A TEMPORAL PROFILE OF MYOCARDIAL ZINC CHANGES AFTER ISOPROTERENOL INDUCED CARDIAC NECROSIS

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Changes in myocardial zinc level was followed up over a period of 5 days after producing myocardial necrosis in albino rats by subcutaneous injection of isoproterenol, 85 mg/kg body weight. Myocardial necrosis was confirmed by ECG (Lead II), histology and serum enzyme studies (SGOT, SGPT and LDH). A decrease in myocardial zinc level was observed on all the five days. The possible mechanism and the therapeutic implications of these changes are discussed.

By virtue of its association with a number of enzymes, the availability of zinc may critically influence the tissue reparative processes. This will be of special significance in the healing of acute cardiac infarct. A decrease in zinc level has been reported in the injured heart tissue after myocardial infarction. However, the changes in myocardial zinc levels after myocardial infarction have not been followed up over a period of time.

In 1959, Rona, et al., demonstrated that subcutaneous injection of isoproterenol hydrochloride in rats produces gross and microscopic myocardial necrosis. It is now generally accepted that isoproterenol induced cardiac necrosis can be taken as a fairly satisfactory animal model of myocardial infarction, and it has been widely used to study the functional, biochemical and histological changes after cardiac necrosis.

This work was done to study the changes in myocardial zinc after isoproterenol induced cardiac necrosis over a period of 5 days.

MATERIAL AND METHOD
Male albino rats weighing 100–150 grammes (obtained from Indian Drugs and Pharmaceuticals, Rishikesh) were selected for the study. The animals were kept under controlled conditions of temperature and humidity and were given commercial rat food. The animals were divided into three groups as follows:

1. Group A: It consisted of 30 animals. Isoproterenol was administered subcutaneously by a single injection of 85 mg/kg body weight.
2. Group B: It consisted of 6 animals which served as controls.
3. Group C: It consisted of 6 animals for histological examination of heart for confirmation of myocardial necrosis after administration of isoproterenol in a single dose of 85 mg/kg body weight.

From Group A, 6 rats were sacrifice every day after 24, 48, 72, 96 and 120 hours of the administration of isoproterenol. The control group (consisting of 6 rats) was sacrificed on the first day. The animals were anaesthetized with anaesthetic ether and Lead II ECG was taken before opening the chest. The chest was then opened by a midline incision and blood was collected directly from the heart for the estimation

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<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength Å°</th>
<th>Slit width Å°</th>
<th>Lamp current m.a.</th>
<th>Air flow Lit/Mt.</th>
<th>Acetylene flow Lit/Mt.</th>
<th>Gain arbitrary Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>2139</td>
<td>7</td>
<td>15</td>
<td>24</td>
<td>4.2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Standard for Zn was run along with the test sample.

of serum glutamic oxaloacetic transaminase (SGOT) serum glutamic pyruvic transaminase (SGPT) and serum lactic dehydrogenase (SLDH). The heart including the auricles was removed, dried and weighed. The heart tissues were kept in separate specimen tubes containing 5 ml of a mixture of concentrated nitric acid: sulphuric acid in the ratio of 4:1. All the instruments used for collecting the specimens and the specimen tubes were previously immersed in 10% nitric acid solution for 24 hours, rinsed with demineralised water and finally dried in a vacuum desicator.

SGOT and SGPT were estimated by the method of REITMAN and FRANKEL and serum LDH by the method of WROBLEWSKI and LA DUE.

Digestion of tissues

The tissues were digested in Kjeldhal apparatus at 60°C for 2 hours. 1% hydrochloric acid was added and the volume made to 10 ml. This solution was then subjected to atomic absorption spectrophotometry.

Atomic absorption spectrophotometry

Perkin Elmer Model 303 atomic absorption spectrophotometer equipped with Bolding burner and Null recorder read out accessory was used. The operating conditions for Zinc was determined. The operating parameters for maximum sensitivity is given below:

Precision of the analytical techniques and detection limits

Recovery experiments and replicate analysis were performed for the determination of the precision of the technique. The standard deviation for the replicates analysed for zinc was between 0.5 to 1.1 per cent at 10 ppm and between 1.0 to 4.8 per cent at 1 ppm level. The recovery for Zinc was 99.4 per cent. Limit of detection for zinc was 0.1 ppm.

Histology

The rats in group C were sacrificed after 24 hours of subcutaneous injection of isoproterenol 85 mg/kg body weight. The heart was removed and blood squeezed out. The tissue was fixed in 10% formal saline. After 48 hours of fixation, a gross examination was done. 3 micro meter sections were cut and stained with haematoxylin and eosin stain. It was examined by light microscope for evidence of cardiac necrosis.

OBSERVATIONS

ECG

The commonest change in ECG was tachycardia, and elevation of ST Segment. The other findings included flattening of T waves, Q waves and complete heart block.

Enzymes

SGOT: The SGOT was found to be 50.90 ± 13.33 micro mole/litre in the normal controls. It increased to 95.24 ± 11.60 micro mole/litre (P < 0.001) on the first day and 86.36 ± 7.33 micro mole/litre (P < 0.001) on the second day after isoproterenol administration.

