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Original Article

Early histopathological effects of fluid versus foam sclerotherapy on great saphenous vein endothelium

Köpük skleroterapiye kıyasla sıvı skleroterapinin büyük safen ven endotelyum üzerinde erken dönem histopatolojik etkileri

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ABSTRACT

Objectives: This study aims to compare the intensity and severity of histopathological changes occurred following the injection of different concentrations of polidocanol in human great saphenous vein samples.

Patients and methods: Vein samples were collected from a total of 22 patients (17 males, 5 females; mean age: 52.4±6.9 years; range, 38 to 63 years) undergoing surgical stripping of the great saphenous vein. We used 0.5%, 1.0% and 2.0% concentrations of lauromacrogol 400 (Aethoxysklerol, Cem Farma, Ankara, Turkey). Nine samples were treated with fluid polidocanol, nine were treated with foam polidocanol, and four for used as controls without being treated at all. Each sample was, then, cut into three 2 cm long segments. Of a total of 66 segments (27 were treated with fluid, 27 with foam, and 12 controls), 63 were found to be eligible for the histopathological examination.

Results: No significant differences were found among six different experiment groups in terms of focal endothelial swelling (p=0.23), necrosis (p=0.66), and intimal thickening (p=0.19), whereas tunica media edema was significantly less common in 2.0% foam sclerotherapy group, compared to all other intervention groups (p=0.03). Total damage scores were not significantly different between the samples exposed to liquid and foam (p=0.33).

Conclusion: Higher concentrations of the sclerosing agent produce a slight, non-significant increase in total damage to the vein wall, indicating that sclerotherapy may be effective even with low doses.

Keywords: Damage; sclerotherapy; vein.

ÖZ

Amaç: Bu çalışmada insan büyük safen ven örneklerinde farklı polidokanol konsantrasyonlarının enjeksiyonu sonrasında görülen histopatolojik değişikliklerin yoğunluğu ve şiddeti karşılaştırıldı.

Hastalar ve Yöntemler: Büyük safen vene cerrahi stripping yapılan toplam 22 hastadan (17 erkek, ? kadın; ort. yaş 52.4±6.9 yıl; dağılım, 38-63 yıl) ven örneği alındı. Loromakrogol 400'ün (Aethoxysklerol, Cem Farma, Ankara, Türkiye) %0.5, %1.0 ve %2.0 konsantrasyonları kullanıldı. Dokuz örneğe polidokanol, dokuzuna köpük polidokanol kullanıldı ve dördü kontrol grubu olarak tedavi edilmedi. Her örnek ardından 2 cm uzunluğunda segmentler olacak şekilde üç parçaya kesildi. Altmış altı segmentin (27'sine sıvı, 27'sine köpük uygulandı ve 12'si kontrol grubu idi) 63'ü histopatolojik inceleme için uygun bulundu.

Bulgular: Fokal endotel ödemi (p=0.23), nekroz (p=0.66) ve intima kalınlaşması (p=0.19) açısından altı farklı deney grubu arasında anlamlı bir fark bulunamaz iken, tunika media ödemi, diğer tüm girişim gruplarına kıyasla, %2.0 köpük skleroterapi grubunda anlamlı düzeyde daha az görüldü (p=0.03). Total hasar skoru, sıvı ve köpük uygulanan örnekler arasında anlamlı düzeyde farklı değildi (p=0.33).

Sonuç: Sklerozan ajanın yüksek konsantrasyonları ven duvarında total hasarı hafif düzeyde ve anlamlı olmayacak şekilde artırır; bu da, skleroterapinin düşük dozlarda dahi etkili olabileceğini göstermektedir.

Anahtar sözcükler: Hasar; skleroterapi; ven.

