

Original Article

Effects of moderate pressure distention on the proximal and distal sections of the saphenous vein

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Abstract: Studies have shown that the distention and traction of a vein leads to the loss of cells and the function of the endothelia. Here, we aimed to compare the effects of pressure distention on the proximal and distal parts of the saphenous vein. Twenty patients were enrolled in this study. The proximal and distal saphenous vein segments were distended to three different pressure levels for two minutes: 100 mmHg, 200 mmHg, and 300 mmHg. In addition, the proximal and distal parts of the saphenous vein were compared with the immunohistochemical examinations and the organ bath system. The endothelial cell loss was similar in the proximal and distal segments at 300 mmHg. However, the endothelial cell loss rate was greater in the proximal segments than the distal segments at 100 mmHg and 200 mmHg ($P=0.02$ and $P=0.06$, respectively). The relaxation response of the proximal samples distended to 100 mmHg and 200 mmHg was significantly decreased when compared to the distal segment samples ($P=0.049$ and $P=0.047$, respectively). Furthermore, there was no relaxation response in the segment samples distended to 300 mmHg. While preparing the graft, inflations with pressures lower than 100 mmHg result in less endothelial damage, and are relatively protective on the graft functions. In this study, we have shown that the endothelial tissue of the distal saphenous vein is more resistant to moderate pressure.

Keywords: Saphenous vein, pressure, segment, endothelial cell loss, relaxation response

Introduction

Despite the increased attention to arterial grafts, saphenous vein grafts (SVGs) are still the most commonly used type of graft in coronary artery bypass grafting surgery (CABG), due to their wide diameter, ease of preparation, and length [1-3].

During the first year after surgery, between 10% and 15% of venous grafts become occluded. In 10 years, only 50% of vein grafts remain free from significant stenosis [4, 5]. There are many theories for the failure of saphenous veins, and the reasons for premature graft closure include: biological, conduit quality, unsatisfactory harvest/preparation, and inappropriate operative strategy or poor surgical technique [6]. Currently, much of the research being performed on graft failure has led to the hypothesis of early thrombosis and neo-intimal

hyperplasia as the physiological basis for graft failure, although the exact mechanism is not well established.

The theory accepted by the majority of surgeons suggests that the endothelial and media damage caused by high pressure for the relaxation of spasms during the preparation of the graft is responsible for the graft occlusion [7]. Previous studies have reported that endothelial-derived nitric oxide synthase levels are decreased, and functional deterioration can occur in grafts distended with high pressure [8, 9]. However, there were a few studies comparing the proximal and distal portions of the saphenous vein [10].

In this study, we aimed to compare the effects of pressure distention on endothelial damage and graft function deterioration in the proximal and distal parts of saphenous vein grafts.

Material and methods

Patient selection

After obtaining informed consent and the Ethical Committee's approval, the SVGs of 20 patients [17 male, 3 female, mean age: 59.52 ± 9.09 years old (44-75)] who underwent CABG surgery at our institution between March of 2015 and May of 2015 were included in this study. The patients were divided into 2 groups according to the use of the proximal or distal part of the saphenous vein. In each group, the vein segments were divided into four experimental groups: Group 1-Control, Group II-Distension to 100 mmHg for 2 minutes, Group III-Distension to 200 mmHg for 2 minutes, and Group IV-Distension to 300 mmHg for 2 minutes. Those patients with a history of deep vein thrombosis, venous insufficiency, peripheral arterial disease, and macroscopic varicosities were excluded from this study.

Graft harvesting

All of the grafts were harvested by the same surgeon using a routine complete skin incision with scissors. Electrocautery was not used for this area. The distal end of the vein was cannulated at the ankle level, all of the branches were ligated, and the graft was prepared without any pressure. No vasodilator agent was used while harvesting the grafts, and after harvesting, the grafts were prepared and cannulated according to the appropriate legend for each target artery. If there was enough saphenous vein, the patient was added to the study.

