Vasoreactivity of arterial grafts in the patient with diabetes mellitus: investigations on internal thoracic artery and radial artery conduits

Olaf Wendler, Peter Landwehr, Doris Bandner-Risch, Thomas Georg, Hans-Joachim Schäfers

Department of Thoracic and Cardiovascular Surgery, University Hospital Homburg, Homburg/Saar, Germany
Institute for Medical Biometrics, Epidemiology and Medical Informatics, University Hospital Homburg, Homburg/Saar, Germany

Abstract

Objective: Arterial revascularization with either internal thoracic artery (ITA) or radial artery (RA) appears to be particularly attractive in diabetic patients. Previous investigations have shown that endothelial dysfunction and atherosclerosis are seen more often in these patients. The aim of this study was to compare the vasoreactive properties of ITA and RA grafts in diabetic and non-diabetic patients. Methods: Arterial rings were harvested from 57 patients who underwent complete arterial revascularization. The patients were divided into a non-diabetic group (I: n = 30) and patients with diabetes mellitus (II: n = 27). Arterial rings of the ITA (I: n = 30; II: n = 27) and RA (I: n = 28; II: n = 19) were mounted on a strain gauge in oxygenated, normothermic Krebs’s-Henseleit solution at optimal resting tension. With KCl (80 mM) serving as the control, assessment of force of contraction (norepinephrine), endothelium-dependent relaxation (acetylcholine) and smooth muscle-dependent relaxation (glyceroltrinitrate) were obtained. Results: After KCL, the RA showed a trend to lower maximum contraction forces in diabetics (I: 76 ± 25 mN; II: 69 ± 29 mN), which was pronounced in patients with diabetes of more than 10 years duration (55 ± 23 mN; P = 0.1). Maximum contraction force of the ITA was similar in both groups (I: 41 ± 20 mN; II: 34 ± 19 mN) and not influenced by the duration of diabetes. The two groups showed no significant differences of the relative vasoconstriction after norepinephrine in RA (I: 53 ± 18%; II: 61 ± 19%) and ITA rings (II: 70 ± 23%; II: 69 ± 25%). Also, endothelium-dependent relaxation with acetylcholine in RA (I: 53 ± 14%; II: 57 ± 16%) and ITA rings (II: 42 ± 17%; II: 44 ± 20%), and smooth muscle relaxation with glyceroltrinitrate of RA (I: 72 ± 8%; II: 73 ± 12%) and ITA rings (II: 64 ± 12%; II: 58 ± 20%) was comparable in both groups. No influence of duration of the diabetic disease was noted. Conclusions: Although RA rings of patients with a long duration of diabetes have decreased maximum contraction forces, their relative vasoconstriction after norepinephrine, endothelium-dependent relaxation and smooth muscle relaxation was similar to non-diabetic patients. We thus conclude that the RA is an adequate arterial conduit in the patient with diabetes mellitus.

Keywords: Arteries; Endothelium; Radial artery; Internal thoracic artery; Coronary artery bypass surgery

1. Introduction

Coronary artery bypass grafting (CABG) using left internal thoracic artery (ITA) and vein grafts is well established in the treatment of coronary artery disease (CAD), particularly in patients with diabetes mellitus [1]. Recently, an increased number of arterial conduits – right ITA anastomosed to the left anterior descending (LAD) and left ITA to the circumflex coronary artery (CX) – has been correlated to improved long-term results in the diabetic patient [2]. Thus arterial revascularization achieves the goal of further advantages in the long term [3]; an increased perioperative morbidity particularly after bilateral harvesting of the ITA remains an associated obstacle [4]. Using skeletonized ITA grafts, the radial artery (RA) as a second graft and the ‘T-graft’ approach, the perioperative risk of total arterial CABG in diabetic patients is not increased compared to non-diabetics [5]. However, vasospasm remains a specific risk factor after CABG using only arterial conduits [6].

It is generally agreed that the RA has a higher potential of vasoconstriction compared to the ITA [7–9], but no data are available on the influence of diabetes mellitus on this problem.

