Possible Role of Cyclooxygenase-2 in Remote Aortic Preconditioning Induced Cardioprotection in Rat Heart

Harlokesh Narayan Yadav, Manjeet Singh, P.L. Sharma, Dhiraj Mittal, Tapan Behl and Atinder Pal Kaur
Department of Pharmacology, ISF College of Pharmacy, Moga 142001, Punjab, India

Abstract: Background: Recently it has been noted that Cyclooxygenase-2 (COX-2) is involved in early phase of ischemic preconditioning (IPC) induced cardioprotection. The present study was designed to investigate the role of COX-2 in the cardioprotective effect of remote aortic preconditioning (RAPC) in rat. RAPC was given by 4 times of 5' occlusion of abdominal aorta. Materials and Methods: Isolated perfused rat heart was subjected to global ischemia of 30 min., followed by reperfusion for 120 min. Coronary effluent was analyzed for LDH and CK release to access the degree of cardiac injury. Myocardial infarct size was estimated macroscopically using TTC staining. Results: RAPC produced a significant decrease in LDH and CK release and decrease in the myocardial infarct size, as compared to ischemia/reperfusion (I/R) control group. Pretreatment with celecoxib, a selective COX-2 inhibitor and glibenclamide, a blocker of K\textsubscript{ATP} channels, given separately significantly attenuated the RAPC-induced cardioprotection. The cardioprotective effect of celecoxib and glibenclamide, administered in combination was almost equal to the sum total of the effect produced by these drugs when administered singly. Conclusion: On the basis of these results it was concluded that the cardioprotective effect of RAPC may be mediated through upregulation of COX-2 and subsequent activation of K\textsubscript{ATP} channels.

Key words: Ischemia, reperfusion, celecoxib, glibenclamide

INTRODUCTION

The endogenous cardioprotective phenomenon of ischemic preconditioning (IPC) is well documented. Brief episodes of ischemia followed by reperfusion of tissue at a distance from target organ offered protection from ischemia reperfusion induced injury (Gho et al., 1996; Singh and Chopra, 2004) and this phenomenon has been termed as remote preconditioning (Gres et al., 2002; Weinbrenner et al., 2004). The IPC mediated cardioprotection is biphasic. The early phase (classical IPC) is immediate in onset and lasts 2-3 h (Murry et al., 1991; Van Winkle et al., 1991; Berekhart et al., 1995). The cardioprotective response, however, reappears after 12-24 h, lasts for 3-4 days (Kuzuy et al., 1993; Marber et al., 1993) and is known as late phase of IPC. Early phase of IPC is mediated through different existing cellular kinases i.e. protein kinase C (PKC), Glycogen synthase kinase-3β (GSK-3β) (Garg et al., 2010; Yadav et al., 2010a, b) where as the late phase of IPC exerts its cardioprotection by synthesizing new proteins viz. inducible nitric oxide synthetase (NO), (Guo et al., 1999) aldose reductase (Shimura et al., 2002), superoxide dismutase (Yamashita et al., 1994) and cyclooxygenase-2 (COX-2) (Guo et al., 2000).

Cyclooxygenase-2 is an inducible form of enzyme (Shimura et al., 2000; Bolli et al., 2002) is upregulated during ischemia, hypoxia and oxidative stress (Shimura et al., 2000; Bolli et al., 2002). It has been reported that vascular endothelium is the major source of COX-2 (Bryant et al., 1998; Schror, 2009). Involvement of COX-2 in the cardioprotective effect of late phase of IPC is well documented (Liu et al., 2006). Upregulation of COX-2 produces cardioprotection through prostanoids receptor i.e. PTG (Shimura et al., 2002) and by making a complex with Nitric Oxide (NO) (Wang et al., 2004). Recently, Booth and Lucchesi, (2008) have reported that COX-2 produces early phase of IPC induced cardioprotection, without mediating through prostanoids receptor. RAPC is noted to protect the myocardium against I/R induced injury (Sharma, 1999; Weinbrenner et al., 2002; Singh and Sharma, 2004) but the nature of this endogenous protective mechanism remains controversial (Dickson et al., 1999; Takaoka et al., 1999; Weinbrenner et al., 2002, 2004; Moses et al., 2005). Upregulation of COX-2 is noted to activate the ATP sensitive potassium channel (K\textsubscript{ATP}) (Hide et al., 1995; Shimura et al., 2004; Shibata et al., 2005). Moreover short occlusion of abdominal aorta-induced cardioprotection is mediated through K\textsubscript{ATP} channels.
MATERIALS AND METHODS

The experimental protocol used in the present study was approved by Institutional Animal Ethics Committee. Drugs and chemicals: Celecoxib and glibenclamide (Sigma Aldrich [P] Ltd., Bangalore, India) were dissolved in minimum quantity of PEG-400 (Ranbaxy fine chemicals Ltd) just before use. All other reagents used in this study were of analytical grade and always freshly prepared before use.

