EVALUATION OF ANTIHYPERLIPIDEMIC AND ANTIATHEROSCLEROTIC POTENTIAL OF RIMONABANT IN EXPERIMENTAL ANIMALS

KIRAN NARKHED*, ASHISH MAHAJAN, SAVITA D. PATIL

Department of Pharmacology, G. N. College of Pharmacy, Sawarde, Dist-Ratnagiri, Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education & Research, Near Karwand Naka, Shirpur, Dist-Dhule, Maharashtra, India 425405.

Email: kpnarkhede@gmail.com

ABSTRACT

Objectives: The objective of present investigation was to evaluate antihyperlipidemic and antiatherosclerotic potential of Rimonabant in experimental animals.

Materials and Methods: Hyperlipidemia was induced by High Cholesterol Diet (HCD). Wistar albino rats were used for the study and were divided in 6 groups of 6 rats each. They were treated with high fat diet containing 4% cholesterol, 1 mL coconut oil, 1% cholic acid for 30 days. The test (Rimonabant) and standard drugs were administered orally for the period of 30 days. The test drug administered at the doses levels of 2.5, 5 and 10 mg/kg and Simvastatin was used as standard drug at the dose level of 4mg/kg b.wt./day. The diet consumed by animals and body weight was recorded and evaluated on daily basis. The Aorta was isolated from all the animals and subjected to histopathological evaluation. Biochemical investigations were carried out to assess serum level of total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein and atherogenic index. Determination of Cholesterol in Feces was also evaluated. In vitro anti atherosclerotic activity was evaluated by Platelet anti-aggregation method and protein denaturation evaluation.

Result: High fat diet showed significant increase in body weight, lipid profile in serum. The Rimonabant and Simvastatin treated groups showed significant increase in HDL levels and decreased total cholesterol, triglyceride, LDL, VLDL, HDL ratio and AI (atherogenic index) significantly.

Conclusion: It could be concluded that Rimonabant posses significant antihyperlipidemic and antiatherosclerotic potential.

Keywords: Rimonabant, Lipoprotein, Hyperlipidemic diet, Obesity, Anti-atherosclerosis.

INTRODUCTION

Coronary artery disease (CAD) is one of the most important causes of death all over world. Hyperlipidemia is one of the risk factors for CAD. Data show that 25–30% risk of CAD is reduced by treating hyperlipidemia[1]. The increasing morbidity and mortality from coronary heart disease is the biggest challenge to nutritionists and medical scientists all over the world.[2]

Hyperlipidemia, hypertension, obesity, raised coagulation factor and homocysteine are modified risk factors for atherosclerosis. Dyslipidemia is most common risk factor causes IHD in elderly population. The underlying mechanism of IHD involves the deposition of serum lipids in coronary arteries, and its resulting in decreased blood flow to cardiac muscles.[3] Literature survey has shown that Rimonabant have potent antiobesity activity[4], helps in cessation of smoking[5] and possesses anti lipogenesis property[6].

Considering all above properties of Rimonabant we have designed the model for evaluation of antihyperlipidemic and antiatherosclerotic activity, which is not scientifically proved yet.

MATERIAL AND METHODS

Drug and Chemicals

Cholesterol, cholic acids (sigma chemicals, USA), Rimonabant (Zydus health care, Ahmadabad, India), Cholesterol, triglyceride, HDL-cholesterol estimating kits (RFCL Pvt. Ltd, Gudeion, India), Citric acid, Sodium citrate, Dextrose, Adenosine di phosphate (ADP), Heparin (S.D. Fine chemicals, India).

Animals

Healthy Adult Wistar albino rats of both sexes, eight weeks old, weighing 150-200g were used in present investigation. The animals were maintained in groups in large specious propylene cages in the departmental Animal House Facility with 12 hrs light and dark cycles. Temperature was maintained at 25±3 °C. Feeding schedule consisted of rat pellet diet and water ad libitum. Daily intake of food was quantitated precisely. Prior to initiation of experiments, the entire experimental protocol was submitted to the Institutional Animal Ethical Committee, reviewed and the approval obtained as per CPCSEA guidelines (Registration No.651/02/C/CPCSEA).

