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Effects of harvesting technique on endothelial inflammation and nitric oxide production in saphenous vein grafts

Ven çıkarma tekniklerinin safen ven greftlerinde endotel inflamasyonu ve nitrik oksit sentezi üzerine etkileri

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Background: In this study, we investigated the effects of harvesting technique in saphenous vein grafts (SVG) (either conventional or endoscopic) on endothelial integrity, inflammation, and nitric oxide (NO) synthesis.

Methods: Segments of 20 SVGs were harvested from 20 patients (23 males, 17 females; mean age 65.6±6.3 years; range 43 to 78 years) undergoing coronary artery bypass graft (CABG) surgery with the endoscopic (group 1) or conventional (group 2) technique. Saphenous vein specimens were stored in heparinized blood for one hour at the room temperature. As a marker of preserved endothelial function, nitric oxide (nitrate, NO_3 and nitrite, NO_2) levels in the SVGs were measured by means of the Greiss method. Saphenous tissual myeloperoxidase (MPO) activity, as a marker of neutrophil infiltration into the saphenous vein graft endothelium, was also measured in each group. This measurement revealed the extent of SVG endothelial damage and inflammation resulting from neutrophils.

Results: Nitric oxide formation, as NO_3 plus NO_2, (group 1=34.18±5.43 μM versus group 2=25.73±2.52 μM, p<0.001) was higher in endoscopically harvested SVGs as compared to conventionally harvested SVGs. Saphenous tissual MPO activity (group 1=6.71±0.86 nm/min versus group 2=9.11±0.94 nm/min; p<0.001) was significantly lower in endoscopically harvested SVGs as compared with conventionally harvested grafts.

Conclusion: Neutrophil infiltration into the vascular endothelium and neutrophil-induced endothelial injury is reduced in endoscopically harvested SVGs. Also, endothelial NO synthesis is better preserved in endoscopically harvested SVGs. These results suggest that endoscopic harvesting techniques can be used without major detrimental effects on vascular endothelial function and integrity in SVGs.

Key words: Coronary artery bypass grafting; endoscopic graft harvesting; endothelium; nitric oxide; venous graft.

Amaç: Bu çalışmada konvansiyonel veya endoskopik safen ven greft (SVG) çıkarma tekniklerinin endotel bütünlüğüne, inflamasyona ve nitrik oksit (NO) sentezi üzerine etkileri araştırıldı.

Çalışma planı: Koroner arter baypas greft (KABG) cerrahisi sırasında endoskopik (grup 1) ya da konvansiyonel (grup 2) teknikle 20 hastanın (23 erkek, 17 kadın; ort. yaş 65.6±6.3 yıl; dağılım 43-78 yıl) 20 SVG segmenti alındı. Safen ven örnekleri oda sıcaklığında bir saat süre ile heparinize kan içerisinde muhafaza edildi. Endotel fonksiyonunun belirleyicisi olarak, endoskopik ve konvansiyonel tekniklerle çıkartılan SVG'lerdeki nitrik oksit (nitrat-NO_3 ve nitrit-NO_2) düzeyleri Greiss yöntemiyle ölçüldü. Safen ven greft endotelinde oluşan nötrofil infiltrasyonun düzeyinin bir göstergesi olarak, safen doku myeloperoksidaz (MPO) aktivitesi de her iki grup için ölçüldü. Bu ölçüm nötrofilin neden olduğu safen ven endotel hasarını ve inflamasyon düzeyi belirlemede kullanıldı.

Bulgular: Nitrik oksit oluşumu, NO_3 ve NO_2 olarak, (grup 1=34.18±5.43 μM, grup 2=25.73±2.52 μM, p<0.001) endoskopik olarak çıkartılan SVG'lerde, konvansiyonel olarak çıkartılan greftlere kıyasla, daha yüksek seviyede bulundu. Safen ven doku MPO aktivitesi (grup 1=6.71±0.86 nm/dk., grup 2=9.11±0.94 nm/dk.; p<0.001) endoskopik olarak çıkartılan SVG'lerde, konvansiyonel olarak çıkartılanlara kıyasla, anlamlı olarak daha düşük düzeydeydi.

