

Studies on the involvement of bradykinin using enalapril and 2-mercaptoethanol in ischemia-reperfusion induced myocardial infarction in albino rats

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The effects of bradykinin were evaluated using the ACE inhibitor enalapril and the APP inhibitor, 2-mercaptoethanol alone and in combination in rats with experimental myocardial infarction. Myocardial infarction was produced by occlusion of the left anterior descending coronary artery for 30 min followed by 4 h of reperfusion. Infarct size was measured by the TTC stain method. Lipid peroxide levels in serum and heart tissue were estimated by the methods developed by Yagi and Ohkawa et al., respectively. A lead II electrocardiogram was monitored throughout the experiment. With the combined inhibition of both the enzymes ACE and APP, a better cardioprotection was observed when compared to individual inhibition of the enzymes, suggesting the involvement of bradykinin during experimental myocardial infarction.

1. Introduction

Myocardial infarction is the most common clinical manifestation of ischemic heart disease. The complete occlusion of a branch of coronary artery with almost no blood supply results in severe myocardial ischemia. Myocardial ischemia when severe and sustained for prolonged periods results in irreversible damage i.e. myocardial infarction [1]. However with early reperfusion, damage is reversible. Reperfusion of ischemic myocardium can result in some new cellular damage that blunts the beneficial effects of reperfusion itself, such damage is called “reperfusion injury”. Reperfusion mediated injury is separate from the ischemic damage that occurs during the period of coronary artery occlusion [2]. Attenuation of this reperfusion injury could improve the benefits of reperfusion therapy [3]. Angiotensin converting enzyme (ACE) inhibitors have been shown to reduce myocardial ischemia-reperfusion injury. This protective effect is produced through an action to preserve the peptide bradykinin (BK) from rapid degradation, rather than by decreasing production of angiotensin II [4–6].

BK is a small vasoactive peptide which is involved in a variety of biological processes. Kinins participate in the regulation of blood pressure, renal and cardiac functions which have been studied intensively using specific kinin receptor antagonists [7]. Because of the very short half-life of kinins, their specific efficiency depends on their local formation by tissue Kallikrein-kinin systems and is restricted by the spectrum and potency of local kinin degrading enzymes [8]. There is increasing evidence that part of the cardiovascular actions of ACE inhibitors, including reduction of infarct size, improvement of performance and energetic state of ischemic myocardium and

prevention of ventricular hypertrophy and remodelling are due to the potentiation of the effects of kinins [9]. ACE is not the only kinin-degrading enzyme and recent studies focus on the role of other peptidases [10]. A number of peptidases have been identified to possess kininase activity, the inhibition of which increases the availability and effectiveness of kinins [8]. In rats, aminopeptidase P (APP) has been shown to participate in myocardial kinin metabolism to the same extent as ACE whereas neutral endopeptidase (NEP) only plays a minor role [10]. Inhibition of APP may therefore be a sufficient means to potentiate cardiovascular effects of kinins. A combination of inhibitors may provide superior protection to that given by a single agent [11]. Hence in the present study, the cardioprotective actions of ACE inhibitor, enalapril and a non selective APP inhibitor, 2-mercaptoethanol were studied alone and in combination in an *in vivo* rat model of acute ischemia and reperfusion to evaluate the involvement of bradykinin.

2. Investigations and results

Percent left ventricle necrosis (PLVN) was found to be 50.49 ± 1.43 in control group animals. It was decreased with enalapril and 2-mercaptoethanol individual treatments (Table 1) and the difference observed was statistically significant ($P < 0.05$). PLVN was further reduced with the combined treatment of enalapril and 2-mercaptoethanol when compared to individual treatments (Table 1). Malondialdehyde (MDA) levels in serum of control group animals were found to be 26.05 ± 0.79 nmol mL⁻¹. Statistically there was no significant difference in serum MDA levels in 2-mercaptoethanol treated animals whereas the

Table 1: Percent left ventricle necrosis (PLVN) in control and in experimental animals

Experimental group	PVLN
Group 1	50.49 ± 1.43
Group 2	27.33* ± 0.87
Group 3	44.07* ± 0.47
Group 4	19.38** ± 0.46

Values represent mean ± SEM from 6 animals in each group. Group 1, saline treated control animals. Group 2, treated with enalapril (3 mg kg⁻¹). Group 3, treated with 2-mercaptoethanol (3 µL kg⁻¹). Group 4, treated with enalapril (2 mg kg⁻¹) and 2-mercaptoethanol (2 µL kg⁻¹). * P < 0.05 compared to Group 1. ** P < 0.05 compared to Group 2

