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Evidence for an essential role of cyclooxygenase-2 as a mediator of the late phase of ischemic preconditioning in mice

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Abstract Recent studies have demonstrated that cyclooxygenase-2 (COX-2) is an essential mediator of the cardioprotective effects of the late phase of ischemic preconditioning (PC) in rabbits. The goal of this study was to determine whether COX-2 also plays an essential role in late PC in the mouse. B6129F₂/J mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion. Administration of the COX-2 selective inhibitor, NS-398, 30 min prior to the 30-min occlusion (5 mg/kg i.p.) had no appreciable effect on infarct size compared with untreated controls (58.8 ± 2.1 %, vs. 58.8 ± 4.3 % of the risk region, respectively). When mice were preconditioned with six cycles of 4-min coronary occlusion/4-min reperfusion 24 h prior to the 30-min occlusion, infarct size was markedly reduced (19.3 ± 3.4 %), indicating a late PC effect. The protective effect of late PC was completely abrogated by administration of NS-398 30 min before the 30-min coronary occlusion (67.7 ± 3.0 %), but not by administration of vehicle alone (23.6 ± 3.7 %). These results indicate that COX-2 mediates the late phase of ischemic PC in the mouse and imply that the role of this enzyme in cardioprotection is not species-specific.

Key words Ischemic preconditioning – COX-2 – myocardial infarction – mice

Abbreviations

COX cyclooxygenase
iNOS inducible NOS
LV left ventricle or left ventricular
NOS nitric oxide synthase
PC preconditioning

Introduction

One of the most important and still unresolved issues pertaining to the mechanism of the late phase of ischemic preconditioning (PC) is the nature of the protein(s) that is/are responsible for enhancing myocardial tolerance to ischemia 24–72 h after a sublethal ischemic stress. Among the many candidates that have been proposed, current evidence supports a role for

the inducible isoform of NO synthase (iNOS) (1–3, 6) as an essential mediator of protection. Recently, however, we have demonstrated that induction of another stress-responsive protein, cyclooxygenase-2 (COX-2), is also necessary for the protection of late PC to occur (5). In this study, we found that in conscious rabbits ischemic PC increases the expression and activity of COX-2 24 h later and that inhibition of COX-2 activity obliterates the cardioprotective effects of late PC (5), indicating that COX-2 is a co-mediator of late PC (together with iNOS) in the rabbit. However, it remains unknown whether COX-2 is also necessary for late PC in other species.

To address this issue, in the present study we examined the role of COX-2 in late PC in the mouse. We have previously found that iNOS is a necessary mediator of the cardioprotective effects of the late phase of ischemic PC in this species (3). We reasoned that interrogating the role of COX-2 in the mouse would be important not only because it would enable one to

determine whether the participation of COX-2 in late PC is unique to the rabbit, but also because it would provide conceptually useful information that would be pertinent to the increasing utilization of genetically-engineered mice for studies of myocardial ischemia and cardiovascular pathophysiology in general.

Methods

Mice

The study was performed in male B6129F₂/J mice, 25–35 g (age, 8–16 wk) purchased from Jackson Laboratory (Bar Harbor, ME). All mice were maintained in microisolator cages under specific pathogen-free conditions in a room with a temperature of 24 °C, 55–65 % relative humidity, and a 12-h light-dark cycle.

Experimental preparation

The experimental preparation has been described in detail (3, 4). Briefly, mice were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and ventilated by using carefully selected parameters (3, 4). After administration of antibiotics, the chest was opened through a midline sternotomy, and a nontraumatic balloon occluder was implanted around the mid-left anterior descending coronary artery by using an 8–0 nylon suture. To prevent hypotension, blood from a donor mouse was given during surgery. Rectal temperature was carefully maintained between 36.7 and 37.3 °C throughout the experiment.

