Sustained Reduction in Myocardial Reperfusion Injury with an Adenosine Receptor Antagonist: Possible Role of the Neutrophil Chemoattractant Response

MERYN B. FORMAN, JOÃO V. VITOLA, CARLOS E. VELASCO, JOHN J. MURRAY, RAGHVENDRA K. DUBEY, and EDWIN K. JACKSON

Center for Clinical Pharmacology, Departments of Pharmacology (E.K.J.) and Medicine (R.K.D., E.K.J.), University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania; and Departments of Medicine and Pharmacology, Divisions of Cardiology (M.B.F., C.E.V., J.V.V.) and Clinical Pharmacology (J.J.M.), Vanderbilt University School of Medicine, Nashville, Tennessee

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ABSTRACT

Recent studies have demonstrated that three membrane-permeant A1 receptor antagonists reduced infarct size in a model of ischemia followed by brief reperfusion. However, it was not determined whether cardioprotection was mediated by non-specific intracellular effects of these highly lipophilic drugs and whether the antagonists only delayed myocardial necrosis without affecting the ultimate infarct size. In the present study, closed-chest dogs were subjected to 90 min of left anterior descending coronary artery occlusion and 72 h of reperfusion and received either a nonmembrane-permeant adenosine receptor blocker that is devoid of direct intracellular effects and is 6-fold selective for the A1 receptor [1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX); n = 11] or vehicle (n = 12). DPSPX was administered as three 200-mg boluses 60 min before and 30 and 120 min after reperfusion. The area of necrosis was determined histologically and expressed as a percentage of the area at risk. Baseline predictors of infarct size were similar in the two groups. The ratio of the area of necrosis to the area at risk was less in the DPSPX group (17.8 ± 4.3% versus 35.0 ± 1.9%; P = .012), and DPSPX improved regional ventricular function. Under both basal and stimulated (formyl-Met-Leu-Phe) conditions, suspensions of human neutrophils generated extracellular adenosine levels (approximately 50 nM) sufficient to activate A1 receptors. Moreover, both DPSPX and 1,3-dipropyl-8-cyclopentylxanthine, a selective A1 receptor antagonist, significantly reduced the chemoattractant response of neutrophils to formyl-Met-Leu-Phe. We conclude that blockade of A1 adenosine receptors attenuates myocardial ischemic/reperfusion injury, possibly in part by decreasing the chemoattractant response of neutrophils.

Although the timely restoration of coronary blood flow to an ischemic region of myocardium reduces the extent of irreversible myocardial necrosis in various animal models and in humans, the act of reperfusion per se may significantly limit the amount of potentially salvageable myocardium by inducing various deleterious and cytotoxic effects on ischemic but potentially viable cells: so-called myocardial reperfusion injury (Reimer and Jennings, 1979; Connelly et al., 1982; Gibbons et al., 1994; Forman and Murray, 1997). Numerous mechanisms have been postulated to explain this phenomenon, including neutrophil- and oxygen free radical-mediated injury, microvascular injury resulting in the “no-reflow” phenomenon, abnormalities of calcium homeostasis, and further depletion of intracellular high-energy compounds (Forman and Murray, 1997).

Three distinct P1 receptors, A1, A2, and A3, are well characterized by pharmacological and molecular biological criteria (Tucker and Linden, 1993; Dalziel and Westfall, 1994). Activation of these receptors produces various physiological effects that could attenuate numerous of the proposed mechanisms responsible for myocardial reperfusion injury; these include preservation of microvascular flow in the reperfusion bed, inhibition of various neutrophil functions, reduction in cytotoxic free radical formation, and modulation of intracellular calcium hemostasis (Ely and Berne, 1992; Forman et al., 1993; Kitakaze et al., 1993; Mentzer et al., 1993; Forman and Murray, 1997). Numerous experimental studies support the concept of adenosine as a cardioprotective agent in the setting of myocardial ischemia and reperfusion. The administration of exogenous adenosine i.v. or directly into the coronary circulation significantly reduces infarct size in various experimental models of regional ischemia (Olafsson et al., 1987; Norton et al., 1991; Pitarys et al., 1991). Similar ben-

ABBREVIATIONS: DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; DPSPX, 1,3-dipropyl-8-p-sulfophenylxanthine; LAD, left anterior descending coronary artery; PMN, polymorphonuclear neutrophil; FMLP, formyl-Met-Leu-Phe; SPT, 8-sulfophenyltheophylline.
Efficial effects are observed with the administration of selective A₁, A₂, and A₃ receptor agonists (Norton et al., 1992; Liu et al., 1994). Moreover, these experimental studies are corroborated in humans; i.v. adenosine administered for 3 h to patients with an acute anterior myocardial infarction who are reperfused with thrombolytic therapy results in significant reduction in infarct size as measured with radionuclide techniques (Mahaffey et al., 1997).