Remote aortic preconditioning: Rats were anaesthetized with thiopental sodium (40 mg kg⁻¹, i.p.). A 2 cm long incision was given on the abdomen. Lower portion of abdominal aorta was exposed below the point of origin of renal artery and a silk suture (numbered 5/0) was used to make a shoelace knot to occlude the abdominal aorta and knot was untied for reperfusion. Four episodes of ischemia and reperfusion, each comprising of 5 min occlusion and 5 min reperfusion, were used to produce RAPC.

Isolated rat heart preparation: Hearts from heparinized rats (500 IU, i.p.) were rapidly excised and immediately mounted on Langendorff's apparatus (Langendorff, 1895). The heart was encosed by a double walled jacket, the temperature of which was maintained by circulating water heated to 37°C. The preparation was perfused retrogradely at a coronary flow rate of 7-9 mL min⁻¹ at a constant pressure of 80 mmHg, with Kreb's-Henseleit (KH) buffer (NaCl 118 mM; KCl 4.7 mM; CaCl₂ 2.5 mM; MgSO₄, TH₂O 1.2 mM; NaHCO₃ 25 mM; KH₂PO₄ 1.2 mM; C₃H₆O₃, 11 m M), pH 7.4, maintained at 37°C, bubbled with 95% O₂ and 5% CO₂. Global ischaemia was produced for 30 min by blocking the inflow of Kreb's-Henseleit solution. It was followed by reperfusion for 120 min. Coronary effluent was collected before ischemia, immediately, 5 min. and 30 min. after reperfusion for estimation of Lactate Dehydrogenase (LDH) and Creatine Kinase (CK). Two silver electrodes fixed at ventricular apex and origin of aorta were employed to record ECG (BPL MK801, Bangalore, India) in order to monitor heart rate.

Assessment of infarct size: Heart was removed from Langendorff’s apparatus. Both the auricles and the root of aorta was excised and ventricles were kept overnight at 4°C. Frozen ventricles were sliced into uniform sections of 2-3 mm thickness. The slices were incubated in 1% triphenyltetrazolium chloride (TTC) at 37°C in 0.2 M Tris buffer (pH 7.4) for 30 min. The normal portion of myocardium was stained brick red while infarcted myocardium remained unstained. Infarct size was measured both by volume and weight method (Fishbein et al., 1981).

Assessment of myocardial injury: To determine the extent of myocardial injury, release of lactate dehydrogenase (LDH) and CK was measured in the coronary effluent using 2-4 DNPH (King, 1959) and Hughes method (Hughes, 1962), respectively. Values were expressed in international units (IU) per liter.

Experimental protocol: Nine groups of Wistar rats were employed in the present study. Diagrammatic representation of experimental protocols is shown in Fig. 1. Group I (Sham control; n = 6): Rats were subjected to surgical procedures to isolate abdominal aorta and to pass ligature beneath it but aorta was not occluded. Hearts were excised 40 min after isolation of aorta and were perfused for 160 min without subjecting them to global ischemia. Group II (I/R Control group; n = 6): After the surgical procedures as describe in group I, heart was subjected to global ischemia for 30 min followed by reperfusion for 120 min. Group III (Remote aortic preconditioning Group; n = 6): Rats were subjected to four episodes of remote aortic preconditioning and just after the last episodes heart was excised and mounted on Langendorff's apparatus and were subjected to global ischemia for 30 min followed by reperfusion for 120 min. Group IV (Celecoxib treated control Group; n = 6): Rats were administered celecoxib (30 mg kg⁻¹, i.p.,) 4h before induction of RAPC. Rest of protocol was same as described in group II. Group V (Glibenclamide treated control group n = 6): Rats were administered glibenclamide (1 mg kg⁻¹ i.p.) 4.5 h before induction of RAPC. Rest of protocol was same as described in group II. Group VI (Celecoxib treated remote aortic preconditioning group; n = 6): Celecoxib (3 mg kg⁻¹, i.p.) was administered to rats 4 h before induction of RAPC followed by RAPC as described in group III. Group VII (Celecoxib treated remote aortic preconditioning Group; n = 6): Celecoxib (30 mg kg⁻¹, i.p.) was administered to rats 4 h before the induction of RAPC followed by RAPC as described in group III. Group VIII (Glibenclamide treated remote aortic preconditioning group; n = 6): Rats were administered glibenclamide (1 mg kg⁻¹, i.p.,) 4.5 h before induction of RAPC followed by RAPC as described in
Fig. 1: Diagrammatic representation of experimental protocol. I represents Ischaemia, R represents Reperfusion and S represents Stabilization.

