Influence of MER-29 on Experimental Atherosclerosis of Cholesterol-Fed Cockerels

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With the technical assistance of Frances Farrow, Rose Shim, and David VanEndt

It has been reported that MER-29, 1-
[(4-diethylaminoethoxy) phenyl]-1-(p-tolyl)-
2-(p-chlorophenyl) ethanol, significantly lowers both serum and tissue cholesterol in rats and humans.1-4

Further investigation has demonstrated that drug-inhibited cholesterol synthesis in the body was due primarily to a block in a late stage of cholesterol synthesis.4 The present study was undertaken to study the effects of MER-29 on the blood lipids and on aortic and coronary atherosclerosis of cockerels placed on either a regimen of plain mash, or on an atherogenic diet which consisted of 2 per cent cholesterol plus 5 per cent cottonseed oil added to plain mash.

Methods

Forty-six ten-week-old Hy-Line Leghorn cockerels were used in the experiment for ten weeks. They were divided into six groups of cockerels as follows: (1) controls on plain mash; (2) cockerels on plain mash treated with 12.5 mg of MER-29 per Kg of body weight; (3) cockerels on plain mash treated with 25.0 mg of MER-29 per Kg of body weight; (4) cockerels fed an atherogenic diet only, and groups (5) and (6) similarly treated as the previous group (4) with the exception of the administration of 12.5 and 25.0 mg of MER-29 per Kg of body weight, respectively. The cockerels on plain mash and those on an atherogenic diet which were not injected with MER-29 received olive oil only. MER-29 was dissolved in olive oil and injected subcutaneously into the thigh, 0.5 cc per Kg body weight, five times a week, for ten consecutive weeks. The body weight and blood samples were taken when the experiment began and bimonthly thereafter. The blood samples were analyzed for total cholesterol by a modification of the methods of Sackett5 and Sperry and Webb6 and the phospholipids by Stewart.7

Upon the sacrifice of these cockerels, the aortae were opened longitudinally and gross grading of the degree of severity of aortic atherosclerosis was made by the method of Katz, which was from zero to four.8 Sections of the thoracic portion of the aortae and coronary arteries were taken at random and stained by the oil-red-O method.9

Results

Figure 1 shows the effects of MER-29 on the body weights of the various groups of cockerels on plain mash or on an atherogenic diet. From our data, it seems that the body weights of the cockerels did not change significantly on either plain mash or on an atherogenic diet when treated with either 12.5 or 25.0 mg of MER-29, as compared with the controls on plain mash or on an atherogenic diet only.

Figure 2 summarizes the changes in the blood cholesterol of cockerels treated with MER-29 on plain mash or on an atherogenic diet. The cholesterol values of cockerels on plain mash treated with MER-29 at the 12.5- or 25.0-mg. dose levels were not significantly different from those of the controls on plain mash. However, cockerels on an atherogenic diet showed a marked elevation in blood cholesterol when compared with those on plain mash, whether treated with MER-29 or not. The administration of MER-29 to cockerels on an atherogenic diet did not cause a significant difference in blood cholesterol, whether they had been treated with MER-29 or not.

Figure 3 indicates that MER-29 did not have any significant influence on the phospholipid patterns of cockerels on plain mash or...
on an atherogenic regimen, when compared with a similar group on the same diet.

Table 1 depicts the comparison of MER-29 on gross and microscopic grading of the aortae and coronary arteries of cockerels on plain mash or on an atherogenic diet.

No gross aortic atherosclerosis was observed in the groups on plain mash, except for the group injected with 12.5 mg. of MER-29, which showed that two of eight birds had atherosclerosis.

Cockerels on an atherogenic diet showed that four of eight had aortic atherosclerosis, while those similarly fed and treated with 12.5 or 25.0 mg. of MER-29 showed a slight increase in atherosclerosis which could be seen grossly. Microscopically, five of eight birds treated with the higher dose of MER-29 on plain mash had aortic atherosclerosis, as compared with one case in the controls on plain mash or cockerels treated with 12.5 mg. of the drug. All birds had microscopic lesions when fed an atherogenic regimen, whether treated with MER-29 or not. The groups treated with 25.0 mg. of MER-29 showed a marked infiltration of lipid into the intima and media, as compared with the controls on an atherogenic diet. Cockerels on plain mash only showed no coronary lesions, whereas one case was found in each group of cockerels treated with 12.5 or 25.0 mg. of MER-29 on plain mash. The birds fed on the atherogenic diet of 2 per cent cholesterol plus 5 per cent cottonseed oil added to plain mash showed an increase in coronary lesions when compared with the groups on plain mash. The group on an atherogenic regimen only showed seven of eight birds with coronary lesions. The administration of 12.5 mg. of MER-29 to the atherogenic birds resulted in all having coronary lesions, while at the 25.0-mg. level, five of seven cockerels had lesions.

