Cytosolic Phospholipase $A_2\alpha$ Protects against Ischemia/Reperfusion Injury in the Heart

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Abstract

Studies with $sPLA_2$ Group X, and $cPLA_2\alpha$ gene-targeted mice suggest that absence of $sPLA_2$ Group X results in protection from ischemia/reperfusion (I/R) injury in the heart, and absence of $cPLA_2\alpha$ Group IV is protective in the brain. Although latter studies might suggest a similar deleterious role for $cPLA_2\alpha$ in I/R injury in the heart, the pathophysiology of stroke is intricately related to excitotoxicity and cannot necessarily be extrapolated to the heart. We report here that unlike findings in the brain, $cPLA_2\alpha^{(-/-)}$ mice have exaggerated injury following I/R *in vivo*. In contrast, there is no difference in injury induced by simulated ischemia in cardiomyocytes isolated from $cPLA_2\alpha^{(-/-)}$ versus $cPLA_2\alpha^{(+/+)}$ mice. This suggests that $cPLA_2\alpha$ does not have an important cardiomyocyte autonomous effect on ischemic injury. Prostaglandin E_2 (PGE_2) levels are significantly reduced in the hearts of the $cPLA_2\alpha^{(-/-)}$ mice, and the enhanced injury is ameliorated by treatment with the PGE analog, misoprostol. We demonstrate that $cPLA_2\alpha$ is cardioprotective *in vivo*, and this is likely via $cPLA_2\alpha$-mediated production of cardioprotective eicosanoids. These studies are the first to identify a protective role for $cPLA_2$ in I/R injury in any organ and raise concerns over long-term inhibition of $cPLA_2$. Clin Trans Sci 2011; Volume 4: 236–242

Keywords: ischemia-reperfusion injury, cytosolic phospholipase A_2alpha, prostaglandin E2, cell death

Introduction

Phospholipases A_2 catalyze the release of arachidonic acid (AA) from the sn-2 position of phospholipids, thereby producing free AA and lysophospholipids.[1–4] There are three classes of these enzymes based on structure, cofactor requirements, and function. Secretory phospholipases A_2 ($sPLA_2$) are low molecular weight enzymes that contain an N-terminal secretion signal peptide. They are believed to play important roles in inflammatory processes, but roles in the heart are less clear. DeWindt et al. reported no effect of deletion of $sPLA_2$ IIA on ischemic injury in an isolated heart model.[5] However, Nijmeijer et al. reported that $sPLA_2$s bound to ischemic cardiomyocytes and contributed to cell death via both direct cytotoxic effects and by facilitating inflammatory responses.[6] In addition, antibodies directed against $sPLA_2$ have been reported to be effective against ischemia/reperfusion (I/R) injury.[7] Recently, Fujioka et al. have clarified the role of one subgroup of $sPLA_2$, Group X, in I/R. Deletion of the gene encoding $sPLA_2$ Group X, which is exclusively expressed in neutrophils, significantly decreased infarct size following I/R.[8] Thus $sPLA_2$ X may mediate many of the pathologic consequences of neutrophil infiltration into the infarct zone. Recently, two clinical trials with a nonselective $sPLA_2$ inhibitor, varespladib, have been completed, one in patients with acute coronary syndromes and the other in patients going to percutaneous coronary intervention.[9,10] Neither of these trials were positive. A third trial is recruiting patients with vaso-occlusive crisis from sickle cell disease. Results of a trial with varespladib examining plasma lipoproteins was recently completed and suggested usage of the agent in prevention of atherosclerosis.[11]

The calcium-independent $iPLA_2$ family consists of two isoforms, $iPLA_2\beta$ and γ. $iPLA_2$ activity has been reported to account for 80% of total PLA_2 activity in the normal myocardium, and the enzyme rapidly associates with the membrane following ischemia.[12,13] Furthermore, the putative $iPLA_2$-selective inhibitor, bromo-enol lactone, was protective in the ischemic isolated, perfused heart.[14] Of note, transgenic mice over-expressing $iPLA_2\beta$ developed lethal ventricular tachyarrhythmias with ischemia, and this was associated with a marked increase in lysophospholid release.[15] These authors also reported that deletion of $iPLA_2\gamma$, which is localized predominantly to mitochondria and peroxisomes, led to profound abnormalities in bioenergetic mitochondrial function that resulted in increased mortality in response to myocardial stressors, including thoracic aortic constriction.[16]

The calcium-dependent cytosolic phospholipase A_2 ($cPLA_2$) family consists of three members, α, β, and γ. The catalytic activity of the enzymes is calcium-independent but submicromolar concentrations of intracellular calcium are necessary for the enzyme's translocation to sites of activity (e.g., Golgi, endoplasmic reticulum and nuclear membrane), mediated by the calcium and lipid binding domain.[4] Activity is modulated by phosphorylation by members of the mitogen-activated protein kinase family, particularly p38-MAPK, making $cPLA_2$s responsive to extracellular signals.[17,18] Based primarily on studies performed in the $cPLA_2\alpha$ knockout mouse, $cPLA_2\alpha$ has been implicated as central to the production of eicosanoids including prostaglandins (e.g., PGE_2 and PGI_2) and leukotrienes as well as platelet-activating factor.[2,3,19,20] This is due in part to the fact that $cPLA_2$s, as opposed to other PLA_2s, are relatively selective for AA (the precursor of eicosanoids) at the *sn*-2 position of phospholipids. $cPLA_2$ is also known to activate neutrophils, and prostaglandin production in these cells relates to NADPH oxidase activation.[21]

