Lomitapide affects HDL composition and function

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Word count (main text):1392 (max 1500)
Abstract word count: 150 (max 150)
References: 22 (max 25)
Number of figures and tables: 2 (max 2)

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Abstract (150 words, max 150)

Background: Lomitapide reduces low-density lipoprotein-cholesterol (LDL-C) but also high-density lipoprotein-cholesterol (HDL-C) levels. The latter may reduce the clinical efficacy of lomitapide. We investigated the effect of lomitapide on HDL-C levels and on cholesterol efflux capacity (CEC) of HDL in patients with homozygous familial hypercholesterolemia (HoFH).

Methods and results: Four HoFH patients were treated with increasing dosages of lomitapide. Lomitapide decreased LDL-C (range -34 to -89%). Total HDL-C levels decreased (range -16 to -34%) with a shift to buoyant HDL. ABCA1-mediated CEC decreased in all patients (range -39 to -99%). The changes of total, ABCG1- and SR-BI-mediated CEC were less consistent.

Conclusion: Lomitapide decreased LDL-C and HDL-C levels. Our report raises the hypothesis that the anti-atherogenic potential of HDL seems to be unaffected as total CEC did not seem to change consistently. Combined with the reduction of atherogenic lipoproteins, the net effect of lomitapide appears to be beneficial in HoFH patients.

Key words: lomitapide, homozygous familial hypercholesterolemia, HDL, cholesterol efflux capacity, cholesterol lowering drugs

Introduction

Homozygous familial hypercholesterolemia (HoFH) is a rare disease caused by mutations in the *LDLR* gene [1, 2]. Untreated patients with HoFH are characterized by extremely raised low density lipoprotein-cholesterol levels (LDL-C) often exceeding 13 mmol/L, rendering them susceptible to unparalleled premature atherosclerotic cardiovascular disease (CVD) and extensive aortic valve calcification and stenosis [3, 4]. Without treatment the majority of patients with HoFH do not survive beyond their twenties. Early diagnosis and treatment of HoFH is therefore essential [4].

A new treatment option for HoFH patients has become available with the microsomal triglyceride transfer protein (MTP) inhibitor, lomitapide, which resulted in 38% reduction of LDL-C levels in a phase III trial in 29 HoFH patients [5]. However, high-density lipoprotein-cholesterol (HDL-C) levels were reduced by 12% [5-7]. Although HDL-C levels show an inverse correlation with CVD risk, there is increasing evidence that HDL-mediated cholesterol efflux capacity (CEC) is a better predictor of CVD risk compared to HDL-C [8, 9]. HDL removes cholesterol from the arterial wall by mediating cholesterol efflux via different pathways involving ABCA1, ABCG1, SR-BI, or aqueous diffusion of free cholesterol [8].

In the present study, we determined the effect of lomitapide treatment on the capacity of HDL to promote cholesterol efflux from macrophages in four HoFH patients.

Methods

Study participants

Four patients with HoFH receiving lomitapide as additional therapy in a clinical setting were included in the present study. They were amongst the first patients to be treated in a named-patients-program worldwide. The diagnosis HoFH was based on genetic analysis and clinical phenotype (LDL-C>13mmol/L) [4].

Three patients were recruited from the Erasmus Medical Center in the Netherlands and one from Palermo University Hospital in Italy, and were treated according to the prescribed protocol [10].

All patients provided written informed consent. This study was approved by the Medical Ethical Committees of the Erasmus Medical Center in the Netherlands and Palermo University in Italy.

Blood analysis and measurements

Venous blood was obtained after a 10-hour overnight fast, prior to treatment with lomitapide and every three or four weeks during the titration period. Plasma and serum obtained after centrifugation were stored at -80ºC. All samples from different timepoints were analyzed in one run.

