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Cardioprotection induced by olprinone, a phosphodiesterase III inhibitor, involves phosphatidylinositol-3-OH kinase-Akt and a mitochondrial permeability transition pore during early reperfusion

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Abstract
Purpose. Ischemic preconditioning is mediated by the activation of phosphatidylinositol-3-OH kinase-Akt (PI3K-Akt) and by the inhibition of the opening of a mitochondrial permeability transition pore (mPTP) during early reperfusion. Preischemic administration of the phosphodiesterase type III inhibitor olprinone protects the myocardium against infarction, but its mechanism has not been fully clarified. We hypothesized that this olprinone-induced cardioprotective effect was mediated by the activation of PI3K-Akt and by the inhibition of mPTP during early reperfusion.
Methods. Pentobarbital-anesthetized rats (n = 42) subjected to 30-min coronary occlusion followed by 2-h reperfusion, received olprinone (20 μg·kg^{-1}) or saline (control) in the preischemic phase in the presence or absence of the PI3K-Akt inhibitor wortmannin (0.6 mg·kg^{-1}) or the mPTP opener atractyloside (5 mg·kg^{-1}) before 5 min of reperfusion. The myocardial infarct size was expressed as a percentage of the area at risk. All values were expressed as means ± SD. Statistical comparisons within groups were made using repeated-measures analysis of variance (ANOVA), followed by a paired t-test, and comparisons among groups were analyzed using a two-way ANOVA, followed by the Tukey-Kramer test.
Results. Mean arterial pressure and heart rate showed no significant differences within or among groups. The preischemic administration of olprinone significantly reduced the infarct size (12 ± 4%) as compared with that in the control group (43 ± 4%). Wortmannin or atractyloside abolished the protective effect of olprinone (42 ± 11% or 41 ± 10%).
Conclusion. The olprinone-induced cardioprotective effect could be exerted via the activation of PI3K-Akt and the inhibition of mPTP during early reperfusion.

Key words Myocardial infarction · Olprinone · PI3K-Akt · mPTP

Introduction

Phosphodiesterase type III inhibitors (PDEIs) are used for the treatment of severe heart failure, because these compounds have vasodilatory and inotropic effects via the increase of intracellular cyclic adenosine monophosphate (cAMP) levels in cardiomyocytes [1] and they act to increase myocardial contractile force with less cardiac oxygen demand than catecholamines [2]. In addition, PDEIs are useful in patients who have beta-adrenoreceptor downregulation associated with long-term beta-adrenergic stimulation [3], because the effect of PDEIs is not mediated by beta-adrenoreceptors. Therefore, PDEIs are used commonly in cardiac surgery.

It is well known that transient episodes of ischemia and reperfusion markedly protect the myocardium during a prolonged episode of ischemia and reperfusion. This phenomenon has been termed "ischemic preconditioning". An increase in the concentration of cAMP was shown to be involved in this ischemic preconditioning [4]. It has also been reported that the pre-ischemic infusion of olprinone, milrinone, or amrinone (PDEIs); or cAMP analogs, leads to the activation of protein kinase A (PKA) and decreased myocardial infarct size [5,6], but the mechanisms involved have not been fully clarified.

Recently, Hausenloy et al. [7] demonstrated that myocardial protection conferred via ischemic preconditioning was mediated by phosphorylating the prosurvival kinases, phosphatidylinositol-3-OH kinase-Akt (PI3K-Akt) and extracellular-signal regulated kinases 1 and 2, at reperfusion during early reperfusion. These prosurvival kinase cascades have been referred to as the reperfusion injury salvage kinase (RISK) pathway [8,9]. Moreover, it was reported that ischemic or pharmacological preconditioning was inhibited by atractyloside, a mitochondrial permeability transition pore (mPTP) opener, administered during early reperfusion [10,11].

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The activation of PKA by an increase in cAMP would lead to the inhibition of Rho kinase [12]; inhibition of Rho kinase leads to the activation of PI3K-Akt and myocardial protection against ischemia-reperfusion injury [13]. However, it is not clear whether PI3K-Akt or mPTP is involved in the PDEI-induced cardioprotective effect.

In this study, we hypothesized that olprinone-induced pharmacological cardioprotection was mediated by the activation of PI3K-Akt or by the inhibition of mPTP.

Methods

All experimental procedures and protocols described in this study were approved by the Institutional Animal Care and Use Committee of the Nagasaki University School of Medicine.

