Cholesterol-lowering therapy evokes time-limited changes in serotonergic transmission

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Abstract

A number of studies have reported an increased risk for violent deaths and depression in subjects with reduced serum cholesterol concentrations. Links with hypothesized impairment of serotonin neurotransmission have not been satisfactorily tested. In this investigation, the serum and membrane cholesterol, microviscosity of erythrocyte membranes, platelet serotonin uptake, and clinical parameters were determined during pharmacotherapy of 17 hypercholesterolemic patients. A significant decrease in serum cholesterol and a nonsignificant decrease in membrane cholesterol concentration were found after 2 months of simvastatin therapy. Serotonin transporter (SERT) activity was significantly increased following 1 month of simvastatin; the tendency to decrease the initial increase in SERT activity was evident following 2 months of therapy. Both membrane cholesterol and SERT activity returned to pre-treatment levels after more than 1 year of therapy. Microviscosity of plasma membranes, impulsivity, empathy, adventure, sensation seeking, and depressed mood were not markedly changed. These data indicate that long-term therapy has different effects on serotonin transmission from short-term (1- to 2-month) therapy. A significant increase in SERT activity was detected only during the first month of simvastatin therapy. This finding suggests that within this period some patients could be vulnerable to depression, violence, or suicide.

Keywords: Serotonin uptake; Microviscosity; Depression; Impulsivity

1. Introduction

Since Muldoon et al. (1990) reported that cholesterol-lowering interventions are associated with a significant increase in male deaths from accidents, violence or suicide, a large body of
studies have confirmed a relationship between lower cholesterol levels, depression, and suicide (Balon, 2000; Huang et al., 2003; Vevera et al., 2003). However, contradictory results have also been reported (Tanskanen et al., 2000). Increased cholesterol levels have been found in patients with pain-related syndromes (Krikava et al., 2004). Other randomized trials on hypercholesterolemic patients have not found adverse effects on mood of cholesterol-lowering treatment (Muldoon et al., 2001; Deisenhammer et al., 2004).

Cholesterol is the main membrane-active sterol, which has an effect on cell growth and on the function of many membrane proteins. The functioning of a receptor or a transporter could be directly modulated by specific molecular interactions (Scanlon et al., 2001) or indirectly affected by cholesterol induced changes in membrane microviscosity and permeability. The widely accepted theory of Engelberg (1992; modified by Terao et al., 2000) states that central serotonin (5-hydroxytryptamine, 5-HT) neurotransmission is decreased via the altered microviscosity of plasma membranes caused by lowered blood cholesterol levels. Serotonin pathways function as a behavioral restraint system that inhibits impulsive behavior (Volavka, 2002). Reduced cholesterol levels could thus facilitate such complex behavior as violence towards the self or others.

In the maintenance of normal serotonergic neurotransmission, a crucial role is played by the serotonin transporter (SERT), which controls the concentration of free serotonin in the brain. An important mechanism for the modulation of SERT activity is the density of transporter molecules at the cell membrane and their affinity to 5-HT. Kinetic parameters of serotonin uptake, maximal velocity ($V_{\text{max}}$) and apparent Michaelis constant ($K_M$) are used to characterize SERT activity. Previous findings indicate that SERT is regulated both by membrane cholesterol (Scanlon et al., 2001) and several regulatory proteins (Haase et al., 2001).

Brain cholesterol synthesis could be affected by cholesterol-lowering medication that can cross the blood–brain barrier. Simvastatin (an inhibitor of the hydroxyymethylglutaryl-coenzyme A reductase) penetrates the blood–brain barrier (Saheki et al., 1994), and it has been demonstrated that treatment with simvastatin decreases levels of cholesterol in synaptosomal membranes, affects transbilayer cholesterol distribution (Kirsch et al., 2003), and reduces cholesterol turnover in the brain (Locatelli et al., 2002).

Given the hundreds of studies discussing the role of cholesterol-lowering therapy on serotonin transmission (the Engelberg theory was cited in 187 articles indexed in Web of Science—January 2004), there have been surprisingly few studies examining the underlying biological mechanisms. Furthermore, the studies that have been conducted have yielded conflicting results (Ringo et al., 1994; Delva et al., 1996; Hibbeln et al., 2000; Sarchiapone et al., 2001; Papakostas et al., 2003).

The aim of our study was to elucidate the effect of cholesterol-lowering therapy on membrane microviscosity and serotonin uptake and behavioral changes (depression, impulsivity, empathy, adventure). SERT activity in platelets was used as a model of SERT function in the brain (Tuomisto et al., 1979). Changes in the lipid composition of erythrocyte membranes were used to estimate changes in brain lipid composition (Chin et al., 1978; Agrawal et al., 1995).