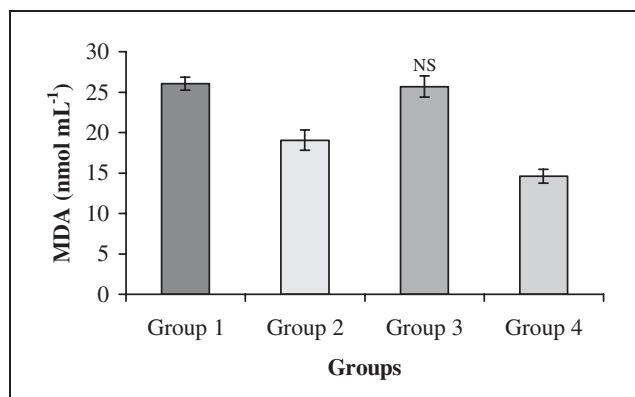


Fig. 1: Levels of lipid peroxides in serum of control and experimental animals. Values represent mean ± SEM from 6 animals in each group. Group 1, saline treated control animals. Group 2, treated with enalapril (3 mg kg⁻¹). Group 3, treated with 2-mercaptoethanol (3 µL kg⁻¹). Group 4, treated with enalapril (2 mg kg⁻¹) and 2-mercaptoethanol (2 µL kg⁻¹). NS, statistically not significant compared to group 1

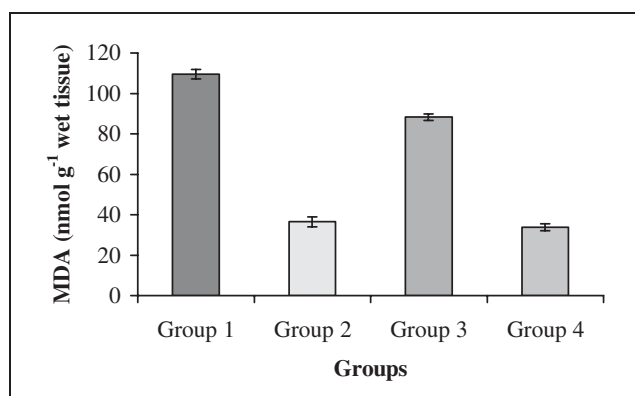


Fig. 2: Levels of lipid peroxides in heart tissues of control and experimental animals. Values represent mean ± SEM from 6 animals in each group. Group 1, saline treated control animals. Group 2, treated with enalapril (3 mg kg⁻¹). Group 3, treated with 2-mercaptoethanol (3 µL kg⁻¹). Group 4, treated with enalapril (2 mg kg⁻¹) and 2-mercaptoethanol (2 µL kg⁻¹)

difference was significant with enalapril treatment and with the combined treatment of enalapril and 2-mercaptoethanol compared to control (Fig. 1). MDA levels in the heart tissue of control group animals were found to be 109.52 ± 2.27 nmol g⁻¹ wet tissue. Levels of lipid peroxidation in the heart tissue was reduced significantly (P < 0.05) with the individual and combined treatments of enalapril and 2-mercaptoethanol (Fig. 2). The heart rates for all the above groups at various stages of occlusion and reperfusion were given in Table 2.

3. Discussion

There is increasing evidence that kinins possess cardioprotective actions in the ischemic heart [12]. In isolated rat hearts with ischemia-reperfusion injuries BK reduced the duration and incidence of ventricular fibrillations and reduced the release of cytosolic enzymes [12]. In anaesthetised animals intracoronary infusion of BK was followed by comparable beneficial changes and decreased the infarct size [13]. Infarct size reduction by ACE inhibitors and BK in anaesthetised animals is reversed by HOE 140 [14] and beneficial effects of ACE inhibitors on cardiac function were not observed in kininogen deficient rats [15]. There are a number of enzymes that possess kinase activity [7]. ACE and APP are the two predominant kininases found in the rat myocardium. ACE and APP form a metabolic barrier which effectively reduces kinin concentrations in the interstitium. ACE is identical to kinase II, an enzyme involved in the degradation of bradykinin [16]. APP activity is responsible for most of the remaining metabolism of BK and this was confirmed by the inhibitory action of 2-mercaptoethanol and apstatin [17, 18]. In the present study, the ACE inhibitor enalapril and the APP inhibitor 2-mercaptoethanol attenuated the myocardial necrosis caused by ischemia-reperfusion when administered individually to different groups of animals. The degree of cardioprotection was higher with enalapril than with 2-mercaptoethanol. Recent studies proposed that ACE inhibitors potentiate BK beyond blocking its hydrolysis by inhibiting desensitization of B₂ receptors [19]. This may be the reason for the enhanced cardioprotective action observed with enalapril. A combination of inhibitors may provide superior protection to that given by a single agent [11]. Inhibition of both ACE, APP and potentiating the BK effect could be an interesting approach in the treatment of ischemia-reperfusion induced myocardial infarction. Previous studies have shown that there was no significant difference in the cardioprotection with the combined treatment of apstatin and ramipril compared to their individual treatments [20, 21]. Previous studies in our laboratory using a non-selective APP inhibitor, 2-mercaptoethanol have shown the significant difference in the cardioprotection with the combined treatment of 2-mercap-

