Mechanisms of Action of Cholestyramine in the Treatment of Hypercholesterolemia

By D. J. Nazir, Ph.D., L. Horlick, M.D., B. J. Kudchodkar, Ph.D., and H. S. Sodhi, M.D., Ph.D.

SUMMARY

Cholestyramine (12 g/day) was administered to four subjects with familial hypercholesterolemia (type II) for 12 to 15 days. Plasma cholesterol values fell by 24 to 28% in all subjects. Total endogenous fecal steroids increased from 2.0 to 2.5 times over the control values. This increment was mainly in the acidic fraction which increased from 1.4 to 6.5 times during treatment. The neutral steroid fraction showed a slight increase (nonsignificant) in two subjects and a significant increase (P < 0.05) in one subject. The total fecal steroid increment was considerably in excess of the decrement in plasma cholesterol, thus indicating either (a) a substantial increase in cholesterol synthesis, (b) a transfer of cholesterol from depots, or (c) both. Plasma cholesterol specific activity time curves showed a sharp increase in the slope immediately after the commencement of treatment, reflecting an increase in the rate of entry of unlabeled cholesterol into the readily miscible pool. Since cholestyramine did not change the absorption of the dietary cholesterol, the increase in the contribution of unlabeled cholesterol could only be from an increase in endogenous synthesis. In accordance with previous findings from this laboratory, no evidence of degradation of the steroid nucleus was detected during its passage through the gastrointestinal tract.

Additional Indexing Words:

Plasma cholesterol  Cholesterol synthesis  Fecal neutral steroids
Cholesterol absorption  Cholesterol degradation  Fecal bile acids

Cholestyramine is an insoluble chloride salt of a basic anion-exchange resin which contains quaternary ammonium groups attached to a styrene-2% divinylbenzene skeleton. It is neither digested nor absorbed, and it binds bile acids in the gastrointestinal tract, thereby interfering with their enterohepatic circulation. Its use in the treatment of hypercholesterolemia in man was first reported in 1959 by Bergen and associates and subsequently confirmed by a number of other investigators. Carey and Williams were the first to demonstrate that cholestyramine increased the fecal excretion of bile acids in man. This sequestration of bile acids from the enterohepatic circulation results in an enhanced conversion of cholesterol to bile acids by the liver.

Only a small number of individuals treated with cholestyramine have been studied by rigorously controlled steroid balance technics. We wish to report the results of such a study in four hypercholesterolemic individuals. Our observations on absorption and degradation of neutral steroids in these subjects are also presented.

Methods

Clinical data on the four subjects studied are given in table 1. Fifty µCi of 4-14C-cholesterol or 100 µCi of 1,2-3H-cholesterol dissolved in 1 ml ethanol were dispersed in 100 ml of saline for intravenous
The former was given to L.M. and the latter to P.K. Subject R.T. received 100 μCi of nL-mevalonic acid 2-3H-lactone intravenously. To permit equilibration of radioactive cholesterol between plasma and other body pools and also equilibration of the specific activity (SA) of the bile and fecal metabolites of plasma cholesterol,12 4 to 6 weeks were allowed to elapse from day 0 (the day of injection) before beginning the studies.

The subjects received a diet of natural food of constant composition which closely resembled their habitual diet. The diet was prepared from a single pool of food and sufficient daily servings were prepared and frozen to last for the duration of the study. A trial period of 7 days was allowed before beginning the metabolic studies so that the subjects might become accustomed to the diet and corrections for weight changes might be made. During the studies the weights of all subjects were maintained at steady levels. Balance studies were performed both during control (6 to 12 days) and treatment (12 to 15 days) periods. The treatment consisted of cholestyramine 4 g three times per day.

Cholesterol and β-sitosterol contents of the diet were determined by the method of Miettinen and associates.13 Chronic oxide (100 mg) was given with three meals daily, 2 weeks prior to and during the metabolic studies to correct for variations in stool flow.14

Fasting blood samples were collected three times a week for determination of the specific activity of plasma cholesterol.15 Feces were collected in 3-day pools for the extraction of neutral11 and acidic16 steroids for determining the amounts of fecal metabolites of cholesterol as described previously.15 Time taken for the duodenal contents to appear in the feces was assumed to be 36 hours. The SA of plasma cholesterol was assumed to be equal to the SA of fecal bile acids 56 hours before the midpoint in time of collection of fecal pools (unpublished data from this laboratory).