Despite the well known cardioprotective effects of adenosine receptor agonists, Neely et al. (1996) report that the administration of the selective A₁ adenosine receptor antagonists 1,3-dipropyl-8-cyclopentylxanthine (DPCPX), bamilifyline, or xanthine amine congener before coronary occlusion or the administration of DPCPX during and after coronary occlusion significantly reduces infarct size in a cat model subjected to myocardial ischemia followed by 2 h of reperfusion. This interesting study raises several important questions: 1) Are the counterintuitive results of Neely et al. (1996) reproducible? 2) Do adenosine receptor antagonists cause a permanent reduction in infarct size or only delay the process of myocardial necrosis? 3) Are the effects of adenosine receptor antagonists, which are generally highly lipophilic drugs that easily accumulate within cells, the result of nonspecific intracellular actions (e.g., inhibition of intracellular enzymes such as phosphodiesterases)? 4) What is the mechanism by which blockade of adenosine receptors causes cardioprotection? The objective of this study was to address these questions.

Accordingly, we investigated the cardioprotective effects of 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX) in a well characterized model of regional ischemia subjected to a prolonged period of reperfusion. DPSPX is an 8-phenyl derivative of theophylline with a 20- to 60-fold greater potency for adenosine receptors compared with theophylline, and although DPSPX blocks both A₁ and A₂ receptors, it is approximately 6-fold selective for A₁ receptors (Daly et al., 1985; Jacobson and van Rhee, 1997). Moreover, DPSPX contains a sulfonic acid group that is negatively charged at physiological pH (Heller and Olsson, 1985). This property prevents it from entering intracellular spaces and conveys high water solubility making it a suitable agent for in vivo studies. Our previous studies demonstrated that DPSPX does not penetrate the blood-brain barrier, distribute into red blood cells, or gain access to membrane-delimited microsomal metabolizing enzymes (Tofovic et al., 1991). Moreover, numerous studies by Jackson and coworkers (Kuan et al., 1990; Dubey et al., 1998) confirm the efficacy of DPSPX as a blocker of adenosine-induced physiological responses in various in vivo and in vitro experimental models. Because we found that the administration of DPSPX did indeed reduce infarct size and
improve regional ventricular function, we investigated the mechanism of this effect. In this regard, because $A_1$ receptors are known to stimulate chemotaxis of inflammatory cells (Cronstein et al., 1990) and because neutrophil activation may play an important role in myocardial reperfusion injury (Forman and Murray, 1997), we determined whether suspensions of neutrophils generate adequate levels of adenosine to activate $A_1$ receptors and whether blockade of $A_1$ receptors with either DPSPX or DPCPX, a highly selective $A_1$ receptor blocker (Jacobson and van Rhee, 1997), inhibits the neutrophil chemoattractant response.

**Materials and Methods**

**Surgical Procedure.** Dogs of either gender weighing 17 to 28 kg were quarantined for 2 weeks to exclude common canine diseases. Approximately 1 week before coronary occlusion, a left thoracotomy was performed under general anesthesia with sodium pentobarbital (30 mg/kg b.wt.). The heart was exposed via a pericardiotomy, and a surgical monofilament ligature was placed around the left anterior descending coronary artery (LAD) proximal to the first diagonal branch and enclosed in a polyethylene tube. A Silastic catheter was inserted into the left atrial appendage and filled with heparin. Both the LAD snare and the left atrial line were buried in a s.c. pouch, the pericardium was loosely approximated, and the left thorax was closed. Animals were administered one dose of penicillin and dihydrostreptomycin (Combiotic) and allowed to recover. The entire protocol conformed to the guiding principles set forth by the American Physiological Society and was approved by the Vanderbilt University Institutional Animal Care and Use Committee; the studies were conducted at Vanderbilt University.

