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Lovastatin in X-linked adrenoleukodystrophy

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(published in abbreviated form)
Abstract

Background
X-linked adrenoleukodystrophy (X-ALD) is a peroxisomal disorder and is characterized by rapidly progressive cerebral demyelination or a gradually progressive myelopathy with or without adrenocortical insufficiency. The disease is caused by mutations in the ABCD1 gene resulting in impaired peroxisomal beta-oxidation and the subsequent accumulation of very long-chain fatty acids (VLCFA; >C22) in plasma and tissues. In 1998 it was reported in this Journal that lovastatin reduces total plasma VLCFA in X-ALD patients. This finding led to treatment with lovastatin in many patients with X-ALD despite contradicting data on the effect of lovastatin on plasma VLCFA. The aim of this clinical trial was to confirm that in patients with X-ALD lovastatin treatment can reduce C26:0 levels in plasma, but also in lymphocytes and erythrocytes.

Methods
The study was designed as a randomized double-blind placebo controlled cross-over trial (ISRCTN31565393). With a total of 14 patients, a 50% reduction of C26:0 levels can be detected with a power of 80%. The primary outcome measures were reduction of LDL-cholesterol in plasma, C26:0 levels in plasma and LDL-lipoprotein particles, lymphocytes and erythrocytes.

Results
Fourteen male patients with X-ALD were enrolled in the trial. LDL-cholesterol in plasma was reduced by 40%. Plasma C26:0 levels were reduced by almost 20% (but this effect was also observed for non-VLCFA). There was no effect on C26:0 levels in lymphocytes, erythrocytes and LDL-lipoprotein particles.

Conclusion
The reduction of plasma C26:0 is non-specific and linked to a reduction in LDL-lipoprotein particles. Lovastatin is not a treatment option for X-ALD.
Introduction

X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder with a birth incidence of 1:17,000 (Bezman et al. 2001). It is characterized by impaired peroxisomal beta-oxidation of very long-chain fatty acids (VLCFA; > C22:0), which accumulate in all tissues (Moser et al. 2001). X-ALD is caused by mutations in the ABCD1 gene (see also the X-ALD database at http://www.x-ald.nl) resulting in the absence or dysfunction of a peroxisomal transmembrane protein, the ALD protein (ALDP) (Mosser et al. 1993). The disease is characterized by a highly variable clinical expression, even within families (Kemp et al. 2001). Often, the first manifestation of the disease in childhood is isolated adrenocortical insufficiency (“Addison-only” phenotype) without neurological dysfunction. About 40% of affected male patients develop rapidly progressive cerebral demyelination causing severe disability and death usually within 2 years after symptom onset (Moser et al. 2001). This usually occurs between the age of 3 and 10 years (childhood cerebral ALD; CCALD), but is more rarely also seen in adolescence (adolescent cerebral ALD; AdolCALD), or adulthood (adult cerebral ALD; ACALD) (Moser et al. 2001). In adulthood the most common phenotype is adrenomyeloneuropathy (AMN), a gradually progressive myelopathy and peripheral neuropathy, causing severe disability. Symptoms usually appear in the 3rd or 4th decade. Patients with AMN can also develop secondary cerebral demyelination (“AMN cerebral”) (Van Geel et al. 2001).

Currently, treatment options are very limited and are mostly symptomatic. Only in a very early stage of CCALD progression can be halted or reversed by hematopoietic stem cell transplantation (Peters et al. 2004). Treatment with Lorenzo’s oil, a 4:1 mixture of C18:1 and C22:1 in combination with a low-fat diet rapidly normalizes plasma VLCFA, but seems to have no effect on disease progression in several open-label clinical trials (Aubourg et al. 1993; van Geel et al. 1999).

Previously, it was reported that lovastatin lowers VLCFA in patients with X-ALD (Singh et al. 1998b; Pai et al. 2000). This finding, however, could not be reproduced with simvastatin (Verrips et al. 2000). Subsequently performed animal experiments showed that lovastatin has no effect on brain and adrenal VLCFA levels in Abcd1 knockout mice (Yamada et al. 2000), and simvastatin even caused accumulation of VLCFA in these tissues (Cartier et al. 2000). In vitro experiments with cultured skin fibroblasts grown in cholesterol-depleted culture medium showed a reduction in VLCFA levels (Weinhofer et al. 2002). We recently demonstrated that this reflects a shift to increased synthesis of mono-unsaturated VLCFA (Engelen et al. 2008).

