Pravastatin and Simvastatin Differently Inhibit Cholesterol Biosynthesis in Human Lens

Alfons C. J. de Vries,* Mario A. Vermeer,* Hans Bloemendal,† and Louis H. Cohen*

Purpose. In the current study, the hypocholesterolemic drugs pravastatin and simvastatin were compared for their influence on cholesterol biosynthesis in the human lens.

Methods. For measurements of cholesterol and fatty acid synthesis rates, human lenses were incubated for 20 hr in the presence of [14C]-acetate, and pravastatin or simvastatin. Radiolabeled [14C]-cholesterol and [14C]-fatty acids were determined. To avoid the influence of individual differences, one lens from each donor was incubated without drug (control) and the other lens was incubated in the presence of drug. For each lens pair, the percentage inhibition of the cholesterol synthesis caused by the drug was calculated. Fatty acid synthesis was not influenced by the drugs. By comparing the fatty acid synthesis rate of the drug-incubated with the control lens of a pair, a predefined exclusion criterion was used to eliminate lens pairs in which the lenses had no comparable biosynthetic capacities.

Results. Using various concentrations of the drugs, a dose–response curve was constructed for the inhibition of the cholesterol synthesis. The IC50 values (drug concentration giving 50% inhibition) were 0.5 μmol/l and 0.004 μmol/l for pravastatin and simvastatin, respectively. 3-Hydroxy-3-methylglutaryl coenzyme A reductase activity in microsomal membranes from human lens cortex was inhibited by simvastatin and pravastatin to the same extent.

Conclusions. Under the conditions used in this study, cholesterol synthesis in human lenses is inhibited by simvastatin 100-fold more effectively than by pravastatin. This difference was likely due to differences in the intracellular exposure of the reductase to the drugs in intact human lenses. Invest Ophthalmol Vis Sci. 1993;34:377-384.

The relationship between increased risk for coronary heart disease and elevated total and low-density lipoprotein cholesterol has been firmly established.1–3 Treatment of hypercholesterolemia by diet modification may require additional pharmacologic intervention.

The vastatins, pravastatin,4 simvastatin,5 and lovastatin,5 have been found to be very effective inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol synthesis pathway. Reducing the rate of cholesterol synthesis, this new class of drugs upregulates LDL receptor activity and as a result decreases the plasma cholesterol concentration.7 Vastatins produce a cholesterol-lowering effect that varies from 25% to 40%, depending on the dose of drug used and the type of patient being treated. All three drugs resemble the transition state of the conversion of HMG-CoA to mevalonate by HMG-CoA reductase. At the 6-position of the decalin ring, however, simvastatin
and lovastatin contain a lipophilic methyl group, whereas pravastatin in the same position contains a hydrophilic hydroxyl group. It has been suggested that the difference in hydrophilicity affects the cellular uptake of the drugs in various tissues.

It is desirable that statins be selectively taken up into the liver, because the liver is the major site of cholesterol synthesis in the body. The liver, as the primary organ for the control of circulating cholesterol in the body, is therefore considered to be the target organ for statins to reduce elevated plasma cholesterol concentrations. Inhibition of cholesterol synthesis in extrahepatic tissues should be avoided, because it is not possible to exclude undesirable side effects by disturbing sterol metabolism in these tissues. Evidence obtained with in vitro, ex vivo, and in vivo experiments on various animal species has indicated that pravastatin spares cholesterol synthesis in extrahepatic tissues more than other HMG-CoA reductase inhibitors.11,12

Tissue-selective inhibition of cholesterol synthesis is especially relevant for avascular tissues that are unable to obtain cholesterol from the circulation to meet intracellular demands. These tissues rely on de novo cholesterol synthesis for normal cell growth and differentiation. The ocular lens is noteworthy in that it is completely avascular. The major source of cholesterol for lens membrane synthesis is considered to be de novo synthesis, although high-density lipoprotein-like particles in aqueous humor as an additional extralenticular source cannot be excluded.9 Mosley et al10 have shown in rat studies that although pravastatin is almost as effective at inhibiting HMG-CoA reductase activity in the liver, it is 100-fold less potent in lens ex vivo compared to simvastatin and lovastatin.

