JOURNAL OF PLASTIC SURGERY AND HAND SURGERY
2020, VOL. 54, NO. 6, 358–364
https://doi.org/10.1080/2000656X.2020.1788042

ARTICLE

Effect of perivascular low dose ethanol on rat femoral vessels: Preliminary study

Soysal Bas[a], Seyhan Hascicek[b], Ramazan Ucak[b], Alican Gunenc[c] and Aysin Karasoy Yesilada[d]

[a]Department of Plastic Reconstructive and Aesthetic Surgery, Sisli Hamidiye Etfal Training and Research Hospital, University of Health Sciences, Istanbul, Turkey; [b]Department of Pathology, Istanbul Training and Research Hospital, University of Health Sciences, Istanbul, Turkey; [c]Department of Plastic Reconstructive and Aesthetic Surgery, Yalova State Hospital, Istanbul, Turkey; [d]Department of Plastic Reconstructive and Aesthetic Surgery, Medipol University, Istanbul, Turkey

ABSTRACT

Vasospasm is one of the important causes of morbidity in free flap and replantation surgery. In secondary Raynaud's phenomenon, nearly half of the patients experience digital ulceration, pain and loss of function at least once in their lifetime. The aim of this study is to investigate the vasodilation effect of ethanol-mediated chemical denervation on peripheral vessels by topical administration. In this study, 27 Wistar albino male rats weighing 250-300 grams were used. The rats were randomly divided into three groups: saline (group S, $n = 8$), lidocaine (group L, $n = 9$) and 96% ethanol (group E, $n = 9$). According to group, 0.1 mL saline, 0.1 mL lidocaine and 0.1 mL ethanol were applied around the rat femoral neurovascular bundle. After the application, on the 0th day and 3th weeks, femoral artery and vein diameters were measured. After 3. weeks, histopathological samples from femoral artery, vein and nerve were evaluated. On the 0th day, the mean diameter of the femoral artery and vein was similar in group E and L and higher than group S. After three weeks, the vasodilatation effect of ethanol was increased in group E. In Group L and S, the vasodilatation effect was lost. Histopathological examination showed that ethanol significantly caused perivascular inflammation and nerve degeneration compared to other agents and did not cause endothelial damage. Vasodilatation obtained by ethanol is a rapid onset and long-lasting effect. It is also inexpensive and effective for peripheral vasodilatation.

ARTICLE HISTORY
Received 28 January 2020
Revised 7 June 2020
Accepted 22 June 2020

KEYWORDS
Ethanol; microsurgery; Raynaud's phenomenon; vasodilatation; vasospasm

Introduction

Vasospasm, one of the body's defense system, is affected by many factors and is caused by smooth muscle contraction in the tunica media. Iatrogenic or traumatic intimal injury leads to smooth muscle activation by local mediators such as endothelin-1 and thromboxane A2 secreted from the endothelium [1]. Vascular preparation, handling and anastomosis tension trigger myogenic response as a result of smooth muscle depolarization and cause vasoconstriction [2]. The sympathetic system mediates vasoconstriction caused by cold exposure both in digital ischemia in Raynaud's phenomenon (RF) and in reconstructive microsurgery. Increased sympathetic activity as a result of systemic cold exposure is mediated by norepinephrine, and local cold exposure causes vasoconstriction by directly stimulating alpha 2 adrenergic receptors [3]. Systemic hormones include the renin-angiotensin-aldosterone system triggered by hypovolemia. Angiotensin 2 (AT-2) causes contractions by stimulating AT-2 receptor type 1 on vascular smooth muscle [4].

Due to these factors, vasospasm may occur by reducing local blood flow and in case of prolonged stasis and thrombus, causing tissue loss. In RF associated with Systemic Sclerosis, nearly half of patients experience digital ulceration, pain and loss of function at least once in their lifetime [5]. Vasospasm, which affects between 5 and 10% of free flap and replantation surgery, is one of the most important reasons for morbidity [6].

Vasospasm therapy is focused on relaxation of vascular smooth muscles. However, the ideal treatment model is still unclear. Pharmacological agents include many agents such as local anesthetics, sympatholytic agents, calcium channel blockers, botulinum toxin-a, phosphodiesterase and prostaglandin E inhibitor. Surgical treatment is based on the elimination of sympathetic innervation. Options include cervical sympathectomy, lumbar sympathectomy and peripheral sympathectomy.

