

## Use of Vasodilators to Overcome Perioperative Spasm of the Left Internal Mammary Artery and Saphenous Vein in Coronary Artery Bypass Grafting: Comparison of Papaverine and Glyceryl Trinitrate/Verapamil Combination

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### ABSTRACT

**Background:** Left Internal Mammary Artery (LIMA) and Saphenous Vein (SV) are two of the most commonly used conduits in Coronary Artery Bypass Grafting (CABG). Perioperative spasm of these conduits, especially LIMA, is associated with perioperative morbidity and mortality. Papaverine is the conventional vasodilator used intraoperatively to overcome the conduits spasm. Accumulating evidence linking papaverine to endothelial damage has prompted the search for alternative vasodilators. **Objectives:** This in vitro experimental study aimed to compare the effects of a combined solution of glyceryl trinitrate and verapamil (GV) and papaverine on isolated human LIMA and SV.

**Materials and Methods:** Isolated segments of LIMA and SV from 14 patients undergoing CABG were cut into 3 mm rings and suspended on wire hooks in organ bath chambers. The rings were stretched to their physiological resting tensions and were then contracted by Norepinephrine (NE)  $10^{-6}$  M. Cumulative concentrations of either GV or papaverine were applied to the contracted LIMA and SV rings (n = 14 for each) and relaxation responses were recorded. When maximal relaxations were achieved, the vasodilators were washed out of the chambers and NE was reintroduced to the chambers after 1 h to assess the residual relaxing effects of the vasodilators.

**Results:** GV was more potent compared to papaverine in fully (100%) relaxing both LIMA and SV (-log [half-maximal inhibitory concentration] (pIC<sub>50</sub>):  $6.54 \pm 0.10$  vs.  $4.58 \pm 0.05$  -log M and  $6.35 \pm 0.08$  vs.  $4.62 \pm 0.05$  -log M;  $P < 0.001$  for both). It also had a faster onset of effect ( $2.9 \pm 0.8$  vs.  $8.1 \pm 1.0$  min;  $P = 0.004$  and  $2.4 \pm 0.6$  vs.  $8.0 \pm 0.09$  min;  $P < 0.001$ ). NE-induced contractions after vasodilator treatment were significantly suppressed compared to the initial contractions in LIMAs treated with either GV or papaverine ( $0.64 \pm 0.05$  vs.  $0.31 \pm 0.04$  g and  $0.56 \pm 0.03$  vs.  $0.08 \pm 0.00$  g;  $P < 0.001$  for both), but only in SVs treated with papaverine ( $0.57 \pm 0.02$  vs.  $0.40 \pm 0.06$  g;  $P = 0.014$ ). Residual relaxations in the vessels treated with GV were not significantly different from those treated with papaverine after removing the drugs.

**Conclusions:** The GV solution represented a potent, rapid-acting, and safe alternative to papaverine for reversal of spasm of LIMA and SV conduits and for inhibition of postoperative spasm in LIMA.

#### ► Implication for health policy/practice/research/medical education:

Pharmacological treatment of perioperative spasm of coronary artery bypass conduits with vasodilators significantly improves both short- and long-term outcomes of coronary artery bypass graft surgeries. Papaverine has been the conventional vasodilator used in this regard in many cardiac surgery centers. However, recent evidence associating papaverine with vascular wall damage in bypass conduits has prompted the search for safe and effective alternative vasodilators. This study aimed to determine whether the combination of glyceryl trinitrate/verapamil could be an appropriate substitute for papaverine or not.

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## 1. Background

Patency of vascular conduits used in Coronary Artery Bypass Grafting (CABG) plays a central role in determining both short- and long-term survival rates of patients. Spasm of grafted vessels in the perioperative period of CABG surgeries can result in left ventricular failure, acute myocardial infarction, hemodynamic collapse, and even death (1-3).

