Original Article

The in vitro Effects of Iloprost with Other Vasodilators on the Human Internal Thoracic Artery

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Purpose: We aimed (a) to analyze the effects of iloprost as a vasodilator on the human internal thoracic artery (ITA) and (b) to compare these effects with the effects of other vasodilators now being used in the clinic.

Methods: Following transfer into only Krebs solution or into Krebs solution containing papaverine or iloprost, human ITA strips were then incubated only in Krebs or in Krebs with vasodilators that are generally used in clinical practice, such as diltiazem or glyceryl trinitrate. Cumulative concentration-contraction curves for noradrenaline (NA) and KCl were then established for these strips. Student's t-test and one-way analysis of variance followed by Tukey-Kramer tests were used to compare differences between groups. A $p < 0.05$ was used to indicate significance.

Results: Among the transfer solutions, papaverine (6.50 ± 0.20) and iloprost (7.33 ± 0.13) were significantly more potent than Krebs (8.46 ± 0.75, $p < 0.001$ and $p < 0.05$) with regard to preventive effect on precontracted ITA with NA. Iloprost significantly relaxed the NA-induced precontracted ITA strips in the Krebs, papaverine, and iloprost groups. Diltiazem significantly relaxed the precontracted ITA with KCl in all storage groups.

Conclusion: Iloprost may also prevent perioperative ITA spasm, but should be tested in the clinical setting. (Ann Thorac Cardiovasc Surg 2010; 16: 78–84)

Key words: internal thoracic artery, vasospasm, iloprost

Introduction

The internal thoracic artery (ITA) is routinely used for myocardial revascularization; however, perioperative vasospasm of this artery may become a serious problem.[1)] ITA can be affected by the method of harvesting, involving potential arterial wall damage from clamping and vasoreactivity to circulating vasoconstrictors during coronary bypass operations.[2,3)] Therefore during graft bypass surgeries, vasodilatory agents such as papaverine, nitroglycerin, or calcium channel blockers are topically and/or systemically used to prevent spasm of the arterial graft.[4,5)]

Iloprost is a stable analog of prostacyclin (PGI_2) and mimics the pharmacodynamic properties of this compound, which include inhibition of aggregation, vasodilatation, and cytoprotection.[6)] The major biological activities of prostacyclin and its stable analog iloprost are mediated by specific cell surface prostanoid (IP) receptors (for iloprost especially at the EP_1 receptor subtype), which are known to be coupled to adenylate cyclase via the guanine nucleotide regulatory protein G_s.[7)] Moreover, iloprost induces relax-

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ation, in part through the activation of K^+ channels (Ca^{2+}-activated K^+, channel-BK_{Ca}–, and ATP-sensitive K^+ channel-K_{ATP}–).[8,9]

The aim of the present study was to analyze and compare (a) the effects of iloprost and papaverine as initial vasodilators on the ITA, and (b) the effects of other vasodilators such as diltiazem and glyceryl trinitrate (GTN) that are used for maintenance therapy following the administration of initial vasodilators.

Materials and Methods

General

Human ITAs from 34 patients aged 61 ± 1 years undergoing coronary artery bypass grafting (CABG) surgery were studied regardless of preoperative drug therapy (Table 1). Approval to use discarded ITA tissue was given by the Ethics Commitee of the Istanbul University Hospital (Cerrahpaşa Medical Faculty, Date: 01.07.2003, Prot. No: 16387), and informed consent forms in accordance with the Helsinki principles were obtained from all patients who participated in the study.

Organ bath experiments

The discarded ITAs were immediately placed into containers with only Krebs solution or with initial vasodilator solutions containing papaverine (1 mg/mL 0.9% saline; pH: 5.75) or iloprost (1 μg/mL 0.9% saline). The ITAs were then maintained at 4°C and transferred to the laboratory. The time from harvesting to experimentation was 30 minutes. After this initial 30-minute period, the vessels were placed in a glass dish and dissected free from surrounding adipose and connective tissues. Each vessel was opened longitudinally and divided into rings, each 3 mm in length, which were then fixed on wires in organ baths (volume 25 mL). The Krebs solution had the following composition (in mmol/L): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4.7H_2O$ (1.2), KH_2PO_4 (1.2), $NAHCO_3$ (25), and glucose (11.1). The solution was bubbled with 95% O_2 and 5% CO_2 at 37°C. For tension testing, the strips were connected to a force transducer (MAY, FDT 10A), and changes in isometric tension were recorded (COMMAT, MAY, FDA 97 polypraph). After resting tension (2 g), the strips were allowed to stabilize for 45 minutes. The strip preparations were then precontracted with KCl (40 mM), washed several times with fresh buffer, and then allowed to equilibrate for an additional 45 minutes. Each strip of artery was exposed to a single constrictor agent.