Antihyperlipidemic activity

Hyperlipidemic diet model: The rats were divided into the following 6 groups each consist of 6 animals. The hyperlipidemia was induced by feeding high fat diet (4% cholesterol, 1% cholic acid, 1mL coconut oil) for 30 days[7].

Group I: rats received 0.5% c.m.c with high fat diet for 30 days

Group II: rats received only 0.5% c.m.c. For 30 days

Group III: rats were treated orally with Rimonabant 2.5mg/kg b.wt./day along with high fat diet for a period of 30 days.

Group IV: rats were treated orally with Rimonabant 5mg/kg b.wt./day along with high fat diet for a period of 30 days.

Group V: rats were treated orally with Rimonabant 10mg/kg b.wt./day along with high fat diet for a period of 30 days.

All animals had free access to diet and water. The daily diet consumed by animals was calculated by subtracting the leftover diet the next day from the previous day's added diet. The body weight of each animal was recorded every day.

Collection of blood samples and biochemical analysis from serum

At the end of the experiments on the 30th day, blood samples were collected 4 h after the last dose of administration using light ether anaesthesia. Blood samples were collected separately from retro orbital sinus puncture into sterilized dry centrifugation tubes. Samples were allowed to stand for 30 min at 37 °C. The clear serum was separated at 2500 rpm for 10 min using Remi centrifuge. The biochemical investigation was carried out to assess total cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, very low density lipoprotein[8] and atherogenic index[9].
**Determination of Cholesterol in Feces**

During the last 3 days of the experiment, the rats were transferred to metabolic cages. Feces were collected, separated from the adhering hair and diet residue and stored[10] then dried at 60 °C for 12 h and pulverized with a mill. Resultant powdered fecal matter was extracted with chloroform: methanol (2:1). This extract was then analyzed for cholesterol content in similar manner of the serum[7].

**Histopathological study**

After the decapitation of the animals, the aorta were removed and fixed in 10% neutral-buffered formaldehyde solution. Fixed tissues were embedded in paraffin, cut into sections and placed on microscope slides. Slides were stained with hematoxylin and eosin for the histomorphological examination which was performed under light microscopy.

**In vitro anti atherosclerotic activity**

Platelet rich plasma (PRP) was prepared by centrifugation (1000 rpm for 5 min) of blood collected from normal aspirin free blood bank donors. 1.5 ml of acid citrate dextrose was used as anticoagulant for every 8.5 ml of blood. PRP was taken into siliconized glass cuvettes. Platelet poor plasma (PPP) collected by centrifugation was kept for every 8.5 ml of blood. PRP was taken into siliconized glass cuvettes. Platelet poor plasma (PPP) collected by centrifugation (1000 rpm for 5 min) before the addition of ADP.

Commercial heparin (20μg/ml) was used as reference standard. The aggregation was recorded for 5 min at 600 nm. The effect of different concentrations (50 μg/ml) of PPE was studied by incubation with PRP at 37 °C for 5 min before the addition of ADP. Inhibition of platelets aggregation activity shown in Table 4. Significant increase in HDL cholesterol (56.75% [p<0.01]), LDL-cholesterol (46.37% [p<0.01]), triglyceride (45.82% [p<0.01]), atherogenic index (73.43% [p<0.01]) and an increased in HDL-cholesterol (6.3% [p<0.01]) and increased fecal cholesterol excretion (10.9% [p<0.05]) was observed in dose dependant manner and significant as shown in (Table 1). There was also significant reduction in body weight of rats those received different doses of Rimonabant along with High Fat Diet. (36% [p<0.01]) at 2.5 mg/kg/p.o. dose, 28.57% [p<0.01] at 5 mg/kg/p.o. dose and 44.18% [p<0.01] at 10 mg/kg/p.o. dose (Table 2). There was also significant reduction in food intake of the hyperlipidemia induced animals, who received simultaneously administration of Rimonabant at a dose of (2.5 mg/kg/p.o., 5 mg/kg/p.o., 10 mg/kg/p.o.), 45.83% [p<0.01], 54.16% [p<0.01], 54.56% [p<0.01] resp (Table 3). Significant increase in HDL level was found in 2.5, 5 and 10 mg/kg Rimonabant treated group.

