 Chronic dipyridamole therapy produces sustained protection against cardiac ischemia-reperfusion injury

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Figueroed, Vincent M., Ivan Diamond, Hui-Zhong Zhou, and S. Albert Camacho. Chronic dipyridamole therapy produces sustained protection against cardiac ischemia-reperfusion injury. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2091–H2097, 1999.—Sustained protection against ischemia-reperfusion injury is not available for patients at risk for myocardial infarction who may require emergent reperfusion therapy. Whereas ischemic preconditioning and adenosinergic agents reduce myocardial injury, they are only effective when given immediately before ischemia or reperfusion. We recently found chronic ethanol exposure, an adenosine uptake inhibitor, produced sustained cardioprotection against ischemia-reperfusion injury. We now ask whether chronic dipyridamole therapy, a clinically usable nucleoside transport inhibitor, induces similar cardioprotection. Perfused hearts from guinea pigs, given dipyridamole (4 mg·kg–1·day–1) in their water for 2–6 wk (n = 10 for each group), underwent ischemia-reperfusion. Injury was assessed by recovery of left ventricular developed (LVDP) and end-diastolic (LVEDP) pressures and creatine kinase release. During reperfusion, hearts from dipyridamole-treated animals (6 wk) had 74% higher LVDP, 28% lower LVEDP, and 61% lower creatine kinase release versus controls. Adenosine A1-receptor antagonism (8-cyclopentyl-1,3-dipropylxanthine; 200 nM) abolished the protection of dipyridamole but A2 antagonist (3,7-dimethyl-1-propargylxanthine; 10 mM) did not. Dipyridamole therapy produces sustained protection against ischemia-reperfusion injury in guinea pigs. This cardioprotection requires adenosine A1 receptor signaling at the time of ischemia.

guinea pig; heart; adenosine

EMERGENT REPERFUSION THERAPIES, including thrombolysis and primary coronary angioplasty, improve survival after myocardial infarction (MI) (29). Unfortunately, these reperfusion therapies are rarely carried out before considerable myocardial infarction has occurred. Moreover, reperfusion after prolonged ischemia also produces a paradoxical myocardial injury (ischemia-reperfusion injury) (1, 14), potentially limiting the efficacy of reperfusion therapies. This has provided impetus for identifying therapies that protect against ischemia-reperfusion injury. Acute interventions, including ischemic preconditioning (18, 27) and infusion of adenosinergic agents (10, 13), reduce ischemia-reperfusion injury in animal models and human myocardium. Unfortunately, the timing of an MI cannot be predicted clinically. Thus we need to develop therapies that produce sustained protection against ischemia-reperfusion injury in vulnerable patients.

Several lines of evidence suggest that nucleoside transport inhibitors, which increase extracellular adenosine levels by inhibiting uptake into myocytes and endothelial cells, might offer sustained protection against ischemia-reperfusion injury. For example, we recently found that chronic exposure to ethanol, an adenosine uptake inhibitor, reduces ischemia-reperfusion injury in guinea pig hearts, requiring adenosineA1 receptor signaling at the time of ischemia to effect cardioprotection (23). Other nucleoside transport inhibitors, including dipyridamole (2, 3, 15), also reduce ischemia-reperfusion injury when infused immediately before experimental MI. However, it is unknown whether these agents offer sustained protection against ischemia-reperfusion injury when given chronically.

Because dipyridamole is clinically available, we asked whether chronic therapy with this adenosine uptake inhibitor produces sustained protection against ischemia-reperfusion injury. Hearts, isolated from guinea pigs given dipyridamole in their drinking water for 2, 3, or 6 wk (4.0 mg·kg–1·day–1), were subjected to global ischemia and reperfusion. We found that dipyridamole therapy protects against ischemia-reperfusion injury. Furthermore, the cardioprotection of dipyridamole requires adenosine A1 receptor signaling at the time of ischemia.

