IJMS
Vol 32, No 4, December 2007

Original Article

The Effect of Verapamil Administred before the Reperfusion Insult in Isolated Preconditioned Rat Heart on the Microsomal ATPase and Mitochondrial Enzyme Activities

G.A. Kurian, J. Paddikkala[1]

Abstract

Background: Calcium overload and free radical mediated damage in the mitochondria is the most important pathological changes associated with myocardial ischemic-reperfusion injury. The verapamil post-treatment has been previously reported to prevent reperfusion-induced myocardial injury but functional recovery may be delayed due to the drug's inherent direct myocardial depression effect. In the present study the effect of verapamil on mitochondrial enzymes and sarcoplasmic ATPase system was examined during the myocardial preconditioning and ischemic reperfusion in rat heart.

Methods: Four groups of isolated rat hearts were perfused with KH buffer in a retrograde manner by Lagendroff apparatus. A time controlled ischemia, ischemic reperfusion and classical precondition was produced by restoring the flow of KH buffer in ischemic rat heart. The hearts were then processed to isolate the mitochondria and sarcoplasmic reticulum for the biochemical estimation.

Results: Mitochondrial enzyme and sarcoplasmic ATPase activities were diminished in the ischemic period and further decreased during reperfusion. However, preconditioning the rat heart before the insult of ischemia and reperfusion improved the enzyme activities. The preconditioning procedure consisted of 4 cycles of 4 min. short ischemic periods followed by 4 min. KH buffer perfusion applied before 30 min. global ischemia and reperfusion caused an improvement in the mitochondrial enzyme activities. On the other hand, sarcoplasmic ATPase enzymes required a precondition procedure of 7 cycles of 2 min. short ischemic periods followed by 2 min. reperfusion. The activities of the above enzymes were improved further when verapamil was administered before the insult of ischemic reperfusion.

Conclusion: This study shows the beneficial effect of classical preconditioning with verapamil.
Iran J Med Sci 2007; 32(4): 227-233.

Keywords ● Mitochondria ● ischemia ● reperfusion injury ● verapamil and microsomal ATPase

School of Chemical and Biotechnology,
SASTRA Deemed University,
Thanjavur
Tamil Nadu, India. 613402
[1]Department of Plant Biotechnology
Amala Cancer Research Center
Amalanagar
Trichur
Kerala

Correspondence:
Gino A Kurian
Lecturer Medical Biochemistry
School of Chemical and Biotechnology
SASTRA Deemed University
Thanjavur
Tamil Nadu
India. 613402
Tel: +91 04362 246485
Email: ginokurian@hotmail.com

Introduction

Reperfusion of coronary flow is necessary to resuscitate the ischemic or hypoxic myocardium. Mitochondrial dysfunction is one of the cardinal features of ischemic reperfusion, which leads to myocardial injury.[1] Changes in mitochondrial function have been described during ischemia,[2] which have been further exacerbated by reperfusion.[3] In normal myocardium ATP synthesis and maintenance for Ca^{2+} homeostasis are the two essential roles of mitochondrial.[4] The reduced mitochondrial function and the amount of oxidative stress have an inverse correlation to the functional myocardial impairment.[5]

Myocardium may be protected ischemic reperfusion injury by administration of exogenous cardioprotective agents or by classical ischemic preconditioning (1P).[6] Verapamil, a calcium channel blocker has been used widely for the treatment of angina pectoris,[7] and supra-ventricular tachy-arrhythmia. Calcium channel antagonists exert their beneficial effects on the myocardium by inhibiting the action of slow Ca^{2+} inward current through L-type Ca^{2+} channels into cardiac cells.[8] There are reports suggesting that verapamil post-treatment can prevent reperfusion-induced myocardial injury but functional recovery may be delayed.[9] Moreover, although verapamil could prevent episodes of acute ischemic ventricular fibrillation in certain patients, its beneficial effect on the overall mortality was not as much as that of β-blockers and angiotensin-converting enzyme (ACE) inhibitors.[10]

Hausenloy and Yellon,[11] proposed that ischemic preconditioning acts to prevent the opening of the mitochondrial permeability transition pore (MPT) during the reperfusion period. This large conductance pore, which spans the inner and outer membranes of the mitochondria, forms in response to the elevated calcium ion concentration, presence of oxidative stress or both, as occurs in reperfused myocardium. Ischemic preconditioning can be performed at the time of reperfusion, which may be feasible in patients undergoing reperfusion by direct angioplasty. However, clinical intervention of this procedure cannot be done perfectly due to the complexity of the method. Therefore, the present study was focused on the possible synergic effects of classical pre conditioning and post ischemic treatment of verapamil in animal model of myocardial ischemic reperfusion. This study also intended to find the activities of mitochondrial and sarcoplsmic enzymes during myocardial ischemia and reperfusion.

