ROLE OF DEFERASIROX, AN ORAL IRON CHELATOR IN PREVENTION OF ISCHEMIC REPERFUSION MYOCARDIAL INJURY IN ALBINO RABBITS

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ABSTRACT: An experimental study was carried out after getting approval from institutional animal ethical committee to study the role of oral iron chelator, deferasirox in prevention of myocardial ischemic reperfusion injury in albino rabbits. Albino Rabbits of either sex were divided into two groups control (n=5) and test group (n=5), receiving deferasirox. Experiments were done using isolated heart perfusion apparatus (Langendorff apparatus). The experiment was divided into three phases, perfusion (15 min) followed by ischemia (10 min) followed by reperfusion (15 min). LDH level of perfusate and coronary flow were taken as biochemical and physiological marker of myocardial reperfusion injury respectively and were measured at 5, 10 and 15 min of post ischemic period. Control and test groups were compared. The result shows that in the group receiving oral iron chelator, deferasirox, the decrease in post ischemic LDH level was significant (p< 0.01) at 5, 10 and 15 min while there was significant increase in post ischemic coronary flow at 5 min (p< 0.01) as compared with control. Thus oral iron chelator, deferasirox has protective role in prevention of myocardial ischemic reperfusion injury in albino rabbits.

INTRODUCTION: Coronary heart disease is the leading cause of death and disability worldwide. According to WHO 7,254,000 deaths worldwide resulted from coronary heart disease (CHD) in 2013. The effects of CHD are usually attributable to the detrimental effects of acute myocardial ischemia- reperfusion injury (IRI). IRI typically arises in patients presenting with acute ST-segment elevation myocardial infarction (STEMI).

The most effective therapeutic intervention for reducing acute myocardial ischemic injury is timely and effective myocardial reperfusion using either thrombolytic therapy or primary percutaneous coronary intervention (PCCI).

However, the process of myocardial reperfusion can itself induce further cardiomyocyte death, a phenomenon known as myocardial reperfusion injury.

Although the process of myocardial reperfusion continues to improve with more timely and effective reperfusion and with advances in primary percutaneous coronary intervention technology, antiplatelet and antithrombotic agent for maintaining the patency of the infarct related...
coronary artery, there is still no effective therapy for prevention of myocardial reperfusion injury.\textsuperscript{3}

A growing amount of evidence indicates that oxygen derived free radicals are important in pathogenesis of cardiac tissue destruction during ischemia and reperfusion.\textsuperscript{4-7} Most of the evidence is indirect and based on the studies that have reported protective effects of superoxide dismutase, mannitol or other free radical scavengers.\textsuperscript{8-11} The generation of superoxide ion or hydrogen peroxide produced during ischemia and reperfusion damages cardiac tissue.\textsuperscript{12,13} This damage is increased when a transition metal is present that can catalyse hydroxyl radical formation from superoxide and hydrogen peroxide (Haber Weiss reaction).\textsuperscript{14,15}

There have been very few studies on parenteral and oral iron chelator in prevention of myocardial reperfusion injury. Our aim was to study the protective effect of oral iron chelator, deferasirox in prevention of myocardial reperfusion injury.

MATERIAL AND METHODS:
Present study was conducted on healthy albino rabbits of either sex weighting 1.5-2.0 Kg from October 2014 to November 2015 after getting approval from institutional animal ethical committee. The animals were made available in the animal house. Department of Pharmacology, GSVM Medical College, Kanpur. Animal were maintained on Standard diet ad libitum. Animals were divided into two groups consisting of five animals in each group. Control group was given no drug and was maintained on standard diet ad libitum for 7 days and test group received oral deferasirox 50mg/kg/day and standard diet ad libitum for 7 days. Experiments were done using isolated heart perfusion apparatus (Langendorff apparatus).

Rabbits were given Heparin i.v 750IU/kg via marginal ear vein. After 40 minutes of heparinization rabbits were anesthetized with i.v sodium thiopentone 20 mg/kg by reconstituting in distilled water.

