

EXPERIMENTAL / LABORATORY STUDIES

Effects of ACE Inhibition and AT1 Receptor Blockade on Cardiac Ischaemia-Reperfusion Induced Mortality and Cardiac Markers in Rats*

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Abstract: Many studies have established the therapeutic benefits of angiotensin-converting enzyme (ACE) inhibitors such as reducing reperfusion arrhythmias, and angiotensin II type 1 (AT1) blocker may have similar effects to ACE inhibitors. In this study, it was aimed to compare the effects of an ACE inhibitor captopril and AT1 receptor blocker losartan on death from arrhythmias and biochemical markers such as cardiac troponin T and I (cTnT, cTnI), myoglobin, creatin kinase (CK), creatine kinase-MB isoenzyme (CK-MB) and aspartate aminotransferase (AST) after cardiac ischemia/reperfusion in an *in vivo* rat model. Study design and methods: sixty four male rats were divided into four groups: Control, captopril (3 mg/kg), losartan (2 mg/kg) and sham. The drugs were administered intravenously 10 min before ischemia under anesthesia. Except for the sham group, the left coronary artery was occluded for 7 min and followed by 10 the min of reperfusion. Blood pressure, heart rate and ECG were monitored throughout the experiment. Biochemical markers were evaluated from the blood samples obtained at the 10th min of reperfusion. Captopril significantly decreased total ventricular fibrillation (VF) and death due to irreversible VF, while losartan did not. cTnT, myoglobin, total CK and CK-MB levels were higher in the control and drug administered groups than in the sham group. cTnT and cTnI levels were significantly increased after captopril administration in comparison with the control group, while losartan administration had no effect. In conclusion, captopril is more effective than losartan, especially for decreasing death from irreversible VF. In addition, captopril may increase the biochemical cardiac markers in the blood during early reperfusion.

Key Words: Myocardial Ischemia-Reperfusion, Arrhythmia, Cardiac Marker, Troponin, Captopril, Losartan.

Introduction

Acute myocardial infarction (AMI) is a major public health problem in the industrialized world and is becoming an increasingly important problem also in the developing countries. Occlusion of coronary arteries induces myocardial changes from ischemia to infarction. Prevention of myocardial infarction in ischemia is the primary and immediate purpose of therapy. Reperfusion may successfully prevent infarction development, but it often exacerbates myocardial damages. Short-term myocardial ischemia/reperfusion (I/R) leads to life threatening arrhythmias in most species (1). Two main

factors concerned with reperfusion arrhythmias are free oxygen radicals and cytosolic calcium overload, although both factors may actually be acting together (2).

The classic World Health Organization (WHO) criteria for the diagnosis of AMI include the history, typical electrocardiographic changes and serum cardiac markers such as creatine kinase (CK), the MB isoenzyme of creatine kinase (CK-MB), myoglobin and cardiac specific troponin T and I (cTnT and cTnI). Troponins exists in different tissues and their levels increase in several clinical conditions such as exercise, convulsions, intramuscular injections, pulmonary embolism, trauma, e.g. (3-5), but

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cTnT and cTnI are specific to myocardial cells. Accordingly, detection of cardiac troponins in serum is very important for detection of myocardial cellular injury. Reperfusion of ischemic myocardium accelerates the washout of intracellular proteins producing an exaggerated and early peak value of substances (3).

Accumulating evidences support the therapeutic benefits of angiotensin-converting enzyme (ACE) inhibitors in many clinical conditions and experimental studies such as hypertension, congestive heart failure. They also limit infarct size and reduce reperfusion arrhythmias in rats and dogs *in vivo*, additionally in isolated rat heart and arrhythmias in post infarction heart failure patients (6-11). ACE inhibitors have been shown to attribute blockade of angiotensin II (Ang II) production and decrease breakdown of bradykinin, which may stimulate the production of prostaglandin and nitric oxide (NO) (6,9,11). Ang II affects the target tissues by means of Ang II receptors, and there are two types of these receptors, AT1 and AT2. Most of the well-known Ang II functions in the cardiovascular system are mediated through AT1 (12). The clinical benefits of AT1 receptor antagonists are thought to be similar to those of ACE, and since they do not increase bradykinin, their cough incidence is lower than ACE inhibitors (13). On the other hand, in previous studies (6,11,14), the effect of AT1 antagonist losartan on cardiac I/R induced mortality has been controversial.

