RESEARCH PAPER

Accepted 19 December 2001
Revised 26 November 2001
Received 24 June 2000
Indian J. Pharm. Sci., 2002, 64(2): 151-154

Possible Role of Nitric Oxide in the Cardioprotective Actions of Enalapril and Lisinopril in Rats

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ACE inhibitors are effective in the treatment of ischemia and reperfusion-induced myocardial infarction. It was reported that in hypercholesterolemic state, myocardial infarction was more. This may be due to decreased synthesis or release of nitric oxide (NO) or enhanced degradation of NO in the coronary bed in hypercholesterolemic state. Hence the cardioprotective effects of enalapril and lisinopril were studied along with L-arginine (NO donor) in hyperchloesterolemic rats. Myocardial infarction was produced by occlusion of left coronary artery for 30 min followed by 4 h of reperfusion. Infarct size was measured by using triphenyl tetrazolium chloride (CTTC) stain method. Infarct size, expressed as percent left ventricle necrosis (PLVN) was found to be significantly reduced with lisinopril and enalapril in normal rats as compared to control untreated animals, the degree of cardioprotection offered by enalapril and lisinopril was reduced in hyperchloesterolemic rats. When ACE inhibitors are combined with NO donor L-arginine, the degree of cardioprotection was improved in hyperchloesterolemic rats. These results suggest a possible role of NO in cardioprotection mediated by ACE inhibitors.

Most of the heart problems result from reduced coronary circulation due to blood clots, fatty atherosclerotic plaques, or spasms of the smooth muscle in coronary artery walls[1]. Among them, the most serious one is myocardial infarction (MI). This is the commonest single cause of death in many parts of the world[2]. Aim of the therapy must include attempts to prevent the complications of acute miyocardial infarction and to provide reperfusion of the ischemic tissue.

Reperfusion of ischemic myocardium can result in some additional cellular damage that blunts the beneficial effects of reperfusion itself, such damage is called reperfusion injury[3]. Cardioprotective drugs which can reduce the ischemia and reperfusion-induced injury may be valuable adjuncts in patients subject to myocardial ischemia and reperfusion during coronary bypass grafting, primary angioplasty or thrombolysis for acute myocardial infarction.

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ACE inhibitors produce cardioprotective effects by several mechanisms such as preventing the formation of angiotensin II, increasing bradykinin levels, promoting the release of nitric oxide in the coronary beds[4]. In addition, altered prostaglandins production or oxygen free radical scavenging properties of ACE inhibition have been postulated to reduce myocardial infarction[4]. Acute hypercholesterolemia increases the severity of myocardial ischemia in a model of coronary occlusion with or without reperfusion[5]. Moreover endothelium-dependent vasodilation is reduced, along with a reduction in the synthesis/release of 'NO, or enhanced degradation of NO during hypercholesterolemia[6].

Present study was carried out to evaluate the cardioprotective actions of lisinopril and enalapril in myocardial infarction induced by ischemia and reperfusion in both normal and rats fed on high cholesterol diet. It was reported previously that NO donor reverses myocardial injury in rabbits with acute hypercholesterolemia[7]. Hence the cardioprotective effects of enalapril and lisinopril were stud-

ied along with L-arginine (NO donor) in cholesterol-loaded rats.

MATERIALS AND METHODS

All the reagents used were of analytical grade. Enalapril, a gift from Dr. Reddy's Laboratories, Hyderabad and lisinopril, a gift from NATCO Pharmaceuticals, Hyderabad were used throughout the study. TTC (triphenyl tetrazolium chloride) LR Grade (BDH) was used.

Experimental design:

Sprague dawley rats of either sex, weighing 150-250 g were used. Rats were divided into 12 groups of 5 animals each. Six groups were daily supplied with 1% cholesterol-rich feed for one week and served as hypercholesterolemic (HC) animals. Group I and Group II served as normocholesterolemic (NC) and HC control groups respectively and these groups received saline only. Group I_E, Group I_L and Group I_A were NC animals treated with enalapril (3 mg/kg, i.v), lisinopril (3 mg/kg, i.v) and L-arginine (200 mg/kg, i.v), respectively. Group II_E, Group II_L and Group II_A were HC animals treated with enalapril, lisinopril and L-arginine, respectively. Group I_{A+E} and Group I_{A+L} were the NC animals treated with enalapril and lisinopril along with L-arginine (200 mg/kg, i.v) respectively. Group II_{A+E} and Group II_{A+L} were the HC animals treated with enalapril and lisinopril along with L-arginine (200 mg/kg, i.v), respectively. All groups of animals were similarly subjected to 30 min of left coronary artery occlusion and 4 h of reperfusion.