Experimental design and standardization of pressure

The SVGs were distended with previously prepared heparinized saline solution (5000 units of unfractionated heparin were diluted in 1000 mL of 0.9% saline solution), with the help of a pressure infusion cuff with a sphygmomanometer (ERKA D-83646, Berlin, Germany) for 2 minutes. The tension was continuously measured with an anisometric force transducer (Pressure Monitoring Set-W/2 Transducer; Bicakçılar A.S., İstanbul, Turkey), which was connected to a computer based data acquisition system (BSM-2301K; BSM-2303K Life Scopei Bedside Monitors, Rosbach, Germany). Afterwards, 0.5-1 cm of the graft segment was pre-

pared for pathological examination; however, the clamped parts of the vein were not used for the study. The same procedure was repeated with 200 and 300 mmHg pressures to the saphenous vein, respectively.

By using a specific mechanism, the SVGs were distended to four different pressure levels for two minutes: 0 mmHg (control group), 100 mmHg, 200 mmHg, and 300 mmHg. Then, 120 saphenous vein graft segment (4 different pressure levels in both the proximal and distal groups) samples from each pressure group were examined.

Tissue bath system

The saphenous vein segments were transferred to the vascular laboratory in a 4°C Krebs solution as in our previous study [9]. Each graft was sliced into rings of 3 mm in width, and the vascular rings were suspended in the classical tissue bath system via steel hooks. An active tension of 1 to 4 g was applied to all of the samples, and the vascular rings were suspended under this tension for a minimum of 60 minutes. The samples were kept alive using a 37°C oxygenated Krebs solution bath every 20 minutes. In order to measure the relaxation response, the samples were first exposed to phenylephrine (Sigma) (10^{-6} M) for submaximal constriction; afterwards, carbachol (Sigma-Aldrich®) was used to induce nitric oxide (NO)-mediated vasodilatation. While the phenylephrine was still in the environment, the carbachol was administered to the tissue bath every two minutes, starting at a concentration of 10^{-8} M, and increasing in logarithmic increments to a concentration of 10^{-4} M. The vasodilatation response curves were obtained and recorded as described above, while the data were transferred to the computer with the help of the transducer acquisition system (MAY IOBS 99; FDT 05, Ankara, Turkey), and stored with the MAY-MASTER MP36 analysis program.

CD31 immunostaining

In this study, CD31 immunostaining was performed to evaluate the endothelial cell loss, since CD31 is a surface marker of endothelial cells. The proximal and distal SVG segments, which represent the lesions, were made available for immunohistochemical study and were selected. The immunohistochemistry was per-

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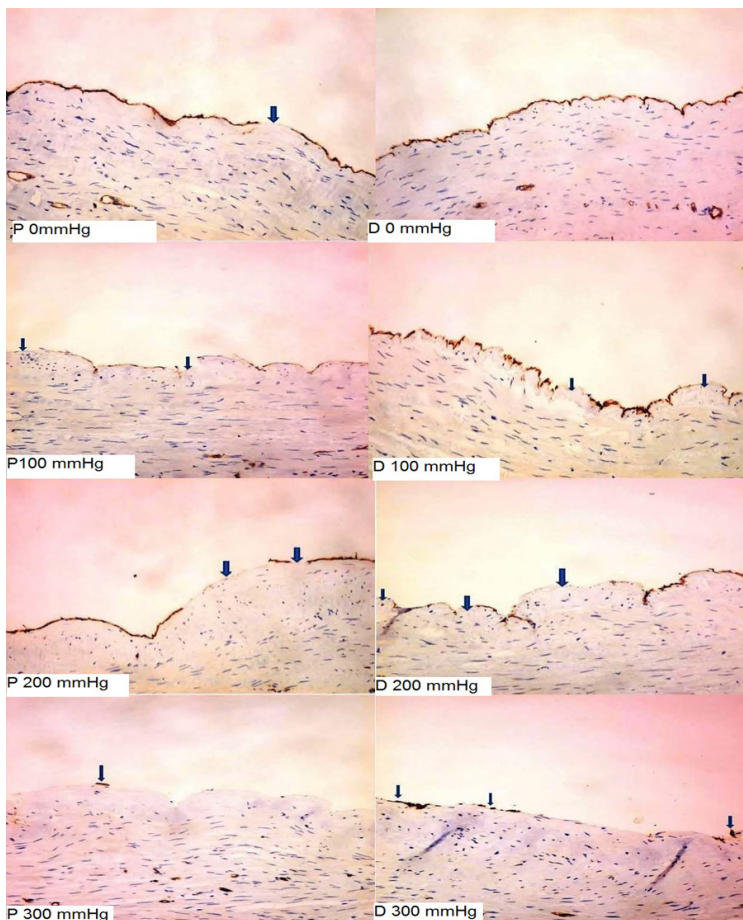


Figure 1. View of the proximal and distal saphenous vein and the endothelial cell loss.

formed on formalin-fixed, paraffin-embedded 5 μm -thick tissue sections, and three cross-sectional slices from different parts of each SVG segment were analyzed in order to obtain representative results.

