Inhibition of Intestinal Cholesterol Absorption by Ezetimibe in Humans

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Background—Ezetimibe has been shown to inhibit cholesterol absorption in animal models, but studies on cholesterol absorption in humans have not been performed thus far.

Methods and Results—The effect of ezetimibe (10 mg/d) on cholesterol absorption and synthesis, sterol excretion, and plasma concentrations of cholesterol and noncholesterol sterols was investigated in a randomized, double-blind, placebo-controlled, crossover study in 18 patients with mild to moderate hypercholesterolemia. Treatment periods lasted 2 weeks with an intervening 2-week washout period. Fractional cholesterol absorption rates averaged 49.8±13.8% on placebo and 22.7±25.8% on ezetimibe, indicating a reduction of 54% (geometric mean ratio; \(P<0.001\)). Cholesterol synthesis increased by 89% from 931±1027 mg/d on placebo to 1763±1098 mg/d on ezetimibe (\(P<0.001\), while the ratio of lathosterol-to-cholesterol, an indirect marker of cholesterol synthesis, was increased by 72% (\(P<0.001\)). Bile acid synthesis was insignificantly increased (placebo: 264±209 mg/d, ezetimibe: 308±184 mg/d; \(P=0.068\)). Mean percent changes from baseline for LDL and total cholesterol after ezetimibe treatment were −20.4% and −15.1%, respectively (\(P<0.001\) for both), whereas campesterol and sitosterol were decreased by −48% and −41%, respectively.

Conclusion—In humans, ezetimibe inhibits cholesterol absorption and promotes a compensatory increase of cholesterol synthesis, followed by clinically relevant reductions in LDL and total cholesterol concentrations. Ezetimibe also reduces plasma concentrations of the noncholesterol sterols sitosterol and campesterol, suggesting an effect on the absorption of these compounds as well. (Circulation. 2002;106:1943-1948.)

Key Words: ezetimibe • cholesterol absorption • cholesterol synthesis • lathosterol • plant sterols

The benefits of lipid-lowering therapy on coronary heart disease (CHD) risk have been clearly established in many large-scale primary and secondary prevention trials (see review). Although statins (HMG-CoA reductase inhibitors) have been shown to be effective in lowering low-density lipoprotein (LDL) cholesterol, many patients do not achieve standard treatment goals as defined by The National Cholesterol Education Program (NCEP).1,2 Although higher doses of statins are more effective in lipid lowering, the risk of serious side effects seems to be dose dependent.3 Thus, the need of additional lipid-lowering compounds not acting as HMG-CoA reductase inhibitors has focused attention on other mechanisms of action such as inhibition of intestinal cholesterol absorption (see review). Ezetimibe (formerly known as SCH 58235) is a compound of the 2-azetidinone class that has been shown to produce a marked inhibition of intestinal cholesterol absorption (up to 96%) in animals.5 Ezetimibe (and/or its phenolic glucuronide) acts at the brush border of the small intestine and inhibits the uptake of dietary and biliary cholesterol into the enterocytes6,7 but does not appear to affect the absorption of triglycerides or fat-soluble vitamins. Although several clinical trials in humans have revealed LDL cholesterol–lowering effects in the range of 17% to 20% at a dose of 10 mg per day,8–10 as yet, no studies have directly tested the effect of ezetimibe on the intestinal cholesterol absorption in humans. Thus, the present trial was designed to investigate the influence of ezetimibe on cholesterol absorption, as well as cholesterol synthesis, sterol excretion, and plasma concentrations of cholesterol and noncholesterol sterols in patients with mild to moderate hypercholesterolemia.

Methods

Study Design
The study was designed as a randomized, double-blind, placebo-controlled, 2-period, crossover trial (see Figure 1). After a 2-week placebo run-in period, patients were randomized to 1 of 2 treatment sequences: either ezetimibe (10 mg/d) in treatment period 1 followed by placebo in treatment period 2 or placebo in treatment period 1 followed by ezetimibe (10 mg/d) in treatment period 2. Treatment periods lasted 2 weeks each with an intervening 2-week washout period. The primary objective of the study was to evaluate the effect of ezetimibe on intestinal cholesterol absorption in patients with mild

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to moderate hypercholesterolemia. Secondary endpoints included cholesterol synthesis, sterol excretion, and plasma concentrations of cholesterol and noncholesterol sterols. The protocol was approved by the local ethical committee, and all patients gave written informed consent.