In each experiment, male Sprague-Dawley rats (body weight, 448 ± 38 g [mean ± SD], and aged 13 ± 1 weeks) were anesthetized with sodium pentobarbital (50 mg·kg^{-1} IP). Briefly, tracheotomy was performed at a midline incision. The rats were ventilated with an SAR-830, a volume-cycled ventilator (CWE, PA, USA), using pure oxygen (fractional inspired oxygen concentration, 1.0). Next, catheters were inserted into the right internal carotid artery and right external jugular vein for measuring blood pressure and administering fluids or drugs. Normal saline solution was administered during each experiment. After catheter placement, a thoractomy was performed at the left fourth and fifth intercostal spaces, and the ribs were removed to expose the heart. After pericardiotomy, the left anterior descending coronary artery (LAD) was identified, a 7-0 Prolene (Ethicon, NJ, USA) ligature was placed around the LAD, and the ends of the tie were threaded through a small plastic tube for reversible LAD occlusion. Body temperature was maintained constant at 37°C to 38°C with a heating blanket. Hemodynamics were monitored with a transducer (blood pressure monitor link sck-9082; Becton Dickinson, Tokyo, Japan) and an AP-641G blood pressure amplifier (Nihon-Kohden, Tokyo, Japan), and shown on a Polygraph system (Nihon-Kohden).

The experimental design is shown diagrammatically in Fig. 1. All rats were subjected to 30-min LAD occlusion followed by 2-h reperfusion. The rats were allocated to one of six groups (n = 7 per group) randomly. Group O received olprinone (20 μg·kg^{-1}) as an i.v. bolus 30 min before LAD occlusion and group C received the same volume of saline. Group OW received olprinone (20 μg·kg^{-1}) as an i.v. bolus 30 min before LAD occlusion, and the PI3K inhibitor wortmannin (0.6 mg·kg^{-1}), while group OA received the olprinone bolus given in the same way as in group OW, plus the mPTP opener atractyloside (5.0 mg·kg^{-1}) i.v. given 5 min before reperfusion. Group W received only wortmannin 5 min before reperfusion, while group A received only atractyloside 5 min before reperfusion. The doses and administration times of olprinone [5], wortmannin [14,15], and atractyloside [16] were based on the doses and the times used in our previous study. Furthermore, abolition of the cardioprotective effect of sevoflurane in this experiment was regarded as occurring under the same conditions as in our previous experiment, because the quantities of wortmannin and atractyloside were the same as those used in our previous study.

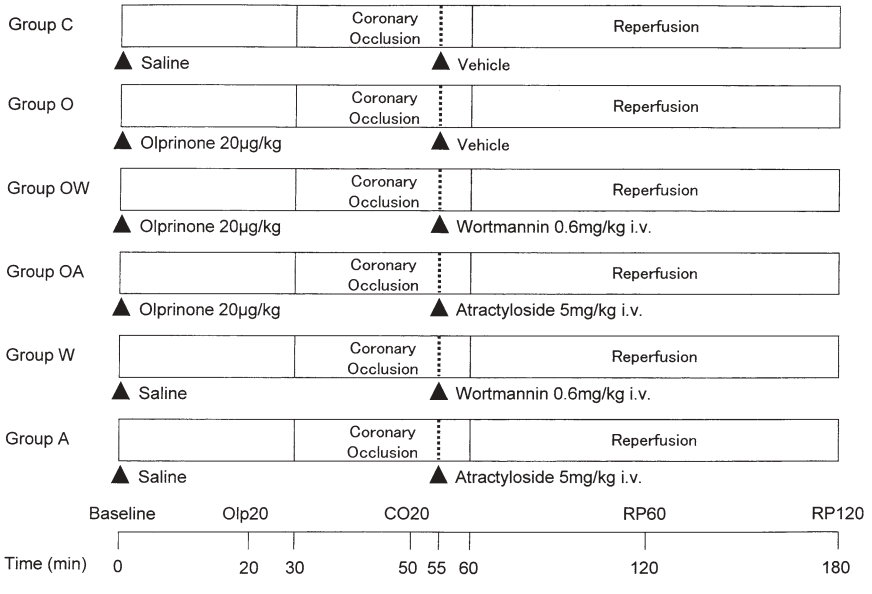

Fig. 1. Schematic illustration of the experimental protocol. *Group C*, control group; *group O*, olprinone group; *group OW*, olprinone + wortmannin group; *group OA*, olprinone + atractyloside group; *group W*, wortmannin group; *group A*, atractyloside group; *Olp20*, 20 min after olprinone bolus; *CO20*, 20 min after coronary occlusion; *RP60* and *RP120*, 60 and 120 min after reperfusion

