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Původní sdělení | Original research article

# The possibility of reducing the Lp-PLA2 mass level using simvastatin monotherapy and combination therapy with ezetimibe

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ARTICLE INFO

*Article history:*
Received: 2. 2. 2015
Received in revised form: 23. 3. 2015
Accepted: 25. 3. 2015
Available online: 24. 4. 2015

*Klíčová slova:*
Ezetimib
Ischemická choroba srdeční
Simvastatin
S lipoproteinem asociovaná fosfolipáza A2

SOUHRN

**Cíl:** Srovnat účinek kombinační léčby simvastatinem a ezetimibem s účinkem monoterapie samotným simvastatinem na masu s lipoproteinem asociované fosfolipázy A2 (Lp-PLA2) u pacientů s ischemickou chorobou srdeční.
**Metody:** Studie se zúčastnilo 100 pacientů s koronarograficky prokázanou aterosklerózou koronárních tepen. Masa Lp-PLA2 a frakce cholesterolu byly stanoveny při vstupu do studie a po šesti měsících léčby. Masa Lp-PLA2 byla stanovena testem PLAC Test.
**Výsledky:** Ve srovnání s léčbou samotným simvastatinem vedla kombinační léčba ezetimibem a simvastatinem k významnému poklesu hodnot Lp-PLA2 i lipidového profilu ($p < 0,05$). Kombinační léčba ezetimibem a simvastatinem v dávce 20 mg/den se ukázala být stejně účinná jako monoterapie samotným simvastatinem v dávce 80 mg/den při ovlivnění masy Lp-PLA2 i hodnot lipidů ($p < 0,05$). Masa Lp-PLA2 byla u pacientů s postižením tří koronárních tepen zpočátku vyšší než u pacientů s postižením jedné tepny, zatímco vstupní hodnoty lipidů a hsCRP se významně nelišily.
**Závěry:** Kombinační léčba se simvastatinem v poloviční dávce vedla k většímu poklesu masy Lp-PLA2 i hodnot celkového a LDL cholesterolu než při monoterapii simvastatinem. Vzhledem k pozvolnému poklesu cílových hodnot LDL cholesterolu, jenž vede k předepisování vyšších dávek statinů (což není kvůli přítomnosti komorbidit vždy možné), představuje kombinace statinu a ezetimibu spolehlivou alternativu umožňující nejen významné snížení hodnot LDL cholesterolu, ale i tak významných faktorů podílejících se na rozvoji aterosklerózy a markerů zánětu, jakými jsou Lp-PLA2 a C-reaktivní protein (CRP).


ABSTRACT

**Aim:** To assess the impact of combined treatment with simvastatin and ezetimibe or treatment with simvastatin only on lipoprotein-associated phospholipase A2 mass level in patients with coronary heart disease.
**Methods:** One hundred patients with angiographically documented coronary atherosclerosis took part in the investigation. Lp-PLA2 mass level and cholesterol fractions were determined at baseline and after 6 months of treatment. Lp-PLA2 mass was determined by PLAC Test; DiaDexus, Inc.

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**DOI:** 10.1016/j.crvasa.2015.03.012

**Please cite this article as:** S. V. Miklishanskaya, et al., The possibility of reducing the Lp-PLA2 mass level using simvastatin monotherapy and combination therapy with ezetimibe, Cor et Vasa 27 (2015) e257–e264 as published in the online version of *Cor et Vasa* available at http://www.sciencedirect.com/science/article/pii/S0010865015000399

**Results:** Combined treatment with ezetimibe and simvastatin led to significantly greater declines in Lp-PLA2 and lipid profile compared with treatment only with simvastatin ($p < 0.05$). Combination therapy with ezetimibe and simvastatin 20 mg/day proved to be as effective as monotherapy with simvastatin 80 mg/day on the effect on Lp-PLA2 mass level and lipids ($p < 0.05$). Lp-PLA2 mass level was initially higher in patients with 3-vessel coronary artery disease, compared with patients with 1-vessel coronary artery disease while baseline levels of lipids and hs-CRP did not differ significantly.

