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Short communication

Vasorelaxant effect of a phosphodiesterase 3 inhibitor, olprinone, on isolated human radial artery

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Abstract

The radial artery is currently used as a viable arterial conduit for myocardial revascularization. The aims of this study were to identify phosphodiesterase 3 isoenzyme in the human radial artery isolated for coronary artery bypass grafting, and to examine the vasorelaxant effect of a cardiotonic and vasodilating phosphodiesterase 3 inhibitor, 1,2-dihydro-6-methyl-2-oxo-5-(imidazo[1,2-*a*]pyridin-6-yl)-3-pyridine carbonitrile hydrochloride monohydrate (olprinone). The phosphodiesterase 3 isoenzyme was separated from the radial artery by DEAE-Sepharose chromatography. Olprinone inhibited the phosphodiesterase 3 activity with an IC_{50} value of 1.25 μM. Olprinone relaxed the phenylephrine-induced contractions of endothelium-denuded arterial strips with an EC_{50} value of 0.107 ± 0.029 μM ($n = 5$). These findings indicate that the human radial artery possesses phosphodiesterase 3 isoenzyme activity and olprinone causes potent relaxation of the arterial strip in vitro through inhibition of phosphodiesterase 3 isozyme activity.

Keywords: Radial artery; Phosphodiesterase 3; Olprinone; Phenylephrine

1. Introduction

The radial artery had been used as a graft for coronary revascularization for many years. However, vasospasm of the artery was observed in about 5% of patients despite intraoperative and postoperative treatment with an antispastic agent such as diltiazem (Acar et al., 1992). Manasse et al. (1996) have reported that there were irregular aspects in 10% of the radial artery grafts, which suggests a higher incidence of segmental spasm. Accordingly, new antispastic drugs should be expected to lessen the risk of arterial narrowing and occlusion, and to expand the opportunities to use the radial artery for coronary artery bypass grafting.

Olprinone, 1,2-dihydro-6-methyl-2-oxo-5-(imidazo[1,2-*a*]pyridin-6-yl)-3-pyridine carbonitrile hydrochloride monohydrate, is one of the cardiotonic and vasodilating phosphodiesterase 3 inhibitors, including amrinone and milrinone, that have been used in patients with acute heart failure. The IC_{50} value of olprinone for phosphodiesterase 3 activity was about 30 times more potent than that of amrinone, and was almost comparable to that of milrinone (Ogawa et al., 1989). Olprinone produced a positive inotropic effect on guinea pig papillary muscle (Ogawa et al., 1989), while it inhibited the norepinephrine-induced contractions of endothelium-denuded rat aortic strips (Itoh et al., 1993). Its vasodilating effect has also been shown in in vivo studies using anesthetized dogs (Ohhara et al., 1989) and conscious pigs with heart failure (Adachi and Tanaka, 1997). The other phosphodiesterase 3 inhibitor, milrinone, has been reported to be useful in preventing spasm of the internal mammary artery, which is utilized as one of the conduit vessels in human coronary bypass graft surgery (Thorin-trescases et al., 1993; Liu et al., 1997). However, it has not been clarified whether olprinone may be effective for the treatment of perioperative spasm of the radial artery for coronary artery bypass grafting.

The objectives of the present study were to identify the phosphodiesterase 3 isoenzyme in radial arteries obtained

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from patients undergoing coronary bypass graft surgery, and to examine the vasorelaxant effect of olprinone on isolated arterial preparations.

2. Materials and methods

2.1. Isolation of phosphodiesterase 3 isoenzyme from human radial arteries and inhibition of phosphodiesterase 3 activity

The procedures and handling of human tissue were approved by the Human Ethics Committee of the Sakakibara Heart Institute. Written informed consent was given by each patient or each patient's family. Human radial arteries were removed from 29 patients undergoing elective coronary bypass surgery.

The organs were quickly frozen and stored at −80°C until use. All subsequent procedures were performed at 4°C. Human radial artery weighing 11 g was minced, and homogenized in 10 volumes (v/w) of buffer A (20 mM Tris–HCl (pH 7.4), 0.1 mM EGTA, 2 mM Mg acetate, 10 mM 2-mercaptoethanol) containing a protease inhibitor cocktail tablet (Boehringer Mannheim, Mannheim, Germany). The homogenate was centrifuged at $1800 \times g$ for 10 min, then the supernatant was centrifuged at $100{,}000 \times g$ for 60 min. The supernatant from the second step was applied to a DEAE-Toyopearl 650S column (1.6 i.d. × 30 cm, TOSOH, Tokyo, Japan) pre-equilibrated with buffer A. After application, the column was washed with buffer A. Phosphodiesterase isozymes were eluted using a linear gradient from 0.08 to 0.3 M NaCl in buffer A and fractions were collected. cAMP hydrolytic activities in the fractions were determined in the presence or absence of cGMP. Further purification of phosphodiesterase 3 was conducted as follows. The peak fractions containing cAMP hydrolytic activity were collected and re-applied to a DEAE-Toyopearl 650S column pre-equilibrated with buffer A. After the second application, the column was washed with buffer A and a linear gradient from 0.15 to 0.24 M NaCl in buffer A was carried out. The fractions, in which cAMP hydrolytic activity was inhibited in the presence of cGMP, were pooled as the phosphodiesterase 3 fraction. The phosphodiesterase 3 fractions were dried by dialyzing against buffer A containing 20% polyethylene glycol (w/v) and then resuspended with buffer A containing 40% ethylene glycol (v/v). The phosphodiesterase 3 isozyme was stored at −30°C until use.

