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β-Blocking activity of PP-34, a newly synthesized aryloxypropanolamine derivative, and its cardioprotective effect against ischaemia/reperfusion injury in laboratory animals

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Abstract

β-Adrenoceptor antagonists are widely used in cardiovascular medicine. However, the main side effect of these drugs is due to antagonism of β_2-adrenoceptors in the airways, resulting in bronchospasm. Therefore, more cardioselective β-blockers have been developed to offer a lower side effect profile. We have studied a new aryloxypropanolamine derivative (PP-34) with more cardioselectivity and efficacy against ischaemia/reperfusion injury in rats. Oxalate salts of 1-(*tert*-butylamino)-3-(5-*tert*-butylaminomethyl-2-methoxyphenoxy) propan-2-ol (PP-34) is a novel β-adrenoceptor antagonist. In-vitro studies in rat isolated right atria, guinea-pig trachea and rat distal colon preparations were carried out to investigate the potency of PP-34 towards different β-adrenoceptor subtypes. pA_2/pK_B values of PP-34 for β_1, β_2, and β_3 adrenoceptor were 7.89 ± 0.15, 6.13 ± 0.09 and 6.30 ± 0.19, respectively. The β_1/β_2 selectivity ratio calculated was in the order of PP-34 > atenolol > propranolol. Pre-ischaemic administration (20 min before coronary occlusion) of PP-34 (0.3 or 1 mg kg^{-1}) showed cardioprotective effects against ischaemia/reperfusion injury in rats and significantly reduced arrhythmias, infarct area and necrosis induced by ischaemia/reperfusion injury. The efficacy of PP-34 was found to be greater then atenolol. In conclusion, PP-34 is a cardioselective β-adrenoceptor antagonist, possessing potent anti-arrhythmic and cardioprotective effects against ischaemia/reperfusion injury in rats.

Introduction

Cardiovascular diseases affect the proper functioning of the heart and blood vessels (WHO 2003). It is one of the leading causes of death in developed and developing countries. Among the various cardiovascular diseases, myocardial infarction, transient ischaemic attack, stroke, hypertension, peripheral vascular diseases and coronary heart diseases are the major burden to health care professionals.

Ischaemia plays a central role in development of diseases like myocardial infarction and stroke (Gao et al 2002). The development of myocardial ischaemia and infarction is a dynamic process that occurs due to an imbalance between myocardial demand and coronary supply of oxygen, substrates and fluids (Opie et al 1980). Sympathetic drive or sympathetic stimulation increases the incidence and severity of the myocardial infarction. β_1-Adrenoreceptors mediate the effects of sympathetic nerve stimulation and circulatory catecholamines in the heart (Nisoli & Carruba 1997). Blockade of sympathetic stimulation with β-blockers is beneficial for the treatment of cardiovascular diseases, such as angina pectoris, acute and post-myocardial infarctions, tachyarrhythmias, congestive heart failure, left ventricular diseases and hypertension (Hjalmarson 2000), and non-cardiovascular diseases such as migraine, hyperthyroidism, anxiety, tremor and glaucoma (Hoffman 2001).

There is evidence that non-selective and cardio-selective (β_1-selective, when given intravenously) β-blockers reduce mortality equally in patients treated either in the first few hours or long-term after myocardial infarction (Yusuf et al 1985). The main cardiovascular use of

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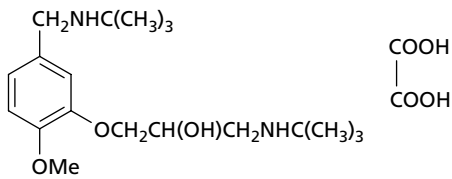
β-blockers is due to the antagonism of cardiac β-adrenoreceptor response. In addition, non-selective β-blockers pose risk of bronchoconstriction and are contraindicated in asthmatics and diabetics. Cardioselective β-blockers, having 20-times greater affinity for β_1-receptor than β_2-receptor, theoretically pose less risk to cause bronchoconstriction (Wellstein et al 1987).

Well controlled and designed clinical trials have now demonstrated that cardio-selective β-blockers did not clinically produce any adverse respiratory effects in patients with mild to moderate reversible airway diseases (Salpeter et al 2002a, b). Moreover, catecholamine-induced myocardial injury appeared to rise from stimuli of the β_1-receptor via a cAMP dependent process, whereas stimuli of the β_2-receptor surprisingly inhibited apoptosis via a G_i-coupled pathway (Communal et al 1999). These advantages have initiated the search for some novel potential cardio-selective β-blockers of greater selectivity towards β_1-receptors. We have been involved in the development of new β-blockers for the past few years (Jindal et al 2002, 2003a, b; Nandakumar et al 2005a, b). Recently we have developed some new compounds having an *ortho* methoxy group with *meta* substitution to the phenyl ring having the propanolamine group. In this study, we report the pharmacological investigation of the oxalate salt of 1-(*tert*-butylamino)-3-(5-*tert*-butylaminomethyl-2-methoxyphenoxy)propan-2-ol (PP-34, Figure 1) for its cardioprotective effect against ischaemia/reperfusion injury and its selectivity for different β-adrenoreceptor subtypes in laboratory animals.

Materials and Methods

Animals and general considerations

Studies were performed using male Wistar rats (250–350 g; National Toxicological Center, Pune, India) and guinea-pigs of either sex (300–350 g; Serum Institute of India Ltd, Pune, India). The animals were housed under 12:12 h light/dark conditions in a temperature controlled environment (24 ± 1°C) and had free access to food and water. Experimental procedures and protocols used were reviewed and approved by the Institutional Ethical Committee of Poona College of Pharmacy (Pune, India) and were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India. Animals for the in-vitro experiments were killed after mild anaesthesia with ether.



PP-34

Figure 1 Structure of PP-34.

