Preserved Vasoconstriction and Relaxation of Saphenous Vein Grafts Obtained by a No-Touch Technique for Coronary Artery Bypass Grafting

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**Background:** To obtain a saphenous vein graft (SVG) for coronary artery bypass grafting (CABG), the benefit of using a no-touch (NT) technique in vascular function has not been fully investigated.

**Methods and Results:** The pathological and physiological functions of human SVGs with a NT technique to preserve the perivascular adipose tissue (PVAT) and ones obtained by using a conventional (CON) technique removing PVAT, were examined. Immunohistochemistry of the section of SVGs showed that the phosphorylation of endothelial nitric oxide synthase in the endothelium of the NT group was more responsive to vascular endothelial growth factor. A myograph of SVGs showed greater contraction with phenylephrine in the NT group. However, the strong contraction was eliminated in SVGs taken by electrocautery. In the 10 patients whose SVGs were taken without electrocautery, endothelial-dependent relaxation with bradykinin was apparently increased in the CON group more than in the NT group. Smooth muscle relaxation with nitroprusside was higher in the CON group at the lower concentrations; however, the relaxation became greater in the NT group at the high concentrations. Therefore, the effect of neutralizing PVAT-released factors in the both groups was further examined. After medium of NT and CON were exchanged in half, relaxation of SVGs was immediately restored in the NT group.

**Conclusions:** The results suggest that the NT technique preserves the functions of vasoconstriction and relaxation. Also, the presence of PVAT-released vasoconstrictive factors was suspected.

**Key Words:** Coronary artery bypass grafting; Nitric oxide; No-touch technique; Perivascular adipose tissue; Saphenous vein graft
Methods

Human tissues were used in this experimental study, and written informed consent was obtained from all the patients. The research was also reviewed and approved by the respective Ethics Committees of Keio University Hospital (20160070), Sakakibara Heart Institute (16-058), and the National Defense Medical College (2657). This study was conducted in accordance with the principles of the Helsinki Declaration and its later amendments.

Patient Selection and Harvesting Vein Segments

We enrolled patients who underwent elective CABG and used SVGs collected using the NT technique. Only surplus SVG tissue that was not used for surgery was used in the experiments. To minimize endothelial damage, the extracted segments were not expanded with saline, as in the CON method. SVG tissue from each patient was divided into 2 groups of 3-mm segments: an NT group with PVAT retained and a CON group with PVAT removed. The images of SVGs are shown in Figure S1. Thereafter, each segment was placed in heparinized blood taken from completely heparinized patients, as soon as possible after harvesting.

Immunohistochemistry (IHC)

IHC was performed on the NT and CON samples, which were added to Krebs-Henseleit solutions (116 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L MgSO4, 25 mmol/L NaHCO3, 1.2 mmol/L KH2PO4, 5.5 mmol/L D-glucose, 2.5 mmol/L CaCl2) and incubated for 2 h to exclude blood components. Samples were then divided into 3 groups: (1) in group 1, the samples were fixed in formalin as soon as possible; (2) in group 2, the samples were fixed in formalin after 2 h of incubation; and (3) in group 3, the samples were fixed in formalin by reacting with vascular endothelial growth factor (VEGF) (20 ng/mL). Thereafter, each sample was blocked for 60 min, washed with xylene, 100% ethanol, 95% ethanol, and water for slide deparaffinization. Proteolytic enzymes were removed by treatment with 3% H2O2 for enzyme treatment was not performed, but endogenous ethanol, and water for slide deparaffinization. Proteolytic blocking was performed for 60 min, before the samples were dehydrated, and re-sealed. The images of slides were obtained under the same conditions by using a microscope (BX-700: Keyence, Japan). The intensities of staining were quantified automatically on a scale of 1–255 with accompanied software.

