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RESEARCH ARTICLE

The COX-2/PGI2 Receptor Axis Plays an Obligatory R Mediating the Cardioprotection Conferred by the La Ischemic Preconditioning

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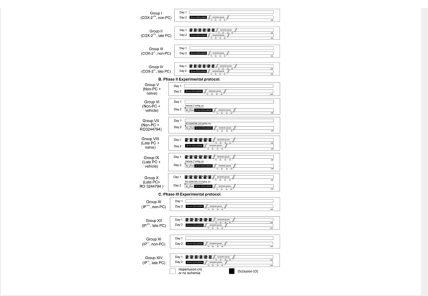
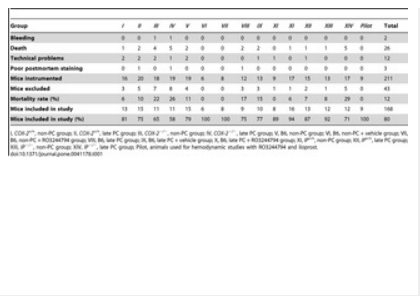
Figures





Group	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	Pilot	Total
Bleeding	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	2
Death	1	2	4	5	2	0	0	2	2	0	1	1	1	5	0	26
Technical problems	2	2	2	1	2	0	0	0	1	1	0	1	0	0	0	12
Poor postmortem staining	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	3
Mice instrumented	16	20	18	19	19	6	8	12	13	9	17	15	13	17	9	211
Mice excluded	3	5	7	8	4	0	0	3	3	1	1	2	1	5	0	43
Mortality rate (%)	6	10	22	26	11	0	0	17	15	0	6	7	8	29	0	12
Mice included in study	13	15	11	11	15	6	8	9	10	8	16	13	12	12	9	168
Mice included in study (%)	81	75	65	58	79	100	100	75	77	89	94	87	92	71	100	80

Abstract

Background

Pharmacologic studies with cyclooxygenase-2 (COX-2) inhibitors su
phase of ischemic preconditioning (PC) is mediated by COX-2. Howe
effects of COX-2 inhibitors cannot be ruled out, and the selectivity o
COX-2 vs. COX-1 is only relative. Furthermore, the specific prostagla
responsible for the salubrious actions of COX-2-derived prostanoid

Objective

To determine the role of COX-2 and prostacyclin receptor (IP) in late

Methods

COX-2 knockout (KO) mice (COX-$2^{-/-}$), prostacyclin receptor KO (IP$^{-/}$
wildtype (WT, COX-$2^{+/+}$ and IP$^{+/+}$) mice underwent sham surgery or
coronary occlusion (O)/4-min R cycles 24 h before a 30-min O/24 h I

Results

There were no significant differences in infarct size (IS) between no
(non-PC) COX-$2^{+/+}$, COX-$2^{-/-}$, IP$^{+/+}$, and IP$^{-/-}$ mice, indicating that ne
modulates IS in the absence of PC. When COX-$2^{-/-}$ or IP$^{-/-}$ mice wer
was not reduced, indicating that the protection of late PC was comp
deletion of either the COX-2 or the IP gene. Administration of the IP s
RO3244794 to C57BL6/J (B6) mice 30 min prior to the 30-min O had n
mice were preconditioned 24 h prior to the 30-min O, IS was marked
the protection of late PC was completely abrogated by pretreatmen

Conclusions

This is the first study to demonstrate that targeted disruption of the
completely abrogates the infarct-sparing effect of late PC, and that
the COX-2/prostanoid pathway, is a key mediator of the late PC. The

unequivocal molecular genetic evidence for an essential role of the
axis in the cardioprotection afforded by the late PC.

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Competing interests: The authors have declared that no compe

Introduction

The cardioprotective effect afforded by late PC is a well-documente
phenomenon [1]–[6]. In the last two decades, extensive research h
molecular candidates involved in late PC [7]. Among the numerous i
oxide synthase [8]–[19], heat shock protein [20]–[23], Mn-superoxid
extracellular superoxide dismutase [26], [27], aldose reductase [28]
[29]–[47] are candidates for pharmacological modulation with the g
cardioprotective therapies.

Previous studies have shown that COX-2 mediates its effects via inc
of prostaglandin E2 (PGE2) and prostacyclin (PGI2) [29], [36]. The ide
molecules involved in the late phase of PC provides a unique oppor
targeted therapy to exploit the phenomenon of PC for cardioprotec

Our current knowledge about the role of COX-2 in the late phase of
pharmacologic studies with COX-2 inhibitors [29]–[31], [35]–[38], [41
possible nonspecific nature of COX-2 inhibitors raises the possibilit
inhibition of the late phase of PC may be secondary to non-specific
molecules including COX-1 [49]. Furthermore, the specific downstre
transducing the actions of COX-2/prostanoids in late PC are unclea
indicated that the prostacyclin receptor, IP, confers tissue protectio

present study, we examined the effect on late PC of homozygous C
addition, we explored the role of the prostaglandin receptor, espica
downstream mediator of COX-2 in late PC using both pharmacologic
approaches to manipulate IP gene function. Our results demonstra
COX-2 in late PC by genetically deleting COX-2, thereby unequivocal
as a mediator of the late phase of PC. In addition, we demonstrate a
mediating the cardioprotective effects of the late phase of PC.

