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Improved endothelial function after a modified harvesting technique of the internal thoracic artery☆

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Abstract

Objective: One of the most important factors in bypass surgery is the preservation of endothelial function in the arterial graft. It was of interest, therefore, whether a slightly modified preparation procedure during surgery could contribute to improved endothelial function of the graft. We compared the functional activity of internal thoracic arteries (ITA) prepared according to the traditional harvesting method with occlusion by a clip, dissection at the distal end and storage of the artery in papaverine until its implantation (CA) with the functional activity of arteries which were also prepared and wrapped in papaverine, but were left perfused and dissected immediately before their anastomoses (PA). **Methods**: Samples of ITA were obtained from a total number of 28 patients, undergoing bypass surgery, and randomly distributed into two groups. The arteries were cut into rings and suspended in organ baths, containing Krebs-Henseleit solution, for isometric tension recording. Cumulative concentration response curves were determined for the contractile agents endothelin-1 (ET-1), 5-hydroxytryptamine (5-HT), noradrenaline (NA) and potassium chloride (KCl) and the relaxant compounds acetylcholine (ACH) and sodium nitroprusside (SNP) during active tone induced by 30 mM KCl. **Results**: ET-1 and 5-HT stimulated rings from both groups within the same concentration ranges but elicited significantly ($P < 0.05$) higher contractile responses in CA compared to PA. By contrast, concentration response curves for KCl and NA where nearly superimposable. On the other hand, maximal endothelium-dependent relaxant responses to ACH proved to be significantly stronger in PA (0.84 ± 0.20 g) as compared to CA (0.31 ± 0.05 g, $P < 0.05$) while endothelium independent relaxant responses to SNP where similar in both groups. **Conclusion**: These data suggest that leaving the ITA perfused during harvesting might improve considerably the endothelial function of the graft.

Keywords: Arteria thoracica interna; Harvesting; Functional activity in vitro; Endothelial function

1. Introduction

The left internal thoracic artery (ITA) is one of the most commonly used arterial bypass graft in myocardial revascularization since the early eighty's. Its clinical superiority has been shown to be tremendously in terms of long term patency and low incidence of graft degeneration as compared to saphenous vein grafts [1–3], even in end-stage coronary artery disease [4]. However, incidental vasospasm of the ITA in the early postoperative period [5,6] and development of intima proliferation/atherosclerosis in the grafted vessel years after operation [7,8] have been reported and may lead to hazardous situations for the patient.

The endothelial layer plays a key role in the regulation of vascular tone and homoeostasis [9], and preservation of these structures during bypass surgery seems to be crucial for long term survival of the graft. The aim of the present study was, therefore, to investigate, whether a slight modification of the ITA-harvesting procedure might modify endothelial function of the graft. In the conventional harvesting technique the ITA is carefully prepared in a pedicle from the chest wall and finally, after heparinization, the ITA is clipped and dissected at its distal end, wrapped in a papaverine cloth and stored underneath the sternum until its implantation [10]. The advantage of this procedure is an immediate information for the surgeon about the quality of

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the vessel concerning its inner diameter and flow. The disadvantages of this procedure, however, are: (1) a high wall tension combined with shear stress due to a sudden boost of resistance/pressure following the distal occlusion; and (2) a prolonged stasis of blood inside the vessel.

To our knowledge, the consequences of a sudden elevation of wall tension and blood stasis for the vascular function of the graft have never been investigated before. Therefore, we performed the following study, comparing the functional activity of arterial rings from two groups, namely the control group with the conventional harvesting technique as described above (clipped artery, CA) and the test group in which the ITA was prepared as described for the control group but kept perfused in situ being cut immediately before anastomosing to the target.

2. Materials and methods

Leftover of the ITA from 28 patients who underwent elective operative myocardial revascularization were used. Only men with a mean age of 64 years (49–75 years) and a mean weight of 84 kg (60–124 kg) were included. The groups were comparable with respect to general patient data, preoperative hemodynamics and medication (Table 1) and none of the patients had taken any medication containing acetylsalicylic acid during the last 10 days. Informed consent was obtained from the patients and permission for using the leftover of the ITA was obtained from the local ethical committee.