**Pilot Studies.** Before we began the protocol, pilot studies were performed in six animals to determine the dose of DPSPX. DPSPX was synthesized by Jack N. Wells (Vanderbilt University) and provided as a generous gift. We have previously evaluated its chemical identity by comparing it with authentic DPSPX (Research Biochemicals International, Natick, MA) with the use of HPLC, ultraviolet spectroscopy, infrared spectroscopy, and NMR (Kuan et al., 1989).

Using an identical surgical preparation to the infarct study, an ultrasound transit-time flow probe (Transonic Systems, Ithaca, NY) was placed around the mid-LAD and anchored by suturing a silicone phalange to the myocardium as previously described (Babbit et al., 1989). After 5 to 7 days, animals were reanesthetized and ventilated, and femoral artery and vein cutdowns were performed for hemodynamic measurements and drug infusions. The flow probe cable was removed from the s.c. pocket, and the flow probe was connected to a flowmeter (model T101; Transonic). Adenosine (Sigma Chemical Co., St. Louis, MO) infused in a dosage of 140 μg/kg/min into the inferior vena cava resulted in a maximal increase in LAD blood flow within 6 min. Blockade of adenosine receptors was then determined by measuring the effect of adenosine infused for 6 min every 30 min on LAD flow after a bolus injection of DPSPX. These studies demon-
strated that DPSPX in a dose of 200 mg totally abolished the coronary vasodilatory effects of i.v. adenosine for at least 90 min.

**Experimental Protocol.** The dogs were reanesthetized with sodium pentobarbital, intubated, and mechanically ventilated with room air to maintain arterial blood gases within the physiological range. Intravenous diazepam (mean dose, 5 mg) and morphine (mean dose, 5 mg) were administered periodically throughout the protocol as required to maintain an appropriate level of anesthesia and analgesia, respectively. Using sterile technique, both femoral arteries and the right femoral vein were exposed and cannulated with a 7F sheath (Cordis, Miami, FL). A 7F Gensini (USCI, C.R. Bard, Tewksbury, MA) catheter was positioned with its distal tip in the proximal inferior vena cava for the infusion of DPSPX or Ringer’s lactate. A 7F pigtail catheter was placed in the right femoral artery and used for monitoring mean and phasic systemic arterial blood pressure and left ventricular end-diastolic pressure. Electrocardiographic leads I, aVF, and aVL were monitored continuously throughout the protocol (model VR-12; Electronics for Medicine, P.P.G. Biomedical Systems, Pleasantville, NY). The LAD snare and left atrial line were retrieved from the s.c. pocket. Before initiation of the protocol, the animals were randomly assigned to receive either Ringer’s lactate or DPSPX. Baseline hemodynamic measurements were then obtained, and regional myocardial blood flow was determined with a bolus injection of 15–m$\text{m}$ microspheres into the left atrium. Microspheres were labeled with radioisotopes and injected serially ($2 \times 10^6$/injection) in the following order: iodine-125, cerium-141, iodine-123, cerium-147, and iodine-131. The microspheres were then collected from the right atrium, and the distribution of the microspheres was quantified to determine the blood flow to each region.
chromium-51, strontium-85, niobium-95, and scandium-46 (3M Company, St. Paul, MN). Reference samples were withdrawn from the left femoral artery via the Cordis sheath at a rate of 7.85 ml/min to allow calculation of myocardial blood flow.

Selective injection of contrast into the left coronary artery via a modified Judkin’s catheter confirmed patency of the LAD. Left ventriculography in the right anterior oblique projection with 8 to 10 ml of meglumine diatrizoate (Renografin 76) injected during 1 s through a power injector was performed to confirm normal and uniform wall motion. After each animal received a bolus injection of lidocaine (2 mg/kg) followed by a maintenance infusion at 0.1 mg/kg/min during the occlusion period, the snare was tightened to occlude the proximal LAD and maintained for 90 min. Total occlusion was confirmed by selective coronary angiography. In the treatment group, a 200-mg bolus of DPSPX was administered through the Gensini catheter in the inferior vena cava after 30 min of coronary occlusion and after 30 and 90 min of reperfusion. Hemodynamic measurements, left ventriculography, and regional myocardial blood flow were repeated after 60 min of occlusion. After 90 min of LAD occlusion, the snare was released, followed by selective coronary angiography and determination of regional myocardial blood flow to confirm vessel patency. Hemodynamic measurements and regional blood flow were determined at 1, 2, and 3 h after reperfusion. Left ventriculography and coronary angiography were again performed at 3 h of reperfusion after determining regional blood flow. The LAD snare and left atrial catheter were then reembedded into the s.c. pocket, femoral lines.