In vitro vascular relaxation of ethanol and alcohol has been demonstrated in studies of aortic ring and mesenteric vascular bed [7,8]. In addition, ethanol performs vasodilation in peripheral vessels by affecting central vasomotor control mechanisms with oral intake [9]. Ethanol metabolized in vascular smooth muscle and endothelial cells as well as liver, activates endothelial nitric oxide synthase (eNOS) by metabolizing to free oxygen radicals. Thus, NO release, which is a strong vasodilator, is increase and peripheral vasodilation effect occurs [8]. Ethanol, which is cytotoxic at high doses and concentrations, leads to protein denaturation and Wallerian degeneration in nerve cells.

Ethanol with the approval of United States Food and Drug Administration for the purpose of therapeutic neurolysis is used in the treatment of chronic pain syndromes [10]. It is also used for spasticity, hyperhidrosis, Morton's neuroma and trigeminal neuralgia [11–14]. It can be preferred as a sclerosing agent up to 1 ml/kg dose in vascular malformations, causing endothelial damage and fibrosis in intravascular applications [15]. In recent years, ethanol has been used in the treatment of renal hypertension with the effect of chemical neurolysis on periarterial sympathetic fibers. The aim of this study is to investigate the vasodilation effect of ethanol-mediated chemical denervation on peripheral vessels. In recent years, ethanol has been used in the treatment

CONTACT Soysal Bas soysalbas@gmail.com Sisli Hamidiye Etfal Egitim ve Arastirma Hastanesi, Halaskargazi Cad. Etfal Sk, Sisli/Istanbul, 34371, Turkey

of renal hypertension with the effect of chemical neurolysis on periarterial sympathetic fibers. The aim of this study is to investigate the vasodilation effect of ethanol-mediated chemical denervation on peripheral vessels by topical administration.

Materials and methods

The study was conducted after approval from the Animal Experiments Local Ethics Committee (ethical approval number: 2019/03). 27 Wistar male albino rats weighing 250–300 grams were used in the study. Rats were kept in separate containers in a temperature-controlled room of 22-24 °C for 12 h light and 12 h dark cycle. They were access to *ad libitum* standard rodent food and water. The rats were randomly divided into three groups: saline (group S, $n = 9$), lidocaine (group L, $n = 9$) and ethanol (group E, $n = 9$). One rat from the saline group died on the second day of the experiment and eight rats were continued.

All surgical procedures were performed by the same surgeon under general anesthesia, in the supine position and on a temperature-controlled table. The surgical procedure was performed and photographed under the microscope (S100/OPMI pico; Carl Zeiss Meditec AG, Jena, Germany). In anesthesia, 50 mg/kg Ketamine (Ketalar; Pfizer, New York, NY) and 5 mg/kg Xylazine (Rompun 2%; Bayer, Leverkusen, Germany) were used intraperitoneally. The skin was cleaned with povidone–iodine after the one-sided inguinal regions were shaved. After longitudinal skin incision, the inguinal fad pad was removed, and femoral neurovascular bundle was exposed. The femoral artery and vein were meticulous dissected, and the adventitial layer remained intact. Vasospasm was provoked with surgical manipulation. According to groups 0.1 ml saline, 0.1 ml lidocaine hydrochloride (Aritmal® 2%, 100 mg/5 ml Osel drugs, industry and trade AS., Beykoz, Istanbul, Turkey) and 0.1 ml of ethanol (99.6%, Merck, Darmstadt, Germany) were topical administered around vessels. Administration of ethanol, the perivascular region became non-translucent white. No discoloration was observed in the application of other agents. After the administered of agents, photos were taken with millimeter sizers and then the skin was primary sutured with 5/0 polyprolene. Three weeks later, the same experimental procedures were repeated by entering the same incision and the femoral neurovascular bundle was resected for histopathological evaluation. After the procedure, the animals were euthanized with high dose anesthesia. Ethanol-induced systemic effects were not observed in the rats for three weeks.

In order to determine the acute effect, the femoral artery and vein diameters were measured for five minutes after the application. Photographs were taken from microscope camera with millimeter sized. Three weeks later, femoral artery and vein were dissected again, and vessel diameters were photographed to determine the long-term effect. Digital photos were measured by Image J software (U. S. National Institutes of Health, Bethesda, MD, USA) program and their vessel diameters were measured in millimeters (mm).

