Venous and arterial coronary artery bypass grafts in a pharmacological perspective
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CHAPTER 5

Functional properties of the saphenous vein harvested by minimally invasive techniques


**Introduction**

In recent years new techniques have been developed to harvest the saphenous vein (SV). These minimally invasive surgical, or skin-bridging, methods are used to diminish the wound surface, and hence reduce postoperative morbidity at the site of explantation.

Surgical techniques have been shown to affect the functional properties and patency of venous grafts. Besides changes in endothelial integrity, the surgical manipulation of SV can result in vascular smooth muscle cell damage. Of the processes explaining venous graft failure, namely thrombosis, intimal hyperplasia and atherosclerosis, the first is triggered by phenomena like surgical-induced endothelial injury. Injury of vascular smooth muscle cell may affect responses to circulating vasoactive agents of the graft.

Since new techniques became available for harvesting the SV, it seemed of interest to investigate the influence of these minimally invasive methods on the functional properties of the vein. The two skin-bridging techniques investigated in the present study consist of the mediastinoscope-assisted venectomy (MV) and the endoscope-assisted venectomy (EV), respectively. We studied the vasoconstrictive and vasodilatory characteristics of saphenous vein remnants harvested by means of the new skin-bridging methods and compared them with those of conventionally dissected vein segments, in vitro.

**Materials and Methods**

**Surgical techniques**

For each venectomy method investigated, one surgeon was involved per method, except for the conventional venectomy (CV). Harvest of the SV by the two skin-bridging methods was followed by cannulation, distension, preservation, and grafting as described for the conventional method.

*Conventional venectomy.* Through a small incision at the medial side of the ankle, the SV was visualized and followed along its course by cutting the skin with a pair of scissors. Subsequently, the vein was cannulated at the distal end and distended with a cold heparinized Ringer-Lactate solution to ensure patency, to check for leaks and to surmount vascular spasm. The vein was dissected free from the surrounding tissue down to the adventitia, and side branches were ligated, followed by excision. Until grafting the prepared vein segment was stored in cold heparinized Ringer-Lactate
solution. The coronary anastomosis and flow were checked by flushing the graft with St. Thomas cardioplegic solution.

Mediastinoscope-assisted extraluminal venectomy. The proximal part of the SV was identified after an incision of the skin in the lower inguinal region. Through a standard mediastinoscope and/or "Langenbeck" hook the skin was lifted and the vein visualized, followed by dissection with assistance of a mediastinoscopic suction cannula. Side branches were clipped by a 3-mm clip applicer (Horizon Medical Inc, Weck Closure Systems, Santa Ana, CA, U.S.A.). Additive skin incisions were made when the length of the mediastinoscope failed to bridge the segment necessary for grafting.

Endoscope-assisted extraluminal venectomy. For endoscopic vein harvesting the Vasoview balloon dissection system (Origin Medsystem Inc, Menlo Park, CA, U.S.A.) was applied as described by Morris and colleagues.3

Isolated vessel preparations
Saphenous vein remnants were obtained from 15 patients, with an age range of 51 to 73 years, who were subjected to aortocoronary bypass operation, and without vein-pathology in their medical history. All patients had chronic stable angina, except for two patients with instable angina pectoris. The antianginal medication the patients received consisted mainly of β-blockers, calcium antagonists and (long-acting) nitrates. The patients gave written informed consent and the study was approved by the Ethics Committee of the Academic Medical Center. Saphenous vein remnants were obtained immediately after the last coronary anastomosis was completed and preserved in University of Wisconsin (UW) solution at 4°C for 2 days.

Experimental protocol
Each piece of SV was cut into 4 rings of approximately 5-mm length. The rings were mounted between two L-shaped stainless steel hooks, in 8 mL organ baths filled with oxygenated Krebs-Henseleit solution of 37°C (pH 7.4). Each preparation was fixed, using a silk thread, to an isometric force transducer (ADI Instruments, Castle Hill, Australia), and force was recorded with a MacLab/8 computer system (ADI Instruments, Castle Hill, Australia). Each ring was subjected to a pretension of 40 mN, which was maintained throughout the experiment. After an equilibration period of 60 minutes the vascular rings were primed and tested for viability, by exposing them twice to an isotonic KCl solution (KCl) of 123.8 mM. After wash out and one hour of
equilibration cumulative concentration-response curves were constructed for phenylephrine (Phe), the nitric oxide (NO)-donor sodium nitroprusside (SNP) and acetylcholine (ACh) (1 nM - 0.1 mM), respectively, with 1 hour intervals. For the relaxations to SNP and ACh, the rings were precontracted with Phe (10 μM). Since binding of ACh at the endothelial cell provokes the release of NO, which induces relaxation of vascular smooth muscle, the responses of the preparations to ACh were taken as evidence for the presence or absence of functional endothelium. Appropriate controls were run simultaneously in different ring preparations obtained from the same vascular segments.

