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Influence of mild heat and restrictive external support on functional changes in vein grafts implanted into arterial circulation

Experimental study

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Abstract

Introduction. *Vein grafts placed in the arterial circulation undergo a set of morphological and functional changes. The aim was to investigate the effects of external mild heat combined with internal cooling and external restrictive support on vascular reactivity of the venous grafts implanted into 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. The rings from all the explanted grafts as well as from jugular veins before implantation were taken and tension study was performed. Contractions to norepinephrine (NE), phenylephrine (Phe), 5-hydroxytryptamine (5-HT) and relaxation to acetylcholine (Ach), calcium ionophore A23187 (A23187) and sodium nitroprusside (SN) were assessed.*

Results. *After pre-treatment with mild heat reaction to the maximal concentrations of NE (37.8 ± 1.9 g/mm^2 before vs. 12.0 ± 1.6 g/mm^2 after), Phe (20.2 ± 1.6 g/mm^2 vs. 2.0 ± 0.4 g/mm^2) were markedly ($p < 0.001$) diminished. Vein grafts before implantation were insensitive to 5-HT. Only endothelium-independent relaxation to SN was preserved in the grafts after mild heat employment, whereas Ach, A23187 did not produce any endothelium-mediated reaction. Three months after implantation markedly lower contractile responses to maximal doses of NE (1.4 ± 0.2 g/mm^2, 2.1 ± 0.3 g/mm^2 and 15.4 ± 1.6 g/mm^2 for H, S and C respectively), and Phe (0.4 ± 0.2 g/mm^2, 1.3 ± 0.2 g/mm^2 and 12.3 ± 1.2 g/mm^2 for H, S and C respectively) were noted. The maximal examined dose of 5-HT provoked 66.2% of the maximal reaction to NE in group H, 66.5% in group S and 53.2% in group C. The grafts in group H and S were insensitive to endothelium-dependent relaxants, but in C the maximal responses to A23187 were significantly weaker ($p < 0.05$) than before implantation (40.7 ± 3.8% vs. 67.4 ± 2.3%). SN-induced endothelium-independent relaxation was observed in all groups.*

Conclusion. *Mild heat of the venous grafts functionally destroys endothelium and significantly impairs smooth muscle cells' function. Employment of mild heat combined with external support may produce venous conduits less sensitive to vasoactive chemicals including also mitogens involved in neointima formation.*

Key words: venous grafts, tension study, endothelium-dependent and independent relaxation, mild heat, external support

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Introduction

Autologous saphenous vein (SV) bypass grafts are employed widely in patients with coronary (CABG) or peripheral arterial occlusive disease [1, 2]. Exposure of veins to arterial circulation subject the grafts to reperfusion injury and new hemodynamic forces (including increase static and pulsatile deformations, static and pulsatile stresses and altered shear stress at the blood--intima interface) [3, 4]. Their subsequent morphological alterations, termed "arterialization", including concentric media and intima thickening, fibrosis and subendothelial cell proliferation represent adaptation [5]. On the other hand, they are commonly believed to display degenerative changes [6]. Morphological alterations are accompanied by functional (biochemical) changes comprising altered production of prostacyclin [6], different reactions to endothelium-dependent (e.g. thrombin, ADP) or — independent (e.g. norepinephrine, sodium nitropruside) factors [2, 7]. It was demonstrated that some functional changes associated with "arterialization" of vein grafts were reversible, proving their adaptative nature [2]. Adaptation is a positive feature of our body, but if the adaptation-demanging state lasts too long then this process becomes mainly negative. Unfortunately venous bypasses are wished to function for long-time, thus many years after SV grafts implantation we can observe mainly negative aspects of "arterialization", often leading to reduction in diameter of the graft lumen and ultimately occlusion [1].