The CD31 antibody (Dako, Glostrup, Denmark; 1:30 dilution in 0.05 mol/l Tris-HCl buffer with 1% albumin) was applied, and the sections were deparaffinized and rehydrated in graded alcohols. The antigen retrieval was performed in a microwave oven for 15 minutes in a 10 mM citrate buffer (pH 6.0), while the endogenous peroxidase activity was blocked with a 3% H_2O_2 methanol solution, and the slides were incubated in 10% normal goat serum for 30 minutes to prevent non-specific staining. They were then incubated for 2 hours at room temperature with an appropriately diluted primary antibody; CD31 mouse monoclonal antibodies were used. Thereafter, the sections were incubated with biotinylated goat anti-polyvalent (Lab Vision) for 15 minutes, and streptavidin

peroxidase (Lab Vision) for 15 minutes. In addition, 3-amino-9-ethyl carbazole-AEC (Lab Vision) was used as a chromogen, and the sections were counterstained with Mayer's hematoxylin. The preparations were evaluated under light microscopy, and the CD31+ endothelial cells on the intimal surface were identified and quantified.

The ratio of the CD31+ endothelial cell surface to the total intimal surface of the cross-section (CD31+ endothelial cell surface and de-endothelialized tunica intima) demonstrated the percentage of the endothelial cell coverage. The reciprocal value gave the percentage of the endothelial cell loss, and the endothelial cell loss results for a distinct distention pressure are expressed as the median value of three measurements in different parts of the proximal and distal SVG segments. The evaluation of the immunohistochemistry was performed by an experienced pathologist in a blinded setting.

Statistical analysis

The program GraphPad Prism 6 Version was used for analyzing tissue bath data. Concentration-response graphs were obtained by using statistical properties of the same program. Non-linear regression analysis (variable slope) and one-way ANOVA analysis were preferred for graphics. Intra-group analyses of groups were performed by using the t-test.

Immunohistochemical data were expressed as numbers and percentages for the categorical variables and as the mean \pm standard deviation for the continuous variables. The Kolmogorov-Smirnov test was used for the distribution of the variables. The normally distributed data were compared using the Student's test and the paired samples t test, while the data with a skewed distribution, were compared with the Kruskal Wallis test. The repeated measures model was also used to determine the difference, and the results were assessed within a 95% confidence interval and at a level of $P < 0.05$ significance. The statistical ana-

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Table 1. Effects of pressure on saphenous vein endothelia according to the graft location

Pressure Groups	Location	Damaged endothelial area ratio (mean)	P	Vasodilatation responses% (mean)	P*
Control	Distal	4.50±4.2	0.296	49.3±12.4	0.546
	Proximal	6.50±4.1		37.9±13.2	
100 mmHg	Distal	27.50±6.3	0.002	48.2±10.4	0.049
	Proximal	51.00±13.7		21.7±7.3	
200 mmHg	Distal	50.50±9.2	0.006	38.5±8.7	0.047
	Proximal	63.50±9.4		15.1±4.7	
300 mmHg	Distal	63.00±26.8	0.641	No response	-
	Proximal	74.00±6.9		No response	

*: Vasodilatation responses with organ bath system.

