Relationship between internal diameter and vasoconstriction in human varicose veins

Atsuko YOKOTA[1], Takayuki MATSUMOTO[2], Takayuki NAGANO[3],
Masachika KUWABARA[4], Eisaku NAKAMURA[5], Ryuichi YAMAMOTO[3] and
Naoko TANAKA-TOTORIBE[3]

[1]Department of Cardiovascular Surgery, Miyazaki City Medical Association Hospital, Miyazaki
880-2102, Japan
[2]Second Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University
of Medical Science, Nobeoka, Miyazaki 882-8508, Japan
[3]First Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University of
Medical Science, Nobeoka, Miyazaki 882-8508, Japan
[4]Kuwabara Clinic, Miyazaki 880-0852, Japan
[5]Department of Cardiovascular Surgery, Miyazaki Prefectural Miyazaki Hospital, Miyazaki
880-8510, Japan

Abstract

Varicose veins are common lower extremity venous disorders characterized by dilated veins and incompetent valves. Although maintaining the required vein wall tone for adaptive responses depends on a proper contractile function of the human saphenous smooth muscle, the contractile properties of varicose veins are mostly unknown. We investigated the relationship between contractile responses and the internal diameter of human saphenous varicose veins. The absolute contractile forces induced by potassium chloride (KCl, 60 mmol/l), serotonin (5-hydroxytryptamine [5-HT], 10 µmol/l), and noradrenaline (NAd, 10 µmol/l) were similar between normal saphenous veins (control) and varicose veins. When the contractile forces were normalized to the internal diameter in each preparation, the contractile responses to these stimuli were significantly lower in varicose veins than in the control veins. Furthermore, varicose veins were divided into three groups according to their internal diameter (group 1, 3–4.5 mm; group 2, 4.5–6 mm; group 3, >6 mm). The contractile responses induced by KCl, 5-HT, and NAd did not differ between groups 1 and 2 and the control group, while the contractile responses in group 3 were significantly lower than those in the control group. Moreover, the contractions induced by KCl and NAd in Group 3 were smaller than those in group 1 or group 2. This trend was also observed in 5-HT-induced contractions, although the results were not statistically significant. In conclusion, contractile responses in varicose veins may be altered by an increase in internal diameter, although adequate contractile responses are preserved in some diameters.

Key words: contraction, internal diameter, noradrenaline, serotonin, varicose veins

Corresponding author: Naoko Tanaka-Totoribe, First Department of Pharmacology, School of Pharmaceutical Sciences, Kyushu University of Medical Science, Nobeoka, Miyazaki 882-8508, Japan
Phone: +81-982-23-5548 Fax: +81-982-23-5550 e-mail: naoko@phoenix.ac.jp

Introduction

Varicose veins are a common disorder affecting lower-extremity veins, with an estimated prevalence of 5–30% in adults (1). Their severity ranges from the undesirable appearance of telangiectasia to large tortuous varicosities with or without related to cutaneous ulcerations, dermatitis, pigmentation, or swelling (2–4).

Varicose veins commonly manifest as dilated veins and incompetent valves (5). Maintaining the required tone in the vein wall during adaptive responses, such as standing and exposure to cold (6), depends on the proper contractile function of human saphenous smooth muscle. Vein wall alterations with disturbances in smooth muscle cells and/or extracellular matrix organization and dysfunctional contraction play important roles in the development of varicose veins (5, 7, 8). The main factor contributing to their development and progression is sustained venous hypertension, which increases the diameter of superficial veins, resulting in further valve incompetence (5). Although the theoretical basis and clinical aspects of varicose veins have been widely discussed and the contractile responses of varicose veins induced by various stimuli have been reported (9–18), the contractile properties and signaling remain insufficiently understood.

Hence, this study aimed to test the hypothesis that the degree of vasoconstriction is altered in human varicose veins with different internal diameters.