Sonuç: Endoskopik olarak çıkartılan SVG'lerde, damar endoteline olan nötrofil infiltrasyonu ve nötrofilin neden olduğu damar endotel hasarı daha azdır. Ayrıca endoteliyal NO sentezi, endoskopik olarak çıkartılan SVG'lerde daha iyi korunur. Bu bulgular, endoskopik çıkartma tekniğinin, SVG'lerde endotel hücre fonksiyonu ve greftin bütünlüğü üzerinde önemli zararlı etkileri olmaksızın kullanılabileceğini göstermektedir.

Anahtar sözcükler: Koroner arter baypas; endoskopik greft çıkarılması; endotelyum; nitrik oksit; venöz greft.

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Endoscopic saphenous vein harvesting during coronary artery bypass grafting (CABG) surgery has been steadily gaining popularity over the last several years. Previous studies have demonstrated that this type of approach reduces the incidence of wound complications associated with conventional harvesting while providing superior aesthetic results[1-5] combined with acceptable and comparable early and long-term graft patency rates.[6,7] Additionally, histopathological studies have failed to depict considerable differences between endoscopically and conventionally harvested vein grafts.[1,8-11] Although the endothelial layer appears grossly intact after minimally invasive harvesting techniques,[8-11] the evaluation of the endothelial function of saphenous veins with more reliable markers has been limited. Neutrophil-endothelial cell interaction plays an important role in endothelial injury and the pathogenesis of saphenous vein graft (SVG) occlusions.[12,13] It has been demonstrated that among other factors, decreased endothelial nitric oxide (NO) synthesis due to endothelial injury[14] can significantly influence adhesion molecule expression and subsequent neutrophil-induced endothelial injury. In fact, leukocyte adhesion to the endothelium is regulated by the presence of leukocyte-endothelial cell adhesion molecules on the surface of endothelial cells, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), P-selectin, and circulating neutrophils (CD11b/CD18).[13,15] Under in vivo physiological conditions, none of the adhesion molecules, or only a relative few, are expressed on the intact SVG endothelium,[13] although enhanced adhesion molecule expression has been observed in SVGs undergoing late occlusion.[16]

Nitric oxide, generated from L-arginine in the endothelial cells, is a reliable marker of endothelial function[17,18] since it activates guanylyl cyclase and increases intracellular concentrations of cyclic guanine monophosphate (cGMP) in platelets and smooth muscle cells.[17,18] In addition, NO plays a significant role in leukocyte-endothelium interactions[14,17,18] and in vascular tone regulation.[18] Moreover, it is believed that endogenous NO and cGMP might contribute in the reduction of inflammation in vivo through the suppression of endothelial adhesion molecule expression. Previous studies have demonstrated that surgical harvesting of saphenous veins[15,18-20] as well as storage-related hypoxia-ischemia[15,16,21] impairs or completely abolishes the endothelial NO synthesis in SVGs.

The present study was therefore designed to evaluate the effects of both the endoscopic and conventional harvesting techniques on SVG endothelial function and integrity by measuring the endothelial NO synthesis and the activity of myeloperoxidase (MPO), an enzyme occurring in neutrophils, monocytes, and macrophages.

PATIENTS AND METHODS

Study population

Forty patients undergoing elective, non-emergency, on-pump CABG surgery were prospectively randomized into either the endoscopic vein harvest group (group 1, n=20; mean age 66.2±5.7 years) or the open vein harvest group (group 2, n=20; mean age 64.6±6.9 years). The criteria for exclusion from the study were diabetes mellitus (DM), peripheral arterial obstructive disease (PAOD), varicose veins, or the recent use of steroids. This study was conducted in accordance with approval from the Columbia University Institutional Review Board (IRB 0988) and in conformance with the Declaration of Helsinki. The procedures were also performend with the patients' informed consent.

Surgical techniques

In group 1, all patients' legs were circumferentially prepared with a povidone- iodine solution, and their feet were placed in sterile stockinettes. Before surgery, 5000 units of intravenous heparin were administered to each patient. The endoscopic vein harvesting was performed with the VASOVIEW Endoscopic Vessel Harvesting System (Guidant Corporation, Santa Clara, California, USA), which uses carbon dioxide (CO_2) insufflation for visualization and dissection. To begin the procedure in group 1, a 1.5 to 2 cm incision was made medially above or below the knee, depending on the length of the vein required. Harvesting was directed toward the groin region. The side branches were divided by using bipolar cauterizing scissors or a bisector. A small puncture was then made under endoscopic guidance proximally over the saphenous vein, which was then clamped and divided, and the proximal end was ligated. After removing the vein from the leg, the side branches were ligated with 4/0 silk ties. Finally, the incisions were closed with absorbable subcutaneous and subcuticular sutures and wrapped with an elastic Ace bandage for 24 hours.