Discussion

From our findings, it seems that the administration of MER-29, at the 12.5- and 25.0-mg. dose levels, did not markedly reduce the blood cholesterol or phospholipids of cockerels placed on plain mash or on an atherogenic diet. This is contrary to the reports of Blohm and Hollander that MER-29 administration lowers blood cholesterol on studies in rats and human subjects, respectively. This is probably due to a difference in species. It is interesting to note that injections of MER-29 at the 25.0-mg. level resulted in an increase of aortic atherosclerosis in the groups fed.
plain mash or on an atherogenic diet. Microscopically, the coronary lesions of cockerels on plain mash were enhanced by MER-29 at the 25.0-mg. level. The group fed an atherogenic diet and treated with 12.5 mg. of MER-29 showed an increase in coronary lesions as compared with a similar group on the same diet. However, the group treated with 25.0 mg. of the drug showed a slight decrease in coronary lesions.

It has been reported by Avigan et al. that the administration of MER-29 inhibits the conversion of 24-dehydrocholesterol (desmosterol) to cholesterol. The increased incidence of aortic atherosclerosis of cockerels treated with MER-29 may be due to the fact that there was an increase of 24-dehydrocholesterol. Since then, the aortae have been analyzed, and it has been found that cockerels injected with 12.5 and 25.0 mg. of MER-29 per Kg. of body weight had 12 per cent and 30 per cent, respectively, of desmosterol in their aortic sterols after ten weeks, as compared with little or none in those cockerels on plain mash without the drug. Analogous results in rabbits were recently reported by Avigan and Steinberg. The apparent difference between these results and those reported by Blankenhorn, who found little desmosterol in human arterial lesions after MER-29 treatment, is probably due to the fact that arterial lesions in humans must have been well-established before the beginning of therapy.

There have been questions raised as to the accuracy of blood cholesterol determinations by Bloor's or Sackett's method, and as a result, there has been a tendency to dismiss these methods for blood cholesterol. The

<table>
<thead>
<tr>
<th>Group</th>
<th>Regimen</th>
<th>Number of cockerels</th>
<th>Aortic Gross grading</th>
<th>Microscopic grading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Aortic No. of lesions</td>
<td>Per cent</td>
</tr>
<tr>
<td>1</td>
<td>Plain mash</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Plain mash + 12.5 mg. of MER-29/Kg.</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Plain mash + 25.0 mg. of MER-29/Kg.</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>A.D.</td>
<td>8</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>A.D. + 12.5 mg. of MER-29/Kg.</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>6</td>
<td>A.D. + 25.0 mg. of MER-29/Kg.</td>
<td>7</td>
<td>5</td>
<td>71.4</td>
</tr>
</tbody>
</table>

*Zero to four.

1 Atherogenic diet = 2 per cent cholesterol + 5 per cent cottonseed oil added to plain mash.
Sackett method, with a modification, was used to analyze our blood samples. For comparison, these same samples were analyzed by Dr. Harold Orvis of George Washington University School of Medicine, using the Sperry-Webb method. The data showed no differences. The modification of Sackett's method made by us was to allow the serum specimens to stand overnight to precipitate out the protein rather than raise the temperature of the contents in the flask to boiling by placing over a water bath as outlined by Sackett. Duffle, using Bloor's method and Sperry-Webb's method for cholesterol determinations, reported comparable data.

Summary

The effects of MER-29 were studied to determine whether this drug will lower the blood lipids as well as the severity of aortic and coronary atherosclerosis of ten-week-old cockerels on a regimen of plain mash or on an atherogenic diet consisting of 2 per cent cholesterol plus 5 per cent cottonseed oil added to plain mash. After ten weeks of treatment with 12.5 mg. or 25.0 mg. per Kg. of MER-29, it was observed that the drug had no significant influence on the blood cholesterol or phospholipid levels. It was interesting to note that cockerels treated with 25.0 mg. of MER-29 per Kg. body weight, whether on plain mash or on an atherogenic diet, demonstrated an increase of aortic atherosclerosis. The drug did not seem to lower the incidence of coronary atherosclerosis of the group fed an atherogenic diet. It appears that MER-29 causes aortic atherosclerosis of cockerels fed plain mash primarily by blocking the conversion of 24-dehydrocholesterol to cholesterol, thus increasing the amount of 24-dehydrocholesterol which was found in the aortae, as compared with little or none in the aortae of cockerels on plain mash.

Acknowledgment

We are grateful for the MER-29 supplied by the Win. S. Merrell Company, Cincinnati, Ohio, and for the assistance of Dr. Harold Orvis, George Washington University School of Medicine, Washington, D. C., in analyzing blood samples by using the Sperry-Webb method.

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