2-Factor ANOVA
DPSPX: p=0.090
Period: p<0.001
DPSPX x Period: p=0.002

CONTROL (n=11)
DPSPX (n=10-11)

2-Factor ANOVA
DPSPX: p=0.098
Period: p<0.001
DPSPX x Period: p=0.822

CONTROL (n=10)
DPSPX (n=10-11)

2-Factor ANOVA
DPSPX: p=0.082
Period: p<0.001
DPSPX x Period: p=0.779

CONTROL (n=10)
DPSPX (n=10-11)
were removed, and the vessels were ligated; the animals were weaned from the ventilator, administered antibiotics, and allowed to recover.

After 72 h, the animals were reanesthetized with pentobarbital. Hemodynamic measurements and left ventriculography were performed, and patency of the LAD was confirmed by selective coronary angiography. A left thoracotomy was performed, and the snare was tightened under direct vision. Monastral blue dye (DuPont, Wilmington, DE) at a dose of 1 mg/kg was injected into the aortic root through a pigtail catheter within 60 s after occluding the vessel. After a lethal dose of pentobarbital and potassium chloride, the heart was rapidly explanted and rinsed with tap water to prevent counterstaining.

Analysis of Area at Risk and Necrosis. The hearts were sectioned into six 1-cm segments parallel to the atrioventricular groove from apex to base and photographed with Ektachrome (Kodak) for later determination of the area at risk (unstained by Monastral blue dye). The slices not used for blood flow (slices 1, 3, 5, and 6) were then dehydrated. All slices were embedded into paraffin (with the slice facing the apex to be used as the cutting surface) according to the method described by Reimer and Jennings (1985). Microscopic sections that were 7 μm thick were cut from the apical end of each slice and stained with H&E and Mallory's trichrome stain. Using magnified tracings ×5 from each slide with the aid of a microscopic slide projector, the area at risk and the area of necrosis (stained purple by Mallory's) were then determined by computerized planimetry of the tracings drawn by an observer blinded to the treatment groups. The ratio of the infarcted area to the area at risk for each heart was calculated according to a method previously described (Forman et al., 1985).

Analysis of Ventricular Function. Regional ventricular function was determined from end-diastolic and end-systolic left ventricular endocardial contours during a well opacified normal sinus beat according to the chord method described by Sheehan et al. (1986).
Regional wall motion in the anterior wall was calculated as the mean motion of half of the most abnormally contracting contiguous chords and expressed in standard deviations (S.D.) per chord from a normal data bank (Velasco et al., 1991).

**Determination of Regional Myocardial Blood Flow.** Myocardial samples for the determination of regional blood flow were obtained from the second and fourth transverse myocardial sections. Tissue from the endocardium, midmyocardium, and epicardium (0.15–0.55 g) in the nonischemic zone (posterior wall) and central and lateral regions of the ischemic zone (unstained by Monastral blue dye) were used. These samples and arterial reference samples were counted for 5 min in a multichannel autogamma scintillation spectrometer (model 5986; Packard Instruments). Background contamination and overlapping radioactivity from other isotopes were accounted for by using a matrix correction method (Compusphere Software; Packard Instruments) and corrected for microsphere loss and tissue edema (Reimer et al., 1985). Myocardial blood flow was calculated in milliliters per minute per gram wet weight as previously described by researchers in our laboratory (Forman et al., 1997), and expressed in standard deviations (S.D.) per chord from a normal data bank (Velasco et al., 1991).

**Studies in Isolated Neutrophils.** Human polymorphonuclear neutrophils (PMNs) were isolated from heparinized venous blood from healthy adult volunteers with the use of Ficoll-Hypaque (Histopaque; Sigma Chemical Co.) density gradient centrifugation, as previously described (Gay et al., 1997), and suspended in Hanks’ balanced salt solution (Life Technologies, Grand Island, NY), pH 7.4. Chemotaxis was assessed by radioassay (Gallin et al., 1973). Briefly, PMNs were labeled with 51Cr (New England Nuclear, Boston, MA) for 1 h and then preincubated with DPSPX, DPCPX, or buffer containing the solvent DMSO for 10 min before placing the PMNs in the upper compartment of a chemotactic chamber that was separated from a lower compartment containing formyl-Met-Leu-Phe (FMLP; 10^{-6} M) or buffer. After a 10- or 60-min incubation, the cells were quickly removed via centrifugation, and the supernatant was frozen until analysis. Adenosine levels in the samples were analyzed by HPLC as we described previously in detail (Jackson et al., 1996). The levels of adenosine in the unknown samples were quantified as the area under the chromatographic peak, and the absolute amount in each sample was calculated from a standard curve. The concentration of DMSO was ≤0.1% in all incubations and had no effect on cell viability or control responses. Each experiment was performed in triplicate, and the data are expressed as the mean ± S.E. of the average of the triplicate determinations.