Reduced production of these signaling intermediates likely leads to the many phenotypes identified in the $cPLA_2\alpha^{(-/-)}$ mice, including enhanced recovery from allergen-induced bronchoconstriction, reduced injury following administration of a dopaminergic-selective neurotoxin, less severe anaphylaxis,

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acute lung injury, and arthritis, and reduced intestinal polyposis in a mouse model of hereditary colon cancer.[2,3,22–25] cPLA$_2$α has also been demonstrated to play a role in cardiomyocyte hypertrophy induced by aortic constriction.[26] Indeed, the broad group of disease states mediated, at least in part, by cPLA$_2$α, has made the enzyme a target of drug development, albeit with little success to date.

Of most relevance to this paper, however, the cPLA$_2$α$^{(-/-)}$ mouse was markedly protected from ischemic injury of the brain.[19,27] Although as noted, this process is importantly driven by excitotoxicity, we reasoned that the cPLA$_2$α KO mouse might also be protected from I/R injury in the heart. To our knowledge, no studies to date have addressed this issue *in vivo*, and those that have examined the issue *in vitro*, employed inhibitors with variable selectivity. The studies *in vitro* have led to the conclusion that cPLA$_2$α inhibition is protective.[28,29]

Herein, we examine the role of cPLA$_2$ in I/R injury in the heart. We employ the cPLA$_2$α$^{(-/-)}$ mouse to determine the response to I/R *in vivo* and also examine simulated ischemia in cardiomyocytes isolated from the mouse. Our studies identify a complex role for cPLA$_2$α but, surprisingly, the net effect *in vivo* of cPLA$_2$α deletion is worsened I/R injury. These studies suggest that while cPLA$_2$ inhibitors may be of value in stroke and many inflammatory disease states, they may be detrimental in the setting of ischemic heart disease. Given the common scenario of patients with coronary artery disease and coexisting cerebrovascular or inflammatory diseases, our findings raise theoretical concerns about the concept of cPLA$_2$ inhibition as a long-term therapeutic strategy.

Methods

Animals

Experiments were carried out according to National Institutes of Health Guidelines on the Use of Laboratory Animals, and all procedures were approved by the Thomas Jefferson University Committee on Animal Care. cPLA$_2$ gene-targeted mice were generated and bred as previously described.[19]

Determination of blood pressure in conscious, unrestrained mice

Mice were anesthetized with ketamine (50 mg/kg i.p.) and xylazine (2.5 mg/kg i.p.). A fluid-filled catheter (DSI Instruments, St. Paul, MN, USA) was inserted into the left carotid artery, and the transducer with battery was placed in the subcutaneous layer of the subscapular region. Mice were allowed to recover and 4 days later, blood pressure was measured via telemetry in conscious, unrestrained animals on two successive days. Measurements were made in all mice at approximately the same time of day to minimize diurnal variations in blood pressure.

In vivo I/R

Surgical procedures were performed as previously described.[30,31] Briefly, mice (8–10 weeks old) were anesthetized with 2% isoflurane inhalation. The heart was exposed and exteriorized through a left thoracotomy at the level of the fifth intercostal space. A slipknot was made around the left anterior descending (LAD) coronary artery 1–2 mm from its origin with a 6–0 silk suture. Sham-operated animals were subjected to the same surgical procedures except that the suture was passed under the LAD but was not tied. Following 30 minutes of ischemia, the slipknot was released and the myocardium was reperfused for 24 hours. A single dose of buprenorphine (0.3 mg/kg s.c.) was administered for pain treatment. At the end of the experiment, animals were euthanized by CO_2 asphyxiation. For studies with the specific PGE_2 analogue, misoprostol (13820, Cayman Chemical, Ann Arbor, MI, USA) compound was administered into the peritoneum at a dose of 150 μg/kg/day (two injections of 75 μg/kg in 0.2 cc of saline) during the 3 days before and during the I/R protocol.

Determination of area at risk and infarct size

Infarct size was determined as previously described.[30,31] Briefly, at the end of the 24-hour reperfusion period, mice were reanesthetized and the ligature around the LAD was retied through the previous ligation site. Following this, 2% Evans blue dye was injected, the heart was then quickly excised, and cut into five sections from apex to base. Sections were then incubated in a 1% 2,3,5-triphenyltetrazolium chloride (TTC, Sigma, St. Louis, MO, USA) solution and digitally photographed. The area not at risk (ANAR, Evan's blue-stained area), and the area at risk (AR, including both TTC-positive [noninfarct] and TTC-negative [infarct] staining area) were measured using the computer-based image analyzer SigmaScan Pro 5.0 (SPSS Science, Chicago, IL, USA). Myocardial infarct size was expressed as a percentage of the AR (I/AR), and the AR was expressed as the percentage of total left ventricle (AR/[AR+ANAR]).

TUNEL analysis

For quantification of cell death, we employed terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL); we used a kit from Chemicon (Billerica, MA, USA) according to the manufacturer's instructions. Images were viewed with a Nikon Eclipse 80i microscope (Nikon Inc., Melville, NY, USA). NIS elements software was used to record immunofluorescence images.

