells (SMCs) located closer to the adventitia were affected by heat pretreatment. After 3 months all grafts were patent. Intimal hyperplasia was observed in group S and C, but not in H. Intimal area was markedly higher ($p < 0.05$) in group S (1.97 ± 0.57 mm^2) and C (1.51 ± 0.77 mm^2) than in H (0.38 ± 0.08 mm^2). Scanning scans 3 months after implantation showed the luminal surface of all grafts was mostly covered by ECs. Smoth muscle cells were present in the intima of all grafts in group C and S, not in H. Some of them were active synthetic type SMCs with many mitochondria and well developed Golgi apparatus (TEM). The media was atrophic in group H and S, where collagen bundles were dissociated, the collagen fibers disrupted and in random orientation in the matrix. Media area was significantly higher ($p < 0.05$) in group C (2.64 ± 0.32 mm^2) than in S (1.71 ± 0.45 mm^2) and H (1.74 ± 0.48 mm^2).*

Conclusion. *Mild heat pre-treatment and external sleeving may mitigate the formation of intimal hyperplasia and reduce medial thickening after implantation in the arterial circulation.*

Key words: venous grafts, neointima formation, media thickening, mild heat, experimental

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Introduction

Coronary artery bypass grafting (CABG) is most commonly performed with either autologous saphenous vein (SV) or internal mammary artery (IMA) [1]. Contrary to the SV grafts, the arteries function better as aorto-coronary bypass, because of their biological properties (e.g. higher production of endothelium-derived relaxing factor — EDRF and prostacyclin — PGI_2 in IMA) [2]. Only one out of four venous grafts remains intact during the first decade [3], and up to 20% of patients require further revascularization procedure (redo bypass grafting or angioplasty) [4]. Most grafts to the left anterior descending artery (LAD) are made with IMA, but only a few percent to circumflex artery (Cx) or right coronary artery (RCA) are arterial [1]. The technical case of SV application and availability and an increasing need for repeat surgery means that SV will continue to play a major role as a conduit for CABG [5].

Early venous graft occlusion results from thrombosis [3]. A slow flow in the vein segment and turbulences at the anastomosis caused by size mismatch between larger saphenous vein segment and the coronary artery, may enhance thrombus formation [6, 7]. The Hearten Saphix (HS) device is designed to reduce the diameter of the SV grafts to size of the recipient artery. It may also ease the making of vein to artery anastomosis, thus avoid poor runoff that is related to impaired endothelial function [8]. The device subject the graft to mild heat, causing shrinkage of the collagen to the predominated size of the calibrating mandrel inside the vein. This mandrel is continuously cooled to preserve the endothelium living. Afterwards an external sleeve is applied around the vein to keep the vein at the desired size. It is known that higher pressure in the arterial system causes vein dilatation and increases the wall tension [9]. This may lead to venous wall thickening [10], narrowing of graft's lumen and ultimately to its occlusion many years after implantation [1].

We investigated the effect of the HS treatment on morphology of the vein grafts interposed in the arterial system in dog for 3 months.

Material and methods

Animals

Eighteen mongrel dogs of either sex weighing 20 to 34 kg underwent reversed jugular vein interposition in the carotid artery. Animal care and surgery complied with the Principles of Laboratory Animal Care and Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23).

Experimental protocol — implantation

The animals (n = 18) were split into three groups. In group H (n = 6) the venous grafts were exposed to mild heat and an external Dacron® sleeve was placed around prior to implantation, in group S (n = 6) the grafts were supported only by the sleeve and in group C (n = 6) untreated jugular vein grafts were used.

The dogs were premedicated with intramuscular injections of ketamine hydrochloride (6.5 mg/kg) and piritramide (1 mg/kg). Anesthesia was induced with sodium pentobarbital (10–20 mg/kg) administered intravenously. The animals were intubated endotracheally, and an arterial line was placed in a femoral artery for pressure monitoring and arterial blood sampling. Anesthesia was maintained with halothane (0.5–1.0 vol. %). The dogs were mechanically ventilated, with oxygen-enriched room-air and ventilatory parameters were adjusted according to the arterial blood gasses, checked every 15 min. ECG was monitored continuously throughout the procedure. A balanced salt solution (Ringer solution) was slowly administered through an intravenous line. Antibiotics (Albipen LA 2.5 mL/15kg) were always used prophylactically.