To prevent some adaptative as well as degenerative alterations in venous grafts, mild heat pre-treatment and a restrictive external wall support are proposed by Hearten Medical, Inc. The device "Hearten Saphix™" (HS) uses well-known ability of collagen to shrink when subjected to mild heat. We hypothesise that shrunk collagen fibres preventing from pronounced and rapid vein dilatation lowering mean wall tension, and a dacron sleeve placed over vein influencing the mean shear stress [7] may both slow the "adaptation" and increase durability of SV coronary and peripheral artery bypasses.

In this study we investigated the influence of mild heat combined with subsequently placed external support on functional alterations of SV grafts implanted into the arterial system in a dog model.

Material and methods

Animals

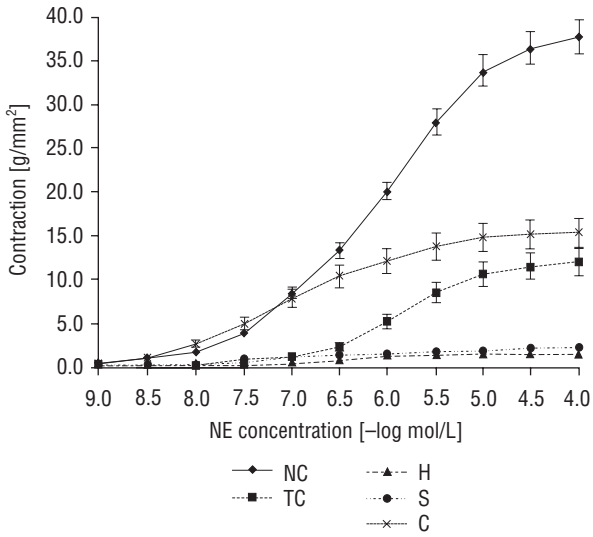
Eighteen mongrel dogs of either sex weighing 20 to 34 kg underwent reversed jugular vein interposition in the carotid artery. Animal care and surgery complied with the Principles of Laboratory Animal Care and Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23).

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 the external 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.

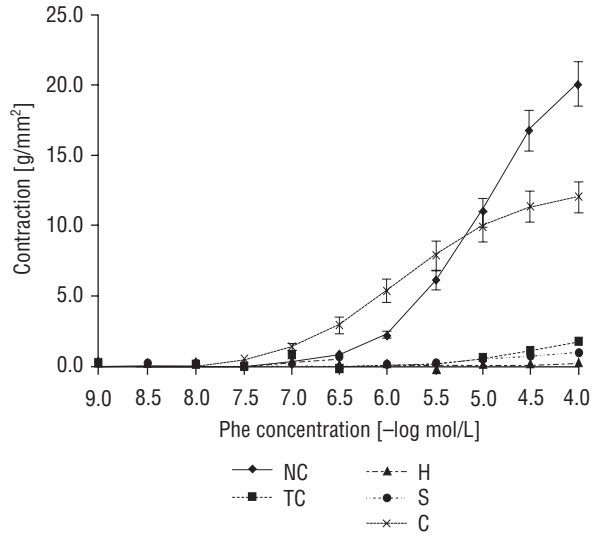
The dogs were premedicated with intramuscular injections of ketamine hydrochloride (6.5 mg/kg) and piritramide (1 mg/kg). Anaesthesia was induced with sodium pentobarbital (10–20 mg/kg) administered intravenously. The animals were intubated endotracheally, and an arterial line was placed in a femoral artery for pressure monitoring and arterial blood sampling. Anaesthesia 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. Antibiotics (Albipen LA 2.5 mL/15 kg) 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 minutes. We always used the mandrels 3 mm in diameter. Saline at room temperature was continuously running through the hollow mandrel. On the rings (4–5 mm in length) cut perpendicularly to a long axis of the harvested non--implanted vein (all groups) (NC) as well as venous grafts after treatment (TC) with HS (group H) *in vitro* tension study (TS) was performed. 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 penicillin (Albipen LA) were injected.



