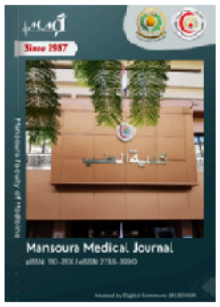
MANSOURA MEDICAL JOURNAL

ISSN - Print: 1110-211X - Online: 2735-3990

journal homepage: mmj.mans.edu.eg



Volume 35 | Issue 1 Article 7

ROLE OF VERAPAMIL (CALCIUM CHANNEL BLOCKER) AND LISINOPRIL (ANGIOTENSIN CONVERTING ENZYME INHIBITOR) ON MYOCARDIAL TOLERANCE TO ACUTE ISCHAEMIA

Hanaa Serougy
Department of Physiology, Faculty of Medicine, Mansoura University

Gad Abd Aziz
Department of Physiology, Faculty of Medicine, Mansoura University

Soheir Bassiony
Department of Physiology, Faculty of Medicine, Mansoura University

Sabry Gad
Department of Physiology, Faculty of Medicine, Mansoura University

Ahmed Gendy
Department of Physiology, Faculty of Medicine, Mansoura University

ROLE OF VERAPAMIL (CALCIUM CHANNEL BLOCKER) AND LISINOPRIL (ANGIOTENSIN CONVERTING ENZYME INHIBITOR) ON MYOCARDIAL TOLERANCE TO ACUTE ISCHAEMIA

By

Hanaa G. Serougy, Gad El. Abd Aziz, Soheir F. Bassiony, Sabry M. Gad and Ahmed A. Gendy

From

Department of Physiology, Faculty of Medicine, Mansoura University

ABSTRACT

This work aims to clarify the role of calcium channel blocker (verapamil) and angiotensin-converting enzyme inhibitor (lisinopril) on myocardial ischaemia reperfusion injury. The study was done using isolated rabbits hearts and Langendroffs apparatus for recording myocardial contractility, heart rate and coronary flow, also glucose uptake by coronary slices was estimated by glucose enzymatic kit. The work included 4 groups; Group A to study effect of ischaemia and reperfusion on mentioned parameters, Group B to study effect of verapamil and lisinopril on tested parameters, Group C to study the effect of 5 minutes preischaemia administration of verapamil and lisinopril on tested parameters and Group D to study the effects of administration of verapamil and lisinopril with reperfusion on parameters mentioned before. Results concluded that global ischaemia decreased the myocardial contractility and heart rate but increases glucose uptake, verapamil is a potent drug used to decrease myocardial contractility, heart rate and increase coronary flow in ischaemic hearts. Lisinopril is a drug of choice to improve contractility and heart rate when administered preischaemic or with reperfusion. Verapamil is a drug of choice to improve coronary flow when administered pre-

ischaemic or with reperfusion. There is no preference between tthe 2 drugs when given preischaemic on increasing glucose uptake. So calcium, angiotensin and O2 free radicals are important mediators of ischaemic reperfusion injury as their modulation by the verapamil and lisinopril significantly improve ischaemia reperfusion induced changes in myocardial contractility, heart rate, coronary flow and glucose uptake.

INTRODUCTION

Acute myocardial ischaemia initiates a complex series of metabolic changes that lead to structural changes in myofibrils which are associated with depletion of high energy nucleotides and the onset of cell death (34), Zaloga, 1992 (66) claimed that sarcolemmal disruption has been implicated due to disturbance in calcium metabolism since calcium is an essential ion for union of actin and myosin. The depletion of ATP, alteration of ATPase system and delayed calcium pump account for intracellular calcium ions overload (66). However, the alteration in myocardial calcium metabolism can be modified by the use of calcium channel blockers (39).

In myocardial ischaemia, both the circulating renin-angiotensin system (RAS) and local tissue ACE (angiotensin converting enzyme) can be activated (14), suppose that ACE inhibitors decrease the frequency of ischaemic events and improve the outcome of ischaemia-reperfusion injury.

In this work, we try to clarify the effect of calcium channel blocker (verapamil) and ACE inhibitor (lisinopril) as a suggestive line for protection against myocardial injury.

MATERIALS AND METHODS

The present work was designed to study the effect of calcium channel blocker (verapamil) and angiotensin converting enzyme inhibitor (lisinopril) on myocardial ischaemia-reperfusion injury.

** Experimental Plan :*

Group (A) non ischaemic control group, included 14 rabbit's hearts divided into 2 subgroups (A1 & A2) each included 7 rabbit's hearts to study the effect of calcium channel blocker (Verapamil) (A1), angiotensin-converting enzyme inhibitor (Lisin-

opril) (A2) on heart rate (beat/ minutes), myocardial contractility (cm) and coronary blood flow (ml/min) after 5 minutes of administration of the drug. Measurement of glucose uptake was also recorded after perfusion of myocardial slices with the drug.

Group (B), included 7 rabbit's hearts to study the effects of ischaemia and reperfusion on the tested parameters (myocardial contractility, heart rate and coronary blood flow) before ischaemia (preischaemia), 5 minutes after ischaemia and 5, 15 minutes after reperfusion. The duration between induction of ischaemia and stoppage of the heart was also recorded. The global ischaemia was induced by stopping delivery of the perfusion fluid mechanically by clamping the rubber tube which leads to cannula for 30 minutes, then the coronary artery perfusion was reinitiated for another 30 minutes. The glucose uptake by myocardial slices was also estimated 5, 15 minutes of ischaemia and 5, 15 minutes of reperfusion.

Group (C), included 14 rabbit's hearts subdivided into 2 subgroups (C1 & C2) each include 7 rabbit's hearts to study the effect of 5 minutes preischaemic administration of the reported drugs on the tested parameters during ischaemia and reperfusion. Also, the duration till stoppage of the ischaemic heart was recorded.

Group (D), included 14 rabbit's hearts subdivided into 2 subgroups (D1 & D2) each included 7 rabbit's hearts to study the effects of administration of the tested drugs with reperfusion on the tested parameters of ischaemia and reperfusion.