Materials

The drugs used were: (−)-isoprenaline hydrochloride, carbachol and propranolol hydrochloride (all from Sigma Chemical Co., St Louis, MO); urethane (Fluka Chemika GmbH, Buchs, France); atenolol (Khandelwal Laboratory Ltd, Mumbai, India). Chemicals used in the physiological salt solutions were of analytical grade procured from S.D. Fine Chemicals Ltd (Mumbai, India). Isoprenaline solutions were prepared in saline (0.9% NaCl) containing 0.5% ascorbic and 0.05% disodium edetate. Test compounds and other drug solutions were dissolved or diluted to appropriate concentration with saline.

In-vitro studies

Rat isolated right atria

β_1-Antagonism was studied in rat isolated right atria. In brief, male Wistar rats were killed after mild anaesthesia and their hearts were quickly excised. Right atria were dissected and mounted in a 20-mL organ bath containing Krebs solution of the following composition (mM): NaCl 113, KCl 4.8, $CaCl_2$ 2.2, KH_2PO_4 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11.0, ascorbic acid 0.03 and sodium salt of EDTA 0.03. Tissues were maintained at temperature of 37 ± 1°C, aerated with 95% O_2 and 5% CO_2. A resting load of 0.2 g was applied and the tissue was allowed to equilibrate for 60 min. Spontaneous atrial frequency was measured with a help of force displacement transducer (T-305) connected to Student's Physiograph (Bio-Devices, Ambala, India). Concentration–response curves to isoprenaline were constructed from the increase in atrial frequency produced by cumulative addition of isoprenaline in the absence or presence of antagonists. Antagonists were added to the bath after 30-min isoprenaline response alone. Antagonists were incubated with the atrium for 30 min before the addition of cumulative concentrations of isoprenaline.

Guinea-pig isolated tracheal rings

β_2-Antagonism was studied in guinea-pig isolated tracheal rings. Guinea-pig tracheal rings were suspended in organ baths filled with 20 mL Krebs solution (composition and conditions as described in rat right atria) maintained at (37 ± 1°C) and aerated with 95% O_2 + 5% CO_2. To achieve a steady spontaneous tone level, an initial tension of 2 g was applied for 1 h. Rings were then contracted by addition of 200 nM carbachol for 15 min followed by a 30-min wash. Tissues were then incubated with appropriate concentrations of antagonist for 30 min with control tissues receiving saline treatment. The tissues were contracted again with a sub-maximal concentration of carbachol (150 nM) and allowed to stabilize for 15 min. Cumulative concentration–response curves to the relaxant effects of isoprenaline were obtained in the absence or presence of a test agent (1-h incubation time).

Rat isolated distal colon

Distal colon (3 cm) from male rats was mounted in organ baths containing Krebs solution (composition and conditions as described in rat right atria). Each tissue was placed under 1 g resting tension and equilibrated for 40 min before contraction with 50 mM KCl for 15 min followed by a 30-min wash.

Tissues were again contracted with a sub-maximal concentration of KCl (30 mM) and washed for 30 min. Tissues were then incubated with the appropriate concentration of antagonist for 30 min, with control tissues receiving saline treatment. The tissues were contracted again with KCl (30 mM) and cumulative concentration–response curves to relaxants were obtained, after contractions were stabilized. The affinity of antagonists to block β_3-receptor was estimated from this experiment.

In-vivo studies

Production of coronary ischaemia/reperfusion injury in rats

Male Wistar rats (300–350 g) were anaesthetized with urethane (1.20 mg kg^{-1}) and the left coronary artery ligation (LCA) was performed as reported by Himori & Matsuura (1989). Briefly, a tracheotomy was performed, and the trachea was intubated, ventilated with room air supplemented with O_2 at 60–65 strokes min^{-1} using a rodent ventilator (INCO, Ambala, India). Body temperature was maintained at 37 ± 1°C. The mean arterial pressure (MAP) was monitored via a catheter inserted into the carotid artery and a standard limb lead II electrocardiogram (ECG) was continuously monitored with an ECG electrode lead set. The MAP and ECG were recorded digitally through a four-channel data acquisition system (MP30, BIOPAC Systems Inc., Santa Barbara, CA). A catheter was cannulated in an external jugular vein for administration of test compounds. The chest was opened by left thoracotomy at the fourth intercostal space. After opening the pericardium, the heart was exteriorized by gentle pressure on the chest wall and a thin silk thread (Ethicon 1.5 metric, 4–0) attached to an atraumatic needle was placed around the left coronary artery, approximately 2–3 mm distal of the origin of the left coronary artery for later ligation. The heart was placed back into the chest and the animal was allowed to stabilize. Transient regional myocardial ischaemia was induced by passing the threads through a small plastic tube and pressing the tube against the coronary artery, and reperfusion was initiated by releasing the ligature and removing the plastic tube. The coronary artery was finally occluded for 30 min followed by 120-min reperfusion. The severity of arrhythmias during the 30-min occlusion was assessed and compared with control. Ischaemia and reperfusion were confirmed as described by Lawson et al (1993). In short, successful occlusion was confirmed by the increase in height of the R wave voltage during the first few seconds of each occlusion (Carbonin et al 1980) and a 20–30% reduction in the arterial blood pressure compared with the pre-ischaemic values. Successful reperfusion was confirmed by the return of the height of the R wave voltage and the arterial blood pressure to the pre-ischaemic values.

Study groups and experimental protocol

Rats were randomly assigned to seven groups including one sham-operated group. All groups, other than the sham-treated group, underwent a 30-min coronary artery occlusion and 2-h reperfusion. The control group underwent saline infusion before 30-min coronary artery occlusion and 2-h reperfusion. Test drug PP-34 (0.1, 0.3 or 1 mg kg^{-1}) or atenolol (0.3 or 1 mg kg^{-1}) were infused for 20 min before prolonged ischaemia and reperfusion. Different dose of drugs were used to see dose-dependent effect of drugs. For the control group n was 19, while for all other groups n was 12. From each group, six animals were selected for determining area at risk and infarct area; the remaining six animals were selected for myocardial necrosis score determination.