NE	NC		TC		H		S		C	
[–log mol/L]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
9.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.0
8.5	0.6	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0.8	0.2
8.0	1.7	0.2	0.2	0.0	0.0	0.0	0.3	0.1	2.5	0.4
7.5	3.6	0.3	0.4	0.1	0.0	0.0	0.7	0.1	4.8	0.7
7.0	8.4	0.7	0.9	0.2	0.2	0.0	1.0	0.2	7.8	1.0
6.5	13.3	0.9	2.1	0.4	0.6	0.1	1.2	0.2	10.3	1.3
6.0	20.4	1.2	5.1	0.8	1.0	0.1	1.4	0.2	12.1	1.4
5.5	28.1	1.5	8.4	1.2	1.2	0.2	1.6	0.2	13.7	1.6
5.0	34.0	1.8	10.6	1.4	1.3	0.2	1.8	0.2	14.8	1.7
4.5	36.5	1.9	11.5	1.5	1.4	0.2	2.0	0.3	15.2	1.7
4.0	37.8	1.9	12.0	1.6	1.4	0.2	2.1	0.3	15.4	1.6

Figure 1. NE-induced contraction



Phe	NC		TC		H		S		C	
[–log mol/L]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.1
7.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.3
6.5	0.7	0.1	0.0	0.0	0.0	0.1	0.1	0.0	2.9	0.5
6.0	2.2	0.2	0.0	0.0	0.0	0.1	0.2	0.0	5.5	0.8
5.5	5.9	0.7	0.2	0.0	0.0	0.2	0.3	0.0	8.0	1.0
5.0	11.1	1.0	0.7	0.1	0.1	0.2	0.7	0.1	10.2	1.1
4.5	16.9	1.4	1.3	0.3	0.2	0.2	0.9	0.1	11.5	1.1
4.0	20.2	1.6	2.0	0.4	0.4	0.2	1.3	0.2	12.3	1.2

Figure 2. Phe-induced contraction

Explantation of the grafts

After 3 months the animals were sacrificed. Premedication, anaesthesia induction and maintenance were performed as described for the implantation procedure. The incisions were reopened, the grafts exposed (Fig. 2). After heparinisation (3 mg/kg) the grafts with adjacent segments of the carotid artery were removed. Rings (4––5 mm) from the central segments of the grafts were excised perpendicularly to the vessels' long axis and TS was carried out soon after explantation.

In vitro isometric tension study

Rings were suspended by 2 stainless steel clips passed through the lumen, in organ chambers filled with 40 ml of Krebs-Ringer physiological solution with final concentrations [mmol/L]: NaCl 118.3, KCl 4.7, $MgS0_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1, Na_2Ca-EDTA 0.026, maintained at 37°C and continuously bubbled with a 95% O_2/5% CO_2 gas mixture. One stainless steel clip was anchored to the bottom of the organ chamber, the another was connected to a force transducer (F30, HSE, March-Hugstetten, Germany) and changes in isometric tension were recorded on a multichannel polygraph (WT-6456, Nikon Kohden, Tokyo, Japan). The optimal resting tension for each ring was determined by their maximal response to a modified oxygenated Krebs solution containing [mmol/L]: NaCl 66.7, KCl 60, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1, Na_2Ca-EDTA 0.026 at tensions ranging from 1–3 g with increments of 1 g. The tissues were then placed under this optimal tension and allowed to equilibrate in the physiological Krebs-Ringer solution for 1 h. Indomethacin (10^{-5} mol/L), desipramine (10^{-6} mol/L), deoxycorticosterone (5×10^{-5} mol/L) and propranolol (10^{-6} mol/L) were administered to the physiological solution throughout the experiment. Indomethacin was intended to prevent from potential interference due to release of vasoactive prostanoids, desipramine and

