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PTEN inhibitors cause a negative inotropic and chronotropic effect in mice

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Abstract

Inactivation of phosphatase and tensin homologue deleted on chromosome ten (PTEN) decreases cardiac contractility under basal conditions and induces cardioprotection against ischemia-reperfusion injury. However, the pharmacological effect of PTEN inhibitors on cardiac contractility has not been studied before. In the present study, we investigated the hypothesis that PTEN inhibition decreases cardiac contractility in mice. We first exposed isolated mouse hearts to the PTEN inhibitor bpV(phen) (40 μ M), the phosphoinositide-3 kinase inhibitor wortmannin (1 μ M), and the PTEN-resistant PIP3 analog 3-phosphorothioate-PtdIns(3,4,5)P3 (3-PT-PTP, 0.5 μ M) for 10 min. Left ventricular pressure was measured by a Mikro-tip pressure catheter. We then inhibited PTEN in mice by intra-peritoneal injection of VO-OHpic (10 μ g/kg) 30 min before ischemia and then exposed them to 30 min of ischemia and 120 min of reperfusion. At the end of the experiments, hearts were isolated for measurement of myocardial infarct size by 1.5 % triphenyltetrazolium chloride. Left ventricular systolic pressure and heart rate were significantly decreased by bpV(phen). Consistent with the result, the maximal rate of left ventricular pressure increase or decrease was significantly decreased by bpV(phen). 3-PT-PIP3 mimicked the effect of bpV(phen), and the opposite effect on cardiac contractility was seen with wortmannin. Moreover, inhibition of PTEN in vivo by VO-OHpic decreased left ventricular systolic pressure and heart rate before ischemia, but resulted in an increase in cardiac functional recovery and a decrease in myocardial infarct size after ischemia-reperfusion. In conclusion, PTEN inhibition causes a negative inotropic and chronotropic effect while inducing cardioprotection against ischemia-reperfusion injury.

Keywords

PTEN; PI3K; cardiac contractility; reperfusion injury; myocardial infarction

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1. Introduction

Coronary artery disease is a common disease in developed countries, with many patients dying each year due to myocardial infarction (Lloyd-Jones et al., 2010). Deaths resulting from ischemia and reperfusion injury may be prevented with the development of novel cardioprotective agents. The phosphatase and tensin homologue deleted on chromosome ten (PTEN) has been reported to regulate cell growth and survival in the heart (Schwartzbauer and Robbins, 2001). The PTEN gene knockdown induces cardioprotection against ischemia and reperfusion injury in isolated mouse hearts (Ruan et al., 2009). PTEN inhibitors have been shown to generate similar cardioprotective effects; however, the pharmacological effects of PTEN inhibitors on cardiac hemodynamics are still not fully understood (Keyes et al., 2010).

Under basal conditions, PTEN is heavily phosphorylated and localized mainly in the cytoplasm. After dephosphorylation, PTEN moves to the plasma membrane where it removes the 3-phosphate of phosphatidylinositol-3,4,5-phosphate (PIP3) to produce PIP2, thereby acting as an antagonist of phosphoinositide-3 kinase (PI3K) (Oudit et al., 2004). PTEN inactivation increases intracellular PIP3 levels, resulting in activation of protein kinase B (or Akt) either directly or through PIP3-dependent kinase 1 (Sun et al., 1999). Akt has been shown to promote cell survival in various cell types including cardiomyocytes (Fujio et al., 2000; Matsui and Rosenzweig, 2005). PIP3 is very sensitive to PTEN at the plasma membrane (Das et al., 2003); however, its analog 3-phosphorothioate-PtdIns (3,4,5)P3 (3-PT-PIP3) is resistant to PTEN enzymatic activity and generates insulin-like effects (Zhang et al., 2006).

PTEN inhibitors are derivatives of vanadium (Rosivatz et al., 2006; Schmid et al., 2004). The active site of PTEN is a large and deep cleft. The PTEN inhibitors fit well into the cleft but are too large for other cysteine-based phosphatases (Lee et al., 1999; Schmid et al., 2004). They specifically inhibit PTEN activity in fibroblasts and activate Akt in cardiomyocytes (Keyes et al., 2010; Rosivatz et al., 2006). In the present study, our goal was to determine the effect of PTEN inhibitors on cardiac contractility and myocardial injury in mice exposed to ischemia and reperfusion. We found that PTEN inhibitors cause a negative inotropic and chronotropic effect with the mechanism most likely being through PIP3.