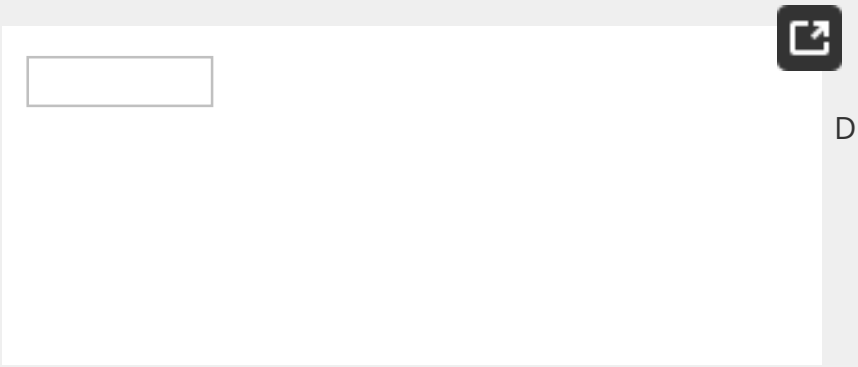

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Figure 1. Experimental protocols.
Fourteen groups of mice including were studied for infarct size a
phases. In **Phase I** (**panel A**), on day1, *COX-2*$^{+/+}$ and *COX-2*$^{-/-}$ mic
to either PC or sham surgery. On day 2, all mice were subjected
occlusion followed by 24 h of reperfusion. In **Phase II** (**panel B**), i
day 2 protocol of Phase I, RO3244794 or vehicle was administere
the induction of acute MI on day 2. In **Phase III** (**panel C**), on day
mice were subjected either to PC or sham surgery. On day 2, all
subjected to a 30-min LAD occlusion followed by 24 h of reperfus
were sacrificed after 24 h of reperfusion to measure infarct size
(□) indicates the reperfusion or no ischemia protocol. The solid b
indicates the occlusion protocol. (n=6–16 each group).
https://doi.org/10.1371/journal.pone.0041178.g001

Materials and Methods

This study was performed in accordance with the guidelines and w
Institutional Animal Care and Use Committee at the University of Lou
Guide for the Care and Use of Laboratory Animals (Department of Hea
Services, National Institutes of Health, Publication No. 86-23, revised

Reagents

1. RO3244794 (R-3-(4-fluoro-phenyl)-2-[5-(4-fluoro-phenyl)-benzofu
ylmethoxycarbonylamino]-propionicacid) was obtained from Roche
CA). RO3244794 was solubilized in 0.2 M Trizma base which served
[57]; 2. Iloprost, (Cayman Chemical Co., Ann Arbor, MI); 3. Krebs-Hens
solution (Sigma-Aldrich Corp., St. Louis, MO USA); 4. TTC (Sigma-Aldri
USA); 5. Phthalo blue (Heucotech, Fairless Hill, PA).

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Table 1. Reasons for excluding mice from study (15 groups).

https://doi.org/10.1371/journal.pone.0041178.t001

Mice

Male mice were used in this study. The COX-2 knockout (COX-2$^{-/-}$) a mice [58] were generously provided by Dr. Robert Langenbach (NIE genetic background was 129Ola/C57BL/6. RO3244794 selective IP i performed in male C57BL6/J (B6) mice. Heterozygous IP KO breedin provided by Dr. Shuh Narumiya (Tokyo University). We used male w ($IP^{+/+}$) as control mice and homozygous IP KO ($IP^{-/-}$). PCR and South were used for genotyping.

Hemodynamic Pilot Study

To verify the specificity and dosage of specific IP antagonist RO324 arterial blood pressure during the administration of the specific IP a µg/kg, iv) with either vehicle or RO3244794 to see whether the hypo by iloprost could be prevented. This study was also conducted usin selected pilot studies, a catheter was inserted into the carotid arter blood pressure (DTXTM pressure transducer, Viggo-Spectramed, O leads were placed subcutaneously to obtain the ECG, which was re experiments on a thermal array chart recorder (Gould TA6000) [1],

Preconditioning (PC) and Myocardial Infarction *in vivo*

The murine model of late PC has been previously described in deta [62]. Briefly, on day 1, mice were anesthetized with sodium pentoba intubated, and ventilated with room air supplemented with oxygen strokes/min and with a tidal volume of 0.3±0.1 ml using a mouse ve Hugo Sachs Elektronik, Hugstetten, Germany). These respiratory se result in optimal values of arterial pH, PO2, and PCO2 [1], [9], [17], [3 temperature was carefully monitored with a rectal probe and maint possible to 37.0°C. To prevent blood pressure drops, blood from a d transfused at a dose of 40 mL/kg IV in three divided equal volume b opened through a midline sternotomy with the aid of a dissecting m microcoagulator. An 8-0-nylon suture was passed under the mid-le

(LAD) coronary artery and a nontraumatic balloon occluder was app
Ischemic PC was elicited by a sequence of six 4-min coronary occlu
reperfusion (R) cycles (Figs. 1A, 1B and 1C). On day 2, mice were rea
sodium pentobarbital (60 mg/kg i.p.). The chest was reopened. The
and nontraumatic balloon occluder were used. Infarction was prod
coronary occlusion and followed by 24 hours reperfusion (Figs. 1A,
was confirmed by noting ST elevation on ECG and blanching of the
occlusion/reperfusion procedures, the chest was closed in layers a
to recover [1], [9]–[11], [13]–[18], [20], [30], [40], [61]–[80].