** Animal preparation :*

Experiments were done on healthy rabbits of both sexes, each weighing about 1.5-2 kilograms. These animals were allowed free access of food and water. The rabbit was sacrificed; its chest wall was opened. The heart was dissected and the fat and connective tissue wore removed. The heart was excised along with an attached length of at least 1 cm aorta and then rapidly removed and placed in dish containing mammalian Ringer-Locke's solution which has the following composition in gm/L (sodium chloride 9.0 potassium chloride 0.42, cal-

cium chloride 0.24, sodium bicarbonate 0.5 and dextrose 1.0).The temperature was adjusted at 37oC and pH at 7.4. The heart was then gently squeezed several times so as to expel as much blood as possible. The aorta was dissected free and cut just below the point where it divides. The heart was then transferred to the Lagendroff perfusion apparatus where the aorta was cannulated to 5 ml cannula. The cannula was then connected to a reservoir delivery oxygenated mammalian Ringer-Locke's solution at a thermostatically constant temperature (37°C). A hook was then passed through the verntricular wall and was attached to a lever for recording (a) ventricular (myocardial) contractions (cm) and (b) heart rate (beats/minute), on a slowly moving smoked drum. Reperfusion of the isolated rabbit's heart was done at a constant flow rate of 11-17 ml/minute. With gentle tension, pull the heart slightly to one side. The fluid leaving the heart now follows the threads and is easily collected in a measuring cylinder to estimate coronary blood flow (The heart was allowed to equilibrate for 15-30 minutes [8].

** Drugs used :*

1- Verapamil (Isoptin): It is supplied in the form of ampoules (5 mg/2 ml). This calcium channel blocker is used in a concentration of (0.18 mg/ml) [44].

2- Lisinopril: It is supplied in the form of white powder by Sedico company (A.R.E.).It was dissolved in distilled water immediately prior to its use. This angiotensin-converting enzyme inhibitor was used in a concentration of 2.33 ug/ml [25].

STATISTICAL METHODS

Data were processed and statistics were carried out using the "t" test for unpaired comparison and "t" paired test for paired comparison.

** Limits of significance (3) :*

- Non-significant (N.S.) at $P > 0.05$.
- Significant (*) at $P < 0.05$.
- Highly significant: at $P < 0.01$.
- Very highly significant: at $P < 0.001$.

RESULTS

The effect of ischaemia and reperfusion on myocardial contractility, heart rate, coronary flow and glucose uptake :

From table (1) and fig. (1) it can be

observed that there was a significant decrease in the height of myocardial contractility at 5 minutes ischaemia (84.91%), 5 minutes reperfusion (84.9) and 15 minutes reperfusion (27%). It has to be noted that there was no significant change between the heights of myocardial contractility at 5 and 15 minutes of reperfusion.

There was a significant decrease of heart rate at 5 minutes ischaemia (73.3%), 5 minutes reperfusion (20.9%) and 15 minutes reperfusion (15%). Also it has to be noted that there was insignificant change in heart rate between 5 & 15 minutes of reperfusion.

There was insignificant decrease of coronary flow at 5 min reperfusion (16%), there was also a decrease in coronary flow at 15 min reperfusion (24.3%). A significant increase in glucose uptake at 5 minutes ischaemia (100.8%) and 5 minutes reperfusion (35.8%). There was also a significant decrease in glucose uptake at 15 min reperfusion (34.2%).

The effect of Verapamil and Lisinopril on myocardial contractility, heart rate, coronary flow and glucose uptake in non ischaemic control group :

From table (2) it was observed that Verapamil caused significant decrease (65.1%) in the height of myocardial contractility and significant decrease (49.9%) in heart rate while significant increase (152.3%) in coronary flow. There was insignificant decrease (10.37%) in glucose uptake.

From table (3) it was observed that lisinopril caused insignificant decrease (24.5%) of the height of myocardial contraction and decrease (2.3%) of heart rate while significant increase (87.1%) of coronary flow and a significant increase (120.7%) of glucose uptake.

The effect of 5 minutes preischaemic administration of the drugs on the tested parameters during ischaemia and reperfusion :

Table (2) and figure (2) showed that the preischaemic administration of verapamil caused a significant decrease (88.5%) in the height of myocardial contractility at 5 min ischaemia, 15 min reperfusion(16.8%) while insignificant decrease (28.4%) at 5 min reperfusion.

The preischaemic administration of verapamil caused significant decrease in heart rate at 5 min ischaemia (85.5%) and at 5 min reperfusion (60.7%) and 15 min reperfusion (28.4%) as compared with control group.

There was significant increase of coronary flow at 5 min reperfusion (62.5%) and at 15 min reperfusion (119.9%) from control values. Verapamil caused significant increase (144.9%) in glucose uptake.

The preischaemic administration of lisinopril (fig. 3) caused non-significant decrease (39%) in the height of myocardial contractility at 5 minutes ischaemia from control value, while a significant increase in height of myocardial contractility at 5 minutes (20.5%) and 15 minutes (65.5%) reperfusion. A significant increase in height of myocardial contractility at 15 minutes reperfusion as compared with 5 minutes reperfusion was observed.

A significant decrease in heart rate at 5 minutes ischaemia (63.8%) 5 minutes reperfusion(17.1%) and 15 minutes reperfusion(11.5%). An insignificant increase in heart rate at 15 minutes reperfusion as compared with 5 minutes reperfusion was recorded.

There was non-significant increase in coronary flow at 5 minutes reperfusion (25.1%) from control value. Also, a significant increase (59.7%) in coronary flow at 15 minutes reperfusion.

Lisinopril caused an insignificant increase in glucose uptake at 5 minutes ischaemia (8.3± 0.9) as compared with 5 minutes ischaemia without Lisinopril (7.4±0.6 mg/gm tissue/ hour).

The effect of the Verapamil and Lisnopril administered during early reperfusion on the tested parameters :

From table (4) and figures (4 & 5) it can be observed that a significant decrease in myocardial contractility at 5 minute ischaemia (84.3%) and 5 minutes reperfusion (76.7%) as compared with control group while an insignificant decrease in height of myocardial contractility at 15 minutes

reperfusion (53.5%) during early reperfusion with verapamil.