2. Materials and methods

2.1. Animals

All experiments were performed with male C57BL6 mice. At the time of the experiment, mice were 2 – 3 months old and weighed 21 – 25 g. All procedures were approved by the Johns Hopkins University Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Drugs

The following drugs were used. bpV(phen), potassium bisperoxo(1,10-phenanthroline)oxovanadate (V) from EMD inc. (San Diego, CA, USA); VO-OHpic (VO), vanadyl hydroxypicolinic acid 5-hydroxypyridine-2-carboxyl (a generous gift of Dr. Rosivatz Erika); 3-PT-PIP3, 3-phosphorothioate-PtdIns(3,4,5)P3 from Cayman Chemical Co. (Ann Arbor, MI, USA); wortmannin and other chemicals from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Mouse Langendorff preparation

Isolated mouse hearts were perfused as described previously (Cai et al., 2008b). Briefly, mice were anesthetized by intraperitoneal injection of pentobarbital (70 mg/kg). Hearts were isolated and perfused with Krebs-Henseleit buffer (in mM, glucose 17, NaCl 120, NaHCO₃ 25, CaCl₂ 2.5, KCl 5.9, MgSO₄ 1.2, and EDTA 0.5). Global ischemia was induced by cessation of perfusion, followed by reperfusion. bpV(phen), wortmannin, and 3-PT-PIP3 were added into the perfusion line right above the aorta through a syringe pump. The final concentration was obtained by adjustment of pump rate relative to coronary flow rate. The perfusion buffer was not circulated.

2.4. In-vivo ischemia and reperfusion mouse model

Mouse ischemia and reperfusion were induced as described previously (Cai et al., 2008a). Briefly, mice were anesthetized with pentobarbital (70 mg/kg). The left coronary artery was occluded about 1–2 mm below the left auricle. Reperfusion was accomplished by loosening the ligature. The PTEN inhibitor VO was administered by intra-peritoneal injection at the dosage of 10 µg/kg once 30 min before ischemia. Saline was used as control. At the end of the experiment, the animals were euthanized by transecting the aorta and removing the heart for infarct size determination.

2.5. Assessment of area at risk and infarct size

Myocardial infarct size was measured as described previously (Cai et al., 2008a). The heart was transected into five sections, incubated in 1.5 % triphenyltetrazolium chloride (TTC) for 15 min at 37 °C. In the left ventricle, infarcted myocardium (white), area at risk (red), and area at non-risk (blue) were measured by computerized planimetry (Image J, NIH, Bethesda, MD, USA). Myocardial infarct size was calculated as a percentage of area at risk: total weight of white tissue/total weight of white tissue plus red tissue × 100%.

2.6. Measurement of left ventricular pressure

After the heart was exposed in anesthetized mice or 15 minutes after initiation of perfusion of isolated hearts, a Mikro-tip catheter (SPR671, Millar Instruments, Houston, Texas, USA) was inserted into the left ventricle as described previously (Cai et al 2008a). Left ventricular pressure was directly measured with the Powerlab Data Acquisition System and displayed on a computer. Left ventricular systolic pressure and end diastolic pressure, heart rate, positive maximal left ventricular pressure derivative (+dp/dt_m), and negative maximal left ventricular pressure derivative (−dp/dt_m) were automatically calculated using Chart 5 software (ADInstruments, Colorado Springs, Colorado, USA).

2.7. Immunoblotting assay

Cardiac tissues were homogenized in lysis buffer (in mM: pH 7.5 Tris 20, NaCl 150, EDTA 1, EGTA 1, PMSF 1, Na₃VO₄ 1, 1% Triton). Proteins were detected by using primary antibodies, followed by horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence. Antibodies against p-Akt (S-473) and Akt were purchased from Cell Signaling Technology (Danvers, Massachusetts, USA).

2.8. Statistical analysis

Data are presented as mean ± standard error of the mean. Statistical comparisons were performed with the use of analysis of variance with Student-Newman-Keuls post hoc test. Differences were considered significant if $P < 0.05$.

