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Lipoprotein-associated phospholipase A_2 activity and low-density lipoprotein subfractions after a 2-year treatment with atorvastatin in adolescents with type 1 diabetes

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Abstract

Background: The objective of the study was to assess the effect of atorvastatin on inflammation markers and low-density lipoprotein (LDL) subfractions.

Methods: In a prospective, randomized, double-blind pilot study involving 28 adolescents with type 1 diabetes (T1D), lipoprotein-associated phospholipase A_2 (Lp-PLA2) activity, high-sensitivity C-reactive protein (hsCRP), and subfractions of LDL were measured at baseline, after 1 year and 2 years of treatment with atorvastatin (10 mg/day) vs. placebo.

Results: For the atorvastatin group, we found posttreatment reductions of Lp-PLA2 activity ($p < 0.001$), LDL cholesterol ($p = 0.001$), non-small dense LDL cholesterol ($p < 0.001$), total cholesterol ($p < 0.001$), and apolipoprotein B (apo B) ($p < 0.001$), whereas small dense LDL cholesterol and hsCRP did not change significantly.

Conclusions: In adolescents with T1D, long-term treatment with atorvastatin is safe and may reduce cardiovascular risk by significant decreases of Lp-PLA2 activity and LDL cholesterol.

Keywords: atorvastatin; inflammatory markers; LDL subfractions; type 1 diabetes.

Introduction

The incidence of Type 1 diabetes (T1D) is increasing, with a number of acute and chronic complications. Atherosclerosis is one of the most important long-term consequences of T1D [1, 2]. In addition, it is recognized that inflammatory processes are an essential component of both atherogenesis and T1D [3, 4]. Lipoprotein-associated phospholipase A_2 (Lp-PLA2) is secreted by inflammatory cells including macrophages and represents an important marker of vascular inflammation [5]. Its local coronary production was demonstrated in patients with early coronary atherosclerosis [6]. Two-thirds of circulating Lp-PLA2 is linked to low-density lipoprotein (LDL) and one-third to high-density lipoprotein (HDL) [7]. In the general population, Lp-PLA2 activity has been found to be associated with increased cardiovascular risk and cardiac mortality [8, 9]. Also in adults with type 2 diabetes, Lp-PLA2 activity has been identified as an independent risk factor and predictor for coronary heart disease and cardiovascular outcome [10, 11]. Although coronary heart disease is a major cause of long-term mortality in adults with T1D [12], there is comparatively little data available about the influence of Lp-PLA2 on the development of atherosclerotic vascular disease in these patients [13, 14]. Moreover, it must be noted that information from the literature on a relationship between Lp-PLA2 and atherosclerosis for children and adolescents with T1D is weak or lacking so far. Due to the close interrelation between Lp-PLA2 activity and increased cardiovascular risk, suitable therapeutic

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agents have been evaluated in order to mitigate the risk. Thus, it could be shown that various lipid-lowering drugs and a novel Lp-PLA2 inhibitor are able to reduce apolipoprotein B (apo B)-associated Lp-PLA2 activity [15–17].

The primary objectives of the present study were to assess Lp-PLA2 activity, high-sensitivity C-reactive protein (hsCRP), LDL subfractions, and the effect of a 2-year treatment with atorvastatin relative to these parameters in adolescents with T1D.