**Conclusions:** Combined treatment, using half the dose of simvastatin, led to greater reduction of Lp-PLA2 mass level total cholesterol and LDL-C, compared to monotherapy with simvastatin. Due to the steady decline target levels of LDL-C, which leads to prescribing high doses of statins (and it is not always possible because of the presence of co-morbidities), combination therapy with statin and ezetimibe is a reliable alternative, which allows not only to largely reduce LDL-C but also to significantly reduce such important participants of atherosclerosis process and markers of inflammation, as Lp-PLA2 and CRP.

*Keywords:*
Coronary heart disease
Ezetimibe
Lipoprotein-associated phospholipase A2
Simvastatin

## Introduction

One of the key factors in the development of atherosclerosis is a chronic systemic inflammation with local specific manifestations in the intima of the vessels. Inflammation plays a major role in the genesis and progression of atherosclerotic plaques, its evolution in the vulnerable plaque rupture and loosening tire [1]. In recent years, lipoprotein-associated phospholipase A2 (Lp-PLA2), which is a marker of intravascular inflammation, has attracted the attention of scientists. Numerous studies have demonstrated the role of Lp-PLA2 as a risk factor for cardiovascular disease and a direct participant in the development and progression of atherosclerosis.

Lp-PLA2 is associated mainly with low-density lipoproteins (LDL), whereas a small proportion of circulating enzyme is also associated with high-density lipoproteins (HDL) and lipoprotein-a. Mechanistically, Lp-PLA2 hydrolyzes oxidatively altered phospholipids that have shortened sn2 fatty acids to produce oxidized fatty acids and lysophosphatidylcholine, a function that deems Lp-PLA2 a pro-atherogenic agent [2–5]. Increased Lp-PLA2 testifies not only to coronary artery disease [6], but can further define prognosis and the risk of vascular complications [6–16]. Lp-PLA2 reflects the presence and intensity of intravascular inflammatory, the marker of which it is.

Using the classic risk factors cannot account for all cases of coronary events in patients with normal lipid profile [17]. That is why the FDA approved a definition Lp-PLA2 as a screening test that predicts a patient's risk for future CHD events. ACC/AHA and ESC have recommended Lp-PLA2 definition for asymptomatic or moderate and high risk patients to clarify the risks and decide begin or increase lipid-lowering therapy [18–21].

The ability to influence intravascular inflammation (Lp-PLA2) as well as non-specific inflammation (hs-CRP) relates to the pleiotropic effects of statins, and it is realized, sometimes, regardless of the lipid-lowering effect of statins.

Information that hypolipidemic drugs, particularly statins, reduce the level of Lp-PLA2 suggests, that Lp-PLA2 can be considered as a target for the therapy action to suppress inflammatory processes and achieve stabilization of atherosclerotic plaque formation [22]. It has been shown that statins – pravastatin, atorvastatin, rosuvastatin and simvastatin – significantly lowered Lp-PLA2 levels [22–35], but is it possible for combination therapy: ezetimibe with lower doses of statins? As a powerful lipid-lowering effect of the combination of ezetimibe and a statin is known, our aim was to evaluate the possibility of reducing the intravascular inflammation using combination therapy with a lower dose of the statin.

## Materials and methods

One hundred patients with angiographically documented coronary atherosclerosis took part in the investigation. All patients included in the study, even with previously established CHD had not taken lipid-lowering drugs for at least 6 months before inclusion in the study. Patients were randomly assigned into two treatment groups: a group of active treatment (combination therapy group) – took ezetimibe 10 mg/day in combination with simvastatin, the control group (group monotherapy) took simvastatin only. The distribution of patients in the two treatment groups was performed by using envelopes. No other randomisation criteria were used. The distribution of patients did not influence the results of the previous coronary angiography or the lipid profile. The results of coronary angiography were used to confirm the presence of CHD and in more detail were evaluated after enrollment.