This trial was designed to investigate if lovastatin indeed has a biochemical effect in vivo in patients with X-ALD, and to help us decide whether a large scale trial with clinical outcome parameters is warranted.

Methods

Eligible patients

Patients were recruited from the AMC neurology outpatient clinic which is a referral center for peroxisomal disorders and through the patient support organization. Men with the AMN phenotype of X-ALD, confirmed by plasma VLCFA analysis and/or ABCD1 mutation analysis, were eligible. Exclusion criteria were the use of Lorenzo’s oil and/or cholesterol lowering therapy, contraindications for lovastatin use (liver- or kidney failure), or impairment so se-
were that the patient was unable to visit the outpatient clinic. The study protocol was approved by the local institutional review board. The trial was registered with the Dutch Trial Register (#852) and Current Controlled Trials (ISRCTN31565393).

Treatment
The study was designed as a randomized double-blind cross-over placebo controlled trial (Figure 1). Eligible patients were randomized and started on a run-in phase with a low fat diet. A randomization list was created by the Biostatistics department. Prepackaged medication sets with identical appearance were created by the Stichting Haarlemse Ziekenhuisapotheken (GCP certified foundation of hospital pharmacies). Patients entering the trial were given consecutive trial numbers, matching a set of prepackaged medication (twelve bottles numbered consecutively). After a one month run-in phase in which a standard low fat diet was administered by the dietician (as recommended by the American Heart Association) (Lichtenstein et al., 2006), medication or placebo was started, followed by washout and switch to the other treatment arm. Adverse effects were recorded. Treatment assignments were concealed from all investigators. Blood samples were taken at 0, 4, 12, 26, 30, 38 and 52 weeks. The code was broken after all patients had completed the follow-up visits and the data analyzed.

Laboratory studies
At each visit four standard Vacutainer tubes (2 with heparin, 2 with EDTA) were collected. Routine measurements (creatine kinase and transaminase activities in serum, and plasma lipid spectrum) were performed at the Department of Clinical Chemistry. The samples for VLCFA measurements were processed the same day at the Laboratory for Genetic Metabolic Diseases. Blood was centrifuged in Leukoprep tubes for 20 minutes at 2000 rpm. The plasma fraction was stored in separate tubes at -80 °C after snap freezing in liquid nitrogen. The lymphocyte pellet was resuspended in lysisbuffer twice to remove erythrocytes, and then washed with cold saline and stored at -80 °C. The erythrocytes were washed twice with cold saline and stored at -80 °C. VLCFA were measured as described previously (Kemp et al 2005). Lipoprotein fractions were isolated from plasma as described previously (Innis-Whitehouse et al 1998).

Outcome measures
Endpoints were plasma total cholesterol, LDL-cholesterol, C18:1, C24:0 and C26:0 levels, lymphocyte and erythrocyte C26:0 levels, and C18:1, C24:0 and C26:0 content of the LDL-
lipoprotein fraction at 22 weeks after start of treatment. For plasma total cholesterol, LDL-cholesterol, C18:1, C24:0 and C26:0 levels, and lymphocyte C26:0 levels intermediate endpoints at 8 weeks after start of treatment were assessed as well.

**Sample size**
It was assumed that the mean baseline C26:0 level prior to treatment would be about 2.94 µmol/L with a standard deviation of 0.87 µmol/L (Valianpour et al 2003). It was further assumed that lovastatin treatment might result in a 50% drop of this level. Based on the crossover design, all patients were to be analyzed within their randomization groups by comparing the change scores over the treatment periods (see below). The change scores in the groups would then be plus 1.47 (placebo minus lovastatin) and minus 1.47 (lovastatin minus placebo) respectively. Assuming a conservatively estimated zero correlation between the outcome measurements for the first and the second treatment period within each group, the standard deviation of the change scores in each group would be 1.23. Using a two groups t-test with a 0.05 two-sided significance level to detect a difference in these change scores of 2.94 assuming a common standard deviation of 1.739, 80% power would be achieved with a sample size of 7 in each group of the 2x2 cross-over design (or 14 in total).

