Inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase by mevinolin in familial hypercholesterolemia heterozygotes: Effects on cholesterol balance

(cholesterol balance/low-density lipoprotein receptors/fecal neutral and acidic steroids)

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ABSTRACT Patients with heterozygous familial hypercholesterolemia (FH) have a deficiency of receptors for plasma low-density lipoprotein (LDL) that impairs removal of LDL from plasma. In these patients, mevinolin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase [mevalonate:NAD+ oxidoreductase (CoA-acylating), EC 1.1.1.88], increases receptors for LDL and decreases LDL concentrations. To determine whether mevinolin also causes severe decreases in total body synthesis of cholesterol, fecal excretions of neutral steroids and acidic steroids were determined in five FH heterozygotes before and during treatment with mevinolin. The drug produced an average decrease in plasma total cholesterol of 23% and in LDL cholesterol of 24%. Mevinolin caused a significant decrease in the output of neutral and acidic steroids in three patients, but it caused no alterations in two others. Changes in fecal output of steroids did not correlate with the degree of lowering of the patients' LDL-cholesterol level. In none of the patients did the output of fecal steroids fall below the values seen in normal subjects studied under similar conditions. One patient had a previous ileal exclusion operation and had a massive output of acidic steroids in the control period; mevinolin therapy caused a slight decrease in excretion of acidic steroids, but the output was still markedly above normal. We conclude that the LDL lowering action of mevinolin does not appear to require a severe decrease in cholesterol synthesis that might lead to depletion of vital body stores of cholesterol.

An exciting class of drugs for treatment of hypercholesterolemia consists of fungal metabolites that are competitive inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase [HMG-CoA reductase; mevalonate:NAD+ oxidoreductase (CoA-acylating), EC 1.1.1.88], a rate-controlling enzyme in cholesterol synthesis. The prototype compound, compactin, and its analogue, mevinolin, decrease the levels of low-density lipoprotein (LDL) in normal animals (1, 2), normal humans (3), and in heterozygotes with familial hypercholesterolemia (FH) (4–7). Recently, we demonstrated that mevinolin achieves this effect by increasing the fractional rate of removal of LDL from the circulation of FH heterozygotes (7). The higher clearance rate appeared to be due to enhanced receptor-mediated catabolism of LDL.

A theoretical basis for the ability of mevinolin to stimulate receptor-mediated catabolism of LDL comes from studies of human and animal cells in tissue culture. These cells have a dual source of the cholesterol required for synthesis of new membranes. Cells can synthesize cholesterol de novo in a pathway that requires the action of HMG-CoA reductase; or, the cells can obtain cholesterol from plasma LDL through endocytosis mediated by a specific LDL receptor (8).

When human fibroblasts or cultured liver cells are incubated with compactin or mevinolin, HMG-CoA reductase is competitively inhibited, and the de novo synthesis of cholesterol is blocked (9, 10). This block triggers a dual regulatory response that seems designed to provide more cholesterol for the cell: there is a simultaneous increase in HMG-CoA reductase and in the number of LDL receptors (9, 11, 12).

In dogs, mevinolin has been shown to decrease plasma LDL by causing an increase in hepatic receptors for LDL (2); the same response probably occurs in humans (7). Mevinolin thus seems to have the same effect in vivo as in cultured cells; i.e., it inhibits HMG-CoA reductase and triggers a regulatory response that increases LDL receptors and probably also increases the amount of HMG-CoA reductase.

The question that arises is whether HMG-CoA reductase can increase sufficiently to return cholesterol synthesis to normal or whether cholesterol synthesis must remain depressed for mevinolin to maintain an increase in LDL receptors. In the current study, we have attempted to distinguish between these two possibilities in humans by use of the cholesterol-balance technique.

Normal humans excrete more steroids in the feces than can be accounted for by the cholesterol that they ingest. These steroids emerge as neutral steroids and bile acids. Since the steroid nucleus is not degraded in the body, and since the feces are the predominant route of steroid excretion, the net fecal steroid excretion (cholesterol intake minus steroid output) in the steady state approximates the total amount of cholesterol synthesized in the body (13).

In the current investigation we treated five heterozygotes for familial hypercholesterolemia with mevinolin and measured the net excretion of fecal steroids. This cholesterol balance study was conducted as part of another study in which turnover of LDL was measured (7). The results indicate that mevinolin can cause a moderate decrease in total-body synthesis of cholesterol, but a marked overall decrease in synthesis seemingly is not necessary for mevinolin to enhance the activity of LDL receptors.