Evaluation of myocardial neutrophil infiltration

Paraformaldehyde-fixed portions of the heart were embedded in paraffin and cut into 5-μM thick sections. The neutrophil clone 7/4 (Abcam, Cambridge, UK) primary antibody was added in blocking solution (2% BSA, 0.2% horse serum in PBS supplemented with 0.2% NP-40) and incubated overnight. Vectastin Elite ABC kit (Vector Laboratories, Burlingame, CA, USA) followed by DAB Plus Kit (Invitrogen, Carlsbad, CA, USA) were used. NIS elements software was used to record images. For determination of number of neutrophils in the infarct area, neutrophils were counted by an observer blinded to the genotype.

Immunoblotting

Heart tissue lysates were matched for protein concentration, loaded on SDS-Page (10–15%) and transferred to nitrocellulose membranes (Whatman, Kent, UK). The membranes were then blocked with 5% nonfat milk for 1 hour and incubated overnight with primary antibodies as indicated. The following day, membranes were washed three times and incubated with appropriate secondary antibody for 1 hour at room temperature. Protein expression levels were quantified by determining band density with Quantity One analysis application from Bio-Rad (Hercules, CA, USA). The signals were normalized to that of glyceraldehyde 3-phosphate dehydrogenase to correct for potential differences in loading.

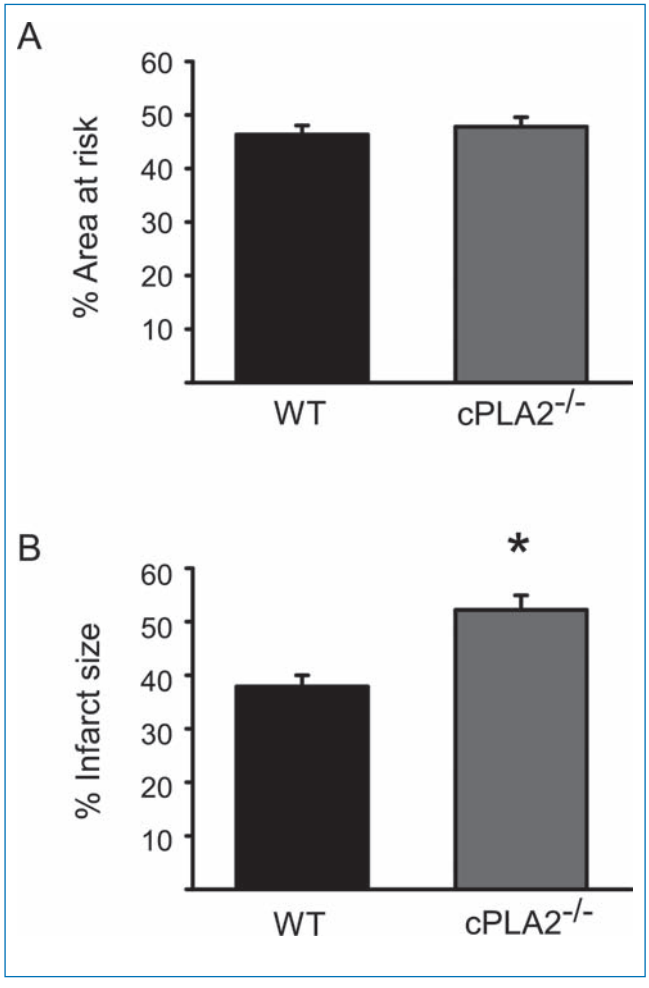


Figure 1. Deletion of cPLA$_2$α increases cardiac I/R injury *in vivo*. WT and cPLA$_2$α$^{(-/-)}$ mice were subjected to I/R as described in the Materials and Methods section. Area at risk was then determined by Evans Blue dye and infarct size by TTC staining. (A) Quantification of area at risk. (B) Quantification of infarct size expressed as a percent of area at risk. n = 12 WT and 13 cPLA$_2$α$^{(-/-)}$. *$p < 0.001$.

Simulated ischemia in isolated adult mouse cardiomyocytes

Adult mouse myocytes were isolated from wild-type (WT) and cPLA$_2$α$^{(-/-)}$ mice as previously described.[32,33] Simulated ischemia (termed metabolic inhibition with anoxia) was provoked by incubating the cells in hypoxia buffer (modified Krebs buffer [137-mM NaCl, 3.8-mM KCl, 0.49-mM $MgCl_2$, 0.9-mM $CaCl_2$, 4.0-mM Hepes] supplemented with 20-mM sodium lactate, 2.75-mM 2-deoxyglucose, 12-mM KCl, 1-mM sodium dithionate at pH 6.7) for 45 minutes.[34,35] Thereafter, cells were incubated in normal growth medium for rest of the experiment. Cell death was evaluated by measuring adenylate kinase release 16 hours following the hypoxia.

Measurement of PGE$_2$ levels

After surgery, hearts were divided into control and ischemic regions, and snap frozen in liquid nitrogen. Harvested heart tissues were homogenized in cold PBS, centrifuged 5 minutes at 300 rpm and supernatants were used for protein analysis and PGE$_2$ assays. PGE$_2$ assay was conducted according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Assay was performed by using 10 μg of protein per reaction, which was in the range providing the maximum sensitivity for the assay. Each assay sample as well as the PGE$_2$ standards were measured in triplicates. PGE$_2$ values were determined by using the standard curve.