Table 1. Patient characteristics

	ITA
Age (years) (n = 34)	61 ± 1
Sex Male:	25 (74%)
Female:	9 (26%)
Diabetes mellitus	8 (23%)
Hypertension	18 (53%)
Hyperlipidemia	11 (32%)
Statin	10
β-blocker	4
Ca antagonists	3
ACE inhibitors	1
Diuretics	7
Aspirin	3
Antidepressants	2
Antidiabetics	6
Insulin	2
α-blockers	1

ITA, internal thoracic artery; ACE, angiotensin coverting enzyme.

The role of initial vasodilators on the noradrenaline (NA) and KCl-induced contractions

The ITA strips preincubated in Krebs, papaverine, or iloprost solutions were all subsequently incubated in Krebs solution for 30 minutes. Strips preincubated in Krebs solution and incubated in Krebs solution with no vasodilator were used as the control group (NC). Contraction of the strips was then induced using NA (10^{-9}–10^{-3} M) and KCl (20–80 mM) to study the possible role of these initial vasodilators (papaverine and iloprost) on the NA- and KCl-induced contractions in the ITA strips. Cumulative concentration-contraction curves for NA and KCl were established in these strips (Fig. 1). The effects of papaverine (P-K) and iloprost (I-K) on ITA spasm may reflect their prophylactic effects.

The effects of maintenance vasodilators on the NA- and KCl-induced contractions

To show whether vasodilators (diltiazem and GTN) could affect the contraction response to the vasoconstrictors (NA: 10^{-9}–10^{-3} M and KCl: 20–80 mM), the preincubated strips were further incubated for another 30 minutes with these maintenance vasodilators. Cumulative concentration-contraction curves were similarly established in these strips. The dose response curves to NA and KCl were also repeated in the presence of diltiazem (1 μmol/L) or GTN (0.1 μmol/L) (Fig. 1). All combinations of conditions were then compared to the control group (NC) and to each other. The effects of the combinations of papaverine (P-D,

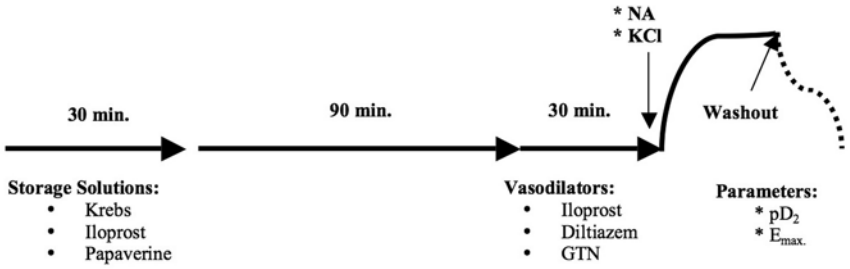

Fig. 1. The protocol of the present study.

Table 2. Maximal contraction and pD_2 values of noradrenaline and KCl in human internal thoracic artery strips with incubation of storage solutions

Groups	Noradrenaline		KCl	
	pD_2	$E_{max.}$ (mg tension/mg ww)	pD_2	$E_{max.}$ (mg tension/mg ww)
Krebs (K)	n = 8 8.33 ± 0.37	n = 8 197 ± 36	n = 6 2.00 ± 0.20	n = 5 192 ± 25
Iloprost (I)	n = 8 7.33 ± 0.13[1]	n = 12 222 ± 26	n = 14 1.74 ± 0.13	n = 9 165 ± 26
Papaverine (P)	n = 6 6.50 ± 0.20[2]	n = 6 210 ± 76	n = 5 1.79 ± 0.11	n = 5 199 ± 69

[1], I vs. K, $p < 0.05$; [2], P vs. K, $p < 0.001$.

