ELSEVIER

European Journal of Pharmacology 387 (2000) 101–105

ejp

www.elsevier.nl/locate/ejphar

Short communication

Intravenous AMP 579, a novel adenosine A_1/A_{2a} receptor agonist, induces a delayed protection against myocardial infarction in minipig

Heping Meng *, Mathew McVey, Mark Perrone, Kenneth L. Clark

Cardiovascular Drug Discovery, Mail Stop NW4, Rhône-Poulenc Rorer, Collegeville, PA 19426, USA

Received 12 August 1999; received in revised form 10 November 1999; accepted 12 November 1999

Abstract

The aim of the study was to probe if acute administration of [1*S*-[1*a*,2*b*,3*b*,4*a*(*S*∗)]]-4-[7-[[2-(3-chloro-2-thienyl)-1-methylpropyl]amino]-3*H*-imidazo[4,5-*b*] pyridin-3-yl] cyclopentane carboxamide (AMP 579) could provide a delayed protection against myocardial ischemia–reperfusion injury after 24 h. Anesthetized Yucatan minipigs were given an intravenous (i.v.) loading dose (3 μg/kg) of AMP 579 in 2 min followed by a 68-min infusion (0.3 μg/kg/min) and were allowed to recover. After 24 h, the animals were subjected to an open-chest operation and the left anterior descending coronary artery was occluded for 40 min, followed by 3 h of reperfusion. Results indicated that there were no significant differences in hemodynamic parameters between vehicle- and drug-treated groups either during drug infusion or ischemia–reperfusion. Both groups had similar area at risk (24.9% for vehicle and 25.1% for AMP 579-treated). However, the infarct size was 36.5% of area at risk in vehicle group ($n = 8$) and 12.5% in AMP 579 group ($n = 8$), representing a 66% reduction of infarct size by AMP 579 ($p = 0.011$). This is the first report to demonstrate that in a large animal model, a hemodynamically silent, single i.v. dose of an adenosine receptor agonist can result in a delayed protection against myocardial infarction.

Keywords: Myocardial infarction; Adenosine receptor agonist; Protection, delayed; (Minipig)

1. Introduction

Brief ischemic preconditioning can result in an immediate protection against a subsequent and delayed ischemia–reperfusion episode (Murry et al., 1986). This protection may last for 1–3 h. When examining the time course of the protection after ischemic preconditioning, Kuzuya et al. (1993) and Marber et al. (1993) found that there was a second phase of protection which appeared 24 h after the initial ischemic preconditioning. This phenomenon has been termed as delayed protection or second window of protection. The delayed cytoprotection is generally accepted as a cellular adaptation process, involving multiple factors such as gene expression, stress protein, and antioxidant protein expression (Yellon and Baxter, 1995).

A number of observations suggest the mechanism underlying ischemic preconditioning is, at least in part, via an adenosine receptor-related mechanism. Adenosine A_1 receptor activation has been shown to induce protection against myocardial ischemia–reperfusion injury acutely (Lasley and Mentzer, 1992; Hale et al., 1993) and in a delayed fashion (Baxter and Yellon, 1997; Baxter et al., 1994; Dana et al., 1998). Adenosine receptor antagonists could abolish the beneficial effects of ischemic preconditioning (Thornton et al., 1993; Baxter et al., 1994; Liang, 1996). A number of studies indicated that pretreatment with intracoronary infusion of exogenous adenosine (Olafsson et al., 1987; Liu et al., 1991; Yao and Gross, 1994) or intravenous (i.v.) adenosine A_1 receptor agonist (Lasley and Mentzer, 1992; Thornton et al., 1992; Hale et al., 1993) could mimic the effects of ischemic preconditioning and was regarded as pharmacological preconditioning. Similar results were observed in large animal model of ischemia–reperfusion, such as pig (Louttit et al., 1999).

Further studies suggested that adenosine receptor activation by an adenosine agonist (Baxter et al., 1994) may also provide delayed protection for up to 24–72 h which can be blocked by an adenosine receptor antagonist, 8-(*p*-

* Corresponding author. Tel.: +1-610-454-2795; fax: +1-610-454-8740.