3. Results

3.1. Cardiac contractility is decreased by bpV(phen) and 3-PT-PIP3 in isolated mouse hearts

Cardiac contractility is an indicator of cardiac function and is associated with oxygen consumption (Wesslén et al., 1992). To determine the effects of bpV(phen) on cardiac contractility, we added bpV(phen) (40 μ M) into the perfusion line for 10 min. Left ventricular pressure and heart rate were continuously measured in isolated mouse hearts. Compared with pre-treatment (S), bpV(phen) significantly decreased left ventricular systolic pressure (30 ± 2 vs. 62 ± 2 mm Hg), heart rate (302 ± 22 vs. 383 ± 11 beats/min), $+dp/dt_m$ (1137 ± 81 vs. 2310 ± 172 mm Hg/s), and $-dp/dt_m$ (594 ± 74 vs. 1648 ± 135 mm Hg/s), $n = 6$, $P < 0.01$; Fig. 1. Left ventricular end diastolic pressure was unchanged after bpV(phen) treatment (6 ± 0 vs. 6 ± 0 mm Hg; $n = 6$, $P > 0.05$, Fig. 1). Thus, bpV(phen) produced a negative inotropic and chronotropic effect. Since PIP3 is a target of PTEN, the effects of PTEN inhibition may result from increased PIP3. To determine whether 3-PT-PIP3 mimics the effects of bpV(phen), we treated isolated mouse hearts with 3-PT-PIP3 (0.5 μ M) for 10 min. 3-PT-PIP3 significantly decreased left ventricular systolic pressure (31 ± 4 vs. 55 ± 3 mm Hg, $P < 0.01$), heart rate (252 ± 18 vs. 338 ± 17 beats/min, $P < 0.05$), $+dp/dt_m$ (1149 ± 117 vs. 1839 ± 65 mm Hg/s, $P < 0.05$), and $-dp/dt_m$ (692 ± 85 vs. 1272 ± 61 mm Hg/s, $P < 0.05$); $n = 4$, Fig. 1. 3-PT-PIP3 had no effect on left ventricular end-diastolic pressure (2 ± 2 vs. 3 ± 2 mm Hg, $n = 4$, $P > 0.05$; Fig. 1).

3.2. Cardiac contractility is increased by wortmannin in isolated mouse hearts

PIP3 levels are decreased by wortmannin, a specific PI3K inhibitor (Higaki et al., 1996). To determine whether wortmannin produces a positive inotropic effect, we treated isolated mouse hearts with wortmannin for 10 min. Wortmannin significantly increased left ventricular systolic pressure (77 ± 3 vs. 52 ± 3 mm Hg, $P < 0.01$), $+dp/dt_m$ (2587 ± 182 vs. 2063 ± 239 mm Hg/s, $P < 0.05$), and $-dp/dt_m$ (1950 ± 116 vs. 1360 ± 172 mm Hg/s, $P < 0.05$); $n = 4$, Fig. 1. Wortmannin did not significantly alter heart rate (252 ± 24 vs. 341 ± 25 beats/min) or left ventricular enddiastolic pressure (4 ± 2 vs. 2 ± 0 mm Hg), $n = 4$, $P > 0.05$; Fig. 1.

3.3. PTEN inhibition results in Akt activation and induces cardioprotection in mice

Akt phosphorylation is increased by PTEN inactivation and PIP3 production (Sun et al., 1999). To determine whether PTEN inhibition increases Akt activity and induces cardioprotection, we pretreated mice with VO, considered as the most potent PTEN inhibitor, for 30 min (Rosivatz et al., 2006). Myocardium from the left ventricle was collected for measurement of p-Akt and total Akt. P-Akt was significantly increased in the heart (145 ± 10 vs. 76 ± 8 AU, $n = 4$, $P < 0.01$; Fig. 2A and 2B). Then, we exposed VO-treated mice to 30 min of ischemia, followed by 120 min of reperfusion. At the end of the experiment, myocardial infarct size was measured by TTC. Myocardial infarct size was significantly decreased in VO-treated mice (25 ± 6 vs. 56 ± 5 %, $n = 7$, $P < 0.01$; Fig. 3A and 3B). There was no difference in the area at risk between these two groups (46 ± 3 vs. 57 ± 3 %, $n = 7$, $P > 0.05$; Fig. 3A and 3B).