In group 2, a longitudinal incision was made over the course of the saphenous vein starting at the groin region. Once exposed, the side branches were ligated with 4/0 silk ties and then divided. The wound was closed in layers by using absorbable sutures and also wrapped with an Ace bandage for 24 hours. All veins were gently distended manually with autologous heparinized blood, and any avulsed branches were repaired by carefully approximating the adventitial layer with 7/0 polypropylene sutures. The veins were then placed in a

heparinized blood solution until they were needed. Both the open and the endoscopic harvesting of the venous conduit were performed by a surgeon with consistent experience with these techniques.

In both groups, a 2 cm sample was taken from the groin end of the vein immediately after its removal from the leg and before any further manipulation, such as distention after harvesting. Tubes containing 5 mL of heparinized autologous blood were prepared, and the samples were then stored at room temperature for 60 minutes, which is comparable with the average storage period for a harvested vein before implantation as a bypass conduit. Each specimen was rinsed briskly in normal saline solution three times, divided into two approximately equal parts (one segment for nitrite and nitrate measurements and one for MPO activity determination), snap-frozen in liquid nitrogen, and stored separately in a –80 °C freezer until analysis.

Nitrite/nitrate assay

The tissue samples were diluted with equal volume of phosphate buffered saline (PBS) and filtered by centrifugation using Amicon 30 kDa cut-off filters (Millipore Corporation. Bedford, Massachusetts, USA). For the determination of nitrite plus nitrate concentration in the filtrates, the Greiss method using the nitrate reductase catalyzed conversion of nitrate to nitrite was adopted.[22] After the conversion of nitrate into nitrite, the Greiss reagent was added to the samples, and the absorbance was measured at 540 nm. Then the nitrite and nitrate standards were studied to calculate tissue nitrite and nitrate concentration.

Myeloperoxidase assay

To determine the neutrophil adhesion to SVG endothelium after harvesting and the one-hour storage period, SVG tissue MPO activity was measured using the method described previously by Mullane et al.[23] The MPO enzymatic activity was measured spectrophotometrically at 460 nm using a PowerWaveX microplate reader (Biotek Instruments, Winooski, Vermont, USA), and the result was expressed as ΔAbs 460 nm/min. The MPO value was expressed as the mean ± SD of duplicate determinations, and all assays were measured without prior knowledge as to the group origin of each of the vein samples.

Statistical analysis

All statistics were obtained using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, Illinois, USA) version 10.0 software program. All continuous variables were expressed as mean ± standard deviation. Comparisons between groups were made by either Student's t-test or the Mann-Whitney U test.

RESULTS

In this study, endoscopic saphenous vein procurement was performed without complications in all patients. The time to harvest the saphenous vein (harvesting time plus skin closure time) was significantly shorter in group 1 (38.46 minutes) versus group 2 (50.47 minutes) ($p<0.001$).

Nitric oxide formation, nitrate plus nitrite release

Greiss assays were performed to quantify the NO level of the saphenous veins, and the total NO (nitrate plus nitrite) levels were higher in group 1 (34.18±5.43 μM than in group 2 (25.73±2.52 µM) ($p<0.001$) (Figure 1).

Myeloperoxidase activity

The mean MPO activity was significantly lower in tissue segments from group 1 (6.71±0.86 nm/min) compared with those in group 2 (9.11±0.94 nm/min; $p<0.001$) (Figure 2).

DISCUSSION

The saphenous vein is still extensively used as a bypass conduit in CABG surgery even though the long-term patency rate is inferior to that of internal mammary artery (IMA) grafts. The poor long-term patency of SVGs, a primary reason for the current trend toward the use of arterial grafts, has been attributed to the structural and functional characteristics of the vascular wall and the endothelium of saphenous veins.[24,25] Therefore, the importance of an atraumatic preparation

