A comparative evaluation of various vasodilators for the anti-spasm effect on radial artery

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A comparative evaluation of various vasodilators for the anti-spasm effect on radial artery

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Abstract

The radial artery (RA) is used as an important graft for coronary artery bypass. This vessel readily develops spasms, and various vasodilators are used as anti-spasmogenic agents. This in vitro study evaluated the anti-spasm effects of various vasodilators on vasoconstrictor-induced spasms in RA graft samples isolated from patients undergoing coronary artery bypass (n=10). The specimens were treated for 15 min *in vitro* with several anti-spasm agents: verapamil-nitroglycerin (VG solution), a verapamil-nitroglycerin-olprinone mixture (VGO solution), olprinone, milrinone, and papaverine. Then, each vasodilator was tested for its anti-spasm effect against five vasoconstrictors as spasm inducers: KCl, norepinephrine, phenylephrine, angiotensin II and Thromboxane agonist.The VG solution and VGO solution exhibited a significantly stronger anti-spasm effect compared to olprinone and milrinone($p<0.05$). A significant difference between the VG and VGO solutions was not noted with any of the vasoconstrictors. With respect to angiotensin II, there was no significant difference between the VG solution and papaverine; that said, the VGO solution appeared to be more potent. These findings may support the use of a mixed anti-spasm agents like as VGO solution, which appears to exhibit a stronger anti-spasm effect, in coronary artery bypass grafting.

Key words coronary artery bypass grafting, radial artery, spasm, vasodilator

The intra-thoracic artery (ITA) and saphenous vein graft (SVG) have been used as vascular grafts for coronary artery bypass grafting (CABG) since the 1960s. The ITA is used as the gold standard in CABG because of its superior patency. Recently, the gastroepiploic artery and radial artery (RA) have been chosen as arterial conduits. The RA was used by Carpentier et al., but its use was later discontinued because of its low early patency compared to venous grafts[1]. The RA is a type III artery (limb artery), with a highly vascular smooth muscle layer compared to type I arteries (somatic arteries including the ITA as well as the gastroepiploic artery) and is known to readily develop spasms[2)3)]. Twenty years later, the patency of RA grafts was reevaluated, and the condition previously considered to be obstruction was found to be a result of arterial spasms. Subsequent studies have shown that RA spasms can be overcome by careful harvesting techniques and the use of anti-spasm agents. Following reports of the RA having relatively higher patency than an SVG and similar patency to the ITA, RA became, following ITA, the second most frequently used graft for CABG[4)5)].

During graft removal, vasospasms are induced by various stimuli that may come in mechanical, physical, or pharmacological form. Adequate use of anti-spasm agents is crucial to relaxing induced spasms and improving patency during grafting. Various agents including papaverine, a verapamil-nitroglycerin (VG) solution, and phosphodiesterase (PDE) III inhibitors have been reported to be effective at eliminating spasms[6)-9)]. However, no reports have systematically compared the range of vasodilators, from those traditionally used in clinical settings to their newer cousins.

This study evaluated the anti-spasm effects of various vasodilators, including a new verapamil-nitroglycerin-olprinone (VGO) solution proposed by the author, on vasoconstrictor-induced spasms in RA graft samples isolated from patients.

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Abbreviation: ARB, angiotensin II receptor blockers; CABG, coronary artery bypass grafting; ITA, intrathoracic artery; PDE, phosphodiesterase; RA, radial artery; SVG, saphenous vein graft; VG, verapamil-nitroglycerine; VGO, verapamil-nitroglycerine-olprinone

Materials and Methods

I. Preparation of specimens

Specimens were collected from 10 patients who underwent CABG using an RA graft at this hospital. Written informed consent was obtained from each patient before the study.

