The Effect of Vasodilator Drugs on Intima Damage in Preparation of the Saphenous Vein Graft

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ABSTRACT

Introduction: The most important issue in the success of coronary bypass surgery is the quality of the grafts used. In this study, the effect of vasodilator drugs for intimal damage on the harvested saphenous veins of coronary bypass patients was investigated.

Patients and Methods: A total of 10 patients who underwent coronary bypass surgery, had 2-cm long segments of saphenous veins harvested and divided into four study groups. Isotonic solutions in Group I (control group) and Group II, verapamil in Group III, and nitroglycerin in Group IV were applied to the saphenous veins at equal times, in the control group under 20 mmHg and in the other groups 100 mmHg pressure. Upon the completion of the preparation, saphenous vein grafts were studied with light and electron microscopy. Endothelial cell loss and intimal and medial edema were considered in evaluations. Scoring was done from 0 to 3. A median score was assessed for each group.

Results: There was a statistically significant difference between the control and saline groups in terms of endothelial and media damage (0.7 ± 0.48, 2.8 ± 0.42) ($p< 0.001$), while there was no statistically significant difference between the control group and the verapamil and nitroglycerin groups (0.7 ± 0.48, 1.4 ± 0.54) (0.7 ± 0.48, 1.2 ± 0.44) ($p> 0.05$). Likewise, no statistically significant difference was found between verapamil and nitroglycerin (1.4 ± 0.54, 1.2 ± 0.44) ($p> 0.05$). It was determined that the verapamil and nitroglycerin groups had the closest score to the control group, the endothelial and medial damage was minimal in the verapamil and nitroglycerin group, like the control group, and a large amount of endothelial and media damage was observed in the saline group.

Conclusion: The use of vasodilators (verapamil or nitroglycerin) in the preparation of the saphenous vein graft increases the early and late success of vein grafts by protecting the tunica intima and media in the graft, preventing the formation of thrombus in the early period and the development of branchendothelial fibromuscular hyperplasia and atherosclerosis in the late period.

Key Words: Saphenous vein graft; endothelium damage; vasodilators; verapamil; nitrogliserin

Safen Ven Greftinin Hazırlanmasında Vazodilatör İlaçların İntima Hasarına Etkisi

ÖZET

Giriş: Bu çalışmada koroner bypass hastalarında alınan safen venlerde intimal hasara yönelik vazodilatör ilaçların etkisi araştırıldı.

Hastalar ve Yöntem: Koroner bypass cerrahisi geçiren toplam 10 hastadan 2 cm uzunluğunda safen ven segmentleri alındı ve dört çalışma grubuna ayrıldı. Grup I'de (kontrol grubu) izotonik solüsyonlar 20 mmHg basınçla, Grup II'de izotonik solüsyonlar, Grup III'te verapamil, Grup IV'te nitrogliserin safen ven greftlerine eşit sürelerde ve 100 mmHg basınçla verildi. Safen ven greftleri hazırlanmalarının tamamlanmasının ardından, ışık ve elektron mikroskobu ile incelendi. Değerlendirmelerde endotel hücre kaybı, intimal ve medial ödem dikkate alındı. Puanlama 0'dan 3'e kadar yapıldı. Her grup için bir medyan skor değerlendirildi.

Bulgular: Endotelyal ve medya hasarı açısından kontrol ve salin grupları arasında istatistiksel olarak anlamlı fark varken (0.7 ± 0.48, 2.8 ± 0.42) ($p< 0.001$) kontrol grubu ile verapamil ve nitrogliserin arasında istatistiksel olarak anlamlı fark yoktu. Gruplar (0.7 ± 0.48), 1.4 ± 0.54) (0.7 ± 0.48, 1.2 ± 0.44) ($p> 0.05$). Benzer şekilde verapamil ile nitrogliserin arasında istatistiksel olarak anlamlı bir fark bulunmadı (1.4 ± 0.54, 1.2 ± 0.44) ($p> 0.05$). Verapamil ve nitrogliserin gruplarının kontrol grubuna en yakın puana sahip olduğu, verapamil ve nitrogliserin grubunda kontrol grubu gibi endotelyal ve medial hasarın minimal olduğu, endotelyal ve medial hasarınsa salin grubunda yüksek oranda olduğu belirlendi.

Sonuç: Safen ven greftinin hazırlanmasında vazodilatörlerin (verapamil veya nitrogliserin) kullanılması greftteki tunika intima ve mediayı koruyarak erken dönemde trombüs oluşumunu ve geç dönemde dalendotelyal fibromüsküler hiperplazi ve aterosklerozu engelleyerek ven greftlerinin erken ve geç dönem başarısını arttırır.