Variable information is reported on endothelial function of ITA and RA conduits. Some studies on human arterial
Non-insulin-dependent DMa (I: Patients were divided into a group of non-diabetic patients and diabetic patients undergoing CABG. Table 1 shows the demographic data of these patients. Preoperative medication was stopped 24 h before the operation. Patients gave their informed consent to this investigation. Preoperative medication was stopped 24 h before the operation. All patients underwent arterial revascularization with bilateral ITA grafts or left ITA and RA at our institution. All patients have shown impaired endothelial function [13,14]. In vitro experiments using aortic rings from diabetic rats described impaired endothelial function correlating to the duration of diabetic disease [15]. There is, however, limited knowledge about the influence of diabetes mellitus on the function of ITA and RA conduits.

The purpose of this study was to determine the vasoreactivity of ITA and RA conduits with respect to diabetes mellitus. Maximum vasoconstriction, endothelium-dependent relaxation and endothelium-independent relaxation of human ITA and RA rings were measured from non-diabetic and diabetic patients undergoing CABG.

2. Material and methods

2.1. Patients

Arterial segments were obtained from 57 patients who underwent arterial revascularization with bilateral ITA grafts or left ITA and RA at our institution. All patients gave their informed consent to this investigation. Preoperative medication was stopped 24 h before the operation. Table 1 shows the demographic data of these patients. Patients were divided into a group of non-diabetic patients (I: n = 30) and those with diabetes mellitus (II: n = 27). A total of 57 arterial rings of the ITA (I: n = 30; II: n = 27) and 47 rings of the RA (I: n = 28; II: n = 19) were used for organ bath experimentation.

ITA and RA conduits were harvested in skeletonized fashion as described previously by our group [16]. Directly after dissection of the graft, the distal vessel segment (around 10 mm) was cut off and immediately stored in a container with ice-cold, oxygenated modified Kreb’s-Henseleit solution. To prevent early vasospasm of the segments, glyceroltrinitrate in a concentration of 10^-6 M was added into the solution.

Transfer to the laboratory took an average of 9.3 ± 3 min. The vessels were cleaned from surrounding tissues and cut into rings of 3 mm length with a sharp scalpel. Care was taken not to disrupt the integrity of the endothelium. Time between harvesting and experimentation varied between 24 and 37 min (31 ± 9 min).

2.2. Organ bath technique

The arterial rings were mounted on two stainless-steel wires in a 20 ml silicon-coated water-jacketed custom-made glass organ bath. The organ bath was filled with modified Kreb’s–Henseleit solution at 37°C. The lower wire was fixed and the upper wire was attached to a force transducer (K30 type 351, Hugo Sachs, March, Germany), which was connected to a micrometer (type 850 N, Hugo Sachs, March, Germany). The force transducer was coupled to a bridge amplifier (type 660, Hugo Sachs, March, Germany).

Rings were given a preload of 3 mN, which was increased to 15 mN over a time of 60 min. During this time of equilibration, washing periods with Kreb’s–Henseleit solution were performed every 15 min until measurements were started.

2.3. Kreb’s–Henseleit solution and drugs

Kreb’s–Henseleit solution had the following composition (mM): NaCl, 118; KCl, 4.7; NaHCO3, 24.88; NaH2PO4, 2.0; MgSO4, 1.64; KH2PO4, 1.18; CaCl2, 2.52; d(+)-glucose, 5.55. The solution was aerated with a gaseous mixture of 95% O2 and 5% CO2 at 37°C.

Potassium-substituted Kreb’s–Henseleit solution had the following composition (mM): NaCl, 44.75; KCl, 80; NaHCO3, 24.88; NaH2PO4, 2.0; MgSO4, 1.64; KH2PO4, 1.18; CaCl2, 2.52. The chemical ingredients were bought from Sigma (Steinheim, Germany) and Merck (Darmstadt, Germany).

The drugs used were norepinephrine (Hoechst AG, Frankfurt, Germany), acetylcholine (Sigma, Steinheim, Germany) and glyceroltrinitrate (Schwarz, Monheim, Germany). Solutions were prepared daily and discarded after each experiment. Initial solubilization and dilutions were performed with distilled water.