Lipoprotein profiles were generated with density-gradient ultracentrifugation using the method described by Proudfoot et.al [11]. Lipoproteins were separated according to their densities into HDL_3 (1·125-1·21 g/ml), HDL_2 (1·062-1·125 g/ml), LDL(1·019-1·063 g/ml), and IDL+VLDL (<1·019 g/ml) [12]. Cholesterol and triglycerides were measured by an enzymatic method using Selectra E (DDS Diagnostic system, Istanbul, Turkey). Lipoprotein(a) [Lp(a)] plasma levels were measured using the Diasys immunoturbidimetric assay [13].

ApoB and ApoA-I levels were measured by immunoturbidimetry on a c311 automatic analyzer (Roche Diagnostics). HDL subclasses were separated by non-denaturing two dimensional (2D) electrophoresis, as previously described [14]. The content of preβ-HDL was calculated as percentage of total ApoA-I signal by densitometric analysis.

Cholesterol loading capacity

Cholesterol loading capacity (CLC) was measured as previously described [15] and defined as macrophage cholesterol content after exposure of cells to serum and expressed as µg cholesterol / mg protein.

Cholesterol efflux capacity .

Serum was depleted of apoB-containing lipoproteins in order to isolate the serum HDL fraction as previously described [16]. ApoB-depleted serum CEC was determined in human monocytes-derived macrophages THP-1 cultured in the presence of 100 ng/ml PMA for 72 hours to allow differentiation into macrophages. The apoB-depleted serum CEC specific for the three cholesterol efflux pathways (ABCA1, ABCG1, SR-BI) was evaluated in established cell culture models, as previously described [17, 18]. Cellular cholesterol content before and after serum exposure was measured fluorimetrically as previously described [15].

Statistical analysis

We performed descriptive analyses at baseline and during lomitapide treatment values and we present data as percentage change from baseline. The number of participants did not allow statistical inference. We used Microsoft Excel and Prism Graphpad 5 for the drawing of statistical graphs and data analysis.

Results

Baseline characteristics

The baseline characteristics of the 4 patients are shown in Table 1. Patients 1, 3, and 4 had a history of CVD. All patients had some gastrointestinal-symptoms during lomitapide treatment but complaints were minimalized by a low-fat diet. Lomitapide treatment was interrupted in patient 1 because of non-adherence and in patient 4 because of persistent liver enzymes elevations >5 times upper limit of normal during treatment, which returned to normal after discontinuation of lomitapide.

Atherogenic lipoproteins

As expected the triglyceride levels (measured in intermediate density lipoprotein and very low density lipoprotein (IDL+VLDL)) decreased strongly in all 4 patients (range -78 to -30%). LDL-C and apoB levels decreased in a dose-dependent manner (range -34 to -89% and -42 to -89%, respectively). Patient 2 and 3 achieved the LDL-C treatment target levels on maximum tolerated lomitapide dose. Patient 3 was treated with LDL-apheresis once every 1-2 weeks. This frequency was reduced to once every 8 to 10 weeks during lomitapide treatment. Lp(a) decreased in patient 1-3 (-20% to -74%), but remained unchanged in patient 4.

The CLC of sera of the patients decreased by an average of 20% at maximum lomitapide dose in comparison to baseline (from 53.6 ± 18.0 to 42.8 ± 12.3 ug cholesterol/mg cell protein).

HDL, ApoA-I and cholesterol efflux capacity

Figure 1A shows the individual cholesterol levels in total HDL-C and in HDL subclasses with increasing dosages of lomitapide of the 4 patients individually. The change in HDL-C levels (range -11 to -34%) was observed during treatment with lomitapide 5 mg/day. In all patients, HDL-C levels remained stable with increasing lomitapide dosage. The reduction in HDL-C levels varied per HDL subclass, the HDL_3/HDL_2 ratio remained stable in patient 1 and decreased in the others

(range -16 to -68%). Apo-AI levels and the content of Preβ-HDL decreased with lomitapide treatment (range -9 to -47% and -6 to -40%, respectively). This decrease was most pronounced with 5 mg/day lomitapide treatment.