Group III: Group IX (Glibenclamide and celecoxib treated remote aortic preconditioning group; n = 6): Rats were administered glibenclamide (1 mg kg⁻¹ i.p.) 4.5 h and celecoxib (30 mg kg⁻¹ i.p.) 4 h before induction of RAPC followed by induction of RAPC as described in group III.

Statistical analysis: Values are expressed as Mean±SEM. One-way ANOVA followed by Studentised range test and Dunnett's test were employed as post-hoc tests for multiple comparisons between groups and comparisons with control group, respectively. Value of p<0.05 was considered to be statistically significant.

RESULTS

Effect of remote aortic preconditioning on ischaemia and reperfusion-induced myocardial injury: Global ischaemia for 30 min followed by reperfusion for 120 min significantly increased myocardial infarct size measured both by volume and weight method (Fig. 2) and the release of LDH (Fig. 3) and CK (Fig. 4) in coronary effluent. The coronary flow rate (8.3±0.25 to 1.5±0.30) and heart rate (222±17 to 98±15) were decreased and this decrease persisted for entire period of 120 min of reperfusion (Table 1, 2). RAPC significantly attenuated I/R induced increase in myocardial infarct size (Fig. 2), release of LDH (Fig. 3) and CK (Fig. 4) but had no significant effect on the decreased coronary flow rate (Table 1) and heart rate (Table 2).

Effect of Celecoxib and Glibenclamide on cardioprotective effect of remote aortic preconditioning: Both celecoxib (30 mg kg⁻¹ i.p.) and glibenclamide (1 mg kg⁻¹ i.p.) per se produced no significant effect on I/R induced increase in myocardial infarct size (Fig. 2), release of LDH (Fig. 3) and CK (Fig. 4) in coronary effluent but significantly attenuated
Table 1: Effect of Remote Aortic Preconditioning (RAPC) and Pharmacological Interventions on Coronary Flow Rate (mL min⁻¹) in isolated rat heart subjected to global ischemia (30 min) and reperfusion (120 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>RAPC</th>
<th>Control</th>
<th>Glibenclamide per se</th>
<th>Cececoxib per se</th>
<th>30 mg kg⁻¹ Cececoxib+RAPC</th>
<th>Glibenclamide 1 mg kg⁻¹+Cececoxib</th>
<th>30 mg kg⁻¹+RAPC</th>
<th>Glibenclamide 1 mg kg⁻¹+RAPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Rate (mL min⁻¹)</td>
<td>8.4±0.20</td>
<td>8.1±0.34</td>
<td>8.3±0.25</td>
<td>8.4±0.34</td>
<td>5.5±0.40*</td>
<td>8.7±0.37*</td>
<td>4.2±0.50*</td>
<td>2.6±0.48*</td>
<td>4.1±0.43*</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 5). Coronary flow rate measured after stabilization (basal) 5, 15, 30, 45, 60, and 120 min of reperfusion. *p<0.05 vs Sham

Table 2: Effect of Remote Aortic Preconditioning (RAPC) and pharmacological interventions on heart rate (beats min⁻¹) in isolated rat heart subjected to global ischemia (30 min) and reperfusion (120 min)

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>RAPC</th>
<th>Control</th>
<th>Glibenclamide per se</th>
<th>Cececoxib per se</th>
<th>30 mg kg⁻¹ Cececoxib+RAPC</th>
<th>Glibenclamide 1 mg kg⁻¹+Cececoxib</th>
<th>30 mg kg⁻¹+RAPC</th>
<th>Glibenclamide 1 mg kg⁻¹+RAPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Rate (beats min⁻¹)</td>
<td>230±25</td>
<td>207±24</td>
<td>205±12</td>
<td>199±18</td>
<td>194±26</td>
<td>182±30</td>
<td>237±23</td>
<td>212±21</td>
<td>209±25</td>
</tr>
</tbody>
</table>

Values are expressed as Mean±SEM (n = 5). Coronary flow rate measured after stabilization (basal) 5, 15, 30, 60, and 120 min of reperfusion. *p<0.05 vs Sham

Fig. 2: Effect of remote aortic preconditioning (RAPC), cececoxib, glibenclamide and cececoxib plus glibenclamide, treatment in RAPC on ischemia-reperfusion-induced myocardial infarct size in isolated rat heart. Values are expressed as mean SEM of six animals. a = p<0.05 vs sham control, b = p<0.05 vs IR control, c = p<0.05 vs RAPC.