The initial dose of simvastatin in all patients included in the study was 20 mg/day, regardless of the chosen strategy of therapy (monotherapy group or combination treatment group). The dose of simvastatin was titrated up to 40 or 80 mg/day every 6 weeks, if it was necessary, according to the achievement of the target level of LDL-C < 2.5 mmol/L. After achieving the target level of LDL-C and confirming measurements, the dose of simvastatin was unchanged for 6 months (until the end of the study). Simvastatin dose titration results are shown in Figure 1.

The study excluded patients with acute coronary syndrome, myocardial infarction, which are less than 6 months, family hyperlipidemia, severe liver disease and kidney disease, congestive heart failure, acute inflammatory diseases. There were no significant differences between groups in age, gender, body mass index, risk factors, comorbidity, severity of angina, the severity of coronary lesions and conducted concomitant therapy. More detailed characteristics of patients are presented in Table 1.

Lp-PLA2 mass level, hs-CRP and cholesterol fractions were determined at baseline and after 6 months of treatment. Lp-PLA2 mass was determined by use of a dual monoclonal antibody immunoassay standardized to re-

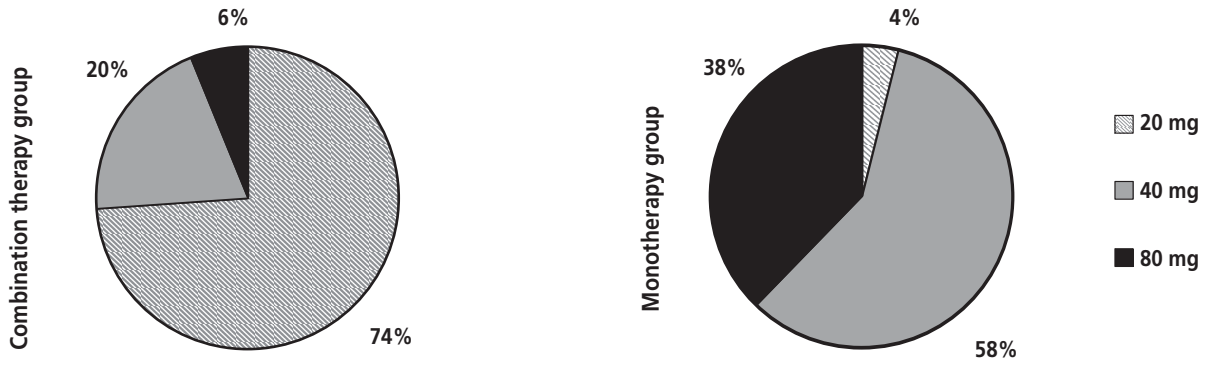



**Fig. 1 – Distribution of simvastatin doses in combination therapy and monotherapy.**

combinant Lp-PLA2 (PLAC Test; DiaDexus, Inc) [2,36]. High sensitivity C-reactive protein was determined by automated analyzer nephelometric method by measuring side scattering of laser radiation at a wave length of 840 nm.

Blood samples for determining Lp-PLA2 mass level and hs-CRP, which have taken in the beginning of the study and at the final stage, have been kept for 1.5 years in temperature below –70 °C. Then we measured Lp-PLA2: the blood samples from one patient were determined using the same test kit (for doublets too).

Statistical processing of the results of the study carried out in accordance with standard methods of statistics. Statistical analysis were performed using STATISTICA 6.0 softpack. Data are presented as the median and range. To test the normality of the distribution of quantitative traits, we used the Shapiro-Wilk test. The significance of differences of all parameters of quantitative traits were determined using paired and unpaired analysis methods for nonparametric variables by Mann-Whitney U test for independent groups and Wilcoxon matched pairs test for dependent parameters. The significance of differences parameters qualitative characteristics was assessed using two-tailed Fisher exact test. To assess the relationship of quantitative traits Spearman Rank Order Correlations

