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

By
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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 Langendorffs 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-
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 were 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-

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

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

Control Ischaemia with Lisinopril

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

Fig. (3): Effect of Preischaemic Administration of Lisinopril 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 Ca2+ is due to impaired pump activity of sarcoplasmic reticulum (54). This Ca2+ 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 Ca2+ overload leads to inhibition of calcium release channels in the sarcolemma and sarcoplasmic reticulum by calcium - calmodulin (31), inhibition

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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.
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 AI, decreased OFRs generation by AI (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 cells (41). Also ACE-I, by decreasing AI, inhibits the increased myocardial O2 demand by AI (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


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