Human Lens Cholesterol Concentrations in Patients Who Used Lovastatin or Simvastatin

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Objective: To determine whether long-term therapeutic use of the hypocholesterolemic drugs lovastatin and simvastatin significantly alters the distribution and concentration of cholesterol in the human lens. Such changes might precede observable alterations in lens structure.

Methods: Pairs of lenses (9-13 pairs) from patients (age range, 46-81 years) who had been taking lovastatin or simvastatin before their death (estimated for the previous 2-4 years) and lenses from similarly aged control subjects were divided into outer cortex and inner cortex plus nucleus by dissolution in a detergent-containing buffer. Ten minutes of dissolution removed 17% to 19% of the lens total volume, which accounted for about 20% of the width of the equatorial cortex and 75% of the width of the sagittal cortex. This fraction plus the residual lens was homogenized, saponified, and assayed for cholesterol by gas-liquid chromatography.

Results: The cortex of adult control lenses contained about 4 µg of cholesterol per cubic millimeter of volume. This concentration increased to 10 to 15 µg/mm³ in the adult nucleus and decreased to about 6 µg/mm³ in the juvenile and fetal nucleus. Treatment with neither lovastatin nor simvastatin significantly altered the concentration of cholesterol in either the cortex or nuclear fractions.

Conclusions: Variations in concentration of cholesterol along the radii of the lens reflect differences in the density or packing of fiber cell membranes. The observed distribution of cholesterol supports the recent model of the adult lens structure, which, from surface to center, is the cortex, adult nucleus, juvenile nucleus, fetal nucleus, and embryonic nucleus. Finding no significant changes in concentration of cholesterol in the cortex formed during treatment with lovastatin or simvastatin reinforces the results of clinical studies that indicate a high lenticular safety of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Nevertheless, caution is encouraged in assuming a similar ocular safety in newer drugs that inhibit cholesterol synthesis at later metabolic steps.

Clinical Relevance: Does clinical use of hypocholesterolemic drugs alter lens cholesterol?


The cell membrane of the human lens contains the highest relative concentration of cholesterol in nature.1,2 This cholesterol is believed to be important for adjusting the fluidity of cortical and nuclear membranes to similar points and for antagonizing excess binding of crystallin proteins to this membrane.3,4 Because the lens must synthesize the cholesterol needed to support its lifelong growth, an inhibition of lens cholesterol synthesis with drugs can produce cataracts in animals and humans.3,4 The cataractogenic potential of hypocholesterolemic drugs became evident in 1962 when use of triparanol (Mer 29; W. S. Merrell Co, Cincinnati, Ohio) was reported to induce cataracts in patients.5,6 Triparanol blocks the reduction of desmosterol to cholesterol, which results in accumulation of desmosterol.9 In 1963, von Sallmann10 showed that use of triparanol produced cataracts in rats, providing perhaps the classic example of reverse toxicologic assessment.

The current era of hypocholesterolemic drugs began with the introduction of lovastatin (Mevacor) in 1987, the first of a series of statin 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors that now includes simvastatin (Zocor), pravastatin sodium (Pravacol), atorvastin (Lipitor), and others. Collectively, these are probably the most prescribed drugs in the United States. In view of the large patient population, the negative experience with triparanol, and the report that lovastatin use can produce cataracts in dogs,11 it seemed reasonable that annual slitlamp examination of the lens was recommended when lovastatin was initially introduced.
PATIENTS AND METHODS

SOURCE OF LENSES

Intact lenses from individuals who had been taking lovastatin or simvastatin before their death or who had not been taking hypocholesterolaemic drugs (controls) became available from a tissue donor bank (National Disease Research Interchange, Philadelphia, Pa). Between 9 and 13 pairs of lenses from lovastatin- and simvastatin-treated individuals and controls (age range, 46-81 years) were received in 3 years. Encapsulation was reported to have occurred typically between 2 and 6 hours after death. Lenses were excised, stored at -70°C, and shipped on dry ice. On arrival, lenses were examined and stored at -80°C. Lenses that were disrupted when received were not used. Although our protocol requested information about the duration of statin use before death, this information was rarely available. When available, it stated treatment durations of 2 to 4 years. The reported cause of death was cardiovascular or cerebrovascular disease for 70% to 80% of controls and treated patients.