Left Internal Mammary Artery (LIMA) is currently the vessel of choice for CABG due to its high long-term patency rates (4-7). Despite the increasing use of Radial Artery (RA) to supplement LIMA (8-10), Saphenous Vein (SV) continues to be frequently used for revascularization of multiple coronary arteries. Perioperative spasm of bypass conduits occurs in both LIMA and SV (11-14) although less commonly in SV due to its smaller bulk of medial smooth muscles and lesser sensitivity to circulating vasoconstrictors (15). To overcome vasospasm, a variety of vasodilators from different pharmacological categories, including calcium channel blockers, nitrates, potassium channel openers, and phosphodiesterase inhibitors, has been proposed (16-23). Despite the continuing controversy regarding the optimal vasodilator, papaverine has been commonly applied to bypass conduits during CABG in many cardiac surgery centers worldwide. However, growing evidence of vascular wall damage with papaverine use and its subsequent negative impacts on CABG outcomes has prompted the search for alternative vasodilators in the recent years (24, 25). Since its introduction by He et al. (26), the mixture of Glyceryl Trinitrate (GTN) and verapamil (GV) has received attention from investigators as a potential substitute for papaverine (27-29). Its lack of adverse effects on the graft endothelium and its activity against a wide range of spasmogenic factors are among the main features that made GV solution a promising vasodilator for preparation of vascular conduits (30). Nevertheless, clinical experience with this vasodilator mixture is still limited and thus far, it has not been generally accepted as the preferred antispasmodic agent in CABG surgeries.

## 2. Objectives

The present in vitro study aims to compare the vasodilative effects of GV solution and papaverine on LIMA and SV with respect to efficacy in reversing an established spasm, potency, time of onset of relaxation, and residual relaxation after removing the drugs. The results will help determine the better choice for clinical application.

## 3. Materials and Methods

### 3.1. Tissue Collection and Preparation

In this in vitro experimental study, discarded segments of LIMA and SV were collected from 14 consecutive CABG patients operated at Nemazee and Shahid Faghihi hospitals, Shiraz, Iran. All vessels were isolated from patients undergoing elective operations using both LIMA and SV. Emergency operations and/or surgeries using only one of the conduits were excluded from the study. It should be noted that using the discarded segments was approved by the Ethics Committee of Shiraz University of Medical Sciences. The isolated vessel segments were immediately

transferred, with an average transfer time of 10 min, to the pharmacology laboratory in a container of ice-cold physiological salt solution. Then, the surrounding tissues of LIMA and SV were stripped off. The vessels were cut into 3 mm-long ring segments. Each patient provided two rings of LIMA and SV that were suspended between two wire hooks in a four-chamber organ bath containing 20 mL of physiological salt solution of the following composition (values are mmol/L (mM)): KCl, 4.7;  $\text{KH}_2\text{PO}_4$ , 1.2;  $\text{CaCl}_2$ , 2.5; NaCl, 118;  $\text{NaHCO}_3$ , 25;  $\text{MgSO}_4$ , 1.2; D-glucose, 11; EDTA, 0.026; and ascorbic acid, 0.1, with a pH of 7.40. The chambers were supplied with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ , and the temperature was set at 37.0°C. The upper hook was connected to a force transducer (Hugo Sachs Elektronik, model K30, Germany), which measured the rings tensions. Tensions were plotted against time by a four-channel polygraph (Hugo Sachs Elektronik, model 705/1) using Windows® software HSEACAD® (Hugo Sachs Elektronik). The normalization process was done by passively stretching the rings to their physiological resting tensions of 2 g for LIMA and 1 g for SV. The rings were then allowed to stabilize for at least 60 min. The solutions within the chambers were renewed every 15 min during this period. Complete details of the organ bath protocol have been elaborated previously (26, 31).