De novo cholesterol synthesis seems to play a critical role in the maintenance of lens transparency. In studies with dogs, it was shown that simvastatin and lovastatin induce cataracts in a dose-dependent manner.11-13 Pravastatin, however, does not cause cataracts in dogs or in any other species in which it has been tested.14 In humans, little is known of the ophthalmologic effects of statins. Although clinical studies with simvastatin,15 lovastatin,16 and pravastatin17 do not exhibit any significant short-term cataractogenic potential, chronic drug exposures should be assessed to define adequately a long-term tolerability profile. It should be remembered that previous studies with other cholesterol synthesis inhibitors, such as triparanol,18,19 have induced cataracts in humans.

The current study investigates the inhibitory potencies of pravastatin and simvastatin on cholesterol biosynthesis of human lens in organ culture. As we have recently reported,20 cholesterologenesis in human lens remains unchanged from birth to death, and, as a result, we observed a marked increase in the cholesterol content of the lens with aging.

**MATERIALS AND METHODS**

**Materials**

Pravastatin (sodium salt) was obtained from Bristol-Myers Squibb (Princeton, NJ). Simvastatin (lactone form) was donated by Merck, Sharp and Dohme ( Rahway, NJ). The lactone form of simvastatin was converted into the open acid form by a 30 min incubation in 0.1 mol/l NaOH followed by neutralization with HCl. The inhibitor solutions always were freshly prepared just before use. In the current study, the open acid forms of pravastatin and simvastatin were used. [2,14C]-acetate (specific radioactivity 1.94 G Bq/mmol), [7(n)-3H]cholesterol (specific radioactivity 200 G Bq/mmol), and [9,10(n)-3H]-palmitic acid (specific radioactivity 2.24 TBq/mmol) were obtained from Amersham International (Amersham, United Kingdom). Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Flow Laboratories (Irvine, United Kingdom). The other chemicals used were of analytical grade.

**Human Lens Organ Cultures**

Human lenses from anonymous donors (aged 13 to 90 yr) were obtained within 24 hr postmortem from the Cornea Bank (Amsterdam, The Netherlands) by mediation of Eurotransplant. The methods for acquiring the human tissue included proper consent and approval, and complied with the tenets of the Declaration of Helsinki. Donor lenses exhibiting opacities as determined by split-lamp examination were excluded from this study. After removal, intact clear lenses were kept at 37°C in DMEM, supplemented with 10% fetal calf serum.

**Cholesterol and Fatty Acid Synthesis in Human Lens Ex Vivo**

For determination of lipid biosynthetic rates, excised human lenses were used within 2 to 5 hr after removal. The lenses were refreshed with 1 ml of the same culture medium per lens. To determine cholesterol and fatty synthesis, [14C]-acetate was added to the medium to a final concentration of 160 μmol/l and the lenses were incubated for 20 hr at 37°C. Pravastatin or simvastatin were added 1 hr before the addition of the label. The isolation of radiolabeled nonsaponifiable lipids was performed as described by Mosley et al,19 except that [3H]-palmitate was used as an internal recovery standard. The values were corrected using the recovery of [3H]-cholesterol or [3H]-palmitate for the cholesterol or fatty acid biosynthetic rate, respectively. Biosynthetic rates were expressed as 14C-dpm/lens per 20 hr incubation. Incubations of human lenses for 3
and 20 hr showed that sterol and fatty acid synthesis were linear with time for this period.