**Results**

Exclusion criteria were established before the start of the study; these included intractable ventricular arrhythmias, failure to survive for 72 h after reperfusion, and absence of adequate myocardial ischemia. The latter was defined as failure to develop akinesia or dyskinesia on ventriculography during coronary occlusion, area at risk volume of <15% of the left ventricle, or transmural collateral blood flow in the ischemic bed of >0.5 ml/min/g. Thirty dogs were entered into the study. Twenty-three dogs completed the protocol and were included in the final analysis; 11 animals were randomized to DPSPX, and 12 animals were randomized to the control group. There were no trends with regard to dogs being excluded at a different frequency for a particular reason from the DPSPX-treated group versus the vehicle-treated group.

No significant differences in serial pH or pO_2 were noted between the two groups throughout the protocol (data not shown). Mean arterial blood pressure, heart rate, rate-pressure product (an indirect measurement of myocardial oxygen consumption), and left ventricular diastolic pressure were not significantly different between the two groups throughout the protocol (Figs. 1 and 2). During occlusion and immediately and at 1 h after reperfusion, left ventricular end-diastolic pressure was increased in both groups.

### TABLE 1

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>10-min Incubation without FMLP</th>
<th>60-min Incubation without FMLP</th>
<th>10-min Incubation with FMLP (10^{-6} M)</th>
<th>60-min Incubation with FMLP (10^{-6} M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>42 ± 6</td>
<td>59 ± 14</td>
<td>49 ± 7</td>
<td>85 ± 29</td>
</tr>
<tr>
<td>DMSO</td>
<td>39 ± 6</td>
<td>78 ± 24</td>
<td>42 ± 6</td>
<td>79 ± 28</td>
</tr>
<tr>
<td>DPSPX (10^{-8} M)</td>
<td>37 ± 3</td>
<td>49 ± 10</td>
<td>39 ± 5</td>
<td>83 ± 28</td>
</tr>
<tr>
<td>DPCPX (10^{-8} M)</td>
<td>41 ± 4</td>
<td>53 ± 16</td>
<td>41 ± 6</td>
<td>64 ± 19</td>
</tr>
</tbody>
</table>
Occlusion and reperfusion had little effect on regional myocardial blood flows in the nonischemic zone of either group of animals (Fig. 3). In contrast, regional myocardial blood flow in the central ischemic zone was markedly reduced in both groups during occlusion and was followed by reactive hyperemia at reperfusion (Fig. 4). In both groups, the hyperemia was followed by a decrease in blood flow that reached statistical significance in the midmyocardium and epicardium by 1 h and in the endocardium by 3 h. Regional blood flows in the ischemic zone were similar in DPSPX versus control animals, with the exception that the reactive hyperemia immediately at reperfusion was significantly greater in the endocardium of DPSPX-treated dogs.

Although the area at risk, expressed as a percentage of the total left ventricle, tended to be larger in the DPSPX group, a marked and highly significant decrease in myocardial infarct size was demonstrated when expressed as a percentage of the area at risk (35.0 ± 1.9% in controls versus 17.8 ± 4.3% in DPSPX-treated animals, \( P = .012 \); Fig. 5). Because area at risk, a baseline predictor of infarct size, tended to be greater in the treatment group and left ventricular mass was similar, infarct size was not significantly different between the two groups when expressed as a percentage of the total left ventricle (10.8 ± 1.5% in controls versus 7.7 ± 2.6% in DPSPX-treated animals). The relationship between infarct size expressed as a percentage of the area of risk and endocardial blood flow in the central ischemic zone 60 min into occlusion is shown by linear regression in Fig. 5. An inverse relation between infarct size and collateral flow was noted; dogs with high collateral blood flow had smaller infarcts. DPSPX-treated animals invariably manifested enhanced myocardial salvage independent of the degree of collateral blood flow compared with control animals.