deoxycorticosterone to inhibit neuronal and extraneuronal uptake of NE, and propranolol to block β-adrenoceptors. During the equilibration period the physiological solution as well as mentioned above drugs were replaced every 15 min. Afterwards, NE, Phe, 5-HT were added in cumulative manner (10^{-9}–10^{-4} mol/L) to each organ chamber. Additionally, ketanserin (Ket) (10^{-6} mol/L) (a selective 5-HT_2-receptor) or methiothepin (Met) (10^{-6} mol/L) (a non-selective 5-HT_1, 5-HT_5, 5-HT_6, 5-HT_7-receptors blocker) were used to reveal more details of a complex 5-HT-induced reaction. All antagonists were added to the organ chambers 30 minutes prior to agonists' injections. The isometric tension developed by the tissue was recorded. The grafts were washout and re-equilibrated at least 45 min after each examined agent. Then rings were precontracted with the estimated ED_{50} concentration of NE, and cumulative doses of the endothelium-dependent relaxants Ach, A23187 (10^{-9}–3×10^{-6} mol/L) and an endothelium-independent relaxant: SN (10^{-9}–10^{-4} mol/L) were administered. Prior to each injection of the relaxants the rings were rinsed and re-equilibrated.

The contractile responses to NE, Phe, 5-HT, 5-HT + + Ket, 5-HT + Met are expresses as [g] of isometric tension normalised for cross-sectional area [mm^2], calculated according to a formula:
weight of tissue/(length × specific gravity),
where value of specific gravity is 1.0 g/cm^3.

Relaxation to A23187, Ach and SN are expressed as a percentage of tension changes after precontraction with ED_{50} of NE.

Drugs

We used following drugs: indomethacin, desipramine hydrochloride, deoxycorticosterone acetate, DL-propranolol hydrochloride, (–)norepinephrine, phenylephrine hydrochloride, 5-hydroxytryptamine creatinine sulfate, acetylcholine chloride, sodium nitroprusside dihydrate, calcium ionophore A23187 (all from Sigma Chemical Co., St. Louis, MO, USA). All drugs were dissolved and diluted in distilled water, except indomethacin dissolved in 10% solution of $NaHCO_3$, deoxycorticosterone in 95% ethanol and calcium ionophore in DSMO (Sigma Chemical Co., St. Louis, MO, USA) prior to final dilution in distilled water.

Data management and statistical analysis

The results of TS are expressed as the mean ± one standard deviation (SD). The ED_{50} is reported as the negative decimal logarithm of the molar concentration of NE, Phe or 5-HT [–log mol/L], which produced 50% of response to 10^{-4} mol/L of NE, Phe or 5-HT, considered the maximal contractions. Two-way repeated-measures analysis of variance (ANOVA) was employed to compare contractile and relaxing responses between groups. If a statistically significant *F* value was found, a multiple-comparison procedure (Fisher's PLSD post-hoc test) was used to determine which individual group differences were significant. Additionally, contraction to estimated ED_{50} of NE, Phe, 5-HT and responses to the maximal doses of all drugs were compared between groups using factorial ANOVA. Differences were considered to be signiticant with $p < 0.05$. Data management and statistical analysis were done with StatView 4.0 software (Abacus Concepts, Inc., USA).

Results

The effects of mild heat pre-treatment on vascular reactivity

Optimal resting tension did not change after mild heating in the chamber of HS (Table I).

Reaction to the contractile agents. The dose/response curves to NE, Phe, 5-HT in NC veins and to NE and Phe in TC veins were sigmoid-shaped (Fig. 1, Fig. 2). NE was a significantly stronger vasoconstrictor than Phe ($p < 0.001$). Mild heating downregulated contractility and sensitivity of venous grafts to NE (Table II). Reaction to Phe was markedly diminished after pre-treatment with HS device, but without any changes in vascular sensitivity (Table II). We did not observe any contraction after 5-HT administration to NC and TC veins.

Relaxation and endothelial function. Ach, A23187 caused endothelium-mediated relaxation in concentration-dependent manner only in NC veins. The maximal percent relaxation of precontracted tension were 78.7 ± 2.7% and 40.6 ± 3.8% for Ach and A23187 respectively (Table III). Low concentrations of Ach (3×10^{-9}–3×10^{-7} mol/L) produced relaxation with maximal response at 10^{-7} mol/L, while higher concentrations (10^{-7}, 3×10^{-6} mol/L) provoked contraction (Fig. 3). Endothelium-independent relaxation to SN was signiticantly stronger in NC ($p < 0.001$, NC *vs.* TC) (Table III).