Table 1
Composition of both study groups including general patient data, preoperative hemodynamics and medical treatment

	Clipped arteries	Perfused arteries
Number of patients	14	14
Age (years)	64 (50–75)	63 (49–74)
Height (cm)	174 (163–184)	175 (164–182)
Weight (kg)	81 (60–124)	87 (64–109)
BMI (kg/m^2)	27 (22–37)	28 (20–38)
Time: prep. to implant. (min)	71 (50–90)	67 (40–80)
Flow of ITA (ml/min)	64 (20–140)	50 (25–90)
Smoker	4	8
Hypertension	8	10
Diabetes	3	2
Hypercholesterolemia	11	10
Beta-blocker therapy	12	10
Calciumantagonists	3	4
ACE Inhibitors	5	6
Aggregation inhibitors	13	13
Anticoagulants	7	4
Coronary vasodilators	7	9
Diuretics	2	1

BMI = body mass index. ECC = extracorporal circulation. Time prep. to implant. = time between the end of preparation and implantation of the ITA (min).

2.1. Preparation during surgery

2.1.1. Clipped arteries (CA)

The ITA was dissected and prepared in a pedicle. Following administration of heparin, the artery was clipped, wrapped in a cloth containing papaverine and stored under the manubrium sterni until use. After installation of the extracorporal circulation and cardioplegic arrest of the heart the peripheral vein anastomosis with the coronaries were accomplished. Thereafter the ITA was prepared about 2 min before implantation and the redundant part of the artery lying proximal to the clip was dissected for the in vitro study. The first flow was determined by measuring the volume of blood expelled from the end of the freely bleeding artery in a 30-s period, mean arterial pressure and the flow on the extracorporal circulation were recorded. The time between the end of ITA-preparation (clip setting and dissection) and reopening of the vessel by dissection of the distal end (duration of stasis) was determined.

2.1.2. Perfused arteries (PA)

In this group the arteries were also prepared in a pedicle, but remained connected to the blood flow in situ until implantation. After dissection immediately before anastomosing flow measurement from the freely bleeding artery was performed as described above. The time between end of ITA-preparation and dissection distally was determined. In all patients the ITA was grafted to the left anterior descending (LAD) artery being the last distal anastomosis.

2.2. Organ bath studies

After removal the ITA segments were placed into Krebs-Henseleit (KH) solution (composition mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.25, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, ethylendiamine tetraacetic acid 0.03), pH 7.4 at room temperature and transported to the research laboratory. The arterial segments (10–20 mm long) were cleaned from surrounding tissue and cut into rings (approximately 2 mm long). The arterial rings were mounted between two hooks of stainless steel wire (diameter, 0.15 mm), suspended in 10 ml organ baths containing KH solution at 37°C, and gassed continuously with 5% CO_2 in oxygen. The tension of the rings was recorded isometrically with electromechanical transducers (Statham Model UC 3, Gould. Inc., Oxnard, CA, USA) and a potentiometric recorder (Rikadenki). At the beginning of the experiments the rings were stretched to an initial tension of about 2 g and allowed to relax and equilibrate for approximately 2 h in the bathing medium, which was changed every 15 min. During this time the arterial rings were challenged once with a submaximal concentration of noradrenaline (1 μM). After the maximum of the contraction to noradrenaline was reached the function of the endothelium was tested with acetylcholine (1 μM). The preload of the rings was repeatedly readjusted until a stable baseline tension of 1 g

was achieved. Concentration-response curves for agonists were determined by cumulative additions, the concentration being increased when maximum effect had been produced by the previous concentration. Between two concentration-response curves repeated washouts during a time of about 100 min allowed the rings to relax and the baseline was readjusted to 1 g if necessary. When relaxant responses to acetylcholine and sodium nitroprusside were investigated, active tone was induced by increasing the KCl concentration in the organ bath to 30 mM, a concentration producing about 60% of the maximal KCl response. Each agonist was tested on two arterial rings from the same patient.

2.3. Drugs

The following pharmacological agents were used: endothelin-1 (ET-1, Novabiochem, Läufelingen, Switzerland), 5-hydroxytryptamine creatinine sulfate (5-HT), (−)-noradrenaline hydrogen tartrate, sodium nitroprusside (Sigma, Buchs, Switzerland), potassium chloride (Riedel-de Haen, Selze, Germany), N^G-monomethyl-L-arginine monoacetate (L-NMMA, Calbiochem, La Jolla, CA 92037, USA), acetylcholine chloride (Dispersa, A.G., Hettlingen, Switzerland). Endothelin was dissolved in 5% acetic acid and diluted in distilled water to give a 0.1 mM solution containing 1.6% acetic acid. Aliquots of 100 μl of 100 μM endothelin-1 and 10 mM acetylcholine were stored at −20°C until use.

