

Original Article

Iloprost relaxes phenylephrine-precontracted rat aortic rings

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Abstract: Iloprost is a prostacyclin analogue mainly used in the treatment of pulmonary hypertension. It is also effective for renal dysfunction during renal transplant and coronary artery bypass grafting surgery, but there have been few comparative experimental studies with iloprost. We aimed to compare vasorelaxant properties of iloprost, with those of diltiazem, nitroglycerin and papaverine on rat thoracic aorta following phenylephrine-induced precontraction. Thirty-two young adult female Wistar albino rats were used in the study. After isolation of thoracic aorta distal to arcus, 3 mm aortic rings were placed in an organ bath and phenylephrine (10^{-6} M) was added for precontraction. Subsequently, cumulative concentrations of iloprost, nitroglycerin, diltiazem and papaverine were added to aortic rings on separate chambers and dose-response curves were recorded. The mean (\pm SD) maximal relaxation (E_{max}) was $27.1 \pm 2.9\%$ for iloprost, $111.7 \pm 2.1\%$ for nitroglycerin, $77.4 \pm 2.9\%$ for diltiazem and $147.2 \pm 2.8\%$ for papaverine. The half maximal effective concentration (EC_{50}) values were 2.4×10^{-11} molar (M) for iloprost, 5.6×10^{-8} M for nitroglycerin, 7.0×10^{-7} M for papaverine and 2.1×10^{-5} M for diltiazem. There was a statistically significant difference in E_{max} values and EC_{50} values between four groups ($P < 0.001$ and $P < 0.01$, respectively). Although a vasorelaxant response was observed with iloprost, it was less compared to papaverine, nitroglycerin and diltiazem on the isolated rat aortic rings. Iloprost has also the lowest EC_{50} value which could be valuable in clinical practice.

Keywords: Iloprost, nitroglycerin, diltiazem, papaverine, rat aorta, vasospasm, coronary artery bypass grafting

Introduction

Iloprost is a stable synthetic prostacyclin analogue (PGI_2) mainly used in the treatment of pulmonary hypertension, vasospastic diseases and has been shown to be effective in preventing renal dysfunction in patients undergoing renal transplant and coronary artery bypass grafting (CABG) [1, 2]. Iloprost is hypothesized to increase cAMP production and flux of potassium into cells with a decrease in calcium influx [2]. Iloprost is a vasodilator of arterial system including pulmonary, renal and peripheral arteries. It inhibits vasospasm caused by prostaglandin $F_{2\alpha}$, phenylephrine, angiotensin I, II, thromboxane A_2 and its analogue U46619 [3, 4].

Several topical vasorelaxant agents such as papaverine, nitroglycerin and calcium channel antagonists (especially verapamil, nifedipine,

and diltiazem) are widely used in order to avoid graft spasm during CABG surgery [5, 6]. Severe vascular spasm is reported to occur between 0.8% and 1.3% of CABG procedures [7, 8]. Vasospasm can result in graft failure and subsequently myocardial hypoperfusion. Therefore it is crucial to manage vasospasm during the surgery.

In vitro studies were usually conducted with human internal mammary artery or radial artery rings obtained during CABG. Although the vasorelaxant properties of diltiazem, nitroglycerin and papaverine are well known [9], clinical data showing beneficial effects of the topical and systemic use of iloprost on graft vasospasm during CABG were limited [10-13].

Rat aorta assay provides a low cost and rapid platform. The *in vitro* section focused on the vasotonic signaling pathways in the vessel [14].

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This experimental model on isolated rat aorta was undertaken to investigate the vasorelaxant properties of iloprost and compare with those of diltiazem, nitroglycerin and papaverine following phenylephrine-induced precontraction.

Material and methods

Animals

After the approval of the institutional ethics committee (prot. no: 12411303331052-50), the experimental protocol was performed according to the European guidelines for the handling of laboratory animals. Young adult female Wistar albino rats ($n = 32$) weighing 240-260 g were used in the study. The rats were provided food and water *ad libitum*. No medical treatment or anesthetic agent was given to the rats before sacrifice.