**Table 1 – Clinical characteristics of the study population**

| Characteristics | Simvastatin monotherapy group (n = 50) | Simvastatin + ezetimibe group (n = 50) | *p*-value |
|---|---|---|---|
| Age, years | 61 [54; 65] | 62 [56; 69] | 0.24* |
| Female (%) | 22% | 26% | 0.81** |
| BMI, kg/m$^2$ | 26 [25; 30] | 27.5 [26; 31] | 0.12* |
| Current smoking (%) | 52% | 70% | 0.15** |
| Family history of CAD (%) | 66% | 80% | 0.18** |
| Diabetes (%) | 14% | 14% | 1** |
| History of AMI (%) | 48% | 60% | 0.31** |
| Hypertension (%) | 90% | 94% | 0.71** |
| **Angiography** | | | |
| One-vessel CAD (n = 26) | 30% | 22% | 0.49** |
| Two-vessel CAD (n = 38) | 32% | 44% | 0.30** |
| Three-vessel CAD (n = 36) | 38% | 34% | 0.83** |
| **Medication use (%)** | | | |
| Platelet inhibitor (%) | 100% | 100% | 1** |
| Beta blocker (%) | 96% | 100% | > 0.1** |
| RAAS (%) | 90% | 86% | > 0.1** |
| Previous lipid-lowering therapy | None | None | |
| Simvastatin dose in the study (median) (mg/day) | 40 [40; 80] | 20 [20; 40] | < 0.001* |
| Simvastatin dose in the study (mean dose) (mg/day) | 54.4 | 27.6 | < 0.001* |

* Mann-Whitney U test.
** Two-tailed Fisher's exact test.

was used. For all types of analysis were considered statistically significant at $p$ values < 0.05.

## Results

All included patients have completed the study, 50 person in each group.

In both groups, Lp-PLA2 mass level has exceeded the physiological level (> 200 ng/ml) in 98% of cases, while median Lp-PLA2 mass level was equal to 351.89 ng/ml and 352.97 ng/ml in the combination and monotherapy treatment group, respectively. The median concentration of hs-CRP for combination therapy group was 2.62 mg/L, for monotherapy 1.63 mg/L.

### *Dynamics of lipid profile and inflammatory markers on combined therapy with simvastatin and ezetimibe*

In patients treated with simvastatin and ezetimibe, Lp-PLA2 mass level significantly decreased by 45% after 6 months of treatment (from 351.89 [286.6, 440.1] ng/ml to 200.95 [173.8, 223.7] ng/ml, $p$ < 0.001). hs-CRP level was also significantly reduced by 37% after 6 months of treatment (from 2.62 [1.3, 4.5] mg/L to 0.96 [0.5, 3.5] mg/L, $p$ < 0.001). Combined therapy with simvastatin and ezetimibe led to a significant reduction in total cholesterol by 35% (from 5,95 [5,4; 6,8] mmol/L to 3.85 [3.4, 4.3] mmol/L, $p$ < 0.001) and LDL-C by 50% (from 4.02 [3.4, 4.8] mmol/L to 2.16 [1.8, 2.4] mmol/L, $p$ < 0.001); TG by 23% (from 1.52 [1.2, 2.0] mmol/L to 1.11 [0.9, 1.7] mmol/L, $p$ < 0.001) after 6 months of therapy compared with the baseline (Table 2).

**Table 2 – Effect of the combination therapy with simvastatin and ezetimibe on cholesterol levels and inflammatory markers**

| Parameter | Baseline | Posttreatment | $p$* | Δ% |
|---|---|---|---|---|
| Lp-PLA2, (ng/ml) | 351.89 [286.6; 440.1] | 200.95 [173.8; 223.7] | < 0.001 | –45 |
| hs-CRP (mg/L) | 2.62 [1.3; 4.5] | 0.96 [0.47; 3.54] | < 0.001 | –37 |
| Total cholesterol (mmol/L) | 5.95 [5.4; 6.8] | 3.85 [3.4; 4.3] | < 0.001 | –35 |
| LDL-C (mmol/L) | 4.02 [3.4; 4.8] | 2.16 [1.8; 2.4] | < 0.001 | –50 |
| HDL-C (mmol/L) | 1.15 [0.9; 1.3] | 1.13 [1.0; 1.3] | 0.920 | 1 |
| TG (mmol/L) | 1.52 [1.2; 2.0] | 1.11 [0.9; 1.7] | < 0.001 | –23 |

* Wilcoxon matched pairs test. Represents median levels and interquartile range.