Preservation in UW-solution
All preparations were preserved in UW-solution, prior to the actual experiments. The effect of preservation in UW on the functional properties of the SV preparations was investigated in a pilot study. Vein remnants of CV procedures, obtained from 27 patients after aortocoronary bypass operation, were divided into 3 groups. In the first group the experiments were performed immediately after the remnants became available, thus without being preserved in UW-solution. The second and third groups of preparations were stored for 24 and 48 hours, respectively. The three groups of veins were compared with respect to their responses to a KCl solution, Phe, SNP and ACh, respectively, using the same experimental protocol as mentioned above for the comparison of the venectomy methods.

Drugs and solutions used
L-phenylephrine hydrochloride was obtained from Sigma (St.Louis, MO, U.S.A.); sodium nitroprusside dihydrate GR from Merck (Darmstadt, Germany); and acetylcholine chloride from Sigma (St. Louis, MO, U.S.A.). All drugs were dissolved in distilled water.

The Krebs-Henseleit solution had the following composition (mM): NaCl 118.0, KCl 4.7, NaHCO₃ 25.0, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.1, and glucose 5.6; the heparinized Ringer-Lactate: Na⁺ 146.8, Ca²⁺ 1.8, lactate 26.8, K⁺ 5.4; Cl⁻ 129.0, and 250 IU heparine per 500 mL solution; and the St.Thomas cardioplegic solution: CaCl₂ 2.0, MgCl₂ 16.0, KCl 20.0, NaCl 147.0, and Procainé-HCl 1.0.

The composition of the University of Wisconsin solution (ViaSpan, Du Pont, Wilmington, DE, U.S.A.) has been described previously.⁹
The KCl solution had the same composition as the Krebs-Henseleit solution used, except for the NaCl which had been completely replaced by an equimolar amount of KCl, which resulted in a concentration of 123.8 mM.

**Statistical analysis**

The data were expressed as mean ± standard error of the mean for n observations. The concentration-response curves for the agonists were analyzed by using a computer program (Graph Pad, Institute for Scientific Informatics, San Diego, CA, U.S.A.) and the pD2 value (-log effective concentration that produces 50 % of the maximal effect [EC50]), as well as the maximal effect (E_{max}) were thus obtained. The statistical significance of the differences was analyzed by means of one-way analysis of variance (ANOVA) or Student's t-test. Values of p less than 0.05 were considered significant.

**Results**

The isolated saphenous vein preparations, obtained and preserved, as described above, showed stable responses to the pharmacological stimuli applied in the experiments.

![Figure 1: Influence of preservation in University of Wisconsin solution for 0 (clear bars), 24 (hatched bars), and 48 (black bars) hours of isolated human saphenous vein preparations on the maximal responses of phenylephrine (Phe), sodium nitroprusside (SNP), and acetylcholine (ACh). The maximal effects (E_{max}) of Phe are expressed as relative responses of the contractions to potassium, and for those of SNP and ACh of the maximum responses to the precontraction by Phe (10μM). Experimental points represent means ± standard error of the mean (n = 9).](image-url)
In the study concerning the effect of the storage in UW on the functional properties of the SV segments, no alterations were observed in contractile and dilatory responses of the preparations after preservation, independently of the duration (Figure 1). The absolute values of the contractions to KCl were $21 \pm 4$, $25 \pm 5$, $28 \pm 5$ mN for the preparations preserved at 0 (n = 9), 24 (n = 9) and 48 hours (n = 9), respectively. Phenylephrine added to the organ bath, induced contractions with comparable sensitivity, calculated as the $pD_2$-value from the logistic curve fit analysis ($5.8 \pm 0.1$, $5.9 \pm 0.2$, $5.9 \pm 0.1$ for 0, 24, and 48 hours of preserved preparations, respectively) and maximal effect ($E_{max}$ values of 101 ± 6%, 94 ± 7%, 93 ± 6% for the vein segments analyzed after 0, 24, and 48 hours of preservation, respectively). The dilatory responses to SNP had equivalent $pD_2$-values ($5.9 \pm 0.3$, $6.0 \pm 0.2$, $6.3 \pm 0.1$) and $E_{max}$ values (130 ± 16%, 109 ± 8%, 118 ± 6%) for 0h, 24h and 48h stored preparations, respectively. The endothelium-dependent responses to ACh were not affected as by storage in UW, as shown by a maximal relaxation of the Phe-induced precontraction of segments stored 0, 24 and 48 hours, of 12 ± 3%, 10 ± 4%, 11 ± 4%, respectively.