Materials and methods

Study design and patients

The present article is based on a prospective, randomized, placebo-controlled, double-blind multicenter study entitled "Vasoprotection by atorvastatin in children and adolescents with T1D, a 2-year pilot study" (ATV 03-005G). The protocol was approved by the Ethics Committee at the Albert-Ludwigs-University of Freiburg (registration number: 243/03; German Clinical Trials Register: DRKS00000297, Registry URL: http://www.germanctr.de) and all participants or their parents signed an informed consent. At the centers for Pediatrics and Adolescent Medicine of the University Medical Centers Freiburg, Heidelberg, and the Olgahospital Stuttgart in Germany, 270 children and adolescents with T1D were screened in terms of their eligibility for participation. The diagnosis T1D was made following the guidelines of the American Diabetes Association (ADA) [18]. For ethical reasons and to avoid unnecessary treatment with atorvastatin, only adolescents were enrolled who showed a median carotid intima-media thickness ≥75th percentile (Schmidt-Trucksäss A) compared to healthy controls [19]. Of 270 screened patients, 28 were eligible for inclusion in the study and randomly allocated to two study groups (10 patients received placebo and 18 patients received 10 mg atorvastatin/day). In the course of the study there were four drop-outs in the atorvastatin group due to one fatal traffic accident and three cases of non-compliance (inadequate intake of the study medication, failure to attend the study visits) and two drop-outs in the placebo group, one because of poor glycemic control with HbA_{1c} >9.5% measured twice within a period of 4 weeks and one because of inadequate intake of the study medication. The pharmacy at the University Hospital in Freiburg provided the randomization code assignment as well as packaging, blinding, and labeling of the study medication. During the whole study period of 24 months, 12 study visits were conducted for assessments of clinical, laboratory, and safety parameters.

Study parameters and laboratory analyses

Standard deviation score (SDS) of the body mass index (BMI, kg/m^2) was calculated using normal values of German children and adolescents [20]. Blood pressure level is composed of the average derived from three measurements with a Dinamap pediatric monitor (Critikon, Tampa, FL, USA). Pulse pressure was assessed from the difference between systolic and diastolic blood pressure. Laboratory determinations were done after an overnight fasting period and evaluated centrally at the Department of Clinical Chemistry, University Medical Center Freiburg, Germany.

Lipids and lipoproteins

Cholesterol and triglycerides were determined enzymatically with the CHOD-PAP and GPO-PAP method with commercially available reagents, respectively (Wako Chemicals, Osaka, Japan). The concentration of apo B was assessed by turbidimetry (A 640 analyzer, Olympus, Tokyo, Japan) using polyclonal antiserum (Greiner Biochemica, Flacht, Germany). LDL particles were analyzed by equilibrium density gradient ultracentrifugation (Beckman Optima LE-80K ultracentrifuge; Beckman Coulter Inc., Fullerton, CA, USA) using the following densities: 1.019–1063 kg/L (total LDL), <1.031 kg/L (LDL-1), 1.031–1.034 kg/L (LDL-2), 1.034–1.037 kg/L (LDL-3), 1.037–1.040 kg/L (LDL-4), 1.040–1.044 kg/L (LDL-5), and >1.044 kg/L (LDL-6). Atherogenic LDL-5 and LDL-6 were summarized as small dense LDL (sdLDL) particles and LDL-1–LDL-4 as non-sdLDL [21].

Inflammation markers

High-sensitivity C-reactive protein (hsCRP) was measured using a commercially available immunonephelometric assay kit (Dade Behring GmbH, Eschborn, Germany). Immunonephelometric assays were performed on an Olympus AU680 analyzer (Beckman Coulter Inc., Fullerton, CA, USA).

Lp-PLA2 is also denoted as platelet-activating factor acetylhydrolase (PAF-AH). The activity of Lp-PLA2 was measured on an Olympus AU680 analyzer (Beckman Coulter Inc., Fullerton, CA, USA) using 1-myristoyl-2-(4-nitrophenylsuccinyl)-sn-glycero-3-phosphocholin as substrate releasing 4-nitrophenylsuccinate. The eventually resulting 4-nitrophenol was then determined photometrically. Inter-assay coefficient of variance for Lp-PLA2 was <5% [22].

Statistical analysis

Comparison of study groups was calculated according to the intention-to-treat principle. Data are shown as median with the 95% confidence interval in brackets (CI). Differences between groups were analyzed with the non-parametrical Mann-Whitney U-test. Changes of parameters within a study group in the course of the study were analyzed with the Friedman test comparing data between baseline, 1 year, and study end after 2 years. The rank correlation coefficient was calculated according to Spearman. A p-value <0.05 was accepted for statistical significance. The SPSS 20.0 statistical program (Chicago, IL, USA) was used for all analyses.

