Pediatric implications of heterozygous familial hypercholesterolemia
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Chapter 7

Pharmacokinetics of pravastatin in children with familial hypercholesterolemia

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to be submitted for publication
Abstract

Background

Familial hypercholesterolemia (FH) leads to premature atherosclerosis and can cause early death. Treatment with HMG-CoA reductase inhibitors in adults is very effective in coronary artery disease prevention, resulting in a considerable morbidity and mortality reduction. However, the majority of events cannot be avoided. Treatment with these compounds should therefore probably be started before adulthood. Pharmacokinetic data on these drugs in children are lacking, which makes it difficult to devise a rational dosing scheme for children.

Methods

A two-week, multiple dose, pharmacokinetic (PK) study has been carried out with pravastatin in 24 children with familial hypercholesterolemia aged 8 to 16 years. Half of these children were prepubertal. All children received 20 mg of pravastatin once daily and a plasma-concentration versus time curve was performed on day 14. Nine blood samples were taken during ten hours after dosing. Pharmacokinetic curves for each individual were constructed using non-parametric methods, yielding AUC, $C_{\text{max}}$, and $t_{\text{1/2}}$. Cholesterol lowering was observed on day 14 and 6 weeks after start of pravastatin. We compared the data of these children to previously published studies in adults.

Results

Pravastatin was well tolerated. The $C_{\text{max}}$ in prepubertal children ($52.1 \pm (\text{SEM}) 7.8 \text{ng.mL}^{-1}$) differed non significantly, ($p=0.09$), from the $C_{\text{max}}$ in adolescents ($31.7 \pm 8.4 \text{ng.mL}^{-1}$) and neither did AUC in prepubertal children ($91.3 \pm 11.5 \text{ng.hr.mL}^{-1}$) differ significantly from AUC in adolescents ($69.3 \pm 16.5 \text{ng.hr.mL}^{-1}$). The $t_{\text{1/2}}$ was the same for both groups: $2.5 \pm 0.3$ hr.

A 27 % LDL-C reduction from baseline was achieved at day 14. An inversed correlation was found between $C_{\text{max}}$ and age. No significant correlation was found between weight, height, body surface area or gender, and the PK or effect parameters. There was no relationship between any PK parameter and cholesterol lowering. PK parameters and effect size were not different from previous studies in adults.

Relevance and Conclusion

Based on our findings there are no reasons to treat children with FH between ages of 8 and 16 with dosage regimens that are different from those in adults. However, for prepubertal children half the advised starting dose for adults may be sufficient.
Introduction

Pravastatin is one of the beta-hydroxy-beta-methylglutaryl-coenzyme A (HMG Co A) reductase inhibitors, widely used in the management of hypercholesterolemia. As a member of the statin family, pravastatin exerts its mechanism of action by competitive inhibition of HMG-CoA reductase, the key enzyme regulating the cholesterol biosynthesis in the liver. This leads to an upregulated production of LDL receptors as well as a decreased cholesterol biosynthesis with as a result a lowering of LDL cholesterol (LDL-C). The pharmacokinetics of pravastatin and interactions with various drugs have been extensively studied in healthy adults and in patients with hypercholesterolemia.1-8

After oral administration, pravastatin is rapidly absorbed with the time to reach maximal concentration ($t_{\text{max}}$) of approximately one hour; oral bioavailability is estimated to be 18%,1 based on the comparison between serum concentration after oral dose administration and intravenous dosing. The amount of pravastatin (parent compound and metabolites) recovered in urine and faeces after oral dosing suggests that the low bioavailability can partly be attributed to incomplete absorption and partly to a first pass-effect.1 The incomplete absorption might be explained by the hydrophilic properties of pravastatin.9 The occurrence of the first pass-effect is supported by a much lower time-averaged fraction of total radioactivity in plasma corresponding to intact pravastatin after oral than after intravenous administration.1 Unlike other statins, pravastatin metabolites are mainly produced by non-enzymatic acid-catalyzed isomerisation in the stomach before absorption (this mechanism is thus strictly speaking not a ‘first-pass effect’). A much smaller fraction is formed by cytochrome P450-dependent metabolism in the liver and the intestinal cell. Therefore no clinically important pharmacokinetic interaction exists between pravastatin and common CYP3A inhibitors.4,7,8