Arrhythmia score determination

Anti-arrhythmic properties were analysed by scoring the arrhythmia during a 30-min ischaemic period. Using the ECG signals, ventricular arrhythmias were assessed as described by Fryer et al (2000) and in accordance with the definitions reported in the Lambeth Conventions (Walker et al 1988). Scores were assigned during the 30-min myocardial ischaemia for a period of 3 min (10 recordings). Arrhythmia scores were assigned as follows: 0 = < 10 premature ventricular contractions (PVCs)/3-min period; 1 = 10–50 PVCs/3-min period; 2 = > 50 PVCs/3-min period; 3 = 1 episode of ventricular fibrillation (VF)/3-min period; 4 = 2–4 episodes of VF/3-min period; and 5 = > 4 episodes of ventricular fibrillation/3-min period.

Measurement of area at risk (AAR) and infarct area

On completion of the above mentioned protocols six animals from each group were selected for AAR and infarct area determination. The coronary artery was re-occluded and the AAR determined by negative staining. Patent blue dye was administered via the jugular vein to effectively stain the non-occluded area of the left ventricle. The rat was killed with a 15% KCl solution. The heart was excised, and the left ventricle was removed from the remaining tissue and subsequently cut into six thin cross-sectional pieces and incubated for 15 min with a 2% triphenyltetrazolium chloride (TTC) stain in phosphate buffer (pH 7.4) at 37°C. Tissues were stored in vials of 10% formaldehyde overnight. The images of heart slices were captured using a scanner (HP 1300 scan jet) and analysed for area measurements using Scion Image software (Ver. 4.0.3.2). Blue areas were considered as non-ischaemic areas and the remaining as AAR. Any whitish portion in the AAR was considered as infarct area. Total area at risk was expressed as the percentage of the left ventricle. Infarct area was expressed as the percentage of the area at risk.

Myocardial necrosis score determination

After 2-h reperfusion, six animals from each group were selected for necrosis score determination. The rats were killed with a 15% KCl solution; heart was excised and rinsed in normal saline to remove blood. The hearts were preserved in 10% buffered formalin (pH 7.4) solution and embedded for histological examination to examine necrosis. Necrosis was quantified by using the scoring system used by Vogel et al (2002). After microscopic examination grades were given as follows: grade 0, no change; grade 1, focal interstitial response; grade 2, focal lesions in many sections, consisting of mottled staining and fragmentation of muscle fibres; grade 3, confluent retrogressive lesions with hyaline necrosis and fragmentation of muscle fibres and sequestrating mucoid oedema; grade 4, massive infarct with occasionally acute aneurysm and mural thrombi.

Exclusion criteria

Any animal in which this procedure itself produced dysrhythmias or a sustained fall in MAP to less than 70 mmHg were discarded from the study. A total of 91 rats successfully completed the above-mentioned protocols and 84 rats were analysed for arrhythmia determination. From these animals, 42 animals were analysed for histological investigation of myocardial infarction, and 42 rats were analysed for AAR and ischaemia/reperfusion determination.

Statistics

In-vitro studies

In the in-vitro studies, mean concentration curves to isoprenaline were analysed using non-linear regression (Graph Pad Prism, version 4.0, Graph Pad Inc., San Diego, CA). The EC50 and pEC50 (negative logarithm of EC50) values of isoprenaline were obtained with and without the presence of antagonist. Concentration ratios (CR) were determined from the EC50 values. The plot of log (CR–1) vs log [antagonist] (Arunlakshana & Schild 1959) was analysed by linear regression. Antagonism was considered to be competitive in nature if the slope of the regression line was not significantly different from unity. In such cases a mean pA_2 value was obtained from the equation: $pA_2 = [\log (CR–1) – \log$ molar concentration of antagonist]. In cases where the slope or regression line significantly differed from unity, the value obtained from the above equation was the pK_B rather than the pA_2 value. A statistically significant difference between two means was analysed using repeated two-way analysis of variance followed by the Tukey test, where comparison was made to the same control group. For comparing unpaired data one-way analysis of variance followed by the Tukey test was performed. $P < 0.05$ was considered significant.

In-vivo studies

All values of parametric measures were expressed as mean ± s.e.m. For haemodynamic parameters, one-way analysis of variance followed by Dunnett post-hoc test were used. For non-parametric measures, arrhythmia and necrosis scores Kruskal–Wallis test followed by Dunn's post-hoc test were used. All these statistical calculations were performed by using Instat (GraphPad Software Inc., San Diego, CA). $P < 0.05$ was considered significant.

Results

In-vitro studies

PP-34, propranolol and atenolol shifted the concentration–response curves of isoprenaline towards the right with a change in EC50 values of isoprenaline in all three tissue preparations (Figure 2). The Schild plot yielded a line with a slope

