ROLE OF LIPISTAT IN PROTECTION AGAINST ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN RATS: A BIOCHEMICAL AND HISTOPATHOLOGICAL STUDY

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Abstract: A test drug (Lipistat) comprising of equal proportions of extracts of Terminalia arjuna, Inula racemosa Hook, latex of Commiphora mukul, in three different doses (225 mg/kg; 350 mg/kg; 450 mg/kg) were administered orally daily for 6 days a week for 60 days in rats. Thereafter, the rats were subjected to isoproterenol (ISO) induced (85 mg/kg, s.c. for 2 days) myocardial necrosis. Gross and microscopic examinations (histopathology) were done along with estimations of myocardial tissue high energy phosphates (HEP) stores and lactate content.

Gross examination showed significant (P<0.05) cardioprotection in Lipistat treated animals. On microscopic examination no statistically significant reduction in myocardial damage by 350 and 450 mg/kg of Lipistat were observed although loss of myocardial HEP stores and accumulation of lactate were significantly prevented.

The results of the present study suggest the potential usefulness of Lipistat in the prevention of ischemic heart disease.

Key words: myocardial necrosis rats cardioprotection

INTRODUCTION

Ischemic heart disease (IHD) has emerged as a major world health problem (1). Vigorous global research is underway in an effort to develop pharmacological means to control morbidity and mortality arising from IHD. Lipistat an Indian herbal formulation has several agents which are reported to have cardioprotective, cardiotonic, antianginal and hypcholesterolemic properties (2, 3). It contains equal proportions of the following compounds: a) Terminalia arjuna W&A Bark (Extract of), b) Inula racemosa Hook Root (Extract of) and c) Commiphora mukul Hook exStocks latex.

On the basis of the above properties Lipistat was tested in a model of acute myocardial necrosis after chronic pretreatment.
METHODS

Albino rats (Wistar strain) of either sex weighing between 150-225 g were used.

Lipistat suspended freshly in 2% gum acacia was administered in three doses orally, 6 days a week for 60 days. At the end of this period all the animals except normal untreated rats which served as the control group were administered isoproterenol (ISO) 85 mg/kg, s.c, for two consecutive days to induce myocardial necrosis (4). After 48 hours of the first dose of ISO, the rats were sacrificed, heart excised and immediately frozen in liquid nitrogen for the estimation of adenosine triphosphate (ATP), creatine phosphate (CP) and lactate (5,6) or examined for gross changes after which it was immediately fixed in 10% buffered formalin for histopathological processing and grading according to the method of Rona et al (4).

The following groups were studied:
Group I: Vehicle + saline
Group II: Vehicle + ISO
Group III: Lipistat (225 mg/kg) + ISO
Group IV: Lipistat (350 mg/kg) + ISO
Group V: Lipistat (450 mg/kg) + ISO

Statistical Analysis

One way Analysis of Variance (ANOVA) was used to test for significance of biochemical data and Kruskal Wallis one way ANOVA for significance of the gross and histopathological data. Significance is set at 0.05. All values are Mean ± S.E.

RESULTS

Gross Examination

Group I: Three rats were sacrificed after administration of saline.

Group II: Ten rats were included in the vehicle treated group of which 2 died after administration of ISO. Gross grading in 8 animals was 3.25 ± 0.25 (Table I). In these animals, large infarct like necrosis was noted macroscopically, which involved upto 1/2 of the left ventricle. These infarcts also extended to adjacent areas of interventricular septum and right ventricle.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Extent of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gross Grades (n)</td>
</tr>
<tr>
<td>I</td>
<td>Vehicle + Saline</td>
<td>0 ± 0 (3)</td>
</tr>
<tr>
<td>II</td>
<td>Vehicle + ISO</td>
<td>3.25 ± 0.25 (8)</td>
</tr>
<tr>
<td>III</td>
<td>Lipistat + ISO</td>
<td>2.3 ± 0.4* (10)</td>
</tr>
<tr>
<td>IV</td>
<td>Lipistat + ISO</td>
<td>1.1 ± 0.3* (8)</td>
</tr>
<tr>
<td>V</td>
<td>Lipistat + ISO</td>
<td>2.3 ± 0.6* (6)</td>
</tr>
</tbody>
</table>

Values are mean ± S. E.
*P<0.05 vs Group II
Group III: (Lipistat, dose 225 mg/kg). Twelve rats were initially included, of which 1 died during the course of treatment and 1 was excluded as it developed a lump around the neck. In the remaining 10 animals, gross grading was $2.3 \pm 0.4$ (Table I). Most of the animals in this group had well demarcated necrotic areas limited to the apex, extending to other parts of the left ventricle, adjacent interventricular septum and to the right ventricle only in a few rats.

Group IV: (Lipistat, dose 350 mg/kg). Thirteen rats were included in this group, of which 4 died during the course of treatment and 1 died after ISO and the gross grading in 8 rats was $1.1 \pm 0.3$ (Table I). Animals of this group showed mottling of the apex and distal parts of left ventricle caused by mixed pale and red streaks.