### 3.1.1. Protocol

**3.1.1.1. Precontraction:** The stabilized passive tension in each ring segment was set as the baseline (zero line). In the preliminary study, the contractile responses of the ring segments to incremental doses of Norepinephrine (NE) were measured. The  $\text{EC}^{70-80}$  concentrations of NE (doses that produce 70 - 80% of the maximal contraction response) were calculated based on the obtained data, which was  $10^{-6}$  M for both LIMA and SV. This concentration was then used in the main study to induce submaximal active contractions in the ring segments to reproduce the effects of the spasmogenic stimuli in vivo. Optimal reactivity of the vascular rings to the vasodilators has been shown to occur at these submaximal contractions (29). Contracted ring segments were allowed to stabilize before addition of the vasodilators.

**3.1.1.2. Relaxation:** LIMA and SV rings isolated from each patient were allocated to the four chambers of the organ bath labeled as follows:

- Chamber 1: GV-treated LIMA,
- Chamber 2: Papaverine-treated LIMA,
- Chamber 3: GV-treated SV, and
- Chamber 4: Papaverine-treated SV (n = 14 for all).

Incremental concentrations (1 or 0.5 log M increments) of the vasodilators were then added to the chambers according to their assigned labels. In each chamber, the cumulative concentration of the vasodilator was progressively increased within the range of  $10^{-9}$  to  $10^{-4}$  M until reaching the concentration that completely relaxed the precontracted vessel. The decreased tension of the rings after addition of each vasodilator concentration was recorded as the response to that concentration. Ring segments were allowed to develop their full responses to each concentration before the next concentration was added.

### 3.1.1.3. Contraction of the Vasodilator-Treated Vessels:

After reaching the maximal responses to the last doses of vasodilators, the vasodilators were removed from the chambers by washing the vessels with the physiological salt solution and the rings were allowed to stabilize for another 60 min. During this period, the vessels were washed twice with 20-min intervals. After 60 min, the vessels tensions were again set as the zero line. Then, the same concentration of NE used initially was reintroduced into the chambers. Contraction forces generated by NE in the vasodilator-treated vessels 1 h after removal of the vasodilators were recorded.

### 3.2. Drugs and Solutions

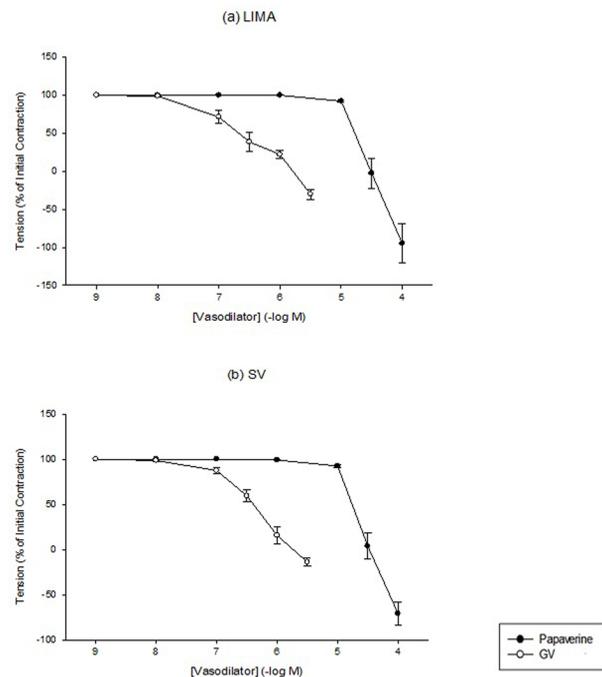
Ampoules of NE (4 mg/4 mL) and papaverine HCl (40 mg/2 mL) were both manufactured by Sterop Laboratories Inc. (Brussels, Belgium). GTN (10 mg/2 mL ampoule) was the product of Caspian Tamin Co. (Tehran, Iran) and verapamil (5 mg/2 mL ampoule) was manufactured by Hexal AG Inc. (Holzkirchen, Germany). GV solution was prepared in our laboratory using equal concentrations of GTN and verapamil (1:1 molar ratio). The vasodilators were serially diluted by distilled water to obtain the concentration range used in our experiment ( $10^{-4}$  to  $10^{-9}$  M).