The purpose of this study was to compare the effects of ACE inhibitor captopril and AT1 receptor antagonist losartan on cardiac I/R induced death due to irreversible ventricular fibrillation (VF) and serum cardiac protein elevations in an *in vivo* rat model.

Material and Methods

Animals and groups

In the experiment, 12 weeks old male Sprague Dawley rats weighing 250-300 g were used. Animals were housed two per cage in the animal quarter with an alternating 12 h light-dark cycle, air-conditioned room with 20-22 °C temperatures and they were given standard rat pellet feed and water *ad libitum*. In the experiments, "Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 85-123, 1985" regulations were followed.

Rats were randomly divided into four groups: (1) Sham operated (n = 10), (2) Control I/R; treated with vehicle (0.09% NaCl, 1 ml/kg) (n = 18), (3) treated with captopril (3 mg/kg; Sigma, USA) (n = 18) and (4) treated with losartan (2 mg/kg; Merck, USA) (n = 18). Drugs and vehicle were administered by intravenous (i.v.) injection 10 min before left coronary occlusion.

Experimental protocols

Male Sprague Dawley rats weighing 250–350 g were anaesthetised with urethane 1.2–1.4 g/kg administered intraperitoneally (i.p.). The jugular vein and trachea were cannulated for drug administration and artificial respiration with room air. Systemic blood pressure (BP) was monitored from the carotid artery by a Harvard model 50-8952 transducer (Harvard Apparatus Inc., South Natick, USA) and displayed on a Harvard model 60-9315 Universal oscillograph (Harvard Apparatus Inc.) together with a standard ECG instrument.

The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs, about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately, using a volume of 1.5 ml/100 g body weight at a rate 60 strokes/min to maintain normal PCO₂, PO₂ and pH parameters.

After the pericardium was incised carefully, the heart was exteriorized by gentle pressure on the right side of the rib cage. A 6/0 silk suture attached to a 10 mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest, and the animal was allowed to recover for 20 min. Any animal in which this procedure produced arrhythmias or a sustained decrease in blood pressure (BP) to less than 70 mmHg was discarded.

The ligature was inserted through a small plastic tube and the tube was placed in contact with the heart. The artery could then be occluded by applying tension to the ligature, and reperfusion was achieved by releasing the tension.

The left coronary artery was occluded for 7 min and then reperfused for a further 10 min before the experiment was terminated. These durations of I/R and number of rats were previously used in the same experimental model successfully and it was reported that

this protocol produced arrhythmic activity in optimum number and severity [7]. ECG, BP and heart rate (HR) were monitored before and during ischemia and 7 min after reperfusion period. Ventricular fibrillation and discharge criteria were assessed according to the diagnostic criteria advocated in the Lambeth conventions [15]. At the end of the experiments, blood samples were collected from the vena cava inferior, centrifuged for 10 min at 3000 r.p.m., and stored at -20°C until biochemical analysis.

Biochemical assays

All serum cardiac markers were measured with autoanalyzers. Total CK, CK-MB and AST were determined with Olympus System Reagent (OSR6179, OSR6153 and OSR6109, respectively; Ireland). cTnT concentration was measured with a Boehringer Mannheim analyzer (Germany). cTnI was quantitatively determined with a Dianostic Products Corporation analyzer (LKT11; England). Myoglobin was determined with a Roche analyzer (20753548, USA).

Statistical analysis

Differences in the incidence of death from irreversible VF were analyzed by Fisher's exact test. Statistical analyses for biochemical parameters and total VF (reversible + irreversible) were carried out by analysis of variance (ANOVA) followed by appropriate *post hoc* tests including multiple comparison tests (LSD). Data were expressed as means \pm standard error (SE). All analyses were made using the SPSS statistical software package and probability value of less than 0.05 was accepted as statistically significant.

Results

After left coronary artery occlusion, all rats exhibited cardiac arrhythmias, such as ventricular ectopic beats, ventricular tachycardia or VF. But, we evaluated only total VF and death from irreversible VF. During the ischemic period, VF and mortality were not observed in any of the rats. In the sham group, no rat exhibit VF during the experiments.

Seven of 18 (38.9%) control rats died due to reperfusion induced irreversible VF ($P < 0.05$). Captopril (5.6%) significantly reduced the incidence of irreversible VF ($P < 0.05$), where as losartan (33.3%) did not affect the incidence of irreversible VF. Besides, captopril significantly reduced incidence of total VF ($P < 0.05$) but losartan did not (Table 1, Figure 1).