The results obtained were corrected for variations in stool flow and losses of neutral steroids during their transit through the gastrointestinal tract by determination of the chromic oxide17 and β-sitosterol and its derivatives in the feces.18

Since subject L.K. was only 16 years old, she was not given radioisotopes. Her feces were collected and processed as described, except that the fecal neutral steroids and bile acids were determined by gas-liquid chromatography (GLC).13 Cholesterol absorption was determined19 in two subjects, one (L.M.) receiving a diet low in cholesterol (150 mg/day) and the

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### Table 1

<table>
<thead>
<tr>
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<th>Sex</th>
<th>Age</th>
<th>Clinical diagnosis</th>
<th>FH†</th>
<th>FH*</th>
<th>CHD*</th>
<th>CHD†</th>
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<tr>
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<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1. R.T.</td>
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<td>CHD; coronary heart disease</td>
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<td>2. L.K.</td>
<td>F</td>
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<td>Mathematically x.blood cholesterol</td>
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<td>3. L.M.</td>
<td>F</td>
<td>49</td>
<td>FH†; FH*; FH*; FH*; FH*</td>
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*FH† = familial history of premature atherosclerotic heart disease; CHD* = coronary heart disease.

†This patient was receiving niacin 1 g three times daily.

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*FH† = familial history of premature atherosclerotic heart disease; CHD* = coronary heart disease.

†This patient was receiving niacin 1 g three times daily.
other (P.K.) receiving a diet high in cholesterol (827 mg/day).

The plasma volume was assumed to be 4.5% of the body weight in kilograms.20 The total cholesterol in the plasma pool was obtained by multiplying its concentrations with the plasma volume. The daily changes in the plasma pool of cholesterol were calculated by dividing the difference between the two values by the number of days during which the observed difference occurred.

Grundy and associates11 noted that following ileal exclusion complete isotopic equilibration between biliary cholesterol and plasma cholesterol was not attained. They attributed this to the marked increase in the rate of cholesterol synthesis after ileal exclusion so that newly synthesized cholesterol was secreted into the intestinal canal before complete isotopic exchange could occur with plasma cholesterol. This would result in underestimation of values for cholesterol absorption using their method I. Since rates of cholesterol synthesis increases markedly in subjects treated with cholestyramine and problems similar to those with ileal bypass may conceivably occur, our data for absorption may be underestimates of the true absorption.

Table 2

Changes in Plasma Cholesterol on Treatment with Cholestyramine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma cholesterol (mg/100 ml ± se)</th>
<th>Decrease in plasma cholesterol (%)</th>
<th>Change in plasma cholesterol content (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>1. R.T.</td>
<td>251 ± 29 (6)*</td>
<td>187 ± 10 (5)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>2. L.K.</td>
<td>373 ± 19 (6)</td>
<td>284 ± 15 (3)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>3. L.M.</td>
<td>373 ± 15 (5)</td>
<td>279 ± 13 (5)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>4. P.K.</td>
<td>403 ± 13 (6)</td>
<td>292 ± 10 (3)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Mean</td>
<td>349 ± 64</td>
<td>254 ± 48</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses give number of determinations.

Results

Plasma Cholesterol Concentrations
(Fig. 1, Table 2)

All the subjects showed a decline in plasma cholesterol concentration. The extent of the decrease in plasma cholesterol concentration was determined by averaging all the pretreatment values and subtracting the average of the last three to five values available for the treatment period (fig. 1, table 2). The fall in plasma cholesterol was significant in each of the four subjects and ranged between 24 and 28%. Fall in concentration did not correlate well with the pretreatment levels of plasma cholesterol (r = 0.39).

Specific Activity of Plasma Cholesterol (Fig. 1)

The decline in the specific activity of plasma cholesterol had become exponential in all subjects before the experimental studies were begun. The fractional turnover rates of the plasma cholesterol (table 3) after the start of

Table 3

Kinetics of Plasma Cholesterol

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fractional turnover rate</th>
<th>Total unlabeled cholesterol* entering pool A (mg/day)</th>
<th>Cholesterol synthesis† (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (%)/day</td>
<td>Treatment (%)/day</td>
<td>Increase during treatment (%)</td>
</tr>
<tr>
<td>1. R.T.</td>
<td>1.98</td>
<td>3.85</td>
<td>94.44</td>
</tr>
<tr>
<td>3. L.M.</td>
<td>2.66</td>
<td>5.33</td>
<td>100.38</td>
</tr>
<tr>
<td>4. P.K.</td>
<td>1.10</td>
<td>2.88</td>
<td>161.82</td>
</tr>
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</table>

*From absorption plus synthesis.
†Total minus absorption. It was assumed that all the newly synthesized cholesterol entered pool A.

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L.M.

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treatment were twice those during the pre-treatment period in R.T. and L.M. and 2.5 times those in the case of P.K. Assuming that all newly synthesized cholesterol entered pool A, the values of endogenous synthesis were estimated from the net turnover rates and daily absorption of exogenous cholesterol. The endogenous synthesis was markedly increased in both subjects examined (table 3).