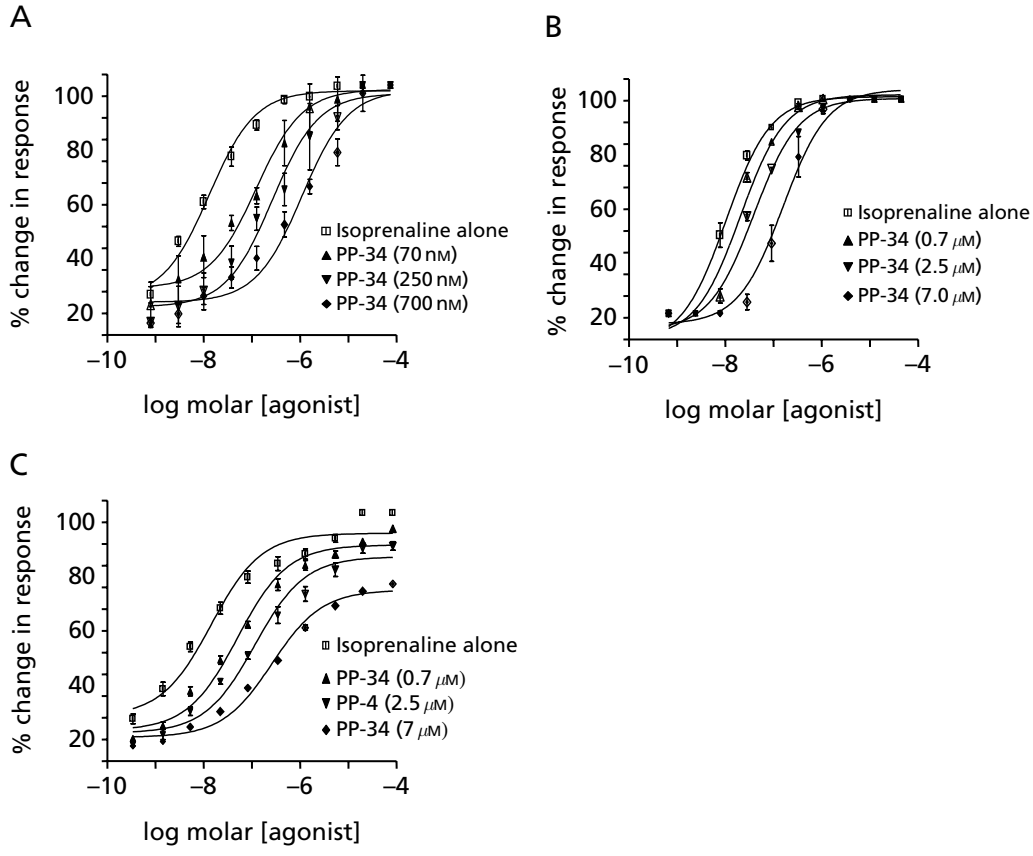


Figure 2 Antagonism of isoprenaline responses by PP-34 in different tissues. A. Rat right atria; B. guinea-pig trachea; C. rat distal colon.

close to unity for the test and standard antagonists in rat isolated atrial and guinea-pig tracheal ring preparations, indicating that the antagonists were competitive in nature for β_1- and β_2-receptor subtypes. However, the slope of the Schild plot was significantly different from unity for the test and standard drugs on rat isolated colon, indicating non-competitive antagonism of β_3-adrenoceptors (Table 1). The pA_2 values of PP-34, propranolol and atenolol are shown in Table 1. The β_1/β_2 ratios were determined. The selectivity to cardiac β-receptors was in the order of PP-34 > atenolol > propranolol. In the in-vitro experiments PP-34 was found to be β_1-adrenoceptor selective, and so propranolol was not included in those experiments.

In-vivo studies

Haemodynamics

Table 2 summarizes heart rate, MAP and pressure rate index (PRI) in all groups determined at baseline, at 15-min coronary artery occlusion, and at 120-min reperfusion. Baseline heart rate and MAP values were significantly different from control with PP-34 (0.3 and 1 mg kg^{-1}) and atenolol (0.3 and 1 mg kg^{-1}) showing bradycardic effects. PRI values for PP-34 and atenolol were found to be significantly different from control. Following left anterior descending artery (LAD) occlusion the MAP values of the animals in the experimental groups consistently and abruptly fell (peak effect at 5 min) and then progressively recovered within 30 min to the levels of 80–85 mmHg. Heart rate was found to be significantly different with PP-34 (0.3 and 1 mg kg^{-1}) and atenolol (0.3 and 1 mg kg^{-1}) after 15-min ischaemia and after 2-h reperfusion. MAP was not found to be significantly different in any group after 15-min ischaemia or after 2-h reperfusion.

Mortality

In the vehicle-treated group mortality was found to be high (37%) i.e. 7 of 19 animals died during the 30-min ischaemia. However, PP-34- and atenolol-treated groups showed no mortality.

Area at risk and infarct size (IS)

There were no significant differences in any group vs control for AAR expressed as a percentage of the left ventricle (LV; Table 3), which indicated that all groups were subjected to a similar degree of ischaemic insult. Control animals (n = 6) exhibited an IS/AAR of 44.9 ± 4.6. PP-34 (0.3 or 1.0 mg kg^{-1}, n = 6) and atenolol (1 mg kg^{-1}, n = 6) reduced IS/AAR significantly (39.9 ± 1.9, 36.6 ± 3.0 and 37.6 ± 2.5, respectively). Similarly PP-34 (0.3 or 1.0 mg kg^{-1}, n = 6) and atenolol (1 mg kg^{-1}, n = 6) reduced IS/LV significantly (23.5 ± 2.5, 20.0 ± 2.4 and 21.9 ± 1.8, respectively) compared with control (28.7 ± 3.7). The data suggested dose-dependent cardioprotection by PP-34 and atenolol.

Arrhythmia score

In vehicle-treated rats, left coronary artery (LCA) occlusion and reperfusion caused consistent ventricular ectopic activity associated with a high degree of mortality. The arrhythmia score in vehicle-treated animals (n = 12, 7 rats died during ischaemia) was 14 (12–17). Non-lethal arrhythmias were significantly reduced via administration of PP-34 0.3 mg kg^{-1} (5 (4–6), n = 12, $P < 0.01$), PP-34 1 mg kg^{-1} (3.5 (3–4), n = 12,

Table 1 Potencies of PP-34 and reference adrenoceptor antagonists on isolated preparations

Group	Rat right atria		Guinea-pig trachea		Rat distal colon		β_1/β_2 selectivity	β_1/β_3 selectivity
	pA_2/pK_B	Slope of Schild plot	pA_2/pK_B	Slope of Schild plot	pA_2/pK_B	Slope of Schild plot		
PP-34	7.89 ± 0.155	0.88 ± 0.03	6.13 ± 0.095	0.90 ± 0.16	6.30 ± 0.199	0.73 ± 0.03	57.41	38.72
Propranolol	8.29 ± 0.02	1.02 ± 0.06	8.18 ± 0.02	0.96 ± 0.01	6.64 ± 0.16	0.54 ± 0.17	1.3	44.2
Atenolol	7.16 ± 0.09	1.18 ± 0.10	5.57 ± 0.06	0.99 ± 0.11	4.62 ± 0.33	0.15 ± 0.05	39.2	346.4

All values were expressed as mean ± s.e.m. $pA_2/pK_B = \log (CR-1) - \log [antagonist]$.