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Chronic venous insufficiency (CVI), a disease known for more than 2,000 years, significantly affects the quality of life and causes important disability. Up to 10 to 35% of adult population in the United States have one form of the disease.[1] Symptoms may vary in nature and in severity, whereas patients may present with a wide variety of complaints ranging from a vague, tingling leg pain to large, and recurrent leg ulcers. Minimal invasive techniques including endovenous ablation therapies and intravenous injection of chemical sclerosing substances have increasingly replaced some surgical operations, as they offer better cosmetic outcomes, earlier return to occupational activities, and similar functional outcomes.[1,2]

Foam sclerotherapy with air-sclerosing substance mixture was first developed by Orbach[3,4] in 1944 and has increasingly become preferred in the treatment of varicose vein disease. The technique was proven to be more effective in the treatment of leg varicose veins, compared to standard sclerotherapy which entails injecting a relatively lower amount of sclerosing substance into the vein lumen.[3,4] Efficacy and tolerability of the foam sclerotherapy are accepted to be directly related to the concentration and the volume of the drug given to the vein lumen. Moreover, several recommendations have been already made upon appropriate dosing and concentration for the procedure. However, more data are needed to reveal to which extent the injection is safe and effective in terms of causing a well-controlled injury limited to the vessel wall without giving harm to nearby tissues.[4]

The main goal of sclerotherapy is to create occlusion within the diseased vein's lumen by producing an organized, non-recanalized thrombus and fibrosis. After injection of the sclerosing substance, the vessel lumen constricts, it is filled with thrombus and eventually becomes blocked due to endothelial injury, vasospasm, and inflammation. Sodium tetradecyl sulphate and polidocanol are the most common sclerosing substances used for sclerotherapy. However, data is limited regarding their half-life in the blood circulation and also to determine about the damage they would make within the vein wall.[3,5] In addition, little is known about the morphological alterations within the vein wall soon after injection of the sclerosing substance.[4]

In this comparative *in vitro* study, we aimed to compare the intensity and severity of histopathological changes occurred after the injection of different concentrations of polidocanol in human great saphenous vein (GSV) samples.

PATIENTS AND METHODS

A written informed consent was obtained from each patient. The study was approved by the Gaziosmanpaşa University Clinical Researchs Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The waste vein tissue samples were collected from a total of 22 patients (17 males, 5 females; mean age: 52.4±6.9 years; range, 38 to 63 years) undergoing surgical stripping of the GSV for the treatment of GSV reflux. Inclusion criteria were as follows: those who were unwilling to receive catheter ablation therapy and those in whom such a therapy was unsuitable due to excessive tortuosity or dilatation of the GSV.

All patients underwent lower extremity venous duplex ultrasound scanning to reveal GSV reflux and dilatation. Ultrasound examination was performed in the standing position, and all patients were assessed by a single radiologist.

Operations were performed under spinal/epidural anesthesia and by a single vascular surgeon. An incision was made about 1 cm lateral to the pulsation of the femoral artery, just below the inguinal crease. The GSV was explored, its proximal end was tied close to the saphenofemoral junction, and its major side branches were also tied and divided. A vascular clamp was applied to the GSV 9 to 10 cm distal to the saphenofemoral junction and a 7 to 8 cm segment of the GSV was gently excised using a 90° Potts' scissors, avoiding any damage to the vein wall tissue. This waste vein sample was placed in physiological saline. The rest of the GSV was removed from the ankle to groin using the standard stripping technique. External manual compression was performed over the course of the GSV for about 5 min to discontinue ongoing bleeding from side branches. Local varicosities were removed as needed, the leg was placed in elastic bandage, and the operation was completed.

We used 0.5%, 1.0%, and 2.0% concentrations of lauromacrogol 400 (Aethoxysklerol, Cem Farma, Turkey, 0.5% 10 mg, 1.0% 20 mg or 2.0% 40 mg). The foam was prepared with the Tessari's technique. For each 1 mL of foam, 0.3 mL of polidocanol was mixed with 0.7 mL of air by connecting tips of two syringes through a three-way stop cock and rapidly injecting

