COX-2: an in vivo evidence of its participation in heat stress-induced myocardial preconditioning

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Abstract

Objective: Heat stress (HS) is known to induce delayed protection against myocardial infarction. We have previously shown that inducible nitric oxide synthase (iNOS), was involved in mediating this form of preconditioning. Since iNOS and cyclooxygenase-2 (COX-2) are co-induced in various cell types, the goal of this study was to investigate whether COX-2 could also participate to the HS-induced cardioprotection.

Methods and Results: A total of 78 male Wistar rats, subjected to either heat stress (42 °C for 15 min) or sham anaesthesia were used for this study. Twenty-four hours later, they were treated or not with a selective COX-2 inhibitor, either celecoxib (3 mg kg⁻¹, i.p.) or NS-398 (5 mg kg⁻¹, i.p.), 30 min before being subjected to a 30-min occlusion of the left coronary artery followed by a 120-min reperfusion, in vivo. HS resulted in a marked increase in myocardial COX-2 protein expression at 24 h, associated with a significant protection against infarction (46.0 ± 1.4% in sham vs. 26.8 ± 3.8% in HS group) (P < 0.05). Administration of selective COX-2 inhibitor 24 h after HS, completely abrogated this delayed cardioprotection (46.4 ± 3.6 and 48.0 ± 2.8%, respectively, in HS + celecoxib and HS + NS-398 groups).

Conclusion: This study provides the first evidence of an implication of COX-2 as a mediator of HS-induced cardioprotection. This suggests that prostaglandins are involved in this type of cardioprotective preconditioning.

Keywords: Shock; Preconditioning; Ischemia; Cyclooxygenase-2

1. Introduction

Environmental stresses, such as heat stress (HS), are known to induce the synthesis of heat shock proteins (Hsps) which seem to play an important role in the cell ability to survive noxious stresses [1]. Heat stress is also known to increase myocardial tolerance to a subsequent period of ischaemia by preserving myocardial function [2].

Abbreviations: COX-2, cyclooxygenase-2; HR, heart rate; HS, heat stress; Hsp, heat shock protein; I, infarct zone; IL-1β, interleukin-1β; iNOS inducible nitric oxide synthase; IP, ischaemic preconditioning; LCA, left coronary artery; LV, left ventricle; MAP, mean arterial pressure; MAPK, mitogen activated protein kinase; NF-κB, nuclear factor-kappa B; PGs, prostaglandins; PKC, protein kinase C; R, risk zone; ROS, reactive oxygen species; TNF-α, tumour necrosis factor-α.

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and reducing myocardial necrosis [3,4]. The development of this late cardioprotection, which occurs 24 to 48 h after HS, implies an immediate activation of triggers followed by the synthesis of proteins which mediate the protection.

Recently, we have demonstrated that inducible nitric oxide synthase (iNOS) is implicated both as a trigger [5] and a mediator [6] of the HS-induced cardioprotection. Indeed, iNOS inhibitors, administered both during HS or 24 h later, abolish the HS-induced reduction of infarct size, in the rat.

Stress-induced iNOS expression seems to be associated with simultaneous induction of cyclooxygenase-2 (COX-2) in various cell types including smooth muscle cells [7] and cardiomyocytes [8]. Cyclooxygenase, also named prostaglandin H₂ synthase, is the enzyme that catalyses the first two steps in the biosynthesis of prostaglandins (PGs) from the substrate arachidonic acid [9]. Exogenous PG perfusion
has been shown to preserve myocardial metabolism [10], decrease infarct size [11] and recent studies support a role for COX-2, the inducible isoform of cyclooxygenase, as an essential mediator of the delayed protection induced by ischaemic preconditioning (IP) [12,13].

Since the HS-induced delayed cardioprotection is similar to that seen with IP, mediators of IP (for a review, see Ref. [14]) might also be responsible for HS-induced cardioprotection. Therefore, the goal of this study was to determine whether COX-2 could contribute to the HS-induced cardioprotection. We thus examined the effect of two selective COX-2 inhibitors, celecoxib and NS-398, on the HS-induced reduction of infarct size, in vivo in the rat.