Rats were anaesthetized with thiopentone sodium (30 mg/kg, i.p) and were ventilated with room air by using a Techno positive pressure respirator. Ventilation parameters were adjusted to maintain satisfactory oxygenation. The chest was opened through fourth intercostal space at left side and the heart was exposed by removing pericardium. Left coronary artery was located. Femoral vein was cannulated to administer saline and drugs.

Coronary artery occlusion and reperfusion:

A silk thread was passed below the left coronary artery and was occluded for 30 min by lifting the thread. Silk thread was removed after 30 min to allow reperfusion of the heart for 4 h. All the drug solutions were prepared in saline and were administered intravenously through femoral vein at 28 min of occlusion i.e., at the beginning of reperfusion.

Quantification of infarct size:

At the end of 4 h of reperfusion, animals were sacrificed. Heart was excised from thorax rapidly and greater vessels were removed. The left ventricle was separated, weighed and sliced parallel to the atrioventricular groove to 0.1 cm thick sections and incubated in 1% TTC solution (prepared in pH 7.4 phosphate buffer) for 30 min at 37°. In viable myocardium TTC is converted by dehydrogenase enzymes to a red formazan pigment that stains tissue dark red[8]. The infarcted myocardium does not take TTC stain where the dehydrogenase enzymes are drained off and remains pale in colour. The pale necrotic left ventricular tissue was separated from the stained portions and weighed. Myocardial infarction was expressed quantitatively in terms of percentage left ventricular infarction (PLVI).

Statistical data analysis:

All values were expressed as mean ± standard deviation. Statistical comparisons were made by using the Student's 't' test. Statistical significance was considered at $P<0.05$.

RESULTS

From the results obtained (Table 1), percent left ventricular necrosis (PLVN) was found to be 62.4±2.1 in saline treated NC animals, where as it was significantly reduced to 23.1±0.9 and 22.3±1.2 with enalapril (3 mg/kg, i.v) and lisinopril (3 mg/kg. i.v) pretreatments respectively ($P<0.05$). In HC animals PLVN was found to 70.5±1.5 in saline treated HC animals, where as it was significantly reduced ($P<0.05$) to 52.5±3.0 and 51 9±2.1 with the enalapril and lisinopril pretreatments respectively. The degree of reduction in PLVN with enalapril and lisinopril pretreatments was more in NC animals compared to HC animals. In L-arginine (200 mg/kg, i.v) treated animals, PLVN was found to be 53.20±3.39 and 60.52±3.42 in NC and HC animals, respectively. PLVN was found to be 19.65±1.19 and 18.01±1.07 by the combined pretreatment of L-arginme (200 mg/kg. i.v) with enalapril and lisinopril, respectively in NC animals. PLVN was found to be 37.5±3.97 and 34.8±0.5 by the combined pretreatment of L-arginine (200 mg/kg, i.v) with enalapril and lisinopril, respectively in HC animals.

DISCUSSION

The results obtained indicate that both the drugs enalapril and lisinopril significantly lowered PLVN and improved percent left ventricular preservation in comparison to untreated NC and HC animals. The degree of cardioprotection with the same doses was decreased in HC animals compared to NC animals. In HC animals, with the combined treatment of ACE inhibitor (enalapril or lisinopril)

TABLE 1: EFFECT OF ENALAPRIL AND LISINOPRIL TREATMENTS ON LEFT VENTRICLE INFARCTION.

Treatment line	Experimental group	% left ventricular necrosis (PLVN)	% cardioprotection compared to saline-treated animals
Saline	Group I (NC)	62.37±1.59	--
	Group II (HC)	70.52±1.34*	--
Enalapril (3 mg/kg, i.v)	Group I_E (NC)	23.13±0.77*	62.92
	Group II_E (HC)	52.51±2.31**	25.54
Lisinopril (3 mg/kg, i.v)	Group I_L (NC)	22.35±0.99*	64.17
	Group II_L (HC)	51.88±1.55**	26.43
L-arginine (200 mg/kg, i.v)	Group I_A (NC)	53.20±3.39*	14.7
	Group II_A (HC)	60.52±3.42**	14.18
L-arginine (200 mg/kg, i.v)+	Group I_{A+E} (NC)	19.65±1.19*	68.49
Enalapril (3 mg/kg, i.v.)	Group II_{A+E} (HC)	37.51±2.95**	46.81
L-arginine (200 mg/kg, i.v)+	Group I_{A+L} (NC)	18.01±1.07*	71.12
Lisinopril (3 mg/kg, i.v)	Group II_{A+L} (HC)	34.79±0.99**	50.67

* Denotes statistical significance compared to saline-treated normocholesterolemic (NC) rats, while, ** denotes statistical significance compared to saline-treated hypercholeterolemic (HC) rats. * Denotes statistical significance compared to saline-treated NC rats. In NC rats, cholesterol levels were in the range of 50-90 mg/100 ml serum, while in the HC group, they were in the range of 130-198 mg/100 ml serum. All values are expressed as mean ± standard deviation of a sample size of 5. Statistical significance was determined at $p<0.05$.

along with NO donor (L-arginine), the degree of cardioprotection was improved but it was not up to the level as with NC animals treated with ACE inhibitors alone.