**Table 3 – Effect of the monotherapy with simvastatin on cholesterol levels and inflammatory markers**

| Parameter | Baseline | Posttreatment | $p$* | Δ% |
|---|---|---|---|---|
| Lp-PLA2 (ng/ml) | 352.97 [272.4; 401.7] | 213.08 [192.2; 235.3] | < 0.001 | –38 |
| hs-CRP (mg/L) | 1.63 [1.2; 3.2] | 0.69 [0.29; 2.0] | < 0.001 | –48 |
| Total cholesterol (mmol/L) | 5.64 [5.2; 6.1] | 4.01 [3.7; 4.3] | < 0.001 | –28 |
| LDL-C (mmol/L) | 3.68 [3.0; 4.1] | 2.19 [2.1; 2.4] | < 0.001 | –40 |
| HDL-C (mmol/L) | 1.28 [1.1; 1.4] | 1.15 [0.9; 1.3] | 0.016 | –8 |
| TG (mmol/L) | 1.50 [1.1; 1.9] | 1.12 [0.9; 1.7] | 0.002 | –17 |

* Wilcoxon matched pairs test. Represents median levels and interquartile range.

### *Dynamics of lipid profile and inflammatory markers on simvastatin monotherapy*

In the monotherapy group, Lp-PLA2 mass level and lipid profile have also significantly decreased. Lp-PLA2 mass level has reduced by 38% (from 352.97 [272.4, 401.7] ng/ml to 213.08 [192.2, 235.3] ng/ml, $p$ < 0.001). hs-CRP level has reduced by 48% (from 1.63 [1.2, 3.2] mg/L to 0.69 [0.29, 2.0] mg/L, $p$ < 0.001). Total cholesterol has decreased by 28% (from 5.64 [5.2, 6.1] mmol/L to 4.01 [3.7, 4.3] mmol/L, $p$ < 0.001), LDL-C has decreased by 40% (from 3.68 [3.0, 4.1] mmol/L to 2.19 [2.1, 2.4] mmol/L, $p$ < 0.001), TG has decreased by 17% (from 1.5 [1.1; 1.9] mmol/L to 1.12 [0.9, 1.7] mmol/L, $p$ = 0.002) after 6 months of therapy (Table 3).

When comparing the two tactics lipid-lowering therapy has been revealed that the Lp-PLA2 mass level, total cholesterol and LDL-C levels decreased to a greater extent in the combination therapy group than in the monotherapy group. The Lp-PLA2 mass level reduction was 45% in the combination treatment group versus 38% in the monotherapy group ($p$ = 0,03). Total cholesterol decreased by 35% in the combination treatment group versus 28% in the monotherapy group ($p$ = 0.003).

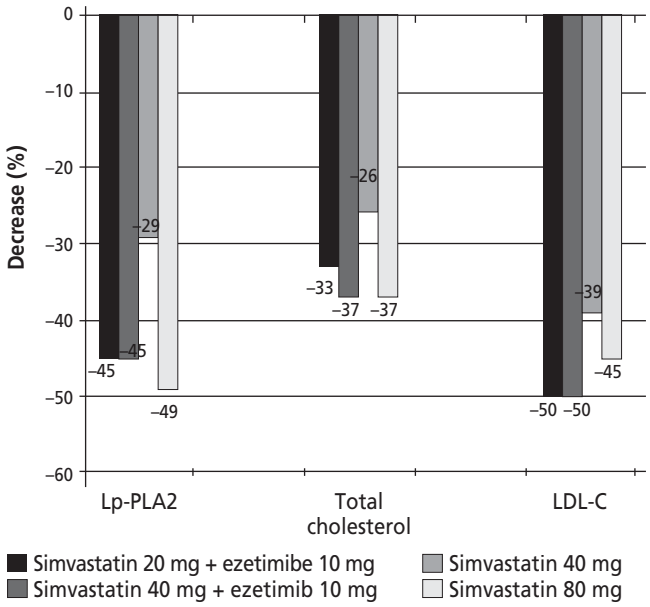
LDL-C reduction was 50% in the combination treatment group versus 40% in the monotherapy group ($p$ < 0.001). With regard to hs-CRP, none of the tactics lipid-lowering therapy showed any benefits, the results of treatment were comparable ($p$ > 0.05).