The $t_{\text{max}}$ is delayed by the administration of food, suggesting that absorption occurs mainly in the upper intestinal tract.5 Also, the AUC was considerably higher after duodenal infusion than after intrajejunal or intralaecal infusion, which strengthens this conclusion.10 After absorption, 43 to 54 % of pravastatin is protein bound.1,6 A steady state volume of distribution of 0.46 L/kg has been determined.1 Intact pravastatin and its metabolites are mainly cleared from the systemic circulation through both renal and non-renal routes: in healthy adults, renal and non-renal routes of elimination account for 47 and 53 %, respectively.1

Prevention of atherosclerosis in FH is most likely successful if started early in life, possibly before adulthood. There is, however, a scarcity of data on the treatment of children with statins. The safety, tolerability and efficacy of pravastatin was previously evaluated in a 12-week, short term double-blind, randomized and placebo-controlled study involving 72 children with heterozygous FH. Pravastatin was well tolerated,
and total cholesterol and LDL-C were significantly reduced from baseline in all pravastatin groups.\textsuperscript{11} Treatment of children with FH seems feasible, although several questions remain unanswered. Most drugs are used in children without adequate age-defined dose recommendations. The American Congress has enacted during the last few years several laws that empower the United States Food and Drug Administration (FDA) to propagate a rule demanding pediatric data from drug manufacturers.\textsuperscript{12,14} No data on the pharmacokinetics of pravastatin in children are currently available. In order to formulate a rational dosing regimen for children with FH, the disposition characteristics of pravastatin have to be studied in the age group in which the drug will be used. Although most kinetic studies in adults are done in healthy subjects it can be and often is argued that such studies in children should be done in patients who can benefit from the drug under study.\textsuperscript{15} Factors that might be of influence are age, body surface area, gender and pubertal development stage. We therefore designed a PK study of pravastatin for a group of children with familial hypercholesterolemia that differed across these characteristics.

Methods

Patients

Eligible were 24 consecutive children between 8 and 16 years, referred to our outpatient clinic over a half year period, who had a molecular diagnosis of heterozygous FH,\textsuperscript{16} and a LDL-C above or equal to 4.0 mmol/L. The study protocol was approved by the Institutional Review Board and all study interventions were performed with informed consent of both the children and their parents. Inclusion criteria were good general health, no evidence of any organ dysfunction now or in the past, normal physical and laboratory examinations (except for their lipid profile), no history of allergies and no use of any drugs in the 30 days prior to enrollment. Girls of child bearing potential had to have a negative serum urine pregnancy test within 24 hours prior to start of study medication.

Study design

This was a single center, open-label, non-randomized, steady state PK study. Children were assigned to group A (prepubertal) or B (pubertal), at least half of them to be assigned to group A. All children followed a fat restricted diet. Subjects received pravastatin 20 mg (tablets supplied by Bristol-Myers Squibb, Princeton, NJ, USA) once daily 30 minutes after breakfast with 250 ml of water for 13 consecutive days. On the morning of day 14, subjects returned to the clinic. Thirty minutes after completion of a regular breakfast, an oral dose of 20 mg pravastatin was administered. Serial blood samples were collected prior to (0 hr) and for 10 hours after dosing at 0.5, 1,
Pharmacokinetics of pravastatin in children with familial hypercholesterolemia

2, 3, 4, 6, 8, and 10 hours. To assess safety and tolerability clinical evaluations including monitoring of vital signs, clinical laboratory tests, and physical exams were performed before and after dosing.

Immediately after collection, the blood samples were placed into chipped ice to allow clotting to occur. Each sample was then centrifuged for 15 minutes at 1000 *g at 5°C. The separated serum was stored frozen below -20°C until analyses. After an overnight fast, at baseline, day 14 and 6 weeks after starting medication, total cholesterol, HDL cholesterol and triglycerides were measured with routine laboratory methods in the AMC department of clinical chemistry; from these values LDL-C was calculated.¹⁷

Analytical methods
Serum samples were assayed for pravastatin content at PharmaLytics, Inc., Saskatoon, Canada, by a validated LC/MS/MS method.¹⁸ Spiked analytical quality control samples (QCs) were analyzed along with the study samples in order to assess the accuracy and precision of each analytical run. The following acceptance criteria for the analyses were applied to each analytical run: (a) the predicted concentrations of at least three-fourth of all calibration standards were to be within ± 15% (± 20% for the lowest concentration in the standard curve) of their respective nominal concentrations; (b) at least one replicate of the lowest concentration in the standard curve was to be within ± 20% of the nominal concentration for that level to qualify as the lower limit of quantitation (LLQ), otherwise the next level standard was subjected to the same test and the LLQ raised accordingly; (c) the predicted concentrations of at least two-thirds of all analytical QC samples were to be within ± 15% of their individual nominal concentrations, with at least one QC sample at each level meeting the acceptance criteria.