MATERIALS AND METHODS

Male Hartley guinea pigs (275–300 g) were divided into dipyridamole and control groups. Animals were fed Lab Diet guinea pig food (PMI Feeds, MO). Dipyridamole was added to the drinking water of the dipyridamole group (11.4 mg/l) for a total of 2, 3, or 6 wk. Guinea pigs drank ~3.5 ml water per 10 g of body weight per day, resulting in a dipyridamole intake of 4 mg·kg–1·day–1. This dose approximates the recommended daily human dose for prophylaxis against thromboembolism after cardiac valve replacement (75–100 mg four times daily) (22a). Animals consumed nearly all their allotted drinking water throughout the study, suggesting level dosing from the initiation to termination of the study protocol. Animals were weighed before being euthanized.

Isolated heart perfusion and measurement of function. Animals were administered heparin (1,000 U ip) and anesthetized with pentobarbital sodium (60 mg/kg ip). Hearts from
each guinea pig were excised and arrested in cold isosmotic saline containing 20 mmol/l KCl. Isolated hearts were cannulated via the aorta and perfused at an initial perfusion pressure of 70 mmHg on a nonrecirculating Langendorff perfusion apparatus, using a Krebs-Henseleit perfusate (mmol/l): 123 NaCl, 6.0 KCl, 2.5 CaCl2, 20.0 NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, 11.0 glucose, 0.5 EDTA, and 20 U/l insulin. The perfusate was continuously bubbled using 95% O2-5% CO2 and maintained at 37°C. Hearts were paced at 240 beats/min using two platinum-tipped electrodes connected to a Grass Instruments SD-5 stimulus generator (Grass Instrument, Quincy, MA).

Left ventricular (LV) pressure was measured using a 2-Fr, high-fidelity micromanometer (Millar Instruments, Houston, TX) passed into a compliant latex LV balloon. The LV balloon was connected to a Y-adapter, one end of which was used to advance the micromanometer to the latex balloon. The other end of the Y-adapter was used to fill the balloon with bubble-free water to set the end-diastolic pressure at 10 mmHg. LV pressure was recorded on a Gould series 8000 chart recorder (Gould Electronics, Hayward, CA). Coronary flow was measured by an in-line flowmeter (Gilmont Instruments, Barrington, IL).

Creatine kinase release. Creatine kinase (CK) release during postischemic reperfusion was measured with a commercially available kit (47–10, Sigma Chemical, St. Louis, MO). Coronary effluent samples were collected every 3 min beginning with initiation of reperfusion for a total of 18 min. Only 7% of the total CK released was present in the final 3-min sample, suggesting that the majority of CK had been released by 18 min of reperfusion. CK release was also measured in four control hearts not exposed to ischemia-reperfusion over the same time period. Values were expressed as units per milliliter per gram dry weight ventricle (U·ml−1·g dry wt ventricle−1).

Experimental protocol. Hearts from guinea pigs treated with dipyridamole for 2, 3, or 6 wk were isolated and perfused as described above. After a 20-min equilibration period, baseline measurements of LV developed pressure (LVDP), coronary perfusion pressure, and coronary flow were made. Hearts were then subjected to 45 min of no-flow ischemia, followed by 48 min of reperfusion. During ischemia, hearts were maintained at 37°C by enclosure in a water-jacketed air chamber. Warmed perfusate kept in the lower part of the chamber saturated the air with humidity and prevented hearts from being cooled by evaporation. Upon reperfusion, hemodynamic measurements were repeated every 6 min for 48 min. Hearts were then cleaned of atria and great vessels, dried for 24 h at 80°C, and weighed.

To determine how long protection against ischemia-reperfusion injury could be maintained after dipyridamole therapy was discontinued, hearts were studied as described above after dipyridamole was stopped for 12 and 24 h before the guinea pig was euthanized. Decline of dipyridamole plasma concentrations has been previously shown to follow a two-compartment model, with initial and terminal elimination phase half-lives of 0.10 and 5.21 h (11).