P-GTN) or iloprost (I-D, I-GTT) with diltiazem (D) or GTN on ITA spasms may reflect their therapeutic effects.

Drugs

The drugs used in this study and their sources were iloprost (Ilomedin, Schering Alman Ilac ve Ecza Tic. Ltd. Sti.; Istanbul, Turkey); papaverine (Galen Ilac San. ve Tic. A.S.; Istanbul, Turkey); diltiazem (Mustafa Nevzat Ilac San. A.S.; Istanbul, Turkey); and GTN (Melusin Ilac ve Saglik Mlz. Paz. ve Tic. Ltd. Sti.; Istanbul, Turkey).

Statistical analysis

The reactivity of ITA was expressed as maximal contraction and sensitivity. The sensitivity of ITA to NA and KCl was expressed as pD_2 and EC_{50}, the effective concentration causing 50% of the maximal contraction. pD_2 or EC_{50} was determined from each concentration-contraction curve by a computerized program (PWS 98, Polwin 98 software). The maximal contraction was then normalized by the mg wet weight strip: mg tension/mg wet weight.

Results were expressed as the mean ± standard error of mean (SEM). The Student's t-test and one- way analyses of variance (ANOVAs) followed by Tukey-Kramer tests were used as appropriate to compare differences between the groups. A p value of less than 0.05 was considered statistically significant.

Results

The effects of initial vasodilators on the NA- and KCl-induced contractions

After exposure to the initial vasodilators, the ITAs exhibited concentration-dependent increases in tension to both NA (10^{-9} to 10^{-3}) and KCl (20–80 mM).

There was a significant difference in sensitivity to NA between strips incubated in papaverine solution (6.50 ± 0.20) and those in Krebs solution only (8.33 ± 0.37, $p < 0.001$). There was also a significant difference in sensitivity to NA between strips incubated in iloprost solution (7.33 ± 0.13) and those in Krebs solution (8.33 ± 0.37, $p < 0.05$). However, there was no significant difference between strips incubated in papaverine solution and those in iloprost solution. These data are shown in Table 2.

There were also no significant differences in maximal

Table 3. The pD_2 values of noradrenaline in human internal thoracic artery strips with incubation of both storage solutions and vasodilators

Storage solutions	Vasodilators		
	Krebs	Diltiazem	GTN
Krebs (K)	NC n =8 8.33 ± 0.37	K-D n = 5 6.38 ± 0.26	K-GTN n = 7 6.61 ± 0.42
Iloprost (I)	I-K n = 8 7.33 ± 0.13[1]	I-D n = 7 6.74 ± 0.35	I-GTN n = 8 7.29 ± 0.24
Papaverine (P)	P-K n = 6 6.50 ± 0.20[2]	P-D n = 6 6.00 ± 0.39[3]	P-GTN n = 6 6.01 ± 0.46[4]

GTN, glyceryl trinitrate.
[1], NC vs. I-K, $p < 0.05$; [2], NC vs. P-K, $p < 0.001$; [3], NC vs. P-D, $p < 0.05$; [4], NC vs. P-G, $p < 0.05$.

Table 4. Maximal contraction values ($E_{max.}$) of noradrenaline in human internal thoracic artery strips with incubation of the storage solutions and vasodilators

Storage solutions	Vazodilators (mg tension/mg ww)		
	Krebs	Diltiazem	GTN
Krebs (K)	NC n = 8 197 ± 36	K-D n = 7 112 ± 40	K-GTN n = 7 187 ± 61
Iloprost (I)	I-K n = 12 222 ± 26	I-D n = 15 232 ± 28	I-GTN n = 8 249 ± 63
Papaverine (P)	P-K n = 6 210 ± 76	P-D n = 6 190 ± 71	P-GTN n = 6 298 ± 66

GTN, glyceryl trinitrate.

contraction force to either NA or KCl or in sensitivity to KCl among strips preincubated in Krebs, iloprost, or papaverine solutions. All groups were incubated in Krebs. These data are also in Table 2.

The order of vasodilatory potency (pD_2) for the three initial vasodilatory solutions is for NA, papaverine ≥ iloprost > Krebs, and for KCl, no significant differences.