There is experimental evidence showing that the ACE inhibitors limit the development of infarct size, reduce the incidence of ischemia and reperfusion-induced arrhythmias and enhance the recovery of contractile function of stunned myocardium[9]. Recent experiments in animals and large clinical trials strongly indicate a role for ACE inhibitors in limiting myocardial ischemia and reperfusion-induced injury[9-11]. Coronary artery occlusion results in the acute activation of the renin-angiotensin system and increases production of angiotensin II, a potent vasoconstrictor and positive inotropic agent. This has raised the possibility that ACE inhibitors might attenuate myocardial injury, dysfunction and necrosis in the event of acute ischemia and infarction[12].

Myocardial ischemia evokes vasoconstrictor neurohumoral activation, which may lead to coronary and systemic vasoconstriction in related coronary segments. The subsequent diminished coronary flow increase in systemic vasomotor tone and after load, unfavourably alter the myocardial oxygen supply/demand ratio. Under laboratory conditions, acute ACE inhibition counteracts this activation in animals[13]. ACE inhibitors suppressed endogenous endothelin-I secretion, which results in improved coronary function and stabilisation of cardiac rhythm after ischemia in rat model. Suppression of endothelin-I results from both removal of endogenous angiotensin II and accumulation of endogenous bradykinin/nitric oxide[13].

The excitation that occurs at and around the ischemic myocardium is thought to be one of the mechanisms underlying for ischemia and reperfusion-induced arrhythmias[14]. ACE inhibitors inhibit the generation of endogenous angiotensin II and are shown to increase coronary flow *in vivo*[13]. ACE inhibitors have been shown to reduce norephinephrine overflow and reperfusion-induced arrhythmias in isolated rat heart[4]. Converting enzyme is also identical to kininase II, an enzyme involved in the degradation of bradykinin. Thus ACE inhibitors increase local tissue concentration of bradykinin

and potentiate its effects[10]. Bradykinin induced vasodilation is shown to be mediated by the production of prostaglandins and NO by vascular endothelial cells[15].

Multiple lines of evidence have indicated the cardioprotective effects of ACE inhibitors in acute ischemia or infarcting myocardium where a role for the intrinsic cardiac RAS is suggested[16]. Numerous protective mechanisms of action have been speculated. Coronary vasodilation due to interference with kininase II or complex changes in systemic hemodynamics that reduce myocardial oxygen demand may account, in part for the cardioprotection. In addition, inhibitors of cardiac angiotensin II formation, altered prostaglandin production or oxygen free radial scavenging properties of ACE inhibitors have been postulated to reduce ischemia and ischemia reperfusion induced myocardial damage[4].

Acute hypercholesterolemia increases the severity of myocardial ischemia in a model of coronary occlusion with or without reperfusion[5]. In HC state, coronary vascular reserve is greatly reduced. This may be caused by reduction in the synthesis or release of NO or enhanced degradation of NO[6]. However, a dual role of NO in cytoprotection or cytotoxicity has also been reported. At relatively lower concentrations NO may act as a pro-oxidant by reacting with O^-_2 resulting in the formation of more reactive oxidant, peroxynitrite ($ONOO^-$), and enhancing lipid peroxidation. In contrast at relatively high concentrations, NO can act as an antioxidant and can inhibit lipid peroxidation[7].

Moreover NO possesses potent antineutrophil activity and thus may exert cardioprotective effects through alteration of PMN mediated reperfusion injury[7]. After myocardial ischemia and reperfusion, though the NO release from coronary endothelium is diminished, the ACE inhibitors and cardioprotective drugs can still offer significant cardioprotection in NC animals. In HC state, severe endothelial dysfunction occurs, and the two ACE inhibitors enalapril and lisinopril alone and in combination with L-arginine were found to offer less cardioprotection in comparison to that in NC animals.

From the results it was found that both the drugs enalapril and lisinopril offered significant cardio-protection in NC and HC rats at a dose of 3 mg/kg (i.v). The degree of cardioprotection was more or less same with lisinopril or enalapril. The percent cardioprotection of these ACE inhibitors was decreased in HC rats compared to NC rats. In HC animals the degree of cardioprotection with the combined treatment of lisinopril or enalapril along with L-arginine was not upto level as with NC animals.

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