Tissue preparation

Rats were stunned before the experimental procedure, and then sacrificed by cervical dislocation method. The thoracic aorta sections distal to arcus were isolated immediately and placed into Krebs-Henseleit solution with a composition of sodium chloride 119 mM, potassium chloride 4.7 mM, calcium chloride 2.5 mM, magnesium sulfate 1.5 mM, sodium bicarbonate 25 mM, potassium dihydrogen phosphate 1.2 mM and glucose 11 mM in a petri dish. The adhering connective tissue around the vessel segment was removed and arterial part was cut into 3 mm wide rings.

The arterial rings were carefully suspended by two stainless steel hooks passed through the aortic lumen to determine the alterations in the tension of the ring. One of the steel hooks was fixed to the base of a 20 ml *in vitro* chamber containing Krebs-Henseleit solution and the other hook was attached to the superiorly located tension transducer. The arterial rings were washed with Krebs-Henseleit solution in every 15 minutes. The solution in the chamber was maintained at 37°C, pH 7.4 and treated with 95% O₂ and 5% CO₂. Tension was determined using an isometric transducer (COMMAT Ltd, Ankara, Turkey), and a computer supported system with ProtoWin v 1.0 software. Before starting to the experimental procedure, the artery rings were stretched with 1.5 g tension for about an hour to allow equilibration, which was optimal for the tension changes measurement [15].

Experimental protocol

After equilibration, phenylephrine, (10^{-6} M) was added to the aortic rings and endothelium dependent nitrous oxide-induced relaxation was checked by cumulative addition of acetylcholine (10^{-5} M) to test the presence of endothelium [16]. Only the rings that respond with vasorelaxation were used in the study. The precontraction of the aortic rings were induced with phenylephrine (10^{-6} M) and the stability of maximal contraction was observed in approximately 10 minutes time. The aortic rings were randomly allocated in different chambers and exposed to cumulative concentrations of iloprost (10^{-11} - 10^{-6} M), nitroglycerin (10^{-10} - 10^{-5} M), diltiazem (10^{-10} - 10^{-4} M) or papaverine (3×10^{-6} , 5×10^{-6} , 1×10^{-5} , 2×10^{-5} , 3×10^{-5} M), separately with 5 minutes intervals. The relevant vasomotor responses to each drug were recorded.

Drugs

Iloprost (20 µg/mL) was purchased from Schering (Berlin, Germany), diltiazem hydrochloride (5 mg/mL) was provided from Mustafa Nevzat Inc. (Turkey) and nitroglycerin (1 mg/mL) was provided from Adeka Inc. (Istanbul, Turkey). Other drugs were purchased from Sigma Chemical (St. Louis, MO, USA).

Statistical analysis

After (10^{-6} M) phenylephrine precontraction, the maximal relaxation (E_{max}) values were obtained from each cumulative dose-response curve produced by iloprost, diltiazem, nitroglycerin or papaverine. The concentrations of the agonists that evoked a 50% maximal response were labeled as the EC_{50} by non-linear regression. Graphpad Prism 4 software (La Jolla, CA, USA) was used for the statistical comparisons. Calculation of the significant differences between the groups was carried out with an analysis of variance (ANOVA). Tukey's multiple comparison test was employed to identify the significance between two groups. A value of $P < 0.05$ was depicted the cut-off point for statistical significance.

Results

The mean maximal relaxation (E_{max}) was separately determined for each ring from the concentration-response curves and this value was $27.1 \pm 2.9\%$ for iloprost, $111.7 \pm 2.1\%$ for nitro-

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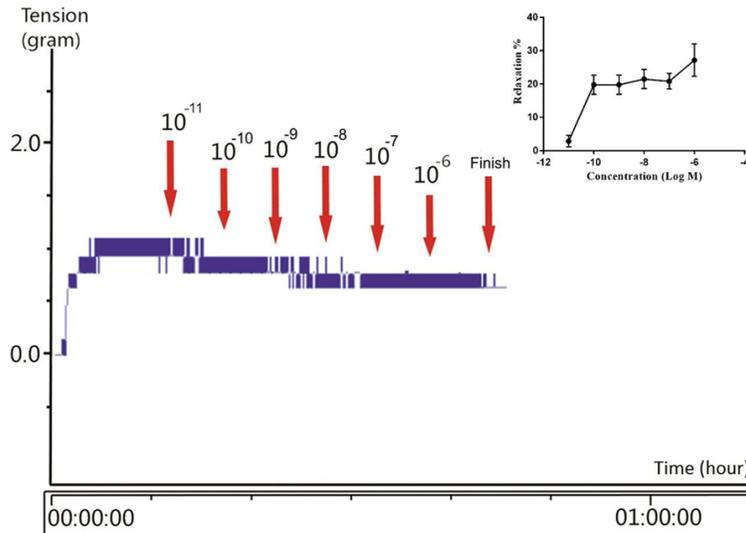