The surgical specimen was fixed in 10% buffered formaldehyde. Sample tissues were embedded in paraffin blocks after processing. 4-micron thick sections were taken from these tissues in paraffin blocks. Tissue sections were prepared as routine hematoxylin and eosin (H&E) and immunohistochemically S100 stained preparations for examination. Immunohistochemical study was performed in Leica Bond Max Fully Automatic immunohistochemistry device. Immunostaining was performed with rabbit polyclonal antibody against the S100 protein using a commercial kit (Leica, S100P-L-CE, RTU,1/200 diluted). Sections were deparaffinized and rehydrated through a series of xylene and ethanol washes. Endogenous peroxidase activity was blocked with hydrogen peroxide. After a 25-min incubation with protease, the diluted antibodies were applied to the slides. Expression was detected with a polymer detection kit (Leica DS9800 Bond Polymer Refine Detection Automated), and the color was developed with diaminobenzidine as the chromogen. Mayer's hematoxylin was used for counterstaining. The preparations were re-hydrated by 95% and 100% alcohols. After cleaning with xylene, it was covered with mounting medium and was ready for examination under a light microscope. S100 positive nerve tissue was detected, which was determined to be light-dark brown with chromogen. The preparations were blindly evaluated by two experienced pathologists under light microscopy. Endothelial proliferation, perivascular nerve injury and inflammation were evaluated. Endothelial proliferation, periarterial inflammation and nerve degeneration were scored from one to three; normal, mild and severe (Table 1). In addition, tunica media damage and inflammatory cell involvement were evaluated.

SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics; number and percentage for categorical variables, mean and standard deviation for numerical variables. When the numerical variables provided the normal distribution condition in the groups, more than two independent group comparisons were made by one-way ANOVA test and when the normal distribution condition did not meet, Kruskal Wallis test was used. Subgroup analyzes were performed by Mann-Whitney U test in nonparametric test and interpreted by Bonferroni correction. Dependent paired group analyzes were performed by paired t-test when the normal distribution condition was satisfied and Wilcoxon test when the normal distribution condition was not satisfied. Statistical significance level was accepted as $p < 0.05$.

Results

The mean diameter of femoral artery on day 0 was similar in group E (1.04 ± 0.05 mm) and L (1.01 ± 0.06 mm) was higher than group S (0.56 ± 0.04 mm) ($p < 0.001$). After three weeks, the vasodilatation effect of ethanol was increased in group E (mean

Table 1. Grading of histopathological findings.

Grade	Nerve degeneration	Endothelial proliferation	Perivascular inflammation
1. Normal	No pathological findings on the nerve	No pathological findings on the vessels	No pathological findings on the perivascular tissue
2. Mild	Thickening of the perineurium, no endoneural fibrosis, partial vacuolization	Intimal fibrin deposition with no endothelial proliferation	Minimal increase of lymphocyte and plasmocyte in perivascular tissue and neovascularization
3. Severe	Endoneural fibrosis with extensive vacuolization and nuclear hypercromasia (ghost cells resulting in hyalinosis, clear cytoplasm, apoptosis in nerve cells)	Endotelial proliferation with thickening of the media	Severe increase of lymphocyte and plasmocyte in perivascular tissue and neovascularization

diameter; 1.11 ± 0.06 mm), in group L the vasodilatation effect was disappeared (mean diameter; 0.54 ± 0.04 mm) and artery diameters in group S was similar to the day 0 (mean diameter; 0.55 ± 0.02 mm). ($p < 0.001$). Ethanol's vasodilatation effect continued to increase with respect to day 0 ($p < 0.001$), the vasodilatation effect of lidocaine was decreased ($p < 0.001$) and the saline was not changed ($p = 0.155$) (Figures 1 and 2).

The vasodilatation effect of the agents on the veins was similar to the arteries. The mean diameter of femoral vein on day 0 in group E (1.24 ± 0.07 mm) and L (1.21 ± 0.08 mm) was higher than group S (0.88 ± 0.09 mm) ($p < 0.001$). After three weeks, the mean diameter of femoral veins in group E (1.29 ± 0.07 mm) was higher than group L (0.87 ± 0.09 mm) and S (0.86 ± 0.08 mm) ($p < 0.001$) (Figure 3).