There was a significant decrease in heart rate at 5 minutes ischaemia (71.7%), 5& 15 minutes reperfusion with verapamil (78.9% and 83% respectively) as compared with control group.

A non-significant increase (16.8%) of coronary flow at 5 minutes reperfusion as compared with control group. However, there was a significant increase (66.4%) of coronary flow at 15 minutes reperfusion. There was insignificant decrease (78.9% and 12.8%) in glucose uptake at 5 and 15 minutes reperfusion when verapamil administered with reperfusion as compared with control group without verapamil at 5 and 15 minutes reperfusion.

From table (5) there was significant decrease (84.9%) in height of myocardial contractility at 5 minutes ischaemia as compared with control. However, non-significant decrease in height of myocardial contractility at 5minutes (7.0%) and 15 minutes (2.0%) reperfusion with lisinopril. 5 minutes reperfusion with lisinopril caused significant decrease (73.6%) in heart rate as compared with control group. Also A significant decrease (28.2%) in heart rate at 15 minutes reperfusion with lisinopril as compared with control one was observed. There was non-significant increase (12.0%) in coronary flow at 5 minutes reperfusion with lisinopril as compared with control group while there was a significant increase (29.3%) in coronary flow at 15 minutes reperfusion with lisinopril. There was significant increase (27.7%) in glucose uptake at 5 minutes reperfusion with lisinopril as compared with 5 minutes reperfusion without lisinopril. While there was significant increase (69.1%) in glucose uptake at 15 minutes reperfusion with lisinopril as compared with 15 minutes reperfusion without lisinopril.

Table (1). Effect of Ischaemia and Reperfusion on Myocardial Contractility, Heart Rate, Coronary Flow, and Myocardial Glucose Uptake.

	Control	Ischaemia	Reperfusion	
		5 minutes	5 min	15 min
Myocardial contractility	3.4±0.2	0.52±0.0 ↓84.9%	2.2±0.3 ↓36.3±	2.52±0.3 ↓27.0
Heart Rate	148.9±6.2	39.7±5.8 ↓73.3%	117.7±6.9 ↓20.9%	126.6±1.6 ↓15.0%
Coronary Flow	7.1±1.2	-	5.9±1.3 ↓16%	5.4±0.8 ↓24.3±
Myocardial Glucose Uptake	3.6±0.4	7.5±0.6 ↑100.8%	5.0±0.6 ↑35.8%	2.4±0.5 ↓34.2%

Fig.(1): Effect of Ischaemia and Reperfusion on Myocardial Contractility and Heart Rate:

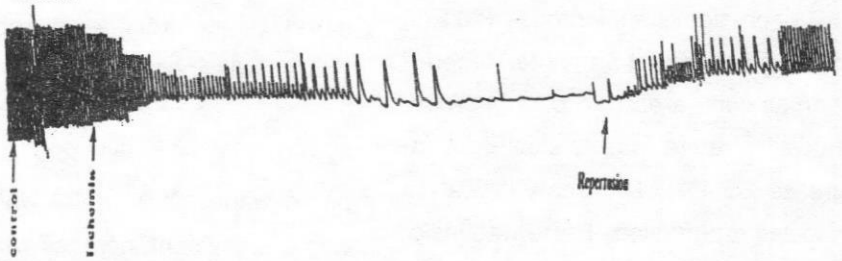


Table (2). Effect of Preischaemic Administration of Verapamil on Myocardial Contractility, Heart Rate, Coronary Flow, and Myocardial Glucose Uptake.

	Control	Preischaemic	Ischaemia	Reperfusion	
			5 minutes	5 min	15 min
Myocardial Contractility	2.6±0.7	0.9±0.2 ↓65.1%	0.3±0.1 ↓88.5%	1.9±0.3 ↓28.4±	2.2±0.4 ↓16.8%
Heart Rate	143.3±13.5	71.6±7.0 ↓49.9V	20.7±4.7 ↓85.5%	56.3±4.3 ↓60.7%	102.0±12.1 ↓28.5%
Coronary Flow	9.2±0.9	23.2±3.3 ↑152.3%	-	14.9±1.3 ↑62.5%	20.2±2.1 ↑119.9%

	Control	Ischaemia	Ischaemia with verapamil
Myocardial Glucose Uptake	3.5±0.5	7.9±0.6 ↑124.3%	7.5±0.7 ↑144.9%

Fig. (2): Effect of Preischaemic Administration of Verapamil on Myocardial Contractility and Heart Rate:

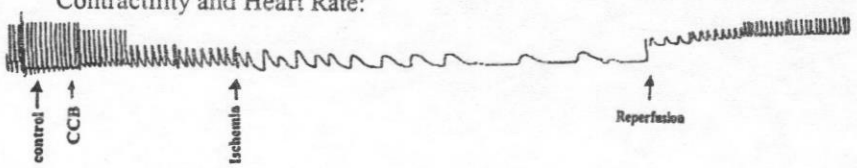


Table (3). Effect of Preischaemic Administration of Lisinopril on Myocardial Contractility, Heart Rate, Coronary Flow, and Myocardial Glucose Uptake.

	Control	Preischaemic	Ischaemia	Reperfusion	
			5 minutes	5 min	15min
Myocardial Contractility	2.0±0.7	1.5±0.6 ↓24.5%	1.2±0.2 ↓39.0%	2.4±0.3 ↑20.5%	3.3±0.5 ↑65.5%
Heart Rate	140.0±9.8	136.7±8.7 ↓2.3%	50.6±5.8 ↓63.8±	116.0±3.6 ↓17.1±	123.9±8.9 ↓11.5%
Coronary Flow	8.0±1.0	14.2±1.8 ↑78.1±	-	10.0±1.2 ↑25.1%	12.7±1.6 ↑59.7%

	Control	Ischaemia	Ischaemia with Lisinopril
Myocardial Glucose Uptake	3.5±0.5	7.4±0.6 ↑120.7%	8.3±0.9 ↑153.0%

Fig. (3): Effect of Preischaemic Administration of Lisinopril on Myocardial Contractility and Heart Rate:

