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***Original Article** / Özgün Makale*

Comparison of vasospasm and vasodilatation response of saphenous vein grafts harvested by conventional and no-touch techniques

Klasik ve no-touch teknik ile hazırlanan safen ven greftlerinde kasılma ve gevşeme yanıtlarının karşılaştırılması

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Background: In this study, we aimed to compare vasoconstriction and vasodilatation responses of saphenous vein grafts (SVG) harvested by conventional and no touch techniques in tissue bath system.

Methods: Between February 2007 and September 2007, 12 patients (10 males, 2 females; mean age 55.6±5.8 years; range 38 to 75 years) who underwent coronary artery bypass graft (CABG) surgery in our clinic with signed informed consents were included. Based on the techniques used during harvesting, the grafts were divided into two groups, including group 1 (n=16) with SVGs harvested by conventional technique and group 2 by no-touch technique. In group 1, SVG was removed completely from adjacent adipose tissues. In group 2, SVG was removed with adjacent adipose tissues, preserving saphenous nerves.

Results: The vasodilatation response of SVGs was significantly higher with the no touch technique, while the vasoconstriction response of SVGs was significantly higher with the conventional technique at lower doses.

Conclusion: We belive that no-touch harvesting technique of SVGs may decrease early graft failure and reduce also postoperative morbidity and mortality rate, contributing to improve graft patency rate in the long-term.

Key words: Coronary artery bypass graft; saphenous vein; vasospasm.

Amaç: Çalışmamızda izole organ banyosu düzeneğinde klasik teknik ve no-touch teknik ile hazırlanan safen ven greftlerinin (SVG) kasılma ve gevşeme yanıtları karşılaştırıldı.

Çalışma planı: Şubat 2007 - Eylül 2007 tarihleri arasında, bilgilendirilmiş onam formu imzalatılarak, kliniğimizde koroner arter baypas greft (KABG) cerrahisi yapılan 12 hasta (10 erkek, 2 kadın; ort. yaş 55.6±5.8 yıl; dağılım 38-75 yıl) çalışmaya dahil edildi. Hazırlık aşamasında kullanılan teknikler göz önünde bulundurularak, greftler iki gruba ayrıldı ve SGV'ler grup 1'de (n=16) klasik teknik, grup 2'de no-touch teknik ile hazırlandı. Grup 1'de SVG çevre yağ dokulardan tamamen temizlenerek çıkarıldı. Grup 2'de SVG, safen siniri korunarak, çevresel yağ doku ile birlikte çıkarıldı.

Bulgular: No-touch teknikle hazırlanan SVG'lerde gevşeme yanıtları anlamlı derecede daha yüksek bulunurken, kasılma yanıtları açısından klasik teknikle hazırlanan SVG'lerde düşük dozlarda daha yüksek kasılma değerleri elde edildi.

Sonuç: Safen ven greftinin no-touch teknikle hazırlanarak kullanılmasının greft yetmezlik oranlarında azalma, ameliyat sonrası morbidite ile mortalite oranlarında azalma sağlayabileceği ve uzun dönemde daha iyi greft açık kalma oranlarının elde edilmesine yardımcı olabileceği kanısındayız.

Anahtar sözcükler: Koroner arter baypas greft; safen ven; vazospazm.

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Coronary artery bypass grafting (CABG) surgery is widely performed and has low mortality and morbidity rates. Although many factors contribute to the outcome of the operation, the choice of the optimal graft and the technique used in the preparation of the grafts are particularly important for the success rate. Mechanical stress and pharmacological agents used during the preparation of the graft are shown to affect both short and long-term graft patency as well.[1,2]

The autologous saphenous vein is widely used as a conduit in CABG operations.[3] While some surgeons prefer to dissect the graft skeletonized without any surrounding tissue, known as the conventional or classical technique, others choose to harvest the graft with minimal handling together with the nearby tissues. The latter alternative is called the "no-touch" harvesting technique.

In this study, we compared the vasodilatation and vasoconstriction responses of saphenous vein graft segments harvested by these two techniques in an in vitro tissue bath system in an effort to reveal the extent of graft injury.

PATIENTS AND METHODS

A total of 12 patients (10 males, 2 females; mean age 55.6±5.8 years; range 38 to 75 years) who underwent a CABG surgery and gave informed consent were included in the study, which conformed to the Declaration of Helsinki and was conducted with local ethics committee approval. Saphenous vein graft segments were used for the purpose of the study, and the techniques used during the preparation of the grafts were classified into two groups.