To determine whether adenosine A1 receptors are important in the protection of dipyridamole against ischemia-reperfusion injury, hearts were studied as described above, except that the adenosine A1 receptor agonist 3,7-dimethyl-1-propargylxanthine (DMPX; 10 mM; Research Biochemicals International) was added to the perfusate 10 min before ischemia. Similar concentrations of DMPX have been used to selectively inhibit adenosine A1 receptors as evidenced by the abolition of increased coronary flow with adenosine boluses (10 mM) in a separate group of four hearts (data not shown).

Statistical analysis. All data are expressed as means ± SE. Comparisons between groups were made using repeated-measures analysis of variance with multiple grouping factors. If differences were observed, a Tukey post hoc test was used to confirm the significance of differences between groups. A value of P < 0.05 was considered statistically significant.

RESULTS

Chronic dipyridamole therapy improves contractile recovery during postischemic reperfusion. Baseline LVDP and coronary flow were similar in perfused hearts from animals treated for 6 wk with dipyridamole and age-matched controls (Table 1). Body weights were also the same in these groups (652 ± 8 vs. 651 ± 8 g).

As shown in Fig. 1A, LVDP recovery was significantly improved during postischemic reperfusion in hearts from animals treated with dipyridamole for 6 wk compared with controls (Table 1; 56% vs. 31% of preischemic values at 48 min; P < 0.05). As shown in Fig. 1B, LV end-diastolic pressure (LVEDP) rise, an indicator of myocardial contracture, was lower in hearts from dipyridamole-treated animals compared with controls (Table 1; 290% vs. 360% of preischemic values at 48 min; P < 0.05). These data suggest that dipyridamole therapy for 6 wk improved contractile recovery and reduced the degree of contracture or irreversible myocardial injury after ischemia-reperfusion.

There were no differences in coronary flow (Table 1) or coronary perfusion pressure before or after ischemia-reperfusion in dipyridamole (70 ± 1 mmHg; 73 ± 1 mmHg) versus control (70 ± 1 mmHg; 74 ± 2 mmHg) hearts. This suggests that differences in coronary flow cannot account for the improved contractile recovery after ischemia-reperfusion in hearts from animals chronically treated with dipyridamole.

Chronic dipyridamole therapy reduces creatine kinase release during postischemic reperfusion. CK release was measured to assess the degree of myocyte injury following global ischemia and reperfusion. Hearts from animals treated with dipyridamole for 6 wk released 61% less CK compared with control hearts (Table 1). CK release was higher in each 3-min aliquot
of perfusate collected in the control compared with the dipyridamole group during the first 18 min of reperfusion. These data suggest that chronic dipyridamole therapy protects against myocyte necrosis after ischemia-reperfusion. Importantly, CK release during this same time period in control hearts not undergoing ischemia-reperfusion was significantly lower (38 ± 6 U·ml⁻¹·g dry wt ventricle⁻¹), suggesting that significant infarct was present in this model of ischemia-reperfusion.

Cardioprotection by dipyridamole requires several weeks to develop. The effect of duration of dipyridamole therapy on contractile recovery and CK release is shown in Fig. 2A and Table 2. There was no evidence of protection against ischemia-reperfusion injury after 2 wk of dipyridamole therapy. However, by 3 wk of dipyridamole therapy, LVDP and LVEDP recovery were improved compared with control hearts; CK release was also reduced after 3 wk of treatment. Thus more than 2 wk of dipyridamole therapy is required to achieve significant protection against ischemia-reperfusion injury.

Cardioprotection was short-lived, however, after dipyridamole therapy was discontinued. Full protection persisted for 12 h after dipyridamole was removed from the drinking water (Fig. 2B and Table 2), as evidenced by similar contractile recovery and reduced CK release compared with hearts from animals receiving dipyridamole until they were euthanized. However, only partial protection was observed after 24 h without dipyridamole. This suggests that continuous exposure to dipyridamole is necessary to sustain its protective effect against ischemia-reperfusion injury.