3.4. PTEN inhibition causes a negative inotropic and chronotropic effect before ischemia but increases cardiac functional recovery after ischemia-reperfusion in mice

To determine the effect of PTEN inhibition on cardiac contractility, we monitored the electrocardiogram and left ventricular pressure after treatment with either VO or CON. VO significantly decreased left ventricular systolic pressure (60 ± 3 vs. 70 ± 2 mm Hg, $n = 7$, $P < 0.01$; Fig. 4A), heart rate (349 ± 12 vs. 427 ± 27 beats/min, $n = 7$, $P < 0.01$; Fig. 4B), and

+dp/dt_m (4061 ± 215 vs. 4818 ± 316 mm Hg/s, n = 7, P < 0.05; Fig. 4C). Ischemia did not cause a further decrease in heart rate or left ventricular systolic pressure in VO-treated mice. After 30 min of ischemia, the difference in heart rate and left ventricular systolic pressure between these two groups was eliminated and a significant increase in +dp/dt_m was seen in the VO-treated mice (4944 ± 376 vs. 3655 ± 101 mm Hg/s, n = 7, P < 0.01; Fig. 4C). After reperfusion, heart rate and cardiac contractility declined over time in control mice. In contrast to these changes, VO-treated mice had better functional recovery. At the end of the 2 hour-reperfusion period, VO treatment led to a significant increase in left ventricular systolic pressure (65 ± 4 vs. 53 ± 4 mm Hg, n = 7, P < 0.05; Fig. 4A), heart rate (476 ± 26 vs. 381 ± 19 beats/min, n = 7, P < 0.05; Fig. 4B), +dp/dt_m (4432 ± 198 vs. 2765 ± 333 mm Hg/s, n = 7, P < 0.01; Fig. 4C), and -dp/dt_m (3699 ± 331 vs. 1921 ± 401 mm Hg/s, n = 7, P < 0.01; Fig. 4C) and caused a significant decrease in left ventricular end-diastolic pressure (-6 ± 2 vs. 2 ± 3 mm Hg, n = 7, P < 0.05; Fig. 4A).

4. Discussion

We report that PTEN inhibitors decrease cardiac contractility in mice, and that 3-PT-PIP3 mimics the effect and wortmannin has the opposite effect of PTEN inhibitors. Therefore, our study suggests that PTEN inhibition causes a negative inotropic and chronotropic effect through PIP3. Moreover, we have shown that the negative inotropic and chronotropic effect of PTEN inhibitors is associated with decreased myocardial ischemia-reperfusion injury. Since a reduction in oxygen consumption helps the heart tolerate ischemia, reducing cardiac contractility may contribute to PTEN inhibitor-mediated cardioprotection.

Muscle-specific PTEN knock-out mice have been shown to have decreased cardiac contractile function; however, the underlying mechanism is not fully understood (Crackower et al., 2002). Cardiac contractility is dependent on intracellular calcium, which is regulated by activity of Gs protein-coupled receptors. After stimulating β1 adrenoceptors, Gα subunit activates adenylate cyclase to catalyze ATP into cAMP, leading to activation of cAMP-dependent kinase (PKA). PKA has been shown to increase cardiomyocyte contractility by phosphorylating phospholamban in the sarcoplasmic reticulum (Soto et al., 2009). Moreover, PKA activity is modulated by phosphodiesterase 4 (PDE4). PDE4 inhibition has been shown to increase myocyte contraction (Soto et al., 2009). It has been reported that cAMP levels are downregulated in PTEN-deficient mice (Crackower et al., 2002). However, it is not known how PTEN deletion leads to a decrease in cAMP in cardiomyocytes. In the present study, we have demonstrated that PIP3 directly decreases cardiac contractility in isolated mouse hearts, suggesting that PTEN inactivation may cause a negative inotropic effect through PIP3. The results are further supported by evidence that the PI3K inhibitor wortmannin increases cardiac contractility, and that Akt downstream of PIP3 is activated by PTEN inhibition in the myocardium. Interestingly, PIP3 has been shown to recruit PDE4 and thereby provide functional proximity to cAMP (Björge et al., 2010). Therefore, the decrease in cAMP levels may be caused by increased intracellular PIP3 in muscle-specific PTEN knock-out mice.

Although PTEN inhibition causes a negative effect on heart rate and left ventricular systolic pressure, decreased cardiac contractility is not always associated with insufficiency of blood supply (Wesslén et al., 1992). β adrenoceptor blockers are negative inotropes and chronotropes, but they are widely known as cardioprotective agents against myocardial infarction recurrence and sudden death (Bangalore et al., 2007). In coronary artery disease, blood supply to the myocardium is commonly compromised. Because oxygen demand is associated with cardiac contractility, a decrease in cardiac contractility can increase tolerance to ischemia (Pepine et al., 1988). Furthermore, the powerful endogenous cardioprotective maneuver ischemic preconditioning, one or several brief periods of

ischemia and reperfusion, induces myocardial stunning and protects the heart from later prolonged ischemia (Cai et al., 2008b). Although different molecular mechanisms may underlie β adrenoceptor blocker-induced cardioprotection and ischemic preconditioning, they both generate a negative inotropic effect, which increases resistance to ischemic injury. From this perspective, decreased cardiac contractility may contribute to PTEN inhibitor-mediated cardioprotection against ischemia-reperfusion injury.