Pharmacokinetic analyses
The serum concentration-time data for pravastatin were analyzed by a noncompartmental method. The peak serum concentration, \( C_{\text{max}} \), and the time to reach the peak concentration, \( t_{\text{max}} \), were obtained from the experimental observations. The slope (\( \theta \)) of the terminal phase of the serum concentration-time profile was determined by the method of least squares (log-linear regression of at least three data points). The apparent terminal half-life, \( t_w \), was estimated as \( \ln(2)/\lambda \). The area under the serum concentration-time curve from time 0 to time of last measurable concentration, AUC(0-T), was determined by summing the areas from time zero to the time of last measured concentration, calculated by using conventional trapezoidal methods. To compare the PK parameters in our children to those in adults, we scanned the literature for studies in which PK parameters of pravastatin were obtained after at least three daily doses of 20 mg.⁵ ¹⁹ For statistical comparisons the two-tailed t-test for unpaired data
was used.

**Cholesterol lowering**

Cholesterol lowering on pravastatin was expressed as a mean change from baseline, in mmol/l as well as in percentage. These values were then correlated with, the previously obtained PK parameters.

**Results**

**Patient demographics**

Twenty-four children (9 boys, 15 girls) entered and completed the study. A total of 12 subjects were prepubertal (Tanner stage = 1), whereas 12 young adolescents.

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics of the Two Study Groups</th>
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<tbody>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td>Prepubertal children</td>
</tr>
<tr>
<td>(n = 12)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Race, white / asian</td>
</tr>
<tr>
<td>Gender, m / f</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>Body Surface Area (m2)</td>
</tr>
</tbody>
</table>

Weight, height and BSA are given as mean ± SEM

![Figure 1. The peak serum concentration, C\text{max}, of pravastatin in children with FH plotted versus age (Lowess splines in red)](image-url)
were included. The demographic characteristics are listed in table 1.

No serious adverse events were reported during the study period and pravastatin was well tolerated in all subjects.

Pharmacokinetics

Plasma drug concentration versus time data of all subjects were described using a non-compartment model. The $C_{\text{max}}$ in prepubertal children (52.1 ± standard error of the mean (SEM) 7.8 ng.ml$^{-1}$) differ significantly from non significantly from the $C_{\text{max}}$ in young adolescents (31.7 ± 8.4 ng.ml$^{-1}$; p=0.09), and neither did AUC in prepubertal children (91.3 ± 11.5 ng.hr.ml$^{-1}$) differ significantly from AUC in young adolescents (69.3 ± 16.5 ng.hr.ml$^{-1}$; p=0.28). The mean $t_{\text{M}}$ was the same for both groups (table 2). An inversed correlation was found between $C_{\text{max}}$ and age (Spearman correlation: $r = -0.52; p = 0.009$), see figure 1. No correlation was found between body surface

Table 2. Pharmacokinetic parameters of pravastatin after daily 20 mg oral administration in children and adults

<table>
<thead>
<tr>
<th>Number (m / f)</th>
<th>Duration (days)</th>
<th>Mean age (years)</th>
<th>AUC (ng*hr/ml)</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$t_{\text{M}}$ (hr)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 / 6</td>
<td>14</td>
<td>9 [ 8-10]</td>
<td>91.3 ± 11.5</td>
<td>52.1 ± 7.8</td>
<td>2.4 ± 0.3</td>
<td>current article</td>
</tr>
<tr>
<td>3 / 9</td>
<td>14</td>
<td>13 [11-16]</td>
<td>69.3 ± 16.5</td>
<td>31.7 ± 8.4</td>
<td>2.5 ± 0.4</td>
<td>current article</td>
</tr>
<tr>
<td>18 / 0</td>
<td>10</td>
<td>27 [19-39]</td>
<td>66.5 ± 8.0</td>
<td>25.2 ± 3.9</td>
<td>-</td>
<td>Triscari 19</td>
</tr>
<tr>
<td>24 / 0</td>
<td>28</td>
<td>50 [24-70]</td>
<td>61.4 ± 5.1</td>
<td>28.0 ± 2.6</td>
<td>2.5 ± 0.2</td>
<td>Pan 5</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM

Figure 2. Percentage of LDL-C reduction on pravastatin plotted versus the peak serum concentration, $C_{\text{max}}$, in children with FH (Lawless splines in red)
area, weight, height or gender, and the PK or effect parameters.