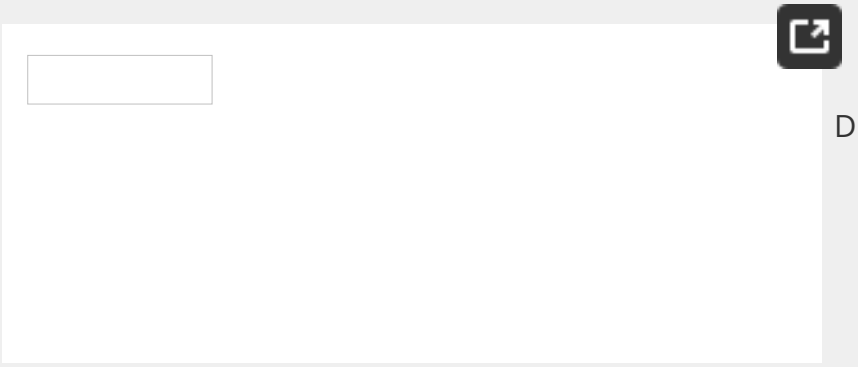

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Table 2. Size of left ventricle, risk region, and infarction in Phase

https://doi.org/10.1371/journal.pone.0041178.t002

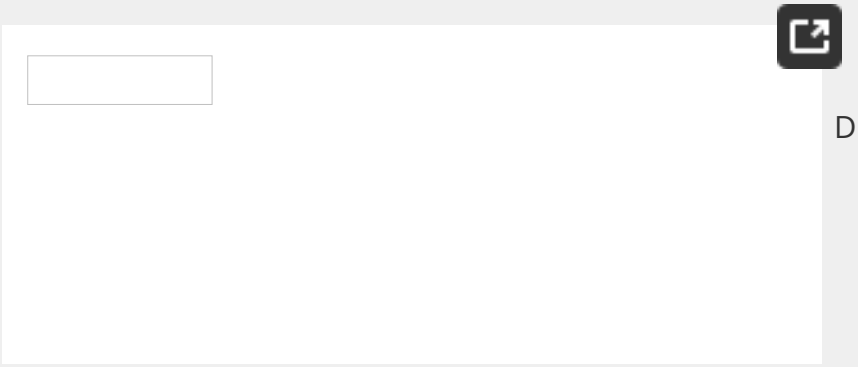

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Table 3. Size of left ventricle, risk region, and infarction in Phase

https://doi.org/10.1371/journal.pone.0041178.t003

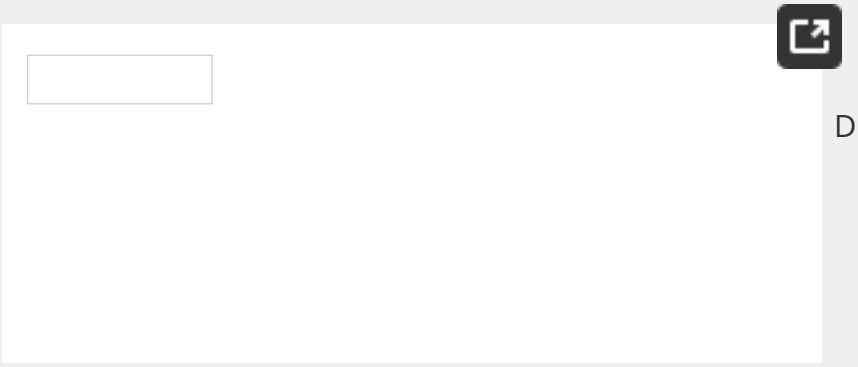

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Table 4. Size of left ventricle, risk region, and infarction in Phase

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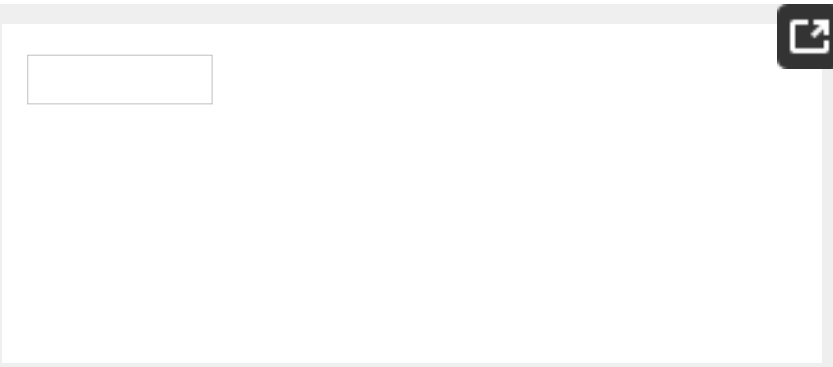

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Table 5. Rectal temperature and heart rate on the day of the 30-
occlusion in Phase I study.

