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Original Research Article

Relaxant effect of the prostacyclin analogue iloprost on isolated human radial artery: An approach for the reversal of graft spasm

Ersoy Engin[a,b], F. İlkay Alp Yildirim[c], Deniz Kaleli Durman[c], Suat Nail Ömeroğlu[a], Deniz Göksedef[a], Önder Teskin[d], Ozan Onur Balkanay[a], Gökhan İpek[a], B. Sönmez Uydeş Doğan[c,*]

[a] Department of Cardiovascular Surgery, Cerrahpaşa Medical Faculty, Istanbul, Turkey
[b] Department of Cardiovascular Surgery, Şişli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey
[c] Department of Pharmacology, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey
[d] Biruni University, Faculty of Medicine, Department of Cardiovascular Surgery, Istanbul, Turkey

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ABSTRACT

Radial artery graft spasm in the perioperative or postoperative period of coronary bypass surgery necessitates urgent treatment due to risk of graft failure and mortality. Herein, we evaluated the effect of iloprost, a prostacyclin (PGI_2) analogue, against the contractions produced by noradrenaline and potassium chloride on isolated human radial artery. Following the determination of endothelial and vascular relaxing capacities of the arteries, iloprost (10^{-9}M-10^{-6}M) was cumulatively applied on rings precontracted submaximally with the spasmogens. In some rings, the response to iloprost was assessed following pretreatment with nitric oxide (NO) synthase inhibitor, L-NAME (3×10^{-4}M,30 min). Iloprost produced complete relaxations on radial artery rings precontracted with noradrenaline whereas, only moderate relaxations against the contractions induced by potassium chloride. Notably, the relaxation to iloprost was remarkably blunted in radial arteries with impaired endothelial function. Moreover, the relaxation to iloprost was unchanged in rings pretreated with L-NAME. Our results demonstrated that iloprost could be a potent relaxant agent in reversing radial artery spasm, particularly initiated by noradrenaline, possibly acting via an endothelium-mediated mechanism unrelated to NO.

1. Introduction

Radial artery has been used as a conduit for coronary artery bypass grafting (CABG) since the early 1970s [1–3]. Similar endothelial function with the internal mammary artery (IMA) suggests a satisfactory long-term patency rate for the radial artery [4]. However, the spastic characteristic of radial artery which is associated with high incidence of vasospasm and failure in the perioperative or early postoperative period, limits its usage in coronary revascularization [5]. This high propensity to vasospasm is possibly related to its muscular nature however, the exact cause of spasm is not clearly defined yet. An increase in the plasma levels of various endogenous spasmogens such as noradrenaline, endothelin-1 angiotensin II, and thromboxane A_2 have been reported in patients undergoing cardiopulmonary bypass surgery [6]. Of note, human radial artery is an α_1-adrenoceptor dominant artery [7] and displays more profound contractions in response to α-adrenoreceptor agonists, such as noradrenaline and phenylephrine compared to IMA [8,9]. In addition, radial artery was found more reactive to several receptor-operated vasoconstrictors, including serotonin, angiotensin II, endothelin-1 and the non-receptor depolarizing spasmogen, potassium chloride in comparison to other arterial grafts, namely, IMA and gastroepiploic artery (GEA) [4,8,9]. Hence, all these vasoconstrictor substances have the potency to increase vascular tone and predispose spasm in the radial artery graft.

The use of effective vasodilators is the current clinical approach for the management of spasm and prevention of low patency rate in the radial artery graft. Several vasodilators including calcium channel blockers [2,10,11], papaverine [2,12], nitrovasodilators [10,13–16], potassium channel openers [15,16], phosphodiesterase inhibitors [17], α-adrenoceptor antagonists [18–21] are recommended for the prevention and/or reversal of vasospasm in radial artery by using either alone or in combination [22–24]. However, the demand for an alternative vasodilator agent is still the subject of current research due to lack of efficacy or undesired side effects of the presently used vasodilators [25–27].

Iloprost is a stable prostacyclin (PGI_2) analogue that produce various pharmacodynamic properties including vasodilatation, inhibition of platelet aggregation and cytoprotection through specific prostanoid

* Corresponding author at: UYDEŞ–DOĞAN, Istanbul University, Faculty of Pharmacy, Department of Pharmacology, Beyazıt, 34116, Istanbul, Turkey.
E-mail addresses: sonmezuydesdogan@gmail.com, sonmezdo@istanbul.edu.tr (B.S. Uydeş Doğan).

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receptors, mainly IP receptor, on the cell surface [28]. In vitro studies on human and animal vessels have shown that iloprost produced more potent vasorelaxation compared to several PGI_2 mimetics [29,30]. In relation to this potent vasodilatory effect, it is widely used in patients with pulmonary hypertension and peripheral arterial disease [28,31]. Recently, a few studies have evaluated the effects of iloprost on the arterial and venous bypass graft materials by using different experimental protocols [32–35]. Regarding to the limited data available to support the possible role of iloprost in the management of graft spasm herein, we aimed to verify the vasorelaxant efficacy of iloprost against the contractions of spasmogens acting by receptor-operated or receptor-independent mechanisms in human isolated radial artery. In parallel, the endothelial and vascular relaxing capacities of the arteries were determined by acetylcholine and sodium nitroprusside, respectively. To elucidate its mechanism of action, the responsiveness to iloprost was assessed in the presence of nitric oxide (NO) synthase inhibitor, L-NAME.

