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Even short-time storage in physiological saline solution impairs endothelial vascular function of saphenous vein grafts

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Abstract

Objectives: A faultless endothelial layer is decisive for vascular function and therewith graft's patency. Functional impairment of the endothelium increases risk of graft thrombosis, intimal hyperplasia, and consecutive accelerated graft atherosclerosis. Storage solutions for intra-operatively harvested saphenous vein segments (SVS) might have significant impact on endothelial function. We investigated the impact of short-time storage in physiological saline solution (PSS) and a potassium-chloride- and N-acetylthiohydroxyamine-enriched storage solution on venous endothelial function. Methods: Intra-operatively isolated SVSs (n = 19) were stored in different storage solutions for 90 min. They were then immediately studied in tissue bath at 36 °C with continuous oxygen insufflation. Following preconstriction with norepinephrine, dose—response relaxation curves of bradykinine (Brad) and sodium nitroprusside (SNP) were determined. We compared developed maximum wall tension, vessel constriction kinetics, endothelial cell- and smooth muscle cell (SMC)-dependent vasodilatory function. Results: Maximum vessel wall tension was reduced significantly in PSS-stored vessels (10.1 ± 9.8 mN mm⁻¹ vs 3.5 ± 3.4 mN mm⁻¹; p = 0.0372). Endothelium-derived vasodilatory function was likewise significantly reduced after short-time storage (20.6 ± 34.4% vs 35.0 ± 27.0%; p = 0.0437). SNP-mediated SMC-vasodilatory function was maintained equally well in both groups (88.2 ± 21.8% vs 83.0 ± 30.6% in PSS; p = n.s.). Conclusion: Even short-time storage in PSS significantly impairs endothelial vascular function. Concerning the essential role of a faultless endothelial layer, the quite common use of PSS as a storage solution for SVS in CABG surgery has to be discussed critically.

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Keywords: CABG; Vein graft; Endothelial vascular function; Preservation; Storage solution

1. Background

Surgical procedures using autologous vessel grafts for myocardial revascularization are providing, today as in the past, the most enduring results concerning recovery rates. Despite the fact that complete arterial revascularization in coronary artery bypass grafting (CABG) has become a proven method for myocardial revascularization with excellent long-time graft patency, venous grafts are still common and frequently used, especially in older patients [1—3]. The graft patency itself is determined by multiple factors, such as the progress of the patient’s coronary artery disease (CAD), quality of target vessel, quality of the anastomosis, bypass run-off, and, of course, the quality of the graft itself. Several aspects of preserving the venous graft’s quality have been the focus of prior discussions. Most important aspects include the method of preparation — conventional, bridging, or endoscopic techniques — to name at least some of them [3], the impact of hydrostatic pressure during intra-operative distensions, and the intra-operative graft storage between harvesting and implantation. Basic considerations are to optimize the graft before implantation and to inhibit activation of the endothelial layer [4,5]. Endothelial activation and injury of the endothelial layer promote leukocyte and platelet adhesion. This is associated with chronic inflammation and an obliteratorive lesion following graft stenosis and occlusion [5]. Concomitant risks are vascular spasm, graft atherosclerosis, occlusive intimal hyperplasia, and early and late graft failure. Regarding the importance of an intact endothelial layer for graft patency, several different storage solutions for intra-operative short-time conservation of free vascular grafts have been tested in the past. Among them heparinized blood, Ringer solution, or physiological saline solution (PSS) are in quite common use in cardiac surgery to date [6—8]. TiProtec® is a recently

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developed N-acetylhistidine-buffered, potassium-chloride-enriched, and amino-acid-fortified storage solution [9,10]. Its superiority in comparison to several different storing solutions in CABG — grafts has been demonstrated and it seems to be a feasible alternative for graft preservation [9,10]. Although several studies have examined the impact of storage solutions on endothelial-cell-mediated vascular function, the data still remain contradictory [11—13]. The aim of our study was to prove the impact of the commonly used PSS on endothelial vascular function in saphenous vein grafts after short-time storage. Our special intent was not to describe morphologic aspects of endothelial cell alteration as other studies have done previously, but rather to test endothelium-dependent vessel function after storage in commonly used PSS. We tested the PSS against the recently developed TiProtec® solution, which has been shown to have highly protective effects during cold storage of arterial vessel segments but has not been assessed before for storage of saphenous vein segments.