Levels of cTnT, cTnI and myoglobin are shown in Table 2, Figure 2 and Figure 3. In all the I/R groups, cTnT and myoglobin values were significantly higher than the sham group ($P < 0.05$). In addition, cTnT and cTnI values of captopril group were significantly higher than the control group at 10 min of reperfusion ($P < 0.05$).

Total CK, CK-MB and AST values are shown in Table 3 and Figure 4. Total CK and CK-MB values of all I/R groups were significantly higher than that of sham group ($P < 0.05$), while AST values was higher than the sham group in only the captopril group. However, there were no significant differences between the I/R groups.

Discussion

Although ACE inhibitors and/or AT1 receptor antagonist can protect the myocardium against I/R injury, the exact mechanisms of the effect have not yet been characterized at the cellular level. Several investigators,

Table 1. Summary of total and irreversible ventricular fibrillation (VF) incidences. Ischemia/reperfusion (I/R)

Groups	Total VF incidence (%)		Irreversible VF incidence (%)	
	Ischemia	Reperfusion	Ischemia	Reperfusion
Control (I/R)	0	14/18 (77.8)	0	7/18 (38.9)
Captopril + I/R	0	7/18 (38.9) * #	0	1/18 (5.6) *
Losartan+ I/R	0	13/18 (72.2)	0	6/18 (33.3)

* $P < 0.05$ significantly different from control group,

$P < 0.05$ significantly different from losartan group.

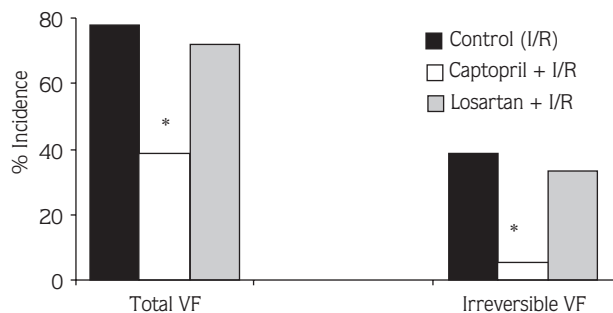


Figure 1. Percentage incidence of total and irreversible ventricular fibrillation (VF) in the reperfusion periods. *P < 0.05, significantly different from control group.

using different ACE inhibitors, have demonstrated a reduction in both incidence and mean duration of malignant arrhythmias in a variety of different experimental models (7,14). In hearts impaired by ischemia, the renin-angiotensin and kallikrein-kinin systems are activated, resulting in increased release of angiotensin II and kinins, especially bradykinin (16). ACE

inhibitors inhibit the accumulation of angiotensin II and accumulate bradykinin in the myocardium. Consequently, antiarrhythmic effects of ACE inhibitor are attributed, among other causes, to inhibition of angiotensin II synthesis and preservation of bradykinin (17,18). Kinins are known to be a potent stimulator of the release of prostaglandins and NO from the endothelium (19,20). So, bradykinin may protect the myocardium by its vasodilator effect. Akula et al (21) demonstrated that the cardioprotective role of bradykinin during experimental myocardial infarction involves prostaglandins and nitric oxide pathways. On the other hand, Kawabata et al (22) was claimed that beneficial effects of ACE inhibitor and/or AT1 receptor antagonist on myocardial I/R were not dependent on NO synthesis. Divisova et al (23) showed that long-term captopril treatment increased the energy potential and had a beneficial effect on tolerance of the isolated heart to ischemia. L-arginine added into the perfusate potentiates the effect of captopril on the NO signaling pathway. Besides, NO was demonstrated to exhibit an inhibitory effect on neutrophils which play an

Table 2. 10th min of reperfusion serum cardiac troponin T, I and myoglobin levels in groups. Values mean ± S.E.M, ischemia / reperfusion (I/R)

Groups	Troponin T (ng/mL)	Troponin I (ng/mL)	Myoglobin (ng/mL)
Sham	0.96 ± 0.08 ^b	0.23 ± 0.01	24.88 ± 2.99 ^b
Control (I/R)	4.26 ± 0.58 ^a	0.32 ± 0.03	44.00 ± 2.08 ^a
Captopril + I/R	8.12 ± 1.38 ^{a,b}	0.70 ± 0.16 ^{a,b}	55.44 ± 6.57 ^a
Losartan + I/R	3.85 ± 0.48 ^a	0.32 ± 0.02	43.67 ± 2.42 ^a

a: P < 0.05 significantly different from sham group, b: P < 0.05 significantly different from control group.