2.4. Protocol

2.4.1. Contraction

Arterial segments were contracted using potassium-substituted Kreb’s–Henseleit solution (KCL 80 mM) to obtain maximum contraction (KCL-100%). After three washing periods with Kreb’s–Henseleit solution over 30 min and stabilization of resting length, dose–response curves for norepinephrine (10^-5–10^-5 M) were registered.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Demographic data</th>
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<tr>
<td></td>
<td>Non-diabetic (I)</td>
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<tr>
<td>Patients (n)</td>
<td>30</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 ± 10</td>
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<tr>
<td>Sex (f)</td>
<td>4 (13%)</td>
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<tr>
<td>Duration of DM (years)</td>
<td>–</td>
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<tr>
<td>DM &gt; 10 years (n)</td>
<td>–</td>
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<tr>
<td>Insulin-dependent DM (n)</td>
<td>–</td>
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<tr>
<td>Non-insulin-dependent DM (n)</td>
<td>–</td>
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<tr>
<td>Triple-vessel-disease (n)</td>
<td>29 (97%)</td>
</tr>
<tr>
<td>Hyperlipoproteinemia (n)</td>
<td>15 (50%)</td>
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<tr>
<td>Hypertension (n)</td>
<td>13 (43%)</td>
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a DM, diabetes mellitus.
2.4.2. Endothelium-dependent relaxation

Kreb’s–Henseleit solution was added in three washing periods over 30 min. After stabilization at resting length, precontraction of the arterial rings to 60–80% of KCL-100-%-contraction was performed using norepinephrine. When a stable level of contraction was obtained, dose–response curves for acetylcholine \(10^{-8}–10^{-5}\) M were registered.

2.4.3. Endothelium-independent relaxation

After three washing periods with Kreb’s–Henseleit solution over 30 min and stabilization at resting length, precontraction of the arterial rings to 60–80% of KCL-100%-contraction was performed using norepinephrine. When a stable level of contraction was obtained, dose–response curves for glyceroltrinitrate \(10^{-8}–10^{-5}\) M were registered.

2.5. Statistical analysis

Forces generated by the arterial rings were registered in milli-Newton (mN) and digitalized by a personal computer using the ACAD 2000 software package (Hugo Sachs, March, Germany).

Contraction to norepinephrine was calculated as percentage of the maximum KCL contraction force. Relaxation to acetylcholine and glyceroltrinitrate was calculated as percentage of the previously established norepinephrine-induced contraction.

Statistical analysis was performed using the StatView software package. Data were reported as mean values ± standard deviation. A comparison between non-diabetic and diabetic rings was made using the Mann–Whitney U-test between RA and ITA conduits with the Wilcoxon signed rank test. The relationship between non-diabetic rings and rings from patients with diabetes longer than 10 years was analyzed using Spearman rank correlations. Confidence intervals (CI) of difference 95% based on normal distributions were calculated using the SPSS (version 10.0) software package and reported for the most important differences. All P-values < 0.2 were noted. A P-value of < 0.05 was considered statistically significant.

3. Results

3.1. Absolute contraction

Maximum KCL contraction of RA rings was higher in non-diabetic compared to diabetic rings (I: 76 ± 20 mN; II: 69 ± 29 mN; CI: –21.5, 10.5), but this did not reach statistical significance. In ITA rings, maximum contraction force was similarly and insignificantly lower in diabetic patients (I: 41 ± 20 mN; II: 34 ± 19 mN; CI: –17, 3.4).

Comparing RA and ITA rings, maximum contraction forces of the RA from non-diabetic and diabetic patients (I: 76 ± 25 mN; II: 69 ± 29 mN) were significantly higher than in ITA rings of the same group (I: 41 ± 20 mN/\(P < 0.0001\); II: 34 ± 19 mN/\(P = 0.003\)) (Fig. 1).

3.2. Relative contraction

Response to norepinephrine in relation to the maximum KCL contraction showed no significant differences between the two groups in RA \(10^{-5}\) M, CI: –3.5, 18.8 and ITA rings \(10^{-5}\) M, CI: –14.1, 11.8 (Figs. 2 and 3).

Comparing RA and ITA rings in group I, maximum response to norepinephrine \(10^{-5}\) M of the RA \(53 ± 18\%\ was significantly lower compared to the ITA \(70 ± 23\%; P = 0.03\). In diabetic patients, this comparison showed no significantly different response of the ITA (RA: 61 ± 19%/ITA: 69 ± 25%).

3.3. Endothelium-dependent relaxation

No significant differences were registered between the dose–response curves of acetylcholine in RA \(10^{-5}\) M, CI:
11.8, 5.4) and ITA rings (10^{-5} M, CI: -12.5, 7.9) from groups I and II (Figs. 4 and 5).