Materials and Methods

Chemicals

DL isocitrate and N-phenyl-P-phenylenediamine were purchased from Acros Organics, New Jersey USA. Cytochrome C and ATP were purchased from Sigma Chemical Co. (St. Louis, MO USA). All other chemicals used were of analytical grade.

Animals

Adult Wistar male albino rats weighing between 250 to 280 g were obtained from King Institute of Preventive Medicine (Chennai, India). They were acclimatized to animal - house conditions and were fed on commercial pelleted rat chow (Hindustan Lever Ltd., Bangalore, India). They had free access to water. This was ethically approved by Ministry of social justices and empowerment Government of India.

Experimental Protocol

The rats were divided into five groups each consist of six animals except group 4 as follows: Group 1, the control group; group 2, ischemic control; group 3, reperfusion, group 4, precondition; and group 5 drug.

Group1

Hearts were perfused for 90 min. with Krebs-Henseleit (KH) buffer.

Group 2

Were perfused with KH buffer for 20min.before insulting 30min. global ischemia.

Group 3

Animals were subdivided into three groups. In IRC group, the 30min. ischemic hearts were subjected to 15min. reperfusion (group 3.1), 30min. reperfusion (group 3.2) and 45min. reperfusion (group 3.3) respectively.

Group 4

After the perfusion of heart with KH buffer for 20 min., the animals in the precondition group were subdivided into eight sub groups (4.1- 4.8) each consisted of 6 animals.

Group 4.1: After equilibrating the heart by 20min. KH buffer perfusion, the heart was preconditioned with seven cycles of two min. global ischemia followed by two min. reflow. Then the heart was subjected to 30min. global ischemia followed by 30min. reperfusion.

Group 4.2: Heart in this group was subjected to the same experimental procedure as 4.1 except that in the final stage, the heart was induced with 45min. reperfusion.

Group 4.3: After equilibrating the heart by

20min. KH buffer perfusion, the heart was preconditioned with five cycles of three min. global ischemia followed by three min. reflow. Then, the heart was subjected to 30min. global ischemia followed by 30min. reperfusion.

Group 4.4: Heart in this group was subjected to the same experimental procedure as 4.3 except that in the final stage, the heart was induced with 45min. reperfusion.

Group 4.5: After equilibrating the heart by 20min. KH buffer perfusion, the heart was preconditioned with four cycles of four min. global ischemia followed by four min. reflow. Then, the heart was subjected to 30min. of global ischemia followed by 30min. reperfusion.

Group 4.6: Heart in this group was subjected to the same experimental procedure as 4.5 except that in the final stage, the heart was induced with 45min. reperfusion.

Group 4.7: After equilibrating the heart by 20min. KH buffer perfusion, the heart was preconditioned with three cycles of five min. global ischemia followed by five min. reflow. Then, the heart was subjected to 30min. of global ischemia followed by 30min. reperfusion.

Group 4.8: Heart in this group was subjected to the same experimental procedure as 4.7 except that in the final stage, the heart was induced with 45min. reperfusion.

Group 5 Drug

A pilot study was conducted to determine the effective dose of verapamil. Three different doses of verapamil (i.e.0.1, 0.2, 0.3 mg/mL) were administered 5min.before the insult of ischemic reperfusion in rats. Since the 0.2 mg/mL dose showed a significant ($P< 0.05$) effect, we used this dose thereafter in the study.

After equilibrating the heart with KH buffer for 20 min., precondition group were randomly divided into two groups

Group 5A-Ischemic precondition and verapamil administered before the insult of global ischemia:

Hearts (n=6) were perfused with KH buffer for 20min. and were preconditioned in the same way as group 4.5. Hearts were infused with 0.2 mg/mL verapamil for five min. and subjected to 30min. of global ischemia followed by 45min. reperfusion.