**Statistical analysis**
Available pre-diet score distributions for plasma total cholesterol, LDL-cholesterol, C18:1, C24:0, C26:0 and lymphocyte C26:0 were assessed for normality by one sample Kolmogorov-Smirnov tests. Given the small number of patients in both trial arms, the pre-diet scores were tested for inequality with two-sample two-sided Student’s t-tests.
Based on the cross-over design, all patients were analyzed within their randomization groups. The analysis was performed with correction for non-significant pre-treatment baseline differences following the run-in phase with standard low fat diet, that were in the same direction as the expected differences between placebo and lovastatin under the alternative hypothesis that there is a significant difference between lovastatin and placebo treatment. The correction was performed by using the changes from baseline in each treatment period. In the group receiving placebo followed by lovastatin, we first subtracted baseline from outcome scores during placebo treatment as well as during treatment with lovastatin. Subsequently, change scores over treatment periods were determined by subtracting the observed changes under the lovastatin regimen from the observed changes under the placebo regimen. In the group receiving lovastatin followed by placebo, we first subtracted baseline from outcome scores during treatment with lovastatin as well as during placebo. Subsequently, change scores over treatment periods were determined by subtracting the observed changes under the placebo regimen from the observed changes under the lovastatin regimen.
Data was analyzed using the SPSS software for Windows, version 16 (SPSS, Chicago, IL). P-values below 0.05 indicated statistical significance. Graphs were made in GraphPad Prism (GraphPad Software, La Jolla, CA).

**Results**

**Patients**
Twenty-two patients were considered for inclusion in the study. Five were excluded from
participation (one was on Lorenzo’s oil, 2 were already on statin therapy, 2 were too severely impaired to complete the trial). Three patients declined to participate. Fourteen patients were randomized. No significant differences were observed for the randomized groups. There were no serious adverse events. All patients completed the study.

Biochemical results

In Table 1 data and change scores from baseline for 8 weeks and 22 weeks following start of treatment are presented for the all outcome parameters. The data is further summarized in scatter plots (Figure 2). At the first (8 weeks of treatment) and second (22 weeks of treatment) time point, there is a significant decrease in total and LDL-cholesterol levels in plasma of 38%. The plasma C24:0 and C26:0 levels are decreased initially by 20% and 18% respectively, although the difference is no longer statistically significant at the second time point for C26:0. There is a reduction of 17% in C18:1 levels at the first and second time point. There is no change in C26:0 levels in erythrocytes or lymphocytes at either time point. The C18:1, C24:0 and C26:0 content of LDL lipoprotein particles are unchanged.

Discussion

This study shows that lovastatin initially reduces C26:0 levels by about 20% in the plasma of patients with X-ALD, but fails to do so in lymphocytes or erythrocytes. It has been reported previously that lovastatin lowers plasma VLCFA in patients with X-ALD (Singh et al 1998b; Pai et al 2000). This prompted many patients worldwide to take lovastatin, even though no clinical trial with clinical endpoints showing a beneficial effect has been performed and both in vitro and in vivo data on results with lovastatin and cholesterol-lowering are conflicting (Singh et al 1998a; Cartier et al 2000; Yamada et al 2000; Weinhofer et al 2002; Engelen et al 2008). A trial with clinical endpoints is difficult to perform, considering the low incidence of X-ALD and the unpredictable disease course, requiring large groups and a long follow-up. It seemed premature to perform this trial for lovastatin since the “biological plausibility” is low, considering the conflicting data in the literature mentioned above. Therefore, our aim was to first perform a “proof of principle” trial with biochemical endpoints. Compliance in the trial was excellent, since there was a clear decrease in LDL-cholesterol levels of almost 40% during the lovastatin treatment period. There was also no drop-out in this small trial with highly motivated participants. There were no adverse effects. We demonstrated a decrease in C26:0 levels in plasma of roughly 20%. However, even with this decrease, levels remain between 2 and 3 times above the control level of 0.67 ± 0.13 µmol/L (Valianpour et al 2003) and the effect seems to diminish over time (the decrease is no longer statistically significant after 22 weeks of treatment). The decrease is not specific for VLCFA since C18:1 (a long chain mono-unsaturated fatty acid) is also reduced in plasma by lovastatin. It is of note that treatment withLovastatin does not affect VLCFA at the cellular level, since C26:0 levels in erythrocytes and lymphocytes were unchanged. Since VLCFA are virtually water insoluble and only a small fraction binds to albumin (Ho et al 1995), most of the plasma VLCFA is transported as cholesterol-esters in lipoprotein particles like LDL. To establish whether plasma VLCFA reduction is linked to a reduction in LDL cholesterol we measured the C18:1, C24:0 and C26:0 content of the LDL lipoprotein fraction. Lovastatin treatment did not decrease the C18:1, C24:0 or C26:0 content of LDL-cholesterol (Table 1). Based on these observations we conclude that lovastatin leads to a small decrease in C24:0 and C26:0 levels in plasma which
Clinical trials has to be considered a non-specific result of the LDL-cholesterol decrease. This is corroborated by the finding that C18:1 is also reduced and further supported by the lack of effect on C26:0 levels in peripheral blood lymphocytes and erythrocytes, and in LDL-lipoprotein fraction VLCFA content.