SGPT: The SGPT level was 67.55 ± 9.39 micro mole/litre in the control group. It increased to 81.73 ± 8.96 micro mole/litre (P < 0.001) on the first day and 86.39 ± 8.95 micro mole/litre (P < 0.001) on the second day after isoproterenol administration.

SLDH: The serum LDH level was 584.0 ± 82.67 micro mole/ml/litre in the control group. The values increased to 1408.0 ± 178.74 micro mole/litre (P < 0.001) on the first day and 1496.0 ± 176.14 micro mole/litre (P < 0.001) on the second day after isoproterenol administration.

Histology: Myocardial necrosis was observed in all the rats which were given isoproterenol. The histological findings consisted of necrosis of muscle fibres, oedema and leukocyte infiltration.

TABLE I  MYOCARDIAL ZINC (Microgram/gram of Tissue)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Normal</th>
<th>1st Day</th>
<th>2nd Day</th>
<th>3rd Day</th>
<th>4th Day</th>
<th>5th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>101.1065</td>
<td>32.8739</td>
<td>48.6242</td>
<td>27.3668</td>
<td>24.3756</td>
<td>22.4711</td>
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<tr>
<td>2.</td>
<td>29.4202</td>
<td>17.9832</td>
<td>29.7498</td>
<td>37.3464</td>
<td>30.3450</td>
<td>21.1018</td>
</tr>
<tr>
<td>3.</td>
<td>35.5231</td>
<td>23.5784</td>
<td>37.9125</td>
<td>27.9668</td>
<td>34.6861</td>
<td>31.9384</td>
</tr>
<tr>
<td>4.</td>
<td>81.0975</td>
<td>23.9786</td>
<td>44.1492</td>
<td>25.8904</td>
<td>23.5395</td>
<td>18.6625</td>
</tr>
<tr>
<td>5.</td>
<td>111.5233</td>
<td>32.8739</td>
<td>49.0751</td>
<td>14.5640</td>
<td>17.9597</td>
<td>15.2781</td>
</tr>
<tr>
<td>6.</td>
<td>37.6304</td>
<td>17.9832</td>
<td>37.9125</td>
<td>25.8904</td>
<td>23.5395</td>
<td>22.1734</td>
</tr>
</tbody>
</table>

Mean 66.0520  24.8785  41.2372  26.5041  25.7408  21.9375  
S.D.  36.3432  6.7148   7.4638   7.2630   5.8875   5.5880   
S.E.  14.8339  2.7407   3.0464   2.9644   2.4030   2.2808   

$t$  8.6294   5.1807   8.4810   8.4810   9.2930   
$P$  <.001    <.001    <.001    <.001    <.001     

S.D.=Standard Deviation;  S.E.=Standard Error

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**Myocardial Zinc** (Table I, Figure 1)

The level of myocardial zinc in the control group was found to be $66.0520 \pm 36.3432$ microgram/gram of tissue. After isoproterenol administration, the zinc level came down to $24.8785 \pm 6.7148$ microgram/gram of tissue ($P < 0.001$) on the first day. There was a rise to $41.2372 \pm 7.4638$ microgram/gram ($P < 0.001$) on the day as compared to the first day value. Later, there was again a fall in the zinc level to $26.5041 \pm 7.2630$ microgram/gram ($P < 0.001$) on the third day; $25.7408 \pm 5.8875$ microgram/gram

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(P < 0.001) on the fourth day and 21.9376 ± 5.5880 microgram/gram (P < 0.001) on the fifth day. At no stage from the 1st to the 5th day of isoproterenol administration did myocardial zinc reach the normal level.

**DISCUSSION**

The results showed a depletion of myocardial zinc after isoproterenol induced myocardial necrosis. Zinc is required for tissue reparative processes. It has been shown to be helpful in tissue repair after myocardial injury as evidenced by an increased uptake of zinc in certain subcellular fractions in injured tissue after coronary artery ligation. The maximum fall in myocardial zinc occurred on the first day. The recovery (over the first day level) seen on the second day may be in an attempt to incorporate more zinc in the myocardium for tissue reparative process. However, the uptake probably could not be sustained because of extensive tissue damage. The myocardial zinc showed an initial fall, then a gradual rise and again a further fall in the level over the five day period studied. This finding correlates well with the changes in serum zinc after isoproterenol induced myocardial infarction in rats reported by Ahmad, et al. who found an initial rise in serum zinc (probably due to release from myocardium) followed by a fall in serum zinc (probably due to an increased myocardial uptake). Apart from the depletion of myocardial zinc occurring due to tissue damage, a fall in myocardial zinc may also be explained by the disappearance of LDH from the infarcted heart tissue because zinc is associated with this enzyme.

Zinc is the metal component or activator of many metalloenzymes including carbonic anhydrase and many dehydrogenases, alkaline phosphatase and proteases. Without trace amounts of this metal, these enzymes can not function or even exist. Availability of zinc governs the tissue concentration and activity of certain zinc metalloenzymes and the rate of synthesis of nucleic acids and proteins suggesting that its availability may critically influence tissue reparative processes. A deficiency of this trace metal may therefore be responsible for poor recovery after a large myocardial infarct. Administration of zinc after myocardial infarction may therefore help in the recovery process.

**Acknowledgement**

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