DISSOLUTION OF LENSES

If therapeutic use of lovastatin or simvastatin inhibited lens cholesterol biosynthesis, one could expect a lower cholesterol content of fiber cell membranes formed during treatment. Because the growth rate of the adult human lens is slow, taking about 40 years to double in size, only the outer cortex should be affected after 2 to 4 years of drug exposure. A simple technique was developed for the uniform and gradual dissolution of the rat lens that consisted of gently stirring the decapsulated lens in a hypotonic buffer (3 mmol of Tris, pH 8.0, 1 mmol of EDTA, and 5 mmol of β-mercaptoethanol) containing 0.2% (weight per volume) sodium decylsulfate. Based on the percentage of total protein removed after various times of dissolution and simultaneous measurement of changes in lens size, the percentage of protein removed at any time could be related to the percentage of lens radii removed. We applied this technique to the dissolution of the human lens. Individual lenses were placed on a glass slide, moistened with a drop of dissolution buffer, decapsulated, and transferred to a preweighed 5-cm culture dish. After reweighing, 8.0 mL of dissolution buffer was added, and the lens was stirred by placing the dish on the surface of a rotatory mixer run at approximately 100 rpm. In a preliminary experiment, a human lens was photographed at various dissolution times to assess changes in the sagittal and equatorial radii and lens volume. Volume was calculated from the formula for an oblate spheroid. Small aliquots of the media were taken at each time for protein measurement. This technique resulted in gradual and uniform decreases in lens radii and volume (Figure 1). The sagittal radius decreased more rapidly than the equatorial radius. Based on results of additional preliminary experiments (see the “Results” section), a standard dissolution time of 10 minutes was used to remove the outer cortex from lenses of controls and treated patients.

MEASUREMENT OF LENSES

CHOLESTEROL CONTENT

After 10 minutes of stirring, the medium (8.0 mL) was collected, and the residual lens was transferred to a preweighed Dounce homogenization vessel and reweighed. The volume of the lens removed (outer cortex) was determined by comparison with the initial weight and was expressed in cubic millimeters to assume a lens density of 1.0. The remaining lens (inner cortex plus nucleus) was homogenized in 8.0 mL of dissolution buffer. After weighing the 2 suspensions to accurately determine volume, aliquots of each were taken for protein and cholesterol assays. Duplicate 2.0-mL aliquots of the outer cortex and 1.0-mL aliquots of the inner cortex plus nucleus were lyophilized in 1.5 × 12.0-cm screw-capped test tubes containing either 20 µg (outer cortex) or 50 µg (inner cortex plus nucleus) of 5-α cholestane added as internal standard. Lipids were saponified for 1 hour at 100°C in 1.0 mL of 1 N potassium hydroxide in 67% ethanol-water. After diluting with 1 mL of water, sterols were extracted into 4 mL of hexane; the hexane layer was recovered, washed once with water, and evaporated, and the residue was dissolved in either 100 or 200 µL of carbon disulfide. Sterols were fractionated by gas-liquid chromatography using a 1.8-m coiled glass column (0.22 mm, inner diameter) packed with 3% OV-17 on 80/100 gas chrom Q and operated isothermally at 265°C in a gas chromatograph (Model 3300; Varian Co, Sugar Land, Tex). Sterols were detected by flame ionization. Cholesterol, the only lens sterol present, was quantitated by comparing its peak area to that of the internal standard. Cholesterol peak area was adjusted for differences in signal response given by equal masses of cholesterol and 5-α cholestane. Cholesterol concentration in the lens outer cortex and inner cortex plus nucleus was expressed in micrograms of cholesterol per cubic millimeter of lens volume and in micrograms of cholesterol per microgram of protein. Expression of data on the basis of protein may be more accurate because it avoids possible errors in determining volume by weight caused by differences in moisture associated with the lens surface before and after dissolution. The concentrations of cholesterol determined for the individual lenses of a given subject were averaged. Differences in the concentration of lens cholesterol between the control and treated groups were analyzed for statistical significance using the unpaired Student test.

Results of clinical safety trials have since indicated that use of lovastatin by older patient populations (>50 years) does not significantly increase the incidence of cataracts or markedly alter visual function. However, results of a trial by Mølgaard et al with simvastatin suggest an increase in cortical opacities after 2 years of treatment (see Cenedella for a review of clinical safety trials).

Although the ocular safety of using statins in older patient populations seems high, information on possible drug-induced changes in the cholesterol concentration of lenses from treated individuals, which might precede structural changes, is not available, to our knowledge. Results of animal studies show that orally administered lovastatin or simvastatin gained access to the lens, inhibited cholesterol synthesis, and completely blocked cholesterol accumulation. De Vries et al demonstrated that, in rats aged 20 to 42 days, oral treatment with simvastatin (50 mg/kg per day) decreased the con-
concentration of lens cholesterol by 45% compared with controls. The aim of this project was to determine whether therapeutic use of lovastatin or simvastatin was associated with a decrease in cholesterol concentration of the human lens. We focused on possible changes in the lens outer cortex because this region would be enriched in fiber cells that formed during the estimated average 2 to 4 years of statin use by the patients before their death.