2. Methods

2.1. Subjects and clinical assessment

The study included 17 consecutively diagnosed patients with familial hypercholesterolemia (8 females, 9 males) with a mean age of 63 (S.D.=10). All patients who were diagnosed with hypercholesterolemia during the period of enrolment participated in the study. None had been diagnosed with a mental disorder. A complete psychiatric and internal medicine examination, including blood sampling, was performed before the first administration of simvastatin, after 1 and 2 months of treatment, and in nine patients, also after 13–16 months. All of the patients were medicated with simvastatin (Simvor, Ranbaxy Laboratories, London) with a 20-mg/day dosage in the evening.

A clinical evaluation of the patients was performed by an experienced psychiatrist (JV). The Structured Clinical Interview MINI 5.0 was used for assessment of patients. Impulsivity, empa-
thy, and adventurousness were evaluated using the Eysenck IVE (impulsiveness/venturesomeness/empathy questionnaire) test, and depressive symptomatology using the 21-point Hamilton Rating Scale for Depression (HRSD). Data from the Zuckerman Sensation Seeking Scale, form V (a 40-item self-report), and the Lecrubier et al. (1995) impulsivity rating scale (IRS) were also collected, but these two scales have not been validated in the Czech Republic.

The study was approved by the ethics committee of the First Faculty of Medicine, Charles University of Prague, Czech Republic. All participants gave written informed consent.

2.2. Blood processing and biochemical determinations

Peripheral blood samples were obtained from the cubital vein of fasting subjects. Seven milliliters of blood were collected between 07:00 and 10:00 h into Vacutainer™ vacuum test tubes (BD Vacutainer), with no additive for cholesterol and triacylglycerol determination. Levels of serum total cholesterol (TC), HDL cholesterol (HDL), and triacylglycerols (TG) were determined using enzymatic kits (Cobas, Mira, ROCHE). LDL cholesterol (LDL) was calculated by Friedewald’s formula.

Four milliliters of non-coagulable blood (BD Vacutainer 9NC with 0.5 ml of 0.129 M trisodium citrate used as anticoagulant) were collected to measure the kinetic parameters of 5-HT uptake into platelets using tritium-labelled 5-HT (Tuomisto et al., 1979) and for erythrocyte ghost preparation (Dodge et al., 1963). Hydrophobic membrane probe 1,6-diphenyl-1,3,5-hexatriene (DPH, Sigma) was used to determine changes in erythrocyte membrane microviscosity (Lakowicz, 1999). Fluorescence anisotropy (r_{DPH}), which reflects the extent of probe movement restriction in an anisotropic membrane environment, was measured. Modified Folch (Koul and Prasad, 1996) methods were used to isolate total lipids from erythrocyte ghosts. Membrane cholesterol was analyzed by thin-layer chromatography (TLC) by chromatographic rods (silica-coated glass rods, type Chromarod SIII) with Iatroscan TH-10 (Iatron Laboratories) flame ionization detection (FID). Total phospholipids were determined in the samples by measurement of phosphorus (Bartlett, 1959) concentrations.

2.3. Data analysis

To calculate kinetic parameters of serotonin uptake, we used AccuFit Saturation Two-Site nonlinear regression analysis software (Beckman). Limiting permeability at low (physiological) 5-HT concentrations was calculated as the $V_{\text{max}}/K_M$ ratio, which more properly reflects the function of the serotonin transporter ($V_{\text{max}}/K_M$ is an efficiency criterion of transport system).

2.4. Statistical analysis

Data are expressed as the arithmetic means. Standard deviations (S.D.) were calculated to characterize group variability. Comparisons between patients before and after cholesterol-lowering therapy were performed using analysis of variance (ANOVA), followed by post hoc Duncan’s test. Pearson correlation coefficients were used to quantify the relation between two quantitative parameters. Statistical analyses were performed with the statistical package Statistica (StatSoft, Inc.).

3. Results

3.1. Clinical evaluation

Seventeen patients were examined before and after 1 and 2 months of cholesterol-lowering therapy, and nine of them were also examined after 13–16 months of therapy. No significant changes were found in impulsivity, empathy, adventurousness, and depressive symptomatology based on the Eysenck IVE, the IRS and the HRSD. The Zuckerman Sensation-Seeking Scale did not reveal any significant changes in Thrill and Adventure Seeking (TAS), Experience Seeking (ES), Disinhibition (Dis), Boredom Susceptibility (BS), or the total score (TS).