After rabbit became unconscious and lost pedal reflex activity, heart was quickly removed from the body and placed in cold tyrode solution. The heart was cannulated in the aorta and perfused by langendorff apparatus. The perfusion was carried out at 37\textdegree C and pH 7.4 with modified tyrode buffer and aeration was maintained.

The perfusion was maintained for 15 minutes. After 15 minutes of perfusion total ischemia was created by closing the gap between perfusion apparatus and heart. Ischemia was maintained for 10 minutes. After 10 min of ischemia, reperfusion was started by opening tap between perfusion apparatus and heart. This was called post-ischemic or reperfusion phase. This phase was maintained for 15 minutes.

Measurements (LDH levels in IU/L and coronary flow in ml/min) in post ischemic phase were done at 5, 10 and 15 minutes after collecting perfusate.

Coronary flow and LDH level were compared in post ischemic phase between control and test group using student t-test for independent variable. p value was calculated. p < .05 was considered as significant.

RESULTS:
Post-ischemic LDH Levels:
In control group the mean post ischemic LDH level at 5, 10 and 15 min were 147.6, 223, 184.8 respectively, while in deferasirox group post ischemic LDH levels at 5, 10 and 15 min were 45.6, 54.6 and 45 respectively Table 1. Comparing observations of control vs deferasirox [Table 1 and Fig. 1] we found that there was significant (p < .01) decrease in post ischemic LDH level at 5, 10 and 15 minutes. Observation indicates that deferasirox caused significant decrease in post-ischemic LDH level as compared to control.

Post Ischemic Coronary Flow:
In control group the mean post ischemic coronary flow at 5, 10 and 15 min were 4.08, 4.54, 4.24 respectively Table 2. In deferasirox group mean post ischemic coronary flow at 5, 10, 15 min were 4.5, 4.6, 4.02 respectively Table 2. Comparing post ischemic coronary flow of control with deferasirox it was found that though there was significant increase in post ischemic coronary perfusion in deferasirox group at 5 min (p < .01) but there was no significant difference at 10 and 15 min Table 2 and Fig. 2.
TABLE 1: STATISTICAL COMPARISON OF MEAN POST-ISCHEMIC LDH (IU/L) LEVELS BETWEEN GROUP RECEIVING ORAL IRON CHELATOR (DEFERASIROX) AND CONTROL GROUP.

<table>
<thead>
<tr>
<th>Iron Chelator + Standard diet (n=5)</th>
<th>Time interval (min)</th>
<th>Mean LDH Level (IU/L)</th>
<th>SE±</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5min</td>
<td>10min</td>
<td>15min</td>
</tr>
<tr>
<td>Mean LDH Level (IU/L)</td>
<td>45.6</td>
<td>54.6</td>
<td>45</td>
</tr>
<tr>
<td>SE±</td>
<td>1.39</td>
<td>2.36</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Control group (only standard diet) | Time interval (min) | Mean LDH Level (IU/L) | SE± |
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<tbody>
<tr>
<td></td>
<td>5min</td>
<td>10min</td>
<td>15min</td>
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<tr>
<td>Mean LDH Level (IU/L)</td>
<td>147.6</td>
<td>223</td>
<td>184.8</td>
</tr>
<tr>
<td>SE±</td>
<td>4.30</td>
<td>16.24</td>
<td>14.21</td>
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‘t’ value       24.23       11.47     10.89
‘p’ value       <0.01     <0.01     <0.01

TABLE 2: STATISTICAL COMPARISON OF MEAN POST-ISCHEMIC CORONARY PERFUSION (ML/MIN) LEVELS BETWEEN GROUP RECEIVING ORAL IRON CHELATOR DEFERASIROX AND CONTROL GROUP.