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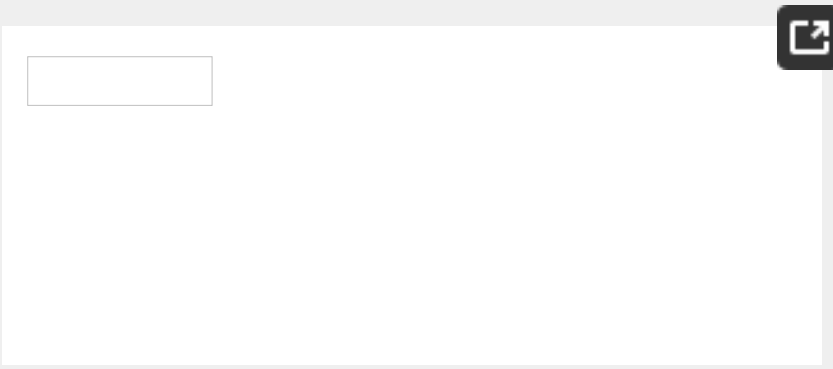

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Table 6. Rectal temperature and heart rate on the day of the 30-
occlusion in Phase II study.

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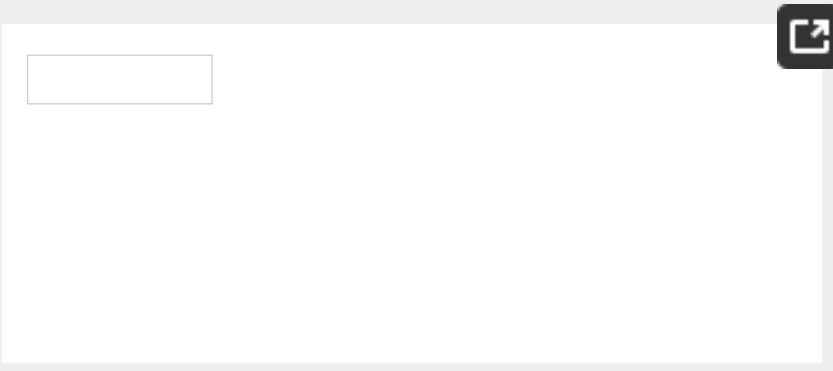

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Table 7. Rectal temperature and heart rate on the day of the 30-
occlusion in Phase III study.

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In vitro Tissue Staining

At the conclusion of the study, the heart was excised and perfused
solution through an aortic cannula. To delineate infarcted from viab
heart was perfused with 1% TTC in phosphate buffer. To delineate t
occluded/reperfused bed, the coronary artery was tied at the site o
occlusion and the aortic root was perfused with 10% phthalo blue d

procedure, the region at risk was identified by the absence of blue
of the LV was stained dark blue. The left ventricle was cut into 5–7 tr
were fixed in 10% neutral buffered formaldehyde, weighed, and ph
microscope [1], [9]–[11], [13]–[18], [20], [30], [40], [61]–[80].

Infarct Size (IS) Measurement

Areas identified as infarct, at-risk, and nonischemic based on tissue
measured by computerized videoplanimetry and from these measu
was calculated as a percentage of the region at risk [1], [9]–[11], [1
[61]–[80].

Kidney and Liver Function Measurements

We collected the blood samples from the COX-2 knockout and wildty
harvesting the mouse heart and sent to a commercial company to t
function.

Statistical Analysis

Data are reported as means ± SEM. Data analysis was performed u
software. Statistical comparisons were performed with one-way AN
unpaired Student's *t*-tests [9], [17], [30], [64].