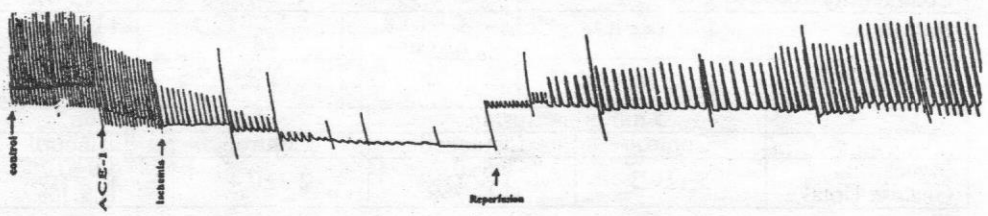


Table (4). Effect of Verapamil Administration with Reperfusion on Myocardial Contractility, Heart Rate, Coronary Flow, and Myocardial Glucose Uptake.

	Control	Ischaemia	Reperfusion	
		5 minutes	5 min	15min
Myocar2.9±0.3dial Contractility	2.9±0.3	0.5±0.1 ↓84.3%	0.7±0.1 ↓76.7%	1.3±0.2 ↓53.5%
144.0±2.3	144.0±2.3	40.7±5.4 ↓71.7%	30.4±3.3 ↓78.9%	24.4±2.9 ↓83.0%
Coronary Flow	7.4±1.4	-	8.6±1.5 ↑16.8%	12.2±2.0 ↑66.4%

	5 min Reperfusion		15 min Reperfusion	
	Control	Verapamil	Control	Verapamil
Myocardial Glucose Uptake	4.9±0.6	4.7±0.6 ↓78.9%	2.1±0.4	1.9±0.4 ↓12.8%

Fig. (4): Effect of Verapamil Administered with Reperfusion on Myocardial Contractility and Heart Rate.

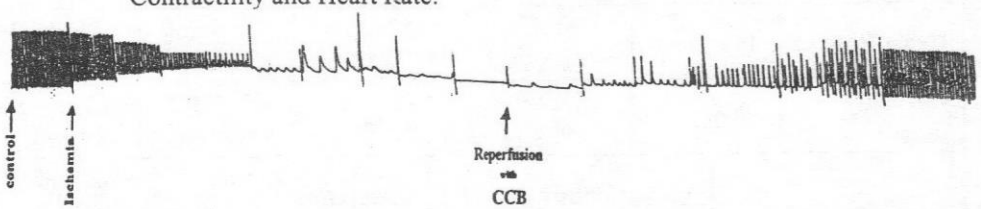


Table (5). Effect of Lisinopril Administrated with Reperfusion on Myocardial Contractility, Heart Rate, Coronary Flow, and Myocardial Glucose Uptake.

	Control	Ischaemia	Reperfusion	
		5 minutes	5 min	15min
Myocardial Contractility	3.6±0.4	0.5±0.1 ↓84.9%	3.3±0.4 ↓7.0%	3.5±0.4 ↓2.0%
Heart Rate	155.0±8.4	40.9±4.8 ↓73.6%	81.3±9.1 ↓47.6%	111.3±9.8 ↓28.2%
Coronary Flow	8.0±1.2	-	9.8±1.4 ↑12.0%	10.3±1.3 ↑29.3%

	5 min Reperfusion		15 min Reperfusion	
	Control	Lisinopril	Control	Lisinopril
Myocardial Glucose Uptake	5.1±0.3	6.5±0.7 ↑27.7%	2.3±0.4	3.9±0.7 ↑69.1%

Fig. (5): Effect of Lisinopril Administered with Reperfusion on Myocardial Contractility and Heart Rate.

DISCUSSION

The ischaemic myocardium of different aetiology is characterized by a reduced availability of oxygen and substrates for metabolism with accumulation of end products [28]. Reperfusion usually precipitates more injury to myocardium and acceleration of cell damage caused by the preceding ischaemic episodes [58].

Our research aimed to study the role of calcium ions and angiotensin-II on myocardial tolerance to acute ischaemia. The study comprised four groups to evaluate the effect of ischaemia and reperfusion on myocardial contractility, heart rate, coronary flow and glucose uptake by myocardial muscle, also to evaluate the effect of calcium channel blockers (verapamil) and the effect of angiotensin converting enzyme inhibitor (lisinopril) administered preischaemic and during reperfusion on the same tested parameters.

The results of the present work regarding the effects of ischaemia and reperfusion, showed deleterious effects on myocardial contractility and heart rate during ischaemia and reperfusion can be explained by decrease intra-cellular ATP and calcium overload: the rapid transition from aerobic to anaerobic metabolism causing an accumulation of protons, lactate and inorganic phosphate [31], depletion of ATP and phosphocreatine (PC) [64], loss of potassium ions to extra cellular fluids, retention of Na+ and depletion of glycogen [2]. The accumulation of Ca^{2+} is due to impaired pump activity of sarcoplasmic reticulum [54]. This Ca^{2+} overload is considered to be a primary contributor to ischaemic reperfusion injury [43]. Alterations in calcium homeostasis may be responsible for myocardial dysfunction [30], arrhythmias resulting from after depolarization [7], uncoupling of mitochondrial oxidative phosphorylation [18], activation of enzymes such as Ca - ATPase of sarcoplasmic reticulum exhausting ATP stores, xanthine oxidase producing super-oxide radicals, modulation of Ca-sensitive nitric oxide synthase activity and phospholipases involved in membrane degradation. Also, the Ca^{2+} overload leads to inhibition of calcium release channels in the sarcolemma and sarcoplasmic reticulum by calcium - calmodulin [31], inhibition

of ATP synthesis and irreversible mitochondrial damage [40] and furthermore, Ca^{2+} overload leads to phosphorylation of cardiac troponin inhibitory subunit (troponin-I) and tropomyosin binding subunit (troponin-T) by cardiac phospholipids sensitive Ca^{2+} dependent protein kinase [29].