Patients
Eighteen patients from the outpatient clinic of the Department of Clinical Pharmacology, University of Bonn, participated in the study. Inclusion criteria for randomization were age between 18 and 55 years, body mass index (BMI = weight (kg)/height (m)^2) between 19 and 30 kg/m^2, plasma LDL cholesterol concentrations ≤130 mg/dL, and dietary cholesterol intake in the range of 200 to 500 mg/d. All patients completed the study (N = 16).

Cholesterol Absorption Measurements
Fractional cholesterol absorption was evaluated during the second week of each treatment period using the continuous feeding dual-isotope method using deuterated markers. For this purpose, patients received tracer capsules containing 3 mg [2H] cholesterol and 3 mg [2H] sitostanol (Medical Isotopes Inc) 3 times per day for 7 days during the second week of each treatment period (days 8 to 14 and days 36 to 42). Daily stool samples were collected over the final 4 days of each treatment period for measurement of fecal isotopic ratios by gas chromatography/mass spectrometry (GC-MS). Fractional cholesterol absorption was calculated from the fecal ratio of deuterium-labeled cholesterol and its bacterial degradation products coprostanol and coprostanone and deuterium-labeled sitostanol and from the ratio of deuterium-labeled cholesterol and sitostanol in the tracer capsules. Because coprostanone was not detectable in the fecal samples, only coprostanol was included into the calculation. The percentage absorption of cholesterol was calculated from the disappearance of cholesterol during the passage through the small intestine compared with sitostanol, which served as nonabsorbable marker by the following formula:

\[ \text{Cholesterol absorption [\%]} = 100 \times \left(1 - \frac{([2H]_\text{cholesterol}}{[2H]_\text{sitostanol}} + [\text{coprostanol}]_\text{coprostanol} + [\text{sitostanol}]_\text{sitostanol})}{([2H]_\text{cholesterol}}{[2H]_\text{sitostanol}} + [\text{coprostanol}]_\text{coprostanol} + [\text{sitostanol}]_\text{sitostanol}})\]

The cholesterol absorption rates were derived from the median of the 4 measurements during each treatment period.

Cholesterol and Bile Acid Synthesis
Fecal excretion of neutral and acidic sterols was measured from the same fecal samples (days 4 to 7) collected for cholesterol absorption using [2H] sitostanol as nonabsorbable marker. Measurements were performed by gas-liquid chromatography in a modified form as described previously. Daily fecal excretion rates were calculated as ratios of neutral and acidic sterols to the [2H] sitostanol in fecal samples multiplied by the daily intake of deuterated sitostanol. Net cholesterol synthesis was calculated as the sum of daily excretion of total fecal sterols minus the mean of dietary cholesterol intake. Dietary cholesterol intake was calculated from the last 7 days of the placebo run-in period and each of the treatment periods based on 7-day daily diaries. Mean daily intake was computed using a nutrition database program (Prodi 4.5, WVG, Germany). Values for neutral and acidic sterol excretion as well as cholesterol synthesis were derived from the median of the 4 measurements during each period.

Plasma Lipids and Noncholesterol Sterols
Blood was drawn for plasma lipid and lipoprotein analysis in the morning after an overnight fast at the beginning and the end of each of the treatment periods. Baseline lipoprotein profiles were recorded after the placebo run-in period. Total cholesterol and triglycerides in serum were measured by enzymatic methods. High-density lipoprotein (HDL) cholesterol was determined in the supernatant after precipitation of apolipoprotein B–containing lipoproteins. Low-density lipoprotein (LDL) cholesterol was calculated by the method of Friedewald et al. Lathosterol, campesterol, and sitosterol were also determined from these blood samples using GC/MS as described previously using epico-prostanol as internal standard.