Comparing maximum relaxation between RA and ITA rings after acetylcholine (10^{-5} M), a significantly increased relaxation of the RA was observed in group I (RA: 54 \pm 14%/ITA: 42 \pm 17%; P < 0.003) and II (RA: 57 \pm 16%/ITA: 44 \pm 20%; P < 0.01).

3.4. Endothelium-independent relaxation

Smooth muscle relaxation after administration of glyceroltrinitrate resulted in a maximum relaxation of all arterial rings. The relaxation of the RA (10^{-5} M, CI: -6.2, 5.3) and ITA (10^{-5} M, CI: -3.3, 14.5) was not significantly different between the two groups (Figs. 6 and 7).

Maximum relaxation of RA rings (I: 72 \pm 8%; II: 73 \pm 12%) after a dosage of 10^{-5} M glyceroltrinitrate was significantly higher than in ITA rings (I: 64 \pm 12%/ P = 0.009; II: 58 \pm 20%/P = 0.01).

3.5. Influence of the duration of diabetes mellitus

To determine the influence of the duration of the diabetic disease, we analyzed only patients with disease for longer than 10 years (n = 10).

Absolute contraction force of RA rings compared to non-diabetic patients tended to be lower in this subgroup (55 \pm 23 mN; CI: -40.3, 0.3; P = 0.1). In contrast, no significant difference in the contraction of ITA rings (40 \pm 24 mN; CI: -15.5, 14.2) was noted.

Relative contraction after norepinephrine of RA and ITA rings was not influenced by the duration of the diabetic disease. However, comparing RA and ITA conduits in these diabetic patients, response to norepinephrine was nearly equal between the two grafts (RA: 65 \pm 15%/ITA: 63 \pm 24%; P = ns).

Endothelium-dependent and endothelium-independent relaxations showed no significant differences between the two groups and arterial conduits.

4. Discussion

The ITA is the conduit of choice in CABG due to its excellent long-term results. This prognostic benefit is
present in diabetic and non-diabetic patients but is less pronounced in the diabetic individual [1]. Using left and right ITA for revascularization of LAD and circumflex artery, further improvement of long-term survival during a follow-up of 12 years has been shown particularly in patients with diabetes mellitus [2]. However, in diabetic patients, bilateral harvesting of the ITA is associated with an increased risk of sternal complications [4].

Complete arterial revascularization achieves the goal of further advantages during the late-term course [3]. Besides left and right ITA, the gastroepiploic artery (GEA) and RA are used for this purpose [3, 16–18]. In the past few years, the RA has been favoured over the GEA due to low morbidity and excellent long-term results [19]. In our own experience – using skeletonized ITA conduits, the T-graft approach and the RA as an additional conduit – the perioperative risk of complete arterial revascularization in the diabetic patient is not increased compared to non-diabetic patients [5]. In around 80% of these patients, the operation can be performed using left ITA and one RA, so that bilateral harvesting of the ITA is avoidable. This raises the question whether the quality of RA and ITA conduits from diabetic patients is comparable to grafts from non-diabetic patients.

In vivo investigations using intravascular flow measurements in T-grafts after complete arterial revascularization have shown no difference of baseline flow and coronary flow reserve 1 week and 6 months postoperatively between RA and ITA conduits [16]. Interestingly, we also found no significant differences between the coronary flow reserve – determined with adenosine – between diabetic and non-diabetic patients after CABG using T-grafts [20].

In vitro investigations on human arterial rings have documented that the RA has a higher potential of vasoconstriction compared to the ITA conduit [7–9]. Information about endothelial-dependent vasodilatation varies between equal [10], decreased [7] and increased [11] dilatation of the RA. These different results are not completely understood up to now. One explanation for this may be the various methodologies used in determining the optimal length and tension during the organ bath experiments [7].

In patients with diabetes mellitus, development of endothelial dysfunction has been documented [12]. One of the explanations is an increase in thickness of the basement membrane in the microvasculature in Type I diabetes, which was demonstrated by microscopic investigations on human skin arteries [21]. Risk factors for early development of endothelial dysfunction are elevated low-density lipoproteins [22] and non-insulin-dependent diabetes [13].