Phosphodiesterase activity of column fractions was determined by modification of a two-step radioisotopic procedure (Saeki and Saito, 1993). The assay buffer for measurement of phosphodiesterase activity contained 40 mM Tris–HCl (pH 8), 1 mM EGTA, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.7 mg/ml bovine serum albumin. One μM [^{3}H] cAMP was used in the absence or presence of 10 μM cGMP to measure the cAMP hydrolytic activities of the fractions. To determine the inhibitory effect of olprinone on phosphodiesterase 3 isozyme, 0.25 μM [^{3}H] cAMP was used as substrate in the absence of cGMP. Olprinone was dissolved in dimethyl sulfoxide (DMSO) to yield a concentration of 10 mM, and serially diluted with DMSO. The solution was further diluted with the assay buffer and added to the reaction mixture (final concentration of DMSO was 1%). Phosphodiesterase activity was expressed as a percentage of the control, and the IC_{50} value of olprinone for phosphodiesterase 3 activity was determined from the sigmoidal curve of phosphodiesterase 3 activity versus log concentration. The IC_{50} value is shown as the mean and 95% confidence interval determined from the results of three experiments.

2.2. Smooth muscle relaxant effects of human radial arteries

Segments of freshly prepared human radial arteries were removed from five patients. After the grafts were trimmed to the appropriate length, the unused portions were immersed in cold physiological salt solution.

The arteries were washed with saline and placed in Krebs–Henseleit solution that contained (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 5.0; glucose, 11.0. The strips were carefully cleaned of fat and extraneous tissue, cut transversely (5 mm) and longitudinally, and denuded of endothelium. The preparations were tied at each end with cotton threads and mounted in organ baths containing 10 ml of Krebs–Henseleit solution. Organ bath solutions were maintained at 37°C and continuously aerated with 95% O_2 and 5% CO_2. The upper thread of each preparation was suspended from an isometric force transducer (TB-611T, Nihon Kohden, Tokyo, Japan), and changes in arterial smooth muscle tension were recorded isometrically via an amplifier (AP-621G, Nihon Kohden). The initial resting tension of each preparation was set at 2 g.

The preparations were washed several times with Krebs–Henseleit solution. Then, organ baths were filled with 10 ml of Krebs–Henseleit solution again and 250 μl of 2 M KCl was added to depolarize the preparations. The depolarization step was performed twice. Then, the preparations were initially contracted by addition of 100 μl of 10 mM phenylephrine (Sigma, St. Louis, MO, USA). Subsequently, organ baths were filled with 10 ml of Krebs–Henseleit solution, 10 μl of 10 mM *N*ω-nitro-L-arginine methyl ester (Sigma) and 10 μl of 10 mM indomethacin (Sigma) was added. The preparations were incubated for 15 min and 100 μl of 10 mM phenylephrine was added to obtain the maximum contraction. When the contraction had stabilized, olprinone solution was cumulatively added to reach a final concentration ranging from