Statistical analysis

All values in the text and figures are presented as mean ± standard error of mean of independent experiments from given n-sizes. Statistical significance of multiple treatments was determined by Student t-test, one-way or two-way analysis of variance followed by the Bonferroni *post-hoc* test when appropriate. P values < 0.05 were considered significant.

Results

Telemetry in conscious animals

We first asked whether there were differences in basal heart rate or blood pressure between cPLA$_2$α$^{(-/-)}$ mice and littermate WT cPLA$_2$α$^{(+/+)}$ mice. These studies had previously been performed in anesthetized mice and had shown no differences. We measured these parameters via radio-telemetry in conscious, unrestrained mice with indwelling pressure transducers and, again, found no differences in either systolic blood pressure (cPLA$_2$α$^{(+/+)}$: 125 ± 10 mmHg; cPLA$_2$α$^{(-/-)}$: 120 ± 15 mmHg) or heart rate (cPLA$_2$α$^{(+/+)}$: 466 ± 10; cPLA$_2$α$^{(-/-)}$: 472 ± 52; n = 3 mice per genotype).

Determination of area at risk and infarct size

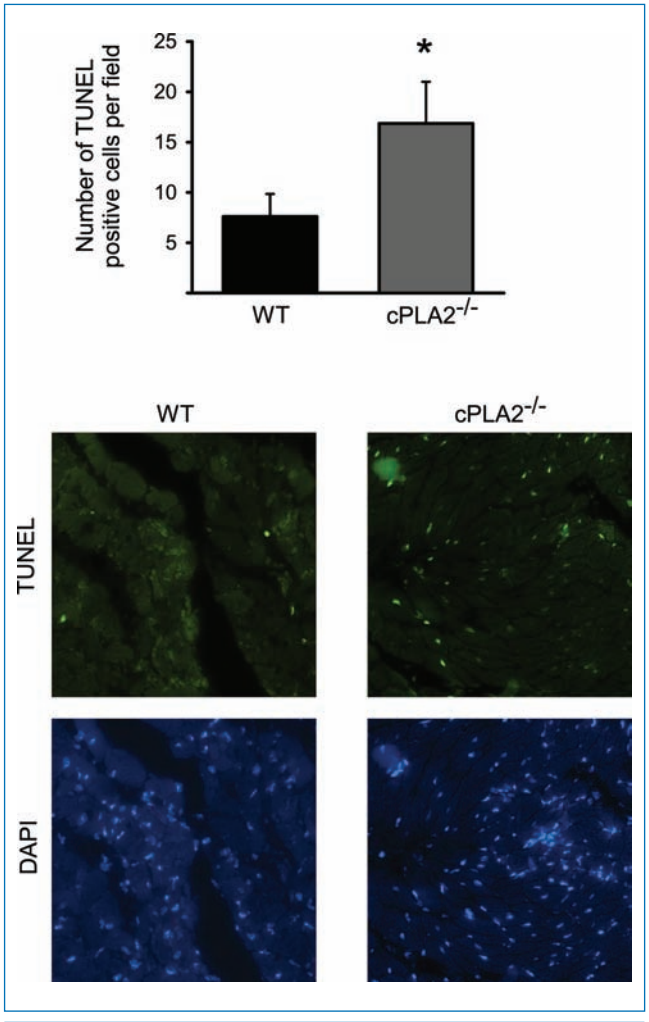
We then subjected cPLA$_2$α$^{(-/-)}$ and littermate cPLA$_2$α$^{(+/+)}$ mice to 30 minutes of ischemia followed by 24 hours of reperfusion. While area at risk, as determined by Evans Blue dye injection, was not different between the genotypes (*Figure 1A*), infarct size, as determined by TTC staining, was significantly increased in the cPLA$_2$α$^{(-/-)}$ mice (37.9 ± 7.3% vs. 52.2 ± 9.8%, *Figure 1B*, $P < 0.001$).

Analysis of cell death and neutrophil infiltration

Cell death, as determined by TUNEL staining, was increased in the ischemic zone of left ventricles in both genotypes at 6 hours post-I/R, but, consistent with the infarct size data, the number of TUNEL-positive cells was higher in cPLA$_2$α$^{(-/-)}$ animals (*Figure 2A*, $P < 0.05$). Of note (and not surprisingly, given the known role of cPLA$_2$α in neutrophil function), enhanced neutrophil infiltration into the ischemic zone did not appear to be the cause of the increased injury since neutrophil number was reduced by 29% in the ischemic zone of hearts of cPLA$_2$α$^{(-/-)}$ mice compared to the cPLA$_2$α$^{(+/+)}$ mice (*Figure 2B*).

Activation of intracellular signaling pathways

We next studied possible involvement of intracellular signaling pathways in mediating the protective effects of cPLA$_2$α. Protein extracts of ischemic zones from hearts of cPLA$_2$α$^{(-/-)}$ and cPLA$_2$α$^{(+/+)}$ mice were analyzed at 30 minutes, 90 minutes, 3 hours, 6 hours, or 24 hours following I/R injury. To our surprise, the only notable difference found was decreased extracellular signal-regulated kinase (ERK) phosphorylation in hearts of cPLA$_2$α$^{(-/-)}$ mice at 3 hours post-I/R (*Figure 3*, $P < 0.05$). However, there was no difference in activity of other major protective or detrimental signaling pathways (such as Akt, Stat3, p38, or JNK) between the genotypes at any of the time points (data not shown).