Statistical Analysis
All data were analyzed in an intention-to-treat approach. An analysis of variance (ANOVA) model appropriate for a 2-period, 2-arm crossover design with terms for treatment, sequence, patient within sequence, and period was used to calculate differences between placebo and ezetimibe treatment using SAS statistic package (SAS Institute Inc). All parameters except plasma lipids and lipoproteins were log transformed to fit the model assumptions. If not otherwise stated, means were expressed as retransformed geometric means. Standard deviations were estimated from the ANOVA model. Percent differences between placebo and ezetimibe treatment were expressed as geometric mean ratios. Lipid parameters (total, LDL, and HDL cholesterol) were expressed as percent change from baseline to endpoint estimated from the LS means of the ANOVA model.

Results
Eighteen male patients were enrolled into the study. Mean age was 25.8 years (range 24 to 58), mean body weight was 85 kg (range 66 to 105), and mean body mass index was 25.5 kg/m^2 (range 20.7 to 29.4). All patients completed the study.

TABLE 1. Fractional Cholesterol Absorption, Neutral and Acidic Sterol Excretion, and Cholesterol Synthesis After 2 Weeks of Treatment With Ezetimibe (10 mg/d) and Placebo in 18 Male Subjects With Mild Hypercholesterolemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo</th>
<th>Ezetimibe</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol intake, mg/d</td>
<td>307±78.5</td>
<td>313±87.1</td>
<td>0.776</td>
</tr>
<tr>
<td>Fractional cholesterol absorption, %</td>
<td>49.8±13.8</td>
<td>22.7±25.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutral sterol excretion, mg/d</td>
<td>999±751</td>
<td>1718±930</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acidic sterol excretion, mg/d</td>
<td>264±209</td>
<td>308±182</td>
<td>0.068</td>
</tr>
<tr>
<td>Cholesterol synthesis, mg/d</td>
<td>931±1027</td>
<td>1763±1098</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Geometric mean ± SD.
and demonstrated excellent compliance with the study protocol based on pill counts and dietary records (data not shown).

Cholesterol Absorption and Synthesis
The results for dietary cholesterol intake, fractional cholesterol absorption rates, fecal excretion of neutral and acidic sterols, and total cholesterol synthesis are summarized in Table 1. Dietary cholesterol intake of all patients was in the range of 200 to 500 mg/d and remained constant in every subject throughout the study. Cholesterol absorption rates ranged from 24.9% to 74.7% on placebo and from 2.3% to 48.7% on ezetimibe. After 2 weeks of treatment, fractional cholesterol absorption was 22.7% on ezetimibe and 49.8% on placebo ($P<0.001$). Based on the geometric mean ratio, ezetimibe reduced fractional cholesterol absorption by 54% compared with placebo. No significant sequence or period effect was detected. Individual fractional cholesterol absorption rates are shown in Figure 2. In all but one patient, cholesterol absorption was lower during ezetimibe than placebo treatment, and in one subject the reduction was marginal (~2%). The fecal excretion of total neutral sterols increased by 72% during treatment with ezetimibe ($P<0.001$; Table 1). A small, but not significant, increase in acidic sterol excretion also was observed ($P=0.068$). Thus, total cholesterol synthesis was increased by 89% on ezetimibe relative to placebo ($P<0.001$).

Plasma Lipoproteins
Baseline mean LDL cholesterol was 142 mg/dL. After 2 weeks of treatment, the mean percent changes from baseline to endpoint were $-20.4\%$ ($P<0.001$) on ezetimibe and $+1.9\%$ ($P=0.640$) on placebo resulting in a $-22.3\%$ treatment difference (Table 2). Similar results were observed for total cholesterol with a baseline value of 220 mg/dL for both groups. The mean percent changes from baseline to endpoint were $-15.1\%$ ($P<0.001$) on ezetimibe and $-1.9\%$ ($P=0.498$) on placebo for a total treatment difference of $-13.2\%$ ($P<0.001$). Changes in HDL cholesterol and triglycerides were not significantly different for ezetimibe versus placebo (Table 2). There was a weak but not significant correlation between percent reduction in total cholesterol and percent change in cholesterol absorption ($r=0.45; P=0.061$).