The patients' backgrounds, risk factors (history of smoking, arteriosclerotic disease, and drugs administered before surgery), and surgical procedure are shown in Table 1. An antiplatelet agent was taken 7 days before surgery; other agents were taken prior to the morning of the day of surgery. All pedicle RA grafts were harvested by one surgeon using electric cautery. The length of RA graft necessary for bypass was cut off on the proximal side, and the remaining distal portion was used for the *in vitro* experiment. The specimen was immediately placed in Krebs-Henseleit solution (Sigma, St. Louis, USA) at 4℃ and transported to the laboratory. The composition of the Krebs-Henseleit solution is as follows: Na^+ 144 mM, K^+ 5.9 mM, Ca^{++} 1.2 mM, Cl^-128.7 mM, HCO_3^- 25 mM, SO_4^- 1.2 mM, $H_2PO_4^-$ 1.2 mM, and glucose 11.0 mM. The surrounding tissues were carefully removed in a glass dish, and the specimen was cut into 5-mm rings. Six rings were obtained from each RA.

Table 1. Patient profile

Age, years	66.5±5.8 y.o.
Male/Female	9/1
Hypertension	8
Diabetes mellitus	6
Hyperlipidemia	5
Medication	
Statins	5
Nitrates	7
ARB	3
β-blocker	2
Ca-antagonist	7
Aspirin	8

For age, the $\bar{x} \pm$ SEM of 10 patients is presented.
For other items, the numbers of patients are shown.
y.o., year old. ARB, angiotensin II receptor blockers.

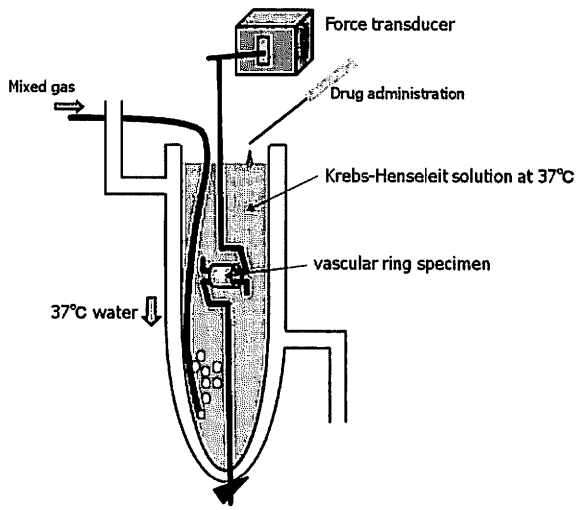

Figure 1. Schema of organ bath
The vascular ring specimen was suspended between the upper and lower hooks. The lower end was fixed, and the upper end was attached to a string connected to an isometric force transducer.

II. Measurement of isometric contraction

The specimens were incubated in an organ bath containing Krebs-Henseleit solution at 37℃ and aerated with a mixed gas consisting of 95% O_2 and 2-5% CO_2 throughout the experiment. The vascular ring specimen was suspended between the upper and lower hooks. After equilibrium for 30 minutes, the reading was taken and used as the baseline. The lower end was fixed, and the upper end was attached to a string connected to an isometric force transducer (TB-611, Nihon Koden, Tokyo). Changes in arterial tension (mmHg) were recorded isometrically via an amplifier (AP-621G, Nihon Koden)(Fig 1).

To minimize the effects of the drugs taken, 60mM KCl was first administered to sufficiently vasoconstrict RA specimens. Then, specimens were rinsed with Krebs-Henseleit solution to sufficiently relax them until they returned to the baseline. Upon restoration of the baseline steady state, one of the following 5 drugs were added: papaverine (Sigma, St. Louis, USA), verapamil(Sigma, St. Louis, USA), olprinone (Eizai, Tokyo), milrinone (Sigma, St. Louis, USA), a verapamil-nitroglycerin (Sigma, St. Louis, USA) (VG) solution, or a verapamil-nitroglycerin-olprinone (VGO) solution. Normal saline solution served as a control. The concentrations of the vasodilators were determined as follows. Verapamil and nitroglycerin were used at 30 μM in accordance with He *et al.*[9]. Olprinone was tested at 16 μM, milrinone at 46 μM, and papaverine 100 μM, which are concentrations used in routine clinical practice. Five mg (1 ampoule) of olprinone, 10 mg (1 ampoule) of milrinone, and 4 mg (1 ampoule) of papaverine were separately dissolved in 100 mL of saline. Each compound was diluted in Ringer's solution to the required concentration at the time of testing.