the mixed content into each other by 20 times. The proximal end (groin end) of the vein sample was tied with silk suture and a vessel cannula was introduced into the distal end (thigh end). The vein lumen was filled with three different concentrations (0.5%, 1.0%, and 2.0%) of fluid and foam polidocanol *in vitro*. Overdistention of the vein wall was avoided by gently palpating the full vein, and the fluid was allowed to drain after 2 min of treatment. Both ends of the vein sample were cut with the Potts' scissors. Nine samples were treated with fluid polidocanol, nine were treated with foam polidocanol, and four for used as controls without being treated at all. Each sample was, then, cut into three 2 cm segments. Of a total of 66 segments (27 were treated with fluid, 27 with foam, 12 controls), 63 were suitable for the histopathological examination.

Pathological examination and assessment

A total of 63 vein segments (25 treated with fluid, 26 treated with foam, 12 controls) were referred for histopathological examination in sealed containers. All pathological assessments were performed by a single pathologist who was blinded to the experiment. The segments were fixed in 10% buffered formaline overnight and, then, separately embedded in the paraffin blocks.

Hematoxylin-eosin staining was performed on 5-micron thick horizontal frames. Microscopic parameters included endothelial swelling, intimal thickening, smooth muscle vacuolization, tunica media edema, and degree of necrosis.

An overall damage scoring scale was constructed to quantify the damage occurred within the vein wall. According to this scale, absence of pathological signs was graded as 0 and presence was graded as 1. Overall damage score was calculated by adding all scores obtained by scoring each parameter (Table 1).

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive data were expressed in means or percentages, where appropriate. Categorical parameters were compared using the chi-square test or Fisher's exact test, where appropriate. Non-normally distributing parameters were compared using the Kolmogorov-Smirnov test. Intergroup comparison of the quantitative parameters was performed using the Kruskal-Wallis test, and

Table 1. Histopathological findings by different concentrations of liquid or foam sclerosing agent

	Foam			Liquid			Controls			0.5		1.0		2.0		0.5		1.0		2.0		
	n	%	Mean±SD	n	%	Mean±SD	n	%	Mean±SD	P value 1/2*	%	Mean±SD	%	Mean±SD	%	Mean±SD	%	Mean±SD	%	Mean±SD	%	Mean±SD	P value
Total number of vein segments	25			26			12				8		8		9		8		9		9		
Focal endothelial swelling	11	44.0		10	38.5		0	0.0		0.68/<0.001	2/8		5/8		4/9		4/8		5/9		1/9		0.23
Necrosis	16	64.0		18	69.2		0	0.0		0.69/<0.001	4/8		6/8		6/9		4/8		7/9		7/9		0.66
Edema of tunica media	13	52.0		20	76.9		8	66.7		0.06/0.17	4/8		7/8		2/9		7/8		7/9		6/9		0.03
Vacuolization of smooth muscle	17	68.0		22	84.6		0	0.0		0.16/<0.001	2/8		6/8		9/9		5/8		8/9		9/9		0.002
Intimal thickening	20	80.0		23	88.4		3	25		0.46/<0.001	5/8		6/8		9/9		6/8		9/9		8/9		0.19
Total damage score			3.1±1.7			3.6±1.3			0.9±0.7	<0.001		2.1±2.1		3.8±1.8		3.3±0.9		3.3±1.6		4.0±1.2		3.4±1.2	0.24

SD: Standard deviation; * p value 1 indicates the difference between foam and liquid group; p value 2 indicates the difference among three groups.

post-hoc analysis was performed using the Mann-Whitney U test. A p value less than 0.05 was considered statistically significant.

RESULTS

The mean diameter of the GSV segments was 5.8±1.7 mm (range, 5 to 7 mm). The distribution of histopathological findings among the samples exposed to different types and concentrations is summarized in Table 1. Although several damage parameters including necrosis, edema of tunica media, smooth muscle vacuolization, and intimal thickening were more commonly present in the liquid sclerotherapy group than those in foam sclerotherapy group, the difference was not statistically significant.