RESULTS

Because lens cholesterol is present exclusively in plasma membrane, differences in the regional concentration of cholesterol should reflect an abundance of fiber cell plasma membranes per unit volume of lens. Distribution of cholesterol in various shells of lens volume from the outer cortex to nuclear regions was determined in 2 groups of control lenses in which up to 60% of the lens was removed in small fractions by gradual dissolution (Figure 2). In both groups, cholesterol concentration over the outer 20% to 25% of the lens volume (100% of total volume down to 70%-75% of total) seemed constant at 3 to 4 µg/mm³. With removal of deeper shells of lens volume, cholesterol concentration sharply increased to between 10 and 15 µg/mm³. This distribution of cholesterol in the older human lens parallels that previously reported by Li et al. Furthermore, this distribution agrees with the current morphologic model for aged healthy human lens described by Taylor et al. Here, the lens is divided from surface to center into cortex, adult nucleus, juvenile nucleus, fetal nucleus, and embryonic nucleus. The abundance of membranes was highest in the adult nucleus, as reflected by the lowest membrane-to-cytoplasm ratio of fiber cells in this region. This zone should have had the highest cholesterol concentration, and that is what we found.

Dissolution of lenses for 10 minutes in the sodium dodecylsulfate–containing buffer removed a relatively uniform 17% to 19% of total volume from all groups of lenses analyzed (Table 1). This should amount to removal of only about 3% of the equatorial radius and 15% of the sagittal radius of the lens (Figure 1 [calculations available from the authors]). Based on the current view of human lens structure (see Figures 5 and 6 in the article by Taylor et al), approximately 20% of the width of the

Figure 1. Dissolution of the human lens in a hypotonic, sodium dodecylsulfate–containing buffer. A 56-year-old lens was stirred in 8.0 mL of buffer, and small aliquots were removed after 1, 2, 4, 8, 15, 30, and 60 minutes for assay of protein content. Photographs of the equatorial and sagittal axes were quickly taken at each time. The estimated equatorial and sagittal radii were used to calculate lens volume from \( V = \frac{4}{3} \pi a^2 b \) (oblate spheroid).

Figure 2. Distribution of cholesterol in the human lens. Lenses from 40- and 41-year-old (top) and 60- and 65-year-old (bottom) individuals were dissolved by stirring in 3.0-mL aliquots of hypotonic buffer containing 0.20% sodium dodecylsulfate. The buffer was removed and replaced with new 3.0-mL aliquots at intervals up to 340 minutes of stirring. Each recovered fraction and residual nucleus was assayed for cholesterol. Lenses were weighed at each media change to estimate changes in lens volume. Each curve is the average of 2 lenses.
equatorial cortex and 75% of the width of the sagittal cortex should have been removed. Because the cortex is estimated to be replaced approximately every 5 years in the 60-year-old lens (the previous cortex is compacted into the adult nucleus during this period), much if not most cortices in the study population should have “turned over” during 2 to 4 years of drug treatment. Thus, if treatment with lovastatin or simvastatin caused major changes in the concentration of cholesterol in the lens membranes, those changes should have been detected.

A novel idea from the laboratory of Joseph Costello, PhD, that the cortex of the adult human lens is replaced by new growth about every 5 years is based on morphometric measurements of cell numbers and growth rates of the various lens regions. The cortex of the 61-year-old human lens was estimated to contain 665,000 cells, with a growth rate of 133,000 cells per year. Thus, in 5 years, 665,000 cells would be displaced and compacted into the adult nucleus by new growth. The authors emphasized that these are rough estimates.

There were no statistically significant differences between concentration of cholesterol in the lens cortex from control subjects and those who had been taking either lovastatin or simvastatin, regardless of whether the results were expressed on the basis of lens volume (Table 1) or protein concentration (Table 2). No significant differences were seen when the drug groups were pooled (lovastatin plus simvastatin) and compared with controls (Tables 1 and 2). No differences were evident between control and treated groups when the concentration data were analyzed on the basis of 3 age groups (46-60, 61-70, and >70 years) (data not shown).