Table I. Values of optimal resting tension

Group	Optimal resting tension (mean ± SD) [g]
NC	1.43 ± 0.57
TC	1.33 ± 0.69
H	2.45 ± 0.50*
S	2.95 ± 0.34#
C	2.75 ± 0.62#

*$p < 0.05$ TC *vs.* group H, #$p < 0.05$ NC *vs.* group S or C

Table II. Maximal contraction (MC) and sensitivity (ST) to vasoconstrictors used in the experiments

Group	NE		Phe		5-HT	
	MC [g/mm^2]	ST [–log EC_{50}]	MC [g/mm^2]	ST [–log EC_{50}]	MC [g/mm^2]	ST [–log EC_{50}]
NC	37.8 ± 1.9	6.27 ± 0.46	20.2 ± 1.6	4.97 ± 0.49	Nr	Nc
TC	12.0 ± 1.6*	5.65 ± 0.53**	2.0 ± 0.4*	4.68 ± 0.39	Nr	Nc
H	1.4 ± 0.2$^{\#}$	6.04 ± 0.43	0.4 ± 0.2$^{\#}$	4.42 ± 0.54	0.9 ± 0.1$^{\#}$	4.43 ± 0.61$^{\#\#}$
S	2.1 ± 0.3$^{\&}$	6.43 ± 1.32	1.3 ± 0.2$^{\&}$	4.87 ± 0.41	1.4 ± 0.3$^{\&}$	3.25 ± 0.46$^{\&}$
C	15.4 ± 1.6$^{\&\&\ddagger}$	6.46 ± 0.59	12.3 ± 1.2$^{\&\&\ddagger}$	5.14 ± 0.70	8.2 ± 0.5$^{\&\&\ddagger}$	5.20 ± 1.30$^{\&\&\ddagger\ddagger}$

All values are expressed as mean ± standard deviation; Nr — no reaction to maximal doses of 5-HT, Nc — not calculated because of no reaction to 5-HT; *$p < 0.001$ group NC *vs.* TC, **$p < 0.05$ group NC *vs.* TC; $^{\#}p < 0.001$ group TC *vs.* H, $^{\#\#}p < 0.05$ group TC *vs.* H; $^{\&}p < 0.001$ NC *vs.* group S or C, $^{\&\&}p < 0.05$ Nc *vs.* group S or C; $^{\ddagger}p < 0.001$ group S *vs.* C; $^{\ddagger\ddagger}p < 0.05$ group S *vs.* C

Table III. Reactions to endothelium-dependent (A23187, Ach) and endothelium-independent relaxant (SN)

Group	Maximal percent relaxation to		
	A23187	Ach	SN
NC	40.6 ± 3.8	78.7 ± 2.7	13.9 ± 5.2
TC	Nr	Nr	59.3 ± 4.8*
H	Nr	Nr	64.4 ± 7.1
S	Nr	Nr	69.5 ± 6.1$^{\#}$
C	67.4 ± 2.3‡	Nr	14.8 ± 4.1$^{\&}$

All values are expressed as mean percentage of tension changes after precontraction with ED_{50} of NE; Nr — no relaxation was observed; $^{\ddagger}p < 0.05$ NC *vs.* group C; *$p < 0.001$ NC *vs.* TC; $^{\#}p < 0,001$ NC *vs.* group S; $^{\&}p < 0.05$ group C *vs.* group H or S