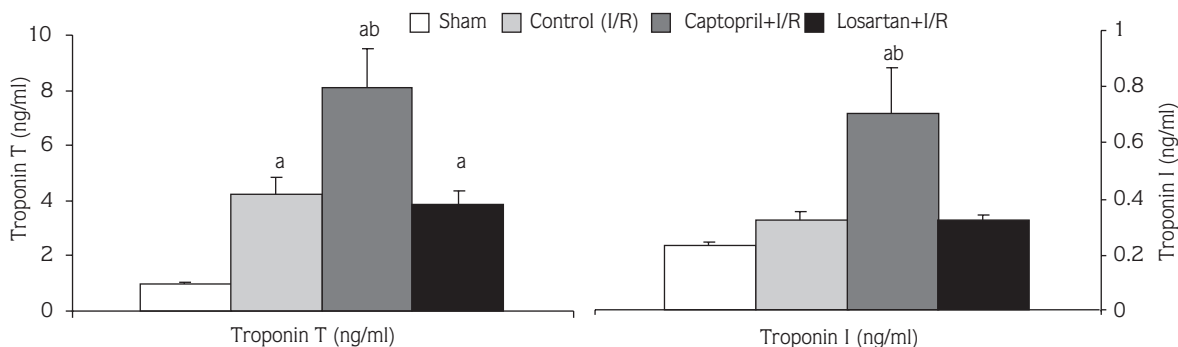


Figure 2. Serum cardiac specific troponin T and I concentrations after ischemia-reperfusion (I/R) procedure in rats. Mean ± S.E.M; a: P < 0.05 significantly different from sham group; b: P < 0.05 significantly different from control group.

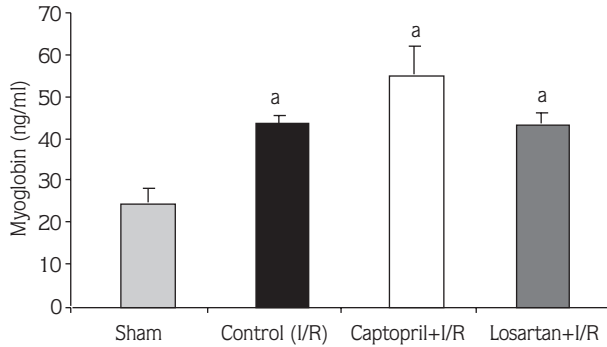


Figure 3. Serum myoglobin concentration after ischemia-reperfusion (I/R) procedure in rats. Mean \pm S.E.M; a: $P < 0.05$ significantly different from sham group.

important role in myocardial I/R injury and/or platelet aggregation. In addition, NO release would potentiate the scavenging of superoxide which might contribute to the myocardial protective effects of ACE inhibitors. The ischemic situation of the myocardium may accelerate

oxygen radical formation, especially during reperfusion, i.e. during reoxygenation, when the oxygen tension in the cell increases. Under circumstances where NO synthesis is enhanced within physiological limits, i.e. in captopril treated rats, with sufficient substrate for NOS by the addition of L-arginine during ischemia, the content of radical forms of mitochondrial respiratory chain compounds decreases, thus reducing the risk of generation of toxic oxygen radicals (24).

Consistent with our findings, Ozer et al (11) indicated that captopril more effectively prevents mortality associated with irreversible VF. On the contrary, Butz et al (25) claimed that losartan but not enalaprilate exerts acute anti-arrhythmic effects during reperfusion. The cardioprotective effect of AT1 antagonists during I/R induced injury has been controversial. Harada et al (26) found that both genetic deletion of the AT1a gene and treatment with the AT1 antagonist CV-11974 significantly attenuate reperfusion arrhythmias.