METHODS

Patients. Five patients with heterozygous FH were studied on the General Clinical Research Center at Parkland Memorial Hospital or in the Metabolic Unit at the Veterans Administration Medical Center (Dallas, TX). Two patients (M.B. and J.P.) were men of ages 36 and 37 yr, respectively; J.P.

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had severe coronary heart disease, but M.B. had no clinical evidence of atherosclerosis. The remaining three patients (C.C., J.C., and M.Ba.) were women of ages 52, 32, and 55 yr, respectively. C.C. had coronary heart disease, but J.C. and M.Ba. were clinically free of ischemic heart disease. J.C. is the daughter of M.Ba.; M.B. and C.C. are first cousins.

**Diets.** The patients were fed a diet of solid food and liquid formula containing 40% of calories as fat, 40% as carbohydrate, and 20% as protein (7). The major fat of the diet was lard, and the polyunsaturated/saturated fat ratio was 0.33. Cholesterol intake varied between 170 and 280 mg per day. Weights were checked daily and caloric intake was adjusted to maintain a constant weight throughout the study.

**Drug Therapy.** Capsules containing mevinolin and placebo were supplied by Merck Sharp & Dohme (courtesy of J. Tobert). Mevinolin was given in doses of 20 mg twice daily. Two patients (J.C. and M.Ba.) received mevinolin first and then placebo; the other three (J.P., C.C., and M.Ba.) were given placebo before mevinolin. The protocol was approved by the U.S. Food and Drug Administration and by the Human Research Review Boards of the University of Texas Health Science Center and the Veterans Administration Medical Center. Informed consent was obtained from each patient.

**Lipid and Lipoprotein Analysis.** In each patient, the kinetics of LDL turnover were measured in each study period, and the results have been reported separately (7). These turnover studies were initiated about 3 weeks into each period. During the kinetic study, which lasted 20 days, blood samples were obtained daily, and total cholesterol was measured on each sample. In this period, samples were taken 6 or 7 times for estimation of triglycerides, LDL cholesterol, and high-density lipoprotein (LDL) cholesterol. Each sample was subjected to ultracentrifugation at density <1.006 g/ml for isolation of very-low-density lipoprotein (VLDL). Cholesterol was measured on the infranatant both before and after precipitation of LDL with heparin-manganese according to Lipid Research Clinic procedures (14). Cholesterol and triglyceride concentrations were determined using a Boehringer Mannheim enzyme kit.

**Sterol Balance.** Cholesterol balance was performed according to described methods (15, 18). Measurements were not made in one patient (T.P.), who had been studied previously (7). Serial stool collections were obtained and combined into 4-day pools during each study period (mevinolin...
FIG. 1. Cholesterol balance studies in five patients treated with mevinolin. Patients J.P., M.Ba., and C.C. received placebo first, and M.B. and J.C. received mevinolin first. Plasma cholesterol levels are those obtained during the LDL turnover (7). These turnover studies generally were started after the patient had been on mevinolin for about 2 weeks. Generally, stool collections were started after allowing 4 days for equilibration on internal markers. However, in M.B., balance studies were not begun until the time of the LDL turnover. Acidic steroids are equivalent to bile acids; neutral steroids include cholesterol and its bacterial conversion products, coprostanol and coprostanone.
Table 3. Comparison of cholesterol balance between current patients and control subjects