### *Effect of combination therapy with simvastatin and ezetimibe on lipid profile and Lp-PLA2 mass level, according to the simvastatin doses included in the combination*

No statistically significant differences were observed on the effect on Lp-PLA2 mass level and lipid profile between different doses of simvastatin (20 or 40 mg/day) in the combination therapy group. Combination therapy with simvastatin 20 and 40 mg/day led to the same reduction of Lp-PLA2 mass level by 45%. During combination therapy with increasing doses of simvastatin from 20 mg up to 40 mg/day, the level of total cholesterol decreased by 33% and 37%, respectively, the level of LDL cholesterol decreased by 49% and 50%, respectively. Thus, the dose-response effect of combined therapy with simvastatin and ezetimibe on Lp-PLA2 mass level and lipid profile (total cholesterol, LDL cholesterol) have not been identified.

### *Effect of simvastatin monotherapy on lipid profile and Lp-PLA2 mass level, according to the simvastatin doses*

After treatment it was found that the maximum reduction in Lp-PLA2 mass level by 49% (up to 207 ng/mL) was




* Mann-Whitney U test
$p < 0.001$ versus baseline for all subgroups. $p > 0.05$ when comparison between subgroups simvastatin 20 mg + ezetimibe 10 mg and simvastatin 40 mg + ezetimibe 10 mg. $p < 0.05$ when comparison between subgroups simvastatin 40 mg and simvastatin 80 mg. $p < 0.05$ when comparison between subgroups simvastatin 20 mg + ezetimibe 10 mg and simvastatin 40 mg. $p > 0.05$ when comparison between subgroups simvastatin 20 mg + ezetimibe 10 mg and simvastatin 80 mg.

**Fig. 2 – Bar graphs illustrating compared effects of combination therapy with simvastatin + ezetimibe and monotherapy with simvastatin on lipid profile and Lp-PLA2 mass level, according to the simvastatin doses included in the combination.**

recorded during therapy with simvastatin 80 mg/day, minimum reduction in Lp-PLA2 mass level by 29% (up to 220 ng/ml) was recorded during therapy with simvastatin 40 mg/day. The maximum decrease by 37% in total cholesterol and by 45% in LDL-C were recorded in the simvastatin 80 mg/day. In group therapy with simvastatin 40 mg/day, total cholesterol and LDL-C reduction was 26% and 39%, respectively. Thus, simvastatin monotherapy was revealed significant dose-dependent effect: monotherapy with simvastatin 80 mg/day was more effective in reducing the Lp-PLA2 mass level and lipid profile (total cholesterol and LDL-C), than monotherapy with simvastatin 40 mg/day ($p < 0.05$). Significant differences on the effect of different doses of simvastatin on HDL-C and TG have not been identified (Fig. 2).

### *Comparison of two tactics lipid-lowering therapy*

When comparing combination therapy with simvastatin and ezetimibe and monotherapy with simvastatin following results were obtained.

Combined therapy with simvastatin 20 mg/day showed significantly greater reductions in levels of Lp-PLA2, total cholesterol and LDL-C, than simvastatin monotherapy 40 mg/day. Lp-PLA2 mass level decreased by 45% in the combination therapy group (from 387.17 ng/mL to 204.5 ng/ml) versus 29% in the monotherapy group (from 319.76 ng/mL to 220.3 ng/ml, $p = 0.003$). Total cholesterol decreased in the combination therapy with simvastatin 20 mg/day by 33% (from 5.84 mmol/L to 3.84 mmol/L) versus 26% in the monotherapy group with simvastatin 40 mg/day (from 5.36 mmol/L to 3.99 mmol/L, $p = 0.001$). LDL-C level in the combination therapy with simvastatin 20 mg/day decreased by 50% (from 3.86 mmol/L to 2.1 mmol/L) versus 39% (from 3 25 mmol/L to 2.16 mmol/L) in monotherapy with simvastatin 40 mg/day ($p < 0.001$).