2. Methods

2.1. Experimental protocol and treatment groups

Male Wistar rats (280–320 g) were used for these studies. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

First, rats were submitted to either heat stress (HS groups) or anaesthesia without hyperthermia (sham groups). Subsequently, all animals were allowed to recover for 24 h. Then, ischaemia-reperfusion was performed in vivo, in anaesthetised rats. Eight experimental groups were studied: sham group—rats were only anaesthetised, sham + vehicle group—animals were given vehicle [20% dimethylsulphoxide (DMSO) in saline, i.p.] 40 min before ischaemia, sham + celecoxib group—animals were treated with the selective COX-2 inhibitor, celecoxib (3 mg kg⁻¹, i.p.) [13], 40 min before ischaemia, sham + NS-398 group—animals were given the selective COX-2 inhibitor, NS-398 (5 mg kg⁻¹, i.p.) [12,13], 40 min before ischaemia. In HS, HS + vehicle, HS + celecoxib and HS + NS-398 groups, rats were similarly treated 24 h after heat stress. Celecoxib and NS-398 were dissolved in 20% DMSO in saline.

The experimental protocol is summarised in Fig. 1.

2.2. Mortality and exclusion

A total of 78 rats were used for this study. Reasons for exclusion are summarised in Table 1.

2.3. Heat stress protocol

Heat stress was achieved, as previously described [6], by placing lightly anaesthetised rats (25 mg kg⁻¹ i.p. sodium pentobarbitone) in an environmental chamber under an infrared light. Their body temperature, recorded with a rectal probe, was increased to 42±0.2 °C for 15 min. Sham control animals were anaesthetised only. All rats were allowed to recover for 24 h.

2.4. Ischaemia-reperfusion protocol

Twenty-four hours after heat stress or sham anaesthesia, rats were re-anaesthetised with 60 mg kg⁻¹ i.p. sodium pentobarbitone and ischaemia was induced by clamping the left coronary artery (LCA) for 30 min. The ischaemic period was followed by 24 h of reperfusion.

Table 1

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
<th>Sham</th>
<th>Sham + vehicle</th>
<th>Sham + celecoxib</th>
<th>Sham + NS-398</th>
<th>HS</th>
<th>HS + vehicle</th>
<th>HS + celecoxib</th>
<th>HS + NS-398</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death during heat stress</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Death during anaesthesia</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bleeding</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Sustained VF during ischaemia</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Total number of rats excluded</td>
<td>–</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Total number of rats included in the study (in vivo+WB)</td>
<td>(8+4)</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>(7+4)</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>63</td>
</tr>
</tbody>
</table>

Sham—Sham anaesthetised, HS—heat-stressed, vehicle—20% DMSO in saline, VF—ventricular fibrillation, WB—Western blot.
pentobarbitone, intubated with a small cannula, and mechanically ventilated using a rodent ventilator (Harvard) at a rate of 55 cycles min⁻¹ and a tidal volume of 1 ml 100 g⁻¹ body weight. Rectal temperature was carefully maintained at 37±0.3 °C throughout the experiment.

Polyethylene tubes were inserted in the penile vein and in the left carotid artery for drug administration and mean arterial pressure (MAP) monitoring (MacLab, eight channels, ADInstrument), respectively.

For temporary occlusion of the left coronary artery (LCA), a 3/0 silk suture (Mersilk W546, Ethicon) was placed around the artery a few millimetres distal to the aortic root. After 20 min of stabilisation, regional ischaemia was induced by tightening the snare around the LCA for 30 min. Thereafter, the heart was reperfused for 120 min, rapidly excised, rinsed in saline and the coronary artery ligature was retied. Infarct size determination was then performed as described below. Unisperse blue dye (Ciba-Geigy) was slowly infused through the aorta to delineate the myocardial risk zone. After removal of the right ventricle and connective tissues, the heart was frozen and then sectioned into 2 mm transverse sections from apex to base (6–7 slices/heart). Following defrosting, the slices were incubated at 37 °C with 1% triphenyltetrazolium chloride in phosphate buffer (pH 7.4) for 10–20 min and fixed in 10% formaldehyde solution to clearly distinguish stained viable tissue and unstained necrotic (46.4±6 1.4% in sham group) tissue. Left ventricular infarct zone (I) was determined using a computerised planimetric technique (Minichromax, Biolab) and expressed as a percentage of the risk zone (R) and of the left ventricle (LV).