Figures 1B, 1C, 1D and 1E show the effect of lomitapide treatment on total CEC, and on cholesterol efflux via the different pathways for the four patients individually. Changes in total, SR-BI-mediated and ABCG1-mediated cholesterol efflux were inconsistent. ABCA1-mediated cholesterol efflux decreased (-39 to -99%) in all patients.

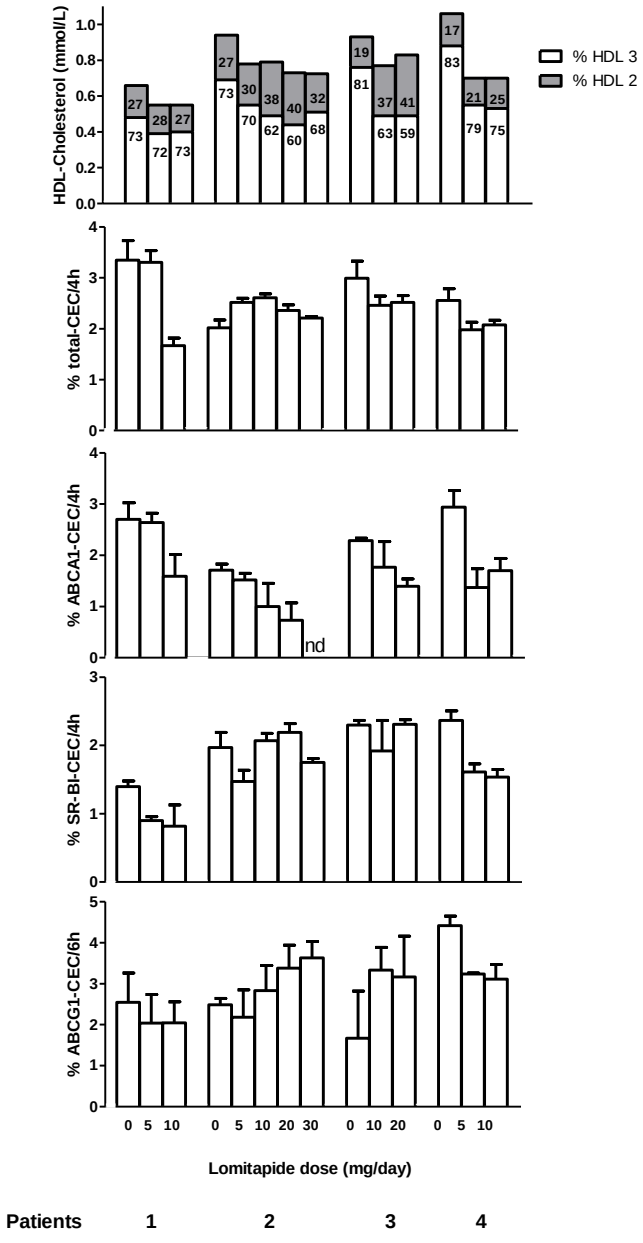
Table 1. Baseline characteristics.

Patient	Age (years)	Sex	Tendon Xanthomas	Mutations	Total cholesterol levels without medication (mmol/L)	LDL levels without lomitapide (mmol/L)	Age of onset CVD (years)	Co-medication (mg/day)	Maximum lomitapide dose (mg/day)	Duration lomitapide treatment (wks)	Discountinuation lomitapide treatment (Yes/No)
1	29	F	+	-G352D exon 8 -2417insG exon 17	20,1	14,5	25	Atorvastatin 80 Ezetimibe 10	10	9.5	Yes
2	20	F	+	-4.4 kb, duplication exon 12 -2.5 kb deletion exon 7, 8	18,9	14,1	-	Atorvastatin 80 Cholestagel 2x 1875	30	36.5	No
3	36	M	+	-G528D exon 11 -G528D exon 11	23,8	3,9*	26	Simvastatin 60 Ezetimibe 10	20	9	No
4	62	F	+	16 kb deletion exon 7-15	16,9	12,9	58	Questran 2x 4gr Modalim 2x 100	10	9	Yes