PK parameters and effect size were not different from previous studies in adults, especially not different in the young adolescent group (table 2). There was no relationship between any PK parameter and cholesterol lowering (figure 2). After 14 days of treatment, a mean LDL-C reduction of 1.8 ± 0.2 mmol/l (27 %) from baseline was achieved (table 3). After six weeks, a daily dose of 20 mg pravastatin reduced the LDL-C from baseline by 32 % (2.1 ± 0.3 mmol/l).

Table 3. Baseline lipids and lipoproteins and mean change from baseline after two and six weeks of daily dose pravastatin 20 mg, respectively.

<table>
<thead>
<tr>
<th>Lipids (in mmol/l)</th>
<th>baseline</th>
<th>change from baseline at 14 days</th>
<th>p-Value</th>
<th>change from baseline at 6 weeks</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>8.2 ± 0.4</td>
<td>-1.8 ± 0.2 (-21%)</td>
<td>0.001</td>
<td>-2.1 ± 0.2 (-25%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-C</td>
<td>6.5 ± 0.4</td>
<td>-1.8 ± 0.2 (-27%)</td>
<td>0.001</td>
<td>-2.1 ± 0.2 (-32%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.3 ± 0.1</td>
<td>0.0 ± 0.0 (+1%)</td>
<td>0.96</td>
<td>0.1 ± 0.0 (+12%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>0.8 [0.5,1.3]</td>
<td>0.0 [-0.4,0.3]</td>
<td>0.96</td>
<td>-0.1 [-0.4,0.2]</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Values are given as mean ± SEM (%)

**Discussion**

Treatment with HMG-CoA reductase inhibitors in children with FH for up to two years of follow up is effective in reducing cholesterol and the intima-media thickness in the carotid artery (this thesis, chapter 8). Serious side effects, as expected from adult studies and those specific for children, have not been observed. However, the dosage in the previously mentioned studies has been chosen on arbitrary grounds, as no specific PK studies in children and young adolescents were available. It could be possible therefore, that lower statin dosages would suffice in children. We now present, to our knowledge, the first data on pharmacokinetics of a HMG-CoA reductase inhibitor in both prepubertal children and young adolescents. According to the present data, dosage regimens of pravastatin should probably be similar in young adolescents and in adults. AUC and C_{max} data might suggest that lower dosages in prepubertal children could be used. As our study is the first pharmacokinetic protocol of a HMG-CoA reductase inhibitor in children, we cannot compare our results to those of other studies.

Of course we have not made any direct comparison to adult patients within the same study. As the studies in adults are relatively homogeneous amongst each other, and as we have studied a relatively large group of children with various characteristics, this does not seem to be a major problem. Extrapolation to children under 8 years of age is of course not possible. It is not certain that pubertal stage by itself is important, as this variable will vary together with age, height, and body surface area; it is possible
that age is more important than pubertal stage perse. Although the differences between prepubertal children and young adolescents in our study were not statistically significant, it is remarkable that our study shows more variation in PK parameters than studies in adults. This probably strengthens the conclusion that smaller (prepubertal) children might be somewhat different from young adolescents.

All but two children were Caucasian. Pravastatin is extensively metabolized, but cytochrome P450 does not seem to be a significant elimination pathway, and ethnic differences due to genetic variance in liver enzyme activity are thus not to be expected. It is remarkable that the PK parameters, notably the very large interindividual variation in AUC, does not give rise to differences in cholesterol lowering. This is in agreement with data in adults, which show essentially the same. Although the relationship between pravastatin concentration and cholesterol lowering is thus complex, the percentage decrease of total cholesterol at 20 mg is exactly the same in our children as in adults.

Can our findings be extrapolated to other statins? Most drugs from this class are cytochrome P450 dependent for elimination, which is not the case for pravastatin. Also, known side effects may differ between various representatives of the class of HMG-CoA inhibitors (as the recent case of cerivastatin shows). Thus, for several reasons, including pharmacokinetics, extrapolation is not possible.

Our conclusion is therefore that pravastatin has identical pharmacokinetics and effect in young adolescents (from age 11 onwards) compared to adults, and may have minor differences in children that are prepubertal. As safety-profile is good and end-organ effects are present in 20-40 mg doses in children, it seems prudent to treat young adolescents as adults, and to start with doses that are somewhat lower in the age from 8 till 11 years.

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Conflict of interest statement: non declared.
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