Although based on limited information, we estimate that treated patients had been taking lovastatin or simvastatin for 2 to 4 years before their death. Because a significant fraction of the cortex would have been replaced in this time, our methods should have permitted detection of major changes in the concentration of cholesterol in this lens region. However, judging from the variability associated with the concentration data, it is unlikely that we could have recognized a concentration difference between control and treated lenses of less than 30%. The absence of an apparent major effect of drug treatment on lens cholesterol concentrations implies that use of neither lovastatin nor simvastatin causes structural changes in lens membranes because of an inhibition of cholesterol biosynthesis. This conclusion supports the results of most clinical toxicology studies showing no increased incidence of cataracts in patients receiving 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors.

The results of the present study should not generally discount concern about the potential lenticular toxic effects of using cholesterol synthesis inhibitors. In contrast to statins, drugs that inhibit at late steps in the cholesterol synthesis pathway may present added risk because of an accumulation of potentially toxic intermediates, in addition to possibly lowering lens membrane cholesterol levels. For example, the oxidosqualene cyclase inhibitor U18666A is highly cataractogenic in animals, and oxidosqualene cyclase inhibitors that are even more potent than U18666A are being considered.

Table 1. Distribution of Cholesterol in Lens Fractions From Control Subjects and Those Taking Lovastatin or Simvastatin Before Their Death*

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients, No.</th>
<th>Age, y</th>
<th>Lens Volume (Outer Cortex), %</th>
<th>Cholesterol Concentration, µg/mm³†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>60.1 ± 8.3</td>
<td>19.4 ± 3.3</td>
<td>4.00 ± 0.7</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>9</td>
<td>65.6 ± 8.8</td>
<td>17.4 ± 6.8</td>
<td>4.34 ± 1.54 (.49)</td>
</tr>
<tr>
<td>Simvastatin</td>
<td>12</td>
<td>67.4 ± 9.6</td>
<td>16.9 ± 8.4</td>
<td>4.82 ± 1.66 (.11)</td>
</tr>
<tr>
<td>Lovastatin + simvastatin</td>
<td>21</td>
<td>66.6 ± 9.0</td>
<td>17.1 ± 7.6</td>
<td>4.62 ± 1.59 (.20)</td>
</tr>
</tbody>
</table>

†Cholesterol concentration is expressed on the basis of lens volume.

Table 2. Distribution of Cholesterol in Lens Fractions From Control Subjects and Those Taking Lovastatin or Simvastatin Before Their Death*

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients, No.</th>
<th>Age, y</th>
<th>Lens Protein (Outer Cortex), %</th>
<th>Cholesterol Concentration, µg/mg†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>60.1 ± 8.3</td>
<td>20.6 ± 3.0</td>
<td>12.4 ± 4.1</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>9</td>
<td>65.6 ± 8.5</td>
<td>19.9 ± 4.0</td>
<td>11.4 ± 3.2 (.54)</td>
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<tr>
<td>Simvastatin</td>
<td>12</td>
<td>67.4 ± 9.6</td>
<td>18.8 ± 3.5</td>
<td>11.8 ± 2.1 (.65)</td>
</tr>
<tr>
<td>Lovastatin + simvastatin</td>
<td>21</td>
<td>66.6 ± 9.0</td>
<td>19.3 ± 3.7</td>
<td>11.6 ± 2.6 (.49)</td>
</tr>
</tbody>
</table>

†Cholesterol concentration is expressed on the basis of lens protein content.
as novel hypocholesterolemic drugs for the treatment of humans.22-24 A practical reason for being concerned about cataracts caused by the use of inhibitors of lens cholesterol synthesis is strongly reinforced by the package insert information provided for the newly approved anti-psychotic drug quetiapine (Seroquel).25 Quetiapine, an antagonist of multiple neurotransmitter receptors in the brain, was stated to induce lenticular changes in patients and cataracts in dogs. The cataracts could be caused by an inhibition of lens cholesterol biosynthesis at a late metabolic site because it was reported in the package insert that treatment of the dog lowered the concentration of 6-8-cholesterol in plasma. Because of these recognitions, the manufacturer currently recommends that lenses of patients undergoing long-term treatment be examined by slitlamp or other methods at 6-month intervals for cataract formation.

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REFERENCES


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A look at the past... 

NGELUCCI reports a case of choked disc dependent upon an intracranial neoplasm or focus of inflammation, which, after the reduction of intracranial pressure by trephining, partially passed off. The patient, a woman of 31, had suffered for a year with various nervous symptoms, when sight began to fail, until finally the right eye became blind and vision in the left was reduced to 1⁄12. The diagnosis of brain tumor was made, the left occipital region was trephined, the dura divided, and an incision made into the presenting lobule of the cerebellum. The symptoms remained stationary for a time, but four months after the operation the swelling of the disc was much diminished and the vision of the left eye still was 1⁄30.