2. Material and methods

All experiments with human tissue were done with written informed consent of the patients. The approval of the ethical committee of the Medical Faculty of Dresden University of Technology, according to the principles expressed in the ’Declaration of Helsinki’ had been obtained previously.

2.1. Patient characteristics

The mean age was 66 ± 8 years, 14 patients were male (74%). Mean body mass index was 28 ± 3 kg m⁻². Arterial hypertension and hyperlipidemia were reported in all patients. Five patients (26%) suffered diabetes mellitus, 14% were current smokers. Patients with a peripheral vascular disease were excluded from the study.

2.2. Preparation of vein segments

Between October 2008 and March 2010, patients undergoing elective first-time CABG with saphenous veins were studied. All patients showed a three-vessel coronary artery disease. Patient’s age, medication and cardiovascular risk factors such as arterial hypertension, hyperlipidemia and diabetes mellitus, were recorded. Harvesting of the veins was uniformly performed via a bridge technique. Intraoperatively proximal adjacent saphenous vein segments were extracted from the thigh in 19 patients. The segments were prepared under direct view and subsequently not distended to minimize preparation-associated endothelial impairment. The extracted vein-segment of each patient was divided into two parts. One part was then stored at 20 °C in PSS, the other one in TiProtec®. The vein segments were then immediately brought to the laboratory for experimental examination. The mean time interval between explantation and experimental examination was about 1.5 h. Vein segments with macroscopic signs of venous insufficiency as well as veins with a diameter lower than 2 mm were excluded from the study.

2.3. Examination of harvested vein segments

Saphenous vein segments were then studied in a four chamber DMT-Myograph®. Vessel rings of 2 mm were placed on tungsten wires and transferred to a tissue bath at 36 °C with continuous oxygen insufflation. Initially, a mechanical preconstriction equivalent to a transmural pressure of 10 mmHg was set. After an accommodation phase of 20 min, when a steady state tension had been reached, receptor-independent vasoconstriction with potassium-enriched PSS (P-PSS 120 mmol l⁻¹) was initiated, and the resulting maximum constriction was recorded. Following replacement of the buffer with a low-potassium containing PSS, preconstriction was triggered with 5 µl 10⁻² mmol l⁻¹ norepinephrine. The main criterion for accepting a vessel to exhibit an undisturbed tension development was the presence of force generation of 70—80% of the maximum tension under 120 mmol l⁻¹ P-PSS for at least 10 min. After another buffer exchange and following 10 min of steady-state preconstriction with norepinephrine, dose—response curves were determined for bradykinine (Brad) and sodium nitroprusside (SNP). We evaluated maximum wall tension as well as constriction kinetics, and in addition, endothelial and smooth muscle cell (SMC)-derived vasodilatory function.

2.4. Data acquisition

DMT Tissue bath system 700MO® was used in connection with a PowerLab Data Acquisition System® Data recording was performed with LabChart® software.

2.5. Solutions

PSS: PSS had the following composition (in mmol l⁻¹): glucose 5.5, Na⁺ 144, Cl⁻ 128.7, H₂PO₄⁻ 1.18, SO₄²⁻ 1.17, HCO₃⁻ 25, K⁺ 5.9, Mg²⁺ 1.17, Ca²⁺ 2.5, and ethylene diamine tetraacetic acid (EDTA) 0.027. Osmolarity was 320 mosmol kg⁻¹.

Potassium-PSS: PSS enriched with potassium chloride (K⁺ 120 mmol l⁻¹) was used.