2.5. Western blot analysis of myocardial COX-2

To determine COX-2 expression, additional animals were submitted to either HS or sham anaesthesia (n=4 in each group). Twenty-four hours later, the animals were re-anaesthetised (60 mg kg⁻¹ i.p. sodium pentobarbitone) and treated with heparin (500 U kg⁻¹, i.v.) before their heart was quickly harvested. Total cellular protein was extracted and samples were loaded at 100 μg/lane. Proteins were separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and electrotransferred to a nitrocellulose membrane. COX-2 was detected using a rabbit antibody (Upstate Biotechnology, Euromedex) at a dilution of 1/250 and visualised with an enhanced chemiluminescence system (Pierce). After scanning, the blots were analysed for optical density using an image analysis program (NIH Image for Windows). Each COX-2 signal was normalised to the corresponding Ponceau signal and presented as arbitrary units (a.u.).

2.6. Products

Celecoxib, NS-398 and 2,3,5-triphenyltetrazolium chloride were obtained from Sigma Chemical.

2.7. Statistical analysis

All data are presented as mean±S.E.M. Comparisons in heart rate and mean arterial pressure were performed using repeated measure analysis of variance (ANOVA) with post-hoc multiple comparison Tukey tests. Infarct size was analysed by a one-way ANOVA. COX-2 protein expression was analysed using a non parametric Mann–Whitney rank sum test. P values ≤0.05 were considered significant.

3. Results

3.1. Haemodynamic data

Table 2 summarises haemodynamic data recorded in the eight experimental groups during the stabilisation period and the ischaemia-reperfusion protocol. Mean arterial pressure and heart rate did not significantly differ among the various groups.

3.2. Myocardial infarct size

Infarct-to-risk ratio (I/R) was reduced from 46.0±1.4% in sham group to 26.8±3.8% in HS group (P≤0.05, Fig. 2). This effect of heat stress was abolished in celecoxib (46.4±3.6%) and NS-398 (48.0±2.8%) treated groups.

In non heat-stressed rats, treatment with celecoxib (49.7±3.5%) and NS-398 (52.9±3.2%) had no effect on infarct size. Similar results were observed with the I/LV ratio (Table 3). Myocardial risk size expressed as the percentage of the left ventricle (R/LV) was similar in the eight experimental groups (Table 3). Therefore, differences in infarct size did not result from variability in the risk zone.

3.3. Myocardial COX-2 protein expression

Western blot analysis of myocardial COX-2 (72 kDa) expression showed a marked increase of this protein, 24 h after HS (1534±193 a.u. in HS group vs. 97±72 a.u in sham group, P=0.029) (Fig. 3).

4. Discussion

Although the protective effect conferred by prior heat stress against subsequent cardiac ischaemic injury is a well known phenomenon (for a review, see Ref. [15]), the exact signalling pathway of this delayed protection remains largely unknown. The pertinent finding of this work is the implication of COX-2 as a mediator of this form of cardioprotection. Indeed, the HS-induced reduction in infarct size was abolished by the administration of selective COX-2 inhibitors, celecoxib and NS-398, prior to ischaemia. Furthermore, we have also shown a marked
increase in myocardial COX-2 protein expression 24 h after HS. These findings imply that COX-2 activity is necessary during ischaemia to mediate the reduction in infarct size, in vivo, in the heat stressed rat.

4.1. Pharmacological treatments: celecoxib and NS-398

To minimise the possibility that the loss of HS-induced protective effect could be due to non specific effects, we chose to evaluate the effect of two very selective COX-2 inhibitors, celecoxib and NS-398. These drugs have been reported to be highly selective for COX-2 (IC$_{50}$: 0.04 and 0.1 μM, respectively) vs. COX-1 (IC$_{50}$: 15 and 16.8 μM, respectively) [9]. Furthermore, since haemodynamic variables and risk zones were similar among all experimental groups (Tables 2 and 3), the loss of late protection in celecoxib and NS-398-treated rats cannot be explained by changes in these parameters.