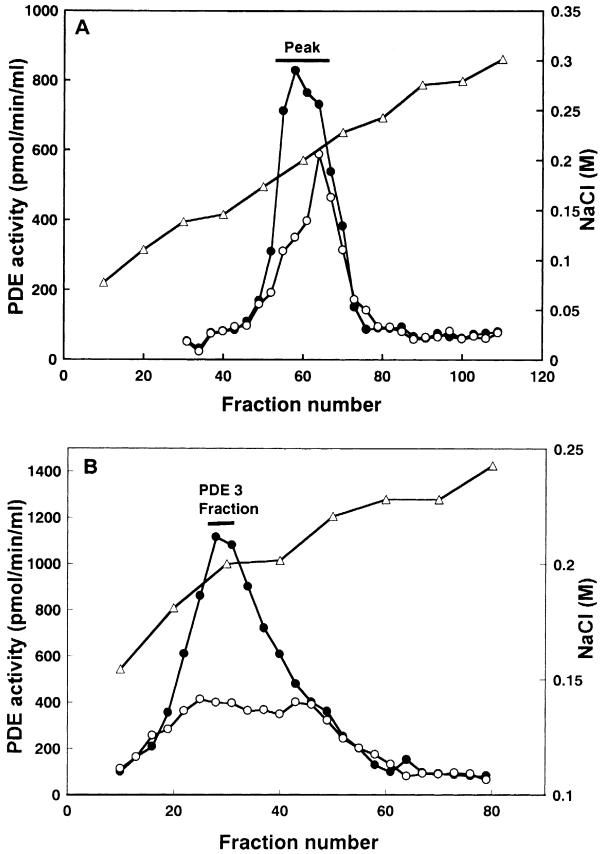

Fig. 1. Elution profile of phosphodiesterase (PDE) isoenzymes in extracts of human radial artery separated using columns of DEAE-Toyopearl 650S. (A) Chromatography of cytosolic phosphodiesterase isoenzymes. (B) Further separation of cytosolic phosphodiesterase isoenzymes of fractions 55–64 (Peak) shown in (A). Fractions were eluted with successive linear gradients of NaCl (open triangles: (A) 0.08 to 0.30 M; (B) 0.15 to 0.24 M). Phosphodiesterase activities were determined using 1 μM cAMP in the absence of cGMP (closed circles) and 1 μM cAMP in the presence of 10 μM cGMP (open circles).

0.01 μM to 100 μM in the organ bath. At the end of the experiment, 100 μl of 10 mM papaverine (Sigma) was added to obtain the maximum relaxation. The relaxation obtained in the presence of papaverine was expressed as 100%. The relaxant response to each olprinone concentration was expressed as a percentage of the maximum relaxation induced by papaverine. All values are shown as the mean ± S.E.M. determined from the results of five experiments.

2.3. Reagents

Olprinone (M.W.: 304.74) was synthesized at the Department of Organic Chemistry (Eisai, Ibaraki, Japan). [^{3}H]cAMP was purchased from Du Pont-New England Nuclear (Wilmington, DE, USA). cAMP and cGMP were purchased from Sigma.

3. Results

3.1. Isolation of phosphodiesterase 3 isozyme from human radial arteries and inhibition of phosphodiesterase 3 activity

Results from chromatograms demonstrating the presence of phosphodiesterase isozymes in the cytosolic fraction of human radial artery are shown in Fig. 1. Fig. 1A presents the elution profile of the phosphodiesterase isozymes from the DEAE-Toyopearl 650S column. Peak fractions 55–64 hydrolyzed cAMP and consisted of two phosphodiesterase isozymes, phosphodiesterase 3 and 4, one inhibited by cGMP and the other not affected in the presence of cGMP, respectively. To separate phosphodiesterase 3 and 4 isozymes, fractions 55–64 were reapplied to the DEAE-Toyopearl 650S column. Fractions 28–31, in which cAMP hydrolytic activity was inhibited in the presence of cGMP (Fig. 1B), were pooled as the phosphodiesterase 3 fraction. Olprinone inhibited the activity of purified phosphodiesterase 3 with an IC_{50} value of 1.25 μM (95% confidence interval: 0.97–1.62 μM, $n = 3$).

3.2. Relaxant response of human radial arteries to olprinone

A concentration–response curve for the relaxant effect of olprinone in endothelium-denuded strips of human radial artery precontracted with phenylephrine is shown in Fig. 2. Olprinone relaxed the arterial strips in a dose-de-

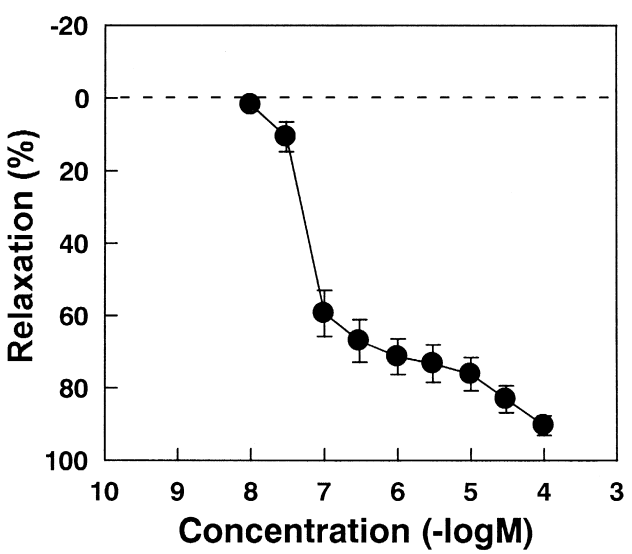

Fig. 2. Concentration–response curve for the relaxant effect of olprinone in endothelium-denuded strips of the human radial artery precontracted with phenylephrine. The maximum contraction was obtained with 100 μM phenylephrine in the absence of olprinone, and relaxation induced by 100 μM papaverine was taken as 100%. All values are the means ± S.E.M. determined from the results of five experiments.

pendent manner. The concentration of olprinone that produced 50% relaxation (EC_{50}) was 0.107 ± 0.029 μM (n = 5). The maximum relaxation induced by the highest concentration of olprinone (100 μM) was 90.4 ± 2.7%.