<table>
<thead>
<tr>
<th>Patient</th>
<th>Period</th>
<th>Cholesterol intake, mg/day</th>
<th>Fecal neutral steroids, mg/kg/day</th>
<th>Fecal acidic steroids, mg/kg/day</th>
<th>Fecal total steroids, mg/kg/day</th>
<th>Cholesterol balance, mg/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.B.</td>
<td>Control</td>
<td>2.5</td>
<td>8.9</td>
<td>8.2</td>
<td>17.1</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>Mevinolin</td>
<td>2.5</td>
<td>7.3</td>
<td>3.4</td>
<td>10.7</td>
<td>8.17</td>
</tr>
<tr>
<td>J.P.</td>
<td>Control</td>
<td>2.3</td>
<td>11.4</td>
<td>4.2</td>
<td>15.4</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>Mevinolin</td>
<td>2.3</td>
<td>8.6</td>
<td>3.9</td>
<td>12.6</td>
<td>10.2</td>
</tr>
<tr>
<td>J.C.</td>
<td>Control</td>
<td>4.0</td>
<td>9.4</td>
<td>3.0</td>
<td>12.4</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>Mevinolin</td>
<td>4.0</td>
<td>8.7</td>
<td>2.8</td>
<td>11.5</td>
<td>7.5</td>
</tr>
<tr>
<td>M.Ba.</td>
<td>Control</td>
<td>1.3</td>
<td>9.2</td>
<td>1.6</td>
<td>10.8</td>
<td>9.5</td>
</tr>
<tr>
<td></td>
<td>Mevinolin</td>
<td>1.3</td>
<td>9.1</td>
<td>1.8</td>
<td>11.2</td>
<td>10.0</td>
</tr>
<tr>
<td>C.C.</td>
<td>Control</td>
<td>2.6</td>
<td>9.2</td>
<td>42.9</td>
<td>52.1</td>
<td>49.4</td>
</tr>
<tr>
<td></td>
<td>Mevinolin</td>
<td>2.6</td>
<td>7.1</td>
<td>33.9</td>
<td>41.9</td>
<td>39.2</td>
</tr>
<tr>
<td>Normal adult mean*</td>
<td></td>
<td>1.8 ± 0.5</td>
<td>6.5 ± 0.5</td>
<td>4.9 ± 0.5</td>
<td>11.4 ± 0.7</td>
<td>9.6 ± 0.6</td>
</tr>
</tbody>
</table>

*These data are expressed as mean ± SEM and have been presented previously (19).

and placebo. On each pool, measurements were made of neutral steroids (15) and acidic steroids (16). These measurements were carried out entirely by chemical means using gas–liquid chromatography for quantification of steroids. Excretions of neutral and acidic steroids were calculated using β-sitosterol (17) and chromic oxide (18) as internal standards. For controls, 14 normal men were studied on a similar diet, and stool samples were analyzed by the same methods. Ages of these men ranged from 29 to 63 yr (mean, 52 yr); their weights were 95–105% of ideal. Sterol balance data from the normal men have been presented (19).

RESULTS

Mevinolin therapy produced a decrease in plasma total cholesterol in all patients (Table 1); the mean decrease for the five patients was 22.6%. Plasma triglyceride levels were not changed for the group, although two individuals (M.B. and C.C.) had sizable decreases. Plasma LDL cholesterol fell during mevinolin therapy by 24.2%, while levels of HDL cholesterol were unchanged. The turnover studies demonstrated that this drop in LDL cholesterol is due to enhanced receptor-mediated removal of LDL from plasma (7).

The effects of mevinolin on cholesterol balance are shown in Table 2 and Fig. 1. One patient (C.C.) had undergone a previous ileal bypass operation and therefore showed a large output of bile acids during the control period. Mevinolin produced significant decreases in outputs of neutral steroids in three patients (M.B., J.P., and C.C.), but no significant changes were noted in two others (M.Ba. and J.C.). Changes in output of acidic steroids paralleled those of neutral steroids in all patients. Two patients (M.B. and C.C.), who were first cousins, had the greatest decreases in total steroid excretion during mevinolin treatment. Patient C.C., who had the ileal exclusion, had a slight decrease in output of acidic steroids when receiving mevinolin, but this output was still markedly above normal despite mevinolin therapy.

Patient J.C. had no overall reduction in steroid excretion when the mevinolin treatment period as a whole was considered, but there was a downward trend in steroid output during the last 12 days of the period (642 ± 31 mg/day); the latter was significantly lower than that of the whole control period (867 ± 118 mg/day, P < 0.005). However, a downward trend was not noted in J.C.’s mother (M.Ba.), and one of the other patients who had reduced steroid outputs during mevinolin treatment showed downward trends in steroid excretion rates during the treatment period (i.e., the mevinolin effect appeared to be maximal within the first week).

In Table 3, the balance data are expressed per kg of total body weight. The results are compared to those for 14 normal men studied in this laboratory under similar experimen-

tal conditions (19). In none of the FH patients did mevinolin cause cholesterol balance to fall below the range seen in normal subjects.

Table 4 compares the percent change in LDL-cholesterol with the percent change in cholesterol balance induced by mevinolin in each patient. Of interest, patient M.B., who had the greatest decrease in cholesterol balance (44%), had the least decrease in LDL cholesterol (12%); overall there was no correlation between the change in cholesterol balance and the fall in LDL cholesterol during mevinolin treatment.