Each vasodilator was tested for its anti-spasm effect against five vasoconstrictors as spasm inducers: KCl (Sigma, St. Louis, USA), norepinephrine (Sigma, St. Louis, USA), phenylephrine (Sigma, St. Louis, USA), angiotensin II (Sigma, St. Louis, USA), and Thromboxane agonist (U-46619, Sigma, St. Louis, USA). The concentrations of vasoconstrictors were established in preliminary experiments[7][8][10]. The concentrations that provided sufficient contraction were 25 mM KCl, 1 μM norepinephrine, 3 μM phenylephrine, 10 nM angiotensin II, and 10 nM U-46619.

After treatment for 15 minutes, the first spasm inducer,

25mM KCl, was administered, and the time required achieving a steady state and the resultant contractile force were measured. The RA sample was washed twice with Ringer's solution and left until the spasms were resolved and the contractile force returned to a stable baseline. The anti-spasm agent was again added to the chamber, and 15 minutes later 1 μM norepinephrine was added as the second vasoconstrictor. The contractile force was measured until it reached the steady state, and measurement was continued for an additional 30 minutes. After washing and returning to the baseline steady state, the same procedures were repeated and the contractile force was measured consecutively for the other vasoconstrictors: 3 μM phenylephrine, 10 nM angiotensin II, and 10 nM U46619 (Table 2).

III. Statistical analysis

Statistical analysis was done with one-way analysis of variance. Furthermore, multiple comparisons were done with Games-Howell's post hoc test. All analyses were done using SPSS version 16 (SPSS, Chicago, IL, USA). The data are presented in $\bar{x} \pm$ SEM. The significance level was set at $p<0.05$.

Results

All spasm inducers induced isometric tension. Table 2 shows the effects of various anti-spasm agents on the isometric contraction rates induced by various vasoconstrictors. For each vasoconstrictor, the maximum contraction without an anti-spasm agent was taken as 100%.

The anti-spasm effect of the VG and VGO solutions was significantly stronger than that of olprinone, milrinone, and papaverine for 25 mM KCl, 1 μM norepinephrine, 3 μM phenylephrine, and 10 nM U46619. With 10 nM angiotensin II, the anti-spasm effect of the VG solution was clearly stronger than that of olprinone and milrinone, but there was no significant difference between its effect and that of papaverine. The effect of the VGO solution was clearly stronger than that of olprinone, milrinone, and papaverine. Under all conditions, there was no significant difference between the effects of the VG and VGO solutions.

Discussion

Following the ITA and gastroepiploic artery, the RA is typically chosen as an autologous arterial graft for CABG. Because it is used as an arterial graft, however, spasms present a major problem in terms of preserving graft patency[2)3)]. To make the best use of the RA as a conduit, spasms should be avoided as much as possible and maximum vascular endothelial function should be conserved during removal of the graft. Arterial spasms during removal are caused by various physical stimuli such as mechanical stimuli resulting from the surgical procedure, temperature stimuli produced by cooling, nervous stimuli, and drug stimuli by vasoconstrictors[10)11)].