Table 1. Systemic hemodynamics

	Baseline	Olp20	CO20	RP60	RP120
Group C					
MAP (mm Hg)	106 ± 9	112 ± 5	109 ± 19	107 ± 14	102 ± 18
HR (bpm)	394 ± 16	396 ± 17	416 ± 26	415 ± 16	408 ± 24
Group O					
MAP (mm Hg)	106 ± 10	93 ± 17	95 ± 11	106 ± 14	102 ± 9
HR (bpm)	404 ± 15	421 ± 23	408 ± 16	421 ± 19	419 ± 24
Group OW					
MAP (mm Hg)	106 ± 10	102 ± 16	109 ± 9	118 ± 16	104 ± 23
HR (bpm)	395 ± 23	411 ± 24	392 ± 17	398 ± 25	412 ± 26
Group OA					
MAP (mm Hg)	107 ± 14	98 ± 18	100 ± 9	113 ± 15	116 ± 14
HR (bpm)	411 ± 21	409 ± 22	394 ± 28	396 ± 22	409 ± 26
Group W					
MAP (mm Hg)	97 ± 13	94 ± 13	105 ± 16	106 ± 19	99 ± 17
HR (bpm)	405 ± 23	405 ± 26	398 ± 28	385 ± 11	403 ± 26
Group A					
MAP (mm Hg)	103 ± 7	98 ± 13	100 ± 18	114 ± 8	113 ± 10
HR (bpm)	399 ± 20	392 ± 14	383 ± 19	407 ± 24	397 ± 16

All values are presented as means ± SD ($n = 7$ per group)
MAP, mean arterial pressure; HR, heart rate; group C, control group; group O, olprinone group; group OW, olprinone + wortmannin group; group OA, olprinone + atractyloside group; group W, wortmannin group; group A, atractyloside group; Olp20, 20 min after olprinone bolus; CO20, 20 min after coronary occlusion; RP60 and RP120, 60 and 120 min after reperfusion

At the end of the experiment, the LAD was reoccluded briefly, and the area at risk was determined by the injection of 1 ml patent blue dye intravenously. The heart was excised, excess-left ventricular tissue was removed, and the left ventricle was cut into six or seven slices transversely. The nonstained area at risk (AAR) was seperated from the blue-stained nonischemic zone. Next, the AAR was incubated in a 37°C 1% solution of buffered triphenyltetrazolium chloride (TTC) for 20 min and immersed overnight in 10% formaldehyde. TTC stains living tissue a deep red color, but necrotic tissue is TTC-negative and appears white. Each slice was read using a scanner. Infarcted and noninfarcted tissue in each slice was measured using NIH Imaging. Myocardial infarct size was expressed as a percentage of the AAR.

Olprinone was purchased from Eisai (Tokyo, Japan). Wortmannin, atractyloside, patent blue dye, and TTC were purchased from Sigma (St. Louis, MO, USA). Wortmannin was dissolved in dimethyl sulfoxide and diluted ten fold with saline. Atractyloside was dissolved in saline. TTC was prepared by dissolving it in phosphate buffer.

All values are expressed as means ± SD. Statistical comparisons within groups were made using repeated-measures analysis of variance, followed by a paired *t*-test. Statistical comparisons among groups were analyzed using two-way analysis of variance, followed by the Tukey-Kramer test. P values less then 0.05 were considered significant.

Results

There were no significant differences in body weight or age among the groups. Fifty-five rats were instrumented, with 42 successful experiments achieved. Four rats were excluded because of technical problems during instrumentation and 9 rats were excluded because intractable ventricular fibrillation occurred during LAD occlusion.

Hemodynamic data for the mean arterial pressure (MAP) and heart rate (HR) are shown in Table 1. There were no significant differences either within groups nor among groups in MAP or HR at any measurement points. A slight fall in MAP was seen with olprinone, but it was not significant. Bolus administration of wortmannin or atractyloside did not cause any hemodynamic changes. Data for the AAR are shown in Table 2; there were no significant differences among the groups.

Myocardial infarct sizes in the study groups are shown in Fig. 2. The preischemic administration of olprinone significantly reduced the infarct size as compared with that in control rats. Neither wortmannin nor atractyloside alone affected the infarct size, but each abolished the protective effect of olprinone.

Discussion

These results indicate that the preischemic administration of olprinone could confer cardioprotection, and

Table 2. Left-ventricular area at risk

	n	Area at risk/left ventricle (%)
Group C	7	55 ± 7
Group O	7	56 ± 11
Group OW	7	56 ± 12
Group OA	7	53 ± 12
Group W	7	53 ± 12
Group A	7	53 ± 12

Data values are presented as means ± SD
Group C, control group; group O, olprinone group; group OW, olprinone + wortmannin group; group OA, olprinone + atractyloside group; group W, wortmannin group; group A, atractyloside group