**Statistical Analysis**

Results are presented as mean ± S.E.M. The data were tested by one way ANOVA, followed by Dunnett’s multiple comparison post test to identify significant difference. All analyses were performed using Graph Pad Prism statistical software. A level of p<0.05 was considered significant.

**RESULTS**

**Body weight analysis and Serum lipid profile**

HFD showed increased serum lipid profile, increased body weight and increased atherogenic index. Administration of Rimonabant at 2.5 mg/kg/p.o. dose to hyperlipidemia induced Wistar albino rats resulted in a decreased of total cholesterol (19% [p<0.01]), triglyceride (17.13% [p<0.01]), LDL-cholesterol (22.7% [p<0.05]), VLDL-cholesterol (1.679% [p<0.05]) (Table 1). With 5 mg/kg/p.o. dose of Rimonabant a further reduction in total cholesterol (23.8% [p<0.01]), triglyceride (35.34% [p<0.01]), LDL-cholesterol (25.57% [p<0.01]) and atherogenic index (41.55% [p<0.01]) was observed as shown in (Table 1). With 10 mg/kg/p.o. dose of Rimonabant a further reduction in total cholesterol (42.2% [p<0.01]), triglyceride (46.37% [p<0.01]), LDL-cholesterol (56.75% [p<0.01]), VLDL-cholesterol (45.82% [p<0.01]), atherogenic index (73.43% [p<0.01]) and an increased in HDL-cholesterol (6.3% [p<0.01]) and increased fecal cholesterol excretion (10.9% [p<0.05]) was observed in dose dependant manner and significant as shown in (Table 1). There was also significant reduction in body weight of rats those received different doses of Rimonabant along with High Fat Diet. (36% [p<0.01]) at 2.5 mg/kg/p.o. dose, 28.57% [p<0.01] at 5 mg/kg/p.o. dose and 44.18% [p<0.01] at 10 mg/kg/p.o. dose (Table 2). There was also significant reduction in food intake of the hyperlipidemia induced animals, who received simultaneously administration of Rimonabant at a dose of (2.5 mg/kg/p.o., 5 mg/kg/p.o., 10 mg/kg/p.o.), 45.83% [p<0.01], 54.16% [p<0.01], 54.56% [p<0.01] resp (Table 3). Significant increase in HDL level was found in 2.5, 5 and 10 mg/kg Rimonabant treated group.

**Platelet anti-aggregation activity**

The Rimonabant (50–150 μg/ml) interestingly inhibited platelet aggregation. Greater inhibition of aggregation was noticed with increased inhibition of platelets aggregation shown in Table 4.

**Inhibition of protein denaturation**

The Rimonabant also inhibited protein denaturation shown in Table 5.

**Histopathological Examination**

No histological alterations in rat aorta were established in any of the six experimental groups.
Fig. A: High fat diet control (HE 40 X): No increase in thickness of aortic valve leaflet in rats fed with high fat diet compared with normal group

Fig. B: Normal (HE 40 X): Cross section of normal rat aorta shows normal thickness. Normal architecture of aorta

Fig. C: High fat diet + Rimonabant 2.5 mg/kg (HE 40 X): Cross section of rat aorta of Rimonabant 2.5 mg/kg treated group shows no degeneration in the inner layer of aortic tissue

Fig. D: High fat diet + Rimonabant 5 mg/kg (HE 40 X): Cross section of rat aorta of Rimonabant 5 mg/kg treated group shows no degeneration in the inner layer of aortic tissue

Fig. E: High fat diet + Rimonabant 10 mg/kg (HE 40 X): Cross section of rat aorta of Rimonabant 10 mg/kg treated group with no change in cellular architecture