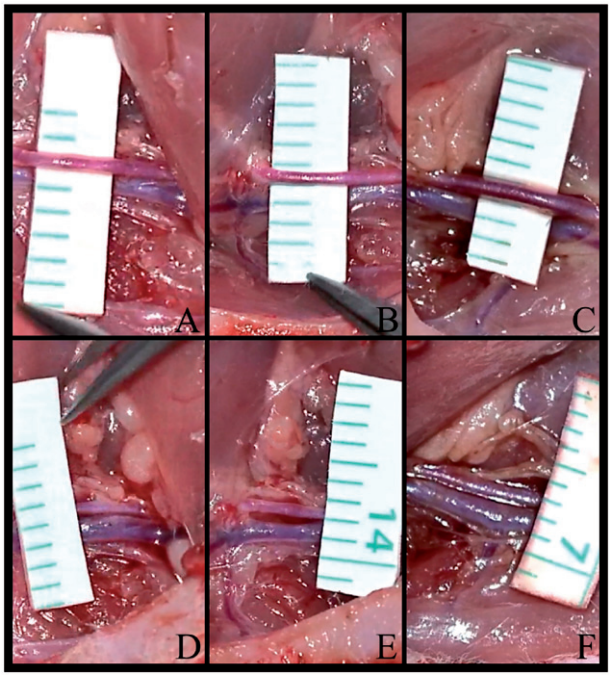


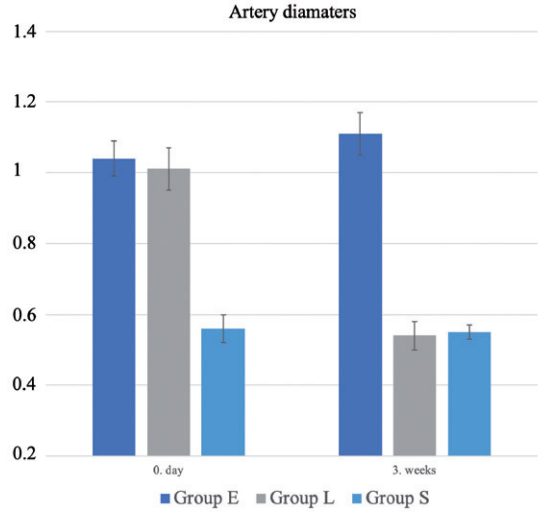
Figure 1. Photos of the femoral artery and veins. 5 min after the application of the agents, (A) saline; (B) lidocaine; (C) ethanol. Images after three weeks after application of agents, (C) saline; (D) lidocaine; (E) ethanol.

The histopathological results and scores of the agents are shown in Figure 4. There was no statistically significant difference between the effects of all three agents on endothelial proliferation (artery, $p = 0.628$; vein, $p = 0.980$), but their effects on perivascular inflammation ($p = 0.004$) and nerve degeneration ($p < 0.001$) were statistically different. Perivascular inflammation was higher in group E and group L than group S. Nerve degeneration was detected only in group E.

Detailed histopathological analysis and sections are presented in Figure 5. Endothelial proliferations detected in all groups were evaluated as non-specific regardless of the agents. In group E, severe endothelial proliferation was detected in only one artery and mild endothelial proliferation in one vein. In Group L, mild endothelial proliferation was detected in an artery and a vein. In Group S, endothelial proliferation was detected in only one vein. In addition, thrombus formation was not detected in any vessels. Perivascular inflammation was evaluated histopathologically according to lymphocyte and plasmocyte infiltration and neovascularization. In group E, severe perivascular inflammation was detected in five specimens and moderate inflammation was detected in four specimens. In group L, severe inflammation was detected in two specimens, moderate in five specimens and normal inflammation in two specimens. In group S, severe perivascular inflammation was detected in one specimen. Histological signs of nerve degeneration (vacuolization and nuclear hypercromasia), severe in six nerves and mild in two nerves, was detected only in group E. Ethanol was found to cause perivascular inflammation and nerve degeneration significantly compared to other agents but not to endothelial damage.

Discussion

The sympathetic denervation effect of low-dose and high-concentration ethanol on the vessel was first reported by Streitparth *et al.* it has been used as an alternative to radiofrequency-mediated renal denervation [16]. In pigs, 0.3 ml and 0.6 ml renal periarterial 95% ethanol caused degeneration of sympathetic fibers, decreased renal norepinephrine levels and decreased systolic blood pressure levels. In the same study, it has been shown histologically that ethanol does not enter the intravascular space and does not cause tunica media and intima damage, renal artery stenosis and aneurysm in perivascular applications [17]. In this



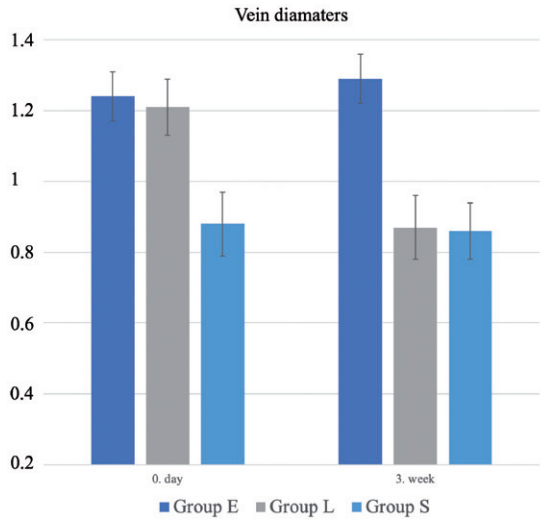
	Grup E (n=9)	Grup L (n=9)	Grup S(n=8)	p
0. day, mm (Mean ± SD)	1.04±0.05	1.01±0.06	0.56±0.04	<0.001
3. week, mm (Mean ± SD)	1.11±0.06	0.54±0.04	0.55±0.02	<0.001
p	<0.001	<0.001	0.155	

*Arterial diameters and statistical results of groups.

