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Addition of omega-3 fatty acid and coenzyme Q10 to statin therapy in patients with combined dyslipidemia

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Abstract

Background: Statins represent a group of drugs that are currently indicated in the primary and secondary prevention of cardiovascular events. Their administration can be associated with side effects and the insufficient reduction of triacylglyceride (TAG) levels. This study aimed to assess the effect of the triple combination of statins with omega-3 fatty acids and coenzyme Q10 (CoQ10) on parameters associated with atherogenesis and statin side effects.

Materials and methods: In this pilot randomized double-blind trial, 105 subjects who met the criteria of combined dislipidemia and elevated TAG levels were randomly divided into three groups. In the control group, unaltered statin therapy was indicated. In the second and third groups, omega-3 PUFA 2.52 g/day (*Zennix fa Pleuran*) and omega-3 PUFA 2.52 g + CoQ10 200 mg/day (Pharma Nord ApS) were added, res//. At the end of the 3-month period (±1 week), all patients were evaluated.

Results: Significant reduction of hepatic enzymes activity, systolic blood preasure, inflammatory markers and TAG levels were detected in both groups in comparison to the control group. Activity of SOD and GPx increased significantly after additive therapy. Coenzyme Q10 addition significantly reduced most of the abovementioned parameters (systolic blood preasure, total cholesterol, LDL, hsCRP, IL-6, SOD) in comparison with the statin + omega-3 PUFA group. The intensity of statin adverse effects were significantly reduced in the group with the addition of CoQ10.

Conclusions: The results of this pilot study suggest the possible beneficial effects of triple combination on the lipid and non-lipid parameters related to atherogenesis and side effects of statin treatment.

Keywords: cardiovascular system diseases; coenzyme Q10; combined dyslipidemia; omega-3 fatty acids; statins.

Introduction

Lowering of low-density lipoprotein cholesterol (LDL-C) levels is one of the main strategies for reducing cardiovascular risk, both in groups of high- or low-risk patients. Studies suggest that the decrease of the LDL-C levels by 1.0 mmol/L lowers the risk of major vascular events by 20% [1]. Statins represent a group of drugs that are currently indicated in the secondary and primary prevention of cardiovascular events due to their ability to lower LDL-C blood levels and suppress atherogenesis. The reduction is carried out by the inhibition of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, which lowers the production of total cholesterol in the liver [2, 3].

However, the effect of statins on triglyceride (TAG) serum levels is marginal and not sufficient [4]. Although elevated levels of LDL-C in plasma is considered as the main predictor and risk factor of coronary heart disease (CHD) progression, strong evidence shows that increased TAGs are an independent risk factor in accelerating the development of atherosclerotic vessel changes [5–7]. A large, randomized prospective study involving patients with coronary artery disease (CAD) reported that statin therapy minimizes the risk of mortality of CAD diseases (myocardial infarction, stroke, etc.) [1], although in many cases despite of an achievement of optimal LDL-C level (with statins in monotherapy), increased cardiovascular risk still persisted. This "residual risk" suggests that,

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except the critical role played by LDL-C in the progression of atherosclerosis, there are almost certainly other lipid factors to increase the cardiovascular risk, especially hypertriglyceridaemia [8].

Omega-3 polyunsaturated fatty acids (PUFA) belong to the group of essential fatty acids, because the human body is unable to synthesize them. The most important source of omega-3 PUFAs is fish oil. The amount of n–3 PUFA varies in different fish species. Unfortunately, it also depends on the processing method; for example, canned tuna compared to fresh one contains only 10% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The same species of fish living in their natural environment contains different levels of n–3 PUFA compared with the same species bred in farms [9].