Also our results showed decreased coronary flow during reperfusion which may be due to increased secretion of angiotensin-II and endothelin during the period of ischaemia leading to coronary vasoconstriction [20]. Increased oxygen free radicals with decrease nitric oxide - dependent smooth muscle relaxation contributes also to the vasoconstrictor effect of ischaemia and reperfusion on coronary arteries [6].

As regards, the effect of ischaemia and reperfusion on glucose uptake by myocardium, the increased uptake during ischaemia could be explained by increase rate of glycolysis during period of ischaemia and 5 minute reperfusion due to rapid shift of metabolism towards anaerobic side and increase expression of GLUT4 transporters [4]. While the decreased rate of glucose uptake during 15 minutes reperfusion was due to inhibition of glycolysis by the more increased levels of Ca^{2+} and free radicals [55] which exert deleterious effects on enzymes responsible for glycolytic pathway [22].

In the present work, we study the role of calcium in ischaemia and reperfusion by using the calcium channel blocker (verapamil). There was significant decrease (65%) in myocardial contractility which is attributed to its direct effect on voltage dependent Ca^{2+} channels, decrease movement of Ca^{2+} in slow Ca^{2+} channels inside myocytes [23], also it blocks Na+ channels [10].It was noted that preischaemic administration of verapamil leads to functional recovery of contractility at 5 & 15 minutes reperfusion as detected by non-significant decrease of contractility (28 & 17% respectively). This was attributed to its protective effect on ATP stores by a reduced ATP use due to lowering of intracellular calcium levels [32], inhibiting generation of protons which decrease ATP hydrolysis in acute ischaemia [15]. Moreover, it decreases injury and myocardial cell necrosis so

considered a contributing factor to protect myocardium against injurious effect of increased Ca^{2+} (1). Verapamil also caused significant decrease in the heart rate; this is attributed to its direct negative chronotropic effect (7). The rise in extra cellular potassium and slowed conduction through ischaemic tissue have been implicated as an important arrhythmogenic factors (52). The ability of verapamil to slow the rise in potassium, by decreasing events of injury, keeping integrity of cell membranes and slowing the development intracellular acidosis provided an antiarrhythmic effect (45).

However Horton, 1980 (26) believed that the ventricular arrhythmias resulted from calcium channel blockers may be due to blocking effect on potassium channels prolonging plateau phase of action potential and causing instability of resting membrane potential of ventricular muscle leading to generation of after depolarization. It was also observed that the calcium channel blocker (verapamil) is significantly more effective in inhibiting contraction of coronary vessels with increasing coronary flow (200%) than inhibiting myocardial contractility (65%). This is because the contraction of vascular smooth muscle, such as that found in the coronary arteries, is slightly different from the contraction of cardiac and skeletal muscles. Myosin must be phosphorylated and calmodulinis regulatory protein to which calcium binds (40). In addition, vascular smooth muscle cells have significantly less intra cellular calcium stores than do so rely more heavily on the influx of extra cellular calcium (56). Verapamil, in addition to reducing the entry of calcium into the cells and therefore inhibiting vascular wall contraction (12), it facilitates effects of endothelial derived relaxing factor (EDRF) and prevents effects of endothelial derived vasoconstrictor substances (24). It is noted in the present work that the preischaemic administration of verapamil is more effective than verapamil administered with reperfusion on improving coronary flow i.e. verapamil inhibits the occurrence of the deleterious effects of ischaemia rather than decreasing them. These effects include decrease nitric oxide; increase Ca^{2+} level, increase free radicals, increase endothelins and increased angiotensin II.

Verapamil exerts non-significant direct effect on glucose uptake by cardiac muscle. However, in vivo studies claimed that calcium channel blockers decrease glucose uptake by myocardial muscle secondary to increase coronary flow rate what is called washing out phenomenon of glucose [38]. The increased glucose uptake (145%) in 5 minutes ischaemia is possibly due to accelerating effect of glycolysis rather than effect of verapamil.

In this study, we examined the role of angiotensin-II (AII) in ischaemia and reperfusion by using the angiotensin-converting enzyme inhibitor (ACE-I), lisinopril, the results showed that lisinopril has direct mild inhibitory effect on myocardial contractility (27%). This direct negative isotropic effect on isolated perfused heart can be explained by inhibition of local cardiac ACE [62], and consequent decrease of A-II which is known to stimulate cardiac contractility [61]. Also, ACE-I exerted this negative inotropic effect by increase the activity of sarcoplasmic reticulum Ca^{2+} ATPase enzyme [57], and can not be explained by a decrease in Ca^{2+} entry through voltage Ca^{2+} channels [49]. On the other hand, ACE-I (lisinopril), when administered preischaemic and during reperfusion, improved the myocardial contractility, this can be attributed to the decrease of the deleterious effects of ischaemia and reperfusion by decrease AII [48], decrease oxygen free radicals, OFRs, increase bradykinin levels [51] and decrease cytosolic Ca^{2+} [16]. So lisinopril can suppress ischaemic injury. Moreover, it is noted that preischaemic administration of lisinopril gives a significant improvement of myocardial contractility than when given with reperfusion indicating the protective effect of lisinopril against ischaemic injury.

Lisinopril also has antiarrhythmic effect, this is in agreement with [37]. And this effect is attributed to decreasing angiotensin-II when is implicated in the genesis of arrhythmia [4], activaiton of bradykinin receptor-2 (BK2) increasing nitric oxide production and prostaglandins which antagonize the effect of AII [13]. Also ACE-I decreased incidence of arrhythmias by decreasing endothelin secretion and action on cardiac myocytes.

Moreover, it decreases production of free radicals [46]. The antiarrhythmic effect of lisinopril was clearly observed when given preischaemic rather than if given with reperfusion.

As regard coronary flow, it was observed that lisinopril improved coronary flow in normal hearts as well as in ischaemic heart when it was administered preischaemic or with reperfusion. This vasodilating effect of lisinopril may be attributed to decreased vasoconstrictor effect of AII, decreased OFRs generation by AII [5] increased level of bradykinin resulting in increase the production of nitric oxide [17], decrease degradation of nitric oxide by OFRs with consequent improvement of endothelial dependent vascular relaxation [50].