Experimental protocol

The coronary occlusion/reperfusion protocols have been described in detail (3, 4). In all groups, myocardial infarction was produced by a 30-min coronary occlusion followed by 24 h of reperfusion (3, 4). Mice were assigned to six groups (Fig. 1). Group I (control group) underwent the 30-min occlusion with no prior PC or any other intervention. Group IV (late PC group) underwent a sequence of six 4-min occlusion/4-min reperfusion cycles on day 1; 24 h later (day 2), the 8–0 nylon suture (which had been left in place after the first surgery) was used to produce the 30-min coronary occlusion (3, 4). Mice in group II (vehicle without PC) were given vehicle (50 % DMSO solution [5 µl/g, i.p.]) 30 min before coronary artery occlusion. Group III (NS-398 without PC) was given the selective COX-2 inhibitor, NS-398 (5 mg/kg i.p.), 30 min prior to the 30-min coronary occlusion, with no prior PC or any other intervention (NS-398 [Cayman Chemicals] was dissolved in 50 % DMSO [v/v = 50 %] in normal saline). Groups V (vehic-

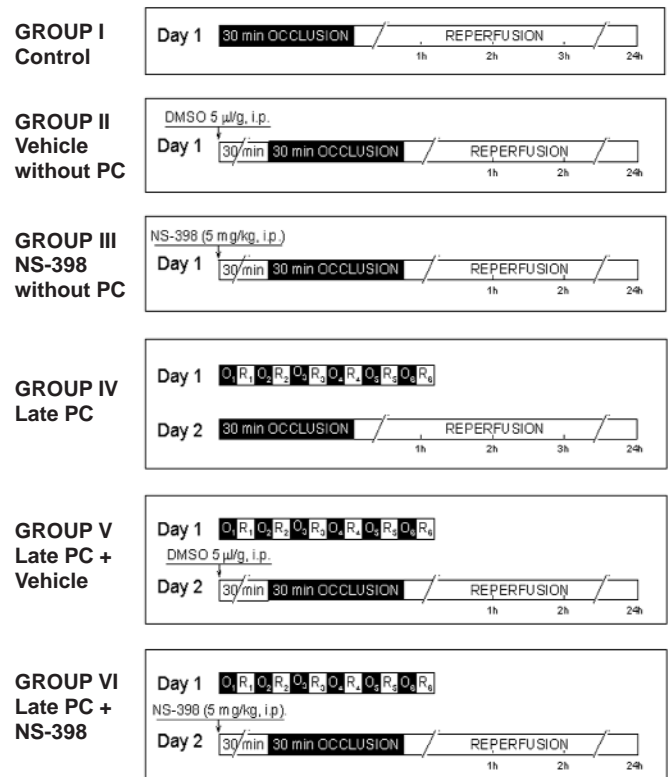


Fig. 1 Experimental protocol. All mice underwent a 30-min coronary occlusion followed by 24 h of reperfusion. Group I (control) did not receive any intervention. Groups II and III received vehicle or NS-398 30 min prior to the 30-min occlusion, respectively. Group IV was preconditioned with six 4-min coronary occlusion/4-min reperfusion cycles on day 1 (24 h prior to the 30-min occlusion). Groups V and VI underwent the same protocol as group IV except that they received either vehicle or NS-398, respectively, 30 min prior to the 30-min occlusion on day 2.

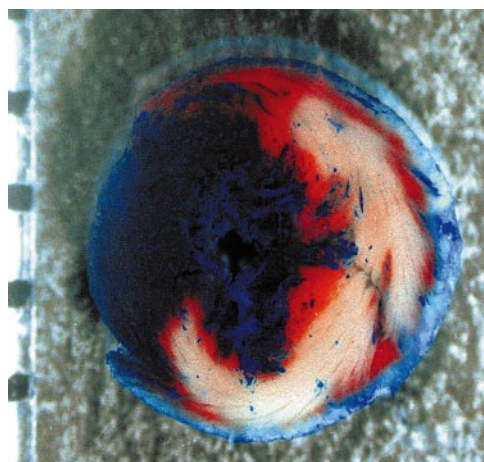
cle + PC) and VI (NS-398 + PC) were subjected to the same protocol as group IV except that the mice were given vehicle (50 % DMSO solution [5 µl/g, i.p.]) or NS-398 (5 mg/kg i.p.), respectively, 30 min before the 30-min coronary occlusion on day 2.