Many clinical or preclinical studies described the positive effects of omega-3 PUFA supplementation on cardiovascular diseases [10]. The beneficial effects of the adequate intake of omega-3 PUFA may be explained by their influence on cellular metabolic functions; by their incorporation into phospholipid cell membranes, which leads to the modulation of enzymes and signaling pathways and molecules [11]; and by the modification of certain gene expressions [12]. Apart from having a direct effect on myocardial contractility, blood pressure, platelet function, coagulation factors and cellular immunity, omega-3 PUFA can also influence the level of pro-inflammatory cytokines [13]. Previous studies have shown that omega-3 PUFA do not affect the level of LDL-C (even though they can cause slight increase), but can significantly reduce TAG and increase high-density lipoprotein cholesterol (HDL-C) [14]. Significant reduction of TAG is mediated by the reduction of the hepatic TAG synthesis and increased beta-oxidation of fatty acids in the liver [15].

Coenzyme Q10 (CoQ10) is the only fat-soluble antioxidant synthesized endogeneously in the human organism. The most important exogenous sources include pork, offal, fish, vegetables, soy and so on. A sufficient amount of CoQ10 is essential for the proper functioning of cell respiration and the efficient production of adenosine triphosphate (ATP). Decrease of CoQ10 was detected in the myocardium of patients suffering from cardiomyopathies. Reduced CoQ10 concentration in the heart muscle has been correlated with the severity of the disease. Reduced levels of CoQ10 have also been detected in a group of patients showing statin side effects. Subsequent supplementation of CoQ10 resulted in the reduction of side effects [16, 17].

The aim of the current study was to determine the possible complementary effects of statin, omega-3 PUFA and CoQ10 combination on lipid and non-lipid parameters, which are associated with the progression of cardiovascular disorders, on the group of patients with combined dyslipidemia and on the presence of side effects associated with statin therapy.

Materials and methods

Study design

This study was designed as a randomized double-blind trial with a statin treatment parallel-group design. This study aimed to investigate the effects of omega-3 PUFA, especially omega-3 PUFA together with CoQ10, on already statin-treated patients with persistent elevations of TG

From October 2012 until September 2014, 342 patients under statin therapy according to current recommendations were examined on the cardiology outpatient clinics in Kosice, Slovakia. A total of 105 eligible patients were selected according to the inclusion and exclusion criteria (see below). Patients were instructed not to change their dietary habits throughout the study. In this study, ethicaly standard therapeutic methods were followed.

All patients who volunteered for this reserach study signed an informed consent. The study was approved by the Local Ethics Committee of the Medical Faculty of Pavol Jozef Šafárik University by the date and number of 25/05/2014. This study was registred at the Medical Faculty of Pavol Jozef Šafárik University and supervised by its Etical Board. Additive therapy was added just beyond standard medical procedure. No other lipid-lowering drugs were allowed.

Inclusion criteria

The inclusion criteria were as follows:

1. Patient treated with statins (according to the current recommendations) for at least 3 months at a dose that led to the achievement of target levels of LDL-C;
2. Increased level of TG in the range 1.7–4.5 mmol/L; and
3. Signed informed consent to participate in the study.

Exclusion criteria

Patients with one or more of the following exclusion criteria were not included in this study:

1. Presence of significant adverse effects of statin treatment requiring dose reduction or discontinuation of the therapy;
2. Previous known intolerance of omega-3 PUFA and/or CoQ10;
3. Evidence of still untreated secondary causes of dyslipidemia (most often hypothyroidism);
4. Presence of other serious comorbidities affecting the prognosis;
5. Possibility of changes of the concomitant therapy during the study period; and
6. All conditions potentially affecting compliance of studied medication.

Procedures

At the beginning of the study, all patients underwent a physical examination including measurement of blood pressure and laboratory examinations (lipid profile, liver enzymes, creatine kinase, glycaemia, creatinine, uric acid, sodium and potassium). The included patients were randomly divided into three groups in double-blind manner (n = 50). In the parallel study control group, continued administration of statins in the unaltered dose was indicated. In the groups with additive treatment, we indicated the continued administration of statins in combination with the following:

a) omega-3 PUFA 2.52 g/day (ZENNIX fa Pleuran, 1 pill min. 520 mg of DHA; 155 mg of EPA) (Pharma Nord ApS. Sadelmagervej 30–32., Vejle, Denmark) (tree times daily).
b) omega-3 PUFA 2.52 g/day (ZENNIX fa Pleuran) (three times daily) together with 200 mg CoQ10 (ubiquinone)/day (Pharma Nord ApS. Sadelmagervej 30–32., Vejle, Denmark) (100 mg, two times daily).