Eight patients did not continue in the study because of time constraints (patients were not compensated for their time). No psychiatric disorder or suicidal attempt was reported for these patients.
3.2. Serum cholesterol

Total cholesterol, TG, HDL cholesterol, and LDL cholesterol were monitored before treatment with simvastatin and during pharmacotherapy (Table 1 and Fig. 1). A decrease of TC was found after 1 month of simvastatin therapy ($P=0.21$), and a highly significant decrease was observed after 2 months ($P=0.000061$) or more than 1 year ($P=0.000053$) of simvastatin therapy compared with pre-treatment values. Similar changes were observed for LDL cholesterol. Neither TG nor HDL cholesterol was significantly changed.

### Table 1
Serum cholesterol and triacylglycerol concentrations before and after treatment of hypercholesterolemia with simvastatin

<table>
<thead>
<tr>
<th>Period of treatment (month)</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.57±1.26</td>
<td>2.08±0.94</td>
<td>1.18±0.13</td>
<td>4.18±0.91</td>
</tr>
<tr>
<td>1</td>
<td>6.20±0.89</td>
<td>1.98±0.74</td>
<td>1.20±0.15</td>
<td>3.94±0.79</td>
</tr>
<tr>
<td>2</td>
<td>***5.10±0.42</td>
<td>2.02±0.60</td>
<td>1.19±0.10</td>
<td>***3.00±0.37</td>
</tr>
<tr>
<td>13–16</td>
<td>***5.02±0.39</td>
<td>1.65±0.37</td>
<td>1.20±0.09</td>
<td>***2.98±0.20</td>
</tr>
</tbody>
</table>

Values are means±standard deviations. The means were calculated from 17 values. TC, total cholesterol; TG, triacylglycerols; HDL, HDL cholesterol; LDL, LDL cholesterol; all in mmol/l.

Marked values are significantly different from values before treatment with simvastatin at ***$p<0.001$.

3.3. Kinetics of platelet serotonin uptake

The kinetics of platelet serotonin uptake were described by the $V_{\text{max}}/K_M$ ratio. When changes in 5-HT uptake during treatment were compared with pre-treatment values (Table 2 and Fig. 1), the $V_{\text{max}}/K_M$ ratio was found to be significantly increased after 1 month of treatment only ($P=0.0082$); no significant increase in this ratio was observed after 2 months ($P=0.067$), and almost the same value as before treatment was observed after more than 1 year of therapy ($P=0.87$). The $V_{\text{max}}$ value was found to have almost doubled after 1 month ($P=0.00027$) and 2 months ($P=0.00013$) of treatment compared with pre-treatment values. The $K_M$ value was significantly increased after 2 months ($P=0.024$) of simvastatin therapy. Both $K_M$ and $V_{\text{max}}$ values returned to nearly the initial value.

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Fig. 1. Parameters measured in hypercholesterolemic patients as percentage of value before treatment with simvastatin. Values are means±standard deviations. The means were calculated from 17 values except for data from more than 1 year, when means were counted from only nine cases. TC, total cholesterol; LDL, LDL cholesterol; all in mmol/l; $K_M$ (nmol/l), apparent Michaelis constant characterizing affinity of serotonin transporter and calculated as extracellular serotonin concentration when the velocity of its transport equals to 50% of $V_{\text{max}}$ (pmol/min·$10^7$ platelets), maximal velocity of platelet serotonin uptake; $V_{\text{max}}/K_M$, ratio representing limiting permeability at low (physiological) extracellular concentrations of 5-HT (ml/min·$10^7$ platelets); $\gamma_{\text{DPH}}$, fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene; CH/PL, molar ratio of membrane cholesterol to total phospholipid. Marked values are significantly different from values before treatment with simvastatin at **$p<0.01$, ***$p<0.001$. 

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Table 2
Platelet serotonin uptake and fluorescence anisotropy of DPH in erythrocyte membranes and molar ratio cholesterol/phospholipid before and after treatment of hypercholesterolemia with simvastatin

<table>
<thead>
<tr>
<th>Period of treatment (month)</th>
<th>Platelet serotonin uptake</th>
<th>Ghosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_M$</td>
<td>$V_{\text{max}}$</td>
</tr>
<tr>
<td>0</td>
<td>189±83</td>
<td>3.68±2.09</td>
</tr>
<tr>
<td>1</td>
<td>224±75</td>
<td>***6.60±1.91</td>
</tr>
<tr>
<td>2</td>
<td>**258±68</td>
<td>***6.85±1.87</td>
</tr>
<tr>
<td>13–16</td>
<td>186±47</td>
<td>3.63±0.69</td>
</tr>
</tbody>
</table>

Values are means±standard deviations. The means were calculated from 17 values except for data from more than 1 year, when means were counted from only nine cases. $K_M$ (nmol/l), apparent Michaelis constant characterizing affinity of serotonin transporter and calculated as extracellular serotonin concentration when the velocity of its transport equals 50% of $V_{\text{max}}$ (pmol/min10^7 platelets), maximal velocity of platelet serotonin uptake; $V_{\text{max}}/K_M$, ratio representing limiting permeability at low (physiological) extracellular concentrations of 5-HT (pmol/min 10^7 platelets); $r_{\text{DPH}}$, fluorescence anisotropy of the membrane probe 1,6-diphenyl-1,3,5-hexatriene; CH, cholesterol; PL, total phospholipid. Values different from values before treatment are marked in case the difference is statistically significant (**$P<0.01$, ***$P<0.001$).