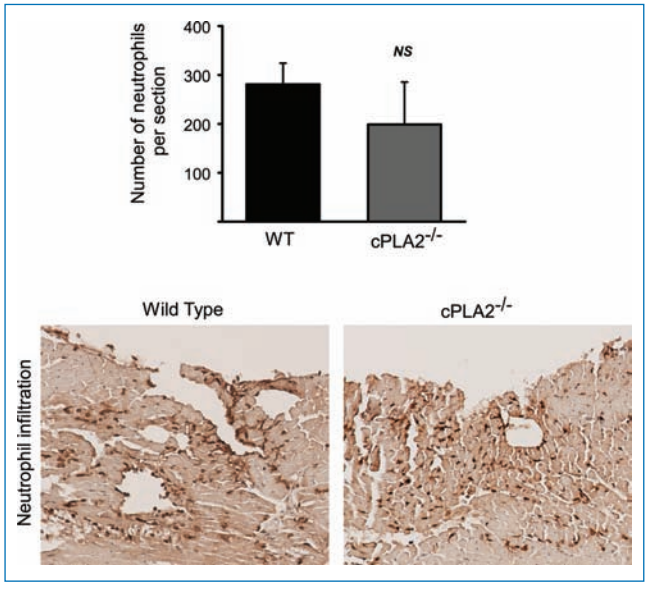


Figure 2. I/R-induced apoptosis and neutrophil infiltration in the WT versus cPLA$_2$α$^{(-/-)}$ mouse. WT and cPLA$_2$α$^{(-/-)}$ mice were subjected to I/R injury as described in the Materials and Methods. At 6 hours of reperfusion, hearts were collected and processed for (A) TUNEL or (B) labeling for infiltrating neutrophils as described in Materials and Methods. n = 9 WT and 7 cPLA$_2$α$^{(-/-)}$ hearts. Between three and seven fields per heart were analyzed, and values are expressed as the average of the number of positive cells per field. Representative images are shown below each graph. *$p < 0.05$ as determined by Mann-Whitney test. NS = nonsignificant.

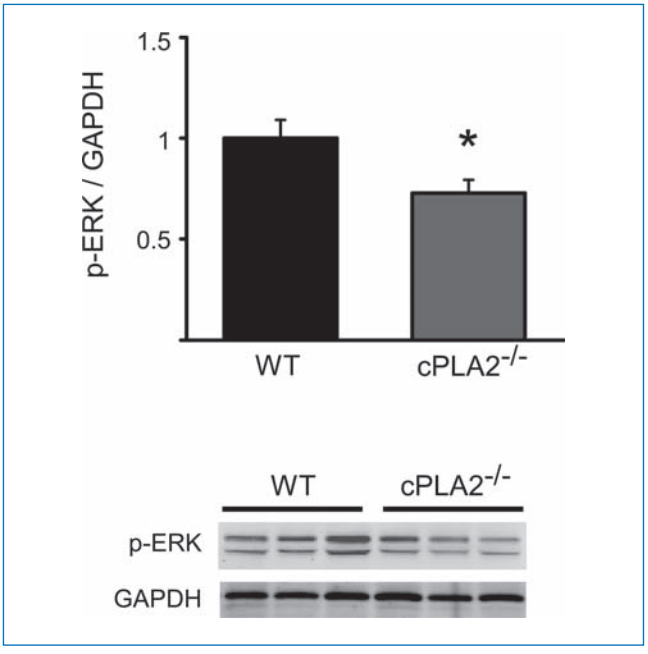


Figure 3. ERK phosphorylation following I/R in the WT versus cPLA$_2$α$^{(-/-)}$ mouse. WT and cPLA$_2$α$^{(-/-)}$ mice were subjected to I/R injury, and heart tissue from ischemic area was collected at 3 hours of reperfusion. Shown is quantification of ERK phosphorylation (upper panel) and representative western blot (lower panel). n = 5 WT and 5 cPLA$_2$α$^{(-/-)}$ hearts. *$p < 0.05$.

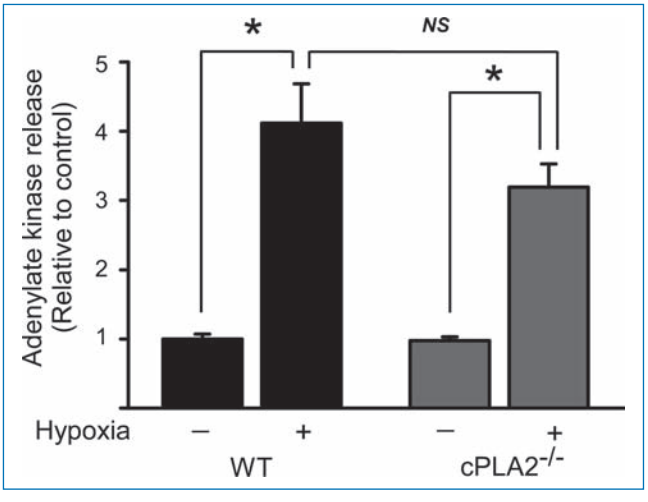


Figure 4. Cell death following simulated ischemia in isolated cardiomyocytes from WT versus cPLA$_2$α$^{(-/-)}$ mice. Cardiomyocytes were isolated as described in Materials and Methods and then were subjected to 45 minutes of hypoxia followed by 16 hours of reperfusion. Quantification of adenylate kinase release (a marker of loss of sarcolemmal integrity) is shown. Data are from three independent experiments, n = 3–5 per experiment. *$p < 0.001$. NS = nonsignificant.

