X. PUBLICATIONS


Hypocholesterolemic Effect of 3-Hydroxy-3-methylglutaric Acid

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In bacterial system, 3-hydroxy-3-methylglutaric acid (HMG) has been evaluated as an antimetabolite of mevalonic acid. Fimognari has shown that HMG inhibits the incorporation of acetate into mevalonate in rat liver preparations. This may explain the HMG inhibition of cholesterol synthesis from acetate as described by Rabenowitz and Gutt. There is certain rationale to the fact that a compound active as an inhibitor of cholesterol biosynthesis may have utility in the treatment of hypercholesterolemia and atherosclerosis, e.g., nicotinic acid, z phenylbutyric acid and α-p-biphenylbutyric acid. This prompted us to investigate the hypocholesterolemic properties of HMG in normal as well as hypercholesterolemic animals.

Materials and methods: 4 groups of young male albino rats, C D R strain, weighing 50 g and each group containing 6 animals were selected for experimental studies (Table I). Group I was kept as control and fed on basal diet. In addition to this diet, groups II, III, and IV received orally, varying doses of HMG in water (as indicated) for 7 or 14 days. At the end of treatment, the total serum cholesterol was determined by Bloor's method. For studies made in Table II, young male albino rats weighing about 100 g were maintained for 2 weeks on an experimental diet (basal diet containing 2% hydrogenated vegetable oils, 20 mg cholesterol and 10 mg sodium cholate as homogeneous water suspension, by intubation) in order to produce hypercholesterolemic conditions. The serum cholesterol level of such rats was found to be significantly elevated (192 ± 19 mg%, p < 0.001) as compared to rats kept on basal diet only (118 ± 5 mg%). The rats were then divided into groups of 5 each. The cholesterol-fed and cholesterol plus HMG-fed groups continued receiving experimental diet. However, in addition, the latter group received 50 mg HMG/kg/day in water. The experimental diet was replaced by basal diet for hypercholesterolemic control.
groups and HMG-fed groups. The latter received 50 mg HMG/kg/day in water for varying periods of HMG treatment (as indicated), the serum cholesterol was determined.

Results: From Table I it is evident that HMG administration to normal rats for 1 or 2 weeks significantly lowers the total serum cholesterol content. From the statistical evaluation of the data presented in Table II, it becomes

<table>
<thead>
<tr>
<th>Group No</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Basal diet</td>
<td>Basal diet + 10 mg HMG</td>
<td>Basal diet + 20 mg HMG</td>
<td>Basal diet + 30 mg HMG</td>
</tr>
<tr>
<td>7 days</td>
<td>151 ± 10</td>
<td>146 ± 2</td>
<td>136 ± 2</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>14 days</td>
<td>164 ± 4</td>
<td>147 ± 3</td>
<td>133 ± 4</td>
<td>130 ± 9</td>
</tr>
</tbody>
</table>

*All figures are average values expressed as total cholesterol content in mg%, with standard error, *dose of HMG/kg/day, * difference as compared with control group statistically significant *p < 0.05, *p < 0.01.

Table II: Effect of HMG and cholesterol + HMG feeding on serum cholesterol of hypercholesterolemic rats (average ± S.E.)

<table>
<thead>
<tr>
<th>HMG treatment (day)</th>
<th>Cholesterol fed group</th>
<th>Cholesterol + HMG-fed group</th>
<th>Hypercholesterolemic control group</th>
<th>HMG-fed group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>207 ± 10</td>
<td>139 ± 5</td>
<td>200 ± 6</td>
<td>133 ± 3</td>
</tr>
<tr>
<td>2nd</td>
<td>203 ± 4</td>
<td>129 ± 13</td>
<td>181 ± 4</td>
<td>138 ± 4</td>
</tr>
<tr>
<td>3rd</td>
<td>208 ± 2</td>
<td>172 ± 8</td>
<td>192 ± 6</td>
<td>161 ± 4</td>
</tr>
<tr>
<td>4th</td>
<td>-</td>
<td>-</td>
<td>181 ± 13</td>
<td>148 ± 3</td>
</tr>
<tr>
<td>5th</td>
<td>211 ± 14</td>
<td>167 ± 5</td>
<td>158 ± 5</td>
<td>162 ± 4</td>
</tr>
<tr>
<td>6th</td>
<td>-</td>
<td>-</td>
<td>139 ± 3</td>
<td>137 ± 3</td>
</tr>
</tbody>
</table>

