Prilozi, Odd. biol. med. nauki, MANU, XXVIII, 2, s. 137‡147 (2007)
Contributions, Sec. Biol. Med. Sci., MASA, XXVIII, 2, p. 137–147 (2007)
ISSN 0351–3254
UDK : 616.153.915-085

EFFECTS OF STATINS (ATORVASTATIN) ON SERUM LIPOPROTEIN LEVELS IN PATIENTS WITH PRIMARY HYPERLIPIDEMIA AND CORONARY HEART DISEASE

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A b s t r a c t: The aim of our study was to investigate the effects of atorvastatin on serum levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein A1 (apoA1), apolipoprotein B (apoB) and lipoprotein (a) in patients with primary hyperlipidemia and established coronary heart disease.

Material and Methods. A group of 96 patients (pts), of both sexes, aged 34–59, with primary hyperlipidemia and coronary heart disease were monitored at baseline and 12 times within 12 months by the measurement of total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Apolipoprotein A1 (apoA1), apolipoprotein B (apoB), and lipoprotein (a) were monitored at baseline, after 6 months and after 12 months of atorvastatin therapy. The analyses were performed in the laboratory of the Clinical Biochemistry Institute of the Clinical Centre in Skopje. Serum levels of TC were evaluated using the calorimetric method, LDL was calculated according to Friedewald's formula, and HDL was determined with a precipitation test. Serum levels of apoA1, apoB and lipoprotein (a) were measured by the immunoturbidimetric method.

The therapeutic doses of atorvastatin used were 40 mg.

Liver enzymes, alanine aminotransferase (AST), aspartate aminotransferase (ALT) and creatine kinase (CPK) level were also monitored in our patients.

Results: According to our results, total cholesterol (TC), low-density lipoprotein (LDL) and apolipoprotein B (apoB) levels decreased after treatment by 43.58%, by

41.46%, and by 6,8%; high-density lipoprotein (HDL) increased insignificantly by 4%, apolipoprotein A1 (apoA1) and lipoprotein (a) remained unchanged.

Conclusion: The significant reduction of total serum cholesterol, LDL, and apoprotein B levels achieved represents the good result of the atorvastatin therapy, as opposed to the effects on HDL values, which showed no significant increase.

Serum apolipoprotein A1 levels and serum lipoprotein (a) levels remainned unchanged at baseline and after 12 months of atorvastatin therapy, and they remained stable over time. This probably means that atorvastatin has no therapeutic effect on the two parameters investigated.

Elevation in alanine aminotransferase and aspartate aminotransferase levels greater than twice the upper limit of normal were seen in only one patient, after 12 months of atorvastatin therapy. Creatine kinase levels remained stable over time.

Key words: dyslipidemia, cardiovascular disease, statins, atorvastatin.

Introduction

Atherosclerotic vascular diseases, which include coronary heart disease (CHD), stroke and peripheral arterial disease (PAD), are a leading cause of death in the general population all over the world [1]. As is known, the process of artherosclerosis represents their underlying etiological factor, which with its step-like and progressive course is the end result of an existing interreaction between genetic factors and factors from the surrounding living conditions. This interreaction, however, includes degenerative, inflammatory and immunological processes.

According to World Health Organization statistics, more than 16 million people die from cardiovascular disease each year, and in 2001 7.2 million deaths were caused by heart disease. By the year 2020 approximately 25 million deaths from cardiovascular disease are expected annually worldwide, and almost half of these deaths (11.1 million) will be from coronary heart disease [1, 2].

Disturbed fat metabolism in affected individuals, either as a primary abnormality or as secondary to other disorders, expressed by pathological lipid blood values (dyslipidaemia), is one of the leading risk factors for the occurrence of cardiovascular disease. Because of this, extensive research has been done in order to provide the possibility of the progressive course of atherosclerosis being prevented by early employment of adequate therapeutic measures in this group of pts. [3].

In epidemiological studies measurements of serum cholesterol have been routinely used. The relationship between cholesterol levels and the inci-

dence of coronary heart disease is almost entirely dependent on low-density lipoprotein (LDL) [4].

Statins can induce relatively large reductions in plasma cholesterol levels and are established drugs for the treatment of hypercholesterolaemia and combined hyperlipidaemia. Clinical trials have demonstrated that they can induce regression of vascular atherosclerosis as well as reduction of cardiovascular-related morbidity and mortality in patients with and without coronary heart disease.