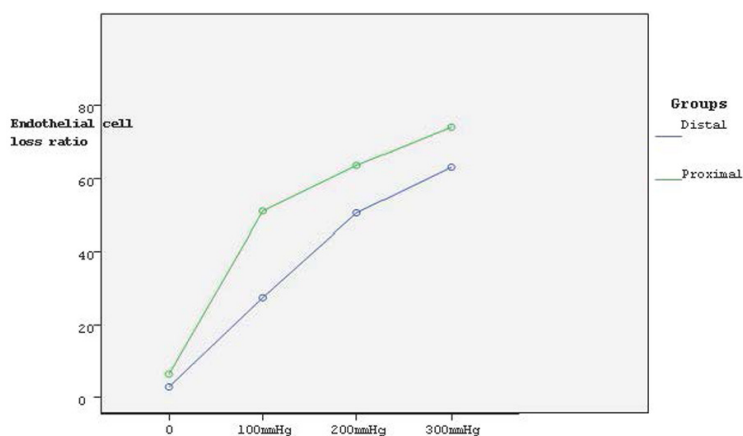


Figure 2. Progressive increase and endothelial cell loss ratios.

Analyses were performed with SPSS 18 for Windows (SPSS Inc., Chicago, IL, USA).

Results

In this study, 40 of both distal and proximal saphenous vein segments were used for histopathological investigation, and 80 graft segments were also used for the tissue bath analysis.

Histopathological findings

The saphenous vein was received from both the distal and proximal parts for each patient; however, the overall endothelial cell loss was higher in the proximal region of the same patient (**Figure 1**). In the statistical analysis of the control group and the 300 mmHg group, the endothelial loss rates were similar in the proximal and distal SVGs ($P=0.296$ and $P=0.641$, respectively). However, in the 100

mmHg and 200 mmHg groups, the endothelial cell loss rate was higher in the proximal saphenous vein segments (**Table 1**). The repeated measures model analysis showed the difference between the groups according to the pressure, while the ANOVA test showed that the endothelial loss rate increased in parallel with the pressure elevation (**Figure 2**).

Organ bath findings and analysis

The carbachol was administered in a cumulatively increasing manner from 10^{-8} to 10^{-4} M concentrations, and the nitric oxide mediated dose-relaxation graphics were obtained separately (**Figure 3**). The saphenous vein was received from both the distal and proximal parts of each patient to analyze the graft relaxation response, and the distal and proximal segment relaxation responses were similar in the control group ($P=0.546$).

The relaxation responses of the proximal samples distended to 100 mmHg and 200 mmHg were significantly decreased when compared to the distal segment samples (**Table 1**). None of the grafts distended to 300 mmHg of pressure were functional in the tissue bath system; thus, a statistical analysis was not possible in this group.

Discussion

The saphenous vein (SV) varies in its structural and functional properties, depending on the regional location [11, 12]. These factors can affect graft performance, and the possible variations along the length of the vessel may be important factors when choosing a segment of the SV as a coronary graft. Pressure induced endothelial loss occurs after tears and damages to the vessel; moreover, when the saphenous vein is inserted in the arterial circulation, it begins to suffer shear frictional force, causing cyclic effort on the vascular wall.

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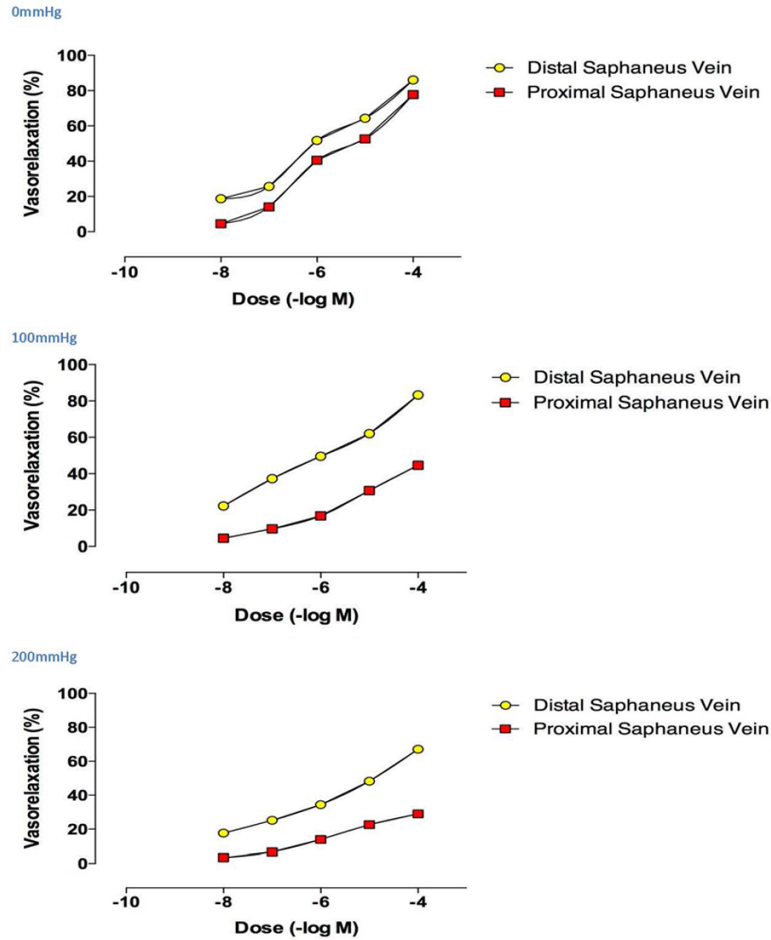