TiProtec® The solution was kindly provided by Dr F. Köhler Chemie (Alsbach-Hähnlein, Germany). The composition of the solution was (in mmol l⁻¹): α-ketoglutarate 2, aspartate 5, N-acetylhistidine 30, tryptophane 2, succrose 20, glucose 10, Na⁺ 16, Cl⁻ 103, H₂PO₄⁻ 1, K⁺ 93, Mg²⁺ 8, and Ca²⁺ 0.05. pH was adjusted to 7.0 and osmolarity was 305.15 mosmol kg⁻¹.

2.6. Vasoactive agents

Norepinephrine was used to induce direct smooth-muscle-mediated vasoconstriction. Bradykinin was used to stimulate endothelial nitric oxide production and thus elicit endothelium-dependent vasodilatation. SNP was applied to induce endothelium-independent vasodilatation by directly reducing the tone of SMCs.

2.7. Statistics

All experiments were performed in a paired manner with two segments taken from each vessel per group. Statistical
analysis was performed with Statistical Package for Social Sciences (SPSS) 12.0®. Software in cooperation with the Institute for Medical Informatics and Biometry. Data are expressed as means ± SD. Data obtained were evaluated for differences of mean values using Student’s t-test. Dose—response curves were analyzed using regression analysis and an analysis of variance (ANOVA) Table. A p value < 0.05 was considered significant. Constriction kinetics were assessed by fitting constriction curves with Sigma Plot®

3. Results

3.1. Patient characteristics and medical treatment

Demographic data such as age, sex, or recorded risk factors for coronary artery disease (CAD) did not gain any significant impact on vascular function in our small collective (n = 19). According to the guidelines for medical treatment of CAD, almost all patients had a preoperative medication consisting of acetylsalicylic acid (100%), angiotensin-converting enzyme (ACE) or AT1 inhibitors (95%), and statins (91%). Thus no significant influence of preoperative medical therapy on vascular function could be recorded.

3.2. Vessel wall tension and constriction kinetics

When vessel segments were stored for a time interval of 90 min in PSS, the maximum wall tension was significantly reduced in this group as compared with the vessels, which had been stored in TiProtec® solution (p = 0.01, Fig. 1). PSS-stored segments reached a mean tension of 3.08 mN mm⁻¹ cells, while that of TiProtec®-stored vessel segments was 8.85 mN mm⁻¹, respectively. In addition to the maximum of the response, the constriction kinetics differed significantly between both groups (p = 0.02). PSS-stored segments developed tension with a delay of approximately 100 ms, as compared with those stored in TiProtec®.

3.3. Endothelium-dependent vasodilatation

The concentration—response curve of bradykinin for PSS-stored vessels showed a decreased slope as compared with the TiProtec® group (Fig. 2). At the highest bradykinin concentration tested, PSS segments reached a significantly lower level of maximal endothelium-dependent vasodilatation (15.2% vs 32.5%, PSS vs TiProtec® storage, respectively; p = 0.048) (Fig. 3).

3.4. Endothelium-independent vasodilatation

Concentration—response curves of SNP did not differ significantly in both groups when expressed as percent relaxation of potassium-induced vasoconstriction (Fig. 4). Mean maximal percent relaxation was similar in both groups and not statistically different (77.4% in PSS vs 90.2% in TiProtec®, p = 0.12, Fig. 5).