Statins are highly effective in lowering LDL and have an excellent tolerability profile. Backed by clear evidence of a reduction in morbidity and mortality, statins are the drugs of first choice for treatment [4].

The aim of our study was to investigate the effects of atorvastatin on serum levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein A1 (apoA1), apolipoprotein B (apoB), and lipoprotein (a) in our patients with primary hyperlipidaemia and established coronary heart disease.

Material and Methods

A group of 96 patients (pts), of both sexes, aged 34–59, with primary hyperlipidaemia and coronary heart disease were monitored at baseline and 12 times within 12 months by measurements of total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Apolipoprotein A1 (apoA1), apolipoprotein B (apoB), and lipoprotein (a) were monitored at baseline, after 6 months and after 12 months of atorvastatin therapy. The analyses were performed in the laboratory of the Clinical Biochemistry Institute of the Clinical Centre in Skopje. Serum levels of TC were evaluated using the calorimetric method, LDL was calculated according to Friedewald's formula, and HDL was determined with a precipitation test. Serum levels of apoA1, serum levels of apoA1, apoB and lipoprotein (a) were measured by the immunoturbidimetric method. The therapeuthic doses of atorvastatin used were 40mg. Liver enzymes, alanine aminotransferase (AST), aspartate aminotransferase (ALT) and creatine kinase (CPK) levels were also monitored in our patients, with the purpose of determining the use of statins in and the manifestation of unwanted side-effects by their use.

Statistical analysis

The results achieved from the diagnostic procedures used were entered in a data-base file by using the Microsoft Excel program and a separate note for each

study case, with multiple variables, was defined in it. Data collected in this way were elaborated by using the Excel Statistics and Statgraf programmers package.

Statistical evaluation was carried out by calculation of means, student *t-test* and *z-test* as well as linear regression for evaluation of dependency of variables.

Results

After a 12-month treatment phase with atorvastatin, the study group with primary hyperlipidaemia and coronary heart disease showed the most significant therapeutic effect on the level on the total cholesterol (TC) and lowdensity lipoprotein (LDL) with a significant reduction of 43.6% ($p < 0.001$). Before initiation of treatment cholesterol levels were 7.48 ± 2.62 mmlo/L and after the 12-month treatment phase cholesterol levels were 4.22 ± 1.12 mmol/L.

A significant effect was observed with levels of low-density lipoprotein, with concentration dropping from 4.92 ± 1.35 mmol/L before initiation to 2.88 ± 0.87 mmol/L after treatment. This was also a significant reduction of 41.16% ($p < 0.001$).

The levels of the "protective or good" cholesterol increased from 0.96 ± 0.22 mmol/L to 1.00 ± 0.35 mmol/L from before to after treatment, respectively. This rise of 4% was statistically insignificant ($p > 0.05$) (Diagram 1).

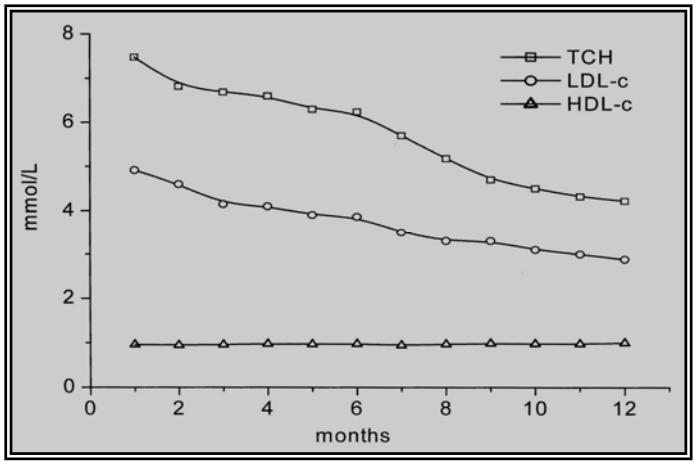

Diagram 1 – Presentation of levels of TC, LDL, and HDL, within 12 months of atorvastatin therapy

Графикон 1 – Приказ на вредноста на ТС, LDL и HDL, во тек на 12-месечната терапија со аторваастатин

Laboratory evaluation of apolipoproteins A1, apolipoproteins B, and Lp(a) may help the diagnosis of dyslipidaemia. By correction of blood values of these components it is possible to slow down the existing process of atherosclerosis and prevent some of its consequences [5].