Table 2: Heart rate (beats min⁻¹) recorded at various stages of occlusion and reperfusion

	Group 1	Group 2	Group 3	Group 4
BO	409.52 ± 6.02	405.95 ± 10.77	392.85 ± 11.29	401.19 ± 9.78
MOP	368.05 ± 6.94	361.60 ± 10.81	296.46 ± 9.17	346.81 ± 6.86
IAR	376.57 ± 11.29	344.23 ± 9.64	316.66 ± 7.45	325.19 ± 7.55
1 h AR	364.37 ± 7.18	331.09 ± 7.44	330.55 ± 15.0	320.18 ± 10.05
2 h AR	361.11 ± 8.78	331.50 ± 9.37	344.29 ± 10.09	320.52 ± 11.22
3 h AR	354.50 ± 10.34	334.42 ± 8.84	347.22 ± 8.78	332.13 ± 11.31
4 h AR	354.50 ± 10.34	350.49 ± 8.34	347.22 ± 8.78	333.44 ± 13.89

Values represent mean ± SEM from 6 animals in each group. Group 1, saline treated control animals. Group 2, treated with enalapril (3 mg kg⁻¹). Group 3, treated with 2-mercaptoethanol (3 µL kg⁻¹). Group 4, treated with enalapril (2 mg kg⁻¹) and 2-mercaptoethanol (2 µL kg⁻¹). BO, before occlusion. MOP, middle of occlusion period. IAR, immediately after reperfusion. AR, after reperfusion.

toethanol and ramipril/lisinopril compared to their individual treatments in an *in vivo* rat model of myocardial ischemia-reperfusion [22, 23]. The difference between the cardioprotective action of apstatin and 2-mercaptoethanol may be due to their selective and non-selective inhibition of the enzyme APP respectively. Similar to our earlier observations, in the present study also, the combined treatment of enalapril and 2-mercaptoethanol, offered more cardioprotection compared to their individual treatments. With the combined inhibition of both the enzymes ACE and APP, degradation of BK may be further inhibited resulting in elevated levels of BK which may account for the enhanced cardioprotection confirming the role of BK in mediating the beneficial cardiac effects in this model.

In conclusion, the cardioprotection achieved by enalapril and 2-mercaptoethanol in an *in vivo* rat model of acute myocardial ischemia and reperfusion may be mediated by bradykinin.

4. Experimental

4.1. Substances

Enalapril was generously supplied by Dr. Reddy's Laboratories Ltd (Hyderabad, India). 2-Mercaptoethanol was purchased from Otto Kemi (Mumbai, India), 2,3,5-triphenyl tetrazolium chloride from BDH Chemicals Ltd. (England) and 1,1,3,3-tetraethoxy propane from Sigma Chemical Co. (Bangalore, India). Thiopentone sodium was generously supplied by Abbott Labs (I) Ltd. (Ankleshwar, India).

4.2. Surgical preparation

Sprague dawley albino rats of either sex weighing 200–400 g each were anaesthetised with thiopentone sodium (30 mg kg⁻¹, i.p), tracheotomized and ventilated with room air by a Techno positive pressure respirator (Crompton Parkinson Ltd., England). The right jugular vein was cannulated in order to inject saline and drugs. A left thoracotomy and pericardiotomy were performed, followed by identifying the marginal branch of the left coronary artery. A silk thread was passed below the artery and was occluded for 30 min by a knot. The silk thread was removed with the help of two knot releasers to allow reperfusion of the heart for succeeding 4 h.