Table 3. 10th min of reperfusion serum creatine kinase (CK), MB isoenzyme of creatine kinase (CK-MB) and aspartate amino transferase (AST) levels. Values mean \pm S.E.M, ischemia/reperfusion (I/R)

Groups	CK (U/L)	CK-MB (U/L)	AST (U/L)
Sham	1136.00 \pm 23.30 ^b	828.62 \pm 30.25 ^b	210.86 \pm 8.01
Control (I/R)	1321.87 \pm 36.78 ^a	1097.75 \pm 42.23 ^a	277.75 \pm 15.69
Captopril \pm I/R	1353.44 \pm 61.23 ^a	1112.78 \pm 50.20 ^a	329.44 \pm 29.44 ^a
Losartan \pm I/R	1311.33 \pm 20.28 ^a	1104.72 \pm 30.84 ^a	266.78 \pm 17.40

a; $P < 0.05$ significantly different from sham group,

b; $P < 0.05$ significantly different from control group.

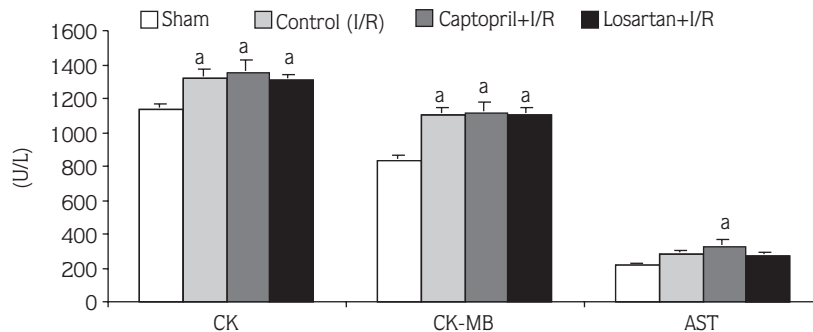


Figure 4. Serum creatine kinase (CK), the MB isoenzyme of creatine kinase (CK-MB) and the aspartat amino transferase (AST) levels after ischemia-reperfusion (I/R) procedure in rats. Mean \pm S.E.M; a: $P < 0.05$ significantly different from sham group.

Reperfusion induced arrhythmias are postulated to be associated with major alterations in intracellular Ca^{2+} levels (27). However, the underlying mechanism of calcium overload remains unknown. Angiotensin II not only increases Ca^{2+} influx through the L-type Ca^{2+} channel but also induces Ca^{2+} release from intracellular stores through AT1 (28). Accordingly, Ang II may play a major role in calcium overload during reperfusion which is closely related to the incidence of I/R arrhythmias (26). In addition, Ang II is known to induce the release of catecholamine, and catecholamine induces arrhythmia. These mechanisms may also contribute to the beneficial effects of ACE inhibitors on I/R induced irreversible VF.

Reperfusion of ischemic myocardium also accelerates the washout of intracellular proteins "serum cardiac markers", producing an exaggerated and early peak value of substances such as CK-MB and cTnT and cTnI (3,4). Serum cardiac markers are performed to confirm the myocardial injury seen in ECG and history. CK and AST have proved to be too unselective for the detection of myocardial injury at an early stage in rat. Although CK-MB is more specific for myocardium than total CK, small quantities of CK-MB isoenzyme are found in tissues other than the heart. Myoglobin is the earliest serum marker in the myocardial infarction but it has no cardiac specificity. Moreover, high serum levels of myoglobin should be supplemented by a more cardiac specific marker (1,29). cTnT and cTnI have been shown to be highly sensitive and specific markers of myocardial cell injury (4,29-32). Cardiac troponins are not detected in the peripheral circulation under normal circumstances. In patients with AMI, cardiac troponins increase after myoglobin and before the other markers of myocardial injury (1).

Recently, the utility of serum markers of cardiac damage has improved dramatically. Fredericks et al (33) confirmed that cTnT and cTnI are cardiac specific markers of myocardial damage in certain common laboratory animals such as rat, dog, pig and monkey. Vorderwinkler et al (34) demonstrated that cTnT and cTnI levels reach peak values in the rat heart perfusate at the 10th min of the reoxygenation period. In this study, myoglobin and cardiac troponin levels were significantly increased after captopril administration in comparison with the control group, while losartan administration had no effect. However, captopril reduced mortality due to the irreversible VF. The contrary finding may arise from the fact that ACE inhibition increases kinin levels in ischemia/reperfused myocardium. Thus, bradykinin induced vasodilatation increases reperfused myocardium and accelerates the washout of serum cardiac markers.

In conclusion; after short time cardiac I/R, a ACE inhibitor captopril reduced VF incidence and mortality due to the irreversible VF, whereas, AT1 receptor blocker losartan had no effect on VF incidence and mortality. In addition, captopril may increase serum cardiac markers in the early reperfusion period.

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