2.4. Data analysis

Computerized analysis of concentration-response curves was performed using the procedure Fit Function in RS/1 (BBN Software Products Corporation, Cambridge, MA, USA) according to the equation $f(x) = A/(1 + B/X)$ where f(x) is the fraction of receptors activated by the agonist concentration X, A represents the maximal response (E_{max}) and B represents the EC_{50} values (concentration of the agonist producing 50% of E_{max}). Illustrations were made by the computer program Origin (Microcal Software, Inc., One Round Plaza Northampton, MA 01060 USA, http://www.microcal.com). Where appropriate, one way analysis of variance was performed, followed by the Bonferroni-corrected *t*-test to assign differences to individual between group comparisons when overall significance ($P < 0.05$) was attained. Data are presented as mean ± SEM. *P*-values less to 0.05 were considered statistically significant.

3. Results

3.1. Contractile responses

In a first series of experiments contractile responses to ET-1, 5-HT, NA and KCl were determined on rings from both clipped (CA) and perfused (PA) arteries. ET-1 and 5-HT stimulated rings from both groups within the same concentration range but elicited significantly higher contractile responses in CA compared to PA ($P < 0.05$, Fig. 1). In additional experiments the effect of nitric oxide synthase inhibition by L-NMMA on contractile responses to 5-HT was investigated on rings from PA. Compared to control rings pretreatment with 400 μM L-NMMA enhanced the maximal contractile effects of 5-HT significantly by 24 ± 9% ($n = 5$, $P < 0.05$) leaving the pD_2 values for 5-HT unchanged. In contrast to that observed with ET-1 and 5-HT, concentration response curves for NA and KCl where nearly superimposable. The calculated parameters for all contractile agonists are summarized in Table 2.

3.2. Relaxant responses

When relaxant agonists were investigated active tone was induced by exposing the arterial rings to KH-solution containing 30 mM KCl. This concentration of KCl evoked a submaximal and nearly similar increase in tone of 1.22 ± 0.13 g ($n = 50$) on CA and 0.95 ± 0.09 g ($n = 50$) on PA, corresponding to about 60% of maximum KCl contraction. Under these conditions the endothelium-dependent relaxant agonist acetylcholine relaxed arteries from both groups within the same concentration range, however it proved to be significantly more effective on arteries from the PA (0.84 ± 0.20 g) as compared to arteries from the CA group (0.31 ± 0.05 g, $P < 0.05$, Fig. 2, left). In contrast, the endothelium-independent relaxant agonist sodium nitroprusside (SNP) relaxed arteries from both groups with similar efficacy (Fig. 2, right). The calculated parameters for both relaxant agonists are summarized in Table 3.

3.3. Flow measurement

The flow measurement revealed similar values of 64 ± 11 ml in CA and 50 ± 6 ml in PA (for each $n = 11$), without reaching statistical significance.

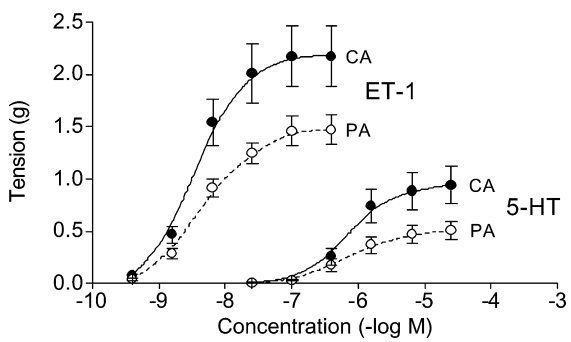

Fig. 1. Contractile responses to endothelin-1 (ET-1, for each curve $n = 20$) and 5-hydroxytryptamine (5-HT, for each curve $n = 16$) on internal thoracic arteries from the perfused (○, PA) and clipped (●, CA) group. Responses are expressed in grams, bars represent mean ± SEM.