Group V: (Lipistat, dose 450 mg/kg). Eight rats were included in this group and 2 died during the course of treatment. Gross grading in the surviving 6 rats in this group was $2.3 \pm 0.6$. A few animals had well demarcated areas in the apical region and the rest had larger infarcts involving at least 1/3 of left ventricle with extensions to the interventricular septum and right ventricle. Since there was not much difference in gross grading between Group II ($2.3 \pm 0.4$) and Group V ($2.3 \pm 0.6$), only one of these i.e. 450 mg/kg was subjected to histopathological and biochemical studies along with Group IV (350 mg/kg).

Light Microscopic Examination

Group I (vehicle): Three animals were sacrificed with exposure to ISO and their hearts preserved in formalin. Light microscopy showed normal myofibrillar structure with striations, branched appearance and continuity with adjacent myofibrils (Fig 1). The histopathological grade (HP) was $0 \pm 0$ (Table I).

Fig. 1: H & E stained light microscopy section of normal rat myocardium (Group I) showing well maintained myofibrillar structure.

Group II (vehicle + ISO). Five animals were included of which 2 died after ISO administration and the microscopic examination showed edema, focal haemorrhage and leukocytic infiltration. The muscle fibres showed vacuolar changes with fragmentation suggestive of necrosis (Fig. 2). The HP grading in this group was $3.7 \pm 0.33$ (Table I).

Group IV (Lipistat dose 350 mg/kg): Microscopic examination showed patchy leukocytic infiltration, mild edema limited to the subendocardial portion with absence of haemorrhage. The morphology of the
cardiac muscle fibres were relatively well preserved (Fig. 3). The HP grading in this group was $2.0 \pm 0.27$ (not significant) (Table I).

**Fig. 2**: H&E stained light microscopy section of rat heart treated with ISO (Group II) showing extensive degeneration of myofibrils with leukocytic accumulation, edema and vacuolization.

**Fig. 3**: H&E stained light microscopy section of rat heart (Group IV) showing relatively less leukocytic infiltration and edema with myocardial architecture being better preserved.

**Group V (Lipistat dose 450 mg/kg)**: Histopathological examination showed extensive leukocytic infiltration, marked degeneration of muscle fibres, edema and haemorrhage (Fig. 4). The HP grading in this group was $3.0 \pm 0.6$ (not significant) (Table I).

**Biochemical studies**

Myocardial ATP was significantly higher in both, 350 mg/kg and 450 mg/kg drug treated groups ($3.0 \pm 0.24$ and $3.8 \pm 0.53$ uM/g wet wt respectively). Similarly, CP was also significantly higher in both the treated groups ($2.7 \pm 0.36$ and $2.7 \pm 0.26$ uM/g wet wt.), in comparison to Group II ($1.4 \pm 0.18$ pM/g wet wt). In both the drug treated groups, HEP contents were not significantly depleted in comparison to Group I. Lactate levels were normal in all the groups except Group II, in which it was significantly elevated (Table II).
TABLE II: Myocardial ATP, CP and lactate contents in rat hearts of different groups.

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>ATP (µM/g wet wt)</th>
<th>CP (µM/g wet wt)</th>
<th>Lactate (µM/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.2±0.25*</td>
<td>3.9±0.35*</td>
<td>9.6±0.8***</td>
</tr>
<tr>
<td>II</td>
<td>1.4±0.18</td>
<td>1.4±0.27</td>
<td>20.9±0.98</td>
</tr>
<tr>
<td>III</td>
<td>3.2±0.24*</td>
<td>2.7±0.36*†</td>
<td>8.4±0.61***</td>
</tr>
<tr>
<td>IV</td>
<td>3.8±0.53*</td>
<td>2.7±0.26*†</td>
<td>10.7±0.87***</td>
</tr>
</tbody>
</table>

*P<0.05 vs Group II
†P<0.05 vs Group I
***P<0.001 vs Group II
Values are Mean ± S.E.

DISCUSSION

The results of this study indicate a cardioprotective action of Lipistat, although microscopic histopathological findings were not statistically significant. From the gross histopathological data of this study it appears that 350 mg/kg might be the most effective dose. The mechanism of such a beneficial action might be through preservation of high energy phosphates (HEPs) and/or enhanced aerobic metabolism. However, the data from this study does not indicate whether preserved HEPs and reduced accumulation of lactate are the cause or effect of cardioprotection by Lipistat.

Although the exact mode of action of T. Arjuna, I. racemosa and gum resin of C. mukul are not clearly known, an interplay of the various postulated mechanisms might be bringing about their cardioprotective action. Enhancement of PGE₂ like activity by T. Arjuna resulting in inhibition of platelet aggregation, hypotension and coronary vasodilation which might aid in prevention of myocardial infarction (7, 8). The second plant, inula racemosa has also similar properties to T. arjuna such as enhancement of PGE₂ like activity, negative inotropic and chronotropic actions, along with catecholamine depleting effects (7, 8, 10).

The third compound i.e. C. mukul Hook is mainly a hypolipidemic agent (11, 12) and this property is unlikely to have contributed significantly to the cardioprotective actions of the drug in this particular model. Nevertheless, it has been made a constituent of the formulation as hyperlipidemia is an important causative factor in the development of atherosclerotic coronary artery disease.

In conclusion, the present study demonstrates a partial effectiveness of Lipistat in the prevention of myocardial necrosis. Further studies are needed to elucidate the exact mechanisms of action of the various constituents and their potential in the treatment and/or prevention of cardiovascular diseases.
REFERENCES