4.2. COX-2 and myocardium: deleterious or salutary effects?

COX-2 is generally thought to be detrimental. Indeed, numerous works have shown an essential role of COX-2 in inflammation, cancer or apoptosis (for a review, see Ref. [9]). A recent study demonstrates that induction of COX-2 increases the production of proinflammatory prostanoids and contributes to dysfunction in ischaemic myocardium [16]. Furthermore, an induction of COX-2 has been shown in myocardium of patients with congestive heart failure [17].

**Table 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Stabilisation</th>
<th>Ischaemia</th>
<th>Reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (beats min$^{-1}$)</td>
<td>5 min</td>
<td>29 min</td>
</tr>
<tr>
<td>Sham</td>
<td>380±13</td>
<td>377±14</td>
<td>355±16</td>
</tr>
<tr>
<td>Sham+vehicle</td>
<td>384±19</td>
<td>374±25</td>
<td>347±21</td>
</tr>
<tr>
<td>Sham+celecoxib</td>
<td>397±14</td>
<td>387±15</td>
<td>369±13</td>
</tr>
<tr>
<td>Sham+NS-398</td>
<td>389±15</td>
<td>388±16</td>
<td>380±3</td>
</tr>
<tr>
<td>HS</td>
<td>413±12</td>
<td>407±12</td>
<td>386±17</td>
</tr>
<tr>
<td>HS+vehicle</td>
<td>378±9</td>
<td>385±13</td>
<td>363±16</td>
</tr>
<tr>
<td>HS+celecoxib</td>
<td>399±17</td>
<td>395±14</td>
<td>394±13</td>
</tr>
<tr>
<td>HS+NS-398</td>
<td>402±15</td>
<td>391±18</td>
<td>361±18</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>104±7</td>
<td>84±6</td>
<td>79±7</td>
</tr>
<tr>
<td>Sham+vehicle</td>
<td>100±4</td>
<td>78±5</td>
<td>77±5</td>
</tr>
<tr>
<td>Sham+celecoxib</td>
<td>94±3</td>
<td>79±4</td>
<td>82±7</td>
</tr>
<tr>
<td>Sham+NS-398</td>
<td>103±5</td>
<td>91±6</td>
<td>91±6</td>
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<tr>
<td>HS</td>
<td>119±6</td>
<td>100±9</td>
<td>86±11</td>
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<tr>
<td>HS+vehicle</td>
<td>116±6</td>
<td>102±8</td>
<td>86±12</td>
</tr>
<tr>
<td>HS+celecoxib</td>
<td>107±6</td>
<td>89±10</td>
<td>85±8</td>
</tr>
<tr>
<td>HS+NS-398</td>
<td>110±6</td>
<td>94±7</td>
<td>81±4</td>
</tr>
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</table>

**Table 3**

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>R/LV</th>
<th>I/LV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>8</td>
<td>58.6±2.5</td>
<td>27.2±1.7</td>
</tr>
<tr>
<td>Sham+vehicle</td>
<td>6</td>
<td>53.8±1.7</td>
<td>24.7±1.0</td>
</tr>
<tr>
<td>Sham+celecoxib</td>
<td>7</td>
<td>53.2±2.9</td>
<td>26.9±3.1</td>
</tr>
<tr>
<td>Sham+NS-398</td>
<td>7</td>
<td>59.5±6.1</td>
<td>31.7±2.6</td>
</tr>
<tr>
<td>HS</td>
<td>7</td>
<td>53.2±2.9</td>
<td>14.4±2.5$^*$</td>
</tr>
<tr>
<td>HS+vehicle</td>
<td>6</td>
<td>53.7±1.3</td>
<td>10.5±0.8$^*$</td>
</tr>
<tr>
<td>HS+celecoxib</td>
<td>7</td>
<td>57.1±0.8</td>
<td>27.1±2.3</td>
</tr>
<tr>
<td>HS+NS-398</td>
<td>7</td>
<td>50.1±1.1</td>
<td>24.5±1.9</td>
</tr>
</tbody>
</table>