In the group 1 (n=16), the grafts were prepared using the conventional method, meaning they were precisely dissected from the surrounding tissues. In the group 2 (n=16), the no-touch technique was applied with minimal handling, and the grafts were dissected en bloc with the nearby nerve and supplying vessels (pedunculated). No grafts in either group were distended with saline nor were they exposed to any pharmacological agent before or after harvesting. The segments were transferred to the vascular laboratory in +4 ºC Kreb's solution (Composition: 122 mmol/l sodium chloride (NaCl), 5 mmol/l potassium chloride (KCl), 1.25 mmol/l calcium chloride ($CaCl_2$), 25 mmol/l sodium hydrogen carbonate ($NaHCO_3$), 1.2 mmol/l magnesium sulphate ($MgSO_4$), 1.0 mmol/l monopotassium phosphate (KH_2PO_4), and 11.5 mmol/l glucose) that was continuously aerated with 95% oxygen (O_2) and 5% carbon dioxide (CO_2). Each graft was sliced into rings of 3 mm in width. The vascular rings were suspended in a conventional tissue bath system on steel hooks attached to an FDT-10A force displacement transducer (COMMAT Ltd, Ankara, Turkey), to which 1-4 g active tension was applied at the upper end for a minimum of 60 minutes while the lower end was kept stable. The samples were kept alive by being bathed in a 37 ºC oxygenated Kreb's solution every 20 minutes. In order to measure the vasodilatation response, samples were first exposed to phenylephrine (Merck & Co., İstanbul, Turkey) to induce submaximal vasoconstriction and then acetylcholine (Sigma-Aldrich®) was used to induce nitric oxide (NO)-mediated vasodilatation. A Keen scale (Mettler Toledo, AB) was used to calculate the molarity of drugs. The agents were dissolved in distilled water, and the injection of drugs into the tissue bath was done with the aid of Eppendorf pipets (BIOTANG Inc. Waltham, MA USA) (10-100 μL, 100-1000 μL).

After the calibration, phenylephrine was administered to the tissue bath every two minutes starting at a concentration of 10^{-9} mol/L and increasing in logarithmic increments to a concentration of 10^{-5} mol/L. Dose-response data for the vasoconstriction response was obtained via a TDA-97 transducer acquisition system (COMMAT Ltd.) and recorded using POLWIN 97 software (COMMAT Ltd). The vascular rings were then washed and recalibrated. In an effort to determine the vasodilatation response of the vascular rings, the samples were first treated with 10^{-6} M phenylephrine to induce submaximal vasoconstriction. While the phenylephrine was still in the environment, acetylcholine was administered to the tissue bath every two minutes starting at a concentration of 10^{-9} mol/l and increasing in logarithmic increments to a concentration of 10^{-4} M. The vasodilatation response curves were obtained and recorded as described above.

Data analysis

Graphpad Prism 4 software (Graphpad Software, La Jolla, California, USA) was used to construct dose-response curves and to perform non-linear regression and one-way analysis of variance of the data (ANOVA). A value of $p<0.05$ represented the cut-off for significance.

RESULTS

The mean age was 55.6±5.8 years with no statistically significant difference between the two groups in terms of medication used (statins, acetylsalicylic acid, nitrates, angiotensin-converting enzyme inhibitors, calcium channel blockers, or diuretics). The wound infection rates at the saphenous vein incision site were similar to the healing rates between the two groups. There was no difference in terms of patient complaints, such as numbness or pain, between the two groups.

Vasodilatation response caused by acetylcholine

In group 2, in which grafts were harvested by the no-touch technique, 10^{-9} M acetylcholine caused a 5.1% vasodilatation response while the same amount of vasodilatation was achieved by a higher concentration ($10^{-7.5}$ M) of acetylcholine in group 1, the classical technique group. A 93.1% vasodilatation response was caused by 10^{-9} to 10^{-6} M of acetylcholine in group 2, but there was a 61.6% vasodilatation response in group 1. Increasing doses of acetylcholine caused more pronounced vasodilatation in group 2 while in group 1, the increasing doses resulted in a Plato-shaped response curve (Figure 1).

