

Lomitapide does not alter PCSK9 and Lp(a) levels in homozygous familial hypercholesterolemia patients: Analysis on cytokines and lipid profile

Maria Giovanna Lupo^a, Diletta Arcidiacono^b, Alice Zaramella^{c, d}, Fabio Fimiani^e, Paolo Calabrò^e, Angelo Baldassare Cefalù^f, Maurizio Averna^f, Laura D'Erasmo^g, Marcello Arca^g, Sara De Martin^a, Alberto Zambon^h, Nicola Ferri^{a, *}

^a Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padua, Italy

^b Digestive Endoscopy Unit, Veneto Institute of Oncology IOV-IRCCS, Padua, Italy

^c Department of Surgery, Oncology and Gastroenterology, University of Padua, Padua, Italy

^d Istituto Oncologico Veneto IOV-IRCCS, Padua, Italia

^e A.O.R.N. Sant'Anna e San Sebastiano, Caserta Department of Translational Medical Sciences, University of Campania "Luigi Vanvitelli", Naples, Italy

^f Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Palermo, Italy

^g Department of Translational and Precision Medicine, Sapienza, University of Rome, Rome, Italy

^h Department of Medicine, University of Padova, Padua, Italy

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ABSTRACT

Lomitapide, a drug for the treatment of homozygous familial hypercholesterolemia patients, reduced total and LDL cholesterol but no significant changes were observed on PCSK9 and Lp(a) plasma levels. Some changes of inflammatory mediators were also observed, including hsCRP, which may suggest an anti-inflammatory effect.

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Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) and Lp(a) are independent risk factors for cardiovascular disease (CVD) and are considered important pharmacological targets for reducing atherosclerotic burden. PCSK9 is a major determinant of cholesterol level, and the latter is a mediator of arterial tissue inflammation by inducing the inflammasome assembly leading to caspase 1 activation of the interleukin-1 β (IL-1 β) family of cytokines [1]. The measurement of high sensitivity C-reactive protein (hsCRP) levels is clinically proven as a method to predict vascular risk [2]. Pharmacological reduction of hsCRP, by statins, provided an additional

cardiovascular protection beyond lipid lowering effect [3], while proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors do not change hsCRP levels [3]. Lomitapide reduces the low-density lipoprotein cholesterol (LDL-c) levels, independently from functional LDL receptor, and it is indicated for homozygous familial hypercholesterolemia (HoFH) patients [4]. However, the effect of lomitapide on proprotein convertase subtilisin/kexin type 9 (PCSK9), Lp(a) and inflammatory mediators has not been determined.

Methods

Subjects

For this prospective study, we selected nine patients under lomitapide therapy from the LIPIGEN cohort [5] with clinical diagnosis of HoFH and/or a Dutch lipid score ≥ 6 [6]. Patients'

* Corresponding author. Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Largo Egidio Meneghetti 2, 35131, Padua, Italy.

E-mail address: nicola.ferri@unipd.it (N. Ferri).

demographics, as well as the genetic mutation(s) they carry and the current hypocholesterolemic pharmacological regimen, are summarized in Table 1. The study complies with the rules of Good Clinical Practice and with the ethical principles established in the Helsinki Declaration, and was approved by local Ethics Committees in each study center. All patients gave written informed consent. The data were collected and analyzed in the year of 2020.

DNA isolation and genetic characterization

Genomic DNA was extracted using the FlexiGene DNA kit (Qia-gen, Milan, Italy) according to the manufacturer's instructions. Appropriate primers were used for the PCR amplification of exons and exon-intron junctions of several FH-linked genes, including *LDLR* and *LDLRAP1*. As internal control, regions of chromosome 21 were amplified. Fifty-eight regions of interest (20 kb) were amplified from genomic DNA in five different multiplex PCR reactions. A second amplification was then performed in order to include an 8 nt index sequence used for sample identification, as well as the adaptors used for sequencing on MiSeq (Illumina) equipment. Before sequencing, purified amplified samples were quantified and diluted to the same concentration. The genetic analysis, including the complete sequence of genes, was performed by Next Generation Sequencing followed by the Sanger sequence to confirm the presence of the identified variant. Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was used to detect copy number variations.

Lipid profile and inflammation markers

Blood samples were collected after an overnight fast and processed. Plasma were stored at -80°C until further analyses. Total cholesterol, LDL-c, HDL-c, Lp(a), triglycerides, and transaminases were measures by certified enzymatic techniques. PCSK9 plasma