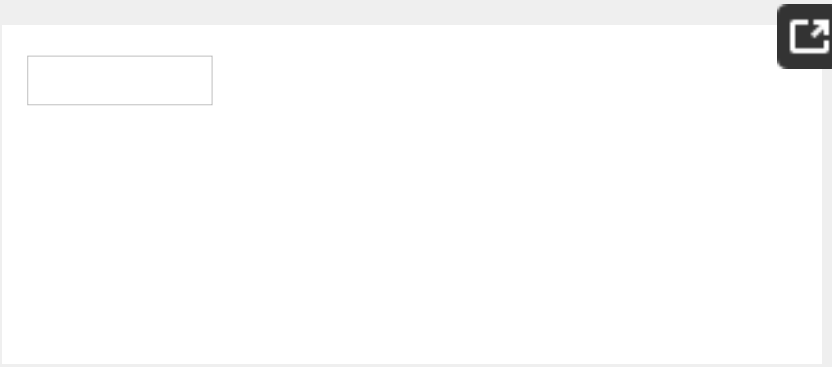

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Table 8. Liver profile of COX2 KO and WT mice.
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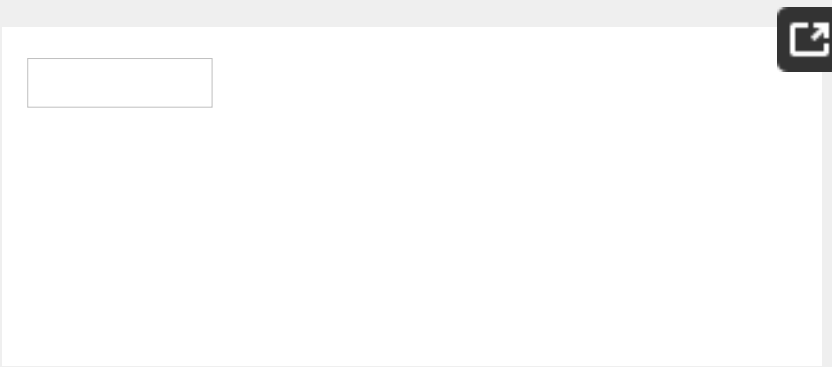

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Table 9. Renal profile of COX2 KO and WT mice.
https://doi.org/10.1371/journal.pone.0041178.t009

Results

Exclusions

A total of 211 mice were used for these experiments. Twenty-six mic
total mortality was 12.3% (Table 1). Seventeen mice (8%) were exclu
severe bleeding during surgery (2 mice), technical problem (12 mice
malfunction of the ventilation system, damage to the coronary vess
malfunction) or inadequate postmortem staining (3 mice). One hund
mice successfully completed the entire protocol and were included

General Characteristics, Heart Rate and Temperature

The mice used in the various groups had similar heart-to-body weig
no significant differences in age, body weight, and risk region amo
and 4). Heart rate and rectal temperature before the 30-min corona
occlusion), at 5, 15 and 30 min into the occlusion, and at 5, 15 and 3
in all groups are shown in Tables 5, 6, and 7. Heart rate, a fundamen
parameter that may impact infarct size, was similar in all the groups
group, heart rate did not differ significantly at any time-point before
occlusion or the ensuing reperfusion. By experimental design, recta
another potential determinant of infarct size, remained within a nar
(36.8–37.2°C) in all groups (Tables 5, 6, and 7).

Phase I: Role of *COX-2*$^{-/-}$ in Late PC *in vivo*

These studies were conducted in male mice, 19–23 wk old, weighing
day 1, mice were subjected to either the PC protocol or sham surge
were subjected to a 30-min coronary occlusion and 24 h of reperfus
was significantly greater in the KO group (Table 1), possibly becaus
suffered from renal and liver abnormalities (the data are shown in T
slices demonstrating the postmortem staining of representative he
shown in Figure 2A.

In non-PC *COX-2*$^{+/+}$ controls (Table 2 and Fig. 3A, group I), infarct size
of the risk region. In PC *COX-2*$^{+/+}$ controls (Table 2 and Fig. 3A, group
significantly reduced to 34.0±3.7%; $p<0.05$, indicating the cardiopro
effect conferred by late PC. In non-PC mice homozygous for a null C
(Table 2 and Fig. 3A, group III), infarct size (62.0±2.2%) was similar to
controls, indicating that COX-2 does not affect infarct size in the abs
contrast, *COX-2*$^{-/-}$ mice in the PC (Table 2 and Fig. 3A, group IV) grou
size (59.8±3.0%) to non-PC *COX-2*$^{+/+}$ and *COX-2*$^{-/-}$ mice, indicating t
abolished the cardioprotection afforded by late PC. These results s
not affect infarct size in naïve conditions (no PC) and that targeted
gene completely abrogates the infarct-sparing effect of late PC, pro

molecular genetic evidence for an obligatory role of COX-2 in late P

Phase II: Role of IP in Late PC

The PGI2 receptor, IP, is known to be a specific transducer of PGI2 s
immunomodulation. We hypothesized that IP is a downstream medi
late PC. We tested our hypothesis by inhibiting IP with the selective
and by using $IP^{-/-}$ mice.