The external jugular vein was exposed through an oblique incision in the neck and dissected free from the surrounding tissue and then harvested. In group H the segments of the harvested vein were mounted over hollow, stainless mandrels that were immersed in a heated saline bath at 85°C for 2 min. We always used the mandrels 3 mm in diameter. Saline at room temperature was continuously running through the hollow mandrel. Small pieces of the harvested non-implanted vein (all groups) (NC) as well as venous grafts after treatment (TC) with HS (group H) were examined by light microscopy (LM), electron microscopy (scanning SEM and transmission TEM). Prior to carotid artery clamping, heparin was administered iv in a dose of 3 mg/kg. The jugular vein grafts were implanted into the carotid arteries as an interposition with a continuous 6–0 polypropylene suture. Additionally, the Dacron® sleeve was placed over the grafts in group H and S (Fig. 1). An external sleeve of 6.0 mm in diameter was used in all experiments. Care was taken to avoid clamping, instrumenting, or disrupting the endothelium of all vessel segments. Hemostasis was performed and the incisions closed in layers. After the animals were extubated, piritramid 10 mg and a long-acting penicilin (Albipen LA) were injected.

Explantation of the grafts

After 3 months the animals were sacrificed. Premedication, anesthesia induction and maintenance were performed as described for the implantation procedure. The

Figure 1. The jugular vein graft with the external support implanted into the carotid artery (group H)

Figure 2. The control vein graft at 3 months after implantation into the carotid artery (group C)

incisions were reopened, the grafts exposed (Fig. 2) and their patency checked by a transonic flowprobe (H3SB987) connected to a flowmeter (T206, Transonic System Inc., Ithaca, NY, USA). When the flow pattern was pulsatile, the graft was considered as patent. After heparinisation (3 mg/kg) the grafts with adjacent segments of the carotid artery were removed. Rings from the central segments of the grafts were excised for examination in LM, EM. The tissue for LM were preserved and stored in 10% solution of formaldehyde, for EM in 2.5% glutaraldehyde.

Histology

For LM, the tissue specimens were embedded in parrafin, cut transversely into 4 mm thick sections, and then stained with hematoxylin and eosin (H + E), Masson's trichrome stain for collagen and elastic-van Gieson stain to identify the intima (area between the lumen and the internal elastic lamina [IEL], and the media (area between the IEL and the external elastic lamina [EEL], the LM sections were photographed and their pictures scanned to PC then the areas of the lumen, inside IEL and EEL were calculated using Canvas™ 3.0.3 software. Intimal area (IA), medial area (MA), and relative intimal area (RIA) were calculated for each microscopic section by the following formulas:

$IA\ [mm^2] = IEL\ area - lumen\ area$

$MA\ [mm^2] = EEL\ area - IEL\ area$

$RIA\ (\%) = (IA/(IA + MA)) \times 100\%$

For SEM, the fixed tissue samples were dehydrated, dried to the critical point with liquid CO_2 and coated with gold. Finally, the surfaces analysis of the inner layer of the vein grafts was performed in Philips XL-40 scanning electron microscope under magnification ranged from 126 to 514.

For TEM, the samples were cut into micrometer-thick section, which were stained with toluidine blue and examined by LM. Fragments including intima and media were chosen from the specimens, cut out and stained with uranylacetate and lead citrate. Sections were then treated with 2% potassium pyroantimonate to demonstrate calcium. Eventually, grids were examined in Philips CM 10 electron microscope. Random pictures were taken.

Data management and statistical analysis

The IA, MA and RIA values are expressed as the arithmetic mean ± one standard deviation. Two-way repeated-measures analysis of variance (ANOVA) was employed to compare the mean values of IA, MA and RIA between groups. If a statistically significant F value was found, a multiple-comparison procedure (Fisher's PLSD post-hoc test) was used to determined which individual group differences were significant. Differences were considered to be significant with $p < 0.05$. Data management and statistical analysis were done with StatView 4.0 software (Abacus Concepts, Inc. USA).

Results

A transonic flowmeter placed over the carotid artery proximal to the grafts proved all grafts were patent 3 months after implantation.