### 3.3. Data and Statistical Analysis

The mean responses of 14 ring segments were used to plot the mean concentration-response curve for each of the four vasodilator/vessel combinations described above using SigPlot® software for Windows®, ver. 11.5. Concentrations were expressed as  $-\log$  M. The half-maximal inhibitory concentrations,  $IC_{50}$ , were calculated for both papaverine and GV by CurveExpert Professional® software, ver. 1.4.0 using the nonlinear regression (curve fit) method.  $pIC_{50}$ s ( $-\log IC_{50}$ ) were used to compare the potency of the two vasodilators. All statistical analyses were done using the SPSS® for Windows®, ver. 16.0. Independent samples t-test was used for comparison of the effects between the two vasodilators and the two vessels. In addition, paired samples t-test was employed to compare reactivity to NE before and after treatment with the vasodilators. Continuous variables were expressed as mean  $\pm$  Standard Error of Mean (SEM), while discrete ones were expressed as number.

## 4. Results

A total of 56 rings of LIMA and SV (28 rings each) were isolated from 14 CABG patients. The clinical characteristics of the patients have been summarized in Table 1. The mean concentration-response curves have been depicted in Figure 1. The vasodilative effects of GV and papaverine compared using mean values have been presented in Table 2.



**Figure 1.** Mean Concentration-Relaxation Curves for GV (Glyceryl Trinitrate/Verapamil Combination) and Papaverine in the Rings ( $n = 14$  for All) of LIMA (Left Internal Mammary Artery); (a) and SV (saphenous vein) (b) contracted by NE (norepinephrine,  $10^{-6}$  M). Symbols represent the mean tensions at each concentration expressed as the percentage of the initial contraction. Vasodilator concentrations are expressed as  $-\log$  M. Vertical bars show 1 standard error of mean tensions. Negative values indicate tensions below the baseline resting tension.

### 4.1. Comparison of the Effects on LIMA

Within the concentration range of  $10^{-9}$  to  $10^{-4}$  M, both vasodilators were able to completely (100%) reverse the initial contraction induced by NE. However, a smaller concentration of GV was required to reliably relax all the 14 ring segments in comparison to papaverine ( $3 \times 10^{-6}$  M vs.  $10^{-4}$  M). Comparison of  $pIC_{50}$ s proved GV to be significantly more potent than papaverine for full reversal of spasm in LIMA ( $P < 0.001$ ).

The maximum dose of papaverine,  $10^{-4}$  M, produced relaxations that were significantly greater in magnitude than those produced by the maximum dose of GV; i.e.,  $3 \times 10^{-6}$  M ( $P = 0.027$ ). The mean time to reach the maximal relaxations in LIMA segments treated with GV and those treated with papaverine was  $60 \pm 3$  and  $52 \pm 2$  min, respectively. Indeed, GV needed significantly less time compared to papaverine to achieve relaxations equal to 50% of the initial contraction ( $P = 0.004$ ). The residual relaxations measured 1 h after

**Table 1.** Clinical Characteristics of the Patients ( $n = 14$ )

| Age (y, mean $\pm$ SEM) |                   | 61 $\pm$ 3 |
|-------------------------|-------------------|------------|
| Sex (n)                 | Male              | 6          |
|                         | Female            | 8          |
| Risk factors (n)        | Hypertension      | 8          |
|                         | Hyperlipidemia    | 8          |
|                         | Diabetes mellitus | 2          |
|                         | Smoking           | 4          |