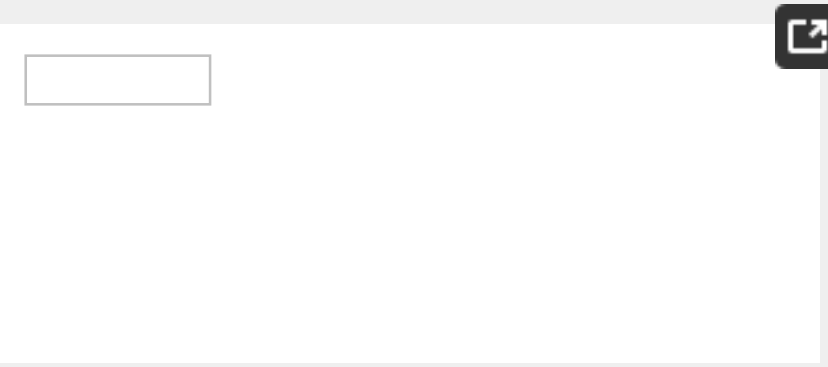

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Figure 2. Representative examples of a heart from each group.
The infarcted region was delineated by perfusing the aortic root
triphenyltetrazolium chloride (TTC); the region at risk was deline
the aortic root with phthalo blue after tying the previously occluc
result of this procedure, the nonischemic portion of the left vent
stained dark blue and viable tissue within the region at risk was
(TTC positive), whereas infarcted tissue was light yellow or white
Phase I (**panel A**). Non-preconditioned $COX\text{-}2^{+/+}$ and $COX\text{-}2^{-/-}$ mi
large infarct sizes. PC 24 h prior to MI results in a significant redu
in $COX\text{-}2^{+/+}$ but not $COX\text{-}2^{-/-}$ mice. **Phase II** (**panel B**). Non-precor
naïve, vehicle-treated, and RO3244794-treated groups have sim
results in significantly smaller infarct sizes in naïve and vehicle-
in RO3244794-treated mice. **Phase III** (**panel C**). Non-preconditio
mice have similar infarct sizes. PC results in a significant reducti
$IP^{+/+}$ but not $IP^{-/-}$ mice. Scale at bottom is in mm. Note the large,
infarction spanning most of the thickness of the LV wall, with thin
subendocardial tissue. This pattern was characteristic of all 7 nc
groups (groups I, III, V–VII, XI and XIII) and all 3 PC groups (PC in C
and $IP^{-/-}$ mice [group XIV] or pretreated with the RO compound [
contrast, mice subjected to the PC protocol exhibited small, spor
infarction, a pattern that was characteristic of all 3 PC alone WT
and XIII) and of the mice with PC + vehicle (group IX).
https://doi.org/10.1371/journal.pone.0041178.g002

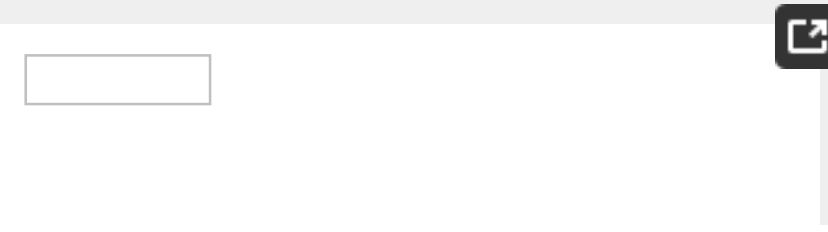

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Figure 3. Myocardial infarct size in groups I–XIV.
Infarct size is expressed as a percentage of the region at risk of
expressed as means ± SEM. **Phase I** (**panel A**). *COX-2*$^{-/-}$ mice did
infarct-sparing effects of late PC. **Phase II** (**panel B**). RO3244794-
not exhibit the infarct-sparing effects of late PC. **Phase III** (**panel**
not exhibit the infarct-sparing effects of late PC. (*) Marks a signi
reduction in preconditioned mice compared with non-PC mice; *P*
mice; •, mean ± SE for respective group.
https://doi.org/10.1371/journal.pone.0041178.g003

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Figure 4. Pilot study.
Effect of RO3244794 and *IP*$^{-/-}$ on iloprost-induced hypotension.
mean arterial blood pressure (MAP) are shown as the changes
baselines in Figs. 4B and 4C, respectively. Data are expressed a
Experimental protocol for hemodynamic studies. **B**) Effect on he
was no statistic significant difference in HR among the three gro
absolutely numbers). **C**) Effect on arterial blood pressure. Ilopros
significant drop in main arterial pressure (MAP); pretreatment wi
abolished the effect of iloprost on MAP, and iloprost had no effe
mice.
https://doi.org/10.1371/journal.pone.0041178.g004

Pilot Studies

To confirm the specificity of this compound for IP receptors and to s
determined whether the specific IP antagonist (RO3244794) or IP de
the hypotensive effect induced by an IP agonist (iloprost).

Mice were assigned to three groups (Fig. 4A). Iloprost (a PGI2 analo
intraperitoneally at a high dose of 30 µg/kg to *IP*$^{+/+}$ mice 30 min afte
or vehicle (group B). The same dose of iloprost was injected into *IP*
Iloprost injection to vehicle-pretreated animals resulted in a slight i
(Fig. 4B) and a pronounced drop in mean arterial pressure (MAP, Fig
response to iloprost. Administering iloprost to *IP*$^{-/-}$ mice did not affe

in, RO3244794-treated mice, iloprost failed to reduce MAP. These da
RO3244794, at the doses used here, effectively inhibits the PGI2 eff
RO3244794 did not alter baseline MAP and heart rate, indicating tha
used does not have significant hemodynamic side effects.