After a period of 3 months (±1 week) medical leaving check and planned sample collection wer conducted again. In all cases, medical history with targeted focus on the possible side effects of statin therapy (including completion of a questionnaire regarding the possible side effects of muscle) was taken. Patients also underwent physical examination and blood sampling for laboratory examination.

Analysis of enzymes and inflammatory markers related to endothelial functional status

Venous blood was collected immediately after randomization, and 3 months (±1 week) after the additive treatment. Blood was withdrawn from the antecubital vein under standard conditions, after at least 12 h of starvation, early in the morning until 8 a.m. Blood samples were collected from clinically stable patients, who did not show signs of acute infectious and inflammatory diseases that may affect the analysed parameters. Blood samples were processed and analyzed at the Institute of Experimental Medicine of the Faculty of Medicine, UPJŠ. Lipid profile was determined by standard clinical laboratory tests from patients' serum.

Glutathione peroxidase (GPx) activity was determined by using the Ransom laboratory kit and an automatic analyzer Dayton (Both from Randox Laboratories, UK) via the spectrophotometric method at 340 nm in whole blood. GPx catalyzes the oxidation of glutathione with the simultaneous reduction of cumene hydroperoxide. In the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), the oxidized glutathione (glutathione disulfide, GSSG) is reduced to glutathione (GSH) and NADPH is oxidized to nicotinamide adenine dinucleotide phosphate ($NADP^+$). A decrease in absorbance at 340 nm has been observed. The results are expressed in U/g Hb (hemoglobin).

The activity of superoxide dismutase (SOD) was measured from washed red cell hemolysates by using the RANSOD superoxide dismutase kit (Randox Laboratories, UK) according to the modified method of Fitzgerald [18]. Measurements were realized at 500 nm on automatic analyzer Daytona (Randox, UK) by spectrophotometric method at standardized 37 °C temperature. Xantineoxidase catalyzes the oxidation of xanthine to uric acid with the contemporary reduction of molecular oxygen to superoxide radical. Superoxide reacts with I.N.T. (color) to form a red-colored formazan (primary reaction). Increase in absorbance was measured at 500 nm. SOD (in the sample) inhibits the formation of formazan, and SOD activity can be calculated based on this inhibition. The enzyme that has an activity of one unit (1 U) reduced the speed of the reaction at 50%.

C-reactive protein (CRP) levels in serum (as well as in plasma) were determined using immunoturbidimetric method laboratory kits high sensitivity CRP (hsCRP) (Randox, UK) by automatic analyzer Daytona (Randox, UK) at 570 nm. Interleukin 6 (IL-6) was determined by ELISA kit (Thermo Scientific Human IL-6 ELISA Kit) on automatic analyzer Dayton (Randox, UK). IL-6 levels are indicated as pg/mL of serum.

Assessment of myopathy symptoms

For the assessment of the possible adverse effects of statin therapy, we used a questionnaire prepared by our team. Patients were given the task to mark their subjective complaints of muscle pain, weakness, cramps and fatigue, on a scale of 0 to 10 (0-no complain; 10-maximum intensity) both at the beginning and at the end of the study.

Statistical analysis

To describe the group, continuous variables were used; the basic descriptive statistics were frequency, mid-worthy the one standard deviation. The statistical analysis was performed using GraphPad InStat version 3.01 (GraphPad Software, San Diego, CA, USA). The quantitative results were determined using one-way ANOVA with a multiple comparison Tukey-Kramer post hoc test. In testing the hypotheses, we considered a significance level of 0.05.