DISCUSSION

In a recent study we demonstrated that mevinolin lowers plasma LDL by enhancing receptor-mediated clearance of lipoprotein (7). This action presumably is due to an increased synthesis of LDL receptors secondary to inhibition of HMG-CoA reductase activity. The latter action should be associated with a decrease in cholesterol synthesis; the degree of inhibition of cholesterol synthesis required to produce the observed effect on plasma LDL is unknown. If a severe decrease in cholesterol synthesis is required, a decreased availability of cholesterol for vital functions—cellular membrane structure and synthesis of steroid hormones and bile acids—might result. The purpose of the present study was to determine the extent to which mevinolin inhibits whole-body synthesis of cholesterol and particularly to learn whether synthesis is seriously decreased.

The method used to estimate cholesterol synthesis was the balance method. This method has the advantage of giving a daily measurement of steroid excretion over a period of several weeks, from which one can estimate cholesterol synthesis. It has the disadvantage of being unable to distinguish between fecal steroids derived from newly synthesized cholesterol and from preexisting tissue cholesterol. Any reduction in total steroid excretion during mevinolin treatment almost certainly would reflect a decrease in total cholesterol.

Table 4. Comparison of changes in plasma LDL cholesterol and cholesterol balance (mevinolin period vs. control period)

<table>
<thead>
<tr>
<th>Patient</th>
<th>% change in plasma LDL cholesterol* (P value)</th>
<th>% change in cholesterol balance* (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.B.</td>
<td>−12 (&lt;0.02)</td>
<td>−44 (&lt;0.001)</td>
</tr>
<tr>
<td>J.P.</td>
<td>−25 (&lt;0.001)</td>
<td>−22 (&lt;0.001)</td>
</tr>
<tr>
<td>J.C.</td>
<td>−28 (&lt;0.001)</td>
<td>−11 (NS)</td>
</tr>
<tr>
<td>M.Ba.</td>
<td>−25 (&lt;0.001)</td>
<td>+5 (NS)</td>
</tr>
<tr>
<td>C.C.</td>
<td>−32 (&lt;0.001)</td>
<td>−24 (&lt;0.01)</td>
</tr>
</tbody>
</table>

*NS, not significant.

*Mevinolin period minus control period.

†P values calculated by Student’s t test.
synthesis. A severe decrease in steroid output would signify a corresponding reduction in synthesis of cholesterol and would be a cause for concern. On the other hand, a severe reduction in cholesterol synthesis might be masked by mobilization of cholesterol from tissue pools, so that cholesterol balance would continue to be normal. This response could not be continued indefinitely, or tissue pools would become totally depleted; eventually fecal steroid excretion would show a decline.

The data of this study indicated that mevinolin does cause a detectable decrease in cholesterol synthesis in some patients. The percentage reductions in cholesterol excretion in three patients (22%, 24%, and 44%) represent a minimum decrease in whole-body synthesis of cholesterol. The findings in these patients thus support the concept that the LDL lowering action of mevinolin is mediated by the action of the drug on cholesterol synthesis.

Although these reductions in cholesterol balance were not marked, we can ask whether cholesterol synthesis might have been reduced severely, and yet this change was obscured by mobilization of cholesterol from preexisting pools. The results in patient C.C. make this very unlikely. This patient had a previous ileal excision operation (20) and thus had a massive increase in synthesis and excretion of bile acids (21). During the period of mevinolin therapy, massive excretion of bile acid continued with no sign of progressive diminution in output. The net loss of steroid during 40 days of mevinolin therapy was 99 g. Since total-body stores of cholesterol are only ~100 g (22) and do not greatly exceed this value even in heterozygotes for FH (23), this patient would have become severely depleted of cholesterol during the mevinolin treatment period if cholesterol synthesis had been profoundly inhibited.

The data from the other patients also are strongly suggestive that their synthesis of cholesterol was not markedly inhibited. Two patients (J.C. and M.Ba.) had no overall change in cholesterol balance on mevinolin and yet showed decreases in LDL-cholesterol of 25% and 28%. It seems highly unlikely that a severe decrease in cholesterol production could have been replaced in fecal steroid excretion entirely by cholesterol mobilized from tissue pools. In J.P. and M.Ba., a total of 30 and 41 g of cholesterol, respectively, were lost in feces with no sign of progressive diminution in output. Furthermore, in none of the patients did cholesterol balance fall below the values seen in normal adult men. Therefore, while we cannot rule out the possibility that a portion of the steroids excreted during mevinolin therapy may have been derived from preexisting pools, it is doubtful that cholesterol synthesis was curtailed to a dangerously low level.

While studies of much longer duration will be required to confirm the current conclusion, we feel that the data strongly suggest that treatment with mevinolin can lower LDL levels without leading to severe depletion of vital stores of cholesterol in familial hypercholesterolemia heterozygotes.

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