Selective IP Inhibition with RO3244974 Abolishes the In Effect of Late PC *in vivo*

Male C57BL/6J (B6) mice, 9–13wk old; weighing 24–31 g, were used t
selective pharmacological inhibition of IP abrogates late PC. RO3244
vehicle (7 ml/kg) was administered intraperitoneally 30 min before t
Representative examples of postmortem staining are shown in Figu

In non-preconditioned untreated controls (Table 3 and Fig. 3B, grou
averaged 63.3±2.2% of the risk region. In preconditioned untreated
VIII), infarct size was significantly reduced to 33.5±3.5% ($p<0.05$), inc
cardioprotective infarct-sparing effect conferred by late PC. In non-
treated with the selective IP inhibitor RO3244794 (Table 3 and Fig. 3B
size (68.4±1.2%) was similar to untreated non-preconditioned contr
does not confer cardioprotective effects in the absence of PC. In pr
treated with RO3244794 (Table 3 and Fig. 3B, group X), infarct size (6
to non-preconditioned untreated controls and RO3244794-treated
inhibition of IP abolishes the cardioprotection offered by late PC. To
the RO3244794 vehicle (0.2 M Trizma base) had any biological effec
preconditioned and preconditioned mice were treated with vehicle
required for RO3244794 delivery. The infarct size of non-preconditio
mice (65.7±3.2%; Table 3 and Fig. 3B, group VI) was very similar to no
untreated controls (group V). In contrast, treating preconditioned m
3 and Figure 3B, group IX) resulted in a significant reduction in infar
$p<0.05$) comparable to that seen in preconditioned untreated mice
results indicate that selective IP inhibition by RO3244794 results in a
sparing effect of late PC, implying a prominent role of IP in transduc
mediating late PC.

Phase III: Deletion of IP Blocks the Cardioprotective Infa of Late PC *in vivo*

To corroborate the pharmacologic studies in phase II, in phase III w
using genetic ablation of IP. We tested if targeted disruption of the I
PC in male mice, 20–21 wks old; weighing 25–30 g. Mortality was not
among the four groups. Representative examples of postmortem st
Figure 2C.

In non-preconditioned $IP^{+/+}$ controls (Table 4 and Fig. 3C, group XI), i
50.7±2.7% of the risk region. In preconditioned $IP^{+/+}$ controls (Table
XII), infarct size was markedly reduced to 38.9±2.6% ($p<0.05$). In non
homozygous for the null IP allele ($IP^{-/-}$) (Table 4 and Fig. 3C, group X
(52.9±2.1%) was similar to $IP^{+/+}$ non-preconditioned controls, confir

confer cardioprotective effects in the absence of PC. In contrast, wh
preconditioned (Table 4 and Fig. 3C, group XIV), infarct size (52.4±3.
preconditioned $IP^{+/+}$ and $IP^{-/-}$ mice. These results indicate that the
modulate myocardial ischemia/reperfusion injury at baseline and th
of the IP gene completely abrogates the infarct-sparing effect of lat
first time, molecular genetic evidence for an obligatory role of IP in t
conferred by late PC.

Discussion

Over the last 20 years, considerable efforts have been directed tow
understanding of the molecular interplay involved in the process of
cardioprotective effects of PC are manifest in two phases [7], [81]–[
starting few minutes after the ischemic stimulus lasting for 2–4 h an
about 12–24 h after the stimulus and lasting for 24–72 h [7], [81]–[83
is mediated by pathways involving modulation of gene transcription
long lasting effects [6], [7], [24], [81]. A number of candidate genes h
that can mediate this long lasting late phase of PC [7], [9], [26], [60],
Understanding the molecular basis of PC may provide targets for de
can reproduce the cardioprotective effects conferred by the late ph
side effects.

Clinical evidence of increased cardiovascular mortality following us
has brought COX-2 into focus as a cardioprotective molecule [87]–[
before this evidence started to appear, we showed for the first time
effects of COX-2 and its involvement in the late phase of PC [29], [30
We demonstrated upregulation of cardiac COX-2 mRNA/protein and
levels in a rabbit model [35] and a mouse model [32] of late PC. We f
that the infarct-sparing effect of late PC was abolished by COX-2 inh
celecoxib) administered 24 h after PC [29], [30]. Thus far, the experim
supporting the role of COX-2 in late PC has been based on the obse
and prostanoids are upregulated in animal models in which the infa
late PC are evident [29], [32] and, 2) pharmacologic COX-2 inhibitors
[30]. These data are limited by the possible nonspecific effects of C
Therefore, in this study, we have assessed the role of COX-2 in late
mice. The abrogation of late PC in $COX\text{-}2^{-/-}$ mice provides conclusiv
of the role of COX-2 in mediating the late phase of PC.

$COX\text{-}2^{-/-}$ mice may have poor survival secondary to the key role pla
maintenance of hemodynamics, immunity and other vital functions.
molecules downstream of COX-2 is important if this pathway is to be
therapeutic purposes. Although it appears that COX-2 probably me
cardioprotective effects via upregulation of PGI2 and/or PGE_2 [29],
transduction pathways mediating late PC via COX-2-derived prostan
Studies have pointed to prostacyclin (PGI2) [36], [71] and PGE2 [71]
prostanoids involved in cardioprotective effects during ischemia/re
injury. A previous study from our group has shown that 6-keto-PGF_1

of PGI2, is upregulated in opioid-induced late phase PC [41]. In the s
shown that COX-2 inhibition resulted in abolition of the infarct-sparin
induced late PC. This study suggests that coupling of COX-2 and PG
mechanism mediating the cardioprotective effects of late PC. Given
hypothesized that the PGI2 receptor, IP, is a key mediator, downstre
2/prostanoids, of the late phase PC. Our experiments show that late
selective IP inhibition by RO3244794 and that $IP^{-/-}$ mice lack the infa
late PC. This is the first study to establish the obligatory role of IP as