Chordal shortening was similar in the ischemic zone at baseline, and both groups exhibited significant worsening of regional function after 60 min of LAD occlusion (Fig. 6). Although the interaction between period and group did not achieve statistical significance (\( P = .116 \)), animals treated with DPSPX manifested improvement in regional ventricular function at 3 and 72 h after reperfusion by post hoc analysis.

Table 1 lists the extracellular adenosine levels in suspensions of human neutrophils incubated for either 10 or 60 min with either buffer alone or buffer with vehicle (DMSO), DPSPX, DPCPX, FMLP, FMLP + vehicle (DMSO), DPSPX + FMLP, or DPCPX + FMLP. Levels of extracellular adenosine ranged from 37 ± 3 to 85 ± 29 nmol/l. Both DPSPX and DPCPX significantly and similarly inhibited the chemoattractant response to FMLP (Fig. 7).

**Discussion**

Results of the present study demonstrate that the administration of the adenosine receptor antagonist DPSPX 30 min after regional ischemia results in a significant and sustained reduction in infarct size in the closed-chest canine model measured 72 h after reperfusion. Moreover, the reduction in infarct size is accompanied by an improvement in regional ventricular function. Importantly, myocardial salvage by DPSPX is not related to changes in the major predictors of infarct size in this model, namely, area at risk, myocardial oxygen consumption, and collateral blood flow. In this regard, linear regression analysis demonstrates that the drug is effective over a broad range of collateral blood flow. Thus, the results of the current study confirm the results of Neely et al. (1996); indicate that adenosine receptor antagonists reduce, not just delay, myocardial necrosis; and prove that cardioprotection afforded by adenosine receptor antagonists is not secondary to nonspecific intracellular actions.

Cronstein et al. (1990) report that activation of A<sub>1</sub> receptors stimulates neutrophil chemotaxis. Therefore, we postulate that part of the cardioprotective effect due to antagonism of the A<sub>1</sub> receptor is mediated by blockade of A<sub>1</sub> receptors on neutrophils, leading to a reduction in the neutrophil chemoattractant response. Importantly, our studies in isolated human neutrophils indicate that levels of adenosine in the extracellular compartment are in the nanomolar range, which is adequate to fully activate A<sub>1</sub> receptors, and demonstrate that both DPSPX and DPCPX markedly inhibit FMLP-induced chemoattractant responses in human neutrophils. However, a major caveat to the above conclusion is that the neutrophil studies were conducted in vitro with human neutrophils. Thus, our conclusion regarding the role of neutrophils, although plausible, is speculative in the context of this study.

To place the current work into perspective, we conducted a Medline search to determine the extent of the relevant literature. In this regard, we searched the intersection of “myocardial reperfusion injury” and “adenosine” (170 citations).
and the intersection of “ischemic preconditioning” and “adenosine” (93 citations). We were able to locate a total of eight studies, in addition to the article by Neely et al. (1996), in which the effects of an adenosine receptor antagonist on myocardial necrosis (determined by tissue staining) after ischemic/reperfusion were assessed in vivo (in vitro studies were not considered). Six studies were conducted in rabbits and two were conducted in dogs. In the present study, we used DPSPX to block adenosine receptors, and DPSPX is approximately 6-fold selective for the A1 receptor (Jacobson and van Rhee, 1997). In the study by Neely et al. (1996), DPCPX, biamfinylaine, and xanthine amine congener were used, which are 739-, 600-, and 52-fold selective for the A1 receptor (Abbracchio and Cattabeni, 1987; Jacobson and van Rhee, 1997). Five additional studies, two in dogs (Kitakaze et al., 1997; Domenech et al., 1998) and three in rabbits (Toombs et al., 1992; Zhao et al., 1993; Haessler et al., 1996), used 8-sulfophenyltheophylline (SPT), an antagonist that is 5-fold selective for the A2 receptor (Jacobson and van Rhee, 1997). These studies did not find cardioprotection with adenosine receptor blockade. Another study in rabbits used PD115,199 (Thornton et al., 1993), an adenosine antagonist that, like SPT, is 5-fold selective for the A2 receptor (Jacobson and van Rhee, 1997). This study also did not observe cardioprotection with adenosine receptor blockade. Another study in rabbits (Todd et al., 1996) examined the cardioprotective effects of polyadenylic acid in the absence and presence of SPT and KW-3902 (292-fold selective for the A1 receptor; Jacobson and van Rhee, 1997). In this latter study, SPT blocked the infarct reducing activity of polyadenylic acid, whereas KW-3902 tended to enhance polyadenylic acid-induced cardioprotection. A final study (Zhao et al., 1994) compared the effects of SPT versus KW-3902 on infarct size in rabbits subjected to myocardial ischemia/reperfusion. Although KW-3902 did not decrease infarct size, SPT markedly increased infarct size compared with either vehicle or KW-3902. Thus, when examining the 10 aforementioned studies (our study, the study by Neely et al. (1996), and the eight additional studies), the common denominator with regard to whether absolute or relative cardioprotection was observed after adenosine receptor blockade seems to be whether the used antagonist was selective for A1 or A2 receptors. This conclusion is consistent with our hypothesis that the cardioprotective effects of DPSPX are mediated possibly in part by blocking the neutrophil chemoattractant response.