**Table 2.** Vasodilative Effects of GV and Papaverine on Contraction of Human LIMA and SV Rings by NE ( $10^{-6}$  M)

| Vessel                           | LIMA        |               |                     | SV                   |               |                     |                      |
|----------------------------------|-------------|---------------|---------------------|----------------------|---------------|---------------------|----------------------|
|                                  | Vasodilator | GV (n = 14)   | Papaverine (n = 14) | P value <sup>c</sup> | GV (n = 14)   | Papaverine (n = 14) | P value <sup>c</sup> |
| Potency (pIC <sub>50</sub> a)    |             | 6.54 ± 0.10   | 4.58 ± 0.05         | < 0.001              | 6.35 ± 0.08   | 4.62 ± 0.05         | < 0.001              |
| Maximum relaxation <sup>b</sup>  |             | 130.23 ± 6.69 | 183.76 ± 23.00      | 0.027                | 114.00 ± 4.57 | 170.95 ± 12.47      | 0.001                |
| Onset of relaxation <sup>c</sup> |             | 2.9 ± 0.8     | 8.1 ± 1.0           | 0.004                | 2.4 ± 0.6     | 8.0 ± 0.9           | < 0.001              |
| Residual relaxation <sup>d</sup> |             | 39.86 ± 2.89* | 41.51 ± 2.86*       | 0.521                | 0.04 ± 4.80   | 8.90 ± 5.84         | 0.192                |

<sup>a</sup> Expressed as  $-\log M$ .

<sup>b</sup> Values are the maximal decrease in contractile tensions, expressed as percent of the initial contractions in response to effective doses.

<sup>c</sup> Values represent time, expressed in minutes, to achieve relaxations equal to 50% of the initial contractions in response to the effective dose of each vasodilator.

<sup>d</sup> Values represent the magnitude of residual relaxation in vasodilator-treated vessels 1 h after removal of the drugs, expressed as percent of the maximal relaxations.

<sup>e</sup> P values were calculated by independent-samples t-test for all comparisons,  $P < 0.05$  was considered to be statistically significant. All variables are expressed as mean ± SEM.

\* denotes significant difference ( $P < 0.001$ ) between LIMA and SV.

washout of the drugs in the vessels treated with GV were not significantly different from those treated with papaverine ( $P = 0.521$ ).

#### 4.2. Comparison of the Effects on SV

All SV rings were fully (100%) relaxed by both GV and papaverine. The effective doses were the same as those observed for LIMA;  $3 \times 10^{-6}$  M for GV and  $10^{-4}$  M for papaverine. Moreover, GV was more potent in comparison to papaverine in relaxing SV segments ( $P < 0.001$ ). SV segments relaxed to a greater extent by papaverine than by GV ( $P = 0.001$ ). Maximal relaxations were produced  $42 \pm 2$  min and  $47 \pm 3$  min after application of the maximum doses of GV and papaverine, respectively. Thus, onset of relaxation was more rapid in the GV-treated SVs than in those treated with papaverine ( $P < 0.001$ ). The results indicated no significant differences between the two vasodilators regarding their residual effects ( $P = 0.192$ ).

#### 4.3. Comparison of LIMA and SV

The results revealed no significant differences regarding the effects of the vasodilators on LIMA and SV ( $P > 0.05$ ), except for residual relaxations that were significantly less prominent in SV than in LIMA regardless of the type of vasodilator ( $P < 0.001$  for both GV and papaverine).

#### 4.4. Suppression of Reactivity to NE

The results of comparison of contraction forces generated in response to NE before and after treatment with the vasodilators have been presented in Table 3. Accordingly, treatment with either GV or papaverine significantly suppressed the reactivity of LIMA to NE 1 h

after the vasodilators were washed out ( $P < 0.001$  for both vasodilators). In SV, papaverine decreased the contractile force produced by NE ( $P = 0.014$ ). However, treatment with GV had no significant inhibitory effects on reactivity to NE after 1 h ( $P = 0.464$ ).