Results

Study population

From the population of 342 patients, 105 patients (30.7%) were randomized. Statins taken by these patients were simvastatin, n = 21 (20%); rosuvastatin. n = 16 (15.23%); atorvastatin, n = 51 (48.57%); and fluvastatin, n = 17 (16.19%). The average doses of statins were 24.4 mg for atorvastatin, 13.65 mg for rosuvastatin, 27.82 mg for simvastatin and 80.0 mg for fluvastatin. The shortest period of statin treatment before the randomization was 6 months.

The average age was 57.24 ± 11.77 years, 53 were women, 52 men. The average weight of the patients was 73.98 ± 7.36 kg, and the calculated average body mass index (BMI) was 27.65 ± 3.59 kg/m^2. Between the randomized groups, no significant difference in basic antropometric and biochomic parameters was detected at the beginning of this study.

In the group with the addition of omega-3 PUFA, there was a significant reduction in aspartate transaminase (AST) ($p = 0.004$), alanine transaminase (ALT) ($p = 0.05$) and alkaline phosphatase (ALP) ($p = 0.005$) activities after 3 months of therapy compared with the control group. In the group with triple combination, significant decrease in AST ($p = 0.03$) and ALP ($p = 0.03$) compared with the control was also observed. Between two groups of additive therapies, no significant differences were observed in liver enzymes (Table 1). Significant decrease in the systolic blood pressure in both groups with additive therapy compared with the control group was observed (addition of omega-3 vs. control $p = 0.03$; omega-3 + CoQ10 vs. control $p = 0.009$). The values of other laboratory parameters did not differ significantly.

In this study, changes in lipid metabolism were determined (Table 2) after 3 months of additive therapy. In the control group, no significant differences in the levels of total cholesterol or in other lipid parameters (LDL-C, HDL-C, TAG) were detected in comparision with the entrance results. In the group with dual therapy, a slight increase in total cholesterol and LDL-C was observed after 3 months of therapy compared with the control group, but this result was not statistically significant. Significant changes in this group were detected only in the TAG levels compared with the control group ($p = 0.004$). The administration of the triple combination therapy induced a significant decrease in total cholesterol ($p = 0.03$) and TAG ($p = 0.01$) as well as in increase in HDL ($p = 0.03$) compared with the control group. Triple combination induced also significant decrease total cholesterol ($p = 0.02$) in comparison to the double combination therapy group. A significant decrease of the statin adverse effects was detected in the triple therapy group compared with the control and double therapy groups (Table 3).

Increased levels of hsCRP were found in patients taking part in the study (Table 4). Significant reduction was detected in both groups with additive therapy compared with the control group (double therapy $p = 0.01$; triple $p < 0.001$). The same level of reduction was observed in IL-6 levels in the double ($p < 0.001$) and triple therapy group vs. control ($p < 0.001$) (Table 4). Significantly lower IL-6 levels were detected in the triple therapy group compared with the double therapy group ($p = 0.02$). Significant increase in SOD ($p = 0.02$) and GPx ($p = 0.008$) activities were observed in the double therapy group compared with the control group (Table 4). Increase of SOD and GPx activities were also detected also in the triple therapy group compared with the control group (both $p < 0.001$) and also in comparison with the double therapy group (SOD, $p = 0.05$).

Table 1: Follow up values in randomized groups.