Table 2 Haemodynamics

Group	n	Baseline			Ischaemia 15-min			Reperfusion 2-h		
		Heart rate	MAP	PRI	Heart rate	MAP	PRI	Heart rate	MAP	PRI
Sham	6	324 ± 9	110 ± 3	36 ± 1	320 ± 10	98 ± 5	31 ± 1	314 ± 8	92 ± 2	29 ± 1
Control	19	317 ± 5	107 ± 3	34 ± 2	384 ± 6	67 ± 4	26 ± 2	375 ± 2	78 ± 3	29 ± 2
PP-34 (0.1 mg kg^{-1})	12	295 ± 14	98 ± 7	29 ± 3*	369 ± 15	67 ± 6	25 ± 3	360 ± 14	85 ± 4	31 ± 2
PP-34 (0.3 mg kg^{-1})	12	272 ± 20**	95 ± 6*	26 ± 2**	359 ± 15*	68 ± 5	25 ± 3	345 ± 12**	83 ± 5	28 ± 2
PP-34 (1.0 mg kg^{-1})	12	255 ± 24**	93 ± 8**	23 ± 3**	335 ± 29**	71 ± 6	24 ± 3	313 ± 19**	87 ± 3	27 ± 3
Atenolol (0.3 mg kg^{-1})	12	280 ± 14**	95 ± 4*	26 ± 4**	346 ± 14**	70 ± 6	24 ± 2	331 ± 11**	79 ± 6	26 ± 3
Atenolol (1.0 mg kg^{-1})	12	260 ± 12**	94 ± 9**	24 ± 3**	330 ± 16**	72 ± 5	23 ± 3	320 ± 19**	84 ± 7	27 ± 2

Values given as mean ± s.e.m. Heart rate, beats min^{-1}; mean arterial pressure (MAP), mmHg; pressure rate index (PRI) mmHg min^{-1}/1000. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 3 Effects of PP-34 and atenolol on myocardial infarct (Inf) size (% LV and % AAR) after 30 min of left anterior descending coronary artery occlusion and 2-h reperfusion compared with vehicle. The mean areas at risk (% AAR/LV) were not significantly different, indicating that the degree of the ischaemic insult was similar

Group	n	% Inf/LV	% Inf/AAR	% AAR/LV
Control	6	28.7 ± 3.7	44.9 ± 4.6	57.3 ± 3.8
PP-34 (0.1 mg kg^{-1})	6	27.5 ± 4.0	43.0 ± 3.6	56.9 ± 3.1
PP-34 (0.3 mg kg^{-1})	6	23.5 ± 2.5*	39.9 ± 1.9*	59.9 ± 4.9
PP-34 (1.0 mg kg^{-1})	6	20 ± 2.4**	36.6 ± 3.0**	60.2 ± 3.7
Atenolol (0.3 mg kg^{-1})	6	25.2 ± 2.6	41.7 ± 2.7	56.0 ± 3.0
Atenolol (1.0 mg kg^{-1})	6	21.9 ± 1.8**	37.6 ± 2.5**	56.2 ± 3.8

*$P < 0.05$, **$P < 0.01$ vs control.

$P < 0.001$) and atenolol 1 mg kg^{-1} (5.5 (5–7), n = 12, $P < 0.05$) (Table 4).

Myocardial necrosis score

Histological examination of hearts of sham-treated animals showed no symptoms of necrosis, while the vehicle-treated group showed confluent retrogressive lesions with hyaline necrosis and fragmentation of muscle fibres and sequestrating mucoid oedema. Pretreatment with PP-34 (0.3 mg kg^{-1}, n = 6, $P < 0.05$ and 1 mg kg^{-1}, n = 6, $P < 0.01$) and atenolol (1 mg kg^{-1}, n = 6, $P < 0.01$) 20 min before LCA occlusion reduced the severity of infarction produced by ischaemia/reperfusion injury (Figure 3).

Discussion

Ischaemic heart failure can have a worse prognosis and a different drug response than non-ischaemic heart failure. Loss of functional myocardial muscle secondary to ischaemia is the leading cause of heart failure (Gilbert et al 1996). Coronary artery ligation in rats is one of the most commonly used models to study the efficiency of new drugs for its cardioprotective effects in myocardial infarction (Janse et al 1998). In our study, pretreatment with PP-34 reduced the arrhythmic score, infarct area, mortality and necrosis score against ischaemic/reperfusion injury. The cardioprotective effect of PP-34 was greater than the standard drug, atenolol. β-Blockers are a class of drug that decrease myocardial oxygen demand and protect the heart against ischaemia. These effects primarily result from the ability of β-blockers to decrease heart rate, myocardial contractility and wall tension (Feuerstein et al 1997). Other beneficial effects of these compounds in myocardial ischaemia are anti-arrhythmic actions (Chadda et al 1986; Gundersen et al 1983) and up-regulation of the β-adrenoceptor density (Heilbrunn et al 1989).