	E vs L (p)	E vs S (p)	L vs S (p)
0. day	0.269	0.001	0.001
3. weeks	<0.001	0.001	0.961

*Statistical results of arterial diameters between two groups.

Figure 2. Average arterial diameters and statistical relationship between groups at 0 days and 3th week.



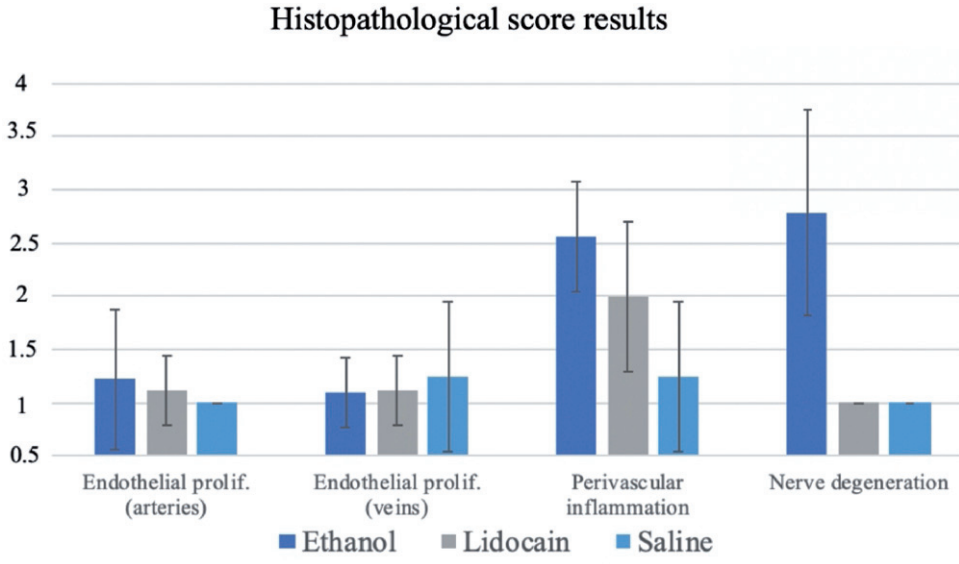
	Grup E (n=9)	Grup L (n=9)	Grup S(n=8)	p
0. day, mm (Mean ± SD)	1.24±0.07	1.21±0.08	0.88±0.09	<0.001
3. week, mm (Mean ± SD)	1.29±0.07	0.87±0.09	0.86±0.08	<0.001
p	<0.001	<0.001	0.155	

*Vein diameters and statistical results of groups.

	E vs L (p)	E vs S (p)	L vs S (p)
0. day	0.894	0.001	0.001
3. weeks	<0.001	0.001	0.963

*Statistical results of vein diameters between two groups.

Figure 3. Average vein diameters and statistical relationship between groups at 0 days and 3th week.



		Ethanol	Lidocain	Saline	p
Endotelial proliferation (Mean ± SD)	A	1.22±0.66	1.11±0.33	1±0	0.628
	V	1.11±0.33	1.11±0.33	1.25±0.70	0.980
Perivascular inflamation (Mean ± SD)		2.56±0.52	2±0.70	1.25±0.70	0.004
Nerve degeneration (Mean ± SD)		2.78±0.97	1±0	1±0	<0.001

*Statistical results of histopathological score. A, artery; V, vein.

Figure 4. Histopathological scoring results and statistical relationship between groups. Endothelial proliferation, periarterial inflammation and nerve degeneration were scored from one to three; normal, mild and severe. Statistical analysis was made according to the ordinal data obtained by scoring.

study, although significant perivascular inflammation and nerve degeneration due to % 99.6 ethanol was detected, there was no inflammation or injury in tunica media. In addition to, endothelial proliferation detected in vessels is thought to be nonspecific and secondary to traumatic vascular manipulation.