Anahtar Kelimeler: Safen ven grefti; endotel hasarı; vazodilatörler; verapamil; nitrogliserin

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INTRODUCTION

Carrel and Guthrie established the principles of vascular anastomoses in 1906 and made the mandatory acceptance of autogenous veins as an alternative material in arterial reconstructions[1]. Kunlin used the saphenous chief for femoral artery occlusion for the first time in 1949[2]. Coronary artery surgery began to be widely performed all over the world, with Favaloro's first use of saphenous vein graft in coronary bypass surgery in 1968[3]. Thanks to the wide diameter of saphenous vein grafts, they are still widely used today due to their adequate blood flow, convenient length, and ease of removal[4,5]. In coronary bypass surgery, the rate of occlusion of the saphenous vein grafts in the early postoperative period was 10-26% and 50% in the late period[6]. Severe atherosclerotic changes were detected in approximately 50% of the grafts in which no obstruction was detected in the late period[6].

Although the reasons for the occlusion of saphenous vein grafts are not fully known, it has been thought that inflation with high pressure to relieve spasms during preparation may be a factor[7]. In studies conducted for this purpose, it has been shown that inflation with high pressure causes endothelial damage and media damage in the saphenous vein graft[8]. It has been determined that endothelial damage in the saphenous vein graft causes thrombus in the early period and lipid accumulation in the late period, leading to occlusion of the graft[9].

The purpose of this study is to use light and electron microscopy to examine the graft endothelial damage caused by the application of solutions such as saline, verapamil, and nitroglycerin and pressure in the saphenous vein graft prepared for coronary bypass, and to find the appropriate solution that reduces endothelial damage.

PATIENTS and METHODS

In this study, 10 cm vein samples, which were taken from saphenous veins and used as grafts in 10 patients who underwent coronary artery bypass graft surgery between January 1999 and March 1999 at Çukurova University Faculty of Medicine, Department of Cardiovascular Surgery were examined. Institutional Ethics committee approval was obtained from Private Epc Hospital Ethics Committee, and numbered 2021/11 project/decision, and written informed consent was obtained from each patient. The saphenous vein was exposed to the standard saphenous vein preparation technique of coronary bypass surgery. A piece of 10 cm was taken with atraumatic intervention, and all the lateral branches were tied one by one with 3/0 silk so as not to cause stenosis in the lumen. The prepared saphenous vein piece was divided into four parts, 2-3 cm in length, and each of them was placed in the solutions to be compared. Four groups consisting of ten samples were formed, one being the control group and the other three being the experimental group.

Saphenous vein fragments were cannulated, and a triple tap was attached to the end of the cannula. A 50 cc syringe was filled with the prepared solution, the saphenous vein part was inflated with 20 mmHg in the control group and with 100 mmHg pressure in other groups to be compared. Here, the pressure application was recorded by transferring it to the monitor via the transducer in the triple tap. After pressure applications, all veins were divided into two parts. In Çukurova University Faculty of Medicine, Department of Pathology, the first pieces were prepared for light microscopy and the second pieces were prepared for electron microscopy.

In Group I (control group); the saphenous vein was inflated with heparinized sem physiological solution (a solution prepared by adding 5000 U heparin in 300 cc 0.9% NaCl) with a pressure of 20 mmHg (equivalent to in vivo lower extremity venous hydrostatic pressure in the foot) for two minutes, then kept in the same solution for one hour. In Group II (sale physiological group); the saphenous vein was inflated with heparinized saline solution at 100 mmHg (equivalent to the mean systemic arterial pressure) for two minutes, then kept in the same solution for one hour. In Group III (Verapamil group); the saphenous vein was inflated with verapamil solution (the solution prepared by adding 5 mg verapamil into heparinized saline solution) at 100 mmHg pressure for two minutes, then kept in the same solution for one hour. In Group IV (Nitroglycerin group); the saphenous vein was inflated with nitroglycerin solution (a solution prepared by adding 2.5 mg nitroglycerin into heparinized saline solution) at 100 mmHg pressure for two minutes, then kept in the same solution for one hour.