The major cardioprotective effect of PP-34 may have resulted due to the decrease in heart rate, myocardial contractility and anti-arrhythmic actions. The lowering of the heart rate, blood pressure and pressure rate index suggested that PP-34 possibly reduced the myocardial oxygen consumption. Earlier studies (Bernauer 1985) showed that the β-adrenoceptor blocking agents pindolol, propranolol, and metoprolol significantly decreased the percentage of necrosis in experiments with permanent ligation of the coronary artery in rats. Kinoshita et al (1988) showed that β-adrenoceptor blockade with atenolol (1 mg kg^{-1}) significantly reduced the incidence of ventricular fibrillation in rats. β-Adrenergic stimulation increases the magnitude of the Ca^{2+} current and slows its inactivation, increases the magnitude of repolarizing K^+ and Cl^- currents (Hume & Harvey 1991; Sanguinetti et al 1991), increases pacemaker current (thereby increasing sinus rate), and under pathophysiological conditions can increase both delayed after-depolarization- and early after-depolarization-mediated arrhythmias. Thus, blockage of β-stimulation

Table 4 Arrhythmia score during 30-min coronary artery occlusion in vehicle-treated animals and animals treated with PP-34 or atenolol

Length of myocardial ischaemia (min)	Treatment groups					
	Vehicle	PP34			Atenolol	
		0.3 mg kg^{-1}	1 mg kg^{-1}	3 mg kg^{-1}	0.3 mg kg^{-1}	1 mg kg^{-1}
	n = 19	n = 12	n = 12	n = 12	n = 12	n = 12
0–3	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
4–6	1 (0–1)	1 (1–2)	0 (0–0)	0 (0–0)	1 (0–2)	0 (0–0)
7–9	1 (1–2)	2 (1–4)	0.5 (0–2)	1 (1–2)	2 (1–4)	1 (1–2)
10–12	3 (3–4)	2.5 (1–3)	2 (1–3)	1.5 (1–2)**	3 (2–3)	2 (1–2)*
13–15	4 (3–4)	2 (1–3)	2 (0–3)	1 (0–1)***	2.5 (1–3)	1 (1–2)*
16–18	3 (3–3)	1 (0–3)	0.5 (0–1)*	0 (0–0)***	1.5 (0–3)	1 (1–2)
19–21	1.5 (1–2)	0 (0–2)	0 (0–0)**	0 (0–0)**	0 (0–2)	0 (0–1)*
22–24	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–1)	0 (0–0)
25–27	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
28–30	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Total score	14 (12–17)	9 (7–12)	5 (4–6)**	3.5 (3–4)***	10 (9–12)	5.5 (5–7)*

Values are given as median (range). Arrhythmia score was calculated as stated in the Materials and Methods. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Kruskal–Wallis test followed by Dunn's post-hoc test).

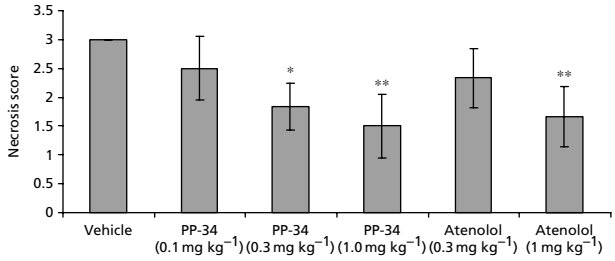


Figure 3 Effects of PP-34 and atenolol on necrosis scoring after ischaemia/reperfusion injury. $*P < 0.05$, $**P < 0.01$ as compared with control group. n = 6 for each group. Kruskal–Wallis test followed by Dunn's post-hoc test.

through β-adrenoceptor antagonist can reduce the severity of arrhythmia in myocardial infarct patients. Results of this study showed that pretreatment with PP-34 reduced the arrhythmic score, and the incidence and severity of arrhythmia. It has been suggested that β-blockade in the CNS might exert part of its anti-arrhythmic effect by counteracting stress-induced vagal (Wikstrand & Kendall 1992) withdrawal and by reducing the level of sympathetic efferent discharge (Lewis & Haeusler 1975). Treatment with hydrophilic β_1-blockers such as atenolol, however, has also been found to increase indices of vagal tone (Coker et al 1984; Cook et al 1991) and to cause a reduction in sympathetic outflow (Scott 1981), suggesting that these effects might be a result of peripheral β-adrenergic blockade. PP-34 is a hydrophilic compound and there are less possibilities of a central action; rather, peripheral β-adrenergic blockade, such as with atenolol, is possible with PP-34.

Since the model adopted in this study was of an acute type, the mechanism of involvement of upregulation of β-adrenoceptor density may less likely be involved in the cardioprotective effect of PP-34. However, in acute myocardial ischaemia catecholamine promoted internalization and functional uncoupling of β-adrenoceptor was abolished (Kurosawa et al 2003). Consequently, the balance of internalization and externalization of receptor was shifted towards an increase in functionally-coupled receptors at the cell surface (Strasser et al 1990). This leads to overload of intracellular free calcium ions which may contribute to myocyte injury (Tani et al 1990). The results showed a significant mean reduction with PP-34 in the infarct size as a percentage of the area at risk in rats subjected to 30-min coronary artery occlusion followed by 2-h reperfusion. These results and the reduction in necrotic scores by pretreatment with PP-34 suggested that PP-34 decreased the overload and maintained a balance in receptor density.

The reduction of heart rate is one of the actions specific to the class of compounds that reduce myocardial oxygen consumption. The cardioprotective effect of PP-34 was found to be greater than that of atenolol. There was increased evidence showing that β_1-blockade was mainly responsible for the significant reduction in mortality, particularly sudden death, in patients with moderate to severe heart failure with bisoprolol and metoprolol (CIBIS II 1999; MERIT-HF Study Group 1999). Based on the literature evidence we hypothesized that cardioselectivity of PP-34 may be one of the components that could be the cause of increased cardioprotection observed in ischaemia/reperfusion experiments.

Tissue experiments were conducted to assess antagonist potency of PP-34 towards different β-adrenoceptor subtypes and its selectivity for β_1-adrenoceptor. The pA_2 values from the tissue in-vitro preparations indicated that the antagonist potency was in the order of propranolol > PP-34 > atenolol to all the β-receptor subtypes (Table 1). The anti-logarithm of the difference between the mean pA_2 values of right atrium and guinea-pig tracheal rings can be used as a quantitative measure of the degree of cardioselectivity (Chiu et al 2000). The selectivity ratio calculated was in the order of PP-34 > atenolol > propranolol. Evidence from other studies, the order of selectivity ratio from this study and the weak antioxidant property of PP-34 (data not presented here) support our hypothesis that the cardioselectivity of PP-34 may be one reason for the potent cardioprotective effect compared with atenolol against ischaemic reperfusion injury in rats.