In SV preparations harvested by different surgical techniques, the absolute values of the contractile responses to KCl were $27 \pm 5.0$ mN, $42 \pm 7.7$ mN and $35 \pm 13$ mN for the CV (n = 6), MV (n = 5) and the EV (n = 4) harvested vein preparations, respectively. The cumulative addition of phenylephrine caused concentration-dependent contractions in the venous segments obtained via the three examined surgical methods (Figure 2,

![Figure 2: Influence of distinct harvesting methods (conventional O, mediastinoscope-assisted • and endoscope-assisted□) on the responses to phenylephrine (Phe). The contractions are expressed as percentages of the response to KCl. Experimental points represents means ± SEM.](image-url)
### Table 1: Effects of harvesting techniques on the sensitivity and maximal effects of the agonists

<table>
<thead>
<tr>
<th>Agonist</th>
<th>pD$_2$</th>
<th>p Value</th>
<th>E$_{\text{max}}$(%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe: CV</td>
<td>6.0±0.1</td>
<td></td>
<td>99±4</td>
<td></td>
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<tr>
<td>MV</td>
<td>5.9±0.2</td>
<td>0.69</td>
<td>104±6</td>
<td>0.46</td>
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<tr>
<td>EV</td>
<td>6.0±0.2</td>
<td>0.80</td>
<td>96±7</td>
<td>0.74</td>
</tr>
<tr>
<td>SNP: CV</td>
<td>6.2±0.1</td>
<td></td>
<td>129±10</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td>6.2±0.1</td>
<td>0.94</td>
<td>106±5</td>
<td>0.12</td>
</tr>
<tr>
<td>EV</td>
<td>5.9±0.4</td>
<td>0.51</td>
<td>127±4</td>
<td>0.81</td>
</tr>
<tr>
<td>Ach: CV</td>
<td></td>
<td></td>
<td>11±5</td>
<td></td>
</tr>
<tr>
<td>MV</td>
<td></td>
<td>11±4</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>EV</td>
<td></td>
<td>5±6</td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

The maximal contraction to Phe is shown as percentage of the maximal response to KCl, the maximal dilation to SNP and Ach is expressed as percentage of the precontraction to Phe (10 μM). Values are given as means ± SEM. Differences between means were compared using a 2-sided Student's t-test for unpaired data, p values are of the comparison with CV.

Ach = acetylcholine, CV = conventional venectomy, E$_{\text{max}}$ = maximal effect, EV = endoscope-assisted venectomy, MV = mediastinoscope-assisted venectomy, pD$_2$ = negative log EC$_{50}$ value, Phe = phenylephrine, SNP = sodium nitroprusside

Table 1). The sensitivity, and the maximal responses to Phe were similar for preparations obtained through the three surgical methods. After submaximal precontraction by means of Phe (10μM) the addition of cumulative concentrations of SNP to the organ bath caused a relaxation below baseline-values (>100%) of all the precontracted vein rings (Figure 3A, Table 1). Neither the maximal responses to SNP, nor the sensitivity proved different, for the distinct harvested vein segments. In the three groups of precontracted preparations the exposition to Ach caused a dilation, with comparable maximal and pD$_2$ values (Figure 3B, Table 1).
Figure 3: Influence of distinct harvesting methods (conventional O, mediastinoscope-assisted ●, and endoscope-assisted ■) on the dilatory properties of SV preparations, investigated by responses to (A) sodium nitroprusside (SNP) and (B) acetylcholine (ACh). The actions of SNP and ACh are expressed as relative responses of the precontraction to Phe (10μM). Experimental points represent means ± SEM.
Discussion