**Determination of the Effects of Vastatins on Human Lens**

To determine the influence of pravastatin or simvastatin on cholesterol and fatty acid synthesis in human lens, one lens of each pair was incubated without drug (control) and the other lens from the same subject with a defined concentration of vastatin. Controls and drug-incubated lenses were randomly allocated. If at least one lens of a pair showed a fatty acid synthesis rate below 5000 dpm/lens • 20 hr, this lens was considered nonvital and was rejected. In addition, some lens pairs were rejected by means of a fatty acid synthesis factor F, as described below. The percentage inhibition of cholesterol synthesis by the drug at the concentration used was calculated from the ratio of cholesterol synthesis rates in the drug-treated and untreated lens.

**Statistical Analysis**

Data for the inhibition of the cholesterol synthesis are expressed as means ± SEM. Differences between mean values were evaluated by unpaired student t-tests.

Lenses pairs were rejected by means of a factor F as a measure to evaluate the difference in fatty acid synthesis rates of the lenses of a lens pair. F was defined by the following equation:

\[ F = \frac{\Delta V}{\bar{V}} = \frac{(V_c - V_d)/(V_c + V_d),}{\bar{V}} = \frac{1}{2}(V_c + V_d) \]

where \( V_c \) and \( V_d \) are the fatty acid synthesis rates of control lens (no drug) and drug-incubated lens from a pair, respectively;

\[ \bar{V} = \frac{1}{2}(V_c + V_d) \]

represents the mean of the fatty acid synthesis rates of the control lens and drug-incubated lens of a pair; and

\[ \Delta V = V_c - \bar{V} \]

represents the deviation of the mean.

From \( F \) (mean value of F of the lens pairs used in this study), a 90% interval around the mean was calculated:

\[ \bar{F} - 1.654 \sigma < F < \bar{F} + 1.654 \sigma \]

Lens pairs with an F value corresponding to the 5% intervals at both extremes of the distribution were rejected.

**Isolation of Human Lens Microsomes and Measurement of the Inhibition of HMG-CoA Reductase Activity by Simvastatin and Pravastatin**

Capsules with adhering epithelial tissue were isolated from freshly obtained human lenses. The remaining tissue was dissected into a cortical and a nuclear region. Capsules and cortical regions were pooled and homogenized in phosphate buffer (0.1 mol/l potassium phosphate [pH 7.4], 0.1 mol/l NaCl, 10 mmol/l EDTA) using a Potter Elvehjem homogenizer (Thomas, Philadelphia). The homogenate was centrifuged at 10,000 \( \times \) g for 15 min, and this step was repeated with the supernatant. Subsequently, the supernatant was centrifuged for 1 hr at 100,000 \( \times \) g. The resulting microsomal pellet was resuspended in the phosphate buffer. All manipulations were performed at 4°C. The microsomal preparation was stored at -80°C. The assay of HMG-CoA reductase activity as described by Shapiro et al was adjusted for determination in lens microsomes. Pilot experiments showed that the conversion of [\( ^{14} \text{C} \)]-HMG-CoA to [\( ^{14} \text{C} \)]-mevalonate was linear with incubation times up to 2 hr and with microsomal protein concentrations up to 300 \( \mu \)g/100 \( \mu \)l incubation volume. The reaction was initiated by the addition of [\( ^{14} \text{C} \)]-HMG-CoA (final concentration, 100 \( \mu \)mol/l, specific radioactivity, 120 dpm/pmol) and proceeded at 37°C for 2 hr. Duplicate incubations with samples containing 300 \( \mu \)g of protein were performed in the presence and absence of cofactors (control) and various concentrations of simvastatin and pravastatin. Values obtained in the presence of cofactors were corrected for the values obtained in the absence of cofactors.

**RESULTS**

To study the influence of pravastatin and simvastatin on the cholesterol biosynthetic rate in human lens, ocular cholesterol synthesis was studied in a population comprising noncataractous subjects ranging from 13 to 90 yr in age. We determined the incorporation of [\( ^{14} \text{C} \)]-acetate into fatty acids and cholesterol by incubating these human lenses in organ culture for 20 hr at 37°C.