Postmortem tissue analysis

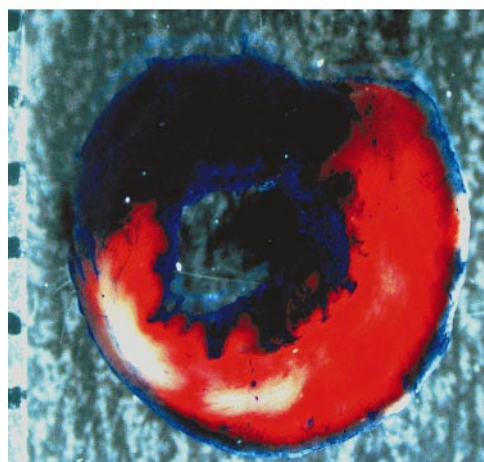
At the conclusion of the study, the occluded/reperfused vascular bed and the infarct were identified by postmortem perfusion of the heart with triphenyltetrazolium chloride and phthalo blue dye (3, 4) (Fig. 2). Infarct size was calculated by using computerized videoplanimetry (3, 4).

Statistical analysis

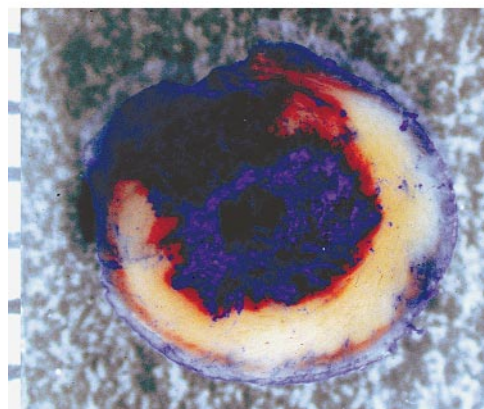
Data are reported as means ± SEM. Measurements were analyzed with a one-way or a two-way repeated-measures



a



b



c

among the six groups with respect to body weight, LV weight, or weight of the region at risk (Table 3). In group I (control group, $n = 10$), infarct size averaged $58.8 \pm 2.1\%$ of the region at risk (Fig. 3). Administration of either NS-398 (group III) or vehicle (group II) had no discernible effect on infarct size in the absence of ischemic PC (Fig. 3). As expected, ischemic PC elicited, 24 h later, a significant reduction in infarct size in group IV ($19.3 \pm 3.4\%$ of the risk region; $P < 0.05$ vs. group

Fig. 2 a Representative example of a heart from group I (control), which was subjected to 30 min of coronary occlusion and 24 h of reperfusion. The occluded/reperfused vascular bed and the infarct were identified by postmortem perfusion with triphenyltetrazolium chloride and phthalo blue dye, as described under Methods. As a result of this procedure, the nonischemic portion of the left ventricle was stained dark blue and viable tissue within the region at risk was stained bright red, whereas infarcted tissue was light yellow. Note the large, confluent areas of infarction with thin rims of viable subendocardial tissue. **b** Representative example of a heart from group IV (late PC group), which was subjected to a sequence of six cycles of 4-min occlusion/4-min reperfusion 24 h prior to the 30-min occlusion. In contrast to **a**, in this heart the 30-min occlusion resulted in small areas of infarction, indicating a cardioprotective effect of late PC. **c** Representative example of a heart from group VI (late PC + NS-398). This mouse underwent the same protocol as that in **b** except that the animal was pretreated with the COX-2 inhibitor NS-398 prior to the 30-min occlusion. In contrast to the pattern observed in **b**, in this mouse the 30-min occlusion resulted in a large infarction indistinguishable from that observed in the control mouse in the left panel, indicating that NS-398 completely abrogated the protective effects of late PC.