* Sham—sham-anaesthetised, HS—heat-stressed, vehicle—20% DMSO in saline. Data are mean±S.E.M. * P<0.001 vs. all the other groups (one-way ANOVA).
However, the present study demonstrates the implication of COX-2 as a mediator of the HS-induced cardioprotection. This is in agreement with recent studies also suggesting a beneficial role of this protein in the myocardium. Indeed, a specific role for COX-2 as a mediator of delayed cardioprotection conferred by other types of preconditioning has been reported. Shimura et al. showed that COX-2 mediates the ischaemic preconditioning (IP)-induced protection against both myocardial stunning and infarction [13], in conscious rabbits [13] or in mice [12]. The role of COX-2 in mediating late cardioprotection has also been described in a pharmacological model of late preconditioning induced by the activation of δ-opioid receptors [18].

4.3. COX-2 upregulation and late preconditioning

In support for the role of COX-2 in the HS-induced preconditioning, we observed a marked induction of COX-2 protein expression in rat myocardium 24 h after hyperthermia. However, our results do not permit us to determine in which cell type (leucocytes, myocytes, others) COX-2 is expressed. This is in accordance with the increase in COX-2 protein level reported 24 h after IP in rabbit myocardium [13]. Other preconditioning stimuli, such as hypoxia [19], oxidative stress [20] or cytokine administration [21] have also been shown to induce COX-2 in different cell types, including cardiomyocytes.

4.4. COX-2 and the HS response

The signalling pathways whereby HS leads to COX-2 expression in the heart are unknown. An interesting observation is that COX-2 is co-induced with iNOS in response to various stresses such as ischaemia, hypoxia or cytokines (for a review, see Ref. [9]). It has recently been demonstrated that the enzymatic activity of newly synthesized COX-2 following IP requires iNOS-derived NO, which implies that COX-2 is located downstream of iNOS in the protective pathway of this form of late preconditioning [22]. Moreover, similarities appear between the signalling pathways that control stress-induced COX-2 and iNOS expression, both involving reactive oxygen species (ROS) [23–25], protein kinase C (PKC) [26,27], and nuclear factor kappa B (NF-κB) [19,28].

We have shown that ROS [6], PKC [29], p38 mitogen activated kinase (MAPK) [30] and iNOS [5] can trigger the HS-induced cardioprotection. More recently, we have
also shown that iNOS, which is up-regulated following HS, is also a mediator of the HS-induced cardioprotection [6].

It has been proposed that cytokine release (in particular IL-1β and TNF-α) upon ROS generation during hyperthermia could activate nuclear factor-kappaB (NF-κB) [31,32]. NF-κB promotes iNOS [33] and COX-2 [34] expression and could thus, along with PKC [26,27,35] and other MAPKs [8,34,35], be responsible for the gene transcription leading to this protein co-induction and their cardioprotective effect upon HS.

4.5. COX-2 mediated cardioprotection

COX-2 catalyses the first two steps in the biosynthesis of PGs from arachidonic acid [9]. PGs, in particular PGI2 and PGE2, are known to be involved in the antiarrhythmic effect [36] and the preservation of endothelial function [37] induced by IP. Moreover, a recent study [13] demonstrated that PGE2 and/or PGI2 are the likely effectors of COX-2-dependent cardioprotection in a rabbit model of IP. An increased production of PGI2 also appears to mediate the opioid-induced late phase of preconditioning [18].

HS stimulates the accumulation of PGE2 [38,39]. Furthermore, PGE2 and PGI2 activate ATP-sensitive potassium (K\textsubscript{ATP}) channels [11,40–42]. These channels are known to mediate the HS-induced cardioprotection [43–45], potentially by protecting cardiac mitochondria at reperfusion [46].

We can thus hypothesise that the COX-2-mediated cardioprotection following HS could, in part, be due to the activation of K\textsubscript{ATP} channels, via PGE2 and/or PGI2 release. Further studies are needed to confirm this hypothesis.

In conclusion, this study provides the first evidence of an implication of COX-2 as a mediator of HS-induced cardioprotection. This suggests that PGs are involved in this protective mechanism, even if their exact nature and their location in the signalling pathway of HS preconditioning remain to be determined.

References