Absorption of Dietary Cholesterol

The intake of dietary cholesterol was constant throughout the period of investigation, and cholestyramine had no effect on its absorption in the two subjects (31 and 29% in L.M. and 62 and 66% in P.K.) investigated. The higher percentage of absorption seen in P.K. who also had a higher (827 mg/day) intake of cholesterol than L.M. (150 mg/day) may reflect either the biologic variations or the effect of other factors such as the differences in their dietary fats.

Fecal Neutral Steroids (Tables 4 and 5)

The excretion of endogenous cholesterol and its bacterial degradation products in the feces were determined by the isotopic method. Although the treatment with cholestyramine increased the excretion in all subjects, the increases were not sufficient to reach the level of significance except in one subject (P.K.). Similarly, increases in the total fecal neutral steroids derived from the endogenous and unabsorbed dietary cholesterol as determined by GLC were also apparent but failed to reach the level of significance (table 5).

The gas chromatographic analysis also permitted us to calculate the fecal recoveries of dietary ß-sitosterol and from this to deduce the extent of steroid degradation. Recoveries were the same (88 to 102%) during both control and treatment periods, indicating that degradation of dietary sterols during the passage through the intestinal tract was not significant.

Table 4

Changes in Endogenous Fecal Steroid Excretion on Treatment with Cholestyramine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Fecal endogenous neutral steroids (mg/day ± sd)</th>
<th>Fecal acidic steroids (mg/day ± sd)</th>
<th>Total fecal endogenous steroids (mg/day ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treatment</td>
<td>Control</td>
</tr>
<tr>
<td>R.T.</td>
<td>498 ± 115</td>
<td>570 ± 139</td>
<td>73 ± 29</td>
</tr>
<tr>
<td></td>
<td>(12:4)*</td>
<td>(15:5)*</td>
<td></td>
</tr>
<tr>
<td>L.K.</td>
<td>277 ± 112†</td>
<td>654 ± 217†</td>
<td>73 ± 29</td>
</tr>
<tr>
<td></td>
<td>(12:4)</td>
<td>(12:4)</td>
<td></td>
</tr>
<tr>
<td>L.M.</td>
<td>265 ± 35</td>
<td>322 ± 51</td>
<td>102 ± 74</td>
</tr>
<tr>
<td></td>
<td>(6:2)</td>
<td>(12:4)</td>
<td>(12:4)</td>
</tr>
<tr>
<td>P.K.</td>
<td>457 ± 52</td>
<td>623 ± 134</td>
<td>119 ± 15</td>
</tr>
<tr>
<td></td>
<td>(12:4)</td>
<td>(12:4)</td>
<td>(12:4)</td>
</tr>
</tbody>
</table>

*Determination by gas-liquid chromatography.

Figure 1

Effect of cholestyramine (12 g/day) on plasma cholesterol, plasma cholesterol specific activity (DPM/mg), body weight, and excretion of fecal neutral and acidic steroids of the four patients. Rx = time when treatment was begun. Day 0 = date of administration of the radioactive isotope. The bars indicating fecal neutral and acidic steroid excretions are superimposed on one another.
Fecal Bile Acids (Fig. 1, Table 4)

The fecal excretion of bile acids was considerably less than the excretion of neutral steroids during the control period. Each of the four patients showed a marked increase in excretion of bile acids when cholestyramine was given. Total fecal neutral and acidic steroids derived from endogenous cholesterol also increased significantly on treatment with cholestyramine.

Total Steroid Balance (Table 6)

Negative balance during the control period indicated that the synthesis of endogenous cholesterol during that period was between 61 and 478 mg/day. After starting treatment with cholestyramine, there were significant increases in the negative balance suggesting (a) increase in synthesis of endogenous cholesterol, (b) mobilization of cholesterol from body pools, or (c) both.

The changes in the specific activity slopes of plasma cholesterol indicated that the entry of unlabeled cholesterol (from synthesis and absorption) into pool A was increased by 94 and 162% (Table 3). The amounts of unlabeled cholesterol derived from absorption were available from cholesterol balance studies, and the amounts derived from the synthesis could be calculated (Table 3). The increases in the negative balance (L.M. and P.K.) could almost be accounted for by the increases in endogenous synthesis and the losses from plasma pool of cholesterol (Tables 2 and 3).