Table 2
Parameters calculated for contractile agonists on rings from clipped and perfused human ITA[a]

	n	Clipped arteries	Perfused arteries
pD_2 values			
Noradrenaline	16	6.40 ± 0.07	6.29 ± 0.09
Endothelin-1	20	8.39 ± 0.06	8.36 ± 0.07
5-HT	16	6.16 ± 0.06	6.00 ± 0.10
KCl	16	1.54 ± 0.04	1.55 ± 0.03
E_{max} (g)			
Noradrenaline	16	1.63 ± 0.27	1.73 ± 0.23
Endothelin	20	2.17 ± 0.29	1.47 ± 0.14*
5-HT	16	0.88 ± 0.19	0.47 ± 0.09*
KCl	16	2.29 ± 0.25	2.35 ± 0.30

[a] Data are presented as mean ± SEM. Difference against clipped arteries significant at $P < 0.05$.

Table 3
Parameters calculated for relaxant agonists on rings from clipped and perfused human ITA during exposure to KH-solution containing 30 mM KCl[a]

	n	Clipped arteries	Perfused arteries
pD_2 values			
Acetylcholine	18	7.13 ± 0.06	7.27 ± 0.07
Sodium nitroprusside	16	6.56 ± 0.14	6.86 ± 0.11
E_{max} (g)			
Acetylcholine	18	0.31 ± 0.05	0.84 ± 0.20*
Sodium nitroprusside	16	1.11 ± 0.19	0.88 ± 0.18

[a] Data are presented in mean ± SEM. *Difference against clipped arteries significant at $P < 0.05$.

4. Discussion

The main finding of the present experiments was the observation that a slight modification of the surgical procedure, avoiding increased wall tension and blood stasis, may improve significantly the endothelial function of the arterial graft. Evidence for this was produced by the contractile responses to ET-1 and 5-HT which proved to be significantly higher in CA than in PA. ET-1, an endothelium-derived 21-amino acid vasoconstrictor peptide, is produced and released by endothelial cells. It exhibits potent vasoconstrictor effects by stimulating ET-A and ET-B receptors located on vascular smooth muscle. At the same time, however, ET-1 may cause release of nitric oxide (NO) and prostacyclin by stimulating ET-B located on endothelial cells, thereby inducing vasodilation [11,12]. Referring to the ET-1 contractile-response curves we found a significantly higher efficacy in CA compared to PA. 5-HT contracts vascular smooth muscle directly through 5-HT_2 receptors [13] and relaxes blood vessels via NO-mediated mechanism(s), through stimulation of 5-HT_{1D} receptors located on the endothelial cells. Evidence for an endothelium-dependent relaxant component in the vascular activity of 5-HT has been produced by a significant enhancement of the efficacy of 5-HT in rings from PA after blockade of nitric oxide synthesis by L-NMMA. These findings are in line with a marked enhancement of contractile responses to 5-HT in endothelium denuded ITA [14].

By contrast, NA and KCl stimulated CA and PA with similar efficacies. NA stimulates the arterial smooth mainly via contraction mediating α_1-adrenoceptors. Relaxation mediating β-adrenoceptors play only a minor role in the ITA, and relaxation mediating endothelial α_2-adrenoceptors are more or less neglectable in this artery [15–18]. Stimulation of α_1-adrenoceptors induces intracellular calcium release from bound intracellular calcium stores, which are triggered by receptor-operated calcium channels on the cell membrane. Potassium chloride induces contraction of the smooth muscle through depolarization of the cell membrane and enhanced calcium influx via voltage-operated calcium channels. In our present experiments both NA and KCl stimulated arterial rings from both groups with similar potency and efficacy producing nearly superimposable concentration-response curves suggesting that the modified harvesting method had not changed the functional activity of the vascular smooth muscle.

Our present experimental results concerning contractile

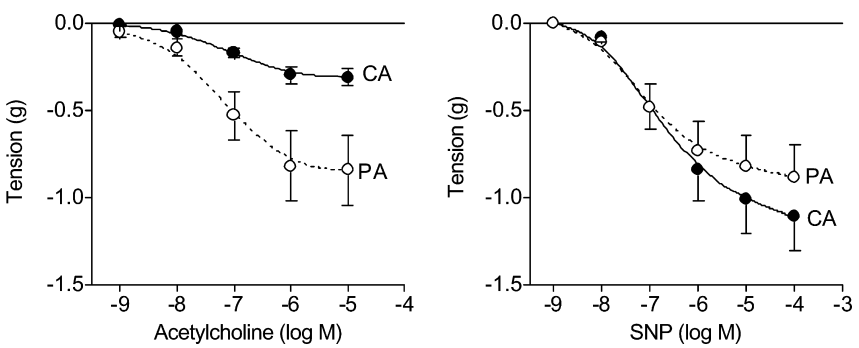

Fig. 2. Relaxant responses to acetylcholine (left, $n = 18$) and sodium nitroprusside (right, $n = 16$) during KCl (30 mM)-induced tone on internal thoracic arteries from the perfused (○, PA) and clipped (●, CA) group. Responses are expressed in grams, bars represent as mean ± SEM.