Simulated ischemia in isolated adult cardiomyocytes

We then asked whether the increased injury was due to a cell-autonomous effect of deletion of cPLA$_2$α in cardiomyoyctes, To address this question, we isolated adult mouse cardiomyocytes from cPLA$_2$α$^{(+/+)}$ and cPLA$_2$α$^{(-/-)}$ hearts and exposed them to simulated ischemia for 45 minutes followed by reoxygenation. We found no difference in rates of adenylate kinase release, a measure of sarcolemmal integrity, between cPLA$_2$α$^{(+/+)}$ versus cPLA$_2$α$^{(-/-)}$ cardiomyocytes (*Figure 4*). These data suggest that

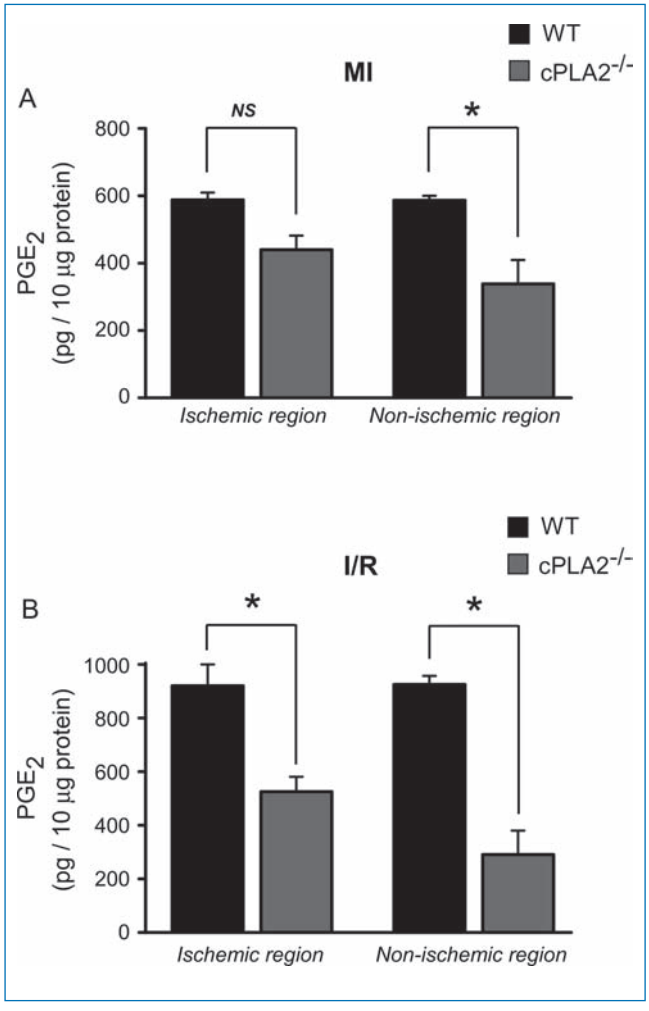


Figure 5. PGE_2 production is decreased in the hearts of $cPLA_2\alpha^{(-/-)}$ *in vivo*. WT and $cPLA_2\alpha^{(-/-)}$ mice were subjected to either (A) 30-minute ischemia without reperfusion (MI) or (B) 30-minute ischemia followed by 20-minute reperfusion (I/R). At the end of the experiment, the ischemic and nonischemic regions of the LV were isolated and snap-frozen in liquid nitrogen. Shown are PGE_2 levels in the ischemic and nonischemic regions of WT and $cPLA_2\alpha^{(-/-)}$ mice. Quantification of PGE_2 production was performed as described in the Materials and Methods section. $n = 3–4$ per experiment, assayed in triplicate. $^*p < 0.05$.