Effect of adding maintenance vasodilators to initial vasodilatory solutions on the NA- and KCl-induced contractions

Strips taken from different initial solution groups were incubated for an additional 30 minutes with Krebs (NC), diltiazem (1 μmol/L), or GTN (0.1 μmol/L). Concentration-contraction curves in response to NA (10^{-9}–10^{-3} M) were established.

There were significant differences in sensitivity (pD_2) to NA among strips incubated in Krebs solution only in the NC group (8.33 ± 0.37). They include those preincubated in papaverine solution and then incubated in diltiazem solution (P-D group) (6.00 ± 0.39, $p < 0.05$), and those preincubated in papaverine solution, then incubated in GTN solution (P-GTN group) (6.01 ± 0.46, $p < 0.05$). The data are shown in Table 3.

There were no significant differences in the maximal contraction force to NA among the preincubated strips incubated in Krebs, diltiazem, or GTN maintenance solutions. The data are shown in Table 4.

The order of vasodilatory potency (pD_2) of the initial then the maintenance solution combinations is P-D = P-GTN = K-GTN.

KCl-induced contractions

Strips taken from the initial preincubated solution group were incubated for an additional 30 minutes with Krebs (NC), diltiazem (1 μmol/L), or GTN (0.1 μmol/L). Concentration-contraction curves in response to KCl (20–80 mM) were established.

There were significant differences in sensitivity (pD_2) to KCl among strips stored in Krebs solution, then incubated in the Krebs solution NC group (2.00 ± 0.20); those stored in Krebs solution, then incubated in the diltiazem solution (K-D group) (1.04 ± 0.17, $p < 0.001$); those stored in iloprost solution, then incubated in the diltiazem solu-

Table 5. The pD_2 values of KCl in human internal thoracic artery strips with incubation of the storage solutions and vasodilators

Storage solutions	Vasodilators		
	Krebs	Diltiazem	GTN
Krebs (K)	NC n = 6 2.00 ± 0.20	K-D n = 5 1.04 ± 0.17[1]	K-GTN n = 6 1.76 ± 0.13
Iloprost (I)	I-K n = 14 1.74 ± 0.13	I-D n = 8 1.06 ± 0.09[2]	I-GTN n = 7 1.54 ± 0.04
Papaverine (P)	P-K n = 5 1.79 ± 0.11	P-D n = 5 1.28 ± 0.14[3]	P-GTN n = 5 1.65 ± 0.12

GTN, glyceryl trinitrate.
[1], NC vs. K-D, p <0.001; [2], NC vs. I-D, p <0.001; [3], NC vs. P-D, p <0.05.

Table 6. Maximal contraction values ($E_{max.}$) of KCl in human internal thoracic artery strips with incubation of the storage solutions and vasodilators

Storage solutions	Vasodilators (mg tension/mg ww)		
	Krebs	Diltiazem	GTN
Krebs (K)	NC n = 5 192 ± 25	K-D n = 6 60 ± 12[1]	K-GTN n = 6 246 ± 56
Iloprost (I)	I-K n = 9 165 ± 26	I-D n = 6 27 ± 4[2]	I-GTN n = 9 214 ± 48
Papaverine (P)	P-K n = 5 199 ± 69	P-D n = 6 28 ± 8[3]	P-GTN n = 5 284 ± 82

GTN, glyceryl trinitrate.
[1], NC vs. K-D, p <0.001; [2], NC vs. I-D, p <0.0001; [3], NC vs. P-D, p <0.0001.

tion (I-D group) (1.06 ± 0.09, p <0.001); and those stored in papaverine solution, then incubated in the diltiazem solution (P-D group) (1.28 ± 0.14, p <0.05). The data are shown in Table 5.

There were also significant differences in the maximal contraction force ($E_{max.}$) in response to KCl among strips stored in Krebs solution, then incubated in the Krebs solution (NC, normal control group) (192 ± 25); those stored in Krebs solution, then incubated in the diltiazem solution (K-D group) (60 ± 12, p <0.001); those stored in iloprost solution, then incubated in the diltiazem solution (I-D group) (27 ± 4, p <0.0001); and those stored in papaverine solution, then incubated in the diltiazem solution (P-D group) (28 ± 8, p <0.0001). The data are shown in Table 6. The rank of vasodilatory potency of the stored solution-systemic solution combinations is, for pD_2, I-D = K-D > P-D, and for $E_{max.}$, I-D = P-D >> K-D.