Dipyridamole-induced protection against ischemia-reperfusion injury requires adenosine A₁ receptor signaling. The selective adenosine A₁-receptor antagonist DPCPX abolished dipyridamole-induced cardioprotection against ischemia-reperfusion injury without changing preischemic LVDP and coronary flow (Table 1). In the presence of DPCPX, recovery of LVDP and LVEDP was the same in hearts from dipyridamole-treated and control animals (Table 1); CK release was also similar between groups (Table 1). Furthermore, these data were similar to those in control hearts studied in the absence of DPCPX. Taken together, these data suggest that adenosine A₁ receptor signaling is required to effect dipyridamole-induced cardioprotection against ischemia-reperfusion injury.

To determine whether the protective effect of chronic dipyridamole therapy was adenosine-receptor specific, hearts from dipyridamole-treated animals were also subjected to ischemia-reperfusion in the presence of the selective adenosine A₂-receptor antagonist DMPX. In contrast to results with the adenosine A₁-receptor antagonist, adenosine A₂ receptor blockade did not abolish cardioprotection produced by chronic dipyridamole therapy (Table 1). These data suggest that the protective effect of chronic dipyridamole therapy reduces ischemia-reperfusion injury through adenosine A₁, not A₂, receptor signaling.

**DISCUSSION**

The major finding in this study is that chronic dipyridamole therapy produces sustained protection against ischemia-reperfusion injury in guinea pig hearts. Furthermore, the cardioprotective effect of dipyridamole requires adenosine A₁ receptor signaling at the time of ischemia-reperfusion. Protection against ischemia-reperfusion injury was present after 3–6 wk of dipyridamole therapy and persisted for at least 12 h after the drug was discontinued. There is no therapy currently available to induce sustained protection against ischemia-reperfusion injury in patients at risk for MI who may require emergent reperfusion therapy. Understanding the mechanisms of the protective effect of dipyridamole may aid in the development of therapies to protect these patients.

Acute dipyridamole therapy mimics ischemic preconditioning and/or enhances the protective effect of preconditioning against ischemia-reperfusion injury (4, 3, 15). Intracoronary dipyridamole improves systolic...
and diastolic ventricular performance in humans during coronary angioplasty (31). Acute exposure to other nucleoside transport inhibitors, including dilazep (17) and ethanol (19), also mimic or enhance the protective effect of ischemic preconditioning against ischemia-reperfusion injury. However, a chronic therapy that produces sustained cardioprotection against ischemia-reperfusion injury for patients at risk for MI is not yet available.

This study was carried out because of our recent findings that long-term exposure to ethanol, which is also an adenosine uptake inhibitor, produced sustained protection against ischemia-reperfusion injury (23, 24). We found that chronic alcohol consumption produces a preconditioning-like effect reducing myocardial ischemia-reperfusion injury and that this effect is attenuated in the presence of selective adenosine A1-receptor antagonism (23). Furthermore, cardioprotection persisted for at least 16 h after ethanol consumption was discontinued (23). We have also found that an important secondary messenger in the adenosine receptor signaling pathway, protein kinase C, is necessary for the cardioprotection of alcohol. Interestingly, recent epidemiological data are consistent with our observations on experimental ethanol consumption; regular drinkers appear to have improved outcomes after acute MI compared with abstainers (12, 26, 33). Unfortunately, it is not always possible to recommend moderate...
ischemia and 48 min of reperfusion. Hearts are from guinea pigs treated with dipyridamole (4 mg·kg⁻¹·day⁻¹) for 2, 3, and 6 wk. Additional hearts were studied from guinea pigs after dipyridamole was discontinued 12 and 24 h before being euthanized after 6 wk of therapy. *P < 0.05 vs. control group; †P < 0.05 vs. 6-wk dipyridamole treatment group.