(above treatment) after more than 1 year of simvastatin therapy.

3.4. Membrane cholesterol and microviscosity

When fluorescence anisotropy changes during treatment were evaluated in comparison to values before treatment (Table 2 and Fig. 1), we found that fluorescence anisotropy of DPH ($r_{\text{DPH}}$) in erythrocyte membranes had not significantly decreased following 1 month, 2 months, or more than 1 year of simvastatin therapy.

To interpret the changes in the relative representation of cholesterol in erythrocyte membranes due to the administration of simvastatin, we used the ratio of cholesterol/total phospholipid (CH/PL, mol/mol). The results are summarized in Table 2. A nonsignificant decrease in CH/PL was found after 2 months of simvastatin therapy ($P=0.17$). The CH/PL ratio returned to a value close to the pre-treatment value after more than 1 year of therapy.

3.5. Mutual relations

Mutual relations between each pair of variables were tested by means of a correlation coefficient matrix. Pearson’s correlations ($r$) were calculated from 57–68 cases consisting of simvastatin-treated patients. There was a significant positive correlation between $V_{\text{max}}$ and $K_M$ ($r=0.48$, $P<0.001$) and between TC and LDL cholesterol ($r=0.94$, $P<0.001$). A negative correlation was found between TC and $V_{\text{max}}$ ($r=-0.29$, $P=0.028$), between LDL cholesterol and $V_{\text{max}}$ ($r=-0.32$, $P=0.015$), and between and LDL cholesterol and $K_M$ ($r=-0.27$, $P=0.040$).

4. Discussion

Our study finds that, in vivo, SERT functioning and membrane composition are influenced by serum cholesterol levels. The microviscosity of plasma membranes was not significantly changed by simvastatin treatment. SERT activity was significantly increased following 1 month of simvastatin therapy. However, longer-term therapy has a different effect on SERT activity (Fig. 1). The tendency for the initial increase in SERT activity to subsequently decline was evident after 2 months of therapy and especially after more than 1 year of therapy (13–16 months). A decreased relative membrane cholesterol representation was observed after 2 months of therapy. Serum cholesterol (TC, LDL) was markedly decreased after 1 month of simvastatin therapy; neither TC nor LDL cholesterol levels changed significantly in the period of 2–16 months of therapy. It is of interest that most of the biochemical parameters showed lower variance after treatment. Psychometric tests did not reveal any changes in depression, impulsivity, empathy, adventurousness, and sensation seeking.

An increase in $V_{\text{max}}$ was used to explain increased serotonin uptake. The $V_{\text{max}}$ value appears to be more significantly modulated by cholesterol than the $K_M$ value. Similar to Scanlon et al. (2001), we found an increase of $K_M$, i.e., a decrease of the affinity of SERT to 5-HT, following depletion of cholesterol; however,
we observed significantly increased $V_{\text{max}}$, and total serotonin uptake was consequently increased. This conflicting finding can be explained by the different designs of the two experiments; we measured in vivo the effect of therapeutic cholesterol-lowering medication in humans, whereas Scanlon et al. used cultured kidney cells and a much greater reduction in cholesterol content. We hypothesize that the direct effect of cholesterol on SERT activity is compensated for by other homeostatic mechanisms that maintain SERT activity in the normal range, e.g., by changes in SERT affinity for serotonin.

Our results suggest that SERT activity is more sensitive to an initial change in cholesterol concentration than to its stable level. This interpretation is consistent with a recent report that found an increase in impulsivity after 4 weeks of cholesterol-lowering therapy that dissipated over a longer (52 weeks) course of therapy (Ormiston et al., 2003). These findings did not support Engelberg’s (1992) hypothesis. Decreased cholesterol did not significantly lower membrane microviscosity as postulated in the theory, and opposite results in serotonin uptake were found. Increased uptake of serotonin was reported.

The fact that changes in SERT activity were observed after 1 month of treatment has important theoretical and practical implications. The implication for clinical practice is that patients could be vulnerable to depression, violence, or suicide during the first month of cholesterol-lowering therapy. In biochemical and behavioral studies, attention should be paid to 1 month of treatment because, at later stages, the changes are no longer apparent.

Acknowledgments

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References