Materials and Methods

Ethics

This study was approved by the ethics committees of both Miyazaki Prefectural Nobeoka Hospital and Kyushu University of Medical Science (formerly Kyushu University of Health and Welfare), with approval number 09-004 (study using human saphenous veins). All patients provided their written informed consent to participate in the study. All experiments conformed to *The Code of Ethics of the World Medical Association (Declaration of Helsinki) for Experiments Involving Humans.*

Preparation of vessels and functional studies

Human saphenous vein samples were collected from 23 patients who underwent coronary artery bypass graft surgery at Miyazaki Prefectural Nobeoka Hospital (Miyazaki, Japan) and 35 patients who underwent surgical varicose vein treatment at Kuwabara Clinic (Miyazaki, Japan). At Miyazaki Prefectural Nobeoka Hospital, portions of each great saphenous vein graft were sectioned to the desired lengths to bypass the occluded coronary arteries. The remainder were used as the control group. At Kuwabara Clinic, portions of the saphenous vein were sectioned from each patient for surgical treatment of lower-extremity varicose veins. Only the non-distended areas were used for the varicose vein group. Subsequently, we transported the saphenous vein segments and measured their contractile responses as previously described (19–21). As previously reported (19–21), handling blood vessels during surgical procedures resulted in varying degrees of damage to the endothelial cells; therefore, we used endothelium-denuded rings. Moreover, we obtained as many rings of saphenous veins as possible from each patient to identify the relationship between the internal diameters and contractions. Briefly, we stretched the rings of the saphenous veins to the optimal tension (2.0 g), quickly measured the diameter of each ring using a Vernier caliper, and allowed them to equilibrate for 60 min by washing them with a fresh buffer solution several times. After stabilization, contractile responses to 60 mmol/l potassium chloride (KCl) were obtained, then they were washed again for restabilization. Subsequently, we obtained the contractile response to 60 mmol/l, 10 μmol/l, and 10 μmol/l concentrations of KCl, serotonin

(5-hydroxytryptamine [5-HT]), and noradrenaline (NAd). Based on previous studies using human saphenous veins, we selected the concentration of each drug to evaluate adequate contractile responses induced by receptor-independent contractions and two potent vasoconstrictors associated with vasospasm (19–24).

Data analysis and statistics

Data are expressed as the mean ± standard error of the mean obtained from different numbers (*n*) of ring preparations. Contractions are expressed in grams (Fig. 1) or g/internal diameter (mm) (Table 1). To evaluate the relationship between internal diameter and contraction, we further divided the varicose vein groups into three groups according to the diameter obtained (group 1, 3–4.5 mm; group 2, 4.5–6 mm; and group 3, >6 mm). The unpaired Student's *t*-test or unpaired Student's *t*-test with Welch's correction was used for two-group comparisons as necessary (GraphPad Prism ver. 8.4; GraphPad Software Inc., San Diego, CA, USA). To compare three or more groups, we used a one-way analysis of variance with Tukey's multiple comparison test (GraphPad Prism). *P*-values of <0.05 were considered to indicate statistical significance.

Results

Contractile responses induced by KCl, 5-HT, and NAd in control saphenous veins and varicose veins

As shown in Fig. 1, the contractions induced by KCl (60 mmol/l), which is a receptor-independent stimulation, cause membrane depolarization and stimulate Ca^{2+} entry via voltage-gated Ca^{2+} channels (25), and two potent vasospasm-associated vasoconstrictors (26), including 5-HT (10 µmol/l) and NAd (10 µmol/l), were all similar between the normal saphenous (control) and varicose veins. When the internal diameter normalized
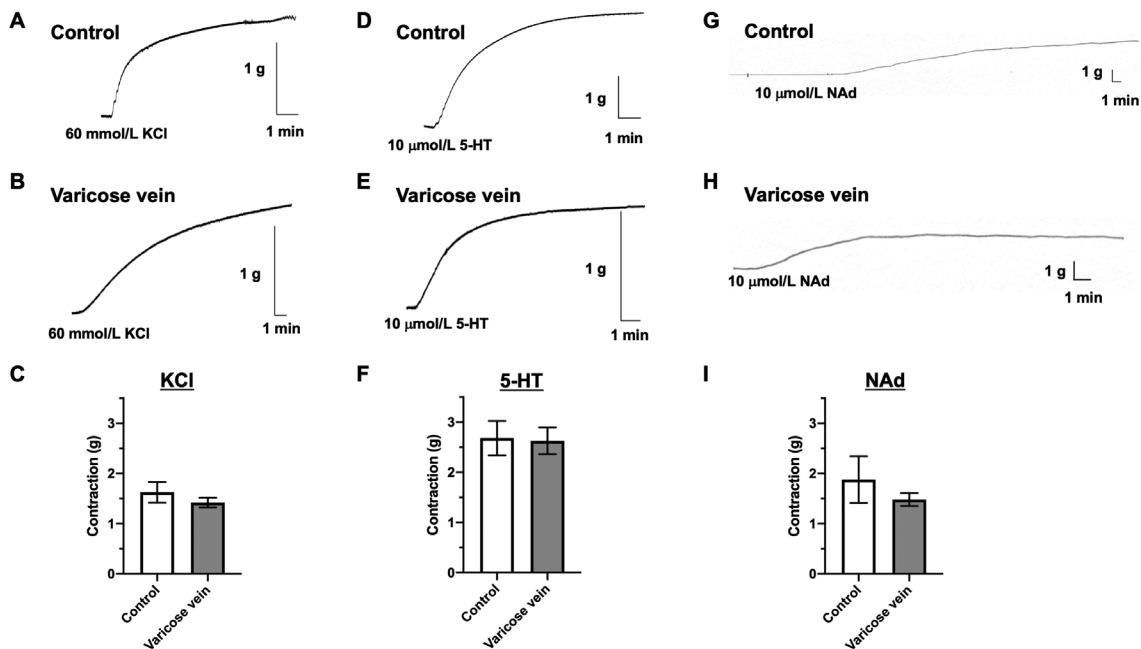