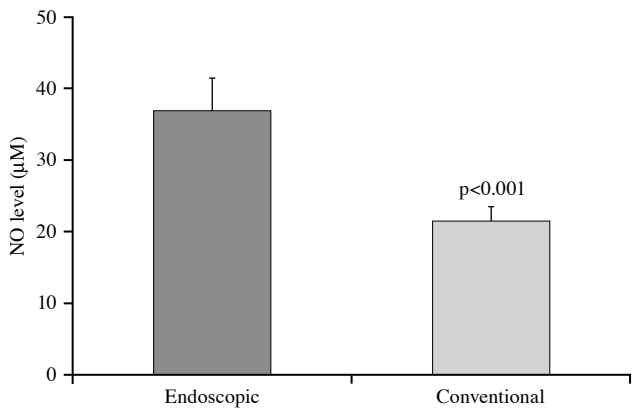

Figure 1. Human saphenous vein graft total nitric oxide (nitrate plus nitrite) synthesis after harvesting and the one-hour waiting period. To evaluate the effects of the harvesting technique (either endoscopic or conventional) on SVGs, endothelial function and nitric oxide (nitrate, NO_3 and nitrite, NO_2) levels in the vein grafts were measured. The data for the samples is shown as group mean ± SD. For each group, n=20.

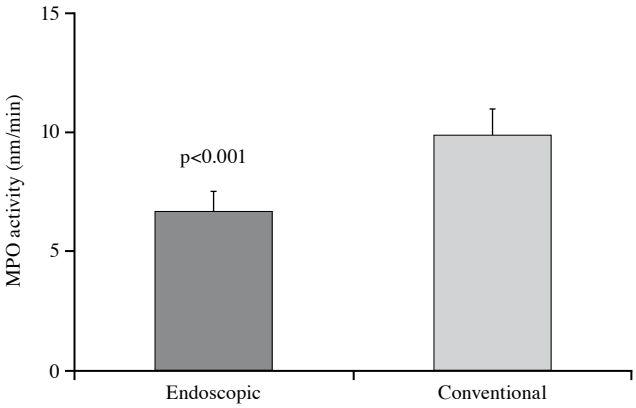

Figure 2. Human saphenous vein graft neutrophil accumulation after harvesting and the one-hour waiting period. Myeloprexidase activity (change in absorbance-ΔAbs at 460 nm/min) was determined to quantify neutrophil deposition in the vein grafts. The data for the samples is shown as group mean ± SD. For each group, n=20.

of the saphenous vein has been recognized for many years. In general, saphenous vein harvesting and the preparation techniques associated with it have been considered as potential sources of trauma that may accelerate the process of graft failure after implantation as bypass conduits.[24,25] Surgical preparation of SVGs for bypass grafting involves exposure, dissection, intraluminal distension, side branch ligation, and storage in a physiological solution; however, this invariably causes a high degree of damage to the endothelium. In particular, excessive surgical manipulation of saphenous veins during surgical preparation injures the endothelial and medial layers,[25,26] reduces NO bioavailability,[25] and results in platelet and leukocyte adhesion and smooth muscle cell migration into the intima.[26] These changes contribute to the accelerated atherosclerotic disease leading to graft failure.[27]

Additionally, the morbidity associated with conventional vein harvesting is high because the open technique requires long incisions and leads to significant wound morbidity, with an incidence rate varying from 3-44%. Additional problems with this technique include hematoma, dehiscence, cellulitis, skin necrosis, neuralgia, and infection.[5]

Several studies have demonstrated that the endoscopic approach avoids traction on the vein while being harvested, significantly minimizes the length of incisions and blood loss, and reduces the incidence of leg wound infection and complications when compared with the traditional open vein harvesting technique.[1-8,28] Moreover, endoscopic harvesting provides major benefits to patients through means of pain reduction and superior aesthetic results[1-8,28] while also having satisfactory early and long-term patency rates.[6,7]