In conclusion, PTEN inhibitors produce a negative inotropic and chronotropic effect, which is likely mediated by PIP3. PTEN inhibition induces a state in which the heart is resistant to ischemic injury. Therefore, PTEN inhibitors may have the potential to be a new class of cardioprotective agents.

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References

- Bangalore S, Messerli FH, Kostis JB, Pepine CJ. Cardiovascular protection using beta-blockers: a critical review of the evidence. *J Am Coll Cardiol.* 2007; 50:563–572. [PubMed: 17692739]
- Björge E, Solheim SA, Abrahamsen H, Baillie GS, Brown KM, Berge T, Okkenhaug K, Houslay MD, Taskén K. Cross-talk between phosphatidylinositol 3-kinase and cyclic AMP (cAMP)-protein kinase a signaling pathways at the level of a protein kinase B/beta-arrestin/cAMP phosphodiesterase 4 complex. *Mol Cell Biol.* 2010; 30:1660–1672. [PubMed: 20086095]
- Cai ZP, Shen Z, Van Kaer L, Becker LC. Ischemic preconditioning-induced cardioprotection is lost in mice with immunoproteasome subunit low molecular mass polypeptide-2 deficiency. *FASEB J.* 2008; 22:4248–4257. [PubMed: 18728217]
- Cai ZP, Zhong H, Bosch-Marce M, Fox-Talbot K, Wang L, Wei C, Trush MA, Semenza GL. Complete loss of ischaemic preconditioning-induced cardioprotection in mice with partial deficiency of HIF-1 alpha. *Cardiovasc Res.* 2008; 77:463–470. [PubMed: 18006459]
- Crackower MA, Oudit GY, Kozieradzki I, Sarao R, Sun H, Sasaki T, Hirsch E, Suzuki A, Shioi T, Irie-Sasaki J, Sah R, Cheng HY, Rybin VO, Lembo G, Fratta L, Oliveirados-Santos AJ, Benovic JL, Kahn CR, Izumo S, Steinberg SF, Wymann MP, Backx PH, Penninger JM. Regulation of myocardial contractility and cell size by distinct PI3K-PTEN signaling pathways. *Cell.* 2002; 110:737–749. [PubMed: 12297047]
- Das S, Dixon JE, Cho W. Membrane-binding and activation mechanism of PTEN. *Proc Natl Acad Sci U S A.* 2003; 100:7491–7496. [PubMed: 12808147]
- Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation.* 2000; 101:660–667. [PubMed: 10673259]
- Higaki M, Sakaue H, Ogawa W, Kasuga M, Shimokado K. Phosphatidylinositol 3-kinase-independent signal transduction pathway for platelet-derived growth factor-induced chemotaxis. *J Biol Chem.* 1996; 271:29342–6. [PubMed: 8910596]
- Keyes KT, Xu J, Long B, Zhang C, Hu Z, Ye Y. Pharmacological inhibition of PTEN limits myocardial infarct size and improves left ventricular function postinfarction. *Am J Physiol.* 2010; 298:H1198–1208.
- Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, Dixon JE, Pandolfi P, Pavletich NP. Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell.* 1999; 99:323–334. [PubMed: 10555148]
- Lloyd-Jones, et al. American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart disease and stroke statistics--2010 update: a report from the American Heart Association. *Circulation.* 2010; 121:e46–e215. [PubMed: 20019324]