Group 5B- Ischemic precondition and verapamil administered before the insult of reperfusion.

Hearts (n=6) were perfused with KH buffer for 20min. and preconditioned in the same as group 4.5. Hearts were subjected to 30min. of global ischemia. Verapamil 0.2 mg/mL for was administered for five min. followed by 45min. of reperfusion.

Heart Preparation

Wistar male rats weighing 250-280 g were anesthetized with administration of 40 mg/kg sodium pentobarbital. After an intravenous injection of 300 U heparin, the heart was rapidly excised via a midsternal thoracotomy and kept in the ice cold KH buffer containing NaCl 118 mM/L, KCl 4.7 mM/L, $MgSO_4$1.2 mM/L, KH_2PO_4 1.2 mM/L, $CaCl_2$ 1.8 mM/L, $NaHCO_3$ 25 mM/L and 11 mM/L $C_6H_{12}O_6$. The heart was attached to a Lagendorff apparatus via its aorta for retrograde perfusion with KH buffer maintained at 37°C and pH 7.4 and saturated with a gas mixture of 95% O_2 and 5% CO_2. The coronary perfusion pressure was maintained at 80 mm Hg.

Tissue Preparation

The heart was excised, rinsed in ice cold isotonic saline, blotted with filter paper, weighed, homogenized in 0.25M sucrose at 4°C by a Polytran homogenizer for five sec. at maximum power. The homogenate was centrifuged for 10 min and the nuclear and cytoskeletal fractions were then discarded. The supernatant was used isolation of mitochondria,[12] and microsomes.[13] The supernatant was centrifuged at 15000 × g for 20 min. The pellet was taken as mitochondria. The mitochondria were suspended in 0.25 M sucrose containing 10mM Tris–HCl and one mM EDTA to a known volume of three mL.

The post mitochondrial fraction (supernatant) was further centrifuged at 105000× g for 60 min. The microsomal pellet was suspended in 50mM Tris-HCl buffer (pH 7.5) containing KCl.

Biochemical assays

Assay of isocitrate dehydrogenase (ICDH),[14] malate dehydrogenase (MDH),[15] succinate dehydrogenase (SDH),[16] α-ketoglutarate dehydrogenase (α-KGDH),[17] NADH dehydrogenase,[18] and cytochrome C oxidase,[19] were carried out in a UV-1601 Shimadzu spectrophotometer. Protein concentration was measured with Folin phenol reagent, following the procedure described by Lowry.[20]

After isolating the sarcoplasmic reticulum, Na^+-K^+-ATPase[21],Ca^{2+}-ATPase,[22] Mg^{2+}-ATPase, [23] and 5'-nucleotidase,[24] were assayed.

Light microscopic study

Myocardial tissue was fixed in 10% formalin, routinely processed and embedded in paraffin. Three µm thick paraffin sections were cut on glass slides and stained with hematoxylin and eosin (H&E), and periodic acid Schiff (PAS) reagent and were examined under a light microscope.

Statistical analysis

All statistics are reported as mean ± SD. Results were analyzed by one-way analysis of variance (ANOVA) by SPSS v 12.00. P< 0.05 was considered statistically significant.

Results

Table 1 shows the changes in mitochondrial enzymes activities during myocardial ischemia, reperfusion and precondition during both the classical and pharmacological preconditioning. Mitochondrial enzymes activities were decreased during ischemic stage as compared to the hearts of normal control group. However, at first, reperfusion of ischemic heart further decreased activities of mitochondrial enzymes. The activities of microsomal ATPase showed a similar pattern as that observed in mitochondrial enzymes (table 2). Decreased enzymatic activities were more pronounced in ischemic reperfusion stage.

A series of short period of occlusion of KH buffer to the isolated heart before sustained ischemia resulted in ischemic preconditioning of the heart. The changes in the mitochondrial enzymes are depicted in table 1. It has been found that a minimum of four min of ischemic preconditioning was required to make the activities of mitochondrial enzyme to return back to a nearly normal level. However, in the case of

Table 1: Activity of mitochondrial enzymes during ischemia, ischemic-reperfusion and classical preconditioning in isolated rat heart.