Plasma Noncholesterol Sterols
Plasma lathosterol concentrations increased by 53% and the ratio of lathosterol-to-cholesterol (a marker of hepatic HMG-CoA reductase activity and total cholesterol synthesis) increased by 72% on ezetimibe (Table 3; $P<0.001$ for both). Significant correlations between the lathosterol-to-cholesterol ratio and cholesterol synthesis rates were observed during both treatment periods (data not shown).

Treatment with ezetimibe lowered plasma concentrations of the noncholesterol plant sterols campesterol and sitosterol in every subject (Figures 3A and 3B). The mean reduction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>n</th>
<th>Baseline</th>
<th>Week 2</th>
<th>% Change From Baseline</th>
<th>% Difference Between Ezetimibe and Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>Ezetimibe</td>
<td>18</td>
<td>220</td>
<td>186</td>
<td>$-15.1$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Placebo</td>
<td>18</td>
<td>220</td>
<td>214</td>
<td>$-1.9$</td>
<td>0.498</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>Ezetimibe</td>
<td>17</td>
<td>142</td>
<td>111</td>
<td>$-20.4$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Placebo</td>
<td>17</td>
<td>142</td>
<td>142</td>
<td>1.9</td>
<td>0.640</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>Ezetimibe</td>
<td>18</td>
<td>47</td>
<td>48</td>
<td>2.7</td>
<td>0.340</td>
</tr>
<tr>
<td>Placebo</td>
<td>18</td>
<td>47</td>
<td>47</td>
<td>0.5</td>
<td>0.905</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mg/dL*</td>
<td>Ezetimibe</td>
<td>18</td>
<td>129</td>
<td>144</td>
<td>$-7.9$</td>
<td>0.702</td>
</tr>
<tr>
<td>Placebo</td>
<td>18</td>
<td>129</td>
<td>118</td>
<td>$-5.2$</td>
<td>0.908</td>
<td></td>
</tr>
</tbody>
</table>

*Values expressed as median.
was \( -48\% \) for campesterol and \( -41\% \) for sitosterol (Table 3; \( P < 0.001 \) for both). Similar results could also be observed for their ratios to cholesterol. The mean decreases were \( -41\% \) for the campesterol-to-cholesterol ratio and \( -34\% \) for the sitosterol-to-cholesterol ratio (Table 3; \( P < 0.001 \) for both).

**Safety**

The tolerability of ezetimibe was excellent and there were no serious clinical adverse events or critical laboratory elevations (including aspartate or alanine aminotransferase elevations 3-fold greater than the upper limit of normal or creatinine phosphokinase elevations 10-fold greater than the upper limit of normal) during either placebo or ezetimibe treatment.

**Discussion**

Although studies in animals have shown that ezetimibe reduces intestinal absorption of cholesterol, no studies in man have been performed to date. Thus, the present study was undertaken to measure the effect of ezetimibe on the intestinal cholesterol absorption in humans. Results showed that ezetimibe inhibited cholesterol absorption by 54\% relative to placebo. This inhibition was associated with a compensatory increase in cholesterol synthesis. On balance, these changes led to a 22.3\% reduction in plasma LDL cholesterol concentrations, consistent with LDL cholesterol reductions observed in previous studies.\(^8\)–\(^{10}\) Marked reductions in plasma concentrations of the noncholesterol plant sterols sitosterol and campesterol also were observed, suggesting that ezetimibe inhibited the absorption of these compounds as well. However, this was not directly tested in the present study.

Compared with animal experiments, the present study revealed a less extensive effect of ezetimibe on inhibition of cholesterol absorption in humans. Studies in cholesterol fed hamsters and rodents showed an inhibition of cholesterol absorption by 92\% to 96\% in a dose range of 1 to 10 mg/kg.\(^5\),\(^{15}\) Although these data are not comparable with the present study due to species differences, the dosage in the present study was 10 to 100 times lower. In comparison to other compounds, intestinal cholesterol absorption by ezetimibe was more pronounced than that observed for other known inhibitors of cholesterol absorption including neomycin and plant sterol and stanol esters in humans. Neomycin has been shown to reduce intestinal cholesterol absorption in a dose-dependent manner by 26\% to 49\%.\(^{16}–^{20}\) Treatment with high-dose plant sterols and stanols has been shown to lower cholesterol absorption by up to 45\%,\(^{21}–^{23}\) but the maximal effects are observed under circumstances in which the active agents are delivered in fat carriers in conjunction with cholesterol meals.