This hypothesis could explain the paradox that both adenosine agonists and A1 antagonists are cardioprotective. Activation of adenosine receptor subtypes early during reperfusion exerts a number of beneficial effects, such as opening potassium channels, dilating the coronary microcirculation, and inhibiting platelet activation (Forman et al., 1993). On the other hand, antagonism of A1 receptors later in the process may attenuate the chemoattractant response. The implication of this hypothesis is that cardioprotection may be optimized by a therapeutic regimen that includes the sequential administration of adenosine agonists and antagonists. Additional in vivo studies are required to address this hypothesis.

In vivo experiments involving the chronic survival of large animals require the utmost attention to ethical considerations. If the animals are in discomfort or if more animals are required than are absolutely necessary to achieve the study objectives, the ethical costs become unacceptable. It is therefore necessary to administer multiple drugs to ensure the comfort and survival of the study animals. In the case of our study, we administered antibiotics to prevent infection, pentobarbital to provide anesthesia, diazepam to enhance the anesthetic effects of pentobarbital, lidocaine to prevent the occurrence of fatal reperfusion arrhythmias, and morphine to prevent discomfort. It is conceivable, therefore, that the reduction in infarct size noted in the present study was due to one or more drug/drug interactions between DPSPX and the coadministered agents. There are two reasons, however, to conclude that this was not the case. First, in the study by Neely et al. (1996), because the experiments were acute rather than chronic, a different anesthetic protocol was used and antibiotics and narcotics were not administered. Also, the adenosine receptor antagonists were different from that used in our study. Despite these differences, identical results were obtained in the two studies. To explain these consistent findings, one would have to postulate that in both studies a different set of drug/drug interactions just happened to afford the same cardioprotective effects. This seems highly improbable. Second, in our in vitro studies provide a plausible explanation for the cardioprotective effects of adenosine receptor antagonists.

Timely reperfusion either pharmacologically or mechanically remains the optimal method of improving myocardial salvage in the setting of acute myocardial infarction. The act of reperfusion per se initiates a number of deleterious effects on the previously ischemic but viable myocardium, partially negating its full beneficial effects: so-called myocardial reperfusion injury (Forman and Murray, 1997). Although a number of mechanisms have been postulated, current evidence suggests that it is mediated in part by microvascular injury in the reperfused bed secondary to the introduction of activated neutrophils (Babbit et al., 1989; Forman and Murray, 1997). Adenosine is an endogenous nucleoside that possesses a number of biochemical and physiological effects that alter many of the postulated mechanisms of reperfusion injury. Previous experimental studies demonstrate that adenosine and adenosine receptor agonists significantly reduce infarct size after regional ischemia, in part by preserving microvascular blood flow (Babbit et al., 1989; Norton et al., 1991, 1992; Pitarys et al., 1991; Forman and Murray, 1997), and a recent clinical study lends support to the results of experimental studies (Mahaffey et al., 1997). However, the administration of adenosine or adenosine A1 and A2 agonists can result in troublesome side effects, which may limit their clinical application. Our study and the study by Neely et al. (1996) demonstrate that the administration of adenosine receptor antagonists that are selective for the A1 receptor may be a safer alternative to adenosine receptor agonists for the treatment of myocardial ischemia/reperfusion injury. A further beneficial effect of A1 receptor antagonists would be in patients with severe left ventricular dysfunction because this class of drugs promotes natriuresis, as opposed to A1 agonists, which produce renal vasoconstriction (Jackson, 1997).

References