Group	ICDH	SDH	MDH	αKGDH	NADH dH	Cyt. C Oxidase
1	735.3 ± 22.1	244.1± 7.3	346.4± 10.3	73.3 ± 2.1	133.2 ± 3.9	30.1 ± 0.9
2	655.6 ± 19.6	171.1 ± 6.2	327.6 ± 9.7 [a]	62.3 ± 1.5	130.5 ± 3.9 [ns]	12.3 ± 0.3
3.1	*533.2 ± 15.9	*136.9 ± 4.8	*203.2 ±6.8	*20.1 ± 0.6	*94.2 ± 2.8	*19.1 ± 0.5
3.2	*571.2 ± 17.1	*110.4 ± 3.3	*212.9 ± 6.3	*28.0 ± 0.8	*88.0 ± 2.6	14.0 ± 0.4
3.3	*588.5 ± 17.6	*112.5 ± 3.3	*220.5 ± 6.6	*30.0 ± 0.9	*91.5 ± 2.7	*15.6 ± 0.4
4.1	679.0 ± 20.3	169.1 ± 5.0 [a]	236.4 ± 7.0 [a]	20.0 ± 0.6 [a]	73.8 ± 2.2 [a]	27.8 ± 0.8
4.2	665.0 ± 19.9 [a]	167.1 ± 5.0 [a]	[ns] 225.2 ± 6.7 [a]	17.0 ± 0.5 [a]	71.1 ± 2.1 [a]	22.2 ± 0.6 [a]
4.3	[ns]599.3 ± 17.9 [a]	169.1 ± 5.0 [a]	[ns] 201.9 ± 6.0 [a]	22.2 ± 0.6 [a]	[ns] 94.2 ±2.8 [a]	25.3 ± 0.7 [a]
4.4	[ns] 582.3 ± 17.4 [a]	163.2 ± 4.8 [a]	[ns] 200.3 ± 6.0 [a]	18.1 ± 0.5 [a]	[ns] 90.3 ± 2.7 [a]	21.7 ± 0.6 [a]
4.5	700.3 ± 21.2	238.5 ± 7.1	326.4 ± 9.7	69.7 ± 2.0	121.1 ± 3.6	27.3 ± 0.8
4.6	690.2 ± 20.2	171.2 ± 5.1 [a]	259.1 ± 7.7 [a]	41.2 ± 1.2	107.2 ± 2.2 [a]	[ns] 15.2 ± 0.4 [a]
4.7	697.2 ± 20.3	161.4 ± 4.8 [a]	254.4 ± 7.6 [a]	[ns] 27.7 ±0.8 [a]	97.1 ± 2.9 [a]	19.1 ± 0.5 [a]
4.8	[ns] 580.0 ± 17.1 [a]	169.3 ± 5.0 [a]	250.1 ± 7.7 [a]	26.2 ± 0.7 [a]	[ns] 95.3 ± 2.8 [a]	12.2 ± 0.3 [a]
5A	[£]319.0± 9.5[€]	[£]210.0± 6.3[€]	[£]626.0± 18.7[€]	[£]57.6± 1.7[€a]	[£]115.0± 3.4[€]	[£]22.3± 0.6[€]
5B	[£]349.2±10.4[€]	[£]240.2± 7.2[€]	[£]715.4± 21.4[€]	[£]63.7 ± 1.9[€]	[£]130.1± 3.9[€]	[£]28.2± 0.8[€]

Results are mean±SD (n=6). Activity is expressed as nmol of NADP reduced per min per mg protein for ICDH, nmol of succinate oxidized per min per mg protein for SDH; nmol of NADH oxidized per min per mg proteins for MDH: nmol of α-ketoglutarate formed per hour per mg proteins for α-KGDH and nmol of NADH oxidized per min per mg protein for NADH dehydrogenase: Change in optical density per min per mg protein for cytochrome C oxidase.
Groups 2 *vs* 3.1; 2 *vs* 3.2 and 2 *vs* 3.3 are significantly (P <0.05)* different
Group 3.2 *vs* 4.1, 4.3, 4.5 and 4.7; 3.3 *vs* 4.2, 4.4, 4.6 and 4.8 are significantly (P <0.05)* different.
ns = not significant, Groups 1.0 *vs* 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8 are significantly (P <0.05)[a] different.
Group 3.3 *vs* groups A and B are significantly (P <0.05)[£] different.
Group 4.6 *vs* groups A and B are significantly (P <0.05)[€] different.