Therapy with atorvastatin showed a marked, but still insignificant, effect on the values of atherogenic apolipoprotein B (apoB), whose values were reduced by 6.8%, from the initial value of 1.63 ± 0.59 to 1.52 ± 0.46, after showing an insignificant effect on the "protective" apolipoprotein A1 (apoA1) and highly atherogenic lipoprotein (a), whose values remained unchanged.

Results from evaluated parameters are shown in Table 1

Table 1 – Табела 1

Mean values of TC, LDL and HDL and apoB, at baseline and after 12 months of atorvastatin therapy
Средните вредности на TC, LDL, HDL и apoB пред започнување со терапијата и по 12-месечната терапија со аторвастатин

	Before	After	p
TC (mmol/l)	7.48 ± 2.62	4.22 ± 1.12	< 0.001
LDL (mmol/l)	4.92 ± 1.35	2.88 ± 0.87	< 0.001
HDL (mmol/l)	0.96 ± 0.22	1.00 ± 0.35	n.s.
apoB (g/l)	1.63 ± 0.59	1.52 ± 0.46	< 0.01
apoA1 (g/l)	1.1 ± 021	1.2 ± 0.18	n.s.
Lp(a)	74 ± 0.46	73.5 ± 0.35	n.s.

Some studies indicate that apoprotein B is a better marker for CAD than LDL. The importance of apoB as a marker for proving a coronary is variable and exclusively dependent on the presence and form and the size of LDL particles [7].

More than 90% of the apoB within the plasma of normolipidemic individuals is contained in the LDL fraction, hence there is little difference between the levels of total plasma apoB and the levels of apoB in LDL.

In our patient study group we performed an analysis of linear dependence between values of apolipoprotein B and LDL and the findings achieved showed that a certain, but not strong, linear dependence exists between them ($R = .57394365$ and $R^2 = .32941131$) (Diagram 2).

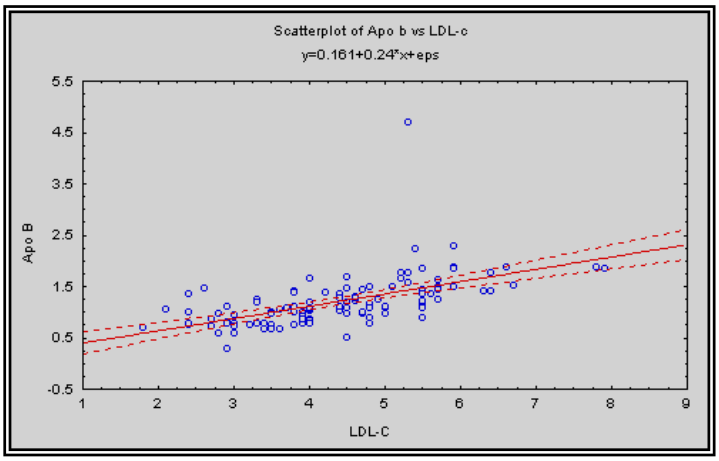

Diagram 2 – Graphical presentation of linear dependence between values of apolipoprotein B and LDL
Графикон 2 – Графички приказ на правата на регресија на испитуваната линеарна зависност помеѓу вредностите на аполипопротеиноt Б и LDL холестеролот

Discussion and Conclusion

Disturbed fat metabolism in affected individuals, expressed by pathological lipid blood values (dyslipidaemia), represents one of the leading risk factors for the occurrence of cardiovascular disease, and that is why the progressive course of artherosclerosis might be prevented by therapeutic correction of existing high blood values [3].

Dyslipidaemia may be secondary to other disorders or a primary abnormality. Common causes of secondary dyslipidaemia include: diabetes mellitus, nephrotic syndrome, chronic renal failure and hypothyroidism. Of the primary causes of dyslipidaemia, the most severe forms are caused by genetic disorders of lipoprotein metabolism [4].

Laboratory tests for the diagnosis of dyslipidaemia are most frequently made by means of laboratory investigations of the total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) values. Laboratory evaluation of apolipoproteins A1, apolipoproteins B and Lp(a) may help diagnosis. By correction of the blood values of these components it is possible to slow down the existing process of atherosclerosis and prevent some of its consequences [5].

In epidemiological studies measurements of serum cholesterol have been routinely used. The relationship between cholesterol levels and the incidence of CHD is almost entirely dependent on low-density lipoprotein (LDL), though oxidised LDL is the most atherogenic form of LDL [6].

So low-density lipoprotein (LDL) has been recognised as a prime target for lipid intervention to prevent CHD.