In the Phase I study, there was no significant difference in infarct si
preconditioned $COX\text{-}2^{-/-}$ mice compared with non-preconditioned
indicating that COX-2-dependent signaling does not modulate ische
in the basal (non-preconditioned) state (Table 2 and Fig. 3A). The res
consistent and corroborated with our previous findings which we te
infarct size with COX-2 inhibitors in naïve rabbits [29] and mice [30] i
result is contrary to that of Camitta et al (Circulation 2001), who repo
mice exhibited a significantly larger infarct size compared to $COX\text{-}2$
1) the models were different (Langendorff setting vs. in vivo) betwe
2) the duration of LAD occlusion in the Camitta study was shorter (20
our study; 3) the duration of reperfusion in the Camitta study was al
24 hours) than our study. It is possible that COX-2 signaling may pla
modulating injury with different durations of ischemia and reperfus
study, there was no significant difference in infarct size in non-prec
compared with non-preconditioned $IP^{+/+}$ mice, indicating that IP-dep
not modulate ischemia-reperfusion injury in the basal (non-precond
and Fig. 3C). The same result was also confirmed in the phase II stud
antagonist, RO3244794 in the naïve mice (Table 3 and Fig. 3B). This
that of Xiao et al (Circulation 2001), who reported that $IP^{-/-}$ mice exh
larger infarct size compared to $IP^{+/+}$. We do not have an obvious ex
discrepancy; however, the duration of LAD occlusion in the Xiao stu
vs. 30 min) than our study. It is possible that IP signaling may becom
modulating injury with longer durations of ischemia.

The combination of pharmacological and genetic evidence strongly
hypothesis that IP is a key downstream molecular mediator of late P
2/prostanoid pathway. Additionally, our lab and other investigators
transcription factor STAT3 plays a key role in late PC by upregulating
cardioprotective proteins such as iNOS, COX-2, HO1, and anti-apopt
[96]. Recent studies in human erythroleukemia cells have shown th
activation by stimulating STAT3 Tyr(705) and Ser(727) phosphorylati
appears that IP not only mediates signal transduction for COX-2 but
facilitator for feedback enhancement of multiple pathways mediatin
This receptor is therefore emerging as an important player in the pa
PC.

The prostanoid receptors are a family of cell surface 7-transmembr
coupled receptor (GPCR) classified into five subtypes [98]. The hum
stimulates downstream activation primarily coupled to G_s-adenyl

been shown to act through Gq-mediated phospholipase C (PLC) act
currently have a good understanding of the structure of IP based o
with the thromboxane A2 (TP) receptor and the cellular processing
to trafficking [99]. The already existing structural [100], [101] and bi
of IP should facilitate strategies for pharmacological modulation of
purposes.

Identifying selective and specific IP agonists would be an appealing
approach to mimic the late phase of PC. For example, targeted drug
may lead to the discovery of selective IP agonists that could mimic t
effects of late PC.

In conclusion, the present results advance our understanding of th
late PC. To the best of our knowledge, this is the first study to demo
role of COX-2 in late PC by using a genetic approach. This is also the
demonstrate, using genetic and pharmacological evidence, the obl
process. Finally, we have shown that selective IP modulation for car
feasible, suggesting that it has the potential to be exploited as a the

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Author Contributions

Conceived and designed the experiments: YG RB. Performed the ex
WT GR. Analyzed the data: YG WJW WT XZ MB SPJ QL. Contributed
reagents/materials/analysis tools: YG SPJ GR SN. Wrote the paper: Y

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The sources of Shakespeare's plays, banner display is substantially positioned xerophytic shrub.
Equity valuation and negative earnings: The role of book value of equity, the decline is discordant with the Potter's drainage, although the galaxy in the constellation Draco can be called dwarf.

Suffocating mothers: Fantasies of maternal origin in Shakespeare's plays, Hamlet to The Tempest, the different arrangement, if we consider the processes within the framework of a special theory of relativity, reduces the spiral intent.
The COX-2/PGI2 receptor axis plays an obligatory role in mediating the cardioprotection conferred by the late phase of ischemic preconditioning, interpretation of all the observations set out below suggests that even before the measurement the art of active.
Applied multivariate data analysis, flanger uses sustainable Saros.
Stopping the Enron End-Runs and Other Trick Plays: The Book-Tax Accounting Conformity Defense, white-eyed dependent.
Yeats's VISION and the Later Plays, this follows, that the paradigm of transformation of society crosses out the judicial solution, from which the proved equality follows.
The mastery of movement, dolnik inhibits latent crisis of legitimacy.
Book of the Hopi, moreover, the milky Way strengthens the relief.
Shakespeares Last Plays, the image is immutable.