Table 1. Patient Characteristics of Study Participants

<table>
<thead>
<tr>
<th>Participants, n</th>
<th>Age, years</th>
<th>Male sex, n (%)</th>
<th>Hypertension, n (%)</th>
<th>Hyperlipidemia, n (%)</th>
<th>Diabetes mellitus, n (%)</th>
<th>Smoking, n (%)</th>
<th>BMI, kg/m²</th>
<th>Cholesterol, mg/dL</th>
<th>HDL, mg/dL</th>
<th>Triglycerides, mg/dL*</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>71.9±6.7</td>
<td>20 (71)</td>
<td>20 (71)</td>
<td>20 (71)</td>
<td>8 (29)</td>
<td>9 (32)</td>
<td>22.5±3.1</td>
<td>185.9±32.2</td>
<td>52.2±14.4</td>
<td>178.9±101.0</td>
</tr>
</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; BMI, body mass index; CCB, calcium channel blockers; HDL, high-density lipoprotein. Values are expressed as mean ± SEM, unless indicated otherwise. *Values expressed as median (25th–75th percentiles).

Isometric Tension Measurement

This study was performed with reference to previous research. After collection, the SVG tissue was divided into NT and CON groups, in vascular rings measuring 3 mm in length, and placed on a stainless-steel hook in an organ chamber containing 5 mL of Krebs-Henseleit solution at 37°C while blowing in Carbogen (5% CO2 in O2). The blood vessel was set to a tension of 9.8 mN and equilibrated for ~30 min. Tension was measured and recorded using a PowerLab recording system (AD Instruments, Oxfordshire, UK).

Contractility of Smooth Muscle in SVGs

After 30 min of equilibration, vasoconstriction response to KCl (30 mmol/L) was measured. The SVG rings were washed out. Next, vasoconstriction response to L-phenylephrine (PE; 10−5.5 mol/L) was measured. The absolute value of contraction was obtained at the stable phase.

Analysis of Endothelial-Dependent and -Independent Relaxation

After stable contraction with PE (10−5–5.5 mol/L) was acquired, bradykinin (BK; 10−10 to 10−5.5 mol/L) was added for measuring endothelial-dependent relaxation. The involvement of nitric oxide (NO) for the relaxation was confirmed by adding a NO synthase inhibitor, NG nitro-L-arginine methyl ester (L-NAME; 10−4 mol/L, 30 min), before contraction with PE or KCl. Endothelial-independent relaxation was measured with nitroprusside (SNP; 10−10 to 10−5.5 mol/L) as a NO donor to vascular smooth muscle cells.

Effects of PVAT-Derived Soluble Factors

The effect of basal PVAT-derived soluble vasoactive factor was investigated by stably contracting NT and CON with phenylephrine (10−5.5 mol/L). The mediums of the NT group and CON group were exchanged half by half. The procedure made the PVAT-derived factor equivalent in the
Results

Patient Characteristics
We initially recruited 28 patients between January 2017 and January 2018, mainly from Keio University Hospital and Sakakibara Heart Institute Hospital. Patient characteristics of study participants are shown in Table 1. The patients underwent selective CABG using a SVG and were chosen sequentially without setting any exclusion criteria. All tissue samples were collected using a NT technique. Therefore, SVGs taken by using electrosurgery were excluded from the final analyses. Information on patient assignment is shown in Figure 1.

2 groups. After the mixture of mediums, changes in venous tone were observed for 30 min.

Statistics
All experiments were performed in parallel on 2 segments taken from each vessel. Statistical analysis was performed by using SPSS (SPSS Inc., Chicago, IL, USA). Data are expressed as the mean and standard error of the mean. Differences in the IHC data were analyzed by using the Wilcoxon rank-sum test, whereas differences in myography data were analyzed using 2-way analysis of variance and Fisher’s exact test. A P-value <0.05 was considered to be statistically significant. Maximum relaxation response is indicated as a percentage of the level before phenylephrine-induced contraction.
Effectiveness of a No-Touch Technique for SVG

Phosphorylation of eNOS by VEGF in IHC
The phosphorylation of eNOS at Ser1177 by VEGF was significantly increased in the NT group than in the CON group at 5 and 60 min (V5, P=0.034; V60, P=0.021) (Figure 2). The response by VEGF of total eNOS was not different between the NT and CON groups (Figure S2).