To characterize the human lens population, the relative frequency distribution of fatty acid biosynthesis in nondrug-treated lenses is shown in Figure 1. Figure 2 depicts the frequency distribution of the cholesterol biosynthesis rate in the population. Both cholesterol and fatty acid synthesis rates show a large variation within the human population and are skewed to the lower values of the distribution. This variation is not due to the age of the subjects but is probably determined by individual factors. In an attempt to circumvent the considerable interindividual variation in
biosynthetic capacity, we decided to analyze separate lens pairs. To determine the influence of pravastatin or simvastatin on cholesterol biosynthesis in human lenses, one lens of a subject was incubated without drug (control) and the other one was drug-treated.

In contrast with rat lenses,10 damage of human lenses by improper explantation caused no opacification. Lenses that were damaged during removal from the eye, however, incorporated almost no labeled acetate into either fatty acids or cholesterol. To eliminate nonvital lenses, we decided to discard lenses with a fatty acid synthesis rate lower than 5000 dpm/lens per 20 hr for further study. Because all damaged lenses had a fatty acid synthesis rate much lower than this arbitrary threshold level, it enabled us to be sure that damaged lenses were not included. Even when working with vital lens pairs, it is still difficult to determine the influence of the drug on the lens when each lens of a pair has a very different cholesterol synthesis rate. A comparison of the lenses in such a pair may show an apparent inhibition or stimulation of cholesterol biosynthesis that is not caused by the drug itself. To ex-

**FIGURE 1.** Frequency distribution of the fatty acid biosynthesis rate in a population of 95 human lenses. The lenses in organ culture were incubated with [2-14C]-acetate in the absence of any drug for 20 hr at 37°C, and the incorporation of label into fatty acid was determined as described in "Materials and Methods."

![Graph of [14C]-Acetate incorporation into fatty acids](image1)

**FIGURE 2.** Frequency distribution of the cholesterol biosynthesis rate in a population of 112 human lenses. The lenses in organ culture were incubated with [2-14C]-acetate for 20 hr at 37°C in the absence of any drug, and the incorporation of label into cholesterol was determined as described in "Materials and Methods."

![Graph of [14C]-Acetate incorporation into cholesterol](image2)
clude pairs of very dissimilar lenses, a factor F depending on the difference in the fatty acid synthesis rates of these lenses was introduced (see "Materials and Methods" for mathematical definition).

To calculate this exclusion factor, the fatty acid synthesis rates of the control lens (no drug) and the drug-treated lens from a pair were measured. Figure 3 shows the frequency distribution of F in the human lens population that was studied. The distribution is nearly normal and the mean of F (F ± SD = −0.075 ± 0.439 (n = 83)) is not significantly different from F = 0 (P > 0.05). This means that the fatty acid synthesis rate is unaffected by simvastatin and pravastatin (stimulation or inhibition of fatty acid synthesis by the drug would give a mean value of F significantly smaller or larger than F = 0, respectively). We observed previously that in individual human lenses cholesterol and fatty acid synthesis are significantly (P < 0.001) correlated with each other in a positive linear way. Because of this correlation, lens pairs with lenses having very different fatty acid synthesis rates probably also will show very different cholesterol synthesis rates. These deviant lens pairs are characterized by the lowest and highest values of the F distribution. Therefore, we rejected lens pairs with values of F corresponding to the 5% intervals at both extremes of the F distribution (F ≤ −0.801 ∧ F ≥ 0.651; the lowest and two highest intervals in Fig. 3). Lens pairs rejected by this exclusion criterion comprised lenses with fatty acid synthesis rates that differed 7 to 119 times between members of individual pairs. In some cases, lens pairs were rejected by both the exclusion factor and the threshold level, as mentioned earlier. In this way we were able to avoid misinterpretation of possible drug effects on ocular cholesterol biosynthesis caused by malfunctioning of one or two lenses of a pair.