ACE inhibitor can also lower vascular OFRs production even insinuations in which angiotensin II is not elevated [68]. This may contribute to the beneficial effect of ACE inhibitors on outcome after myocardial infarction or in heart failure [33]. Inhibition of kinin degradation by the use of ACE inhibitors with increase in nitric oxide formation is important in the control of cardiac O2 consumption. Vasodilatation and control of myocardial O2 consumption may contribute importantly to the therapeutic actions of ACE inhibitors in cardiac diseases [65]. ACE inhibitors can significantly suppress myocardial O2 consumption via stimulation of nitric oxide production from endothelial calls[41].Also ACE-I, by decreasing AII, inhibits the increased myocardialO2 demand by AII (which is due to increased contractility, after load and decreased coronary supply) [11].

The effect of lisinopril on glucose uptake revealed a significant direct increase in glucose uptake as lisinopril promoted more expression of GLUT4 transporter via nitric oxide mediated mechanism and help more glucose uptake [59]. Preischaemic administration of lisinopril or its administration with reperfusion potentiates the increase of glucose uptake. This is because ischaemia also resulted in greater expression of GLUT4 transporter proteins with facilitation of glucose entry and glycolysis [60].

It can be concluded that the global

ischaemia decreased the myocardial contractility and heart rate but increased glucose uptake and these parameters do not return to control levels on reperfusion. Also, Lisinopril proved contractility of myocardium and heart rate but verapamil improved coronary blood flow whether both drugs administered preischaemic or with reperfusion. There is no preference between 2 drugs when given preischaemic on increasing glucose uptake. While lisinopril increased glucose uptake more when administered with reperfusion. Finally, calcium and angiotenin II are important mediators of ischaemic reperfusion injury, as their modulation by the calcium channel blocker (verapamil), angiotensin converting enzyme inhibitor (lisinopril) significantly improved ischaemic-reperfusion induced changes in myocardial contractility, heart rate, coronary flow and glucose uptake.

REFERENCES

(1) **Anand I.S.; Sharma, P.L.; Charravart, R.N.; Wahi, P.L. (1989) :** Experimental myocardial infraction in the US monkeys: verapamil pre-treatment in the reduction of infarct size. Advances in myocardiology; 2: 425-431.

(2) **Anderson T.J.; Overhiser, R.W.; Haber, H.; & Charbonnea F. (2000) :** A comparative study of four antihypertensive agents on endothelial function in patients with coronary disease Am. J. Cell. Cardial.; 31 (suppl.): 327 A.

(3) **Armitage, P. (1974) :** Statistical methods in medical research. Third ed. Blackwell Scientific Publication; 116-120.

(4) **Ball, S.G.; Hall, A.S.; and Murrau, G.D. (1997) :** Angiotensin converting enzyme inhibitors after myocardial infarctions and timing. J. Am. Coll. Cardiol.; 25-65.

(5) **Becker, R.H.A.; Wiemer, G. and Linz, W. (1991) :** Preservation of endothelia function by ramipril rabbit on a long term athrogunic diet.

(6) **Beckman, J.S.T.W.; Beckman, J.; Chen, P.A.; Marshall and Freeman, B.A. (1996)** : Apparent hydroxyl radical for endothelial injury from nitric oxide and super oxide. Proc. Natl. Acad. Sci. USA; 87: 1620-1624.

(7) **Brooks, W.W.; Conrod, C.H. and Morgna, J.P. (1995)** : Reperfusion-induced arrhythmia as following. Ischaemia in intact rat heart: role of intra-cellular calcium. Cardiovasc. Res.; 29-542.

(8) **Burn, J.H. (1952)** : In practical pharmacology. Blackwell-Scientific Publication. Ltd Oxford, p. 37-39.

(9) **Crtee, G.D.; Douen, A.G.; Ranlal, T.; Klip, A.; and Holloszy, J.O. (1997)** : Stimulation of glucose transport in skeletal muscle by hypoxia. J. Appl. Physiol.; 70: 1593-1600.

(10) **Chen, C.M.; and Gettes, L.S. (1981)** : Effects of verapamil on rapid Na+ channel-dependent action potentials of K+-depolarized ventricular fibers. J. Pharmacol. Exp. Ther.; 209: 415 J.P.

(11) **Clugh, O.P.; Collis, M.G.; Conway, J.; Hatton, R. and Keddie, J.R. (1993)** : Interaction of angiotensin converting enzyme inhibitors with function of the sympathetic system. Am. J. Cardiol.; 49: 1410-4.

(12) **Dazau, V.J. and Re, R. (1994)** : Tissue angiotensin system in cardiovascular medicine. Circulation; 89: 493-498.

(13) **De Marco, T.; Daly, P.A.; Lin, M.K.; Kayser, S; Parmley, W.W. and Ghatter Jack, K. (1987)** : Enalapril a new parenteral angiotensin converting enzyme inhibitor: rapid changes is systemic and coronary haemodynamics and human profile.

(14) **Dzau, V.J. (1995)** : Angiotensin converting as a mutimecha-

nite factor in CAD. J. Myocard. Ischaemia; 7: 6-14.

(15) **Ehring, T. (1997) :** Dihydropyridine calcium antagonists beneficial or adverse effects in the setting of myocardial ischaemia reperfusion? Cardiology, Abst.

(16) **Ertl, G.; Kloner, R.A.; Alexander, R.W.; and Braun Wald, E. (1982) :** Limitation of experimental infarct size by an angiotension converting enzyme. Circulation; 65: 40-48.

(17) **Finta, K.M.; Fischer, M.J.; Lee, L.; Gordon, D.; Pitt, B. and Webb, R.C. (2000) :** Ramipril prevents impaired endothelium dependent relaxation in arteries from rabbits fed on atherogenic diet.

(18) **Follette, D.M.; Fey Buckberg, G.D.; Helly, J.J.; Steed, D.L.; Foglia, R.P. and Maleney, J.V.J.R. (1981) :** Reducing postischaemic damage by temporary modification of Perfusate calcium, potassium, PH, and osmolarity. J. Am. Chem. Soc.; 90; 6233.