Histology

Morphological changes after mild heat application

The vein treatment with HS did not disrupt global organization of vein wall. The elastic laminae and the media remained intact. Smouth musle cells within the media looked normal under LM. There were less ECs (recognized by their protruding and bulging nuclei) cov-

ering the intima in TC, compared to NC. Some areas were completely denuded of ECs. The effects of treatment was also seen in TEM scans. Endothelial cells were damaged, with many vacuoli in cytoplasm and pycnotic nuclei. Contact with the basement membrane was lost and ECs changed their shape (from flat to more round), which was consistent with findings in SEM of TC, where fewer ECs were seen, and most of them lost intercellular contact resulting in appearence of the clefts between them. In some areas vascular SMCs were exposed. The basement membrane of TC was also affected by the treatment, mainly as focal disruptions. In the sections closer to the intima, SMCs in the media were well preserved with normal myofilaments, intact caveoli and densities typical for healthy cells, and the structure of collagen was slightly changed (somewhat "cotton wool--like") besides many collagen bundles and elastic fibers without any morphological abnormalities. In the deeper strita (closer to the adventitia) still within the media, SMCs with abnormal cytoplasmatic densities and collagen fibers with lost interconnections and increased interfibrillar spaces were seen. Mean IA, MA and RIA did not differ between NC and TC (Table I).

Morphological changes after 3 months

Group H. The cytoarchitecture of the grafts' wall was well maintained in the LM sections. Their inner surface was mostly covered by cells with bulging nuclei, whose nature as ECs was confirmed in TEM. Transmission electron microscope and SEM showed some areas denuded of ECs, where ECs remnants, rare erythrocytes and fibrin strands were attached. No intimal thickening was observed (IA: 0.34 ± 0.15 mm^2 and 0.38 ± ± 0.08 mm^2 in TC and group H respectively; NS). Because IA did not change, and MA simultaneously decreased (TC 2.75 ± 0.95 mm^2 *vs.* group H 1.74 ± ± 0,48 mm^2; $p < 0.05$), RIA increased significantly (Table I). The atrophic media lost its inner organization because of the fibers' separation in collagen bundles and increased volume of ground substance with some features of edema (TEM study). Contractile type of SMCs in the media showed severe intracellular abnormalities. Nuclei found to be pycnotic, the large electrolucent, perinuclear vacuoli and calcium deposits appeared. In the adventitia a benign fibrohistiocytic reaction (a typical chronic inflammation) around the dacron sleeve was seen.

Table I. Intima (IA), media (MA) and relative intima (RIA) areas calculated from LM sections

	IA [mm^2]	MA [mm^2]	RIA
Prior to implantation			
NC (n = 18)	0.23 ± 0.14	1.97 ± 0.73	0.12 ± 0.06
TC (n = 6)	0.34 ± 0.25	2.75 ± 0.95	0.12 ± 0.08
After implantation (3 months)			
H (n = 6)	0.38 ± 0.08	1.74 ± 0.48*	0.34 ± 0.12*
S (n = 6)	1.97 ± 0.57*#	1.71 ± 0.45	1.20 ± 0.36*#
C (n = 6)	1.51 ± 0.77*†	2.64 ± 0.32‡	0.57 ± 0.18*†

All values are expressed as mean ± standard deviation (SD); *$p < 0.05$ — 3 months after *vs.* prior implantation (ie. group H *vs*. TC, group S *vs*. NC, group C *vs*. NC); #$p < 0.05$ — group S *vs*. H 3 months after implantation; †$p < 0.05$ group C *vs*. H 3 months after implantation; ‡$p < 0.05$ group C *vs*. S 3 months after implantation; Group: NC — non-implanted veins; TC — grafts heated with HS (group H); H — mild heat + sleeve grafts; S — sleeve only grafts; C — control grafts

Group S. In all grafts the luminal surface was mostly covered by ECs. Scanning showed the inner surface was denuded of ECs in some areas. Transmission electron microscope examination revealed that most of present ECs was markedly damaged at the cellular level with many vacuoli as the tokens of the tissue degeneration. They lost intercellular as well as cell-membrane contact resulting in a disruption of the endothelium. The active synthetic SMCs with many mitochondria, well developed rough endoplasmic reticulum (RER) and Golgi apparatus were found in the intima. Examined in light revealed markedly intimal thickening. In the media atrophic changes were noted. Collagen bundles were dissociated by the interstitial edema, their fibers disrupted and in random orientation. In the media, only a few contractile type SMCs were observed and in all of them pathological appearences mainly within nuclei (shrinkage of nuclei and expansion of the nuclear envelopes) were noted. Scattered, focal calcifications located intracellularly in the SMCs and extracellularly within collagen bundles were observed.