E-mail address: harry.meng@rp-rorer.com (H. Meng)

0014-2999/00/$ - see front matter
PII: S0014-2999(99)00804-3

sulfophenyl)-theophylline. However, this delayed effect has not been demonstrated using large animal models, such as minipig or dog.

[1*S*-[1*a*,2*b*,3*b*,4*a*(*S* *)]]-4-[7-[[2-(3-chloro-2-thienyl)-1-methylpropyl]amino]-3*H*-imidazo[4,5-*b*] pyridin-3-yl] cyclopentane carboxamide (AMP 579) is a novel adenosine A_1/A_2 receptor agonist ($K_i = 5$ nM and 56 nM for A_1 and A_{2a} receptors, respectively). We have previously evaluated the immediate protective effect of AMP 579 on ischemia–reperfusion injury in minipig (Smits et al., 1998). When a hemodynamically silent dose of AMP 579 was administered intravenously 40 min prior to ischemia, it resulted in an almost complete suppression of infarct size. When AMP 579 was given intravenously 10 min before reperfusion began, it reduced infarct size by 55%. The results prompted us to further explore if this compound could result in a delayed cardioprotection, for it would be a great benefit clinically, especially to those patients in high risk of myocardial infarction. In the present study, we used a similar large animal model of coronary occlusion and observed that AMP 579 pretreatment 24 h prior to myocardial ischemia could still effectively reduce the infarct size.

2. Materials and methods

2.1. Surgical preparation and instrumentation

All experiments were conducted in accordance with a protocol approved by the Rhône-Poulenc Rorer Animal Care and Use Committee and conform to the NIH Guidelines for the Use and Care of Laboratory Animals.

A 2-day protocol was designed for this investigation. At day 1, fasted female Yucatan minipigs (17.8–30.3 kg) were intramuscularly sedated with 4.4 mg/kg of Telazol (Fort Dodge Laboratories, Fort Dodge, IA) and anesthetized with 2% isoflurane. The trachea was then incubated. An aseptic neck incision was made to cannulate the jugular vein for drug infusion and the carotid artery for measurement of blood pressure. Lead II electrocardiogram was monitored. Body temperature was kept between 36°C and 37°C. After a 15-min stabilization period, a bolus dose of AMP 579 (3 μg/kg) or equal volume of vehicle (0.83 ml/min) was infused in 2 min via the venous cannula. This was followed by a continuous infusion of AMP 579 at 0.3 μg/kg/min or vehicle for 68 min. This dosing pattern was the same as our previous study in which AMP 579 provided immediate cardioprotection in minipig (Smits et al., 1998). At the end of the procedure, the cannulas were removed, the vessels were ligated, and the wounds were sutured. Minipigs were allowed to recover and eat in the afternoon.

The following morning (24 h after i.v. dosing), the pretreated minipigs were fasted, sedated and anesthetized with sodium pentobarbital (Anpro Pharmaceutical, Arcadia, CA). Animals were artificially ventilated via endotracheal tubing. Blood pCO_2 and pH were maintained between 35–45 mmHg and 7.35–7.45, respectively. Blood oxygen saturation was kept above 96%. Polyethylene catheters were placed into the femoral artery and vein for measurement of blood pressure and infusion of 0.9% NaCl (5 ml/h), respectively. A microtip catheter (Millar Instruments, Houston, TX) was inserted into the left ventricle via the carotid artery for measurement of left ventricular pressure, left ventricular end diastolic pressure, and maximum velocity of ventricular pressure elevation (dP/dt).

The heart was exposed with a mid-line sternotomy. A 10-mm section of the left anterior descending coronary artery was isolated just distal to its first major diagonal branch and instrumented with an electromagnetic flow probe (Skalar Medical, Netherlands). An elastic thread was placed around the left anterior descending coronary artery to make a snare loop. Lead II electrocardiogram, mean arterial blood pressure, heart rate, left ventricular pressure, left ventricular end diastolic pressure, maximum velocity of ventricular pressure elevation, coronary blood flow, and rate pressure product were recorded throughout the experiment on an MI^2 data processing system (Modular Instruments, Malvern, PA). Body temperature was maintained between 36°C and 37°C. The pigs were given a bolus i.v. injection of heparin (100 u/kg) and were allowed to stabilize for 1 h. The ischemia was created by tightening the occlusive snare for 40 min. The snare was then released to allow 3 h reperfusion of the ischemic zone. At the end of the experiment, the heart was removed for determination of area at risk and infarct size.