Nasiri et al. [18], they were measured one week later by applying 2 ml of 10% ethanol subcutaneously around the posterior central artery and marginal vein in rabbit ear. They showed the effect of ethanol on vascular dilatation in arteries and veins [18]. In this study, the vasodilatation effect of ethanol on arteries and veins

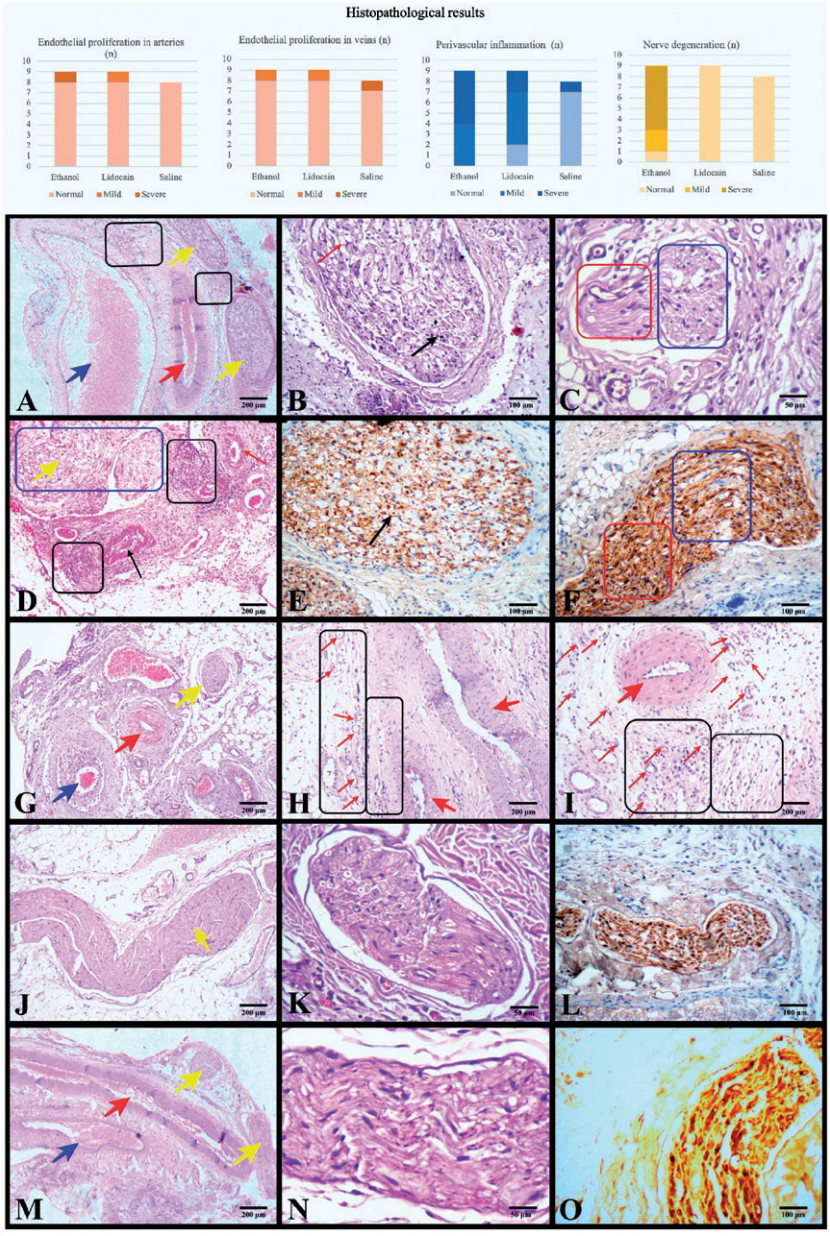


Figure 5. Endothelial proliferation, perivascular inflammation and nerve degeneration results in groups. Histopathological sections of the ethanol (A-F), lidocaine (G-L) and saline (M-O) groups. A-D, G-K, M, N H&E stain, E, F, L, O, S100 stain. Blue arrow, femoral vein; red arrow, femoral artery; yellow arrow, femoral nerve. (A) Mild perivascular inflammation (black rectangles, lymphocyte and plasmocyte infiltration); severe nerve degeneration (extensive vacuolization and nuclear hypercromasia); normal artery and vein. (B) Severe nerve degeneration with extensive vacuolization and nuclear hypercromasia; little red arrow, vacuolization; little black arrow, nuclear hypercromasia. (C) Mild nerve regeneration, partial vacuolization; blue rectangle, vacuolization and nuclear hypercromasia area; red rectangle, normal nerve area. (D) Severe perivascular inflammation (black rectangles, severe lymphocyte and plasmocyte infiltration); severe nerve degeneration (blue rectangle, extensive vacuolization and nuclear hypercromasia area), normal endothelial proliferation (little red arrow), severe endothelial proliferation (little black arrow, tunica media thickening with fibrin depositions). (E) Severe nerve degeneration with extensive vacuolization; ghost cell, little black arrow. (F) Mild nerve regeneration, partial vacuolization; red rectangle, normal nerve area; blue rectangle, vacuolization area. (G) Normal perivascular tissue, nerve and vessels. (H) Mild perivascular inflammation; black rectangles, mild lymphocyte and plasmocyte infiltration; little red arrows, neovascularization. (I) Severe perivascular inflammation; black rectangles, severe lymphocyte and plasmocyte infiltration; little red arrows, neovascularization. (J) Normal nerve. (K) Normal nerve (L) Normal nerve. (M) Normal perivascular tissue, nerve and vessels. (N) Normal perivascular tissue and vessels. (O) Normal nerve.

has been shown to begin within five minutes after administration and the effect continues to increase after three weeks. This rapid effect suggests that the onset time depends on the chemical denervation effect as well as the pharmacological effect on the endothelial and vascular smooth muscle.