For evaluation in light microscopy, saphenous vein graft samples were fixed in 10% buffered formalin solution and passed through ascending alcohol series (70%, 90%, 96%, 100%). Following dehydration with alcohol and clearing with toluene, the samples were blocked after paraffin inclusion, and tissue sections taken with a thickness of 4-5 μm were stained with hematoxylin-eosin and elastic Van Giosen stain to show the general vessel histology, evaluated at light microscopy level, scored and photographed. Samples taken for electron microscopy were fixed in Karnovsky fixative for at least one hour. After washing with 0.1 M cacodylate buffer, postfixation was performed in buffered osmium tetroxide solution. Tissues dehydrated with ethanol were embedded in Epona. First, I micron thick sections were cut and stained with toluidine blue and examined under a light microscope. For electron microscopy examination, 500 A thick sections were taken from the marked samples with a Reichefi OMU 3 ultramicrotome. Sections were collected on 200-300 mesh copper grids and stained with 70%

ethanol saturated solutions of uranyl acetate and Reyolds' lead citrate. The stained sections were examined by electron microscopy and photographed.

Pathological damage was scored by considering endothelial cell loss, exposed basal lamina, and intimal and medial edema. These pathological damages were scored as the following: 0: No damage; 1: Light damage; mild desquamation of the endothelium, minimal exposure of the basal lamina; 2: Moderate damage; moderate desquamation of the endothelium, edema in the intima and media; 3: Severe damage; which was scored as severe desquamation and advanced edema in the endothelium.

In this study, "Student's t-test" was used for statistical evaluation. Pathological findings obtained from four different groups were scored and the mean value was found. The mean scores between the groups were compared, and the "p" value was found. The result was considered statistically insignificant if $p > 0.05$, and significant if $p < 0.05$. Values in the study are expressed as mean ± standard deviation.

RESULTS

In Group I (Control Group) under light microscopy, tunica intima (endothelium, branchendothelium), tunica media, and tunica adventitia were observed to be natural (Figure 1A). A pathological damage score of 0.7 ± 0.48 was detected (Table 1). Under electron microscopy in Group I, the endothelial cells covering the lumen were natural, smooth, and thin (Figure 2A). Tunica intima, tunica media, and tunica adventitia were observed in their natural structure.

In Group II (Sale physiological group) under light mi-

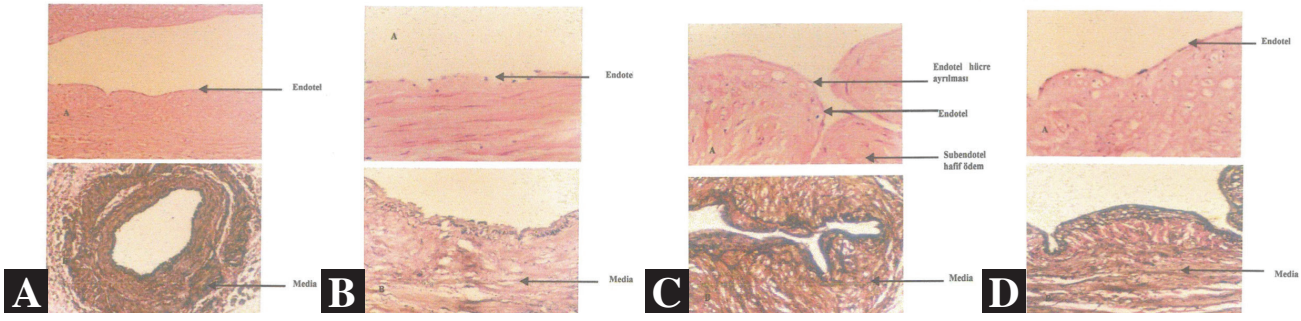

Figure 1. A. Control group (Group I): Endothelium and medise in normal structure are observed, **B.** Saline group (Group ll): Wide areas of desquamation in the endothelium and edema in the media are observed, **C.** Verapamil group (Group III): Mild endothelial damage, mild edema in the subendothelial region and normal media layer are observed, **D.** Nitroglycerin group (Group IV): Mild endothelial damage, mild edema in the subendothelial region and normal media layer are observed (A: Hematoxylin-Eosin X 20, B: Elastic Van Giosen X 10).

Table 1. Pathological mean damage score of the groups

Groups	Solution	Average Score
Group I	Control	0.7 ± 0.48
Group II	Physiological saline	2.8 ± 0.42
Group III	Verapamil solution	1.4 ± 0.54
Group IV	Nitroglycerin solution	1.2 ± 0.44