## 5. Discussion

Based on the results of this in vitro study, both GV solution and papaverine were capable of completely reversing the catecholamine-mediated spasm. However, GV was significantly more potent and more rapid-acting in comparison to papaverine. Yet, the relaxing effects of GV declined at the same rate as those of papaverine during the first hour after removing the agents. Moreover, application of GV solution significantly suppressed the reactivity to NE in LIMA, but not in SV, for at least 1 h after its removal.

Adding verapamil, a slow but long-acting vasodilator (26, 31), to GTN, a rapid but short-acting vasodilator (26, 32, 33), would theoretically produce an ideal vasodilator that can rapidly relax bypass conduits and maintain the relaxation for a relatively long duration. Voltage-gated calcium channel blockers, such as verapamil, have limited efficacy against constrictors that act through mechanisms other than membrane depolarization and opening the voltage-gated calcium channels (17, 32, 34). In contrast, GTN, a nitric oxide donor, inhibits the contractile machinery of the vascular smooth muscles regardless of the type of the constrictor stimulus (15, 32, 35). Therefore, a combination of these two agents should be active against a wide range of vasoconstrictors, which is of paramount value considering the multifactorial etiology of vasospasm in vivo (15, 35).

Papaverine, administered either topically or intraluminally,

**Table 3.** Reactivity of Human LIMA and SV Rings to NE ( $10^{-6}$  M) before Vasodilator Addition and 1 h after Removing the Maximally Affected Vasodilators

| Vessel | Vasodilator          | Reactivity to NE <sup>a</sup>         |                               |                      |
|--------|----------------------|---------------------------------------|-------------------------------|----------------------|
|        |                      | Before vasodilator addition (Initial) | 1 h after vasodilator removal | P value <sup>b</sup> |
| LIMA   | GV (n = 14)          | 0.64 ± 0.05                           | 0.31 ± 0.04                   | < 0.001              |
|        | Papaverine, (n = 14) | 0.56 ± 0.03                           | 0.08 ± 0.00                   | < 0.001              |
| SV     | GV (n = 14)          | 1.06 ± 0.18                           | 1.00 ± 0.17                   | 0.464                |
|        | Papaverine (n = 14)  | 0.57 ± 0.02                           | 0.40 ± 0.06                   | 0.014                |

<sup>a</sup> Reactivity is defined as contractile force, expressed in grams, generated in response to NE.

<sup>b</sup> P-values were derived from paired samples t-tests;  $P < 0.05$  was considered to be statistically significant. Values are expressed as mean ± SEM.

has been associated with induction of apoptosis in both endothelial and vascular smooth muscle cells (24). It decreases the endothelium-dependent relaxation of vessels in response to cholinergic stimuli and impairs the production of prostacyclin, an endothelium-derived vasodilator and antithrombotic agent (18, 30, 34). These functional and morphological derangements, superimposed on the proinflammatory milieu associated with CABG (36), can predispose conduits to vasospasm and increase the risk of thrombosis in grafted vessels. On the contrary, application of GV has not been reported to adversely affect the function and structure of the endothelium and conduits' long-term patency (30, 34, 37).

Activation of the  $\alpha^1$ -adrenoceptors by catecholamines induced strong vasoconstriction in bypass conduits, especially arterial ones (32). Plasma catecholamine concentrations increased significantly during CABG operations, peaked at the end of the Cardiopulmonary Bypass (CPB), and remained elevated during the early postoperative hours (38, 39). Administration of catecholamines as vasopressor agents for postoperative hemodynamic support in CABG patients could further increase their plasma levels. Thus, catecholamines, such as NE, play a significant role in the pathogenesis of both intraoperative and postoperative spasm of the CABG conduits. In the present study, NE was used as a clinically relevant constrictor to reproduce the perioperative vasospasm *in vitro* to assess the efficacy of GV against contractions induced by a non-depolarizing mechanism.