Fig. F: High fat diet + Simvastatin 4 mg/kg (HE 40 X): Showing normal cellular architecture

Table 1: Effect of Rimonabant on various biochemical parameters in hyperlipidemic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>HFD</th>
<th>Control</th>
<th>HFD + Rimonabant 2.5 mg/kg</th>
<th>HFD + Rimonabant 5 mg/kg</th>
<th>HFD + Rimonabant 10 mg/kg</th>
<th>HFD + simvastatin 4 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol Level</td>
<td>250±6.6</td>
<td>88.5±4.5</td>
<td>202.5±12**</td>
<td>190.5±7.9**</td>
<td>144±6.4**</td>
<td>111±5.5**</td>
</tr>
<tr>
<td>Triglyceride Level</td>
<td>258±3.5</td>
<td>125.0±7.9</td>
<td>224.1±11**</td>
<td>166.8±6.9**</td>
<td>138.6±9.4**</td>
<td>128±9.5**</td>
</tr>
<tr>
<td>HDL-Cholesterol Level</td>
<td>26.3±4</td>
<td>42±2.8</td>
<td>29±3.3</td>
<td>30.4±2.1</td>
<td>42.6±3.2**</td>
<td>27.5±5.6**</td>
</tr>
<tr>
<td>LDL-Cholesterol Level</td>
<td>171±5.7</td>
<td>21.3±1.2</td>
<td>132.1±4.3**</td>
<td>127.2±3.2**</td>
<td>74±8.2**</td>
<td>58±2.6**</td>
</tr>
<tr>
<td>VLDL-Cholesterol Level</td>
<td>51.6±1</td>
<td>25±1.3</td>
<td>43±2.1*</td>
<td>33.6±1.3**</td>
<td>28±1.9**</td>
<td>25.2±1.7**</td>
</tr>
<tr>
<td>Atherogenic Index</td>
<td>9.4±0.84</td>
<td>1.3±0.12</td>
<td>8.4±0.5**</td>
<td>5.5±0.4**</td>
<td>2.5±0.37**</td>
<td>0.95±0.16**</td>
</tr>
<tr>
<td>Fecal cholesterol excretion</td>
<td>22±1.2</td>
<td>19±1.6</td>
<td>23±1.3</td>
<td>24±1.5</td>
<td>24±0.9**</td>
<td>25±1.9**</td>
</tr>
</tbody>
</table>

Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett’s Multiple Comparative test *p< 0.05, **p<0.01, as compared with control.

Rimonabant treated groups with high fat diet shows significant as well as dose dependant decrease in serum cholesterol, triglyceride, LDL-C, VLDL-C and Atherogenic index and increased HDL- cholesterol.

Table 2: Effect of Rimonabant on body weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial</th>
<th>5th day</th>
<th>10th day</th>
<th>15th day</th>
<th>20th day</th>
<th>25th day</th>
<th>30th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD</td>
<td>165±2.9</td>
<td>175±1.4</td>
<td>180±1.6</td>
<td>180±15</td>
<td>200±14</td>
<td>210±11</td>
<td>230±18</td>
</tr>
<tr>
<td>Control</td>
<td>166±4.3</td>
<td>167±2.5</td>
<td>160±4.9</td>
<td>160±6.4</td>
<td>160±6.7</td>
<td>170±8.2</td>
<td>160±6.9</td>
</tr>
<tr>
<td>Rimonabant 2.5 mg/kg</td>
<td>171±1.3</td>
<td>163±1.5</td>
<td>150±6.4*</td>
<td>140±11*</td>
<td>130±13**</td>
<td>130±14**</td>
<td>110±11**</td>
</tr>
<tr>
<td>Rimonabant 5 mg/kg</td>
<td>168±1.1</td>
<td>161±2.2</td>
<td>150±4.1*</td>
<td>150±4.5*</td>
<td>140±4.9**</td>
<td>140±4.3**</td>
<td>120±3.5**</td>
</tr>
<tr>
<td>Rimonabant 10 mg/kg</td>
<td>172±1.8</td>
<td>158±1.9</td>
<td>140±6.7**</td>
<td>120±6.5**</td>
<td>120±5.0**</td>
<td>120±6.2**</td>
<td>96±3.2**</td>
</tr>
<tr>
<td>Simvastatin 4 mg/kg</td>
<td>173±2.1</td>
<td>157±5.8</td>
<td>150±4.0*</td>
<td>140±3.7*</td>
<td>130±3.6**</td>
<td>130±4.0**</td>
<td>120±4.7**</td>
</tr>
</tbody>
</table>

Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett’s Multiple Comparative test *p< 0.05, **p<0.01, as compared with control.