* LDL-apheresis once every 1-2 weeks

Figure 1. HDL levels and total HDL-mediated CEC and CEC pathways at baseline and during lomitapide treatment per individual

A. HDL-C levels (HDL_3+HDL_2) **B.** Total cholesterol efflux capacity (CEC) of apoB-depleted serum with increasing lomitapide daily dose. **C.** HDL-mediated cholesterol efflux via ABCA1. **D.** HDL-mediated cholesterol efflux via SR-BI. **E.** HDL-mediated cholesterol efflux via ABCG1.



Discussion

Our data confirm that lomitapide treatment decreases HDL-C levels. In depth analysis show a shift in HDL subclasses to larger buoyant HDL_2. ABCA1-mediated cholesterol efflux decreased in all four HoFH patients, whereas changes in efflux via ABCG1, SR-BI and total cholesterol efflux were less consistent.

Previous studies showed that lomitapide treatment is associated with a moderate decrease of both HDL-C and ApoA-I levels during the titration period of the drug [5-7]. In line, we found a decrease in the levels of HDL-C, ApoA-I, preβ-HDL, and HDL_3-C, which was most prominent on the lowest dose of lomitapide and remained stable thereafter. However, a shift of HDL to larger and more buoyant particles was observed with HDL_2-C levels remaining unchanged or increased. A reduced formation of HDL during lipolysis of predominantly postprandial triglyceride rich lipoproteins (TGRL) may underlie the reduction in HDL and ApoA-I levels and the alterations in HDL subclass levels. Additionally, lomitapide may reduce the levels of HDL derived from the intestine, since MTP-deficiency has been reported to reduce HDL-cholesterol secretion from the intestine in mice [19-21]. In line with this shift in HDL subclasses, the ABCA1-mediated cholesterol efflux was decreased in all patients, whereas changes in the ABCG1- and SR-BI-mediated cholesterol efflux were less consistent.

As expected, lomitapide treatment decreased LDL-C and apoB levels substantially as well as the other atherogenic lipoproteins, i.e. IDL, VLDL, and Lp(a) [5]. Consistently, we found that lomitapide reduced the macrophage CLC of serum of all patients. This reflects the improved anti-atherosclerotic potential despite the moderate decrease of HDL-C [22].

Limitations

The major limitation of this study is the small number of participants. Although two of the patients stopped lomitapide treatment this did not interfere with our analyses.

Conclusions

Lomitapide treatment substantially lowered LDL-C levels, though it moderately reduced HDL-C levels. However, HDL seemed to shift from HDL_3 to the larger and more buoyant HDL_2. In addition, the ABCA1-mediated cholesterol efflux decreased, whereas other pathways did not change consistently. Our report raises the hypothesis that the anti-atherogenic potential of HDL seems to be unaffected as total CEC did not seem to change consistently despite decreased HDL-C levels. Combined with the reduction of atherogenic lipoproteins, the net effect of lomitapide appears to be beneficial in HoFH patients.

Disclosures

RY report a travel grant from Aegerion Pharmaceuticals, outside the submitted work. LC reports grants and personal fees from MedImmune, and grants from The Medicines Company, and BioMarin Pharmaceutical, outside the submitted work. MA reports personal fees from Aegerion, Amgen, Chiesi, MSD, Mediolanum, Sanofi, Astra Zeneca, Mylan-Abbot, outside the submitted work. F. Bernini reports personal fees from Astra Zeneca, Milano, outside the submitted work. JRVL reports grants and fees from Sanofi and Aegerion, attributed to the institution, outside the submitted work. All other authors declare no conflicts of interest.

Acknowledgments

Aegerion Pharmaceuticals, for supplying the lomitapide for the patients

Sources of Funding

None.

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