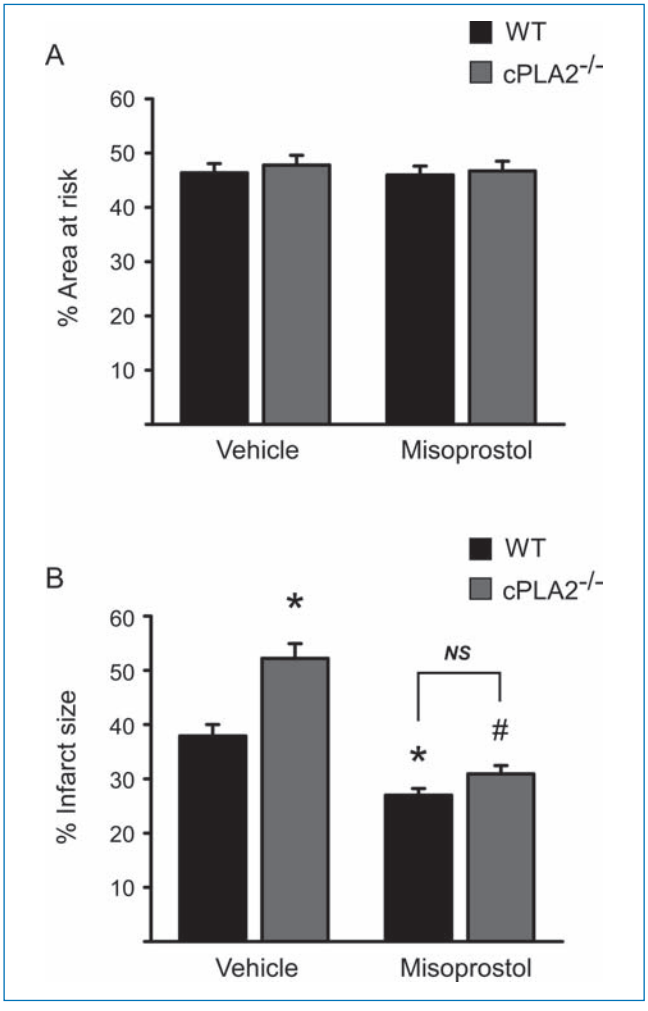


Figure 6. Misoprostol rescues the increased I/R injury in the $cPLA_2\alpha^{(-/-)}$ *in vivo*. WT and $cPLA_2\alpha^{(-/-)}$ mice were pretreated with vehicle or misoprostol as described in Materials and Methods and then were subjected to ischemia (30 minutes) and reperfusion (24 hours) prior to sacrifice for determination of area at risk (panel A) and infarct size (panel B). (A) Area at risk is similar irrespective of genotype or treatment condition. (B) Quantification of infarct size expressed as a percent of area at risk. $n = 12$ WT, 13 $cPLA_2\alpha^{(-/-)}$, 5 WT + misoprostol, and 7 $cPLA_2\alpha^{(-/-)}$ + misoprostol. $^*p < 0.001$ versus WT + Vehicle; $^*p < 0.001$ versus $cPLA_2\alpha^{(-/-)}$ + Vehicle. NS = nonsignificant. The misoprostol (—) mice are the same as those shown in *Figure 1*.

$cPLA_2\alpha$ does not have a major cardiomyocyte-autonomous role in regulating injury.

Analysis of PGE_2 levels

Production of the eicosanoid, PGE_2, is dependent on $cPLA_2\alpha$ in a variety of settings. Ischemia activates $cPLA_2$, AA is released, and PGE_2 synthesis is increased in the heart during I/R.[4,36–38] Furthermore, PGE_2 has been implicated in cardioprotection (see below). Therefore, we asked whether levels of this critical eicosanoid were reduced in the heart of the $cPLA_2\alpha^{(-/-)}$ mouse following ischemic stress. We subjected the $cPLA_2\alpha^{(+/+)}$ and $cPLA_2\alpha^{(-/-)}$ mice to either myocardial infarction (MI) secondary to permanent left anterior descending coronary artery occlusion or transient I/R injury. Analysis of cardiac samples showed that PGE_2 levels were slightly lower in both the ischemic and nonischemic zones of the $cPLA_2\alpha^{(-/-)}$ versus $cPLA_2\alpha^{(+/+)}$ mice following permanent ligation of LAD (*Figure 5A*). With I/R injury, PGE_2 levels were markedly decreased in both the ischemic and nonischemic zones of $cPLA_2\alpha^{(-/-)}$ as compared to $cPLA_2\alpha^{(+/+)}$ mice (*Figure 5B*, $P < 0.05$).

PGE_2 analog misoprostol reduces infarct size

Our data indicate that the increased infarct size in $cPLA_2\alpha^{(-/-)}$ following I/R is associated with decreased PGE_2 levels in the heart. We then asked whether we could reduce the infarct size in the $cPLA_2\alpha^{(-/-)}$ mice with exogenous administration of PGE_2. Prior to inducing I/R injury, $cPLA_2\alpha^{(-/-)}$ and $cPLA_2\alpha^{(+/+)}$ mice were treated with the PGE analog, misoprostol, which interacts with both the EP3 and EP4 receptors. Misoprostol treatment had no effect on area at risk, as determined by Evans Blue dye injection (*Figure 6A*). Remarkably, treatment with misoprostol reduced the infarct size in the $cPLA_2\alpha^{(-/-)}$ mice to the point that the difference in infarct size between the $cPLA_2\alpha^{(-/-)}$ and $cPLA_2\alpha^{(+/+)}$ mice was abolished (*Figure 6B*). These data, taken together, are consistent with PGE_2 being a critical eicosanoid product downstream of $cPLA_2\alpha$ that mediates cardioprotection.

Discussion

Activation of cPLA$_2$s releases AA, which is then metabolized to various prostaglandins and to thromboxane by cyclo-oxygenases, to leukotrienes by lipoxygenases, and to epoxytrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs) by cytochrome P450 enzymes.[39,40] Some of these metabolites (e.g., PGE$_2$ and PGI$_2$ as well as various EETs) are believed to be protective in the heart whereas others (e.g. HETEs) are felt to be detrimental.[39,40] Thus, the net effect of cPLA$_2$ inhibition might reflect the balance of production of these various mediators of protection versus injury. Studies examining the role of cPLA$_2$s in I/R injury have consistently reported that cPLA$_2$ is detrimental, although as noted, many of these studies have relied on inhibitors of questionable selectivity, have been done in isolated cells, or have specifically examined I/R injury in the brain with its inherently different pathophysiology compared to the heart. Taken together, our data demonstrate (1) a protective effect of cPLA$_2$α *in vivo*, (2) no protection by cPLA$_2$α in isolated cardiomyocytes, and (3) amelioration of the deleterious effects of deletion of cPLA$_2$α *in vivo* by administration of a stable PGE analog. Although misoprostol ameliorated injury in the cPLA$_2$α$^{(-/-)}$ mouse, we cannot conclude that PGE$_2$ is the only (or even the primary) mediator of cardioprotection *in vivo* following I/R. Rather, we believe that other eicosanoid products of cPLA$_2$α likely contribute to cardioprotection in the cPLA$_2$α$^{(+/+)}$ mouse.