(Significant weight increase in HFD treated group compared with control and significant weight reduction in Rimonabant treated group compared with HFD treated group)

Table 3: Effect of Rimonabant on food intake in high fat diet treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>5th days</th>
<th>10th days</th>
<th>15th days</th>
<th>20th days</th>
<th>25th days</th>
<th>30th days</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFD</td>
<td>22±2.2</td>
<td>27±1.67</td>
<td>28±2.4</td>
<td>26±0.92</td>
<td>29±2.1</td>
<td>24±1.21</td>
</tr>
<tr>
<td>Control</td>
<td>20±2.2</td>
<td>25±2.33</td>
<td>26±1.8</td>
<td>24±0.86</td>
<td>27±2.4</td>
<td>22±0.97</td>
</tr>
<tr>
<td>Rimonabant 2.5 mg/kg</td>
<td>11±0.71**</td>
<td>10±0.91**</td>
<td>8.6±0.40**</td>
<td>11±1.0**</td>
<td>11±1.2**</td>
<td>13±0.97**</td>
</tr>
<tr>
<td>Rimonabant 5 mg/kg</td>
<td>9±1.91**</td>
<td>8±0.87**</td>
<td>6.6±0.91**</td>
<td>9.4±1.6**</td>
<td>8.8±1.7**</td>
<td>11±0.54**</td>
</tr>
<tr>
<td>Rimonabant 10 mg/kg</td>
<td>7±1.54**</td>
<td>6±0.71**</td>
<td>4.6±0.54**</td>
<td>7.4±1.5**</td>
<td>6.8±0.65**</td>
<td>8.6±0.84**</td>
</tr>
<tr>
<td>Simvastatin 4 mg/kg</td>
<td>21±1.2</td>
<td>22±0.87</td>
<td>21±1.4</td>
<td>23±2.8</td>
<td>21±2.6</td>
<td>22±2.14</td>
</tr>
</tbody>
</table>

Value represents, Mean ± S.E.M. (n=6) ANOVA: Dunnett’s Multiple Comparative test *p< 0.05, **p<0.01, as compared with control. (Significant decrease in food intake in Rimonabant treated group compared with HFD treated group)
rent animal models is possible due to cholesterol feeding substitutes one of the best available tools.\textsuperscript{12} Rimonabant inhibits platelet activation and reduces pro-inflammatory chemokines and leukocytes in Zucker rats.\textsuperscript{19}

The antiplatelet therapy constitutes one of the best available tools for ameliorating the mechanisms related to atherogenesis and Rimonabant interestingly inhibited platelet aggregation.

Atherosclerosis is an inflammatory disease.\textsuperscript{20} In inflammation, protein denaturation occurs\textsuperscript{13} Denaturation affects nearly all physico-chemical properties of protein molecules. To approach an understanding of the behavior of anti-inflammatory agents, several kinds of denaturing conditions should be imposed and several different parameters are measured. In the present study we examined the influence of anti-inflammatory drugs on the denaturation of egg albumin induced by heat.\textsuperscript{21} Rimonabant prevent the denaturation of protein in the present study and shows the dose dependant effect.

**CONCLUSION**

In conclusion, the findings in this study suggest that the Rimonabant possesses antihyperlipidemic and antitherosclerotic Potential against high fat diet induced hyperlipidemia in experimental animals. These properties indicate its usefulness in obese patients with hyperlipidemia and or atherosclerosis.

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**REFERENCES**