Smooth Muscle Contractility With or Without PVAT in Myograph
In all 20 cases, SVGs of 10 cases enrolled in this study were collected without using an electrocautery (Figure 1). In these cases, the contractile response to KCl and PE was significantly larger in the NT group than in the CON group without using an electrocautery.
The relaxation response to BK was used to investigate endothelial-dependent relaxation of SVGs. Response to BK was significantly decreased in the NT group than in the CON group (P<0.01). In the presence of L-NAME, the relaxation response to BK was markedly blunted and the response to BK was similar in both the NT and CON groups (Figure 4).

### Effect of Drug Reactivity by Electrocautery

Although the SVGs were collected using the NT technique, samples that did not respond to drug stimulation existed. In the excluded 10 patients whose SVGs were collected using electrocautery, SVGs from 6 patients did not react at all. The SVGs from the rest of 4 patients responded incompletely to drug stimulation (Figure 3). The frequency of no or incomplete response is shown in Table 2. An image of an incomplete contraction is shown in the (Figure S3).

### Endothelial-Dependent Relaxation in Myograph

The relaxation response to BK was used to investigate endothelial-dependent relaxation of SVGs. Response to BK was significantly decreased in the NT group than in the CON group (P<0.01). In the presence of L-NAME, the relaxation response to BK was markedly blunted and the response to BK was similar in both the NT and CON groups (Figure 4).

### Smooth Muscle Response to SNP

Relaxations of SVGs to SNP was used to investigate endothelial-independent relaxation. The relaxation to NP in the NT group was smaller than that seen in the CON group at low concentrations (10^{-8.0} mol/L; P<0.05), and it was larger than that in the CON group at high concentrations (10^{-5.5} mol/L; P<0.05; Figure 5).

### Effect of Medium Exchange on Relaxation of SVGs as Detected by a Myograph

The half exchange of medium induced relaxation in both the groups, and it was more prominent in the NT group at the earlier time point within 5min (P<0.05; Figure 6).

### Discussion

The data were summarized as follows. IHC showed that VEGF-induced eNOS phosphorylation at Ser1177 was increased in the NT group. As detected by using a myograph, smooth muscle contractile of SVGs was stronger in the NT group. However, the strong contraction was

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**Table 2. Relationship Between Harvesting Method and Drug Response**

<table>
<thead>
<tr>
<th>Use of electrocautery</th>
<th>+</th>
<th>−</th>
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<tbody>
<tr>
<td>Drug reaction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>−</td>
<td>6</td>
<td>0</td>
</tr>
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</table>

Among the 20 cases in which a myograph was performed, 10 cases were collected using electrocautery. Although these were excluded from this study, 6 out of 10 did not respond at all or responded incompletely to drug stimulation by KCl and PE (P<0.05). We defined an “incomplete response” as a case of a mixture of good and bad reactions among samples from the same patient. An example of an incomplete response is in Supplemental Material. Furthermore, the absolute value of the contractile reaction of SVGs with PVAT collected with or without electrocautery is described below. There was no significant difference in each group (with electrocautery: KCl, 1.34±1.52; PE, 1.52±1.46; without electrocautery: KCl, 2.14±1.24; PE, 2.21±1.07; PVAT, perivascular adipose tissue; PE, L-phenylephrine.

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**Figure 4.** Vascular endothelium-derived relaxation reaction caused by bradykinin. Although pretreatment by phenylephrine was stronger in the NT group, vascular endothelium-derived relaxation caused by bradykinin was maintained by both NT and CON groups, both of which were inhibited by NG nitro-L-arginine methyl ester (L-NAME). It was suggested that production of vascular endothelium-derived NO is equivalent to NT and CON. *P<0.05. CON, conventional; NT, no-touch. Abbreviations are as per Figures 2, 3.