Consequently, the vasodilatation response caused by acetylcholine was significantly higher in the group 1 (no-touch technique) than in group 1 (conventional method) ($p<0.001$).

Vasoconstriction response caused by phenylephrine

The dose-response curves of the vasoconstriction response to phenylephrine were obtained by adding increasing doses of phenylephrine to the tissue bath system beginning with a concentration of 10^{-9} M. 10^{-9} M phenylephrine. This caused a 7.6% vasoconstriction in the group 2. An 11.2% vasoconstriction response was obtained by only 10^{-8} M phenylephrine in the same group while an equal amount of vasoconstriction was obtained by a higher concentration of phenylephrine (10^{-7} M) in group 2. The extent of vasoconstriction in the dose interval of 10^{-9} M to 10^{-6} M was 87.9% in group 2 and 55.2% in group 1. Concentrations higher than 10-6 M phenylephrine resulted in a Plato-shaped response curve in group 2, but this Plato was reached at higher concentrations (10^{-5} M and higher) in the group 2 (Figure 2).

Although lower doses of phenylephrine caused larger amounts of vasoconstriction in group 2, this difference did not reach statistical significance in terms of vasoconstriction response ($p>0.05$).

DISCUSSION

Although the saphenous vein is advantageous due to its easy accessibility, rapid preparation and implementation, and resistance to vasospasm, its limited long-term patency rate due to the diameter discrepancy between its proximal and distal ends, varicosity, and susceptibility to sclerosis is a major drawback in CABG surgery.[4]

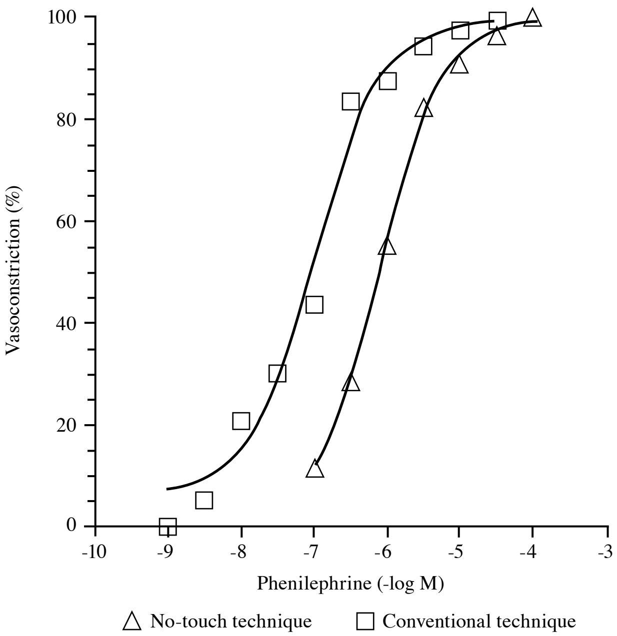

Figure 1. A 93.1% vasodilatation response was caused by 10^{-9} to 10^{-6} M of acetylcholine in the no-touch technique group, but there was a 61.6% vasodilatation response in the group in which the conventional method was used. Increasing the doses of acetylcholine caused more pronounced vasodilatation in the no-touch technique group while in the classical technique group, the increasing doses resulted in a Plato-shaped response curve.

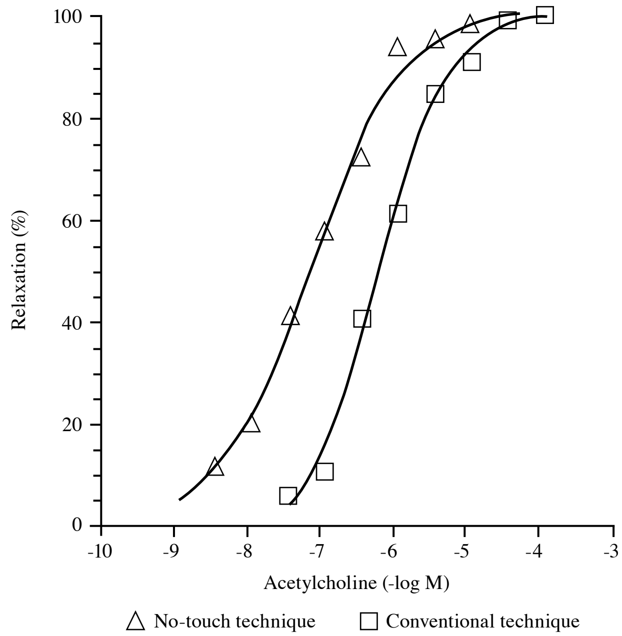

Figure 2. The extend of vasoconstriction in the dose interval of 10^{-9} M to 10^{-6} M was 87.9% in the no-touch technique group and 55.2% in the conventional technique group. Concentrations above 10^{-6} M of phenylephrine were needed to result in a Plato-shaped response curve in the no-touch technique group. This Plato was reached at higher concentrations (10^{-5} M and higher) in the conventional technique group.