When comparing the initial dose of simvastatin 20 mg/day in the combination therapy group with a maximum dose of simvastatin 80 mg/day in the monotherapy group, statistically significant differences in the effects of drugs on the Lp-PLA2 mass level and LDL-C disappeared ($p > 0.05$). Monotherapy with simvastatin 80 mg/day led to a greater reduction in total cholesterol in comparison with combination therapy with simvastatin 20 mg/day: 37% (from 6.11 mmol/L to 4.19 mmol/L) versus 33% (from 5.84 mmol/L to 3.84 mmol/L), respectively ($p = 0.03$).

When comparing the average dose of simvastatin (40 mg/day) in combination therapy group and monotherapy group, following results were obtained. Combined therapy with simvastatin 40 mg/day showed significantly greater reductions in Lp-PLA2 mass level, total cholesterol and LDL-C, than monotherapy with simvastatin 40 mg/day. Lp-PLA2 mass level decreased by 45% in the combination therapy group (from 306.41 ng/ml to 189.64 ng/ml) versus 29% (from 319.76 ng/ml to 220.3 ng/ml) in the monotherapy group ($p = 0.03$).

Total cholesterol decreased in the combination therapy with simvastatin 40 mg/day by 37% (from 6.17 mmol/L to 3.74 mmol/L) versus 26% (from 5.36 mmol/L to 3.99 mmol/L) in the monotherapy group with simvastatin 40 mg/day ($p = 0.003$). LDL-C level in the combination therapy with simvastatin 40 mg/day decreased by 50% (from 4.29 mmol/L to 2.18 mmol/L) versus 39% (from 3.25 mmol/L to 2.16 mmol/L) in the monotherapy group with simvastatin 40 mg/day ($p = 0.002$). When comparing the impact of combination therapy with simvastatin 40 mg/day and monotherapy with simvastatin 80 mg/day on lipid profile and Lp-PLA2 mass level significant differences between the two groups were not found. There was a significant correlation between baseline levels of Lp-PLA2 and total cholesterol (r = 0.28), LDL cholesterol (r = 0.33).

## Discussion

Because most of the studies identified the relationship between elevated level of Lp-PLA2 and the risk of cardiovascular complications [37–45], the possibility of reducing this marker has been actively studied. However, according to the trials, darapladib did not improve cardiovascular outcomes in patients with coronary artery disease, when added to standard of care [46,47]. Perhaps, this is due to the fact, that statins also able significantly reduce Lp-PLA2 mass and activity [22–35]. This can be seen as they pleiotropic effect. Conducting further reduce Lp-PLA2 by darapladib, did not lead to further reduction of the risk of coronary events.

The results of our studies have shown that statin therapy, in addition to lipid-lowering effect, leads to a significant reduction of Lp-PLA2 mass level in patients with coronary artery disease.

Hypolipidemic effect and the reduction of Lp-PLA2 mass level were more pronounced in the combination therapy with simvastatin and ezetimibe compared with simvastatin monotherapy. For example, in the combination therapy group Lp-PLA2 mass level reduced by 45% vs. 38% in the monotherapy group, the reduction in total cholesterol was 35% vs. 28%,

LDL-C reduced by 50% vs. 40%, respectively. It was shown that the maximum decrease in Lp-PLA2 mass level was achieved already at the initial dose of the simvastatin in the combination therapy and dose-response effect has not been obtained. Initial dose of the combination therapy is comparable in efficiency with a maximum dose of simvastatin in monotherapy.