Figure 1. Cumulative dose response curve of iloprost (ProtoWin1.0 software): After the aortic ring was contracted with phenylephrine (10^{-6} M), cumulative increasing doses of iloprost (10^{-11} - 10^{-6} M) were added to the aortic ring at 5 min intervals (left basal side); Log concentration and mean relaxation response graph of iloprost ($n = 7$) (right upper side).

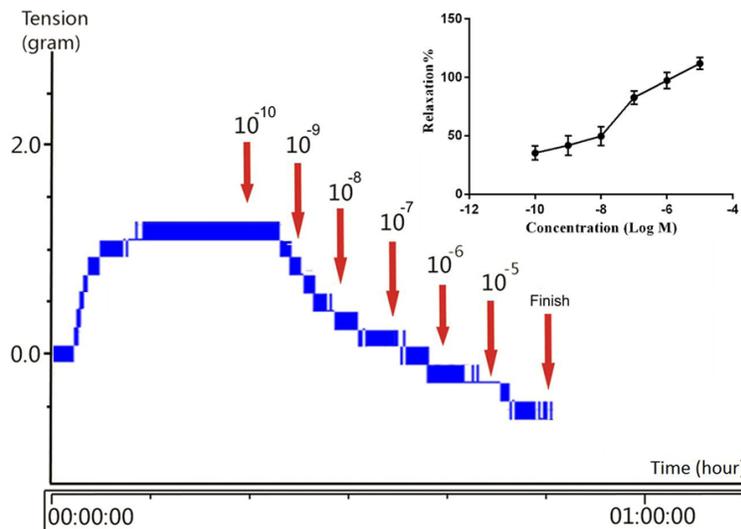


Figure 2. Cumulative dose response curve of nitroglycerin (ProtoWin 1.0 software): After the aortic ring was contracted with phenylephrine (10^{-6} M), cumulative increasing doses of nitroglycerin (10^{-10} - 10^{-5} M) were added to the aortic ring at 5 min intervals (left basal side); Log concentration and mean relaxation response graph of nitroglycerin ($n = 8$) (right upper side).

glycerin, $77.4 \pm 2.9\%$ for diltiazem and $147.2 \pm 2.8\%$ for papaverine (**Figures 1-4** and **Table 1**). There was a statistically significant difference in E_{max} values between four groups ($P < 0.001$). The maximum vasorelaxation produced by papaverine was greater than those of nitroglycerin and diltiazem ($P < 0.01$ and $P < 0.001$, res-

pectively). However, iloprost produced the least vasorelaxation ($27.1 \pm 2.89\%$) compared to other vasorelaxant drugs ($P < 0.001$).

The EC_{50} values were calculated from the concentration-response curve of the drugs (**Figures 1-4**) as 2.4×10^{-11} M for iloprost, 5.6×10^{-8} M for nitroglycerin, 7.0×10^{-7} M for papaverine and 2.1×10^{-5} M for diltiazem. There was a statistically significant difference in EC_{50} values between four groups ($P < 0.01$).

Both nitroglycerin and papaverine showed similar classical sigmoid vasorelaxation curve (**Figures 2** and **4**). The vasorelaxation curve of iloprost showed a remarkable rise at the lowest concentration (10^{-11} M) whereas that was at the highest concentration of diltiazem (10^{-5} M) as presented in **Figures 1** and **3**.

Discussion

Iloprost is used in patients with pulmonary hypertension, vasospastic diseases and have been found to be useful in renal transplant, contrast induced nephropathy and CABG surgery [2]. There are only few studies about topical and systemic use of iloprost to prevent graft spasm and to improve the patency of the grafts during CABG surgery [9, 11, 12].