4. Discussion

The major findings of the present study were that the human radial artery contained phosphodiesterase 3 isoenzyme activity, and that its inhibitor, olprinone, markedly inhibited phenylephrine-induced contraction of endothelium-denuded arterial strip in vitro through the inhibition of phosphodiesterase 3 isozyme activity, suggesting that olprinone could prevent constriction of a coronary artery grafted vessel and ameliorate subsequent regional myocardial ischemia.

Acar et al. (1992) revived the clinical introduction of the radial artery for coronary revascularization by Carpentier et al. (1973), and again proposed the effectiveness of the radial artery as a viable arterial conduit for myocardial revascularization, together with use of calcium channel blockers and aspirin postoperatively. Recently, other vasodilators such as organic nitrates have also been shown to prevent the development of a contraction and to reverse the sustained contraction of human radial artery grafts (Cable et al., 1998). In the present study, we examined the vasorelaxant effect of olprinone, which is effective for cardiohemodynamic improvement in weaning from cardiopulmonary bypass and in post-cardiotomy cardiogenic shock (Orime et al., 1999), in isolated human radial artery.

Up to now, although the distribution of phosphodiesterase 3 isozyme in human tissues such as heart (Sugioka et al., 1994), aorta (Miyahara et al., 1995) and platelet (Ito et al., 1996) has been described, there is no information as to its existence in the human radial artery, a coronary artery graft. We demonstrated for first time that phosphodiesterase 3 isozyme was present in the cytosolic fraction in the artery.

Olprinone produces vasodilation via selective inhibition of phosphodiesterase 3 isozyme and accumulation of cAMP associated to a decrease in intracellular calcium levels of vascular smooth muscle cells (Ohoka et al., 1990). In the present study, olprinone relaxed the phenylephrine-induced contraction of radial artery with an EC_{50} value of 0.107 μM. This value was comparable to that (0.14 μM) for the norepinephrine-induced contraction of rat aortic strips (Itoh et al., 1993). It is known that there is a good correlation between the vasorelaxant effects of the cardiotonic phosphodiesterase 3 inhibitors, including olprinone and amrinone, and their ability to inhibit the activity of phosphodiesterase 3 isozyme (Itoh et al., 1993). In contrast, olprinone inhibited the phosphodiesterase 3 isozyme isolated from the radial artery with an IC_{50} value of 1.25 μM, which is half or one-third of the inhibitory activity from rat aorta (0.6 μM; Itoh et al., 1993) or human heart (0.35 μM; Sugioka et al., 1994), respectively. Unfortunately, we do not know why the EC_{50} value of olprinone for arterial relaxation was about 10-fold smaller than its IC_{50} value for phosphodiesterase 3 isozyme. However, considering the results (4.3-fold difference) from rat aorta preparations (Itoh et al., 1993) as mentioned above, we suppose that the cAMP–phosphodiesterase 3 system may play a functionally important role in adjusting the mechanism of vascular tone in human radial artery and rat aorta. Interestingly, the EC_{50} value of olprinone, 0.107 μM (32.6 ng/ml), was similar to the therapeutic plasma olprinone concentration (almost 20 ng/ml, unpublished data) obtained from a pharmacokinetic study on patients with acute heart failure. Accordingly, olprinone could bring about vasodilation of the radial artery for coronary bypass graft in cardiac surgery.

We selected phenylephrine as the standard vasoconstrictor to produce spasm in vitro, because it induces contraction of both arteries and veins for myocardial revascularization through activation of receptor operating channels (Thorin-trescases et al., 1993; Liu et al., 1997). Under these conditions, olprinone caused endothelium-independent relaxation and exerted its vasodilator effect directly on the smooth muscle, not on the membrane receptors in the human radial artery precontracted with phenylephrine. Therefore, olprinone, having an endothelium-independent vasodilating effect, may be useful in the treatment of spasm arising from endothelial dysfunction caused by atherosclerosis or surgical procedure.

In conclusion, olprinone causes a potent, endothelium-independent dilation of the human radial artery via the inhibition of phosphodiesterase 3 activity present in the artery. These results suggest that this phosphodiesterase 3 inhibitor would be useful as a vasodilator for the short-term treatment of perioperative spasm of coronary bypass grafts.

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