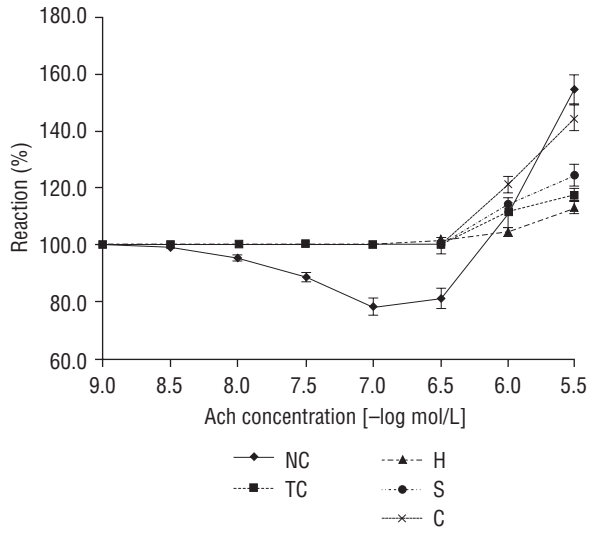


Figure 3. Ach-induced reaction

Functional changes after 3 months

Three months after implantation, calculated optimal resting tension increased markedly in a comparative degree in all groups of the implanted grafts (Table I).

Reaction to the contractile agents. We noted altered concentration-dependent contraction to NE in all grafts, with markedly lower contractile responses to maximal doses but comparable sensitivity (Table II, Fig. 1). Phe-induced contractions in group H, S and C were weaker compared with the non-implanted venous grafts (H *vs.* TC, S and C *vs.* NC) (Fig. 2). 5-HT was the only contractile agent, which caused the reactions in group H markedly stronger than TC, and in group C than NC ($p < 0.05$) (Table II). The maximal examined dose of 5-HT provoked 66.2% of reaction to the highest concentration of NE in group H, 66.5% in group S and 53.2% in group C. Met, but not Ket completely inhibited contraction to 5-HT in group H. In two other groups Met was also a more efficacious inhibitor than Ket. Maximal contractions to 5-HT + Met were 0.6 ± 0.2 g/mm^2 in group S and 1.3 ± 0.1 g/mm^2 in group C, to 5-HT + Ket 1.3 ± ± 0.3 g/mm^2 in group S and 6.3 ± 0.4 g/mm^2 in group C.

Ach	NC		TC		H		S		C	
[–log mol/L]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
9.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
8.5	99.1	0.2	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
8.0	95.9	0.7	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
7.5	88.8	1.6	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
7.0	78.7	2.7	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
6.5	81.3	3.5	100.0	0.0	101.7	0.6	100.0	0.0	100.0	2.6
6.0	110.6	4.3	112.1	1.7	105.0	1.7	114.8	2.1	121.9	2.6
5.5	155.5	5.2	117.7	2.6	113.3	4.6	125.2	3.6	146.0	4.5

Relaxation and endothelial function. A23187 evoked endothelium-dependent relaxation in group C. The time of three months under arterial conditions attenuated A23187-induced relaxation, causing a reduction in the maximal response ($p < 0.05$) (Table III). The rings

from group H and S were insensitive to A23187. SN--induced endothelium-independent relaxation was not affected in group C (*vs.* NC) and in group H (*vs.* TC). However, this reaction was diminished in group S ($p < 0.05$ *vs.* NC) (Table III). In all explanted grafts 3 months after interposition Ach did not evoke relaxation, but in higher concentrations (10^{-6}, 3×10^{-6} mol/L) contraction was seen, the strongest in group C (Fig. 3).

Discussion

In our study we employed a widely used canine model of reversed jugular vein interposition grafting of the carotid artery [2, 9, 10].