Vasodilatation effect of 2% lidocaine in single application starts in the first five minutes and is frequently used in microsurgery. The duration of action is 15-30 min for lidocaine [19]. Ethanol has been shown to perform more vasodilatation at the fifth minute than lidocaine. Although there is no algorithm for postoperative vasospasm in microsurgery, many surgeons prefer topical vasodilatation and adventitial stripping effective in reexploration [20]. Ethanol mimics topical vasodilators with its rapid effect and adventitial stripping with chemical denervation effect. For this reason, ethanol may be an alternative agent in postoperative vasospasm.

Because of local sympathetic denervation effect, Botulinum Toxin (BTX) is used in selected cases for digital sympathectomy in

RF [21]. In addition, it has been demonstrated experimentally that BTX-A and B pretreatment models increase resting vessel diameters. The maximum vasodilatation effect in perivascular BTX-A administration has been demonstrated in rats on the 14th day and on the 28th day the effect is reduced [22]. BTX-B, which has a faster onset of action than BTX-A, has been reported to have anti-vasospastic activity in rats on day 3 [23]. In ethanol, which is relatively inexpensive compared to BTX preparations, the effect starts within five minutes after application. BTX provides temporary and partial denervation by preventing the release of acetylcholine and norepinephrine from presynaptic nerve endings [24,25]. Ethanol causes protein denaturation and nerve degeneration. Therefore, the duration of action is considered to be longer.

Adventitial stripping can be applied in secondary RF in order to reduce distal ulceration and pain [26]. With adventitial stripping, both sympathetic tonus and periarterial fibrosis is removed. Application of ethanol in secondary RF, treats the vasoconstrictive component of the disease, but an increase in perivascular inflammation will accelerate the fibrotic process. Therefore, it is unlikely to be in terms of an alternative agent in secondary RF both BTX application and adventitial stripping.

The effect of ethanol at different doses and concentrations on nerve degeneration has been previously studied. The effect at low concentration ethanol has been reported to be reversible [27,28]. In this study, low dose and high concentration of ethanol was used for peripheral vessels. To the best of our knowledge, the acute effect of different concentration ethanol on perivascular tissue was not found in the literature. We think the effective dose that causes less inflammation and vasodilation should be evaluated, and this study will be preliminary for other studies.

This study has some limitations. Although, ethanol is cytotoxic for endothelium, intravascular permeability of the anastomosis line should be studied, and possible thrombosis risks should be evaluated for use in peroperative. The study was performed on rats and human studies are needed for its use in perivascular administrations.

Conclusions

In this study, it has been shown that high concentration and low dose ethanol do not cause to thrombosis by topically administration to the perivascular area. This is the first study to evaluate *in vivo* acute vasodilation effect of ethanol on peripheral vessels by topical administration. Vasodilatation obtained by ethanol is rapid onset and long-lasting effect. It is also inexpensive and effective for peripheral vasodilatation. Ethanol may be an alternative agent in the treatment of vasospasm if supported by more studies in terms of anastomosis line permeability in microsurgery.

Disclosure statement

The authors report no declarations of interest.

References

[1] He GW, Yang CQ, Starr A. Overview of the nature of vasoconstriction in arterial grafts for coronary operations. Ann Thorac Surg. 1995;59(3):676–683.

[2] Borgström P, Grände PO, Mellander S. A mathematical description of the myogenic response in the microcirculation. Acta Physiol Scand. 1982;116(4):363–376.

[3] Wigley FM, Flavahan NA. Raynaud's Phenomenon. N Engl J Med. 2016;375(6):556–565.

[4] Borland JA, Chester AH, Rooker SJ, et al. Expression and function of angiotensin converting enzyme, chymase, and angiotensin II in the human radial artery and internal thoracic artery. Ann Thorac Surg. 2000;70(6):2054–2063.

[5] Herrick AL. Management of Raynaud's phenomenon and digital ischemia. Curr Rheumatol Rep. 2013;15(1):303

[6] Afridi NS, Paletz J, Morris S. Free flap failures: what to do next?. Can J Plast Surg. 2000;8(1):30–32.