**FIGURE 3.** Frequency distribution of the fatty acid synthesis factor F in a population of 83 human lens pairs. F is defined by the equation:

\[ F = \frac{\Delta V}{\bar{V}} = \frac{(V_c - V_d)}{(V_c + V_d)} \]

where \( V_c \) and \( V_d \) are the fatty acid synthesis rates of control lens (no drug) and drug incubated lens of a pair, respectively;

\[ \Delta V = V_c - \bar{V} \]

(deviation of the mean). Dot shading:

\[ \bar{F} - 1.654 \sigma < F < \bar{F} + 1.654 \sigma \) (accepted lenses).

Cross hatch shading:

\[ F \leq \bar{F} - 1.654 \sigma \land F \geq \bar{F} + 1.654 \sigma \) (rejected lenses).
Figure 4 shows the dose–response curve for the inhibition of cholesterol biosynthesis in human lenses by pravastatin and simvastatin. The percentage inhibition was calculated from the ratio of the cholesterol synthesis rate of the untreated and drug-treated lenses of a pair. Concentrations of up to 0.3 μmol/l of pravastatin gave no significant inhibition (0.01, 0.1, and 0.3 μmol/l pravastatin: P > 0.05). Only at 1 μmol/l of pravastatin was a significant inhibition of cholesterol synthesis (P < 0.001) observed. On the other hand, as little as 0.003 μmol/l of simvastatin caused an inhibition that is significant (P < 0.05). At drug concentrations of 0.01 μmol/l (P < 0.05), 0.1 μmol/l (P < 0.001), and 0.3 μmol/l (P < 0.05), the inhibition of cholesterol synthesis by simvastatin is significantly greater than by pravastatin. The IC₅₀ values (the drug concentration giving 50% inhibition) for [¹⁴C]-acetate incorporation into cholesterol by pravastatin and simvastatin were 0.5 μmol/l and 0.004 μmol/l, respectively. Thus, pravastatin was at least 100-fold less inhibitory in the human lens compared to simvastatin under the conditions used in this study.

To exclude the possibility that the difference in inhibition of lenticular cholesterol synthesis was due to differing sensitivities toward the drugs of the HMG-CoA reductase in the lenses, we determined the extent of inhibition of reductase activity present in microsomes isolated from human lenses. To this end, the HMG-CoA reductase activity in such microsomal preparations was determined in the presence of various quantities of simvastatin or pravastatin. As depicted in Figure 5, both drugs inhibited HMG-CoA reductase activity to the same extent. This demonstrates that the difference in inhibitory potency in lens organ culture probably is due to the difference in the ability of the drugs to gain access to the reductase enzyme.

DISCUSSION

In rat liver, the IC₅₀ values for pravastatin and simvastatin were 48.1 ± 10.9 nmol/l and 18.6 ± 6.0 nmol/l, respectively. This means that in the liver, which is the target organ, both drugs inhibit to a similar extent. In the liver, these HMG-CoA reductase inhibitors are extremely potent when compared to the binding constant (Kₘ) of 4 μmol/l for the substrate HMG-CoA.

In this study, we describe the inhibitory potency of pravastatin and simvastatin on cholesterol synthesis in the human lens, a completely avascular organ in which cholesterol supply probably depends largely on de novo synthesis, as has been shown for neonatal rat lens. We have developed an organ culture system to assess the influence of these drugs. [¹⁴C]-Acetate incorporation into cholesterol and fatty acids was proportional to time (data not shown). Fatty acid biosynthesis in the lens organ culture was unaffected by these drugs. This also has been found in rat lens using pravastatin or lovastatin, and in the human hepatoma cell line Hep G2 using pravastatin or simvastatin. The unaffected fatty acid synthesis enabled us to exclude lens pairs with lenses having very dissimilar biosynthetic capacities. The dose–response curve showed that in human lens, pravastatin is about 100-fold less potent in inhibition of cholesterol biosynthesis than simvastatin. The IC₅₀ values for pravastatin and simvastatin were 0.5 μmol/l and 0.004 μmol/l, respectively.
Inhibition of Human Lens Cholesterol Biosynthesis