Preservation of saphenous vein functionality by means of minimally invasive harvesting might have important implications for the immediate functional integrity and long-term patency rate of venous grafts. Leukocyte-endothelial cell interaction is one of the primary determinants of endothelial injury. Adhesion molecules, expressed on endothelial cells, mediate the binding of leukocytes to endothelial cells through interactions with their counterreceptors on the leukocytes.[13] As previously state, under in vivo physiological conditions, adhesion molecule expression on segments of recently harvested saphenous veins (endoscopic or conventional) is very low or almost absent.[13,29,30] However, during the storage period, SVG endothelial cells retain their ability to synthesize leukocyte chemoattractants such as interleukin-8[1] while the endothelial disruption upregulates the expression of such molecules.[12,21] Therefore, preservation of endothelial integrity during harvesting and storage along with the attenuation of adhesion molecule expression before implantation may be potentially beneficial in preventing leukocyte adhesion to the vessel wall and suppressing neutrophil-mediated endothelial injuries in SVGs after implantation as bypass conduits. In a previous study, we demonstrated that after one-hour storage of endoscopically harvested vein grafts, it is possible to determine visible endothelial cell adhesion molecules, ICAM-1, and VCAM-1 as well as inducible NO synthase-2 (INOS-2) expression.[29,30] Therefore, in this study, we again stored the vein samples for a one-hour period before measuring leukocyte infiltration in the vascular endothelium of the SVGs. By comparing matched pairs of vein tissue, we demonstrated that harvesting and a 60-minute period of storage in heparinized blood results in increased leukocyte infiltration into the endothelium in SVGs harvested by means of both endoscopic and conventional techniques, indicating an increase in the proinflammatory reaction in these vein grafts in the endothelium. In contrast, when compared with conventional harvesting, endoscopic harvesting seemed to provide better endothelial cell function, as depicted by decreased myeloperoxidase activity, indicating less neutrophil infiltration.

Additionally, our study aimed to evaluate the difference in NO production following conventional or endoscopic saphenous vein harvesting. Endothelial nitric oxide synthase (NOs) transcription is increased by hypoxia.[17] Inducible NOs is found in various tissues, such as vein grafts, and its expression is induced by immunoactivation of neutrophils,

macrophages, and monocytes.[17] Their induction generally reflects a pathophysiological cellular response to immunoactivation and elicits vasoplegia, myocardial depression, and cytotoxic effects.[17] The main property of NO is to inhibit the expression of adhesion molecules (such as selectins, GPIIbIIIa, ICAM, and VCAM) in platelets, neutrophils, and vascular tissues.[18] Harvesting and hypoxic storage reduce endothelial NOs expression and decrease NO production in SVGs,[14,16,19,20] causing an upregulation of leukocyte adhesion molecules on SVG endothelial cells.[12,16] It has been previously demonstrated that surgical damage to the endothelium results in a loss of endothelial-derived NO, which increases neutrophil and monocyte adhesion to the vessel wall and vasospasm.[25] Liu et al.[20] investigated the effect of surgical preparation of the conventionally harvested saphenous vein on NO release from the endothelium by direct measurement of NO. They demonstrated that mechanical distention during surgical preparation almost abolishes NO release (both the basal and stimulated NO), thus holding potential implications for the long-term patency rate of the vein graft.[20]

Therefore, to evaluate the effect of endoscopic and conventional harvesting on saphenous vein endothelium, we measured the total NO levels in the endoscopically and conventionally harvested grafts since this serves as a much more reliable marker of endothelial integrity and function. We demonstrated that the levels were significantly higher in endoscopically harvested SVGs compared with the conventionally harvested grafts, which implies a more preserved endothelial function in the endoscopically harvested conduits.

Studies evaluating endothelial function after minimally invasive vein harvesting have been limited; therefore, the impact of endoscopic harvesting on endothelial function is unclear. Griffith et al.[9] showed similar endothelial, elastic lamina, and smooth muscle continuity as well as medial and adventitial connective tissue uniformity between conventionally harvested versus endoscopically harvested veins. In a large group of patients, Crouch et al.[31] reported that there was no consistent decrease in vein integrity after performing the endoscopic harvesting technique. Moreover, Yun et al.[7] and Davis et al.[6] determined the long-term patency rates of endoscopically harvested SVGs and reported that this technique provided a comparable or even higher patency rates in comparison with traditionally harvested veins. These studies clearly prove that the endoscopic harvesting technique does not induce more significant histological trauma than that observed during traditional saphenectomies.

In conclusion, our study demonstrates that endoscopic harvesting reduces neutrophil infiltration and neutrophil-induced endothelial injury into the vascular endothelium of SVGs. Furthermore, our study shows that endothelial NO formation is better preserved with the endoscopic harvesting technique than with conventional harvesting. Finally, better preservation of endothelial NO synthesis and reduction of leukocyte-induced endothelial inflammation by endoscopic harvesting suggests that minimally invasive harvesting improves the quality of saphenous veins and leads to potential benefits in terms of the long-term patency of SVGs.

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Declaration of conflicting interests

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