It was proved that contractile and proliferative responses of vascular smooth muscle cells (SMC) shared common receptor-mediated signal transducing mechanisms including modulation of ion membrane transport, calcium mobilisation, phosphoinositide turnover and activation of adenylate cyclase pathway [11]. Functional responses to vasoactive agents such as NE, Phe, 5-HT or SN are considered to be representative of "stimulus-response coupling" in SMC cells and define many of the common extracellular signals, surface receptor systems and intracellular regulatory mechanisms. Thus, the study of SMC responses can indirectly indicate of the cellular changes to other functional responses such as proliferation, migration or metabolism. It is likely that mild heating affecting the contractile responses of TC veins to NE. Phe and SN, downregulates also proliferative abilities of SMC, which are essential for neointima formation. Furthermore we showed that venous grafts in group H acquired much less ability to contract to 5-HT than the veins in group C or S. This reaction was mediated by 5-HT receptors, but mainly different from 5-HT_2 since Ket was not able to block 5-HT evoked contraction. This is consistent with earlier findings [12]. An increase in 5-HT contractility appeares parallel to the development of intimal hyperplasia [13]. The expression of the 5-HT receptors may identify SMC proliferative phenotype, which is involved in the formation of intimal hyperplasia [11].

The external stenting of saphenous bypass grafts was found to reduce early intimal and medial hyperplasia [14]. The external support eliminating pulsatile strain can induce medial atrophy [15], because circumferential strain is a major factor controlling medial structure and cell number. In our experiments we observed lower maximal contractions to NE, Phe in group S in comparison to group C, which may indicate SMCs dysfunction including their atrophy. Moreover we showed that even stent alone can downregulate reactivity to 5-HT, important factor stimulating proliferation of SMC (see above).

Basal tension. also called optimal resting tension, is higher for arteries than veins [2]. Its value in big canine veins (e.g. jugular, femoral) is slightly above 1 and removal of endothelial cells does not change it [12]. It is thought that arterialization of venous grafts is accompanied by increase in resting tension [2]. In our experiment the optimal resting tension inereased significantly three months after implantation in all groups to a similar degree. We noted the only slight difference between group H, S and C, possibly because of too small groups of a experimental animals.

It is thought that adrenergic supersensitivity is typical for deendothelialized veins, arteries and venous grafts at 6 weeks after implantation [16–18], because endothelial α_2-adrenergically mediated relaxation is lost [16]. In our experiments adrenergic sensitivity was slightly higher in grafts after implantation, but not enough to achieve statistical significance. Fann claimed that the adrenergic sensitivity of veno-arterial grafts at 12 weeks after implantation decreased [2], what may be related to endothelial recovery [19]. We assessed the adrenergic sensitivity of venous grafts at 3 months after implantation (that is, after peak of adrenergic hipersensitivity). Moreover we proved at least partial recovery of endothelium in experiments with A23187, that provoked endothelium-mediated relaxation.

Mild heat employed in HS device was found to be harmful for endothelium. We did not note any reaction to endothelium mediated relaxants (Ach, A23187) in TC as well as three months after implantation (group H). We observed different reaction to Ach and A23187. Ach produced decrease in tension only NC veins and in low concentration (10^{-9} to 3×10^{-7} mol/L), while in other groups and in NC veins higher concentrations evoked contraction. Increase in tension is mediated not by endothelium but by vascular SMC [2]. Davies revealed earlier that the reversed vein grafts did not relax to Ach, because they lose their ability to produce endothelium-derived relaxing factor (EDRF) in response to Ach and histamine even when endothelium is morphologically preserved [11, 20]. Ach needs receptor activation in endotelium to stimulate it for production of EDRF acting through SMCs [7, 21]. In contrast, A23187 releases similar EDRF as Ach by a process not associated with receptor activation [22]. Latest study showed that Ach-produced relaxation required transfer of EDRF from the endothelium to SMCs via gap junctions, whereas A23187 permits release directly into the extracellular space [23].

Conclusions

We conclude that mild heating of the venous grafts functionally destroys endothelium and significantly impairs smooth muscle cell function. Employment of mild

heat combined with external support may produce venous conduits less sensitive to vasoactive chemicals including mitogens involved in neointima formation.