Observed parameters	Entry values	Statin control group after 3 months	p-Value entry vs. control	Statin + omega-3 PUFA th. after 3 months	p-Value omega-3 PUFA vs. control	Statin + omega-3 PUFA + CoQ10 th. after 3 months	p-Value omega-3 PUFA + CoQ10 vs. control	p-Value omega-3 PUFA vs. omega-3 PUFA + CoQ10
Sample size	105	35	NS	35	NS	35	NS	NS
Men/women	52/53	17/18	NS	18/17	NS	17/18	NS	NS
Age	60.71 ± 12.38	59.4 ± 13.5	NS	61.96 ± 12.2	NS	58.4 ± 13.8	NS	NS
BMI, kg/m^2	28.31 ± 3.81	28.79 ± 6.31	NS	29.31 ± 4.13	NS	27.89 ± 6.01	NS	NS
Duration of statin therapy, months	14.7 ± 12.3	13.4 ± 9.4	NS	15.0 ± 10.5	NS	16.3 ± 7.9	NS	NS
Fasting glucose, mmol/L	4.64 ± 1.42	4.41 ± 0.98	NS	4.26 ± 1.19	NS	4.69 ± 1.62	NS	NS
CK, µkat/L	2.19 ± 1.32	2.04 ± 0.79	NS	2.71 ± 1.98	NS	2.11 ± 1.95	NS	NS
AST, µkat/L	0.52 ± 0.23	0.54 ± 0.19	NS	0.38 ± 0.13	0.004	0.41 ± 0.12	0.03	NS
ALT, µkat/L	0.61 ± 0.32	0.59 ± 0.24	NS	0.41 ± 0.14	0.05	0.51 ± 0.34	NS	NS
GMT, µkat/L	0.42 ± 0.24	0.49 ± 0.28	NS	0.52 ± 0.39	NS	0.51 ± 0.32	NS	NS
ALP, µkat/L	0.98 ± 0.32	0.87 ± 0.39	NS	0.59 ± 0.32	0.005	0.65 ± 0.29	0.03	NS
Urea, mmol/L	4.76 ± 1.25	5.2 ± 1.24	NS	4.92 ± 1.19	NS	4.93 ± 1.52	NS	NS
Creatinine, µmol/L	65.76 ± 14.66	74.19 ± 12.33	NS	78.37 ± 14.24	NS	76.13 ± 14.21	NS	NS
sBP, mmHg	135.09 ± 23.19	132.8 ± 17.7	NS	120.08 ± 13.03	0.03	118.14 ± 11.18	0.009	NS
dBP, mmHg	79.98 ± 10.12	81.23 ± 11.12	NS	82.14 ± 9.82	NS	73.92 ± 7.22	NS	NS

th., therapy.

Table 2: Changes of blood lipid parameters.

Observed parameters	Entry values	Statin control group after 3 months	p-Value entry vs. control	Statin+omega-3 PUFA th. after 3 months	p-Value omega-3 PUFA vs. control	Statin+omega-3 PUFA+CoQ10 th. after 3 months	p-Value omega-3 PUFA+CoQ10 vs. control	p-Value omega-3 PUFA vs. omega-3 PUFA+CoQ10
Total cholesterol, mmol/L	5.12±0.86	4.94±0.36	NS	4.98±0.49	NS	4.64±0.55	0.03	0.02
LDL-C, mmol/L	2.56±0.72	2.62±0.56	NS	2.86±0.76	NS	2.63±0.39	NS	NS
HDL-C, mmol/L	1.01±0.29	1.12±0.36	NS	1.23±0.20	NS	1.31±0.28	0.03	NS
TAG, mmol/L	2.34±1.01	2.53±2.21	NS	1.64±0.81	0.004	1.42±0.65	0.01	NS

th., therapy.

Table 3: Intensity of adverse effects of statin therapy according to the subjective questionnaire 0–10 (0 – no presence; 10 – maximum intensity).

Observed parameters	Entry values	Statin control group after 3 months	p-Value entry vs. control	Statin+omega-3 PUFA th. after 3 months	p-Value omega-3 PUFA vs. control	Statin+omega-3 PUFA+CoQ10 th. after 3 months	p-Value omega-3 PUFA+CoQ10 vs. control	p-Value omega-3 PUFA vs. omega-3 PUFA+CoQ10
Myalgia	3.9±1.2	3.7±0.8	NS	3.6±1.6	NS	2.7±0.7	0.002	0.007
Muscle weakness	3.8±1.3	4.1±1.5	NS	2.9±1.7	NS	2.3±1.1	<0.001	<0.001
Muscle cramps	2.8±1.5	2.4±1.3	NS	3.1±1.6	NS	1.6±1.1	0.05	<0.001
Fatigue	3.4±1.2	3.6±1.6	NS	3.2±1.4	NS	2.9±1.8	0.02	NS

th., therapy.