2.2. Determination of area at risk and infarct size

The heart was mounted on a Langendorff perfusion apparatus for double staining. The ischemic zone was infused with 1% solution of triphenyltetrazolium chloride through the coronary occlusion site. The rest of the heart was perfused simultaneously with 0.5% Evans Blue through the aorta. This delineated the non-ischemic from ischemic myocardium (area at risk). The left ventricle was then sliced into six transverse sections from apex to the occlusion site. Each slice was blotted dry and weighed. The non-ischemic zone, area at risk, and infarcted areas were traced onto acetate sheets for planimetric quantitation.

2.3. Exclusion criteria

Minipigs were excluded if: (1) the defibrillation was seven times or more; (2) the ventricular fibrillation (VF) was not converted within 2 min; (3) experiments could not be completed due to pre-existing conditions or technical problems.

2.4. Statistical analysis

Hemodynamic data were analyzed with repeated measures of analysis of variance (ANOVA) followed by Newman–Keuls post-hoc test. Area at risk and infarct size were analyzed with unpaired Student's t test. Differences were considered statistically different if $p < 0.05$.

3. Results

A total of 22 pigs were used in our experiment. Six were excluded: one died of fatal VF; one had seven defibrillation attempts; one had longer than 2 min fibrillation; one pig's heart was not properly stained; one had an unusual left ventricle tumor which would cause inaccurate assessment of infarct size; and one had pre-existing tachycardia and hypotension. Decisions to exclude these pigs were made before the end of the experiments.

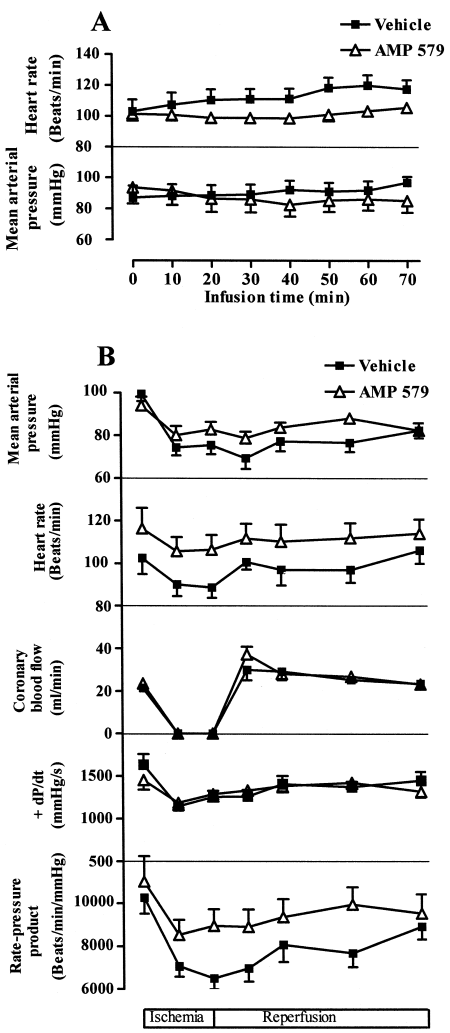

Fig. 1. Hemodynamics of minipigs at (A) day 1 and (B) day 2. Animals were treated with vehicle (■) or AMP 579 (△) intravenously at day 1. At day 2, they were subjected to 40 min coronary occlusion and 3 h reperfusion. Mean ± S.E.M. ($n = 8$ in each group).

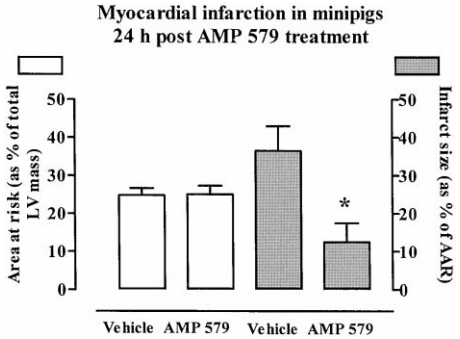

Fig. 2. Area at risk (blank rectangle) and infarct size (shaded rectangle) in vehicle- and AMP 579-pretreated groups. Animals were given vehicle or AMP 579 intravenously at day 1 and subjected to 40 min of coronary occlusion followed by 3 h reperfusion at day 2. Hearts were stained with Evans Blue and triphenyltetrazolium chloride to distinguish the non-ischemic area, area at risk, and infarcted area. Area at risk is the ischemic zone as percentage of the entire left ventricle mass. Infarct size is the infarcted myocardium as percentage of area at risk. Mean ± S.E.M. ($n = 8$ in each group). $^{*}p < 0.05$ vs. vehicle.