Duration of the diabetic disease seems to play a major role in the alteration of the endothelium. Postischemic flow measurements in brachial arteries demonstrated a significant reduction of blood flow in patients with diabetes for more than 10 years [14]. This is confirmed by intravascular flow measurements in diabetic rats, which showed no impairment of endothelium-mediated relaxation at the onset of diabetes [23]. In vitro investigations have been performed on aortic rings from streptozitocin-induced diabetic rats [15]. The author could show that after precontraction with norepinephrine, endothelium-dependent relaxation to acetylcholine was normal for 1 and 2 weeks but was impaired 8 weeks after induction of the diabetic metabolism. Endothelium-independent relaxation using nitroglycerin was unaltered at both the stages. However, preliminary data from resistance vessels of gluteal fat biopsies from Type I diabetic patients showed no significant differences of the acetylcholine-induced relaxation compared to non-diabetic patients [24].

In the present study, we found higher maximum contraction forces in human RA rings compared to the ITA, a phenomenon which is already known. Although absolute maximum contraction of the RA was around twofold higher compared to the ITA vasoconstriction, physiological stimulation with norepinephrine resulted in lower relative contraction of the RA compared to ITA conduits. In addition, endothelium-dependent and endothelium-independent relaxations of the RA were increased compared to rings from ITA conduits. Comparison between RA and ITA rings, however, is complicated by differences in the diameter of the two grafts and the fact that the distal ITA is more prone to spasm than the proximal ITA. In published investigations, different experimental attempts have been made to neutralize differences of the ring diameter, which may explain the discrepancy of the results [7, 10, 11].

Interestingly, we have seen a trend of reduced maximum contraction forces in RA and ITA conduits in diabetic patients. Particularly in patients with diabetes of longer than 10 years duration, this reduction in contraction of the RA rings was pronounced in contrast to ITA rings. From the statistical point of view, this comparison is hampered by the small number of arterial rings from patients with a longer duration of diabetes. The trend of reduced contraction of the RA rings in these patients may become significant if the size of this group were higher and more similar to the non-diabetic group. These differences may be explained by the
higher incidence of calcification in the RA in diabetic patients [5]. However, this showed no negative influence on the reactivity to physiological stimulation with norepinephrine. Surprisingly, endothelium-dependent and endothelium-independent relaxations were not significantly different between diabetic and non-diabetic patients. One may suggest that this conflicts with data from Lekakis et al. [14] who reported impaired flow-mediated vasodilatation in the brachial artery in diabetic patients. Following ischemia of the arm, however, hyperaemic flow in the brachial artery, which is endothelium-dependent, is not only influenced by the vasoreactivity of the brachial artery but also by the vasoreactivity of the distal capillary bed.

In summary, the present study shows an adequate vasoreactivity of ITA and RA conduits. In the presence of diabetes mellitus, risk of vasospasm may be decreased; however, endothelial function, which is a predictor for good long-term prognosis was not impaired. Thus we conclude that the RA is a functionally adequate arterial conduit for complete arterial CABG in the diabetic patient. Further investigations using different stimuli of vasoconstriction and endothelial function are desirable to verify these data in future.

References


Appendix A. Conference discussion

Dr M.A. El Banna (Cairo, Egypt): I want to ask whether you conducted your experiments in ice-cold solution?
Dr Wendler: No. When we cut off the rings, we put them in ice-cold solution for the transport to the laboratory.
Dr El Banna: Just to preserve them?
Dr Wendler: Only for the transportation; the experiment was performed at 37°C.
Dr El Banna: Doesn’t this affect the composition of the vessels?
Dr Wendler: No, this is the normal way to perform these experiments like others have done in the past. If you don’t use the ice-cold medium for transport, you may have endothelium damage by the influence of higher temperature.
Dr F. Wellens (Aalst, Belgium): Can you extrapolate the results of the ITA? You used only the distal part of the ITA, but there are different segments of the ITA which are reacting in a different way.
Dr Wendler: Our aim was to investigate the influence of diabetes. As you mentioned, the comparison between the ITA, and radial artery rings is different because these two grafts have different diameters. We have performed comparisons between these two conduits, but I did not show these data yet because from the experimental point of view comparison of the data for diabetic influence is more exact. In this investigation, we compared the same segments of the two vessels with the same diameter with each other.