References

1. Angelini G, Newby A (1989) The future of saphenous vein as a coronary artery bypass conduit. Eur Heart J, 10: 273.
2. Fann J, Sokoloff M, Sarris G et al. (1990) The reversibility of canine vein-graft arterialization. Circulation, 82 (Suppl.): IV8.
3. Dobrin P, Littooy F, Endean E (1989) Mechanical factors predisposing to intimal hyperplasia and medial thickening in autogenous vein grafts. Surgery, 105: 393.
4. Schwarz L, O'Donohoe M, Purut C et al. (1992) Myointimal thickening in experimental vein grafts is dependent on wall tension. J Vase Surg, 15: 176.
5. Zwolak R, Adams M, Clowes A (1987) Kinetics of vein graft hyperplasia: association with tangential stress. J Vasc Surg, 5: 126.
6. Henderson V, Mitchel R, Kosek J et al. (1987) Biochemical (functional) adaptation of "arterialized" vein grafts. Ann Surg, 203: 339.
7. Miller V, Reigel M, Hollier L et al. (1987) Endothelium-dependent responses in autogenous femoral veins grafted into the arterial circulation of the dog. J Clin Invest, 80: 1350.
8. Angelini G, Izzat M, Bryan A et al. (1996) External stenting reduces early medial and neointimal thickening in a pig model of arteriovenous bypass grafting. J Thorac Cardiovasc Surg, 112: 79.
9. Karayannacos P, Rittgers S, Kakos G et al. (1980) Potential role of velocity and wall tension in vein graft failure. J Cardiovasc Surg Torino, 21: 171.
10. Bush H Jr, Jakubowski J, Curl G et al. (1986) The natural history of endothelial structure and function in arterialized vein grafts. J Vasc Surg, 3: 204.
11. Davies M, Klyachkin M, Svendsen E et al. (1996) A comparative study of endothelium-derived relaxing factor-mediated relaxation and smooth muscle cell function in arterial and venous vein bypass grafts. Cardiovasc Surg, 4: 150.
12. Mawatari K, Komori K, Kuma S et al. (1997) Effects of serotonin and endothelin on the smooth muscle cells of autogenous vein grafts. Br J Surg, 84: 1419.
13. Radic Z, O'Donohoe M, Schwartz L et al. (1991) Alterations in serotonergic receptor expression in experimental vein grafts. J Vasc Surg, 14: 40.
14. Izzat M, Mehta D, Bryan A et al. (1996) Influence of external stent size on early medial and neointimal thickening in a pig model of saphenous vein bypass grafting. Circulation, 94: 1741.
15. Courtman D, Cho A, Langille L et al. (1998) Eliminating arterial pulsatile strain by external banding induces medial but not neointimal atrophy and apoptosis in the rabbit. Am J Pathol, 153: 1723.
16. Miller V, Reigel M, Hollier L et al. (1987) Endothelium-dependent responses in autogenous femoral veins grafted into the arterial circulation of the dog. J Clin Invest, 80: 1350.
17. Seidel C, Lewis R, Bowers R et al. (1984) Adaptation of canue saphenous veins to grafting: Correlation of contractility and contractile protein content. Cir Res, 55: 102.
18. O'Malley M, Hagen P, Mikat E et al. (1986) Increased vascular contraction and sensitivity to norepinephrine after endothelial denudation is inhibited by prazosin. Surgery, 99: 36.
19. Makhoul R, Davis W, Mikat et al. (1987) Responsiveness of vein bypass grafts to stimulation with norepinephrine and 5-hydroxytryptamine. J Vasc Surg, 6: 32.
20. Bush H, McCabe M, Nabseth D (1984) Functional injury of vein graft endothelium. Arch Surg, 119: 770.
21. Furchgott R, Jothianandan D, Cherry P (1984) Endothelium-dependent responses: the last three years. Bibl Cardiol, 38: 1.
22. Singer H, Peach M Calcium nd endothelial mediated vascular smooth muscle relaxation in rabbit aorta. Hipertension (Dallas), 4 (Suppl.): II–19.
23. Hutcheson I, Chaytor A, Evans W et al. (1999) Nitric oxide-independent relaxations to acetylcholine and A21387 involve different routes of heterocellular communication. Role of gap junction and phospholipase A_2. Circ Res, 84: 53.