Table 1: Major outcome measures after 8 weeks (if applicable) and 22 weeks of treatment with lovastatin at a dose of 40 mg once daily*

<table>
<thead>
<tr>
<th></th>
<th>8 wk of treatment</th>
<th>22 wk of treatment</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Mean change</td>
<td>95% CI</td>
<td>P value</td>
<td>Mean change</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.52</td>
<td>1.48 ± 0.25</td>
<td>0.94 to 2.02</td>
<td>&lt;0.001</td>
<td>1.45 ± 0.24</td>
<td>0.92 to 1.98</td>
</tr>
<tr>
<td>LDL</td>
<td>3.54</td>
<td>1.44 ± 0.20</td>
<td>1.02 to 1.87</td>
<td>&lt;0.001</td>
<td>1.35 ± 0.21</td>
<td>0.89 to 1.82</td>
</tr>
<tr>
<td>C18:1</td>
<td>2.37</td>
<td>0.38 ± 0.17</td>
<td>0.0 to 0.76</td>
<td>0.05</td>
<td>0.44 ± 0.17</td>
<td>0.06 to 0.82</td>
</tr>
<tr>
<td>C24:0</td>
<td>77.1</td>
<td>14.2 ± 2.0</td>
<td>9.8 to 18.5</td>
<td>&lt;0.001</td>
<td>10.7 ± 3.80</td>
<td>2.4 to 19.0</td>
</tr>
<tr>
<td>C26:0</td>
<td>2.56</td>
<td>0.39 ± 0.11</td>
<td>0.15 to 0.63</td>
<td>0.004</td>
<td>0.23 ± 0.16</td>
<td>-0.12 to 0.58</td>
</tr>
<tr>
<td><strong>Lipoprotein Fraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 in LDL</td>
<td>1540</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>-84 ± 69</td>
<td>-240 to 80</td>
</tr>
<tr>
<td>C24:0 in LDL</td>
<td>64</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>3.4 ± 2.5</td>
<td>-2.0 to 8.9</td>
</tr>
<tr>
<td>C26:0 in LDL</td>
<td>9.7</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>-1.9 ± 1.1</td>
<td>-4.3 to 0.6</td>
</tr>
<tr>
<td><strong>Cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>C26:0 (lymphocytes)</td>
<td>0.35</td>
<td>-0.01 ± 0.02</td>
<td>-0.04 to 0.03</td>
<td>0.64</td>
<td>0.03</td>
<td>-0.02 to 0.08</td>
</tr>
<tr>
<td>C26:0 (erythrocytes)</td>
<td>0.18</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>0.002 ± 0.003</td>
<td>-0.01 to 0.01</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SE. The mean reduction indicates the absolute change from baseline levels after treatment. P values were calculated with the use of a two-sided, unpaired Student’s t-test. ApoB denotes apolipoprotein B, CI confidence interval, C18:1 oleic acid, C24:0 tetracosanoic acid, C26:0 hexacosanoic acid, NA not applicable, and ND not determined.

† Equal variances were not assumed.
Figure 2: Scatter plots of the effect of lovastatin on levels of plasma total cholesterol (A), LDL-cholesterol (B), plasma C24:0 (C) and C26:0 (D), C24:0 (E) and C26:0 (F) in LDL lipoprotein particles, C26:0 in lymphocytes (G) and C26:0 in erythrocytes (H). TP1 and TP2 are 8 or 22 weeks. Units are mmol/L (A and B), µmol/L (C and D), pmol/mmol ApoB (E and F) and nmol/mg protein (G) and % of total fatty acids (H). Error bars indicate the mean and standard deviation. *** p < 0.001.
Conclusion

It seems unnecessary to invest a great deal of resources and time in a trial with clinical endpoints. Physicians should not prescribe lovastatin as a VLCFA lowering therapy to patients with X-ALD, since evidence does not support it.

Acknowledgements

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