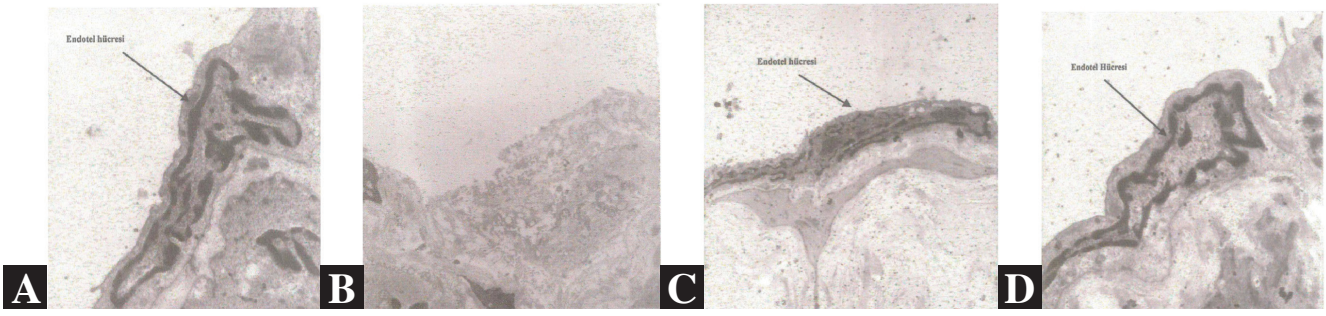

Figure 2. A. Control group (Group I): Endothelial cells observed with thin, straight, smooth, and normal appearance, **B.** Saline group (Group II): Severe endothelial damage, no endothelial cells are observed in the lumen, **C.** Verapamil group (Group III): Normal endothelial cells are observed, **D.** Nitroglycerin group (Group IV): Endothelial cells with normal structure (Uranyl acetate-lead citrate X 24000).

Table 2. Comparison of endothelial and media damage between groups with Student-t test

Groups	p	T
I - II	<0.001	9.82
I - III	<0.05	2.35
I - IV	>0.05	1.80
II - III	<0.001	5.08
II - IV	<0.001	6.31
III - IV	>0.05	0.56

croscopy, large areas of degeneration and loss of endothelial cells were observed in the intima layer (Figure 1B). In the tunica intima, occasional separations were evident. Significant edema between muscle bundles and separation of smooth muscle bundles were observed in the tunica media (Figure 1B). A pathological damage score of 2.8 ± 0.42 was detected (Table 1). Under electron microscopy; severe desquamation of the endothelial cell layer, dense fibrin and cell debris on the intima surface, and vacuolar degeneration of intimal cells were detected (Figure 2B).

In Group III (Verapamil group), a vein wall structure similar to the control group was observed under light microscopy. Vacuolization in a small number of endothelial cells, loss of endothelial cells in some areas, and endothelial cell detachment reflected mild endothelial destruction. In some regions, protrusions of endothelial cell nuclei towards the lumen were noted as findings reflecting mild degeneration. In addition to mild edema in the subendothelial layer, the tunica media and adventitia were normal (Figure 1C). A pathological damage score of 1.4 ± 0.54 was detected (Table 1). Surface scanning performed by electron microscopy revealed that the natural structure of the vein wall was largely preserved, the endothelial cells preserved their normal structures similar to the control group, with slight separations and minimal vacuolization in a few areas (Figure 2C).

In Group IV (Nitroglycerin group), light microscopy showed a slight loss of endothelial cells in the tunica intima, similar to the control group. Edema, which caused the smooth muscle bundles to separate from each other, was observed in the tunica media and tunica adventitia (Figure 1D). A pathological damage score of 1.2 ± 0.44 was detected (Table 1). Surface scanning performed by electron microscopy revealed desquamation and detachment from place to place in the endothelial cells, and the natural structure of the endothelium was preserved, but some endothelial cells showed vacuolar degeneration (Figure 2D).

There was a statistically significant difference between the control and saline groups in terms of endothelial and media damage ($p< 0.001$), while there was no statistically significant difference between the control group and the verapamil and nitroglycerin groups ($p> 0.05$). Likewise, no statistically significant difference was found between verapamil and nitroglycerin groups ($p> 0.05$) (Table 2).

According to these results, it was determined that the verapamil and nitroglycerin groups had the closest score to the control group, the endothelial and medial damage was minimal in the verapamil and nitroglycerin group like the control group, and the saline group had a large amount of endothelial and media damage.

DISCUSSION

In the saphenous vein, which is used as a graft in coronary bypass surgery, spasm usually occurs during the preparation of the chef or rarely in the postoperative period[10,11]. The spasm that occurs during preparation can be relieved with various solutions and by applying these solutions to the saphenous vein graft at certain pressures[12]. In order to determine the ideal graft preparation method, only blood and saline or vasodilators were used at various pressures. It was determined that more wall contraction and endothelial cell loss occurred in the serum physiologic group, and vascular relaxation was better in the saline group[13]. Because of these findings, saline was used instead of blood in our study.