In SV preparations, obtained after different surgical harvesting methods (CV, MV or EV) the in vitro vascular reactivity was not diversely influenced. The contractile responses to KCl and Phe of the venous rings were comparable for the surgical techniques investigated (Table 1, Figure 2). Furthermore, the concentration-dependent dilatory responses to SNP and the endothelium-dependent vasodilator ACh were not affected differently as shown in Table 1, Figure 3.

A special preservation medium was needed to transport the remnants to the laboratory. In order to standardize the procedure all preparations were stored in University of Wisconsin solution for 2 days, prior to the experiments comparing the three harvesting methods. In the past, UW demonstrated to be a suitable storage medium for the preservation of the functional properties of canine arteries as well as human SV. In the present study as well, the responses to the substances utilized in the experiments concerning the comparison of the surgical methods (KCl, Phe, SNP and ACh) remained fully intact after preservation in UW (Figure 1).

To evaluate the viability and quality of saphenous vein used in aortocoronary bypass operation a functional method is preferable, since the presence of an intact structure of the vessel wall, as obtained by morphological studies, does not necessarily implicate a normal function of the tissue. For the functional experiments applied in the present study, all the vein segments were divided into a minimum of four rings. Among patients and rings of one group variance was minimal, and the used method seemed valid to demonstrate differences between small groups of experiments. While using a pharmacological method in vitro, extrapolation to the in vivo situation of the venous conduit can only be done with caution. Jett showed in dogs that graft flow depends not only on the effect of the drug on the conduit, as studied in vitro, but also on the effect of the drug on the regional vasculature, as studied in vivo.

Defects of the endothelium can lead to thrombosis, and thus to graft stenosis or occlusion and are thereby one of the patency determining factors. Subsequently, the assessment of endothelial function by in vitro responses to ACh, could have a certain predictive value for the graft patency, although this matter would require appropriate clinical follow-up studies. In literature reports the maximal dilatory responses to ACh varies between 21% and 34% in norepinephrine-precontracted isolated human SV segments taken after the dissection has been completed or immediately after exposure of the vein, respectively. In the present study the non-preserved, conventionally harvested, phenylephrine precontracted SV preparations demonstrated a maximal response of 12 ± 5% to ACh. In the knowledge that the effector mechanism of NO in
the vascular smooth muscle cell is intact, as proven with responses to SNP (Figures 1, 3A), together with substantial evidence for the preservation of endothelial function in isolated vessels by UW (Figure 1)\textsuperscript{10-12}, the smaller ACh-induced dilatory responses compared to those in the literature, can be explained by injury of the endothelial monolayer as a result of the venectomy. Possible factors influencing endothelial function during surgery, are the exposition to storage solutions (not UW)\textsuperscript{4,15}, the used high pressure distension to overcome spasm of the SV\textsuperscript{15}, conditions of ischemia\textsuperscript{5}, and the surgical handling itself\textsuperscript{16}. Maintaining all other factors for influencing the endothelium constant, with exception of the surgical handling, as in the present study, the found comparable dilatory responses to ACh, indicate that the type of manipulation was not decisive for the demonstrated degree of endothelial injury.

In the present study, functional properties of the vein after minimally invasive harvesting are similar to those after CV. Our findings are in line with those reported in literature\textsuperscript{1-4}, demonstrating comparable responses to 5-hydroxytryptamine, noradrenaline, SNP or ACh after a different harvesting method by use of a Mayo stripper, and endothelial release of vasoactive substances after endoscopic harvesting, respectively. One study demonstrated a graft patency rate between 80 and 94\% at one-year follow-up\textsuperscript{4}.

Upon evaluation of the clinical outcome of the patients treated with the investigated skin-bridging techniques, it appears that these techniques resulted in a reduction of postoperative complications at the site of explantation, compared to the CV procedures. The present study evidenced that the new minimally invasive harvesting techniques, compared to the conventional method, can be applied without significant loss of vascular reactivity.

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References