responses to ET-1 and 5-HT suggested, therefore, that the endothelial function in arteries from the PA group was considerably better preserved as compared to the CA group. Further evidence for this was obtained when relaxant responses to acetylcholine (ACH) and SNP were investigated on rings from both groups. Both agents induce relaxation of the vascular smooth muscle via NO-mediated activation of the soluble guanylate cyclase and enhanced formation of cyclic guanosine-5′-monophosphate. However, while SNP acts directly by donating NO, the action of ACH is endothelium-dependent, i.e. it requires endothelial cells to release NO [19]. In our experiments ACH proved to be significantly more effective in PA compared to CA whereas SNP relaxed arterial rings from both groups with similar efficacy, again suggesting significantly better endothelial function in arteries from the PA group as compared to the CA group.

The endothelium plays a central role in regulating smooth muscle tone by the release of vasoactive substances. Disruption of the critical balance between endothelium derived relaxing and contracting factors is believed to predispose the vascular smooth muscle to increased tone. Sudden occlusion of a vessel due to the surgical harvesting method induces an unphysiological condition, leading to communication between blood cells in static condition and endothelium via various humoral factors. Under these conditions adhesional processes or even apoptosis of endothelial cells may be the consequences.

In conclusion, our experimental data, demonstrating that clipping and occluding the ITA for even a relatively short time period attenuates significantly the endothelial function of the graft, suggest that leaving the ITA perfused during harvesting improves the preservation of the endothelial function and might thereby contribute to short- and long-term patency of the graft.

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Appendix A. Conference discussion

Dr U. Mehlhorn (*Cologne, Germany*): I have a short question. Did you measure flow in the ITA's in the respective groups?

Dr Grapow: Yes, we measured it.

Dr Mehlhorn: Do you have some data on that?

Dr Grapow: Yes. We didn't find any differences among both groups.

Dr J. Svennevig (*Oslo, Norway*): Could you indicate some values of the flow measurement. What was the flow?

Dr Grapow: The flow was actually around 60 ml/min. And we measured the free bleeding ITA for a half minute, and then we doubled the value.

Dr Svennevig: Many of us would rather inject papaverine into the artery after cutting the artery instead of just putting it into soaked tissue as you state. Do you have any comments on that practice?

Dr Grapow: Yes. We didn't investigate the injection of papaverine. We just put a small lump of papaverine around the pedicles during the preparation. So this is something that we would like to investigate in further experiments.

Dr S. Hagl (*Heidelberg, Germany*): Can you say anything about your preparation technique?

Dr Grapow: We just prepared the ITA and pedicle around 2 cm wide. And in the one group, dissection was performed after preparation; and in the

other group, we dissected shortly before anastomosing to the LAD. I think this is the traditional harvesting technique, the first one. We performed the preparation with electrocautery.

Dr D. Harris (*Cape Town, South Africa*): When you clamped the internal thoracic artery, was it clamped proximally? Or was it clipped distally and allowed to pulsate? Do you think that would make a difference?

Dr Grapow: It was just clipped distally, and we didn't occlude it proximally. I think this is maybe one point in the whole story. We have two things.

In the clipped artery, first, we are not on bypass and there is still pulsation on the graft. So I think the quality of pressure in the graft, in the clipped artery, is different to an open artery.

And the second thing is that we have a blood column inside the vessel which doesn't move. There is stasis of the cells. And we all know that stasis of cells leads to communication in between those cells. And macrophages, monocytes, thrombocytes, they all release transmitter substances due to starting coagulation, or perhaps apoptosis, we don't know yet. This has also to be investigated.

Dr S. Hagl (*Heidelberg, Germany*): I have an additional question. You are arguing that this is a mechanical effect, if I understand you well. The pulse characteristics are responsible; is that right?

Dr Grapow: No.

Dr Hagl: It's not right?

Dr Grapow: That's not right. We don't know exactly what mechanism is underlying.