I). This cardioprotective effect was completely abrogated when NS-398 was administered 30 min prior to the 30-min coronary occlusion (on day 2) (group VI; $67.7 \pm 3.0\%$ of the risk region) (Fig. 3). Administration of the vehicle for NS-398 had no effect (groups II and V, 57.1 ± 3.1 and $23.6 \pm 3.7\%$, respectively, of the risk region) (Fig. 3). In groups I, II, III, and VI, the size of the infarction was positively and linearly related to the size of the region at risk ($r = 0.93, 0.97, 0.83$, and 0.78 , respectively). As expected, the regression line was significantly shifted to the right in groups IV and V (late PC group and late PC + vehicle group, respectively) compared with group I (control) (Fig. 4), indicating that for any given size of the region at risk, the resulting infarction was smaller in pre-conditioned mice. In contrast, in the late PC + NS-398 group (group VI), the regression line was indistinguishable from that in control mice (Fig. 4), indicating that the protective effects of late PC were completely abrogated.

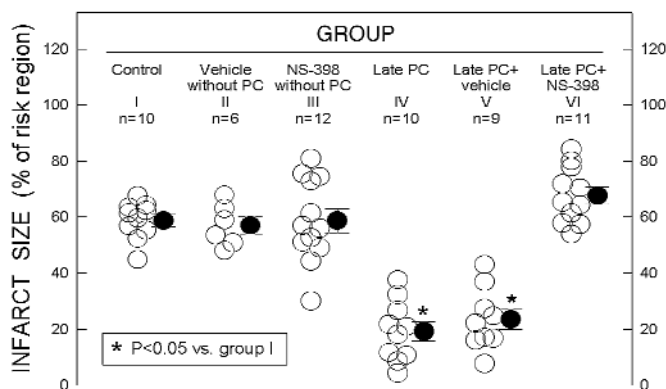


Fig. 3 Infarct size, expressed as a percent of the risk region, in the six groups of mice. Open circles indicate individual measurements, solid circles indicate means. Data are means \pm SEM.

Table 3 Size of left ventricle, risk region, and infarct

		Age (wk)	Body (g)	Heart (mg)	LV (mg)	Risk Region (mg)	Infarct (mg)	Risk Region (% of LV)	Infarct (% of risk region)	Infarct (% of LV)
Group I	n = 10	14 ± 2	28.3 ± 1.1	158 ± 7	114 ± 6	40 ± 3	23 ± 2	34.9 ± 2.3	58.8 ± 2.1	20.6 ± 1.6
Group II	n = 6	12 ± 0	30.1 ± 1.0	159 ± 11	124 ± 7	46 ± 8	27 ± 5	36.2 ± 5.1	57.1 ± 3.1	21.0 ± 3.5
Group III	n = 12	10 ± 0	29.6 ± 0.6	178 ± 6	141 ± 5	46 ± 4	27 ± 3	32.4 ± 2.2	58.8 ± 4.3	19.0 ± 1.8
Group IV	n = 10	18 ± 2	31.7 ± 1.5	145 ± 9	109 ± 6	38 ± 4	6 ± 1*	34.2 ± 2.8	19.3 ± 3.4*	6.2 ± 1.0*
Group V	n = 9	11 ± 0	32.1 ± 0.8	179 ± 7	133 ± 6	39 ± 3	9 ± 4*	29.4 ± 2.2	23.6 ± 3.7*	7.1 ± 1.5*
Group VI	n = 11	11 ± 0	28.5 ± 0.8	170 ± 8	131 ± 7	52 ± 3	35 ± 3	39.7 ± 1.7	67.7 ± 3.0	26.7 ± 1.3

The experimental protocols for the six groups of mice are specified in the legend of Fig. 1. LV left ventricle; Body body weight; Heart total heart weight (ventricles and atria). Data are means ± SEM. *P < 0.05 vs. group I.