**FIGURE 5.** Inhibition of HMG-CoA reductase activity in a microsomal preparation from human lens cortex by simvastatin (circles) and pravastatin (squares). From the cortical regions of 22 human lenses, microsomes were isolated as described in “Materials and Methods.” HMG-CoA reductase activity was determined in samples containing 3 mg/ml microsomal protein, the indicated drug concentrations, and 100 μmol/1 of [14C]-HMG-CoA. The assay was performed in the presence or absence of cofactors at 37°C for 2 hr. Values were corrected for counts formed in the absence of cofactors and are expressed as percentage of control (6 pmol mevalonate formed/mg of microsomal protein • 2 hr). Each data point represents the mean of a duplicate determination, with bars indicating the range.

Vastatin in human lens, 0.5 μmol/1 and 0.004 μmol/1, respectively, are very similar to those reported for rat lens (IC50 for pravastatin and simvastatin: 0.5 μmol/1 and 0.005 μmol/1, respectively).10 In the current study, we could not observe a difference in the effect of the drugs on lenses of various ages. The absence of such a difference could be anticipated because the cholesterol and fatty acid synthesis capacity of human lens does not seem to change with aging.20

The difference in inhibition of cholesterol biosynthesis by pravastatin and simvastatin in the lens does not seem to be unique to this organ. Koga et al8 administered pravastatin, simvastatin, or lovastatin to mice and determined the influence of these drugs on hepatic and extrahepatic cholesterol biosynthesis. All drugs produced 70% to 90% inhibition of sterol synthesis in liver at doses of 5 and 20 mg/kg. In testes, adrenal glands, spleen, and kidney, the drugs lovastatin and simvastatin (acid and lactone forms) significantly inhibited cholesterol synthesis, whereas pravastatin showed no significant inhibition. In addition, based on ex vivo experiments, Tsujiita et al9 reported that pravastatin is more liver-selective than other statins. The mechanism underlying the tissue-selective inhibition of cholesterol synthesis by pravastatin is not totally clear. Although structurally similar to each other, simvastatin is much more lipophilic than pravastatin. The hydrophilic nature of pravastatin appears to be related to a relatively low cellular penetration in extrahepatic cells. Our observations showed that human lenticular HMG-CoA reductase activity was inhibited to the same extent by both drugs (Fig. 5). Hence, the difference in inhibitory potency in human lens in organ culture can be explained by a lower uptake of pravastatin compared to simvastatin, as has been reported for the other extrahepatic cells and tissues by the authors mentioned above.

There are no data available on vastatin concentrations in the human lens in vivo situations. A study using dogs that were chronically dosed with simvastatin13 revealed that the drug could be detected in the aqueous and vitreous humor of the eye. A study of the intralenticular drug distribution showed that as the dosing duration increased, the simvastatin concentration in the lens nucleus and cortex similarly increased. A distribution study by Germershausen et al12 with orally dosed rats claimed that simvastatin and lovastatin are more concentrated in the liver than is pravastatin. As a result, the pravastatin concentration could be three to six times higher in extrahepatic tissues compared to simvastatin and lovastatin. In the latter study, no distinction was made between peripherally distributed and intracellular drug concentrations. The sensitivity of a given tissue to inhibition of cholesterol syn-
thesis, however, will be determined by the actual intracellular drug concentration at the site of HMG-CoA reductase, and not by the extracellular concentration.

We conclude from our investigations that pravastatin spares cholesterol synthesis in the human lens much more than simvastatin does under the conditions used in this study. To sustain our observations, it would be interesting to develop additional studies to determine the cellular uptake mechanisms of pravastatin and simvastatin in the human lens.

Key Words
cholesterol biosynthesis, fatty acid biosynthesis, human lens, pravastatin, simvastatin.

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