No significant differences were found among six different experiment groups in terms of focal endothelial swelling (p=0.23), necrosis (p=0.66), and intimal thickening (p=0.19), whereas tunica media edema was significantly less common in 2.0% foam sclerotherapy group, compared to all other intervention groups (p=0.03). Also, vacuolization of the smooth muscle was significantly less common in 0.5% liquid and 0.5% foam groups, compared to all other groups. Although not statistically significant, there was a steady increase in scores of the intimal thickening, vacuolization of the smooth muscle, and necrosis by increasing concentration of the either substance.

Total damage scores were not significantly different between the samples exposed to liquid and foam (p=0.33). There was also no significant difference in total damage scores among samples exposed to different types and concentrations of the sclerosing agent.

DISCUSSION

We found that severity of the damage caused by foam sclerotherapy was comparable to that produced by liquid sclerotherapy, although the dosage of the liquid required to prepare the same amount of foam sclerosing agent is about one-third of that is given in liquid sclerosing form. Using the foam technique, it may be possible to close a longer or a wider segment of the vein with same amount of the sclerosing agent, or a lesser amount may be sufficient to close a given length segment. Moreover, we observed that increasing the drug concentrations of either technique did not cause a significant increase in the extent of the damage. This finding can hypothetically be translated into clinical practice: using higher concentrations of sclerosing agents would not provide any additional benefit, but rather would increase the risk of procedural side effects, including skin irritation and inflammation.

In this study, we used the histopathological grading system which was constructed and used in a recent *in vitro* study by Erkin et al.[4] These authors sought to determine the minimum effective concentration of foam sclerosant by evaluating the histopathological changes in the vein wall samples after being exposed to four different concentrations of foam polidocanol. In the aforementioned study, endothelial swelling was present in 50% of the samples, focal or widespread necrosis in 64%, vacuolization in 45%, edema in 45%, and intimal thickening in 43%. These findings were more or less in consistent with our findings: the authors reported a median damage score of 2.75 in the foam sclerosing agent group, which is similar to that we found in the samples exposed to foam sclerosing. The aforementioned authors also reported that total damage score was significantly higher, when 1 to 2% concentration were used, although damage scores in the treatment group were significantly higher compared to the controls, even 0.5% concentrations were used.

In another study, Orsini et al.[6] analyzed the immediate pathological effects of sodium tetradecyl sulfate foam sclerotherapy on excised, but not removed saphenous vein samples of six patients undergoing saphenous vein stripping. The authors highlighted that the damage of the foam was extremely rapid with endothelial damage being completed within two min. In consistent with our results, the authors observed intimal edema and reported intimal separation and thrombus adhesion to the tunica media.

Extravascular injection of sclerotherapeutic agents has also been a major concern to avoid side effects, including skin irritation and decreased vascular flow. Sato et al.[7] conducted an animal study where they made absolute ethanol, ethanolamine oleate, and 3% polidocanol injections into the rat femoral and superficial inferior epigastric vessels. The authors demonstrated that the integrity of the vascular lumen, endothelial cells, and vascular patency were not affected by the injection of the sclerosing agents. These findings suggest that inadvertent injection of the sclerosing agent do not harm to the adjacent vessels surrounding the target vessels. Although

dermatological complications of sclerotherapy were rare and resolved in a few weeks,[8] our results support the use of sclerosing agents in low concentrations for foam sclerotherapy in the treatment of lower extremity varicose veins.

Nonetheless, our results cannot be directly translated into clinical relevance due to several reasons. Although the experiment was done as soon as possible after completion of surgery, the *in vitro* preparation is incapable of representing the vital environmental adequacy. The effect of blood coagulation and thrombus formation during the injection of the sclerosing agent was unable to be demonstrated, since the excised samples were kept in physiological saline. In addition, our experiment fell short to represent the real-time sequestration of the sclerosing agent within the wall lumen.

In conclusion, our study demonstrated that higher concentrations of sclerosing agent produce a slight, non-significant increase in total damage to the vein wall, indicating that sclerotherapy may be effective even with low doses.

Declaration of conflicting interests

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