Discussion

The data presented in this paper relate to relatively short periods (12 to 15 days) of treatment with cholestyramine under controlled conditions. The results obtained, therefore, cannot be extrapolated to long-term studies with cholestyramine. During the brief period of observations concentrations of plasma cholesterol declined promptly in all subjects (24 to 28%). One of the subjects (P.K.) had already been treated with nicotinic acid and had reached a plateau when cholestyramine was added to the treatment. Treatment with cholestyramine resulted in a further decline in the concentrations of plasma cholesterol.}

Table 5

<table>
<thead>
<tr>
<th>Patient</th>
<th>Control (mg/day)</th>
<th>Treatment (mg/day)</th>
<th>Net change (mg)</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. L.K.</td>
<td>381 ± 150</td>
<td>522 ± 126</td>
<td>+141</td>
<td>37.0</td>
</tr>
<tr>
<td>3. L.M.</td>
<td>368 ± 92</td>
<td>428 ± 91</td>
<td>+60</td>
<td>16.3</td>
</tr>
<tr>
<td>4. P.K.</td>
<td>769 ± 70</td>
<td>899 ± 240</td>
<td>+130</td>
<td>16.9</td>
</tr>
</tbody>
</table>

Table 6

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cholesterol intake (mg/day)</th>
<th>Cholesterol excretion (mg/d)</th>
<th>Total steroid excretion (mg/day)</th>
<th>Cholesterol balance (mg/day)</th>
<th>Difference in cholesterol balance (mg/day)</th>
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<tr>
<td>2. L.K.</td>
<td>180</td>
<td>658</td>
<td>-478</td>
<td>1176</td>
<td>-996</td>
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<tr>
<td>3. L.M.</td>
<td>150</td>
<td>470</td>
<td>-320</td>
<td>1023</td>
<td>-873</td>
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<tr>
<td>4. P.K.</td>
<td>827</td>
<td>888</td>
<td>-61</td>
<td>1492</td>
<td>-665</td>
</tr>
</tbody>
</table>

*GLC = gas-liquid chromatography.
cholesterol. Reductions in plasma cholesterol concentrations have previously been reported by several investigators, however, patients presumed to be homozygous for familial type II hyperlipoproteinemia usually have failed to show any consistent effect of cholestyramine on plasma cholesterol concentrations. The failure in such instances may be due either to an enhanced synthetic capacity of the liver to compensate for the losses of cholesterol and its derivatives or to mobilization of cholesterol from massive tissue deposits into the plasma, thereby maintaining the plasma concentration.

Prompt increases in the hepatic synthesis of cholesterol have previously been reported by Goodman and Noble and Miettinen in subjects given cholestyramine. Our results support their findings. A marked increase in the rate of decline of plasma cholesterol SA after starting the treatment with cholestyramine reflects an increase in the rate of entry of unlabeled cholesterol into the readily miscible pool. Since there were no changes either in the intake or the absorption of dietary cholesterol, the increased rate of entry of unlabeled cholesterol could only result from an increase in the endogenous synthesis (barring any cholestyramine-stimulated mobilization from pools not exchanging significantly with plasma cholesterol).

The values of the unlabeled cholesterol entering into the readily miscible pool from endogenous synthesis and from absorption of the dietary cholesterol are available in two subjects. In one (L.M.), the absorption was only 47 mg/day and the endogenous synthesis was 320 mg/day whereas in the other (P.K.) the absorption was 515 mg/day and synthesis was only 61 mg/day. The total amounts of entry of unlabeled cholesterol in these two subjects were therefore 367 mg/day and 576 mg/day during the pretreatment periods. The increase in fractional turnover rates in these two subjects were about 100 and 160% (table 3). Since the amounts of cholesterol absorbed remained constant, the values of cholesterol from endogenous synthesis were derived. They were 572 mg in L.M. and 895 mg in P.K. (table 3): an increase of about two times in L.M. and 15 times in P.K. The fecal excretion of total steroids during the treatment period was greater than could be accounted for by the intake and the increased synthesis. The extra cholesterol, 300 to 325 mg/day, must have been derived either from plasma or from tissue pools. The losses from the plasma pool (in L.M. and P.K.) were about 200 mg/day, suggesting that losses from tissue pools, if any, were minor. Decrease in the size of xanthomatosus deposits on long-term treatment with cholestyramine would be in accord with slow mobilization of cholesterol in tissue pools.

The most dramatic effect of cholestyramine treatment was on the fecal loss of bile acids, which increased two- to sixfold. Increases in the fecal excretion of neutral steroids were also seen, but they failed to reach the level of significance. These data are in accord with the previous publications. The concentration of bile acids in portal venous blood is believed to regulate hepatic synthesis of bile acids. Cholestyramine interferes with reabsorption of bile acids in the small intestine and, therefore, results in compensatory increase in the bile acid synthesis from cholesterol. Somehow, this in turn causes a marked increase in the synthesis of cholesterol.

There was no evidence of significant degradation of dietary steroids during their passage through the gastrointestinal tract. This is in accord with previous reports from our laboratories and lends support to the suggestion of DenBesten and associates that the steroid degradation may be specific to formula diets lacking in cellulose.

Acknowledgment

The technical assistance of Mrs. Marcia McNeil is gratefully acknowledged.

References

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