Spasm of bypass conduits can occur at any time from dissection of the conduits to hours or days postoperatively (1, 2, 13, 17). Perioperative spasm of bypass conduits can lead to early graft failure and serious morbidity or even mortality. Yet, established spasm of bypass conduits during the intraoperative and immediate postoperative periods can be reversed effectively by direct application of vasodilator agents to the affected vessels via topical, perivascular, or intraluminal routes. Also, when vasospasm is detected later, during angiographic assessment of a suspected early graft failure, intraluminal injection of potent vasodilators through coronary catheters can promptly relieve the spasm (1, 2). We are not aware of any previous study that directly determined and compared the potency of GV solution in relieving the spasm of CABG conduits. An *in vitro* study reported verapamil to be more potent compared to papaverine in relaxing LIMA contracted by NE (40). Another study proved GTN to be the most potent among a group of vasodilators, including papaverine, for inhibition of NE-induced contraction in LIMA (41). GTN and verapamil, each used alone, have also been shown to be more potent than papaverine in relieving the spasm of SV induced by high concentrations of  $K^+$  (26). In keeping with these previous studies, the current study findings demonstrated that GV solution was nearly 200 times more potent than papaverine for complete reversal of the NE-induced contraction. It should be noted that the effective dose of GV in our study was more than 60 times smaller than that of papaverine. Although the results indicated that the maximal responses to this relatively small dose of GV ( $3 \times 10^{-6}$  M) was significantly smaller than those to the effective dose of

papaverine ( $10^{-4}$  M), it has been reported that application of larger doses of GV ( $3 \times 10^{-5}$  M) produced relaxations similar to those produced by papaverine (28, 42, 43). Furthermore, maximal relaxations in our study were reached after the conduits had been incubated with the vasodilators for at least 40 min. However, such long incubation times are not always practical when trying to treat the perioperative spasm of CABG conduits. Therefore, the maximal relaxations observed *in vitro* may not be achievable *in vivo*. Thus, as far as the full reversal of spasm of bypass conduits is concerned, GV and papaverine can be considered equally effective *in vivo*. When spasm occurs before reversal of cardioplegia and weaning patients from CPB, complications of prolonged CPB, such as acute kidney injury and neurological deficits, can be avoided by using more rapid acting vasodilators (44, 45). Rapid reversal of spasm after termination of CPB allows prompt restoration of the coronary blood supply and, consequently, prevents irreversible myocardial injury and improves the CABG outcome.

GV concentrations  $\geq 10^{-5}$  M were shown to rapidly reverse the spasm induced by thromboxane  $A_2$  analogue or high concentrations of  $K^+$  in both LIMA and SV (26, 42, 46). Our study findings demonstrated the same rapid relaxation by a lower concentration of GV ( $3 \times 10^{-6}$  M) in the vessels contracted by NE. Indeed, GV was proven to be almost 3 times faster than papaverine in relieving the spasm of human LIMA and SV.

Generally, disastrous consequences of delayed detection and reversal of an existing spasm in CABG conduits can be best avoided by using vasodilators to prevent the vasospasm from occurring in the first place. Graft preparation protocols commonly include direct intraoperative application of vasodilators to the bypass conduits, particularly arterial ones (47). Inhibition of intraoperative spasm can be rapidly and efficiently achieved by locally applied vasodilators while avoiding the side-effects and drug interactions associated with their systemic use. However, the efficacy of this technique to suppress vasospasm during the postoperative period when there is no direct access to the grafts may be limited. This relies on the residual effects of the intraoperatively administered vasodilators, underscoring the need to use longer-acting agents. According to previous studies, continuous application of either GV or papaverine to conduits could inhibit intraoperative spasm for up to 2 h (26, 42). By washing the vasodilators out of the chambers and reintroducing the constrictor stimulus to the vessels 1 h later, we provided an *in vitro* model of the immediate postoperative period. Calculating the residual effects as a proportion of the maximal effects enabled us to compare the rate at which the inhibitory effects of the two vasodilators against NE waned during the immediate postoperative period. The results indicated significant suppression of reactivity to NE in LIMA 1 h after treatment with the either vasodilator. Although the inhibitory effects were not evaluated at later time points after the removal of the vasodilators in our research, similar studies on isolated human RA and mouse aorta demonstrated that the inhibitory effect of GV on the  $\alpha^1$ -adrenoceptor-mediated spasm was limited to the first 1-2 h after its removal. It has also been reported to last even shorter in grafted vessels under *in*