Fig. 3: Effect of remote aortic preconditioning (RAPC), cececoxib, glibenclamide and cececoxib plus glibenclamide, treatment in RAPC on ischemia-reperfusion-induced LDH release in isolated rat heart. Value are expressed as mean SEM of six animals. LDH was estimated in sample of coronary effluent collected before global ischemia (Basal), immediately and 30 min after reperfusion. a = p<0.05 vs sham control, b = p<0.05 vs IR control, c = p<0.05 vs RAPC.

the RAPC-induced decrease in myocardial infarct size (Fig. 2), release of LDH (Fig. 3) and CK (Fig. 4). The effect of the combined treatment with cececoxib and glibenclamide was almost equal to the sum total of the effect produced by these drugs when administered singly (Fig. 2). However, cececoxib and glibenclamide administered in combination produce no significant
Fig. 4: Effect of remote aortic preconditioning (RAPC), celecoxib, glibenclamide and celecoxib-glibenclamide treatment in RAPC on ischemia-reperfusion-induced CK release in isolated rat heart. Values are expressed as mean SEM of six animals. CK was estimated in sample of coronary effluent collected before global ischemia (Basal), 5 min after reperfusion. a = p<0.05 vs sham control, b = p<0.05 vs IR control, c = p<0.05 vs RAPC.

change in coronary flow rate (Table 1) and heart rate (Table 2).

DISCUSSION

Brief occlusion of distant organs, has been reported to produce cardioprotection against sustained ischemia, termed as remote preconditioning. Four episodes of occlusion of abdominal aorta significantly decrease the ischemia and reperfusion-induced (IR) increase in myocardial infarct size and release of LDH and CK. This observation is consistent with our previous reports (Sharma, 1999; Singh and Sharma, 2004).

Cyclooxygenase-2 is an inducible form of enzyme (Morham et al., 1995) and it is upregulated during hypoxia, ischaemia and oxidative stress (Schmedje et al., 1997; Guo et al., 2000; Shinmura et al., 2000, 2002). Vascular endothelium is the major source of COX-2 (Bryant et al., 1998). Induction of COX-2 has been reported to mediate the late phase of cardioprotective effect of IPC (Bolli et al., 2002). Recently, Booth et al. (2008) have reported the role of COX-2 in early phase of IPC mediated cardioprotection and this effect was significantly attenuated by pretreatment of nimesulide (5 mg kg⁻¹) a COX-2 inhibitor. In our study, treatment with celecoxib (30 mg kg⁻¹) did not attenuate the IR induced myocardial injury, however, it significantly attenuated the RAPC-induced cardioprotection (decrease the infarct size, release of LDH and CK). Therefore, it may be probable to suggest that cardioprotective effect of RAPC may be mediated through up-regulation of COX-2 by vascular endothelium injury during brief ischemic episodes by occluding abdominal aorta.

Remote preconditioning is also noted to produce cardioprotection by opening of ATP sensitive potassium channels (K_{ATP}) (Kristiansen et al., 2004; Moses et al., 2005; Taliyan et al., 2010). Moreover, opening of K_{ATP} channel by its selective agonist diazoxide produces similar cardioprotection as IPC (Patel et al., 2002). Glibenclamide is reported to block K_{ATP} channels (Pell et al., 1998; Imagawa et al., 1998; Dickson et al., 2002; Wang et al., 2001, Garlid et al., 1997). In the present study, treatment with glibenclamide significantly attenuated RAPC-induced cardioprotection. This result is in agreement with our previous studies (Taliyan et al., 2010). It may suggest that the observed cardioprotective effect of RAPC induced myocardial protection is in part, mediated by opening of K_{ATP} channel.

However, ischemia-induced upregulation of COX-2 is a time consuming process and peak level of COX-2 derived PGE₁ and PG₂ were observed after 6 h of ischemic insult (Shinmura et al., 2002). However, Booth and Lucchesi (2008) reported that COX-2 upregulation does not protect the myocardium in early phase of preconditioning through the increased production of PG₂. In present study, the total RAPC protocol of 40 min was sufficient to induce COX-2 mediated cardioprotection. The attenuation of RAPC-induced cardioprotection against ischemia-reperfusion injury by combined treatment with celecoxib and glibenclamide was almost equal to the sum total of their effects (additive response) when administered singly. This indicates that these two drugs act at different sites of the same pathway.

Thus, it is concluded that the observed cardioprotective effect of RAPC in ischemia-reperfusion-induced injury may be due to upregulation of COX-2 and subsequent opening of K_{ATP} channels.

ACKNOWLEDGMENTS

This work is dedicated to the memory of our esteemed colleague Prof. Manjeet Singh, who expired on 30.3.2009.
REFERENCES