Table 2. Effect of duration of therapy with dipyridamole on reperfusion injury

<table>
<thead>
<tr>
<th>Dipyridamole Treatment Period</th>
<th>LVDP, mmHg</th>
<th>LVEDP, mmHg</th>
<th>CK, U·ml⁻¹·g dry wt⁻¹ (Reperfusion)</th>
</tr>
</thead>
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|                             | Preischemia | Reperfusion | Preischemia | Reperfusion | }
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>120 ± 3</td>
<td>38 ± 5</td>
<td>10 ± 1</td>
<td>36 ± 5</td>
</tr>
<tr>
<td>3 wk</td>
<td>117 ± 4</td>
<td>63 ± 3*</td>
<td>10 ± 1</td>
<td>37 ± 5</td>
</tr>
<tr>
<td>6 wk</td>
<td>120 ± 3</td>
<td>38 ± 5</td>
<td>10 ± 1</td>
<td>22 ± 2*</td>
</tr>
<tr>
<td>12 h off</td>
<td>113 ± 5</td>
<td>65 ± 3*</td>
<td>10 ± 1</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>24 h off</td>
<td>118 ± 4</td>
<td>49 ± 3*</td>
<td>10 ± 1</td>
<td>33 ± 3</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE; n = 10 animals for each group. Hemodynamic data from perfused hearts subjected to 45 min of global ischemia and 48 min of reperfusion. Hearts are from guinea pigs treated with dipyridamole (4 mg·kg⁻¹·day⁻¹) for 2, 3, and 6 wk. Additional hearts were studied from guinea pigs after dipyridamole was discontinued 12 and 24 h before being euthanized after 6 wk of therapy. *P < 0.05 vs. control group; †P < 0.05 vs. 6-wk dipyridamole treatment group.

alcohol consumption given the risks for abuse and alcoholic-related diseases. Furthermore, ethanol, no doubt, has pleotropic effects beyond its inhibition of adenosine reuptake, which may have detrimental effects on the heart. Therefore, we searched for, and found, evidence that dipyridamole, a safer adenosine uptake inhibitor, produced a similar cardioprotective effect. Taken together, our results show that chronic therapy with at least two adenosine uptake inhibitors, dipyridamole and ethanol, produces sustained cardioprotection against ischemia-reperfusion injury. Because protection is continuously present either chronic treatment and still present at least 12-16 h after discontinuation, it is unlikely that protection is due to the continual presence of elevated adenosine levels. Future work will determine the contribution to this cardioprotective effect of transient elevations of adenosine with intermittent intake of adenosine uptake inhibitors.

Although the potential benefits of dipyridamole in protecting against ischemia-reperfusion injury have not been examined clinically, dipyridamole has been already studied as potential therapy to reduce the risk of reinfarction in patients after MI (26a). In the Perisartine-Aspirin Reinfarction Study (PARIS), the efficacy of the combination of dipyridamole and aspirin, compared with that of aspirin alone, versus placebo for preventing subsequent cardiac events in patients who had previously suffered an MI was examined (26a). Dipyridamole and aspirin produced no statistically significant additional benefit against coronary events compared with aspirin alone (subgroup analysis did show that subjects <6 mo from their previous MI benefited from combination therapy).

However, there are two mitigating factors about the PARIS trial that cause us to reconsider this issue. First, although the number of “coronary incidences” (a primary end point defined as fatal coronary disease plus nonfatal MI) were similar in the dipyridamole plus aspirin group (13.8%) and the aspirin group (14.0%), examination of the data suggest that a greater percentage of subjects in the dipyridamole plus aspirin group survived MI (51% vs. 43% in the aspirin group; a 16% improvement; statistics not performed on this end point). Second, the PARIS trial was performed in the prethrombolytic era. Emergent reperfusion therapies, including thrombolysis and primary coronary angioplasty, have markedly improved the outcome after MI. Before the advent of these reperfusion therapies, infarct-related arteries often remained occluded, providing little role for therapies that protected against ischemia-reperfusion injury. In this era of emergent reperfusion therapies, potential preconditioning-like therapies will require reexamination.