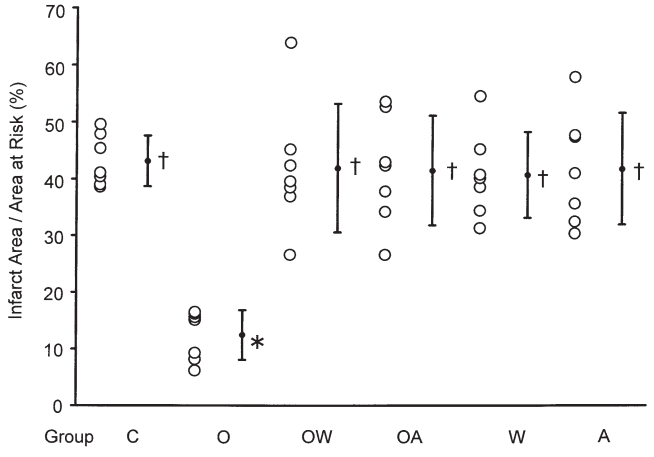

Fig. 2. Myocardial infarct sizes expressed as percentages of the left-ventricular area at risk. *Group C*, control group; *group O*, olprinone group; *group OW*, olprinone + wortmannin group; *group OA*, olprinone + atractyloside group; *group W*, wortmannin group; *group A*, atractyloside. *($P < 0.05$), significantly different from group C; †($P < 0.05$), significantly different from group O

that this cardioprotective effect could be caused by the activation of PI3K-Akt or by the inhibition of mPTP during early reperfusion. The present study results show that pretreatment with olprinone reduced infarct size to one-third of that in controls in a manner independent of the hemodynamic effect in the rat heart in vivo, and that this effect of olprinone was reversed by both wortmannin and atractyloside. The present results on the reduction of infarct size are consistent with previous studies. Sanada et al. [5] reported that olprinone reduced infarct size by 53% in dogs subjected to 30-min ischemia. Nomura et al. [17] reported that olprinone reduced infarct size by 55% in rats subjected to 20-min ischemia.

PDEIs could be used in patients showing down-regulation of beta-adrenergic receptors. Both beta-adrenergic receptor stimulation and the administration of PDEIs could confer cardioprotection through elevation of the cAMP level and activation of PKA [4,5,18]. Such this transient pre-ischemic activation of PKA inhibits Rho-A [12]. Rho-A is a serine-threonine kinase which mediates many important downstream effects of the small granosine triphosphate (GTP)-binding protein [19]. The activation of Rho-A inhibits both Akt and endothelial nitric oxide synthase activities [20]. Akt, a serine-threonine kinase, has been linked to enhanced cell survival [21]. Akt is activated downstream of PI3K, and has been closely observed to play an important role in protecting the heart against ischemic reperfusion injury [9]. Hausenloy et al. [7] demonstrated that ischemic preconditioning resulted in the phosphorylation of PI3K-Akt, and induced cardioprotection. In the present study, a PI3K-Akt inhibitor, wortmannin, inhibited the reduction of myocardial infarct size induced by olprinone. Thus, activation of the PI3K-Akt pathway could play an important role in olprinone-induced cardioprotection.

Furthermore, it was reported that the reduction of myocardial infarct size by ischemic or pharmacological preconditioning was inhibited by atractyloside, an mPTP channel opener, and it was suggested that the inhibition of mPTP during the first few minutes of reperfusion had a cardioprotective effect [11]. In the present study, the administration of atractyloside alone did not influence myocardial infarct size, while the olprinone-induced cardioprotection was reversed by the inhibition of mPTP. The core components of mPTP consist of the adenine nucleotide translocator in the inner mitochondrial-membrane, voltage-dependent anion channels on the outer membrane, and cyclophilin D located in the mitochondrial matrix [22]. The mPTP normally remains closed, but can open under conditions of cellular stress. The opening of mPTP could lead to a loss of mitochondrial membrane potential, swelling of the mitochondrial matrix, and eventually, rupture of the outer mitochondrial membrane and cell death. Because one of the factors involved in mPTP opening is the calcium ion, elevation of the mitochondrial matrix calcium ion concentration could contribute to the opening of the mPTP [23].

Hausenloy et al. [7,8] have reported that Akt is activated during the preconditioning phase and early phase of reperfusion, and this Akt activation during reperfusion is required for ischemic preconditioning. They suggested that the Akt activation during the preconditioning phase was the trigger for activation of the RISK pathway during the early phase of reperfusion. Therefore, we studied whether olprinone-induced pharmacological cardioprotection was reversed by the inhibition of PI3K-Akt and by the opening of mPTP during the early reperfusion phase. In this study, we have provided pharmacological evidence for the roles of PI3K-Akt and mPTP in the cardioprotection conferred by olprinone, but there was no molecular evidence to indicate that

olprinone activated PI3K-Akt and inhibited mPTP opening. Thus, there were no data to suggest that the preischemic administration of olprinone activated Akt before ischemia and during the reperfusion phase, as ischemic preconditioning does. Further study is needed to clarify the mechanisms of olprinone-induced cardioprotection.

In conclusion, the preischemic administration of olprinone leads to a reduction of myocardial infarct size, and this cardioprotective effect could involve the activation of PI3K-Akt and the inhibition of mPTP during the early reperfusion phase.

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