3.1. Hemodynamics

At day 1, mean arterial pressure and heart rate were monitored during the 70-min drug/vehicle infusion. Data are shown in Fig. 1A. AMP 579 i.v. did not result in significant changes in MAP and heart rate throughout the infusion as compared to baseline.

At day 2, the minipigs were subjected to pentobarbital anesthesia for the open-chest operation and ischemia–reperfusion challenge. Hemodynamic data are shown in Fig. 1B. In the AMP 579-pretreated group, MAP, HR, coronary blood flow, and dP/dt did not have significant changes in comparison with vehicle-pretreated group.

3.2. Effect of AMP 579 on infarct size

After 40 min coronary occlusion and 3 h reperfusion, the area at risk (ischemic zone) was defined with Evans Blue/triphenyltetrazolium chloride double staining and expressed as the percentage of the entire left ventricle mass. Fig. 2 indicates that the area at risk was 24.9 ± 1.8% and 25.1 ± 2.3% of left ventricle mass in vehicle- and drug-treated groups, respectively. The amount of the infarcted myocardium is expressed as a percentage of total area at risk. The ischemia–reperfusion procedure resulted at 36.5 ± 6.5% and 12.5 ± 5.0% infarct in vehicle- and AMP 579-pretreated groups, respectively ($p = 0.011$). This represented a 66% reduction of mean infarct size in the AMP 579-treated animals.

3.3. Effect of AMP 579 on VF

VF occurred during ischemia and early stage of reperfusion. Direct current cardioversion was applied immediately to stop VF. If the VF could not be converted, subsequent defibrillation was imposed with higher current (30 J at

maximum). The average number of VF episodes were 1.3 ± 0.3 and 1.8 ± 0.5 in vehicle- and AMP 579-treated groups, respectively ($p = 0.23$). The number of defibrillations applied were 1.6 ± 0.4 and 2.9 ± 0.9 in vehicle- and AMP 579-treated groups, respectively ($p = 0.43$).

4. Discussion

AMP 579 is a novel adenosine A_1/A_2 receptor agonist. Previously it has been shown to have marked cardioprotective activity when given intravenously in rat (Merkel et al., 1998), dog (McVey et al., 1999) and minipig (Smits et al., 1998) models of acute myocardial infarction. In this study, we demonstrated a delayed protective effect of AMP 579 against myocardial infarction in a minipig model of coronary occlusion/reperfusion.

The dose of AMP 579 selected for this study was the same as that used in a previous study by our group which demonstrated its acute cardioprotective effect in minipigs and was hemodynamically silent (Smits et al., 1998). In the present study, a high-performance liquid chromatography (HPLC) assay confirmed that AMP 579 level in plasma after 70 min drug infusion achieved ~ 7 ng/ml. Since the half lifetime of AMP 579 i.v. is 1 h, there was no measurable plasma levels at the time of the coronary occlusion (HPLC data not shown). Therefore, the delayed protective effects to reduce infarct size observed at day 2 were not related to residual AMP 579.

At a 10-fold, higher dose AMP 579 may cause bradycardia and hypotension in rats and pigs (Merkel et al., 1998; Smits et al., 1998). It is noteworthy that the dosage of AMP 579 we used in this study was relatively low and did not result in significant changes in hemodynamic parameters, either in day 1 or day 2. At such a hemodynamically silent dose, AMP 579 could still significantly reduce the myocardial infarct size 24 h after administration. This suggests that cardioprotection is not related to hemodynamic alterations but more likely a direct pharmacological effect of AMP 579 on myocardium.