Results

Table 1 compares characteristics of children and adolescents with T1D who received either atorvastatin (10 mg/day) or placebo. Neither groups differed significantly.

Table 1: Characteristics of children and adolescents with type 1 diabetes.

Parameters	Patients		
	Atorvastatin (n=18)	Placebo (n=10)	p-Value[a]
Age, years	14.4 (13.9–15.8)	15.9 (13.8–16.6)	NS
Diabetes duration, years	4.9 (4.0–7.6)	5.5 (3.5–8.9)	NS
HbA_{1c},%	8.3 (7.7–8.8)	8.2 (7.0–9.0)	NS
Insulin, IU/kg/day	0.70 (0.63–0.95)	0.90 (0.70–1.04)	NS
BMI-SDS	0.65 (0.34–1.03)	0.50 (0.48–0.79)	NS
Systolic blood pressure, mmHg	121 (112–126)	126 (117–132)	NS
Diastolic blood pressure, mmHg	70 (65–73)	69 (65–76)	NS
Pulse pressure, mmHg	51 (43–57)	53 (47–61)	NS

Data are medians (95% CI). [a]Mann-Whitney U-test was used for comparison between groups; NS, not significant; HbA_{1c}, glycated hemoglobin A_{1c}; BMI-SDS, body mass index-standard deviation score.

Table 2 compares the course of lipid and lipoprotein levels between the atorvastatin and the placebo group at baseline (atorvastatin group n = 18, placebo group n = 10), after 1 year (atorvastatin group n = 16, placebo group n = 9), and after 2 years (atorvastatin group n = 14, placebo group n = 8). In the atorvastatin group, total cholesterol, apo B, and total low-density lipoprotein cholesterol (LDL-C) were significantly reduced. Non-sdLDL-C was also significantly reduced but closely parallel to the reduction of total LDL-C. In contrast, the level of sdLDL-C remained more or less the same. In the placebo group, total cholesterol was significantly reduced, but all other analyzed lipid parameters showed only non-significant changes.

Lp-PLA2 was significantly reduced by atorvastatin and increased non-significantly in the placebo group. The ratio of Lp-PLA2 to LDL-C increased ($p < 0.001$) during the course of the study (Table 3).

Correlations between sdLDL and triglycerides and sdLDL and HDL-C were tested using the Spearman rank correlation coefficient. There was no significant

Table 2: Lipoproteins at baseline and during the administration of either atorvastatin (10 mg/day) or placebo in children with type 1 diabetes.

Parameters	Baseline	1 year	2 years	p-Value[a]
non-sdLDL-C, mg/dL				
Atorvastatin	40 (33–50)	29 (22–36)	25 (21–29)	<0.001
Placebo	38 (32–44)	32 (25–44)	33 (28–47)	NS
sdLDL-C, mg/dL				
Atorvastatin	14 (12–16)	12 (10–14)	13 (9–20)	NS
Placebo	16 (12–18)	13 (11–18)	15 (13–21)	NS
Total cholesterol, mg/dL				
Atorvastatin	172 (160–193)	143 (127–153)	134 (124–151)	<0.001
Placebo	164 (145–202)	153 (133–181)	162 (148–205)	NS
LDL-C, mg/dL				
Atorvastatin	85 (77–105)	64 (48–76)	55 (48–71)	<0.01
Placebo	85 (70–99)	75 (58–97)	84 (72–107)	NS
apo B, mg/dL				
Atorvastatin	65 (57–79)	53 (43–62)	46 (40–62)	<0.001
Placebo	66 (56–77)	61 (48–77)	68 (56–81)	NS
HDL-C, mg/dL				
Atorvastatin	57 (53–66)	54 (52–64)	52 (47–60)	NS
Placebo	67(57–83)	50 (40–70)	55 (47–70)	0.030
Triglycerides, mg/dL				
Atorvastatin	73 (59–94)	68 (55–111)	54 (41–98)	NS
Placebo	50 (45–68)	56 (50–64)	65 (56–75)	NS