HDL is the smallest, but most abundant, of the lipoproteins. It returns about 20–30% of cholesterol in the blood to the liver from peripheral tissue for excretion. It does not cause atherosclerosis, but actually protects against its development. As is known, apolipoproteins are the protein constituents of lipoproteins.

Apolipoprotein A1 represents the dominant protein in HDL structure and constitutes approximately 65% of serum values of apoA1 in HDL. Apolipoprotein A1, like HDL, does not cause atherosclerosis, but actually protects against its development.

Apolipoprotein B is the apolipoprotein involved in chylomicron, VLDL and LDL metabolism. The serum concentration of apoB reflects the number of particles of VLDL and LDL in the circulation.

Some studies indicate that apoprotein B is a better marker for CAD than LDL. The importance of apoB as a marker for proving a coronary is variable and exclusively dependent on the presence, form and size of LDL particles [7].

More than 90% of apoB within plasma of normolipidaemic individuals is contained in the LDL fraction, hence there is little difference between the levels of total plasma apoB and the levels of apoB in LDL. Sniderman *et al*. have shown that among premature CAD patients with normal LDL levels, the percentage of apoB within LDL is often extremely elevated [8].

Several retrospective studies have shown a strong independent correlation between LP(a) and atherosclerotic heart disease, especially at an early age, and its values over 0.30 g/l are associated with a doubly increased coronary risk, and consequently the occurrence of cardiac arrest at an early age.

Lipoprotein (a) is considered a genetic variant of LDL cholesterol, which has similar but not identical physical and chemical, imunological and specific functional characteristics. In the last few years many new studies published have been devoted to the role of Lp(a) in the process of thrombogenesis [9–11].

The National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATPIII) has made recommendations for the treatment of hypercholesterolaemia.

The NCEP ATP III guidelines continue to identify LDL as the primary target for CHD prevention and defines the optimal LDL level as < 2.6 mmol/l or lower, compared with < 3.4 mol/l in the ATP II guidelines. The recommended LDL level has been lowered because the risk of developing CHD appears to be less when the LDL is maintained at a level of < 2.6 mmol/l. Also, an LDL goal

of < 2.6 mmol/l is now the target goal for patients with both CHD and CHD-risk equivalents, such as diabetes, carotid or peripheral vascular disease.

Low HDL is a strong independent predictor of CHD, and is defined in NCEP ATP III as < 1 mol/l. In previous guidelines the level was < 0.9 mol/l. By raising the HDL cutting point to < 1 mol/l, more patients with an increased risk of developing CHD are identified [12].

Statins can induce relatively large reductions in plasma cholesterol levels and are established drugs for the treatment of hypercholesterolaemia and combined hyperlipidaemia. Clinical trials have demonstrated that they can induce regression of vascular atherosclerosis as well as reduction of cardio-vascular-related morbidity and mortality in patients with and without coronary heart disease [13–16].

They are used for correction of total cholesterol and the atherogenic LDL, but in patients with reduced values of protective HDL as well.

But there is no further information about the effects of the recently-introduced statins on Apolipoprotein A1, B and lipoprotein (a).

The Scandinavian Simvastatin Survival Study (4S)1 was the first study to demonstrate a benefit from statins in patients with CHD. These patients had angina pectoris or previous MI, and raised cholesterol levels of 5.5–8.0 mmol/L (mean LDL-C 4.87 mmol/L,). Thus, statin treatment has been shown to provide morbidity and mortality benefits in a wide range of patients. These benefits have been seen even in patients at low risk and with average baseline cholesterol levels, supporting the use of aggressive cholesterol-lowering with statins [18]. The Heart Protection Study (HPS) found a benefit in treating subjects at high risk of coronary events with 40mg. simvastatin daily, regardless of baseline LDL levels. The study found no negative effects with 40mg. simvastatin daily [13].

The Myocardial Ischaemia Reduction with Aggressive Cholesterol Lowering (MIRACL) study (with 80mg. atorvastatin daily in pts. with acute coronary syndromes), showed that at the end of the study (after 16 weeks) LDL had decreased by an adjusted mean of 40%. Changes in HDL cholesterol during the study were minor.

In this trial, early treatment with atorvastatin reduced recurrent ischaemic events.

No serious adverse event occurred in a frequency of more than 1% of pts. [14].

The basic mechanism of action of the statins is inhibition of the HMGoA reductase, the essential enyzme in a synthesis of the endogenic cholesterol, but the statins also have effects on body functions different from those associated with the above-mentioned ones. These are called nonlipid or pleotropic effects. They influence a wide range of systems, and include vasodilative

effects through the increase of NO production, antithrombotic, antioxidant, antiproliferative, antiinflamatory and plaque stabilizing effects [17].