Table 2: Activity of sarcoplasmic ATPase during classical preconditioning in isolated rat heart.

Group	Na^+-K^+-ATPase	Ca^{2+}-ATPase	Mg^{2+}-ATPase	5' nucleotidase
1	0.557± 0.01	0.268 ± 0.01	0.469± 0.01	4.21± 0.12
2	0.526± 0.01	0.269 ± 0.02	0.393± 0.01	4.07± 0.12
3.1	*0.490 ± 0.01	* 0.219 ± 0.01	* 0.353 ± 0.02	* 3.90 ±0.11
3.2	0.500± 0.01	0.226± 0.01	0.361± 0.01	3.95± 0.15
3.3	0.525 ± 0.01	0.238 ± 0.01	0.379 ± 0.01	4.01 ± 0.13
4.1	0.529± 0.01	[*]0.266 ± 0.01	[*]0.455± 0.01	4.20 ± 0.17
4.2	0.568± 0.01	0.239 ± 0.01 [a]	[*]0.453± 0.01	4.18± 0.13
4.3	[*]0.560± 0.01	[*]0.163 ± 0.01 [a]	0.386± 0.01 [a]	3.97± 0.11
4.4	0.566± 0.01	0.156 ± 0.01[a]	0.399 ±0.01 [a]	3.96± 0.1 4
4.5	0.518 ± 0.01	[*]0.185 ± 0.01 [a]	[*]0.427 ± 0.01 [a]	4.04 ± 0.12
4.6	0.572 ± 0.01	[*]0.143 ± 0.01 [a]	[*]0.411 ± 0.01 [a]	4.06 ±0.16
4.7	[*]0.676 ± 0.02 [a]	[*]0.142± 0.01 [a]	[*]0.490± 0.01	4.02± 0.13
4.8	[*]0.594± 0.01	[*]0.142 ± 0.01 [a]	[*]0.476 ± 0.01	4.19 ± 0.15
5A	[£]0.91± 0.01[€]	[£]0.69 ± 0.01[€]	[£]0.27± 0.01[€]	[£]4.48± 0.16[€]
5B	[£]0.94 ± 0.01[€]	[£]0.72 ± 0.01[€]	[£]0.28± 0.01[€]	[£]4.49± 0.11[€]

Results are mean ± SD (n=6). Activity is expressed as µmol of phosphorus liberated per sec per g protein for Na^+-K^+-ATPase, Ca^{2+}-ATPase and $Mg2^+$-ATPase; mmol of phosphorus released per mg protein per hr for 5' nucleotidase.
Groups 2 *vs* 3.1; 2 *vs* 3.2 and 2 *vs* 3.3 are significantly (P <0.05)* different
Groups 3.2 *vs* 4.1, 4.3, 4.5 and 4.7; 3.3 *vs* 4.2, 4.4, 4.6 and 4.8 are significantly (P <0.05)* different.
ns = not significant, Groups 1.0 *vs* 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8 are significantly (P <0.05)[a] different.
Groups 3.3 *vs* groups A and B are (P <0.05)[£] different.
Groups 4.2 *vs* groups A and B are significantly (P <0.05)[€] different.

microsomal ATPase, two min of ischemic preconditioning cycle (table 2) was needed.

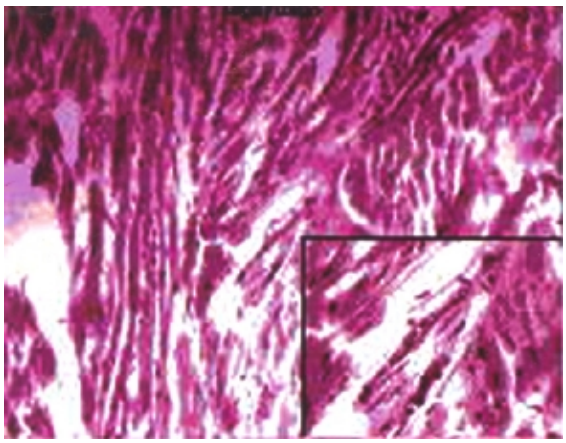
Tables 1 and 2 also show the synergic effects of verapamil and ischemic preconditioning on the mitochondrial and microsomal enzymes. A significant ($P<0.05$) improvement was observed in the activities of both mitochondrial and sarcoplasmic ATPase as compared to reperfusion control and preconditioned groups. The histo-pathologic slides also showed the improvement in the ultra-structure of the reperfused myocardium when treated with verapamil just before the re-flow of KH buffer to the ischemic heart.