Conclusion

PP-34 possessed potent anti-arrhythmic and cardioprotective effects against ischaemia/reperfusion injury in rats. These beneficial effects of PP-34 derived mainly from β-adrenoceptor antagonistic activity with increased cardioselectivity. PP-34 opens the possibility for a broad range of therapeutic applications in cardiovascular diseases, particularly in ischaemia/reperfusion injury-induced arrhythmia and myocardial infarction.

References

Arunlakshana, O., Schild, H. O. (1959) Some quantitative uses of drug antagonists. *Br. J. Pharmacol.* **14**: 48–57

Bernauer, W. (1985) The effect of beta-adrenoceptor blocking agents on evolving myocardial necrosis in coronary ligated rats with and without reperfusion. *Naunyn Schmiedebergs Arch. Pharmacol.* **328**: 288–294

Carbonin, P., Gennaro, M. D., Valle, R., Beranbei, R., Habed, A. (1980) Intracellular calcium and electrogram in ischaemic isolated rat heart. *Am. J. Physiol.* **239**: H380–H390

Chadda, K., Goldstein, S., Byington, R., Curb, J. D. (1986) Effect of propranolol after acute myocardial infarction in patients with congestive heart failure. *Circulation* **73**: 503–510

Chiu, C. C., Lin, Y. T., Tsai, C. H., Liang, J. C., Chiang, L. C., Wu, J. R., Chen, I. J., Yeh, J. L. (2000) Pharmacological effects of an aldehyde type α/β adrenoreceptor-blocking agent with vasodilating properties. *Gen. Pharmacol.* **34**: 391–400

CIBIS II Investigators and Committees (1999) The Cardiac Insufficiency Bisoprolol Study II (CIBIS II): A randomized trial. *Lancet.* **353**: 9–13

Coker, R., Koziell, A., Oliver, C., Smith, S. E. (1984) Does the sympathetic nervous system influence sinus arrhythmia in man? Evidence from combined autonomic blockade. *J. Physiol. (Lond).* **356**: 459–464

Cook, J. R., Bigger, J. T., Kleiger, R. E., Fleiss, J. L., Steinman, R. C., Rolnitzky, L. M. (1991) Effect of atenolol and diltiazem on heart period variability in normal persons. *J. Am. Coll. Cardiol.* **17**: 480–484

Communal, C., Singh, K., Sawyer, D. B., Colucci, W. S. (1999) Opposing effects of $beta_1$ and $beta_2$ adrenergic receptors on cardiac myocyte apoptosis. *Circulation* **100**: 2210–2212

Feuerstein, G. Z., Bril, A., Ruffolo, R. R. (1997) Protective effect of carvedilol in the myocardium. *Am. J. Cardiol*. **80**(11A): 41L–45L

Fryer, R. M., Hsu, A. K., Nagase, H., Gross, G. J. (2000) Opioid induced cardioprotection against myocardial infarction and arrhythmias: mitochondrial versus sarcolemmal ATP-sensitive potassium channels. *J. Pharmacol. Exp. Ther*. **294**: 451–457

Gao, F., Yao, C. L., Gao, E., Mo, Q. Z., Yan, W. L., McLaughlin, R., Lopez, B. L., Christopher, T. A., Mo, X. L. (2002) Enhancement of glutathione cardioprotection by ascorbic acid in myocardial reperfusion injury. *J. Pharmacol. Exp. Ther*. **301**: 543–550

Gilbert, E. M., Abraham, W. T., Olsen, S., Hattler, B., White, M., Mealy, P., Larrabee, P., Bristow, M. R. (1996) Comparative hemodynamic, left ventricular functional, and antiadrenergic effects of chronic treatment with metoprolol versus carvedilol in failing heart. *Circulation* **94**: 2817–2825

Gundersen, T. (1983) Influence of heart size on mortality and reinfarction in patients treated with timolol after myocardial infarction. *Br. Heart J*. **50**: 135–139

Heilbrunn, S. M., Shah, P., Bristow, M. R., Valantine, H. A., Ginsburg, R., Fowler, M. B. (1989) Increased β-receptor density and improved hemodynamic response to catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. *Circulation* **79**: 483–490

Himori, N., Matsuura, A. (1989) A simple technique for occlusion and reperfusion of coronary artery in conscious rats. *Am. J. Physiol*. **256**: H1719–H1725

Hjalmarson, A. (2000) Cardioprotection with β adrenoceptor blockers. Does lipophilicity matter? *Basis Res. Cardiol*. **95** (Suppl. 1): I41–I45

Hoffman, B. B. (2001) Catecholamines, sympathomimetic drugs and adrenergic receptor antagonists. In: Hardman, J. G., Limbird, L. E. (eds) *Goodman and Gilman's The pharmacological basis of therapeutics*, 10th edn. McGraw Hill, New York, pp 215–267

Hume, J. R., Harvey, R. D. (1991) Chloride conductance pathways in heart. *Am. J. Physiol*. **261**: C399–C412

Janse, M. J., Opthof, T., Kleber, A. G. (1998) Animal models of cardiac arrhythmias. *Cardiovasc. Res*. **39**: 165–177

Jindal, D. P., Coumar, M. S., Bruni, G., Massarelli, P. (2002) Synthesis and β_1, β_2-adrenergic receptor binding studies of 4-acyl amino-substituted phenoxypropanolamine and 5-acyl amino-substituted naphthyloxypropanolamine derivatives. *Arzneimittelforschung* **52**: 654–663

Jindal, D. P., Coumar, M. S., Nandakumar, K., Bodhankar, S. L., Purohit, P. G., Mahadik, K. R., Bruni, G., Massarelli, P. (2003a) Synthesis, β adrenergic blocking activity and β receptor binding affinities of 1-substituted-3-(2-isopropyl-5-methyl-phenoxy)-propan-2-ol oxalates. *Il Farmaco* **58**: 557–562