However, the clinical studies performed so far show that aggressive therapeutic decrease of LDL with statins results in lowering morbidity and mortality rates associated with atherosclerotic vascular diseases in humans.

In our study, the significant reduction of total serum cholesterol, LDL, and apoprotein B levels achieved represents the good results of atorvastatin therapy, as opposed to the effects on HDL values, which showed no significant increase.

Serum apolipoprotein A1 levels and serum lipoprotein (a) levels remainned unchanged at baseline and after 12 months of atorvastatin therapy, and they remained stable over time. This probably means that atorvastatin has no therapeutic effect on these two investigated parameters.

In spite of this, irrespective of the fact that statins do not act directly on Lp(a) values, because of their proven therapeutic effect on LDL values, aggressive treatment with this agent, especially in patients with increased values of both Lp(a) and LDL, could achieve a significant decrease of morbidity and mortality from atherosclerosis and vascular diseases.

As to patients with either proven CAD or increased risk of developing such a disease, treatment with statins is recomended not only in pts. with pathological blood values of lipoproteins but also in those with normal values. In our opinion, it could be concluded that statins are agents which contribute greatly to the control and progress (development) of atherosclerosis, with all the consequences this has in humans.

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Р е з и м е

ЕФЕКТОТ НА СТАТИНИТЕ (АТОРВАСТАТИНОТ) НА СЕРУМСКИТЕ ЛИПОПРОТЕИНСКИ ВРЕДНОСТИ КАЈ ПАЦИЕНТИТЕ СО ПРИМАРНА ХИПЕРЛИПИДЕМИЈА И КОРОНАРНА АРТЕРИСКА БОЛЕСТ

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Целта на нашата студија беше да се испита ефикасноста на аторвастатинот, на серумските вредности на тоталниот холестерол, LDL, HDL, аполипопротеинот А, аполипопротеинот Б и липопротеинот а. кај пациенти со примарна хиперлипидемија и докажана коронарна артериска болест.

Материјал и методи: Беа иследени вкупно 96 пациенти, од обата пола, на возраст од 34–59 години, со примарна хиперлипидемија и коронарна

артериска болест и кај истите беа анализирани вредностите на тоталниот холестерол, LDL и HDL, пред започнување со терапијата, а потоа 12 пати во текот на 12 месеци. Аполипопротеинот А, аполипопротеинот Б и липопротеинот а., беа мерени пред започнување со терапијата, по шест месеци и по 12 месеци терапија. Анализите беа извршени во Институтот за Клиничка биохемија, Клинички центар, Скопје.

Вредностите на тоталниот холестерол беа добиени со ензимска калориметриска метода, LDL беше добиен по Friedwaldova формула, а HDL со преципитационен тест. Вредностите на аполипопротеинот А, аполипопротеинот Б и липопротеинот а, беа мерени со имунотурбидиметриска метода. Тераписката доза на аторвастатинот беше 40 мг на ден.

Со цел да се оцени безбедноста на употребата на статини кај пациентите, исто така беа мерени хепаталните ензими, серумските трансаминази (AST и ALT), како и вредностите на креатинин киназата (CPK).

Резултати: Беа добиени следните резултати: вредностите на тоталниот холестерол беа намалени за 43,58% по 12-месечната терапија со аторвастатин, вредностите на LDL беа намалени за 41,46%, на аполипопротеинот Б за 6,8%. Вредностите на протективниот HDL беа наголемени за 4%, додека вредностите на аполипопротеинотА и липопротеинот а. останаа непроменети по 12-месечна терапија со аторвастатин.

Заклучок: Можеме да заклучиме дека по 12-месечната терапија со аторвастатин во доза од 40 мг на ден беше постигната значајна редукција на тоталниот холестерол и LDL холестеролот, ефектот на аторвастатинот на аполипопротеинот Б и протективниот HDL е помал во однос на тоталниот холестерол и LDL. Во однос на вредностите на аполипопротеинот А и липопротеинот а., не беше регистриран тераписки ефект на аторвастатинот.

Елевација на серумските аминотрансферази, повеќе од два пати од нормалната вредност, беше регистрирана само кај еден пациент, по 12-месечна терапија, Вредностите на креатинин фосфокиназата останаа стабилни во текот на целиот период на статинската терапија.

Клучни зборови: дислипидемија, кардиоваскуларна болест, статини, аторвастатин.

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