Protection against ischemic reperfusion injury in verapamil pre treated and post treated rat heart was evident in histo-pathological analysis (figure 1-4). Post treatment of verapamil preserves the ultrastructure of myocardium (figure 3 and 4) indicates the efficacy of therapeutic intervention before the onset of reperfusion.

Discussion

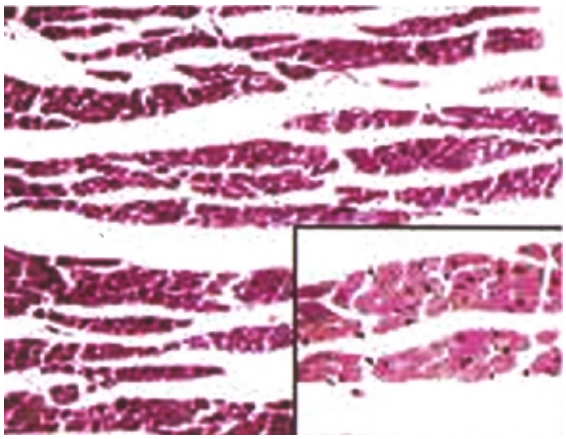
In the present study, we found that the mitochondrial enzymes and microsomal ATPase activities, in the rat hearts, were significantly ($P<0.05$) decreased in ischemia and ischemia reperfusion stage as compared to the normal control rats. During the initial period of ischemia, the utilization of ATP is increased in the cytosol. This can reduce the ATP concentration in the mitochondrial matrix which results in a decline in ATP/ADP ratio. This, in turn, can disturb the overall phosphorylation potential.[25] On the other hand, decrease in ATP can increase the Ca^{2+} level in cytosol and mitochondria with concomitant increase in cytosolic Mg^{2+} level.[26]

It is generally believed that a high Ca^{2+} influx during the ischemia-reperfusion causes pathologic changes observed in the ischemia-reperfusion injury.[27] Therefore, the calcium-mediated inactivation may be one of the reasons for the decreased activities of the mitochondrial



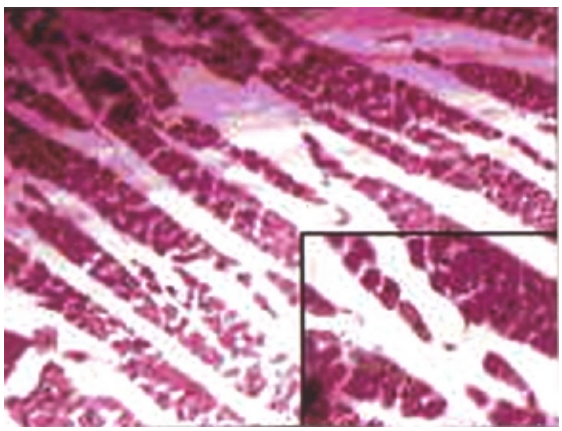
Cell swelling, striated muscles and blood vessels seen

Figure 1: Administration of verapamil before the insult of 30min.global ischemia in preconditioned heart followed by 45min. reperfusion.



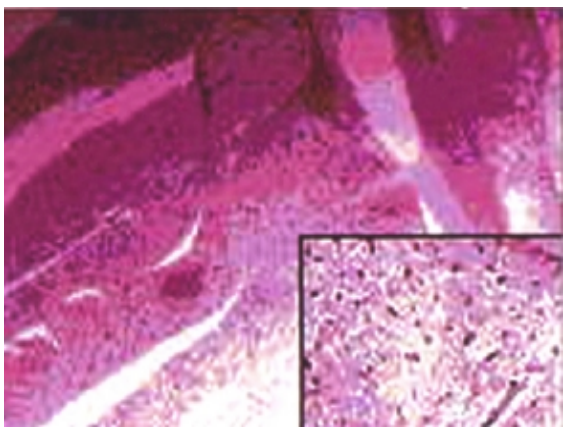
Cell swelling and infiltration of esosinophils

Figure 2: Administration of verapamil before the insult of 30min. global ischemia in preconditioned heart followed by 30min. reperfusion.