This study demonstrated that iloprost showed a weak vasorelaxant response on phenylephrine precontracted rat aortic rings compared to widely used agents such as papaverine, diltiazem and nitroglycerin. Considering the mean maximal relaxation, papaverine had the highest efficacy followed by nitroglycerin and diltiazem whereas iloprost had the lowest efficacy. Our findings were consistent with Ege et

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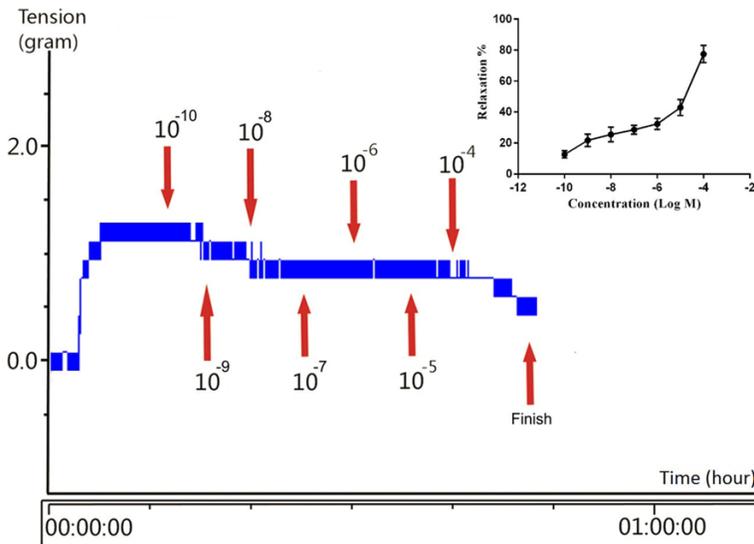


Figure 3. Cumulative dose response curve of diltiazem (ProtoWin1.0 software): After the aortic ring was contracted with phenylephrine (10^{-6} M), cumulative increasing doses of diltiazem (10^{-10} - 10^{-4} M) were added to the aortic ring at 5 min intervals (left basal side); Log concentration and mean relaxation response graph of diltiazem ($n = 8$) (right upper side).

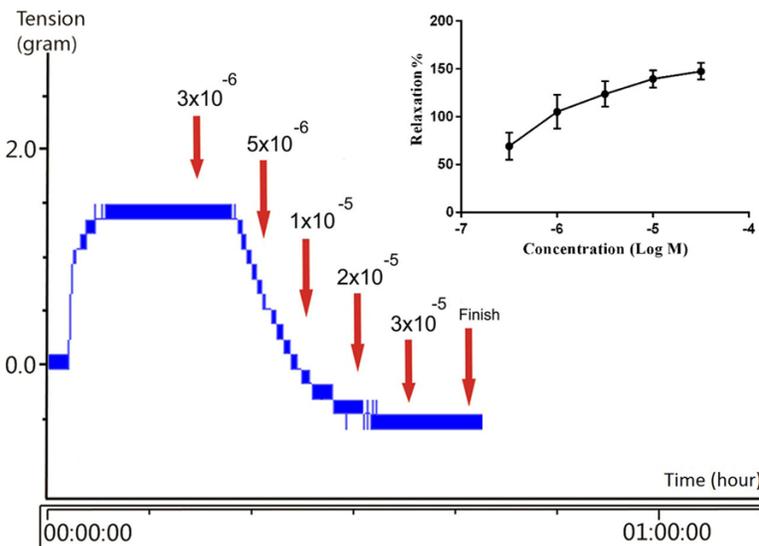


Figure 4. Cumulative dose response curve of papaverine (ProtoWin1.0 software): After the aortic ring was contracted with phenylephrine (10^{-6} M), cumulative increasing doses of papaverine (3×10^{-6} , 5×10^{-6} , 1×10^{-5} , 2×10^{-5} , 3×10^{-5} M) were added to the aortic ring at 5 min intervals (left basal side); Log concentration and mean relaxation response graph of papaverine ($n = 9$) (right upper side).

al. which had similar order of efficacy for papaverine and nitroglycerin *in vitro* on rat thoracic aorta with and without endothelium [17].