The main cause of early graft failure in the first month is graft thrombosis, which occurs in 3-12% of patients.[5] Between the first and fifth years, the most common cause of graft failure is fibrous intimal hyperplasia, occurring at a rate of 2-3%. After the fifth year, the rate of graft failure is 5% per year as a result of atherosclerosis.[6] Early thrombosis of the saphenous vein is strongly associated with endothelial injury resulting from barotrauma, mechanical manipulation and handling delay. Sönmez et al.[7] showed that endothelial damage results in the exposure of the subendothelial tissue to erythrocytes, leading to the formation of mural thrombi, release of cytokines, myointimal hyperplasia, and graft thrombosis. For this reason, the conservation of endothelial integrity is vital in CABG operations.

In a study by Johansson et al.,[8] the saphenous vein grafts prepared by the no-touch technique (118 grafts) and classical technique (112 grafts) were compared angiographically. At the end of 18 months, 13 grafts prepared by the no-touch technique were stenotic (11%) while this was the case in 28 grafts prepared by the conventional harvesting method (25%). The study concluded that grafts harvested by the no-touch technique had improved patency and decreased atherosclerosis rates.

In his recently published review, Sepehripour et al.[9] pointed out that there is clear enhancement in vessel wall properties at a cellular level and angiographical evidence of superior graft patency when the no-touch harvesting technique is used.

In our study, we used phenylephrine to evaluate smooth muscle cell function and obtain vasoconstriction response curves and acetylcholine to evaluate endothelial function and obtain vasodilatation response curves. Phenylephrine exerts alpha 1 agonistic activity by stimulating intracellular calcium influx through calcium channels located on the cell membrane and cytoplasmic reticulum.[10] Acetylcholine causes vasodilatation indirectly by binding to muscarinic receptors and causing the release of NO synthesized by constituted NO synthase (cNOS). The NO, which rapidly penetrates to the vascular smooth muscle, activates guanylate cyclase and increases intracellular cyclic guanosine monophosphate (cGMP), which in turn causes vasodilatation.[11]

Acetylcholine, in the concentration range of 10^{-9} to 10^{-6} M, has caused significantly more potent vasodilatation in vein segments using the no-touch technique. This supports the thesis that endothelial integrity and function are better preserved by this harvesting method.

Phenylephrine, in the concentration range of 10^{-9} to 10^{-6} M, has caused more potent vasoconstriction in the vein segments using the classical technique. This supports the preceding thesis that better endothelial function and NO release from vein segments harvested by the no-touch technique spares the grafts from the effect of phenylephrine.

In addition to better preservation of endothelial integrity by the no-touch technique, another mechanism that has recently been shown to influence vasodilatation of saphenous graft segments collected by this harvesting method is the presence of fat-derived relaxant factors, mainly leptin released by the perivascular tissues. Dashwood et al.[12] showed that unlike conventional vein grafts, the vasa vasorum of grafts harvested by the no-touch technique remain intact, allowing for retrograde blood flow at the completion of the graft insertion. The presence of leptin was demonstrated in these tissues.

In conclusion, we compared the influence of the conventional harvesting technique and the no-touch harvesting method in terms of the vasodilatation and vasoconstriction responses of graft segments in an isolated in vitro tissue bath system. The vasodilatation response caused by acetylcholine was significantly higher in the no-touch technique group ($p<0.001$) while the vasoconstriction response caused by phenylephrine was significantly higher in the classical technique group. The improved vasodilatation and attenuated vasoconstriction responses with the no-touch harvesting technique prove that endothelial integrity and function are better preserved by this method. We believe that the no-touch harvesting technique can decrease early graft failure caused by thrombosis and intimal hyperplasia and improve postoperative morbidity and mortality.

Declaration of conflicting interests

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