4.3. Study protocol

Twenty four rats were randomly assigned to four groups of six in each (Fig. 3). Group 1 was the control group. In this group of rats, saline (0.2 ml) was administered before release of occlusion. Group 2 received enalapril. After control and 15 min occlusion measurements enalapril (3 mg kg⁻¹) dissolved in saline was administered intravenously 5 min before release of occlusion. Group 3 received 2-mercaptoethanol. After control and 15 min occlusion measurements 2-mercaptoethanol (3 µL kg⁻¹)

was administered intravenously 5 min before release of occlusion. Group 4 received enalapril and 2-mercaptoethanol. After control and 15 min occlusion measurements enalapril (2 mg kg⁻¹) and 2-mercaptoethanol (2 µL kg⁻¹) were administered intravenously 10 min and 5 min before release of occlusion respectively.

4.4. Measurement of infarct size

In all the groups after sacrificing the animal, the heart was excised from the thorax rapidly and the greater vessels were removed. The left ventricle was separated from the heart and was weighed. It was sliced parallel to the atrioventricular groove to 2–3 mm thick sections and the slices were incubated in 1% TTC solution prepared in pH 7.4 phosphate buffer for 30 min at 37 °C. In viable myocardium TTC is converted by dehydrogenase enzymes to a red fomazan pigment that stains tissue dark red [24]. The infarcted myocardium that does not take TTC stain where the dehydrogenase enzymes are drained off, remains pale in colour [25]. The pale necrotic myocardial tissue was separated from the stained portions and weighed on an electronic balance (Dhona 200D). Myocardial infarct size was expressed quantitatively in terms of percent left ventricle necrosis (PLVN).

4.5. Biochemical estimations

In all the groups before sacrificing the animal at the end of 4 h of reperfusion, 2 ml of blood sample was collected from the left ventricle, for the estimation of MDA levels in blood serum. Serum MDA levels were estimated by the method developed by Yagi [26]. Lipid peroxide levels in serum were expressed in terms of MDA (nmol mL⁻¹).

Lipid peroxidation in infarcted myocardial tissue was determined by the method of Ohkawa et al. [27]. Lipid peroxide levels were expressed in terms of MDA (nmol g⁻¹ wet tissue).

4.6. Electrocardiography (ECG)

After surgical preparation, rats were allowed 10 min for stabilization and then control measurements of ECG were taken. The rats were then subjected to a 30 min occlusion. At 15 min of occlusion ECG was taken again. Measurements of ECG were repeated immediately after release of occlusion and at 1h, 2h, 3h and 4h intervals for all the groups of animals (Fig. 3). A lead II electrocardiogram was monitored by using Cardiart 408 (BPL) with 20 mm mv⁻¹ sensitivity at a paper speed of 50 mm s⁻¹. Heart rates were expressed as beats min⁻¹.

4.7. Statistical analysis

The results are expressed as mean ± SEM. Differences in PLVN, serum and tissue lipid peroxide levels were determined by factorial one-way analysis of variance. Individual groups were compared using Dunnett's 't' test. Differences with P < 0.05 were considered statistically significant.

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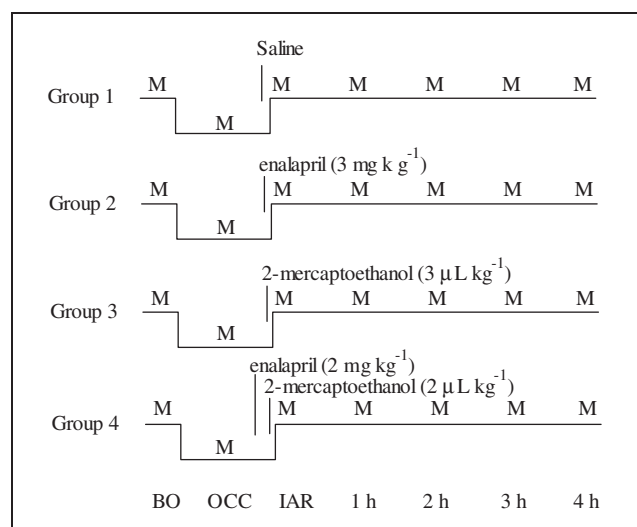


Fig. 3: Illustration of the study protocol. Group 1 (Saline), Group 2 (enalapril), Group 3 (2-mercaptoethanol), Group 4 (enalapril + 2-mercaptoethanol). M indicates ECG measurement. BO, before occlusion. OCC, 30 min occlusion. IAR, immediately after reperfusion

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