- Matsui T, Rosenzweig A. Convergent signal transduction pathways controlling cardiomyocyte survival and function: the role of PI 3-kinase and Akt. *J Mol Cell Cardiol.* 2005; 38:63–71. [PubMed: 15623422]
- Oudit GY, Sun H, Kerfant BG, Crackower MA, Penninger JM, Backx PH. The role of phosphoinositide-3 kinase and PTEN in cardiovascular physiology and disease. *J Mol Cell Cardiol.* 2004; 37:449–471. [PubMed: 15276015]
- Pepine CJ, Hill JA, Imperi GA, Norvell N. Beta-adrenergic blockers in silent myocardial ischemia. *Am J Cardiol.* 1988; 61:18B–21B.
- Petersen JW, Felker GM. Inotropes in the management of acute heart failure. *Crit Care Med.* 2008; 36:S106–111. [PubMed: 18158469]
- Rosivatz E, Matthews JG, McDonald NQ, Mulet X, Ho KK, Lossi N, Schmid AC, Mirabelli M, Pomeranz KM, Erneux C, Lam EW, Vilar R, Woscholski R. A small molecule inhibitor for phosphatase and tensin homologue deleted on chromosome 10 (PTEN). *ACS Chem Biol.* 2006; 1:780–790. [PubMed: 17240976]
- Ruan H, Li J, Ren S, Gao J, Li G, Kim R, Wu H, Wang Y. Inducible and cardiac specific PTEN inactivation protects ischemia/reperfusion injury. *J Mol Cell Cardiol.* 2009; 46:193–200. [PubMed: 19038262]
- Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxovanadium compounds are potent PTEN inhibitors. *FEBS Lett.* 2004; 566:35–38. [PubMed: 15147864]
- Schwartzbauer G, Robbins J. The tumor suppressor gene PTEN can regulate cardiac hypertrophy and survival. *J Biol Chem.* 2001; 276:35786–35793. [PubMed: 11448956]
- Soto D, De Arcangelis V, Zhang J, Xiang Y. Dynamic protein kinase activities induced by beta-adrenoceptors dictate signaling propagation for substrate phosphorylation and myocyte contraction. *Circ Res.* 2009; 104:770–779. [PubMed: 19213958]
- Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, Gavrilova N, Mueller B, Liu X, Wu H. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. *Proc Natl Acad Sci U S A.* 1999; 96:6199–6204. [PubMed: 10339565]
- Wesslén O, Hallhagen S, Ekroth R, Jagenburg R, Joachimsson PO, Nordgren L, Nyström SO, Tydén H. High-dose intravenous beta 1-blockade in patients early after cardiac operations. Negative inotropism versus myocardial oxygen economy. *J Thorac Cardiovasc Surg.* 1992; 104:1672–1678. [PubMed: 1453732]
- Zhang H, Markadiou N, Beauwens R, Erneux C, Prestwich GD. Synthesis and biological activity of PTEN-resistant analogues of phosphatidylinositol 3,4,5-trisphosphate. *J Am Chem Soc.* 2006; 128:16464–16465. [PubMed: 17177371]

Fig. 1. Cardiac contractility is decreased by bpV(phen) and 3-PT-PIP3 but increased by wortmannin in isolated hearts. Isolated mouse hearts were perfused for 15 min prior to treatment with bpV(phen), 3-PT-PIP3, or wortmannin. Data were collected at the start (S) and end (E) time points of the experiments. A: Representative left ventricular pressure (LVP) recordings from each treatment of bpV(phen) (n = 6), 3PT-PIP3 (n = 4), or wortmannin (n = 4). B–E: bpV(phen) decreased left ventricular systolic pressure (LVSP) (B), heart rate (C), $+dp/dt_m$ (D), and $-dp/dt_m$ (E). 3-PT-PIP3 mimicked the effects.

However, wortmannin increased LVSP, $+dp/dt_m$, and $-dp/dt_m$. *: $P < 0.01$ vs. S, #: $P < 0.05$ vs. S.

Fig. 2.

VO increases Akt phosphorylation in the myocardium. Mice were treated with either VO or saline (CON) by intra-peritoneal injection. Cardiac tissues were collected before ischemia.

A: Representative Western blots from each treatment of CON or VO, n = 4. B: Akt phosphorylation was increased in VO-treated hearts. N = 4, *: P < 0.01 vs. CON.

Fig. 3. VO decreases myocardial infarct size after ischemia-reperfusion. Mice were exposed to 30-min ischemia and 120-min reperfusion (I-30/R-120) after treatment with VO or CON. A: Representative cardiac sections from CON or VO group, $n = 7$. B: Myocardial infarct size was decreased in VO-treated mice. $N = 7$, *: $P < 0.01$ vs. CON. IS, infarct size; AAR, area at risk.

Fig. 4. VO decreases cardiac contractility before ischemia but improves cardiac functional recovery after ischemia-reperfusion. Mice were treated with VO or CON 30 min before ischemia, followed by I-30/R-120. A: VO decreased LVSP but not end-diastolic pressure (EDP) before ischemia. However, after I/R, VO increased LVSP in mice. B and C: VO treatment decreased heart rate and $+dp/dt_m$ before ischemia but increased them after ischemia. At the end of the experiment, VO increased both $+dp/dt_m$ and $-dp/dt_m$. N = 7, *: P < 0.01 vs. CON, #: P < 0.05 vs. CON.