Previously, the delayed protection (or late preconditioning) stimulated pharmacologically by adenosine A_1 receptor agonists was investigated only in small animal models, such as the rabbit (Baxter and Yellon, 1997; Dana et al., 1998). The present study is the first to demonstrate that a similar protection can also be stimulated by an A_1 agonist in a large animal model which has coronary circulation much closer to that of a human (Hamburger et al., 1991). Pigs are species relatively deficient in native collateral coronary vessels and these are unlikely to develop within the 24-h time course of the experiments. Therefore, although transmyocardial blood flow measurement is not included, it is more unlikely that increased collateral flow accounts for the delayed cardioprotection.

Baxter and Yellon (1997) reported that another adenosine A_1 receptor agonist, 2-chloro-*N*6-cyclopentyladenosine, can result in cardioprotection for 48–72 h in a rabbit model of coronary occlusion. Although our results are similar to theirs, we have not determined beyond 24 h duration of cardioprotection induced by a single dose of AMP 579 in our porcine model. It is possible, therefore, that the delayed cardioprotective effect of AMP 579 extends beyond the 24-h period examined in this study.

The mechanisms underlying the delayed cardioprotective effect of AMP 579 are not clear. However, since AMP 579 is a potent adenosine A_1 receptor agonist, it is more likely that the activation of adenosine A_1 receptor may play a role in triggering a pharmacological preconditioning as demonstrated by other labs using A_1 agonists (Baxter and Yellon, 1997; Dana et al., 1998). A_2 receptor stimulation may contribute to the ability of AMP 579 to attenuate reperfusion injury (Smits et al., 1998). In the present study, the peak plasma level of AMP 579 at day 1 was 15 nM which is much lower than the in vitro K_i of AMP 579 for A_2 receptors (56 nM), and at day 2, AMP 579 could not be detected in the plasma. Therefore, it is unlikely that A_2 receptor activation may contribute to the delayed protection in this investigation.

In the present model, AMP 579 did not produce a delayed protection against the incidences of VF occurring during ischemia–reperfusion. This contrasts with the ability of AMP 579 to reduce the VF incidence in the similar minipig model when given acutely, just prior to myocardial ischemia (Smits et al., 1998). The reason for this is not clear, but probably reflects the fact that the compound was absent in plasma during the ischemia–reperfusion period. Thus, in order to observe the anti-arrhythmic activity of AMP 579, it seems that the drug must be present and must exert a direct pharmacological action on target cells in the heart.

Evidence that preconditioning may occur in the human myocardium is accumulating (Leesar et al., 1997). It is of great interest to develop new compounds that may provide extended benefits to patients with ischemic heart diseases. The finding that similar to other A_1 agonists, AMP 579 can induce delayed protection may provide more clinical advantages. For example, although it is currently envisaged, AMP 579 will be given acutely just during the acute phase of infarction, these data may suggest that in addition to its acute protective effect, the drug will trigger a delayed protective effect in the myocardium which maintains the heart in a state resistant to ischemia for at least 24 h. This will provide a greater therapeutic window of cardioprotection to patients during the important 24 h post-ischemic reperfusion. Secondly, these data suggest the potential of prophylactical use of adenosine analogs to patients with high risk of myocardial infarction.

In summary, the novel adenosine agonist, AMP 579, at a hemodynamically silent dose, can produce a delayed protection for at least 24 h against myocardial infarction in a large animal model. This effect is probably related to a pharmacological preconditioning of the heart via adenosine

A_1 receptor agonism and may potentially extend the clinical applications of the compound.

References

Baxter, G.F., Yellon, D.M., 1997. Time course of delayed myocardial protection after transient adenosine A_1 receptor activation in the rabbit. J. Cardiovasc. Pharmacol. 29, 631–638.

Baxter, G.F., Marber, M.S., Patel, V.C., Yellon, D.M., 1994. Adenosine receptor involvement in a delayed phase of myocardial protection 24 hours after ischemic preconditioning. Circulation 90, 2993–3000.

Dana, A., Baxter, G.F., Walker, J.M., Yellon, D.M., 1998. Prolonging the delayed phase of myocardial protection: repetitive adenosine A_1 receptor activation maintains rabbit myocardium in a preconditioned state. J. Am. Coll. Cardiol. 31, 1142–1149.