4. Discussion

CABG has been the treatment of choice for symptomatic CAD historically. Recently, percutaneous coronary intervention (PCI), especially since the development of drug-eluting stents (DESs), has become a challenging alternative for CABG. Despite this, CABG remains the standard treatment for patients with severe three-vessel CAD or affection of the left main stem [14]. Although CABG provides excellent
Several studies have indicated the solution, Ringer’s lactate solution, PSS, or blood augmented particular interest [16]. Frequently used options are HTK time point to explain tissue and repair of the graft between the development of graft atherosclerosis [16]. In this regard, it is noted here consecutive inflammatory reactions and is causal in development of endothelial cell injury promotes leukocyte and thrombocyte adhesion with faultlessness of the graft’s endothelial layer. Endothelial the long-term outcome of CABG. Of particular interest is the progress of the patient’s CAD itself, were identified to affect venous grafts, quality of target vessel, run-off, and the sense of unreactive) conduits. An intact endothelial cell layer limit the success of autologous graft transplantation for treatment of CAD [15]. Several factors, for example, methods of vessel preparation, hydrostatic distension of venous grafts, quality of target vessel, run-off, and the morphologic patterns, these results demonstrate that the functional integrity of the endothelial layer was particularly interesting is the faultlessness of the graft’s endothelial layer. Endothelial injury promotes leukocyte and thrombocyte adhesion with consecutive inflammatory reactions and is causal in development of graft atherosclerosis [16]. In this regard, an optimized intra-operative storage of the graft between the time point of explantation and reimplantation is of particular interest [16]. Frequently used options are HTK solution, Ringer’s lactate solution, PSS, or blood augmented with heparin [16]. Several studies have indicated the superiority of HTK solution or heparinized blood in comparison to the reported alternatives [16,17]. However, more recently, a poor preservation of HTK solution with respect to coronary artery function has been reported [20]. Despite these evaluations, storage with saline solutions still not able to do so if an endothelium-dependent stimulus was applied. This supports the view of a rather selective impairment of endothelial function in the PSS group.

An intact endothelial layer plays a decisive role for whole graft function by influencing the vascular reactivity by paracrine secretion of vasoactive substances [17]. Following this, saphenous vein grafts are not simply passive (in the sense of unreactive) conduits. An intact endothelial cell layer is a prerequisite for a wide range of vascular functions, guarding against early and late graft failure. Our results clearly indicate that endothelial function is largely impaired after vessel storage in PSS.

In addition, we tested vessel relaxations with an endothelium-independent vasodilatory stimulus. SNP spontaneously liberates nitric oxide, which affects guanylate cyclase of SMCs directly, and provides vasodilation by stimulation of the SMC layer and bypassing the endothelium. This reaction was maintained in both groups, reflecting a retained vasodilatory vessel capacity. Following this reasoning, PSS-stored vessels had the capacity to relax, but were not able to do so if an endothelium-dependent stimulus was applied. This supports the view of a rather selective impairment of endothelial function in the PSS group.

Our results clearly show the profound reduction of vascular function after a short-time storage in PSS. This is in accord with the results of other groups [16,17]. Zerkowski et al. showed a greater degree of dissociation of the endothelial cells and an altered surface structure in saline solution-stored vessel segments studied with scanning electron microscopy [17]. In this context, it is noted here that the frequently added heparin may exert additional toxic
effects on the endothelial layer and therefore should be avoided [18,19].

5. Conclusion

Short-time PSS storage of venous vessel segments resulted in a significant reduction of baseline vessel tone and of endothelium-dependent vasodilatation. In both groups, the endothelium-independent vasodilatory function was maintained alike the SNP—induced vasodilatation showed. Despite this, PSS-stored vessels did not relax appropriately following endothelium-dependent stimulation with bradykinin. Our results reveal evidence for a negative impact of PSS on vascular and endothelial function even after short-time storage. Following this and with regard to the importance of a faultless endothelial layer for graft patency, PSS should be avoided for intra-operative storage of harvested vein grafts. Alternatives such heparinized blood, HTK, or TiProtec® should be established by default.

6. Study limitations

Our study is certainly limited by the proportional small number of examined segments (n = 19 each group). Anyhow, the negative impact of PSS gained significant relevance. Performing an outcome study to prove the clinical impact would be practically impossible. Hence, we have to follow the leads and rely on the tested surrogate end points, linked logically with the main subject — graft patency.

References