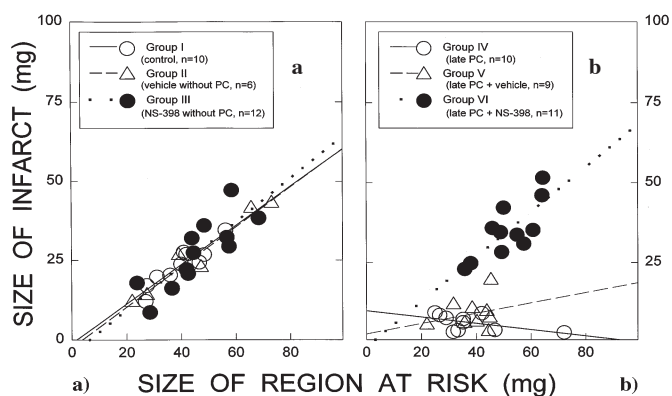


Fig. 4 Relationship between size of the region at risk and size of myocardial infarction. The figure illustrates both individual values and the regression lines obtained by linear regression analysis for the various groups. **a** Nonpreconditioned groups: groups I (Control), II (Vehicle without PC) and III (NS-398 without PC). **b** Studies of late PC: groups IV (late PC), V (late PC + Vehicle), and VI (late PC + NS-398). In all groups, infarct size was positively and linearly related to risk region size. The linear regression equations were as follows: group I, $y = -1.11 + 0.62x$, $r = 0.93$, $P < 0.001$; group II, $y = -2.39 + 0.63x$, $r = 0.97$, $P = 0.001$; group III, $y = -4.45 + 0.69x$, $r = 0.83$, $P < 0.001$; group IV, $y = 9.99 - 0.10x$, $r = 0.54$, $P = 0.106$; group V, $y = 2.78 + 0.16x$, $r = 0.26$, $P = 0.497$; group VI, $y = -1.25 + 0.70x$, $r = 0.78$, $P = 0.005$. ANCOVA demonstrated that the regression lines for groups IV and V were significantly different from that for group I ($P < 0.05$ for each comparison), indicating that for any given risk region size, infarct size was smaller in preconditioned compared with relative control mice. In contrast, the regression line for group VI was not significantly different from that for groups I and III, indicating that inhibition of COX-2 abrogated the late PC effect.

Discussion

This study demonstrates that administration of a COX-2 selective inhibitor, NS-398, results in complete loss of the infarct-sparing protection conferred by the late phase of ischemic PC in mice, indicating that COX-2 is an essential mediator of late PC in this species.

NS-398 was chosen to interrogate COX-2 because it is highly selective for COX-2 vs. COX-1 (IC_{50} for COX-1 and COX-2: 16.8 and 0.1 μ M, respectively) (7). The obliteration

of late PC by NS-398 cannot be ascribed to an inherent detrimental influence of this drug on ischemic cell death, since NS-398 had no effect on infarct size in the absence of ischemic PC (a finding consonant with the notion that COX-2, the target of NS-398, is induced in the heart by stresses such as ischemia (5, 7)). Furthermore, the absence of late PC in NS-398-treated mice cannot be explained by unfavorable differences in determinants of infarct size, such as heart rate, body temperature, or region at risk, for these variables were similar among all groups (Tables 2 and 3). Since the experimental protocol used herein results in normal arterial oxygenation and pH and in normal arterial pressure (3, 4), spurious deviations of these variables from the normal range are unlikely to account for the observed differences in infarct size.

A previous study has identified COX-2 as an essential mediator of late PC in rabbits (5). The present investigation expands and corroborates these findings by demonstrating that COX-2 also plays an indispensable role in late PC in the mouse. The data reported herein support the concept that the role of COX-2 in late PC is not species specific but rather represents a general mechanism of delayed cardiac adaptation to stress. Given the increasing utilization of genetically-engineered mice, the notion that COX-2 mediates the cardioprotection of late PC in this species has important implications for future studies of ischemic PC utilizing gene-targeted or transgenic animals.

Many important questions remain to be addressed regarding the involvement of COX-2 in delayed cardioprotection and the mechanism for COX-2 upregulation. Nevertheless, the present data indicate that, in addition to iNOS (1–3, 6), at least one additional gene, COX-2, plays a crucial role in late PC in the mouse. Our results suggest that late PC is a multigenic phenomenon with greater complexity than heretofore suspected.

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