Discussion

ITA spasm during CABG operation is a well-recognized phenomenon.[1)] Therefore during CABG surgery, different pharmacological agents are topically and/or systematically used either to prevent or to treat ITA spasm, though the best vasodilator agent to use is not known.[6,10)] This is the first report to compare the effects of iloprost and other vasodilators when given as initial vasodilators topically or intraluminally to the ITA, followed by a maintenance vasodilator.

We have used two approaches in this study to investigate the vasoreactivity of ITA in vitro. The investigation of vasoreactivities was performed both in the presence of initial vasodilatory solutions and initial vasodilatory solution plus a subsequent dose of maintenance vasodilators.

Our first results concern the vasoreactivity of ITA in vasodilatory solutions. Among these solutions used in the present study, papaverine (P) and iloprost (I) are significantly more potent than Krebs alone (K-control) with regard to the prophylactic effect in precontracted-ITA exposed to NA. Papaverine delivery to the ITA after dissection treated the spasm effectively and improved blood flow.[11)] Jett et al. also demonstrated that among various

vasodilators (nifedipine, nitroprusside, verapamil, papaverine), papaverine produced the greatest maximal inhibition of both potassium and NA-induced contraction of human ITA.[12] However, some unwanted effects have been associated with these drugs. For instance, although papaverine is a potent vasodilator, exposure to this compound may compromise long-term viability of graft endothelial cells.[13] Papaverine may impair endothelial function and trigger apoptosis of both endothelial and smooth muscle cells of the human internal thoracic arteries.[14] Although it has been recommended that papaverine be applied to the perivascular space into the pedicle of ITA for the highest increase in blood flow, the most effective method of papaverine application is still contraversial.[15] Furthermore, a room temperature solution of papaverine for injection was used in this study. It was demonstrated that normothermic papaverine is superior to room temperature solution when applied topically.[16] The reason for this has been suggested to be because the relative hypothermia of an ambient solution may be vasospastic or may cause it to be a less potent dilator.[17] Our study found no significant differences between papaverine and iloprost. It has also been previously demonstrated that iloprost may inhibit the contractile effects of NA and KCl by decreasing $[Ca^{2+}]_i$ and Ca^{2+} sensitivity of contractile elements through a cyclic adenosine monophosphate (cAMP)-dependent mechanism.[7,18] The hyperpolarization in response to iloprost may play an additional role, and this is blocked by glibenclamide, implicating roles for the ATP-sensitive potassium channel and calcium-activated potassium channels.[8,9,19] Thus iloprost may also be used alone or in combination as a storage solution during harvesting. None of the storage solutions used in the present study affected the vasoreactivity (sensitivity and maximal contraction) of the precontracted ITA with KCl.

The range of vasodilator concentrations mentioned above and therefore our data are consistent with previous studies. Diltiazem as a maintenance vasodilator significantly relaxed the NA-induced precontracted ITA stored papaverine solution. GTN as a vasodilator also significantly relaxed the NA-induced precontracted ITA stored in papaverine and Krebs solutions. None of the vasodilators affected the vasoreactivity (maximal contraction) of the NA-induced precontracted ITA in any of the storage conditions. However, diltiazem did reduce the maximal contraction of ITA to KCl in all storage conditions, and GTN reduced the maximal contraction of ITA to KCl in the verapamil-stored group. He et al. also previously showed that in potassium chloride-precontracted ITA, GTN and diltiazem caused full relaxation.[5] However, it has been reported that diltiazem may play a role greater than that of nitrites in the prevention of vasospasm. Moreover, the occurrence of rapid tolerance to nitrovasodilators can abolish its therapeutic effects.[20] Diltiazem is a calcium channel antagonist and binds to the L-type calcium channel. Blockage of the L-type calcium channel in vascular tissues results in relaxation of vascular smooth muscle. However, blockage of it in the heart results in negative inotropic and chronotropic effects, which is a distinct disadvantage after CABG.[21]

Although there were several limitations, such as the small size of the study group, and thus lower statistical power, we were able to conclude that iloprost may also prevent perioperative ITA spasm, though the finding needs to be confirmed in the clinical setting. Further, a continual dose of iloprost could be more effective during and after operation in the prevention of ITA spasm.

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