Fig. 1. Contractile responses induced by KCl, 5-HT, or NAd in endothelium-denuded saphenous veins. Representative traces of KCl (60 mmol/l) (A, B), 5-HT (10 µmol/l) (D, E), or NAd (10 µmol/l) (G, H) in saphenous veins obtained from patients with normal (control) (A, D, G) and varicose veins (B, E, H). (C, F, I) Data represent the mean ± standard error of the mean of the contraction value (g) (*n*=12–105). KCl, potassium chloride; 5-HT, 5-hydroxytryptamine; NAd, noradrenaline.

Table 1. Contractions induced by KCl, 5-HT, and NAd in the saphenous veins as normalized by the internal diameter

	KCl (60 mmol/l)	5-HT (10 μmol/l)	NAd (10 μmol/l)
Control	0.50 ± 0.06 (94)	0.85 ± 0.12 (62)	0.54 ± 0.13 (12)
Varicose vein	0.25 ± 0.02 (105)[a]	0.55 ± 0.05 (48)[a]	0.21 ± 0.02 (82) [a]
Varicose vein			
Group 1 (ID: 3–4.5 mm)	0.41 ± 0.03 (29)	0.61 ± 0.08 (17)	0.51 ± 0.08 (10)
Group 2 (ID: 4.5–6 mm)	0.41 ± 0.06 (22)	0.69 ± 0.09 (19)	0.42 ± 0.07 (14)
Group 3 (ID: >6 mm)	0.09 ± 0.01 (54) [b, c, d]	0.24 ± 0.09 (12) [b]	0.10 ± 0.01 (58) [b, c, d]

Contractions are expressed as g/internal diameter (mm). Values are presented as the means ± standard error of the mean obtained from different numbers (*n*) of ring preparations shown within parentheses. [a]*P*<0.05 versus control (using the unpaired Student's *t*-test with Welchi's correction). [b]*P*<0.05, control versus group 3. [c]*P*<0.05, group 1 versus group 3. [d]*P*<0.05, group 2 versus group 3 (using a one-way analysis of variance with Tukey's *post hoc* test). KCl: potassium chloride; 5-HT: 5-hydroxytryptamine; NAd: noradrenaline; ID: internal diameter.

contractile forces (Table 1), the contractile responses to KCl, 5-HT, and NAd were all significantly lower in the varicose veins (KCl, *n*=105 from 27 patients; 5-HT, *n*=48 from 17 patients; and NAd, *n*=82 from 18 patients) than those in the control saphenous veins (KCl, *n*=94 from 23 patients; 5-HT, *n*=62 from 18 patients; and NAd. *n*=12 from 5 patients).

To investigate whether the contractile responses induced by these constrictors would alter the internal diameter of varicose veins, varicose veins were divided into three groups according to their internal diameter (Table 1). KCl-, 5-HT-, and NAd-induced contractile responses did not differ between group 1 (KCl, *n*=29 from 14 patients; 5-HT, *n*=17 from 9 patients; and NAd, *n*=10 from 4 patients)/group 2 (KCl, *n*=22 from 9 patients; 5-HT, *n*=19 from 5 patients; and NAd, *n*=14 from 6 patients) and the control group. Conversely, the KCl- and NAd-induced contractile responses in group 3 (KCl, *n*=54 from 15 patients; and NAd, *n*=58 from 15 patients) were significantly lower than those in the control group, group 1, and group 2. Moreover, the contractile responses induced by 5-HT were significantly lower in group 3 (*n*=12 from 8 patients) than in the control group (Table 1).