Table 4: Markers of endothelial functional status.

Observed parameters	Entry values	Statin control group after 3 months	p-Value entry vs. control	Statin + omega-3 PUFA th. after 3 months	p-Value omega-3 PUFA vs. control	Statin + omega-3 PUFA + CoQ10 th. after 3 months	p-Value omega-3 PUFA + CoQ10 vs. control	p-Value omega-3 PUFA vs. omega-3 PUFA + CoQ10
hsCRP, mg/L	5.65 ± 2.28	5.43 ± 2.56	NS	3.84 ± 1.88	0.01	3.31 ± 1.76	<0.001	NS
Il-6, pg/mL	2.11 ± 0.43	2.18 ± 0.23	NS	1.38 ± 0.33	<0.001	1.13 ± 0.26	<0.001	0.02
SOD, U/g	2768 ± 815	2668 ± 675	NS	3183 ± 594	0.02	3638 ± 604	<0.001	0.05
GPx, U/g	31.50 ± 13.28	34.98 ± 14.29	NS	45.32 ± 9.17	0.008	50.91 ± 16.12	<0.001	NS

th., therapy.

Discussion

Statins do not affect the levels of TAG, they induce only a moderate decrease. Nowadays, it has been confirmed that the increased plasma TAG levels (even though accompanied with achieved target LDL-C) may increase the cardiovascular risk and promote atherogenesis [8].

In our study, 150 patients were randomly divided into three groups according to the therapeutic substances. Changes in lipid profile were monitored after the addition of omega-3 PUFA, including the omega-3 PUFAs together with the CoQ10 supplementation. Vascular inflamatory changes were determined by the hsCRP, respectively, IL-6 level quantification and anti-atherogenic factors through the detection of the antioxidant enzyme activities of GPx and SOD.

The administration of omega-3 PUFA has the ability to lower the serum TAG levels. This reduction is due to the decrease of TAG synthesis and increase in the beta-oxidation of fatty acids in the liver [10]. In our study, significant reduction of TAG was observed after the addition of omega-3 PUFA to statin monotherapy compared with the control group. Similar significant reductions in TAG were also observed in other studies [19, 20]. Long-term effects of omega-3 PUFA were studied in JELIS trial [21]. In the mentioned trial, low doses of statins (15–20 mg/day) and the combination of statins (15–20 mg/day) with EPA (1.8 g/day) were administed for 4.6 years. Results of the JELIS study showed no significant differences in the lipid profiles between the two groups. However, in the group with the EPA administration, 19% reduction in the prevalence of coronary events was found [21].

Other clinical studies were designed to monitor the effect of the combination of omega-3 PUFA with statins in patients with combined dyslipidemia, including a recent COMBOS (Combination of Prescription Omega-3 with Simvastatin) study [22]. In the COMBOS study, the administration of simvastatin (20 mg/day) in combination with omega-3 PUFA (4 g/day) induced a significant reduction of TAG and apoprotein E and total cholesterol after 5 weeks of combined therapy. In the COMBOS study, omega-3 PUFA administration induced a decrease in the levels of TAG and HDL-C, without the significant increases in LDL-C [22].

In our study, there were slight increases in total cholesterol and LDL-C but they were not statistically significant compared with the control group. In the group with the addition of omega-3 PUFA and CoQ10 to statin monotherapy, significant decrease in LDL-C compared with the control group and the dual therapy group was detected. Significant increase in HDL-C compared with the control group and the dual combination group was also detected.