Vasoconstrictor agents are classified as follows: vascular endothelium-derived substances such as endothelin, prostanoids such as Thromboxane A2, α-adrenoceptor antagonists such as norepinephrine, α_1-adrenoceptor agonists such as phenylephrine, muscarinic receptor agonists such as acetylcholine, renin-angiotensin system-related substances such as angiotensin II, and depolarizing agents such as KCl[7)]. The degree of reactivity to these vasoconstrictors varies depending on the arterial type. In the gastroepiploic artery, which is a type II artery (splanchnic artery), spasms are readily induced by K^+, Thromboxane A2, and norepinephrine[9)]. The RA is a type III artery (limb artery), and spasms are reported to be induced by norepinephrine, angiotensin, and endothelin-

Table 2. Effect of anti-spasm agents on the contraction of radial artery graft

Vasoconstrictor	Vasodilators contraction rate (%)					
	VG	VGO	Olprinone (16 μM)	Milrinone (46 μM)	Papaverine (100 μM)	F
KCl (25mM)	0.5±1.7※	4.2±1.6※	31.5±3.6	37.8±4.6	23.7±2.5	21.862 (P<0.05)
Norepinephrine (1 μ M)	9.2±1.6※	5.0±1.4※	41.2±6.0	44.6±3.8	39.2±5.7	20.755 (P<0.05)
Phenylephrine (3 μ M)	7.1±2.2※	2.8±0.9※	31.4±3.8	47.1±3.2	34.3±4.0	39.234 (P<0.05)
Angiotensin II (10 nM)	15.9±2.8	12.7±2.8※	42.2±6.6	39.1±5.7	33.0±5.7	7.298 (P<0.05)
U-46619 (10 nM)	44.1±4.9※	41.6±6.1※	80.0±2.2	78.8±3.3	75.5±3.1	20.613 (P<0.05)

The constriction rate with each vasoconstrictor alone is set at 100%.
Isometric contraction rate is shown. Each value represents the $\bar{x} \pm$ SEM (n=10).
One-way analysis of variance showed, the effect of vasodilators is statistically significant ($p<0.05$).
Multiple comparisons (Games-Howell's post hoc test) showed significant differences for VG or VGO solutions versus Olprinone, Milrinone and Papaverine (※$P<0.05$).

1[3]. However, these factors do not necessarily act independently depending on the type of artery; spasms are caused by the combined action of these factors. In light of the variation in spasmodic responses in arteries, the five vasoconstrictors KCl, norepinephrine, phenylephrine, angiotensin II, and U46619, a Thromboxane A2 mimetic, were thus used in this study.

In clinical practice, various vasodilators are used as anti-spasm agents during removal of arteries. Papaverine is a typical vasodilator that is used perioperatively; it acts as a non-specific vasodilating phosphodiesterase inhibitor that relaxes vascular smooth muscle by reducing the intracellular Ca^{2+} level through elevation of cGMP levels. However, it has short-lasting action and is also reported to several disadvantages: it is strongly acidic, so it injures vascular endothelium, and it risks increasing vasoconstrictor sensitivity[12].

The VO solution is a vasodilator consisting of nitroglycerin and verapamil. The mechanism of vascular relaxation induced by nitrovasodilators including nitroglycerin is as follows. NO production increases cGMP via stimulation of guanylate cyclase, resulting in blockage of receptor-operated channels, which decreases intracellular calcium levels and inhibits vascular smooth muscle contraction. The effect is potent and has a rapid onset but soon disappears because resistance is easily acquired. In general, the effect on smooth muscle spasms via voltage-operated calcium channels is weak. These agents are effective at resolving spasms but are less effective at preventing them[13]. Ca^{2+}-antagonists including verapamil close the voltage-operated calcium channels and reduce intracellular Ca^{2+} levels, which inhibits K^{+}-mediated depolarization-induced vasoconstriction. Their effect is slow, but acquisition of resistance is less likely, and the effect is sustained. In general, the effects on receptor-operated channel-mediated constriction, such as those induced by Thromboxane A2, adrenoreceptors, and endothelin, are weak[12].