Data are medians (95% CI). [a]Friedman test; NS, not significant; LDL-C, low-density lipoprotein cholesterol; non-sd LDL-C, non-small dense (LDL 1-4) LDL-C; sdLDL-C, small dense (LDL 5 and 6) LDL-C; HDL-C, high-density lipoprotein cholesterol; apo B, apolipoprotein B.

Table 3: Inflammation markers and risk factors at baseline and during the administration of either atorvastatin (10 mg/day) or placebo in children with type 1 diabetes.

Parameters	Baseline	1 year	2 years	p-Value[a]
hsCRP, mg/L				
Atorvastatin	0.45 (0.35–1.01)	0.40 (0.34–1.20)	0.60 0.37–0.94)	NS
Placebo	0.30 (0.12–1.26)	0.70 0.07–3.03)	0.45 (0.17–2.20)	NS
Lp-PLA2 activity, U/L				
Atorvastatin	403 (364–436)	331 (295–360)	318 (295–358)	<0.001
Placebo	364 (338–427)	397 (343–445)	389 (347–468)	NS
Lp-PLA2 activity/LDL-C				
Atorvastatin	4.6 (4.2–6.0)	5.6(5.0–8.3)	6.1 (5.5–7.9)	<0.001
Placebo	4.4 (4.2–5.0)	5.7(4.3–6.8)	5.2 (3.8–7.9)	NS
$HbA_{1c,}$%				
Atorvastatin	7.9 (7.4–8.5)	8.2 (7.7–8.7)	8.3 (7.7–9.2)	NS
Placebo	8.2 (7.0–9.0)	7.8 (6.9–8.9)	8.7 (7.5–9.3)	NS
Syst. BP, mmHg				
Atorvastatin	121 (113–126)	123 (116–134)	118 (108–125)	NS
Placebo	126 (117–132)	121 (114–130)	120 (114–130)	NS

Data are medians (95% CI). [a]Friedman test; NS, not significant; LDL-C, low-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; Lp-PLA2, lipoprotein-associated phospholipase A2; HbA_{1c}, glycated hemoglobin A_{1c}; syst. BP, systolic blood pressure.

correlation both at baseline (total group) and after 2 years (atorvastatin group).

No adverse event occurred in either the atorvastatin group or the placebo group during the course of the study.

Discussion

The main outcomes of this study were significant reductions of Lp-PLA2 activity and LDL-C during the long-term treatment with low-dose atorvastatin in adolescents with T1D. Statins are increasingly used for the treatment of high LDL levels in children and adolescents, but should not be prescribed before 10 years of age in boys and only after the onset of menses in girls. The recommended daily dose of atorvastatin for children is 10–20 mg and number and type of adverse effects are comparable to those of adults [23]. A similarly positive safety profile of atorvastatin 10 mg and 80 mg has been confirmed in numerous clinical trials in adult populations [24]. With regard to the long-term safety of statins, particular attention shall be paid to elevations of liver transaminases and creatine kinase using regular monitoring. Although cases of rhabdomyolysis are rare, patients and their parents should be informed about the muscle-related side effects of statins [23].