We assume that the administration of CoQ10 with omega-3 fatty acids could affect the structure of unsaturated fatty acids, and that it could protect the double bonds against peroxidation and prolong their acitivity due to their antioxidant effects [23]. On the other hand, CoQ10 is lipid soluble and its absorption is up to three times faster in combination with food. Fatty acids could facilitate the absorption and significantly increase the bioavailability of CoQ10. Recent studies have proven that CoQ10 is capable of modulating lipid parameters, such as LDL particles, through the modulation of gene expression and metabolism [24]. The observed decrease of LDL levels in the triple combination group in our study could be explained by this possible effect of CoQ10 but also probably by another pharmacological interaction of these three active substances.

Our results could be correlated with the study of Mabuchiho et al. [25]. In their study, significant increase in HDL-C in the group with CoQ10 addition compared with the statin therapy was found. Similar significant reduction in LDL-C was also observed as. Based on the similar results between our study and that of Mabuchiho et al. [25], we assume that the omega-3 PUFA with CoQ10, when administered together, increase each other's effect.

The synthesis of cholesterol and the synthesis of CoQ10 depends on HMG-CoA reductase. This means that their synthesis could be significantly reduced and even blocked by statins. Coenzyme Q10 (ubiquinone, resp. ubidecarenone) is an important substance of myocardial energy metabolism; it also influences the stability of cell membranes, including myocytes. Sufficiant decrease of CoQ10 makes the muscles vulnerable to damage with subsequent development of myopathy, resp. myositis and in the worst cases of rhabdomyolysis [26]. Some experimental studies have shown the possible negative pleiotropic effects of statins, such as the reduction of diastolic function of the left ventricle, due to the reduced synthesis of endogenous CoQ10 and subsequent recovery after the CoQ10 replacement therapy. Other symptoms of low plasmatic CoQ10 levels could be fatigue, pain, cramps and muscle weakness. According to some studies, the increased levels of CoQ10 about 200%–300% above the normal concentrations are capable to eliminate these symptoms [16]. The supplementation of CoQ10 is recommended for the prevention of myopathy and other complications of statin therapy [25]. Our previous study has shown that the dose of 300 mg/day is capable of increasing the plasmatic CoQ10 levels by almost four times [27]. Reduction of negative pleiotropic effects of statins was also observed. Moreover, the presence of the statin-associated side effects was determined according to the subjective questionare. With the addition of CoQ10 in the current study, the significant reduction of almost all obesrved statin-associated side effects compared with the control was observed in the double therapy group.

Recent studies have shown that inflammation plays an important role in patients with ischemic heart disease and in those with atherosclerosis in other localization. The most common markers of inflammation and atherogenesis include CRP (measured by high sensitive method, called high sensitivity CRP-hsCRP), IL-6, intracellular adhesion molecule-1 (ICAM-1) and serum amyloid A [28, 29]. Elevated levels of CRP are associated with significant migration of monocytes and increased uptake of LDL by macrophages [30]. The level of CRP, especially hsCRP, correlates with the severity of the atherosclerotic process, especially with its instability and is one of the predictors of subsequent cardiovascular events. According to some authors, it is even more important than LDL-C [31]. Many experimental, randomized, clinical studies have confirmed the ability of statins to significantly reduce hsCRP the levels. According to the PROVE-IT study, significant decrease in the level of CRP was observed after the statin therapy [32]; in that study, the decrease in hsCRP reached 89% in the atorvastatin treated group and 85% in the pravastatin group.

Coenzyme Q10 prevents the oxidation of LDL particles and, through this mechanism, it has significant anti-aterogenic effects and can antenuate vascular inflammatory response. Various studies have shown a direct anti-atherogenic effect in a model of apolipoprotein E-deficient mice [33]. Coenzyme Q10 supplementation at a dose of 150 mg/day has been shown to have the ability to reduce oxidative stress and production of IL-6 in patients with atherosclerosis [34, 35]. Coenzyme Q10 is an intracellular antioxidant that protects membrane phospholipids, mitochondrial membrane protein and LDL lipoprotein particles against free radicals [29].