2. Material and methods

2.1. Sampling and preparation of radial artery

Radial artery samples were obtained from patients undergoing coronary artery bypass operations. Due to the limitations of graft length, only the patients in which radial artery grafts were to be used to bypass the first marginal branch of circumflex or intermediate coronary arteries (ramus intermedius) were enrolled. Use of discarded human radial artery segments was approved by the Institutional Review Board of Istanbul University, Cerrahpaşa Medical Faculty (Protocol No: 2009/17277). Informed consent was obtained from all candidate patients prior to operation. Clinical characteristics of the patients undergoing coronary artery bypass operations and their drug therapies are given in Table 1.

Caution was exercised during harvesting of the vessel in order not to stretch and touch the endothelial surface. The sampled segment of radial artery, which is not exposed to any preparatory solution, was placed into cold (4 °C) Krebs Ringer-bicarbonate solution, then immediately transferred to the laboratory. Composition of the solution was as follows (in mM): NaCl 118.5, KCl 4.8, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4{\cdot}7H_2O$ 1.2, $CaCl_2$ 1.9, glucose 10.1, and disodium EDTA 0.026. Adherent connective tissues were removed and the specimen was cut into rings of 3–4 mm in length. Rings were suspended between two stainless steel L-shaped hooks in a 10 ml jacketed organ bath containing Krebs Ringer-bicarbonate solution at 37 °C and aerated with 95% O_2 and 5% CO_2. One hook was fixed at the bottom of the organ bath while the other was connected to a force displacement transducer (Grass Model FT03) which was fixed to a micrometric manipulator allowing adjustments in the resting tension of the rings. The optimal point of length-tension relation was determined according to our previous study [21] and thus, 4 g, which provided maximal noradrenaline responsiveness in isolated human radial arteries, was used as the resting tension. Two to three rings were obtained from each artery specimen and each ring was subjected to only one experimental protocol with iloprost. Thus, the responses to iloprost were obtained in parallel rings of each radial artery specimen which determined to display similar contractile and relaxant properties in the preliminary experiments.

Table 1
Clinical characteristics of the patients undergoing coronary artery bypass operation.

Parameter	n
Age (year)	58.2 ± 4.93
Sex	
Male	14
Female	0
Blood Creatinine (mg/dl)	0.94 ± 0.22
Ejection Fraction (%)	53.0 ± 6.00
Risk Factors	
Hypertension	10
Angina Pectoris	12
Hyperlipidemia	8
Diabetes Mellitus	5
Smoking	10
Drug therapy	
β-Blockers	9
Calcium Channel Blockers	5
Oral Antidiabetics	5
ACE Inhibitors	7

n: Number of the patients, ACE: Angiotensin-converting enzyme.
Age, blood creatinine and ejection fraction values of the patients are given as (mean ± SEM).

2.2. Experimental protocol

Following the equilibration period of 2 h, viabilities of the vessel segments were checked by potassium chloride (40 mM) and preparations that produced a tension of less than 2 g were discarded. Two consecutive potassium chloride responses were obtained for each ring in order to standardize the reactivity of the preparations. The presence of functional endothelium was confirmed by the relaxation to acetylcholine (10^{-9}–10^{-4} M) in noradrenaline precontracted radial arteries. Vascular smooth muscle relaxation capacity of the radial arteries was tested by the nitrovasodilator, sodium nitroprusside which applied either cumulatively (10^{-9}–10^{-4} M) or at maximal concentration (10^{-4} M) in each experiment.

Experiments were performed to evaluate the effect of iloprost on the contractions induced by the spasmogens, noradrenaline and potassium chloride in isolated human radial artery. For each spasmogen, the concentrations required to produce a similar contractile force (g) were determined in radial artery rings and these concentrations were used to induce a submaximal (70–80%) contraction. Thus, isolated radial arteries were precontracted submaximally with noradrenaline (10^{-6}–5×10^{-6} M) and potassium chloride (40–60 mM). Then, increasing concentrations of iloprost (10^{-9}–10^{-6} M) were applied on rings precontracted with either of these contractile agents. In parallel rings of the radial artery specimen, time-match control experiments were performed to elucidate whether the precontractions elicited by noradrenaline and potassium chloride were stable during the experimental period.

In order to analyze the involvement of NO, an endothelium derived relaxing substance, in the action mechanism of iloprost, human radial artery rings were pretreated with NO synthase inhibitor, L^G-nitro-L-arginine (L-NAME, 3×10^{-4} M) for 30 min [36]. Thereafter, in the presence of L-NAME, the relaxing response to iloprost was obtained on noradrenaline (10^{-6}–5×10^{-6} M) precontracted arteries.

2.3. Statistical analysis

The results are given as mean ± SEM. In all experiments ***n*** is the number of patients from whom the vessels were obtained. The precontractile force induced by noradrenaline was expressed as absolute (g) contraction. The maximal relaxations (E_{max}) to iloprost and sodium nitroprusside were expressed as percent decreases of noradrenaline and potassium chloride induced precontractions. The sensitivities of radial arteries to iloprost, sodium nitroprusside and acetylcholine were expressed as the effective concentration that elicited 50% (EC_{50}) of the maximal response and calculated separately for each concentration-response curve by probit regression analysis. A computerized program was used for the curve-fitting. EC_{50} values were given as $-\log$ M (i.e. pEC_{50}). Statistical analyses were performed by using Student's *t*-test (paired or unpaired) and one way analysis of variance (ANOVA), where appropriate. A ***p*** value less than 0.05 was considered significant.