Discussion

The major findings of this study concern the relationship between the internal diameter and the contractile response of varicose veins. The results showed that when exposed to the three different stimuli, namely, KCl, 5-HT, and NAd, the contractile responses were preserved in varicose veins with an internal diameter of 6 mm but decreased in those with an internal diameter of over 6 mm.

Defective control of venous tone reportedly causes dilatation and subsequent insufficiency in varicose veins (15). Indeed, in clinical observations and experimental studies, varicose veins showed a reduced ability to contract in response to various vasoconstrictors such as α-adrenoceptor agonists, extracellular nucleotides, histamine, 5-HT, and endothelin (12–18). Asbeutah et al. (17) observed the following: 1) 5-HT and NAd induced concentration-dependent contractions of normal and varicose saphenous vein segments; and 2) the potency of 5-HT and NAd showed no significant differences, but the maximal response normalized to tissue weight was lower in the varicose vein segment. In the present study, although the absolute force development induced by stimuli was similar between the control and varicose veins, the contractile responses normalized by the internal diameter were lower in varicose veins with an internal diameter of >6 mm than in normal saphenous veins induced by both receptor-independent (KCl) and receptor-mediated contractions stimulated by 5-HT and NAd. Reportedly, α_{1A}, α_{1B}, α_{1C}, and α_{1D} adrenoceptors are expressed in human saphenous veins

(27–29). Moreover, using a pharmacological approach, Yan et al. (29) observed that α_{1A} and α_{1B} adrenergic receptors are the major subtypes linking the contractile functions in the human saphenous vein. Furthermore, the functional post-junctional α_2 adrenoceptor mediating contractions of the human saphenous vein closely resembles the human recombinant α_{2C} adrenergic receptor ligand-binding site (30). Conversely, 5-HT$_{2A}$ (20, 22) and 5-HT$_{1B}$ (22, 31) receptors are expressed and function in the human saphenous vein. However, to date, no studies have compared these receptors between normal and varicose veins (and their various diameters) obtained from humans have been conducted. Future investigations are required regarding this point, including the relationship among functional changes, receptor expression, and internal diameter in human saphenous veins with and without varicose veins. In addition, receptor-independent KCl-induced contractions are also decreased in varicose veins with an internal diameter of >6 mm relative to contractions in normal saphenous veins, suggesting that reduced contractile responses in hyperdiastolic veins can be attributed to the contractile apparatus in vascular smooth muscle cells rather than receptor downregulation or to morphological abnormalities in the venous smooth muscle. For example, the RhoA-Rho kinase-dependent smooth muscle function is downregulated in varicose veins, which is correlated with the decreased expression of Rho kinase (7). As the human saphenous venous contractions induced by 5-HT and NAd are partly mediated by the Rho kinase pathway (23), and KCl can also cause Ca^{2+} sensitization involving Rho kinase translocation and activation (25), Rho kinase may be related to the reduced contraction in hyperdiastolic veins. Another putative mechanism underlying the decrease in contractions of large-diameter varicose veins may be alterations in matrix metalloproteinase (MMP) activity. Several studies have demonstrated alterations in mRNA and/or protein expression levels and proteolytic activities of MMPs have been detected in varicose veins (10, 32, 33). MMPs induce increased proteolysis of different protein substrates in the extracellular matrix, particularly collagen and elastin, which results in weakening of the vein wall and promotes venous dilation via several mechanisms, including activating potassium channels, thereby leading to smooth muscle hyperpolarization and relaxation (10, 32, 33). However, further research is needed to clarify the relationships between smooth muscle content, MMP activities, contractile responses, and their signaling induced by various stimuli and internal diameters in varicose veins. As contractile properties were preserved in varicose veins of smaller internal diameter, this is significant for preventing an increased internal diameter (e.g., remodeling) in varicose veins before the development of hyperdiastolic veins.

Conclusion

Contractile responses in varicose veins may change after an increase in internal diameter, although adequate contractile responses are preserved for certain diameters. These findings may be instrumental in pursuing further studies on varicose vein management.

Data Availability

The data are available from the corresponding author on reasonable request.

Author Contribution

Conceptualization: NTT
Data curation: AY, TM, TN, RY, NTT

Formal analysis: AY, NTT

Investigation: AY, MK, EN, NTT

Methodology: NTT

Project administration: NTT

Resources: AY, MK, EN, RY, NTT

Visualization: TM, TN, NTT

Writing-original draft: TM, NTT

Writing-review and editing: AY, TM, TN, MK, EN, RY, NTT

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Conflicts of Interest

The authors have no conflicts of interest to declare.

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