Elongated and rod-shaped cells suggesting that the drug was effective

Figure 3: Administration of verapamil after the insult of 30min. global ischemia in preconditioned heart followed by 30min. reperfusion.



The number of rod-shaped cells is decreased

Figure 4: Administration of verapamil after the insult of 30min. global ischemia in preconditioned heart followed by 45min. reperfusion

and sarcoplasmic enzymes during ischemia-reperfusion observed in the present study. Moreover, re-administration of oxygen to the ischemic myocardium triggers the Ca^{2+} entry into the cells, and thus, increases the Ca^{2+} uptake by mitochondria. Since ATP store was depressed in the ischemic myocardium, during reperfusion, sarcoplasmic reticulum is unable to uptake the increased Ca^{2+} load in the mitochondria.

Preconditioning the ischemic myocardium with a series of short periods of ischemia followed by reperfusion (classical preconditioning) significantly restored the activities of the mitochondrial respiratory and sarcoplasmic enzymes back to a nearly normal levels (tables 1 and 2). The beneficial levels of preconditioning depend on several factors and the presence of numerous agents released from the myocardium during preconditioning, including bradykinin,[28] opioids,[29] norepinephrine,[30] and angiotensin,[31] that can initiate one or more signal transduction pathways. It has also been shown in various studies that brief episodes of ischemia can release small amounts of calcium,[32] and induce protein kinase (PKC-) activity, and thus, induce cardio-protection. The present study also showed an improvement in 5' nucleotidase activity—the enzyme for the breakage of AMP—which releases adenosine which is also a cardio-protective agent.

Although protection provided by ischemic preconditioning appears to be robust, a drawback of the majority of preconditioning studies is that protection is not absolute, and when ischemia is severe and prolonged, even those preconditioned areas of myocardium will go further to develop complete infarction.[33] Therefore, possibility of preconditioning the heart with an exogenous agent (e.g., verapamil), as was used in the present study, may provide new avenues to induce preconditioning for cardio-protection, by an external pharmacological intervention. Administration of verapamil to preconditioned heart before the insult of global ischemia produced similar changes as classical preconditioning in mitochondrial and sarcoplasmic enzymes activities. However, a significant improvement in the above-mentioned enzymes activities were observed in the hearts pre-treated with verapamil before the onset of reperfusion as compared to the normal hearts.

The cardio-protective effect of verapamil administration before the global ischemia, as was shown in the present study, suggests the possible role of transient release of Ca^{2+} in brief episodes of ischemia during precondition mechanism. As a calcium-channel blocker, verapamil administered before the preconditioning,[34] attenuates the cardio-protective effect mediated by ischemic preconditioning. This emphasizes the role of calcium-induced protein kinase C (PKC) activation during ischemic preconditioning. Therefore, administration of verapamil before global ischemia blocks the release of further Ca^{2+} from sarcoplasmic reticulum and also uses the calcium which is transiently released during the precondition mechanism into the cell for the activation of PKC. This might explain the similarity of results observed in both verapamil-treated heart (before the insult of global ischemia) and preconditioned hearts.

Pre-treatment of myocardium with verapamil before the onset of reperfusion significantly improved the mitochondrial respiratory and sarcoplasmic ATPase enzymes activities (tables 1 and 2). This indicated that administration of verapamil during the initial period of reperfusion significantly decreased the extent of calcium overload in the mitochondria. Nevertheless, the extent of cardio-protection mediated by this synergic effect of ischemic-preconditioning and verapamil has to be further studied. However, the exact source of Ca^{2+} release during the initial stages of reperfusion may be of much interest to therapeutic interventionists.

We finally, concluded that pre-treatment of ischemic preconditioned rats with verapamil before the onset of reperfusion with KH buffer reduce the extent of mitochondrial and sarcoplasmic damage during ischemic-repefusion and thereby reduces the oxidative stress associated with the ischemic-reperfusion injury.

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