Karabulut et al., examined the endothelial damage that occurred during the preparation of the human saphenous vein graft for coronary bypass with IM and EM, using blood and saline solutions at different pressures. They found that significant endothelial damage occurred in both groups in applications above 100 mmHg, and that less endothelial damage occurred in the blood group in applications below 100 mmHg compared to the saline group[14]. Based on these findings, an average of 100 mmHg pressure was applied in our study.

Many studies have been conducted using nitroglycerin, verapamil, papaverine, diltiazem, nipruss solutions, or combinations as vasodilators. Baumann et al. determined that the solution prepared with papaverine, which is a nonspecific po-

tent vasodilator, provides sufficient relaxation[13]. Roberts et al. showed that prostacyclin decreased, and endothelial damage occurred in human saphenous vein grafts prepared with papaverine[15]. In another study, it was determined that papaverine creates an acidic environment between PH 3-4 and this acidic environment causes damage to the endothelium. Therefore, although papaverine is a strong vasodilator, it was not included in our study.

The effect of the vasodilator on the saphenous vein graft should be rapid and long-lasting. In a study conducted in canine saphenous veins, it was found that the contraction created by potassium was resolved in one minute with nitroglycerin, three minutes with verapamil, and six minutes with papaverine[16]. Verapamil and nitroglycerin have a rapid and long-lasting effect in saphenous vein grafts. For the vasodilator to provide optimal relaxation in the saphenous vein graft, it must be prepared in appropriate concentrations. In their study on human saphenous data, He et al. discovered a substantial vasodilator effect at nitroglycerin concentrations of 7.2 pg/mL, papaverine concentrations of 37.5 g/mL, and verapamil concentrations of 15.7 pg/mL[16]. Considering the practical validity in our study, and in light of the aforementioned literature, saphenous vein grafts were prepared and examined at a concentration of verapamil 16.7 pg/mL, nitroglycerin 8.3 pg/mL, and an average pressure of 100 mmHg.

In our study, endothelial cell loss and edema in the intima and media were observed in the saline group, while endothelial damage was found to be minimal in the nitroglycerin and verapamil group, similar to the control group. Likewise, He et al. reported in their study that they achieved close to maximum relaxation in human saphenous veins with nitroglycerin and verapamil and that these effects were rapid and long-lasting[16]. In our light and electron microscopy study, it was shown that endothelin and media are better preserved as a result of this maximum relaxation, which is consistent with the results of this study. Roubos et al., in their light and electron microscopic examinations, proved that the combination of verapamil and nitroglycerin caused less endothelial and media damage than the saline and papaverine group[17]. These results show a correlation with our study.

Thanks to the developing technology and science, the pursuit of excellence in the field of health continues. There are also studies that delve down to the receptor level in the preparation of saphenous vein grafts. Dashwood et al. in their study investigated the effect of the Endothelin-1 (ET-1) mechanism on vein graft[18]. ET-1 is a peptide with potent vasoconstrictor and cell proliferation properties that plays a role in vein graft failure, which has been suggested to play a role in graft spasm and occlusion[18]. Endothelin antagonists have been shown to reduce neointimal hyperplasia in various in vitro experimental studies, as well as in vivo animal bypass models. More specific and, if necessary, molecular studies would help find the right approach which would require more studies with greater detail.

CONCLUSION

It is suggested that the use of vasodilators (verapamil or nitroglycerin) in the preparation of the saphenous vein graft will protect the tunica intima and media in the graft, prevent thrombus formation in the early period, and prevent the development of branchendothelial fibromuscular hyperplasia and atherosclerosis in the late period, thus increasing the success of vein grafts in the early and late periods.

In this study, it was found that mechanical distension (100 mmHg) applied to relieve spasms during the preparation of the saphenous vein graft in coronary bypass surgery showed a large amount of endothelial and media damage in the saline group, and minimal endothelial and media damage in the nitroglycerin and verapamil group (close to the control group) as evaluated with light and electron microscopy. Therefore, we believe that the saphenous vein graft should be prepared using a vasodilator (verapamil or nitroglycerin).

Ethics Committee Approval: The approval was obtained from EPC Hospital Non-Interventional Clinical Research Ethics Committee (Decision no: 2021/11, Date: 01.08.2021).

Informed Consent: This is retrospective study, we could not obtain written informed consent from the participants.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept/Design - MAP; Analysis/Interpretation - MAP; Data Collection - MAP; Writing - MAP; Critical Revision - MAP; Final Approval - MAP; Statistical Analysis - MAP; Overall Responsibility - MAP.

Conflict of Interest: The authors declared that there was no conflict of interest during the preparation and publication of this article.

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