In studies that assessed the effect of statins on the Lp-PLA2 mass level following results were obtained. Clinically was shown, that statins pravastatin [23], atorvastatin [24,32], simvastatin [25,31] and rosuvastatin [27,33] have significantly reduced Lp-PLA2 mass level at 22–36%. The maximum reduction of Lp-PLA2 mass level was shown in the MIRACLE study with atorvastatin monotherapy 80 mg/day in which decrease of Lp-PLA2 was 35.8% [32]. JUPITER study has shown on 8 901 patients, that treatment with rosuvastatin 20 mg/day led to the Lp-PLA2 mass level reduction on 33.8% [33]. According to the work of E. Schaefer et al. monotherapy with atorvastatin 40 mg/day lowered the Lp-PLA2 mass level by 26% [24]. Simvastatin monotherapy 40 mg/day reduced the Lp-PLA2 mass level on 27% according to the Heart Protection Study [31].

On the effect of statins on LDL-C, our work is consistent with the data of world literature, but to influence the Lp-PLA2 mass level results diverge: in our work has been revealed more significant reduction in Lp-PLA2 mass level as a monotherapy, and in combined therapy. We supposed that this result is due to the fact, that our patients had higher Lp-PLA2 mass level baseline.

Patients from the above studies had Lp-PLA2 mass level lower, it rarely exceeded 320 ng/ml. Confirming our hypothesis, we found in the work of Muhlestein et al., where it was shown, that anti-inflammatory effects of simvastatin depend on the degree of increase of inflammatory markers at baseline. Simvastatin 20 mg/day reduced Lp-PLA2 mass level by 34.5%, LDL-C by 34% and hs-CRP by 16% in a group of 100 patients with type 2 diabetes and hyperlipidemia. In the subgroup of patients with a median of Lp-PLA2 mass level 320.9 ng/ml and more or hs-CRP levels > 2 mg/dl, it was found a significantly greater reduction in inflammatory markers. During the therapy with simvastatin 20 mg/day Lp-PLA2 mass level decreased by 47.5%, and hs-CRP level by 24.8% [34].

In the course of combination therapy with simvastatin and ezetimibe dose-dependent effect on Lp-PLA2 mass level and lipid profile (total cholesterol, LDL-C) has not been identified. While simvastatin monotherapy, reliable dose-dependent effect of the drug was detected on the lipid profile (total cholesterol, LDL-C), and also on its effect on Lp-PLA2 mass level ($p < 0.05$).

In addition, IMPROVE-IT study has showed that combination therapy with simvastatin and ezetimibe have similar coronary event reduction efficacy as simvastatin monotherapy [48]. Combination therapy achieves target level of LDL-C using significantly lower doses of statins. This can be important in elderly patients, in patients with an increased risk of adverse events, in patients with non-alcoholic liver disease or isolated elevation of liver transaminases.

Comparison of the two groups can be considered correct, as both groups started with the same dose of simvastatin 20 mg/day and increase the dose depended on achieving the target level of LDL-C in each patient. The ability to reduce Lp-PLA2 mass level was determined on the background of the main lipid-lowering effects of drugs. Dose titration was performed in accordance with clinical practice. Dose titration of statin taken in many studies in recent years, for example IMPROVE-it study. The later comparisons between subgroups (the population of which is, at least 10 patients) does not contradict the rules of statistical analysis.

In conclusion the present study demonstrates, that combined treatment, using half the dose of simvastatin, led to greater reduction of Lp-PLA2 mass level, total cholesterol and LDL-C, compared to monotherapy with simvastatin. Due to the steady decline target levels of LDL-C, which leads to prescribing high doses of statins (and it is not always possible because of the presence of co-morbidities), combination therapy with statin and ezetimibe is a reliable alternative, which allows not only to largely reduce LDL-C but also to significantly reduce such important participants of atherosclerosis process and markers of inflammation, as Lp-PLA2 and CRP.

**Conflict of interest**

There was no conflict of interests during this study.

**Funding body**

Institute of Clinical Cardiology of Russian Cardiology Scientific and Production Center at the head of I.E. Chazova provided financial support for the conduct of the research preparation of the article.

**Ethical statement**

The research was done according to ethical standards.

**Acknowledgements**

We are grateful to I.E. Chazova, the head of Institute of Clinical Cardiology of Russian Cardiology Scientific and Production Center, for providing financial support for the research preparation of the article and V.P. Masenko, the head of Laboratory of Immunology, for technical assistance.

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