levels were evaluated through a commercial ELISA kit (Human Proprotein Convertase 9/PCSK9, Quantikine® ELISA SixPak, R&D System, MN, USA, cod. SPC900), according to manufacturer's instructions. The assay sensitivity was 0.219 ng/mL , while intra- and inter-precision assay were $5.4 \pm 1.2\%$ and $4.8 \pm 1.0\%$, respectively. PCSK9 sample concentrations were obtained by generating a four parametric logistic curve-fit (GraphPad Prism v5.0). High-sensitivity CRP was measured by a commercial ELISA kit (hsCRP ELISA, apDia, Belgium, cod. 740011), according to manufacturer's instructions. The minimal detectable concentration is approximately $0.02\text{ }\mu\text{g/mL}$. Intra-assay variability was $5.1 \pm 1.6\%$, inter-assay variability was $6.1 \pm 0.3\%$. A linear curve-fit (GraphPad Prism v5.0) was used to obtain hsCRP concentrations. A panel of pro and anti-inflammatory cytokines (ProcartaPlex Human Cytokine Panel 1B 25plex, ThermoFisher, MA, USA, cod. EPX250-12166-901) were determined by Luminex-xMAP® technology from plasma samples, according to manufacturers' instructions.

Statistical analysis

Total cholesterol, triglycerides, LDL-c, HDL-c, Lp(a), PCSK9, hsCRP, cytokines, ALT, AST data are expressed as mean \pm standard deviation. Differences between pre- and post-treatments were analyzed via non-parametric Wilcoxon signed-rank test (GraphPad Prism v5.0) and p-values lower than 0.05 were considered statistically significant.

Results

As previously shown [7], lomitapide reduced total cholesterol (from $317.4 \pm 105.0\text{ mg/dL}$ to $141.8 \pm 62.3\text{ mg/dL}$; -55.3%), LDL-c (from $251.7 \pm 104.2\text{ mg/dL}$ to $86.8 \pm 55.9\text{ mg/dL}$; -65.5%), and triglycerides (from $105.3 \pm 47.5\text{ mg/dL}$ to $61.8 \pm 26.3\text{ mg/dL}$; -41.3%) (Fig. 1A). No significant changes were observed on

Table 1

Patient's demographic, diagnosed disease, pharmacological treatments and biochemical parameters. LDLR: low-density lipoprotein receptor, ex: exon, intr: intron, Rec: receptor; simva: simvastatin, rosu: rosuvastatin, eze: ezetimibe, ali: alirocumab, EFA: essential fatty acids (200 mg linoleic acid, 110 mg icosapentaenoic acid, 210 mg α -linoleic acid, 80 mg docosahexaenoic acid); TC: total cholesterol, LDL-c: low-density lipoprotein cholesterol, HDL-c: high-density lipoprotein cholesterol, TG: triglycerides, Lp(a): lipoprotein (a), PCSK9: proprotein convertase subtilisin/kexin type 9, ALT: alanine aminotransferase, AST: aspartate aminotransferase, Q2W: every 2 weeks.

Patient's demographic, gene mutation and current therapy									
	age (y)	gender	gene	Location	genotype	protein	Phenotypic effect	lomitapide (mg)	concomitant therapy
1	41	M	<i>LDLR</i>	ex11	c.1646G > A	p.G528D	Homozygous Rec negative	40	simva 40mg/eze 10mg/EFA
2	30	F	<i>LDLR</i>	ex11	c.1618G > A	p.A519T	Rec defective	40	ω -3-6-9/EFA
				ex12	c.1775G > A	p.G571E	Rec defective		
3	52	M	<i>LDLRAP1</i>	ex4	c.430_431insA	p.H144fs	—	10	rosu 20mg/eze 10mg/EFA
4	33	F	<i>LDLRAP1</i>	intr1/ex2	c.89-1G > C	p.K30Tfs	—	20	rosu 40mg/eze 10 mg
5	65	F	<i>LDLRAP1</i>	ex4	c.432insA	p.A147Sfs	—	5	simva 40mg/eze 10 mg
6	63	M	<i>LDLRAP1</i>	ex4	c.432insA	p.A147Sfs	—	5	rosu 40mg/eze 10 mg
7	60	M	—	—	—	—	—	20	rosu 40mg/eze 10 mg
8	51	M	<i>LDLR</i>	ex12	c.1775G > A	p.G571E	Rec defective	10	rosu 40mg/eze 10 mg
				ex14	c.2054C > T	p.P685L	Rec defective		
9	74	M	<i>LDLR</i>	ex12	c.1775G > A	p.G571E	Homozygous Rec defective	5	rosu 20mg/eze 10mg/ali 150 mg Q2W
Biochemical parameters									
	pre (mean \pm SD)		post (mean \pm SD)		mean change between pre and post treatment			p-value	
TC (mg/dL)	317.4 ± 105.00		141.8 ± 62.3		-55%			0.0039	
LDL-c (mg/dL)	251.7 ± 104.2		86.8 ± 55.9		-66%			0.0039	
HDL-c (mg/dL)	44.7 ± 10.6		42.7 ± 6.9		=			0.84	
TG (mg/dL)	105.3 ± 47.5		61.8 ± 26.3		-40%			0.016	
Lp(a) (mg/dL)	55.1 ± 47.6		63.6 ± 71.1		=			0.90	
PCSK9 (ng/mL)	567.65 ± 321.61		473.27 ± 78.9		=			0.65	
ALT (U/l)	29.8 ± 21.2		43.7 ± 31.3		$+47\%$			0.26	
AST (U/l)	31.7 ± 17.8		46.9 ± 19.7		$+48\%$			0.07	