(19) **Follette, D.M.; Fey, K.; Buckberg, G.D.; Helly, J.J.; Stad, D.L.; Foglia, R.P. and Maleney, J.V.J.R. (1981) :** Reducing postischaemic damage by temporary modification of reperfusate calcium, Potassium, PH, and osmolarity. J. Thorac. Cardiovasc. Surg.; 82: 221-238.

(20) **Francis, G.S. (2000) :** ACE inhibition in cardiovascular disease. N. Engl. J. Med.; 342: 201-202.

(21) **Grosse, P.; Grellet, J.; Benoren, S.; Tariesse, L.; Basse, P. and Dallecchie, M. (1987) :** Effects du perindopril sur L. hypertrophie ventriculaire, gauche, La reserve. Coronaire et les properties mecaniques du muscle papillaire du rat

avec hypettension arterielle revasculaire. Arch., Mal. Coeur.; 6: 905-910.

(22) **Hall, J.I.; Hernandez, L.A.; Henderson, J.; Kellerman, L.A. and Tandey, W.C. (1994) :** Decreased interstitial glucose and transmural gradient in lactate during ischaemia Basic. Res. Cardiol.; 89: 468-486.

(23) **Hamm, C.W. and Opie, L.H. (1989) :** Protection of infracting myocardium by slow channel inhibitors: comparative effects of verapamil-nifedipine and deltiazem in the coronary isolated working rat heart. Circ. Res.; 52: 1-129.

(24) **Hirosumi, J.; Ouchi, Y.; Wantanabe, M.; Kusuoki, J.; Nakamura, T. and Orimo, H. (1997) :** Effect of super oxide and lipid peroxide on cytosolic free calcium concentration in cultured pig aortic endothelial cells. Biochem. Biophys. Res. Commun.; 152: 301.

(25) **HOPE (1993) :** Heart outcomes prevention evolution study investigators. ACE-I in cardiovascular diseases. Lancet; 342: 821-828.

(26) **Horton, R. (1995) :** Spinning the risk and benefits of calcium antagonists. Lancet; 346: 586-587.

(27) **Hoch, C.E.; Riberiro, L.G. and Lefer, A.M. (1985) :** Preservation of ischaemic myocardial by a new converting enzyme inhibitors enalapritic acid, in acute myocardial infarction.

(28) **Jenning, R.B. and Ganote, C. (1976) :** Mitochondrial structure and function in acute myocardial ischaemic injury. Circ. Resp.; 34 (Suppl. I): 1-80.

(29) **Katoh, N.; Wise, B.C. and Kuo, J.F. (1989) :** Phosphorylation of cardiac troponin inhibition submit (troponin-I)

and Tropomyosin-binding submit (tropnin-T) by cardiac phospholipids-sensitive Ca^{2+} dependent protein kinase. Biochem. J.; 209: 189-195.

(30) **Kusuka, H.; Porterfield, J.K.; Wiesman, H.F.; Wiesfeildt, M.L. and Marbon, E. (1987) :** Pathophysiology and pathogenesis of stunned myocardium depressed Ca^{2+}. Activation of contraction as a consequences of reperfusion-induced cellular calcium over-load in ferrel hearts. J. Clin. Invest.; 79: 950-961.

(31) **Kloner, R.A.; Ganote, C.E.; Whalen, D.A. and Jennings, R.S. (1997) :** Effects of transient period of ischaemia on myocardial cells fine structure during the first few minutes of reflow. Am. J. Pathol.; 74: 399-422.

(32) **Lange, R.; Ingwall, J.; Hale, S.L.; Alker, K.J.; Braunwold, E.; and Kloner, R.A. (1984) :** Preservation of high energy phosphates by verapamil in reperfusion myocardium. Circulation; 70: 734-741.

(33) **Laini, R.; Maggioni, A.P Flatheee, M.; Sleight, P. and Tognoni, G. (1995) :** ACE inhibitor use in patient with myocardial infarction. Circulation; 92: 3132-3137.

(34) Lewis, M.S.R.E.; Whatley, P.; Chain, T.M.; Molntyre, S.M.; Presott;

(35) **Lowo, J.E.; Cunnings, R.G.; Adams, D.N. and Mull-Ryde, E.A. (1983) :** Evidence that ischaemic cell death being in the subendotheral flow or wall tension. Circulation; 68: 190-202.

(36) **Marian, A.J.Y.U.Q.T.; Workman, R.; Greve, G. and Roberts, R. (1993) :** Angiotensin-converting-enzyme polymorphism in

hypertrophic cardiomyopathy and sudden cardiac death. Lancet; 342: 1085-1086.

(37) **Muller, C.A.; Opie, L.H.; Peisoch, M.; and Pineda, C.A. (1997) :** Chonicoralpretreatment with the angiotensin-converting enzyme inhibitor tranddapril decreases ventricular fiberillotionin acute ischaemia and reperfusion. Eur. Heart; Is: 988-96.

(38) **Natali, A.; Santoro, D. and Palombo, C. (1991) :** Impaired insulin action on skeletal-muscle metabolism in essential hypertension. Hypertension; 17: 170-170.

(39) **Nakanishi, J.; Soguchi, M.; Tauchiyo, T.; Yasukouichi, S. and Takee, A. (1990) :** Effect of acidosis on intracellular PH and calcium concentration in the newborn and adult rabbit myocardium.

(40) **Nayler, W.G.; and Elz, J.S. (1986) :** Reperfusion injury: laboratory artifact of clinical dilemma? Circulation; 74: 215.

(41) **O'Driscoll, G.;G reen, D.; Rankin, J.; Stanton, K.; and Taylor, R. (1997) :** Improvement in endothelial function by angiotensin converting enzyme inhibition in insulin dependent diabetes mellitus. J. Clin. Invest.; 100: 678-684.

(42) **Ohishi, M.; Fuji, K.; Minamino, T.; Higahi, J.; Kamitani, A.; Rakugi,H.; Zhoo, Y.; Mikami, H.; Miki, T. and Ogihara, T. (1993) :** A potent genetic risk factor for restenosis. Nat. Genet.; 5: 324-325.