Hale, S.L., Bellows, S.D., Hammerman, H., Kloner, R., 1993. An adenosine A_1 receptor agonist, *R*(−)-*N*-(2-phenylisopropyl)-adenosine (PIA), but not adenosine itself, acts as a therapeutic preconditioning–mimetic agent in rabbits. Cardiovasc. Res. 27, 2140–2145.

Hamburger, S.A., Kopaciewicz, L.J., Valocik, R.E., 1991. A new model of myocardial infarction in Yucatan minipigs. J. Pharmacol. Methods 25, 291–301.

Kuzuya, T., Hoshida, S., Yamashita, N., Fuji, H., Oe, H., Hori, M., Kamada, T., Tada, M., 1993. Delayed effects of sublethal ischemia on the acquisition of tolerance to ischemia. Circ. Res. 72, 1293–1299.

Lasley, R.D., Mentzer, R.M.J., 1992. Adenosine improves recovery of postischemic myocardial function via an adenosine A_1 receptor mechanism. Am. J. Physiol. 263, H1460–H1465.

Leesar, M.A., Stoddard, M., Ahmed, M., Broadbent, J., Bolli, R., 1997. Preconditioning of human myocardium with adenosine during coronary angioplasty. 95, pp. 2500–2507.

Liang, B.T., 1996. Direct preconditioning of cardiac ventricular myocytes via adenosine A_1 receptor and KATP channel. Am. J. Physiol. 271, H1769–H1777.

Liu, G.S., Thornton, J., Van Winkle, D.M., Stanley, A.W., Olsson, R.A., Downey, J.M., 1991. Protection against infarction afforded by preconditioning is mediated by A_1 adenosine receptors in rabbit heart. Circulation 84, 350–356.

Louttit, J.B., Hunt, A.A., Maxwell, M.P., Drew, G.M., 1999. The time course of cardioprotection induced by GR79236, a selective adenosine A_1 receptor agonist, in myocardial ischaemia–reperfusion injury in the pig. J. Cardiovasc. Pharmacol. 33, 285–291.

Marber, M.S., Latchman, D.S., Walker, J.M., Yellon, D.M., 1993. Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. Circulation 88, 1264–1272.

McVey, M., Smits, G.J., Cox, B., Kitzen, J., Clark, K., Perrone, M., 1999. Cardiovascular pharmacology of the adenosine A_1/A_2 receptor agonist AMP 579: coronary hemodynamic and cardioprotective effects in the canine myocardium. J. Cardiovasc. Pharmacol. 33, 703–710.

Merkel, L., Rojas, C., Jarvis, M., Cox, B., Fink, C., Smits, G., Spada, A., Perrone, M., Clark, K., 1998. Pharmacological characterization of AMP 579, a novel adenosine A_1/A_2 receptor agonist and cardioprotective. Drug Dev. Res. 45, 30–43.

Murry, C.E., Jennings, R.B., Reimer, K.A., 1986. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 74, 1124–1136.

Olafsson, B., Forman, M.B., Puett, D.W., Pou, A., Cates, C.U., Friesinger, G.C., Virmani, R., 1987. Reduction of reperfusion injury in the canine preparation by intracoronary adenosine: importance of the endothelium and the no-reflow phenomenon. Circulation 76, 1135–1145.

Smits, G.J., McVey, M., Cox, B.F., Perrone, M.H., Clark, K.L., 1998. Cardioprotective effects of the novel adenosine A_1/A_2 receptor agonist, AMP 579 in a porcine model of myocardial infarction. J. Pharmacol. Exp. Ther. 286, 611–618.

Thornton, J.D., Liu, G.S., Olsson, R.A., Downey, J.M., 1992. Intravenous pretreatment with A_1-selective adenosine analogues protects the heart against infarction. Circulation 85, 659–665.

Thornton, J.D., Thornton, C.S., Downey, J.M., 1993. Effect of adenosine receptor blockade: preventing protective preconditioning depends on time of initiation. Am. J. Physiol. 265, H504–H508.

Yao, Z., Gross, G.J., 1994. A comparison of adenosine-induced cardioprotection and ischemic preconditioning in dogs. Efficacy, time course, and role of K-ATP channels. Circulation 89, 1229–1236.

Yellon, D.M., Baxter, G.F., 1995. A ''second window of protection'' or delayed preconditioning phenomenon: future horizons for myocardial protection? J. Mol. Cell. Cardiol. 27, 1023–1034.