In terms of Lp-PLA2 activity in children and adolescents, it has been reported that obese children and adolescents showed significantly higher levels than healthy controls, [25] and moreover, an association between increased Lp-PLA2 concentrations and higher BMI percentiles [26]. In male schoolchildren, Lp-PLA2 activity was inversely and independently associated with physical activity emphasizing the importance of exercise for the prevention of atherosclerosis [27]. Lp-PLA2 activity was also significantly increased in children and adolescents with heterozygous familial hypercholesterolemia in comparison to unaffected siblings and was significantly reduced by pravastatin therapy, whereby the increase of Lp-PLA2 activity was probably due to the close binding to the markedly increased LDL particles in this disease [28]. However, studies investigating Lp-PLA2 activity in children and adolescents with T1D have not been published yet. In adults with T1D, levels of Lp-PLA2 activity were significantly higher than in controls indicating that it may be related to low-grade inflammation or to the higher risk of developing cardiovascular disease established in patients with T1D [29, 30]. In addition to the significant reduction of Lp-PLA2 and LDL-C, we have also established an increasing ratio of Lp-PLA2 activity to LDL-C indicating that the decrease in LDL-C was proportionally greater than the decrease in Lp-PLA2 activity. In various publications, the reduction of Lp-PLA2 activity by statins is in the range from 20% to 40% [15, 16]. In line with this, our diabetic patients in the atorvastatin group revealed a decrease of Lp-PLA2 activity of 21%, whereas the patients receiving placebo showed no significant change of Lp-PlA2 activity during the study period. Although two-thirds of plasma Lp-PLA2 is bound to LDL particles, only 1 LDL particle in 1000 obtains a molecule of Lp-PLA2 [7]. This could also be a reason for a more effective reduction of LDL-C

compared to Lp-PLA2 activity by atorvastatin. The close association between Lp-PLA2 and LDL-C in the circulation leads to simultaneous reduction through statin therapy. Therefore, the JUPITER trial has shown that on-treatment measurement of Lp-PLA2 no longer predicted cardiovascular risk when the participants were treated with rosuvastatin [31].

Of note is that the reduction of LDL-C in our patients was largely generated by a significant decrease of non-sdLDL-C during the treatment with atorvastatin, while the level of sdLDL-C remained almost unchanged. In a recently published study, similar results were seen after a 6-month treatment with atorvastatin (10–20 mg/day) in adolescents with T1D and elevated LDL-C. In addition to the decrease of atherogenic intermediate-density lipoprotein and very low-density lipoprotein particles, concentrations of large, medium, and small LDL subfractions were reduced more than very small LDL particles by atorvastatin. In contrast to our results, very small dense LDL particles were even increased after 6 months of treatment with atorvastatin [32]. It has been reported that a 3-month treatment with atorvastatin (10 mg/day) in adults with heterozygous familial hypercholesterolemia revealed a statistically significant reduction of both sdLDL-C and large buoyant LDL-C [33]. It should be noted, however, that the latter study can only be compared with ours to a limited extent mainly because of completely different study populations and differing baseline LDL-C profiles. Interestingly, however, treatment with human growth hormone led to a significant reduction of sdLDL-C in children with growth hormone deficiency and children with small for gestational age status [34].

It is known that children and adolescents with good control of their T1D have the same or even a more favorable plasma lipid profile than healthy peers [35]. A study involving 98 reasonably well-controlled children and adolescents with T1D (mean $HbA_{1c} = 8.41\%$) and 57 healthy control subjects showed that large LDL particle concentration was greater in T1D patients than in controls ($p = 0.007$). Moreover, controls had higher small LDL ($p = 0.0067$), medium small ($p = 0.0026$), and very small LDL particle concentrations ($p = 0.0091$) than T1D patients [36]. One might speculate that due to the favorable and rather antiatherogenic baseline concentrations of triglycerides, HDL-C, total LDL-C, and a comparably low proportion of sdLDL-C, no further significant reduction of sdLDL-C by atorvastatin could be expected in our equally well-controlled T1D patients.

A notable strength of our study is the prospective, placebo-controlled, double-blind multicenter study design including very strict criteria for the inclusion of patients. This, in turn, led to the small sample size which is the major limitation of the study.

In summary, with these study results, we present new data about Lp-PLA2 activity and LDL-subfractions in adolescents with T1D that could be helpful in assessing their cardiovascular risk. Furthermore, long-term treatment with atorvastatin is safe and may reduce the cardiovascular risk by significant decreases of Lp-PLA2 activity and LDL cholesterol.

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