(43) **Opie, L.H. (1992) :** In angiotensin converting enzyme inhibitors: Scientific basis for clinical use. 2nd ed. Wiley, T. & Sons, Inc., New York, p. 194.

(44) **Paget, G.E. and Barens, J.M. (1964) : Evaluation** of drug activities. Academic Press, London and New York; 1: 135-166.

(45) **Pelleg, A.; Mitamiura, H.; Price, R.; Michelson, E.L.; Koplonsky, E. and Dreifus, L.S. (1985) :** Peng, C.F.; Murphy, M.L.; Colwell, K.; and Straub, K.D.(1989):

(46) **Pfeffer, M.A. and Braunwald, E. (1990) :** Ventricular remodeling after myocardial infarction. Circulation; 81: 1161.

(47) **Pfeffer, M.A.; Braunwald, E.; Moya, L.A.; Basta, I.; Brown, E.J.; Cuddy, T.E.; Davis, B.R.; Geltman, S. and Flker, G.C. (1990) :** Effect of captopril on mortility and morbidity inpatients with left ventricular dysfunction after myocardial infarction. N. Eng. J. Med.; 322: 669-677.

(48) Raiagopalan, S.; Karz,S.; Munzel,T.; Tarpey, M.; Freeman, B.A.; Griendling, K.K. and Harrison, D.G. (1997): angiotensin II Mediated hypertension in the rat increase super oxide production via membrane NADH/NADPH oxidase activation. J. Clin. Invest.; 97: 1916-1923.

(49) **Rogers, T.B. (1984) :** Highaffinity angiotensin II receptors in myocardial sarcolemmal membranes. J. Biol. Chem.; 259: 810-14.

(50) **Rubayi, G.M.; and Vonhoutte, P.M. (1986) :** Super oxide anions and hyperoxia inactivate endothelium-derived relaing factors. Am. J Physiol.; 250: H822-H827.

(51) **Scholkens, B.A. and Linz, W. (1988) :** Local inhibition of angiotensin II formation and bradykinin Degradation in isolated heart. Clin. Exp. Hypertens. Theory Pract.; 10: 1259-70.

(52) **Senges, J.; Brachmann; Pelzer, D.; Mizutani,T. and Kubler, W. (1979) :** Effects of some components of ischemia on electrical activity and the ventricular conducting system. Circ. Res.; 44: 864.

(53) **Shen, A.C. and Jennings, R.B. (1989) :** Myocardial calcium and magnesium in acute ischaemia injury. Am. J. Pathol.; 67: 417-440.

(54) **Shen, A.C. and Jennings, R.B. (1997) :** Kinetics of calcium accumulation in acute myocardial ischaemic injury. Am. J. Pathol.; 67: 441-449.

(55) **Silverman, H.S and Stern, M.D. (1996) :** Ionic basis of ischaemic cardiac injury insights from cellular studies. Cardiovasc. Res.; 28: 581-597.

(56) **Smith, R.D.;Chin, A.T.; Wing, C.; Herblin, W.F. and Timmermans, P.M.M.W.M. (1992) :** Pharmacology of non peptide angiotensin II receptor. Ann. Res. Pharmacol. Toxicol.; 32: 135-165.

(57) **Spinale, F.G.; deGaspare, M.; Whitebread, S.; Hebbat, L.; Clair, M.J.; Melton, D.M.; Krombach, R.S.; Mukherjee, R.; Lonnini, J.P. and OS, J. (1997) :** Modulation of renin-angiotensin pathway through enzyme inhibition and specific receptors blockade in pacing induced heart failure; effect on left ventricular performance and neurohormonal systems. Circulation; 96: 2385-2396.

(58) **Steenbergen, C.E.; Murphy, J.A.; Walts, R.E. (1996) :** Correlation between cytosolic free calcium, injury in perfused rat heart. Circ. Res.; 66: 135-146.

(59) **Stanley, W.; Hall, J.; Hacker, T.; Hemandez, L. and Whitesell, L. (1997) :** Decreased myocardial

glucose uptake during ischaemia in diabetic swine. Metabolism; 46: 168-172.

(60) **Sun, D.; Nguyen, N.; DeGrade, T.; Schwaigen, M. and Brosius, F.(1994)** : Ischaemia responsive glucose transporter GLUT4 to the plasma member of cardiac myocytes. Circulation; 89: 793-798.

(61) **Takemoto, M.; Egashi, K. and Takeshuta, A. (1997)** : Important role of tissue angiotensin converting enzyme activity in the pathogenesis of coronary and myocardial structural changes induced by long term blockade on nitric oxide synthesis in rats. J. Clin. Invest.; 99: 278-287.

(62) **Weber, K.T. and Billa, C.G. (1994)** : Pathological hypertrophy and cardiac interstitium. Fibrosis and renin angiotensin-aldosteorne system. Circulaiton; 83: 1849-1885.

(63) **Wholfart, G. and Noble, M.I.M. (1982)** : The cardiac excitation contraction cycle. Pharmacol. Ther.; 16: 1-43.

(64) **Woo, K.S.; and White, H.D. (1994)** : Factors effecting income after recovery from myocardial infarction. Annu. Rev. Med.; 45: 325-339.

(65) **Young, J.B.(1995)** : Reduction of ischaemic events with angiotensin converting enzyme inhibitors cardiovasc. drugs. Ther.; 9: 89-102.

(66) **Zaloga, G.P. (1992)** : Hypocalcaemia in critically ill patients. Crt. Core Med.; 20: 251-262.

(67) **Zimmerman, G.A. (1988)** : Hydrogen peroxide stimulates the synthesis of platelet activating factor by endothelin and induces endothelial dependent cell adhesion. J. Clin. Invest.; 48: 2444-2450b.

(68) **Zuanetti, G.; Ltini, R.; Maggioni, A.; Franzosi, M.; Santoro and Tongnomi, G. (1997) :** Effects of the ACE-inhibitor lisinopril on mortality in diabetic with acute myocardial infarction: the data from GISSI-3 study.