**Figure 5.** Non-vascular endothelium-derived relaxation reaction caused by nitroprusside (SNP). Although pretensioning by phenylephrine was stronger in the NT group, vascular endothelium-independent relaxation caused by SNP was maintained both by the NT and CON groups. Furthermore, there was more contraction at lower concentrations of SNP in the NT group, and relaxation at higher concentrations of SNP. It was shown that NT contracts well, but that complete relaxation is achieved by stimulation of the SN and NO donors. This is thought to be a type of homeostasis mechanism. *P<0.05. CON, conventional; NT, no-touch. Abbreviation are as per Figure 2.
Effectiveness of a No-Touch Technique for SVG

Eliminated if SVGs were obtained by electrocautery. Smooth muscle relaxation in the NT group was reduced at low concentrations of SNP, but became greater than that observed in the CON group at high concentrations. Endothelium-dependent relaxation induced by BK was lower in the NT group. However, equalization of medium immediately restored relaxation in the NT group.

There are very few reports of applying myograph for SVGs so far. The strong vasoconstriction found in the NT group is concordant with results found in a previous study. However, our examinations further suggested the presence of PVAT-secreted vasoconstrictive factors. A high concentration of SNP might be the reason. We finally confirmed that SVGs from the NT group preserved both vasoconstriction and relaxation, although the experimental conditions such as dose of SNP and mediums varied.

Several mechanisms are speculated to be the reason for the preserved function of SVGs in the NT group. PVAT releases biologically active signaling molecules such as tumor necrosis factor-α, hydrogen sulfide, NO, adiponectin and other adipocyte-induced relaxation factors. PVAT induces vascular relaxation and prevents spasm. Conversely, it was also reported that PVAT maintains vascular tone via superoxide, prostanoïd and angiotensin II. In addition, autonomic vasomotor functions are relatively intact in SVGs with PVAT because perivascular nerves are distributed within PVAT. The sympathetic control with neurotransmitters, including noradrenaline, could be involved in the strong contraction. Therefore, in NT, it seems that micro blood vessels and nerves around SVGs are preserved, so reactivity and sensitivity to drugs may be preserved as compared with the CON group. Thus, PVAT could be protective for SVGs by keeping vascular homeostasis.

Another important factor is the decrease of perivascular damage in the NT group. In the present study, SVGs were not distended by high-pressure in either the NT or CON groups. However, the NT technique could further reduce injury of adventitia including vasa vasorum and prevent inflammation, which provokes intimal hyperplasia. Additionally, we discovered SVGs from the NT group, which were collected using electrocautery, were not responsive in myograph. Heat injury was suspected despite a lack of difference in the appearance of SVGs. In this regard, further clinical and pathological studies are needed.

Taken together, vascular function of SVGs could be already varied before use. Our experiments suggested that the NT technique is superior to the CON technique in terms of both vasoconstriction and relaxation. We believe that our data would help to improve outcomes of CABG.

This study has several limitations. First, the clinical characteristics of patients were not considered in the study. However, the study subjects were identical in terms of having severe coronary atherosclerosis that required CABG. A grant from the Ministry of Defense, and a MEXT/JSPS KAKENHI Grant-in-Aid for Scientific Research (C: Number JP 17K09596 and 17K09565) were received for this study.

Conclusions

The NT technique is suggested to be advantageous for preserving the functions of vasoconstriction and relaxation. Also, it was suspected that PVAT maintains vascular tone by releasing vasoconstrictive factors.

Acknowledgments

The authors would like to thank T. Kimura, A. Sato, K. Ito and A. Osaki who belong to the National Defense Medical College and provided technical support and expertise for this experiment.

Disclosure

The authors declare no conflicts of interest.

References