As described above, Ca^{2+}-antagonists and nitrovasodilators have different mechanisms of vascular smooth muscle relaxation. He *et al.*[9] reported on the synergistic effect of these two classes of drugs; using a VG solution, they anticipated a combined effect: nitroglycerin would have a rapid anti-spasm effect on smooth muscle contraction via receptor-operated channels and verapamil would have a long-lasting anti-spasmogenic effect via voltage-operated calcium channels. Indeed, the superior anti-spasm effect of a VG solution has been demonstrated.[9][13]

More recently, phosphodiesterase (PDE) inhibitors, which are drugs to treat heart failure, have reportedly been used as anti-spasm agents.[7][8][12][14] Inhibition of PDE III increases the intracellular cAMP level and decreases Ca^{2+} levels, resulting in inhibition of vascular muscle contraction via receptor-operated channels. Eleven isozymes of PDE have been identified, at least 4 of which have been confirmed to be present in human vascular smooth muscle; of these, PDE III has at least 3 isoforms (cAMP-specific PDE)[15]. Accordingly, the target PDE III isozyme varies depending on the type of PDE III inhibitor, resulting in different effects on vasodilatation. Due to differences in the subtype of PDE inhibitor, milrinone and olprinone have been reported to be more effective than amrinone at preventing spasms in RA grafts[2]. The current author has long administered various PDE inhibitors to arterial grafts to prevent spasms. Levy *et al.* reported that intravenous PDE III inhibitor administration in heart surgery yields favorable hemodynamics, including elevation of the cardiac index and reduction of pulmonary blood pressure[16]. In the author's clinical experience, this positive effect on hemodynamics is absent with other vasodilators, suggesting another benefit of PDE III inhibitors in addition to their anti-vasospasm effect.

As described above, the synergistic effect of Ca^{2+}-antagonists including verapamil and nitrovasodilators has been reported. Moreover, the synergistic effect of nitrovasodilators and PDE III inhibitors is such that both exhibit a potent anti-spasm effect via receptor-operated channels Nitroglycerin and PDE III inhibitors exhibit an anti-spasm effect via receptor-operated channels. The strong and rapid effect of nitroglycerin is mediated by NO and resistance is acquired in the early phase, with the effect soon diminishing. In contrast, the effect of PDE III inhibitors is weak and slow but it is sustained. He *et al*[17] reported that a mixture of nitroglycerin and milrinone exhibited a clear additive effect on ITA. The current study also anticipated such a synergistic effect and thus proposed a VG solution with olprinone (VGO solution).

Results of this study indicated that with respect to vasoconstrictors besides AT II the VG and VGO solutions were significantly more potent than milrinone, olprinone, and papaverine indicating the usefulness of the two solutions. A significant difference between the VG and VGO solutions was not noted with any of these vasoconstrictors. With respect to AT II, however, there was a significant difference between the VGO solution and papaverine but not between the VG solution and papaverine; that said, the VGO solution was more potent. A mixture of a nitrovasodilator, Ca^{2+}-antagonist, and PDE III inhibitor was anticipated to have a combined or synergistic effect. A Ca^{2+}-antagonist (verapamil) with a potent anti-spasm effect via voltage-operated calcium channels (VGO solution) was added to this mixture. Therefore, the VGO solution may prove to exhibit an additional synergistic anti-spasm effect on the VG solution *in vivo*. The use of higher concentrations of vasoconstrictors as well as a longer measurement time may further clarify the usefulness of the VGO solution in light

of the characteristic sustained effect of PDE III inhibitors in comparison to the VG solution.

In conclusion, on the radial artery, the VG solution and VGO solution exhibited a significantly stronger anti-spasm effect than olprinone, milrinone, and papaverine with respect to vasoconstrictors besides AT II . With respect to AT II, however, the VGO but not VG solution had a significantly stronger anti-spasm effect than papaverine; that said, the VGO solution was more potent. These findings may support the use of a mixed anti-spasm agents like as VGO solution, which appears to exhibit a stronger anti-spasm effect, in coronary artery bypass grafting.

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