[7] Ru XC, Qian LB, Gao Q, et al. Alcohol induces relaxation of rat thoracic aorta and mesenteric arterial bed. Alcohol Alcohol. 2008;43(5):537–543.

[8] Rocha JT, Hipolito UV, Callera GE, et al. Ethanol induces vascular relaxation via redox-sensitive and nitric oxide-dependent pathways. Vascul Pharmacol. 2012;56(1-2):74–83.

[9] Malpas SC, Robinson BJ, Maling TJ. Mechanism of ethanol-induced vasodilation. J Appl Physiol. 1990;68(2):731–734.

[10] Jang SH, Ahn SH, Park SM, et al. Alcohol neurolysis of tibial nerve motor branches to the gastrocnemius muscle to treat ankle spasticity in patients with hemiplegic stroke. Arch Phys Med Rehabil. 2004;85(3):506–508.

[11] Brock M, Frangakis C, Georgiades CS. CT-guided, percutaneous ethanol sympatholysis for primary hyperhidrosis. Cardiovasc Intervent Radiol. 2018;41(3):477–482.

[12] Kastler A, Cadel G, Comte A, et al. Alcohol percutaneous neurolysis of the sphenopalatine ganglion in the management of refractory cranio-facial pain. Neuroradiology. 2014;56(7):589–596.

[13] Shah SA, Khan MN, Shah SF, et al. Is peripheral alcohol injection of value in the treatment of trigeminal neuralgia? An analysis of 100 cases. Int J Oral Maxillofac Surg. 2011;40(4):388–392.

[14] Perini L, Perini C, Tagliapietra M, et al. Percutaneous alcohol injection under sonographic guidance in Morton's neuroma: follow-up in 220 treated lesions. Radiol Med. 2016;121(7):597–604.

[15] Su L, Fan X, Zheng L, et al. Absolute ethanol sclerotherapy for venous malformations in the face and neck. J Oral Maxillofac Surg. 2010;68(7):1622–1627.

[16] Streitparth F, Walter A, Stolzenburg N, et al. MR-guided periarterial ethanol injection for renal sympathetic denervation: a feasibility study in pigs. Cardiovasc Intervent Radiol. 2013;36(3):791–796.

[17] Fischell TA, Fischell DR, Ghazarossian VE, et al. Next generation renal denervation: chemical "perivascular" renal denervation with alcohol using a novel drug infusion catheter. Cardiovasc Revasc Med. 2015;16(4):221–227.

[18] Nasiri M, Kardar MH. Effect of Ethanol on Micro-Vessels Diameter and Prevention of Thrombosis. World J Plast Surg. 2019;8(2):249–253.

[19] Vargas CR, Iorio ML, Lee BT. A systematic review of topical vasodilators for the treatment of intraoperative vasospasm in reconstructive microsurgery. Plast Reconstr Surg. 2015;136(2):411–422.

[20] Turin SY, Walton RL, Dumanian GA, et al. Current Practices in the Management of Postoperative Arterial Vasospasm in Microsurgery. J Reconstr Microsurg. 2018;34(4):242–249.

[21] Van Beek AL, Lim PK, Gear AJL, et al. Management of vasospastic disorders with botulinum toxin A. Plast Reconstr Surg. 2007;119(1):217–226.

[22] Arnold PB, Campbell CA, Rodeheaver G, et al. Modification of blood vessel diameter following perivascular application of botulinum toxin-A. Hand (New York, N,Y). 2009;4(3): 302–307.
[23] Yoon J, Yoon ES, Lee BI, et al. Anti-vasospastic effects of botulinum toxin B pretreatment in animal models. J Plast Surg Hand Surg. 2019;53(1):25–30.
[24] Turton K, Chaddock JA, Acharya KR. Botulinum and tetanus neurotoxins: structure, function and therapeutic utility. Trends Biochem Sci. 2002;27(11):552–558.
[25] Morris JL, Jobling P, Gibbins IL. Botulinum neurotoxin A attenuates release of norepinephrine but not NPY from vasoconstrictor neurons. Am J Physiol Heart Circ Physiol. 2002;283(6):H2627–H2635.
[26] Hartzell TL, Makhni EC, Sampson C. Long-term results of periarterial sympathectomy. J Hand Surg Am. 2009;34(8): 1454–1460.
[27] Lu X, Li B, Liu S, et al. The dose and volume-response relationship of an ethanol-induced tibial nerve block in a rabbit model. Am J Phys Med Rehabil. 2014;92(8): 138–145.
[28] May O. The functional and histological effects of intraneural and intraganglionic injections of alcohol. Br Med J. 1912;2(2696):465–470.