<table>
<thead>
<tr>
<th>Iron Chelator + Standard diet (n=5)</th>
<th>Time interval (min)</th>
<th>Mean coronary perfusion (ml/min)</th>
<th>SE±</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>5min</td>
<td>10min</td>
<td>15min</td>
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<tr>
<td>Mean coronary perfusion (ml/min)</td>
<td>4.5</td>
<td>4.68</td>
<td>4.02</td>
</tr>
<tr>
<td>SE±</td>
<td>.035</td>
<td>.044</td>
<td>.086</td>
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</table>

Control group | Time interval (min) | Mean coronary perfusion (ml/min) | SE± |
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<tbody>
<tr>
<td></td>
<td>5min</td>
<td>10min</td>
<td>15min</td>
</tr>
<tr>
<td>Mean coronary perfusion (ml/min)</td>
<td>4.08</td>
<td>4.54</td>
<td>4.24</td>
</tr>
<tr>
<td>SE±</td>
<td>.041</td>
<td>.057</td>
<td>.027</td>
</tr>
</tbody>
</table>

‘t’ value       8.57       1.48       1.42
‘p’ value       <0.01     >0.05     >0.05

FIG.1: GRAPHICAL COMPARISION OF MEAN POST-ISCHEMIC LDH LEVEL BETWEEN ORAL IRON CHELATOR (DEFERASIROX) AND CONTROL AT 5 MIN, 10 MIN AND 15 MIN.
FIG. 2: GRAPHICAL COMPARISON OF MEAN CORONARY PERFUSION (ml/min) BETWEEN DEFERASIROX AND CONTROL AT 5 MIN, 10 MIN AND 15 MIN.

DISCUSSION: The aim of present study was to see the preventive effect of deferasirox in myocardial reperfusion injury. LDH is an important biochemical marker of myocardial ischemia as well as myocardial reperfusion injury. Free radical damage caused during ischemia and reperfusion leads to cell necrosis and release of LDH.

Coronary perfusion can be altered in reperfusion injury due to endothelial cell dysfunction. Endothelium dependent vasodilation is impaired, whereas responses to endothelium dependent vasoconstrictors are exaggerated. Increased production of potent vasoconstrictors such as endothelin-1 and oxygen free radicals increases coronary vasoconstriction and reduces blood flow\textsuperscript{16}.

Iron catalyses hydroxyl radical (free radical) formation from superoxide and H\textsubscript{2}O\textsubscript{2}. Iron is considered as the most important transition metal present in cardiac tissue. Normally, all iron is stored in ferritin in which it is unable to catalyse hydroxyl radical formation. Superoxide radicals produced during ischemia mobilizes iron from ferritin\textsuperscript{17}. Thus iron indirectly causes ischemic reperfusion injury by formation of hydroxyl radical which reacts rapidly with various molecules such as lipid, proteins or DNA and destroys their structure.

Thus oral iron chelator deferasirox can have a role in prevention of myocardial reperfusion injury. Till date as per web research data there have been studies on parenteral iron chelator such as desferrioxamine in preventing ischemic and reperfusion injury in experimental models, but pubmed research has scarce data on oral iron chelator, in prevention of myocardial reperfusion injury. In one such study Antonius MM et al (1989) found that administration of oral iron chelator (Deferiprone) and antioxidant cyanidanol-3 protect postischemic cardiac tissue in Wistar rats\textsuperscript{18}.

Our study indicated significant protection by deferasirox in myocardial ischemic reperfusion injury as it significantly reduced post-ischemic LDH level at 5 min, 10 min and 15 min of reperfusion and significant increase in post-ischemic coronary flow at 5 min of reperfusion.
CONCLUSION: Our study indicates that deferasirox provides significant protection against myocardial reperfusion injury in albino rabbits in isolated heart perfusion experiment in post ischemic phase by significantly reducing post ischemic LDH level at 5, 10, 15 min and significantly increasing post ischemic coronary flow at 5 min of reperfusion.

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REFERENCES:


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