Group C. In LM sections, inner surface was partially covered by ECs, what was confirmed by SEM scans. Transmission electron microscope examination revealed that the present ECs was swollen with many vacuoli. Intimal hyperplasia was observed. Intimal area and RIA markedly increased 3 months after implantation and were significantly higher than in group H (Table I). Many active synthetic SMCs were seen in the intima. In the media, contractile type SMCs as well as transitional phenotype SMCs, possessing the features of contractile and synthetic cells, were observed. Extracellular scattered calcifications between dissociated by interstitial edema collagen bundles were noted.

Discussion

In our study we employed a widely used canine model of reversed jugular vein interposition grafting of the carotid artery [11, 12].

An intriguing and encouraging observation in our study is that the vein treatment with HS device (group

H) significantly reduced intimal hyperplasia, which process is a main reason of late venous graft failure [1]. We proved that SMCs ultrastructure (TEM study) was affected by mild heat pretreatment. It is known that they play a crucial role in neointima formation [1, 3]. Several growth factors released from endothelium, monocytes, activated platelets provoke the proliferation of medial SMCs [5], that migrate through internal elastic lamina [13] and continue to proliferate and secrete matrix protein within intima [14] at the same time changing their fenotype from contractile to synthetic [15]. Three months after implantation we did found no synthetic SMCs in media of group H, but they were present in intima and media in the other 2 groups. If especially exuberant, intima hyperplasia can narrow the lumen sufficiently to cause graft thrombosis and utimately occlusion [16]. Three months was not enough to occlude the grafts, but we proved neoitima formation in control grafts (group C).

Surrounding the grafts by the external sleeve has two opposite effects [9]. By the limiting graft expansion, mean wall tension is markedly reduced or even eliminated, but shear stress at the endothelial surface is greater than in unstented grafts [9]. Earlier, it was shown by Dobrin that intimal thickening and medial thickening represent responses to different stimuli [16]. He suggested that intimal thickening was result from abnormal flow characteristics at the blood-intima interface, whereas medial thickening occurred in response to arterial pressure and high wall tension. Zwolak considered that increase in both mean wall tension and mean shear stress might cause SMCs migration into the intima and subsequent proliferation [17]. Morinaga claimed that low shear stress rather than high could mediate intimal thickening through promotion of platelet deposition on the luminal surface of the vein graft and abnormal transport of plasma constituents between the blood and the vessel tissue [18]. In his study elimination of low shear stress region in peripheral arterial reconstruction reduced intimal thickening. Angelini concluded that restrictive external stenting enhanced neointima formation because of reduction of fluid flux, thereby increasing the effective concentration of endogenously produced mitogens [19]. Investigating the net effects of shear stress and wall tension, we proved that rigid external conduit was independent factor enhancing intimal thickening. Moreover, MA in group S, although not different from NC, was comparable to group H and significantly lower than in group C. This means that external support can partially prevent media thickening. We confirmed earlier observations that medial thickening is best associated with deformation of the vein wall in the circumferential direction (increased diameter) and higher wall tension [17, 20]. Thus, group S not only proved the opposite influence of the external stenting on intima and media thickening, but also showed no benefit from the dacron sleeve itself, because enhancing effects on the neointima formation overbalanced protective effects on the media.

Although ECs play a key role in regulating intimal growth through a number of growth-inhibitory mechanisms [21], morphological damage of endothelium caused by HS treatment possibly does not influence the fate of the grafts. It is known that interposition of vein into arterial circulation itself results in intimal ECs loss and spontaneous recovery completed by 2 weeks, likely caused by vascular endothelial growth factor (VEGF) [9, 22]. Reendothelialization was confirmed in all groups in TEM and SEM study.

In our study no thrombosis causing early graft occlusion in humans was observed. Possible explanation is rather big diameter of carotid artery (4–6 mm) and jugular vein (9–12 mm) of dog in comparison to human coronary arteries and SV. Furthermore, after primary heparinization at total dose of 3 mg/kg body weight (the same dose as during CABG) we did not use protamine.

Conclusions

We conclude that vein treatment with the Hearten Saphix device and external sleeving may mitigate the formation of intimal hyperplasia and reduce medial thickening after implantation in the arterial circulation. It might have a beneficial effect on venous grafts' late patency and long-term durability.

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