*Difference as compared to respective control group statistically significant *p < 0.001, *p < 0.01.
Hypocholesterolemic Effect of
3-Hydroxy-3-methylglutaric Acid

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In bacterial system, 3-hydroxy-3-methylglutaric acid (HMG) has been evaluated as an antimetabolite of mevalonic acid. Fimognari has shown that HMG inhibits the incorporation of acetate into mevalonate in rat liver preparations. This may explain the HMG inhibition of cholesterol synthesis from acetate as described by Rabinowitz and Gurin. There is certain rationale to the fact that a compound active as an inhibitor of cholesterol biosynthesis may have utility in the treatment of hypercholesterolemia and atherosclerosis, e.g., nicotinic acid, alpha-phenylbutyric acid and alpha-biphenylbutyric acid. This prompted us to investigate the hypocholesterolemic property of HMG in normal as well as hypercholesterolemic animals. We have shown that administration (both orally and intraperitoneally) of HMG causes significant depression of serum and liver cholesterol levels of normal as well as hypercholesterolemic rats. However, it is interesting to mention that in normal rats there is no decrease in liver cholesterol content. To further strengthen this effect of HMG, the effect of combined feeding of HMG and cholesterol on serum and liver cholesterol content was studied. The data reveal that the combined feeding of HMG along with cholesterol does not permit rise in serum as well as liver cholesterol levels; on contrary, it causes 36 per cent and 40 per cent decrease in serum and liver cholesterol levels respectively, as compared to cholesterol-fed controls. It has also been shown that HMG-feeding not only decreases total cholesterol content but also causes reduction in esterified cholesterol, total esterified fatty acids, and triglyceride content of serum in normal as well as hypercholesterolemic rats.

The histopathological studies show that HMG-feeding to hypercholesterolemic rats did not bring any adverse effect at the cellular organization of the tissue. On contrary, HMG treatment of experimental animals causes quicker reversal of the fatty changes in liver, towards normalcy as compared to those animals in which the physiological lowering of cholesterol took place on its own accord.
The enzyme studies have demonstrated that cholesterol feeding increases HMG-CoA hydrolase activity with no effect on HMG-CoA reductase activity. HMG-feeding does not allow any increase or decrease of HMG-CoA hydrolase activity.

These investigations, coupled with the hypocholesterolemic property of HMG, suggest that HMG, like other inhibitors of cholesterol biosynthesis, may find a use in the treatment of hypercholesterolemia. The possible mechanism for hypocholesterolemic action of HMG will be discussed.

REFERENCES

clear that HMG feeding causes a significant depression in serum cholesterol levels of HMG-fed groups as compared to parallel hypercholesterolemic control groups. It was also observed that combined feeding of cholesterol along with HMG also significantly declines the serum cholesterol levels as compared to parallel cholesterol-fed groups. This further supports the hypocholesterolemic property of HMG. It is interesting to mention that no apparent harmful effects of HMG feeding were noticed on animals during the course of investigation. From other works it is known that, in vivo, HMG arises from HMG-CoA by the action of HMG-CoA hydrolase (EC 3.1.2.5), which strongly and competitively inhibits the HMG-CoA reductase (EC 1.1.1.34) activity in bacterial as well as rat liver preparations. These observations, coupled with the hypocholesterolemic property of HMG, suggest that HMG, like other inhibitors of cholesterol biosynthesis, may find a use in the treatment of hypercholesterolemia provided that in men this substance acts in the same way and is well tolerated. The detailed pharmacological screening of this compound is in progress and will be reported elsewhere in detail.

Zusammenfassung

Bei Futterung mit 3-hydroxy-3-methylglutarsäure (HMG) wurde eine bedeutende Senkung des Serum-Cholesterinspiegels sowohl in normalen, als auch in hypercholesterinämischen Ratten beobachtet. Bei gleichzeitiger Verabreichung von HMG und Cholesterol blieb die erwartete Zunahme beim Serumcholesterin aus und gleichzeitig wurde eine Abnahme des Cholesterinspiegels festgestellt.

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