Figure 3. Progressive decreases in the relaxation function upside of the pressure height.

The variations in the morphology and vascular reactivity of grafts can influence their short and long term performance as bypass conduits [12]. In this study, we investigated the difference in the endothelial damage depending on the location, by applying moderate pressure in the saphenous vein.

Despite the advantages of arterial grafts being well known, the saphenous vein is the most commonly used graft in coronary artery bypass grafting surgery [1-3, 11]. The functional inferiority of saphenous veins has been widely studied, with great importance being attributed to possible traumatic and functional lesions during the harvesting and preparation for coronary artery bypass grafting [1-5, 13]. Moreover, the patency rates may be related to vein lesions during the removal and preparation [8]. The vein is cannulated and pressure is applied to identify and ligate the collateral vessels. The

pressure is usually controlled manually without measurement; thus, endothelial cell loss, tears in the vessel layers, and functional barotrauma occur in those saphenous veins under pressure [8-10]. Therefore, endothelial injury results from the direct mechanical trauma and stretching as a result of luminal distension. The early patency rates of saphenous veins harvested with surrounding tissue are reported to be as high as 95.4% [14-16].

The endothelium takes part in regulating the vascular smooth muscular cell tonus and hemostasis. In addition, the endothelium is not just a barrier for blocking the extravasation of blood cells and elements, but is also an organ with various biological functions. Under normal circumstances, platelet activation, adhesion, and aggregation are inhibited by prostacyclin (PGI₂) and EDRF, which are continuously secreted by the endothelium [13, 14].

Traumatic preparation or the high pressure distention of a saphenous vein graft is considered to be responsible for early graft occlusion. Moreover, the high pressure applied in the harvesting of the graft results in endothelial and media damage. For example, Viaro *et al.* reported that the endothelial-derived nitric oxide synthase (eNOS) levels were significantly lowered in SVGs distended with 300 mmHg [8].

Golbasi *et al.* had shown that in a comparison of the maximal contractile responses and sensitivities to these spasmogens, the reactivity of the proximal and distal SV segments is not significantly different [10]. Gurkan and colleagues showed that the saphenous vein lost contraction and relaxation functions when administered to 300 mmHg, while Viaro *et al.* reported that, from pressures of 200 mmHg, there was a tendency to reduce the CD34 expression,

which became statistically significant at 300 mmHg [8]. Golbasi *et al.* reported that the contractile functions of the proximal and distal segments of the saphenous vein were similar in the control group and the 300 mmHg pressure group.

In the present study, we compared the lower and upper saphenous vein according to the histological changes, and its contractile function after 3 stages of pressure. In the 100 mmHg and 200 mmHg pressures, the proximal saphenous veins had more endothelial cell loss. In addition, the relaxation response of the proximal samples distended to 100 mmHg and 200 mmHg were significantly decreased when compared to the distal segment samples. However, in those exposed to pressures of 300 mmHg, excessive endothelial cell loss was seen in both the proximal and distal segments. Furthermore, at this pressure, the group saphenous graft relaxation response could not take hold in both the distal and proximal segments.

According to this study, graft harvesting techniques without graft distention were protective for the saphenous vein endothelium. In addition, the distal region of the saphenous vein graft function and endothelium were more resistant at a moderate pressure, when compared to the proximal segment. The proximal graft choice can increase the graft failure rate, and additional *in vivo* studies should be conducted on this subject.

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Disclosure of conflict of interest

None.

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