In our study, the levels of the most important inflammatory markers, hsCRP and IL-6, were monitored. Results showed that the control group did not differ significantly in comparison to the entry values. We suggest that this is due to the previous statin treatment and the achievement of the target LDL levels by the optimal statin dosage. In the group with the application of omega-3 PUFA and omega-3 PUFA and CoQ10, significant decrease in the levels of hsCRP was measured compared with the control group. The significant decrease was also detected in the triple combination group compared with the dual combination group.

A significant decrease in levels of hsCRP by the administration of omega-3 PUFA with statins compared with the

group with only statin could be explained by affecting the cellular metabolic functions by the integration of PUFA into the phospholipid membrane, by the modulation of enzymes and signaling molecules [11], the expression of genes [12] and the decrease of the pro-inflammatory cytokine production [13]. According to the American Heart Association, the combination of EPA and DHA in a dose of about 1000 mg/day is recommended to reach the effect of the secondary prevention of CHD [36]. In patients with hypertriglyceridaemia, medium and higher doses of PUFA (2–4 g/day) in combination with other lipid-lowering substances are recommended for the reduction of cardiovascular risk [37].

Excess cholesterol serum levels cause increased oxidative stress, increased production of free radicals and increased affinity of LDL-C to oxidation, which is involved in inflammatory reaction of the vessel wall and the progression of atherosclerosis. The lipid-lowering effect of statins is accompanied by their antioxidant activity [23, 38]. The reduction of oxygen free radicals in the endothelial cells could be caused by inhibition of izoprenylation of a small Ras protein. Ras, which is from the group of geranyl ivanych proteins, is a regulatory protein of oxygenase enzyme NADPH that is involved in stimulating the production of superoxide anion and reactive oxygen species [39]. Potential additional antioxidant mechanism of statins is to increase the bioavailability of nitric oxide (NO), which is involved in reduced synthesis of oxidized forms of LDL-C, reduced cellular oxidative stress and improvement of endothelial dysfunction. Results published in the studies PROVE-IT, REVERSAL and JUPITER only confirmed the anti-inflammatory potential of statins [32, 40, 41].

In our study, we have determined that omega-3 PUFAs significantly increase the activity of SOD and glutathione compared with the statin control group. These enzymes could neutralize ROS. Omega-3 PUFAs are susceptible to oxidation due to the existence of double bonds between the carbon atoms in the molecule. A recent study has determined that the oxidized product of omega-3 fatty acid, despite the oxidation of PUFAs, can reduce the progression of atherosclerosis. For example, the oxidation products of EPA could inhibit human neutrophil and monocyte adhesion to the endothelium, suggesting that the oxidized EPA has anti-inflammatory effects [42]. Coenzyme Q10 may provide a protective effect against lipid peroxidation, as an indicator of damage caused by free radicals during ischaemia [43, 44]. Moreover, CoQ10 supplementaion at a dose of 150 mg compared with 60 mg significantly reduced lipid peroxidation. Antioxidant enzymes, such as catalase (CAT), SOD and GPx, react against reactive free oxygen radicals and reduce their activity, thus contributing to oxidative cell attack [34, 35]. In our study, the significant increase in the activities of SOD and GPx was determined in the group with omega-3 PUFA and CoQ10 as an additive therapy compared with the statin control group. Statistically significant increase of the SOD activity was determined after the addition of CoQ10 to omega-3 PUFA combined with statin therapy, which may confirm the complementary effect of the combination of these three substances on the treatment of combined dyslipidemia.

Limitations

Although carried out in a randomized, prospective and double-blind fashion, the present study involved a relatively small number of patients as a pilot trial. The CoQ10 dosage and plasmatic concentrations were based on our previous study and were not quantified separately.

Conclusions

Our study has shown that the triple combination (combination of CoQ10 and omega-3 PUFA together with statins) should have a higher protective effect than the dual combination and could attenuate the muscle adverse effects of statin therapy.

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