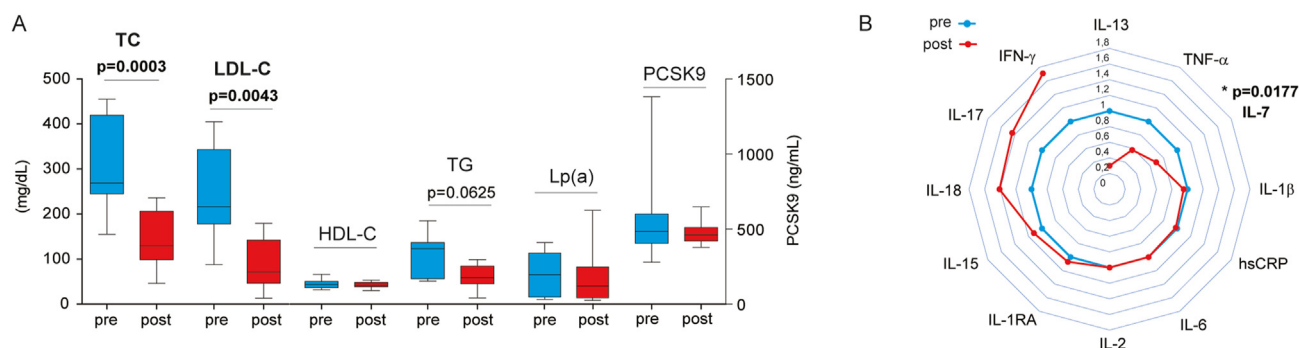


Fig. 1. Lipid and inflammatory profile of HoFH patients ($n = 9$) after 3 months of treatment with lomitapide. A) Lipid parameters are expressed as mg/dL (left Y axis) while PCSK9 as ng/mL (right Y axis). B) Data of post-treatment are expressed as median variation from pre-treatment. p-values were determined by Wilcoxon signed-rank test.

high-density lipoprotein cholesterol (HDL-c) (from 44.7 ± 10.5 mg/dL to 42.7 ± 6.9 mg/dL), Lp(a) (from 63.7 ± 51.4 mg/dL to 58.6 ± 64.4 mg/dL), and PCSK9 plasma levels (from 567.7 ± 321.6 ng/mL to 473.3 ± 78.9 ng/mL) (Fig. 1A).

As previously described [8], hsCRP was not altered after 3 months of lomitapide treatment (Fig. 1B), while a significant reduction was observed in a subset of patients treated for 6 months (from 2.1 mg/L (95% CI, 0.0–4.7) to 0.01 mg/L (95% CI, 0.0–1.7)). Minor reductions in median values in response to lomitapide were observed for TNF- α (–48%), IL-13 (–70%) and IL-7 (–31%) while increased median values were shown in IL-18 (+41%), IL-17 (+44%) and IFN- γ (+71%) (Fig. 1B). However, none of these changes were statistically significant due to the rarity of the pathology and the variability among patients.

Discussion

The main finding of the present study is that lomitapide, an hypocholesterolemic agent that acts in an LDL receptor independent manner, did not affect both PCSK9 and Lp(a) plasma levels and marginally reduced hs-CRP after 6 months of treatment. Regarding the pro inflammatory cytokines, none of the changes were shown to be statistically significant, due to the rareness of the HoFH and therefore the limited number of patients recruited in the study. Nevertheless, we have observed lower levels of TNF- α after lomitapide treatment that may contribute to a protective pharmacological action on CVD. Indeed, TNF- α , IL-1 β and IL-6 are considered to play a causal role in atherogenesis, as recently shown by the CANTOS trial, which found that specifically targeting IL-1 β improves clinical outcomes in patients with a previous myocardial infarction [9]. On the contrary, the changes in IL-18 levels in response to lomitapide may not be considered of clinically significant, since in CANTOS trial, IL-18 baseline levels were not associated with future cardiovascular risk after adjustment for covariates [10], IL-7 and IL-15 increase the number and function of CD28^{null} T cells in patients with acute coronary syndrome [11]. Interestingly, IL-7 was shown to be significantly reduced by lomitapide that also partially affected IL-15 expression, these changes may target the inflammation mediated CD28^{null} T cells. However, not all observed changes indicated a positive effect of lomitapide on inflammatory cytokines; for instance, IL-13 was reduced by lomitapide, an anti-inflammatory cytokine that negatively correlates with intima-media thickness (IMT), a surrogate marker for subclinical atherosclerosis [12].

In conclusion, our analysis demonstrated, for the first time, a lack of a significant effect of lomitapide on Lp(a) and PCSK9 levels

and provide new evidence on changes on inflammatory cytokines profile involved in human atherosclerosis. However, the clinical significance of these variations still needs to be determined.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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