No therapy is yet available that produces a sustained preconditioned state. Cohen and colleagues (8) reported that the protective effect of ischemic preconditioning in rabbits wanes after 3–4 days of continued preconditioning with 40–65 5-min occlusive episodes before prolonged ischemia-reperfusion. Similarly, Tsuichida and colleagues (32) recently showed that continuous infusion of an adenosine A₁ agonist for 72 h prevented subsequent protection by ischemic preconditioning. However, a recent study by Dana and colleagues (9) showed that a preconditioned state could be maintained in rabbits for at least 10 days using intermittent, not continuous, infusions (every 48 h) of 2-chloro-N⁶-cyclopentyladenosine (CCPA), an adenosine A₁ agonist. This is the first evidence of a sustained preconditioning effect beyond 72 h. In our study, protection was observed after 3–6 wk of dipyridamole therapy but was not present at 2 wk. These differences among studies suggest that producing a sustained protective effect against ischemia-reperfusion injury may depend on the type and interval of therapy. Thus, whereas activation of protection by chronic dipyridamole, intermittent CCPA, or acute ischemic preconditioning may be mediated similarly at the time of ischemia (e.g., via adenosine receptor activation), chronic dipyridamole therapy may induce more lasting responses that produce sustained protection, which are not induced with continued ischemic preconditioning. For example, we (24) have recently shown that chronic exposure to ethanol causes sustained activation of e-protein kinase C (e-PKC) in myocytes (24), a PKC isoenzyme implicated in ischemic preconditioning (16). It may be that chronic therapy with an adenosine uptake inhibitor produces sustained translocation of e-PKC, which is then poised to phosphorylate effector proteins of preconditioning if triggered by the adenosine A₁ receptor-signaling cascade at the time of ischemia. Mochly-Rosen (25) has suggested that PKC activation may be a two-step
process. First, PKC isoenzymes must translocate to their receptors for activated kinases (RACKs). They then must be stimulated to an activated state at this site by, for example, adenosine A1 receptor signaling. It is at this time that they can phosphorylate the effector proteins of preconditioning. Thus, adenosine may have two roles in the sustained cardioprotection seen with dipyridamole therapy: 1) as a repetitive stimulator of factors that, with time, produce a sustained preconditioning-like state (e.g., ePKC remains poised at its RACK), and 2) as a trigger of the preconditioning signaling cascade at the time of ischemia-reperfusion.

In our study, ischemia-reperfusion were produced ex vivo in perfused guinea pig hearts, not in vivo. Despite this methodological limitation, the perfused animal heart model is a standard tool for studying ischemia-reperfusion and protection afforded by preconditioning protocols. As we further document and understand the mechanisms underlying the sustained cardioprotective effect of dipyridamole in animal models, we could ultimately proceed with clinical trials using dipyridamole, a drug already shown to be safe for human use.

We do not believe the issue of coronary steal, produced by rapid infusion of dipyridamole via intracoronary or intravenous infusion, is relevant to the potential cardioprotective effect suggested by this study. A “coronary steal effect” has been demonstrated in a canine model of coronary artery disease after intravenous dipyridamole (4). This steal of blood flow from stenosed arteries to normal arteries occurred following a large dipyridamole bolus (1-1.5 mg/kg). Thus coronary steal would be a concern with rapid dipyridamole infusion. However, clinical data do not support a steal syndrome with oral dipyridamole dosing. In addition to the PARIS trial already discussed, a meta-analysis of 11 published trials demonstrated the safety of oral dipyridamole in patients with coronary artery disease (30). The unlikely occurrence of coronary steal with oral dipyridamole dosing is discussed in detail by Picano and Michelassi (28). We did not study whether chronic dipyridamole produces additional protection against ischemia-reperfusion injury via alterations in platelet aggregation (the potential cardioprotective effect studied in the PARIS trial). We chose, in the present study, to focus on the potential cardioprotective effect of chronic dipyridamole therapy at the level of the adenosine A1 signaling pathway, given the involvement of this receptor in cardioprotection produced by ischemic preconditioning (18, 27) and chronic alcohol exposure (23, 24).

There is currently no therapy available to induce sustained protection against ischemia-reperfusion injury in patients at risk for MI who may require emergent reperfusion therapy. Our data suggest that chronic dipyridamole therapy produces sustained protection against ischemia-reperfusion injury. Future studies will determine how chronic exposure to adenosine uptake inhibitors produces this delayed, yet sustained, preconditioning state.

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