In addition to effects on plasma cholesterol concentrations, ezetimibe produced a marked reduction in circulating levels of noncholesterol plant sterols. In fact, effects on plasma concentrations of campesterol and sitosterol were more pronounced than those on cholesterol. This difference may reflect different absorption rates for campesterol and sitosterol versus cholesterol\(^{23}\) and/or the fact that, unlike cholesterol, plant sterols cannot be synthesized endogenously.\(^{24}\)

**Table 3.** Lathosterol, Campesterol, and Sitosterol and Their Ratios to Cholesterol After 2 Weeks of Treatment With Ezetimibe (10 mg/d) and Placebo in 18 Subjects With Mild Hypercholesterolemia

<table>
<thead>
<tr>
<th>Sterol</th>
<th>Placebo</th>
<th>Ezetimibe</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lathosterol, mg/dL</td>
<td>0.258±0.102</td>
<td>0.395±0.131</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Campesterol, mg/dL</td>
<td>0.443±0.181</td>
<td>0.231±0.096</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sitosterol, mg/dL</td>
<td>0.312±0.102</td>
<td>0.183±0.073</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio lathosterol to cholesterol, ( \mu g/mg )</td>
<td>1.103±0.404</td>
<td>1.895±0.609</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio campesterol to cholesterol, ( \mu g/mg )</td>
<td>1.894±0.660</td>
<td>1.110±0.425</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio sitosterol to cholesterol, ( \mu g/mg )</td>
<td>1.332±0.342</td>
<td>0.880±0.293</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Geometric mean±SD.
Indeed, the reduced cholesterol absorption was compensated by an increase in cholesterol synthesis as indicated by an increase in the fecal excretion of neutral sterols and the ratio of lathosterol-to-cholesterol in plasma. Because the ratio of lathosterol-to-cholesterol is an indicator for the hepatic HMG-CoA reductase activity and total cholesterol synthesis, it can be speculated that the increase in cholesterol synthesis is mainly due to de novo hepatic cholesterol synthesis. Although the observed increase in the acidic sterol excretion was small and marginal, we cannot exclude the possibility that during long-term treatment, ezetimibe might lead to a slight increase in bile-acid synthesis.

The observed increase in hepatic cholesterol synthesis might explain the favorable effects of coadministering ezetimibe and statins. Several recent trials (currently either published in abstract form or unpublished) have shown that coadministration of ezetimibe with statins produces an incremental reduction of LDL cholesterol in the range of 12% to 15% independent of kind and dose of the coadministered statin. When ezetimibe was added to on-going statin therapy, LDL cholesterol was incrementally reduced by 21.5% (relative to on-statin baseline values) compared with placebo. Thus, because statins can reduce the compensatory increase in the hepatic cholesterol synthesis induced by ezetimibe, the combination of ezetimibe and statins results in an incremental lowering of LDL cholesterol concentrations.

The inhibitory effects of ezetimibe were characterized by considerable interindividual variation. The basis of these variations cannot be determined from the present study but potentially could involve intrinsic differences in responsiveness to ezetimibe. Importantly, variations in cholesterol absorption did not correlate significantly with changes in total cholesterol and did not correlate with variations in LDL cholesterol reductions after ezetimibe treatment, likely due to the influence of other factors, including compensatory changes in cholesterol synthesis.

In conclusion, 2 weeks of treatment with ezetimibe at 10 mg/d produced a 54% inhibition of cholesterol absorption in mildly to moderately hypercholesterolemic subjects. This was associated with reductions in LDL and total cholesterol and plant sterol concentrations and a increase in endogenous cholesterol synthesis.

Acknowledgments
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