Jindal, D. P, Babita, S., Sharma, N., Coumar, M. S., Bruni, G., Massarelli, P. (2003b) Synthesis of 4-(1-oxo-isoindoline)-, 4-(5,6-dimethoxy-1-oxo-isoindoline) and 4-acetamido substituted phenoxy-3-amino-propane derivatives and their β_1-, β_2-adrenergic receptor binding studies. *Ind. J. Chem*. **42B**: 2808–2813

Kinoshita, K., Hearse, D. J., Braimbridge, M. V., Manning, A. S. (1988) Ischemia- and reperfusion-induced arrhythmias in conscious rats: studies with prazosin and atenolol. *Jpn. Circ. J*. **52**: 1384–1394

Kurosawa, S., Kanaya, N., Niiyama, Y., Nakayama, M., Fujita, S., Namiki, A. (2003) Landilol, esmolol and propranolol protect from ischaemia/reperfusion injury in isolated guinea pig hearts. *Can. J. Anesth*. **50**: 489–494

Lawson, C. S., Coltart, D. J., Hearse, D. J. (1993) Dose-dependency and temporal characteristics of protection by ischaemic preconditioning against ischemia-induced arrhythmias in rat hearts. *J. Mol. Cell Cardiol*. **25**: 1391–1402

Lewis, P. J., Haeusler, G. (1975) Reduction in sympathetic nervous activity as a mechanism for the hypotensive effect of propranolol. *Nature* **256**: 440

MERIT-HF Study Group (1999) Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL randomised intervention trial in congestive heart failure. *Lancet* **353**: 2001–2007

Nandakumar, K., Bansal, S. K., Singh, R., Mohite, A. J., Bodhankar, S. L., Jindal, D. P., Coumare, M. S., Balaraman, R., Bharadwaj, S. H. (2005a) Study of β-adrenoceptor antagonistic activity of DPJ 904 in rats. *Pharmacology* **74**: 1–5

Nandakumar, K., Bansal, S. K., Singh, R., Bodhankar, S. L., Jindal, D. P., Coumare, M. S., Balaraman, R., Bharadwaj, S. H. (2005b) Selective $\beta_1$adrenoreceptor blocking activity of newly synthesized acyl amino-substituted aryloxypropanolamine derivatives, DPJ 955 and DPJ 890, in rats. *J. Pharm. Pharmacol*. **57**: 1–6

Nisoli, E., Carruba, M. O. (1997) Pharmacological properties of β_3 receptor. *Trends Pharmacol. Sci*. **18**: 257

Opie, L. H., Owen, P., Riemersma, R. A. (1980) Relative rates of oxidation of glucose and free fatty acids by ischaemic and non-ischaemic myocardium after coronary artery ligation in the dog. *Eur. J. Clin. Invest*. **3**: 419–435

Salpeter, S., Ormiston, T., Salpeter, E., Pool, P., Cates, C. (2002a) Cardioselective beta-blockers for chronic obstructive pulmonary disease (Cochrane Review). The Cochrane Library, Issue 2. Oxford: Update software.

Salpeter, S. R., Ormiston, T. M., Salpeter, E. E. (2002b) Cardioselective beta-blockers in patients with reactive airway disease: a meta-analysis. *Ann Intern Med*. **137**: 715–725

Sanguinetti, M. C., Jurkiewicz, N. K., Scott, A., Siegl, P. K. (1991) Isoproterenol antagonizes prolongation of refractory period by the class III antiarrhythmic agent E-4031 in guinea pig myocytes. Mechanism of action. *Circ. Res*. **68**: 77–84

Scott, E. M. (1981) The effects of atenolol on spontaneous and reflex activity of the sympathetic nerves in the anaesthetized cat. *Br. J. Pharmacol*. **73**: 609–616

Strasser, R. H., Marquentant, R., Kübler, W. (1990) Adrenergic receptor and sensitization of adenylyl cyclase in acute myocardial ischemia. *Circulation* **82** (Suppl. II): II23–29

Tani, M. (1990) Mechanisms of Ca^{2+} overload in reperfused ischemic myocardium. *Annu. Rev. Physiol*. **52**: 543–559

Vogel, G. H., Vogel, W. H., Scholkens, B. A., Sandow, J., Muller, G., Vogel, W. F. (2002) (eds) In: *Drug discovery and evaluation. Pharmacological assays*. 2nd edn, Springer-Verlag, Berlin, pp 191–192

Walker, M. J. A., Curtis, M. J., Hearse, D. J., Campbell, W. F., Janse, M. J., Yellon, D. M., Cobbe, S. M., Coker, S. J., Harness, J. B., Harron, D. W. G., Higgins, A. J., Julian, D. G., Lab, M. J., Manning, A. S., Northover, B. J., Parratt, J. R., Reimersma, R. A., Riva, E., Russell, D. C., Sheridan, D. J., Winslow, E., Woodward, B. (1988) The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia, infarction, and reperfusion. *Cardiovasc. Res*. **22**: 447–455

Wellstein, A., Palm, D., Belz, G., Butzer, R., Polsak, R., Pett, B. (1987) Reduction of exercise tachycardia in man after propranolol, atenolol and bisprolol in comparison to beta adrenoceptor occupancy. *Eur. Heart J*. **8** (Suppl. M): 3–8

Wikstrand, J., Kendall, M. (1992) The role of beta receptor blockade in preventing sudden death. *Eur. Heart J*. **13** (Suppl. D): 111–120

WHO Cardiovascular Diseases (2003) http://www.who.int/cardiovascular_diseasses/en/ on 12th July 2003

Yusuf, S., Peto, R., Lewis, J., Collins, R., Sleight, P. (1985) Beta blockade during and after myocardial infarction; an overview of the randomised trials. *Prog. Cardiovasc. Dis*. **27**: 335–371