In a comparative study where isolated internal mammalian arterial rings were used, papaver-

ine had still the highest vaso-relaxation response while PGI_2 had stronger relaxation response than prostaglandin E_2 [18]. In another *in vitro* study, Ege *et al.* found the vasorelaxant efficacy of iloprost was similar to that of papaverine but better than diltiazem. In this study, vasodilator effect of iloprost has been found to have a sigmoid curve of relaxation and there was significantly more pronounced vasorelaxation with radial arterial rings compared to internal mammalian artery and saphenous vein rings [10]. However, in a previous study of Serbest *et al.* on isolated human internal mammary arterial rings, iloprost had weaker vasorelaxant effect compared to diltiazem and papaverine. Our results were similar to findings of Serbest *et al.* for iloprost, diltiazem and papaverine [13].

On the clinical side, a randomized 70 patient study showed that iloprost was more beneficial compared to diltiazem during CABG surgery with radial artery graft, [12]. The internal mammary artery has advantage of having better protection against local thrombotic events and development of coronary artery graft disease due to endothelial secretion of NO and PGI_2 [18, 19]. Although certain spasmolytic agents were being used during surgery, there is still a need for better vasodilators since the currently used spasmolytics can cause hemodynamic instability [20].

Papaverine, widely known as a vasorelaxant agent, was found to be the most efficacious agent to resolve phenylephrine precontraction. This nonspecific vasorelaxant agent blocks the distribution of several cyclic nucleotides such as cAMP and cGMP and causes vasodilatation

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Table 1. The mean maximal relaxation and EC₅₀ values for each drug on rat thoracic aortic rings

	n	E _{max} (%) (mean ± SD)	EC ₅₀
Iloprost	7	27.1 ± 2.9% ^{a,c,e}	2.4 × 10 ⁻¹¹
Nitroglycerin	8	111.7 ± 2.1% ^b	5.6 × 10 ⁻⁸
Diltiazem	8	77.4 ± 2.9% ^{a,d}	2.1 × 10 ⁻⁵
Papaverine	9	147.2 ± 2.8%	7.0 × 10 ⁻⁷

n: number of rings, E_{max}: maximal relaxation, EC₅₀: concentration that evoke 50% vasorelaxation; ^aP<0.001 vs. papaverine; ^bP<0.01 vs. papaverine; ^cP<0.001 vs. nitroglycerin; ^dP<0.01 vs. nitroglycerin; ^eP<0.001 vs. diltiazem.

by inhibition of phosphodiesterase [21]. Papaverine had different vasoplegic responses with different vasoconstrictor agents. Its acidic nature and toxicity were the major disadvantages [22] that abandoned its intraluminal usage in our clinical practice as well.

Iloprost on the other hand is clinically in use as a vasorelaxant molecule in various clinical situations during cardiovascular surgery practice; but its use in the management of graft spasm is not clear yet. The use of iloprost for the prevention of graft vasospasm is a novel idea.

In our study, in terms of potency according to log [EC₅₀] values, iloprost was the most potent agonist followed by nitroglycerin, papaverine and diltiazem. Weak vasorelaxant effect of iloprost may be beneficial for the management of graft spasm because high relaxant effect may not be physiological and may cause local harmful effects in the tissue as might be observed with other high-potency vasorelaxants. Also combination of iloprost and other vasorelaxants may be another research field. It may be a promising agent during cardiac surgery in comorbid patients with pulmonary hypertension or renal failure.

There were two limitations of this study. Because of cyclic changes of their hormonal profile, the use of female rats might be a limiting factor. Secondly, the use of the thoracic aorta, as a big conductive vessel was another limiting factor, but the study is still informative because in the literature most studies included the materials from patients with coronary artery disease and other systemic diseases whereas in our study we used the samples from healthy rat thoracic aortas.

We observed a weak vasorelaxant response with iloprost compared to those of papaverine, nitroglycerin and diltiazem with isolated rat aortic rings. Here, we proposed that this weak vasorelaxation effect may be beneficial for the management of graft spasm observed in CABG surgery, but further clinical and *in vitro* studies are needed to support our hypothesis for the use of iloprost as a vasorelaxant agent.

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Disclosure of conflict of interest

None.

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