vivo conditions (29, 48). Previous reports on the duration of action of papaverine on RA vary depending on the applied dose of papaverine (18, 29, 49). However, in none of the studies, the relaxing effects of papaverine lasted for more than 4 h. Thus, to reliably suppress the spasm of arterial conduits beyond the immediate postoperative period, systemic vasodilators may be needed when there are risk factors for spasm, such as using distal portion of LIMA (50), vessel trauma during surgery, perioperative occurrence of spasm, and administration of systemic vasopressors.

In the current study, a significantly faster decline of relaxation was observed in SV than in LIMA rings during the first hour after withdrawal of the vasodilators. Diffusion of the vasodilators through the thick wall of the isolated arteries in the organ bath can take considerable time (15). Therefore, faster diffusion of vasodilators out of the thin wall of the SV can account for the more rapid decline of the vasodilative effects compared to that in the thick-walled LIMA. The insignificant residual relaxations in GV-treated SVs can be additionally explained by the low potency of verapamil on human veins contracted by catecholamines (51). In fact, the residual effects of GV combination are mostly attributable to its long-acting verapamil component rather than the short-acting GTN component (26, 33, 52). The maximal relaxations in papaverine-treated SV segments just before removing the vasodilators were large enough to cause the reactivity to NE to be still suppressed after 1 h. However, in view of the smaller in vivo maximal relaxations, the limited residual effects of papaverine (less than 9%) may not be sufficient to prevent the postoperative spasm of SV graft. Considering the low incidence of SV spasm in this period (15), prophylactic use of systemic vasodilators to prevent spasm in grafted SVs may not be warranted.

A major limitation of our study is that preoperative use of GTN for angina relief in CABG patients may have induced tolerance to this agent (15, 35). By allowing the vessels to stabilize for more than 1 h before adding the vasodilators and frequently washing them (every 15 min), we tried to minimize the effects of any previously administered vasodilator. Nevertheless, tolerance to GTN may have persisted in some of the vessels and contributed to the lower maximal relaxations by GV compared to those by papaverine.

In conclusion, our results suggested that perioperative spasm of the LIMA and SV could be completely and rapidly relieved by direct application of the potent GV solution. Furthermore, intraoperative treatment of the conduits with GV might obviate the need to administer systemic vasodilators during the immediate postoperative period to prevent vasospasm in arterial conduits, such as LIMA that has a greater propensity than SV to develop postoperative spasm induced by circulating vasoconstrictors. Therefore, the GV solution can be presented as an effective and safe alternative to papaverine for preparation of both arterial and venous coronary conduits.

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#### Authors' Contribution

1- Study concept and design: Reza Rafieossadat, Mojtaba Haddad, Hossein Mirkhani, 2- Acquisition of data: Reza Rafieossadat, Mojtaba Haddad, Masih Shafa, 3- Analysis and interpretation of data: Reza Rafieossadat, Mojtaba Haddad, Hossein Mirkhani, 4- Drafting of the manuscript: Reza Rafieossadat, Mojtaba Haddad, 5- Critical revision of the manuscript for important intellectual content: Reza Rafieossadat, Mojtaba Haddad, Masih Shafa, Hossein Mirkhani, 6- Statistical analysis: Reza Rafieossadat, Mojtaba Haddad, 7- Administrative, technical, and material support: Dean of School of Medicine, Shiraz University of Medical Sciences, Reza Rafieossadat, Mojtaba Haddad, Masih Shafa, Hossein Mirkhani 8- Study supervision: Masih Shafa, Hossein Mirkhani.

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