How might cPLA$_2$α mediate protection *in vivo* but not in the isolated cardiomyocyte model? The lack of an effect in isolated adult cardiomyocytes subjected to simulated ischemia suggests that cPLA$_2$α does not play an important role in a cardiomyocyte-autonomous manner. Rather, protective effects may primarily be due to effects on other cell types (e.g., endothelial cells, vascular smooth muscle cells, or even fibroblasts). Answers to this question must await cell-type-specific knockouts of cPLA$_2$α. This conclusion differs from that reached by Engelbrecht and Ellis, who subjected isolated neonatal rat cardiomyocytes to hypoxia/reoxygenation (H/R).[28] There are several possible explanations for this. First, cPLA$_2$α may play different roles in the response of neonatal rat versus adult mouse myocytes to H/R. Alternatively, these authors employed a chemical inhibitor (AACOCF$_3$) that inhibits all three members of the cPLA$_2$ class (and may have some inhibitory activity against other PLA$_2$s). Thus, it is possible that cPLA$_2$β and/or γ may be more important in cardiomyocytes, or that some activity against iPLA$_2$s may play a role in the protection seen with AACOCF$_3$.

cPLA$_2$α is an essential factor in the generation of PGE$_2$ in many cells,[19,41] and we confirmed that cPLA$_2$α plays a key role in PGE$_2$ production *in vivo* following ischemic injury in the heart. PGE$_2$ has important protective effects against I/R injury since germline deletion of the receptor for PGE$_2$ (EP4 receptor) enhanced I/R injury, and an EP4 agonist (4819-CD) was cardioprotective.[37] ERK is one of the key signaling pathways shown to protect the heart from I/R injury.[42–44] PGE$_2$ activates ERK, and the antiapoptotic actions of PGE$_2$ are abolished by inhibition of the ERK pathway,[45,46] which is consistent with the noted decrease in ERK phosphorylation in ischemic regions of cPLA$_2$α$^{(-/-)}$ hearts. Thus, it seems likely that the enhanced infarct size in the cPLA$_2$α$^{(-/-)}$ is due, at least in part, to impaired generation of this eicosanoid. Consistent with this conclusion, treatment with the PGE$_2$ mimetic misoprostol, which binds to both the EP4 and EP3 receptors for PGE$_2$, rescued the phenotype of increased infarct size in the knock out animals. The protective effect of misoprostol was also noted in WT animals, though it was less marked.

The EP4 receptor is expressed in cardiomyocytes. The receptor couples to the pro-survival phosphoinositide-3 kinase pathway in multiple cell types.[47] We considered that either this or activation of STAT3[48,49] may explain the protection seen. However, we saw no significant differences in activation of these signaling pathways in the hearts of cPLA$_2$α$^{(-/-)}$ versus cPLA$_2$α$^{(+/+)}$, as demonstrated by no differences in phospho-Akt or phospho-STAT3 (data not shown). Since such studies largely reflect signaling in cardiomyocytes (the vast majority of cell mass in the heart), these data suggest that reduced PGE$_2$/EP4 signaling in cPLA$_2$α$^{(-/-)}$ *cardiomyocytes* is not an important mechanism of increased I/R injury in the cPLA$_2$α$^{(-/-)}$. Consistent with this, prior studies have shown that cardiomyocyte-specific deletion of the EP4 receptor resulted in no increase in MI size, in contrast to the increase in MI size noted above in the germline KO of EP4.[37,50] Finally, activation of the EP4 receptor by a synthetic ligand only weakly activated signaling in cardiomyocytes but strongly activated it in noncardiomyocytes.[37] These data, which suggest a limited, if any, protective role for PGE$_2$ and the EP4 receptor in regulating ischemic injury specifically in cardiomyocytes, further support the conclusion that PGE$_2$ exerted beneficial effects on cell types other than cardiomyocytes. Thus, we believe our data are most consistent with the detrimental effect of cPLA$_2$α deletion being due to a reduction in PGE$_2$ and, likely, other eicosanoid-mediated protective effects on noncardiomyocytes in the heart.

In summary, cPLA$_2$α is protective *in vivo* in the setting of I/R injury. This does not appear to be due to an important direct action of cPLA$_2$α in cardiomyocytes. cPLA$_2$α-dependent production of PGE$_2$ and, possibly, other prostaglandins (or EETs) appear to be critical to the infarct-reducing effect seen in the knock out *in vivo*. Thus, inactivation of cPLA$_2$α can produce widely divergent effects on I/R injury in various tissues, depending on the major factors driving injury. The variation likely depends, in large part, on the level of expression of cPLA$_2$α, and the specific location of receptors for protective versus deleterious eicosanoids. In any case, our findings suggest that targeting of cPLA$_2$α with small molecule inhibitors for any of the numerous disease states that are regulated by cPLA$_2$α should be balanced against potential deleterious effects in patients with concomitant cardiovascular disease.

Conflict of Interest

None

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