Appreciation efficacy and safety of statin therapy in lowering cardiovascular risk in patients with type 2 diabetes, according to serum levels of inflammatory biomarkers

PhD Thesis Abstract

Key words: atorvastatin, diabetes type 2, high sensitive C-reactive protein, oxidised LDL, cardiovascular disease

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1. The current state of knowledge on statins, therapeutic solutions in the dyslipidaemia of type 2 diabetes.

1.1. Diabetes and cardiovascular risk

In type 2 diabetes mellitus (DM 2) leading cause of mortality is generated by the clinical consequences of atherosclerosis. LDL cholesterol and atherosclerosis are related both to the healthy people and those with diabetes, only those with DM 2 predisposition for atheromatosis is higher, although their levels of LDL are similar to those of healthy people. Atherosclerosis begins with endothelial dysfunction, lipid accumulation in macrophages and an inflammatory response, leading to plaque formation and narrowing of the vascular lumen. Less resistant plates will crack and could lead to myocardial infarction (MI) or stroke. Atherosclerotic cardiovascular disease is the leading cause of morbidity and mortality in patients with type2 diabetes, and the risk of developing the disease is two to four times higher than in non-diabetic subjects [1]. In addition, the risk of cardiac morbidity and mortality in individuals with type 2 diabetes without previous MI has been shown to be similar to that in non-diabetic subjects with a history of MI [1]. Several prospective studies have shown that cross a preponderance of small LDL particles is associated with an increased risk of coronary artery disease (CAD) and Diabetes Atherosclerosis Intervention Study (DAIS, 2003) reported that small LDL particles are significantly associated with progression of the disease [2].

LDL particle size can be used with LDL cholesterol determination, to give an indication of the number of circulating LDL particles. Two
individuals may have the same concentration of LDL-cholesterol, but that with predominantly small LDL particles will need more particles to carry the same amount of cholesterol.

The observation that diabetics have small particles of LDL but normal plasma levels of LDL-cholesterol, shows that particle number is increased in type 2 DM.

1.1.1. LDL oxidation and oxidative stress

LDL oxidation initiates a series of events that ultimately will lead to increased acquisition of LDL by macrophages, the formation of foamy cells and the development board atherom [3]. Oxidized LDL and antibodies against the modified form of the lipoproteins were found in atherosclerotic lesions [4], but not in normal arteries or veins. Some characteristics of oxidized LDL play a role in the development of atherosclerotic plaques: oxidised LDL is toxic for endothelial cells [5], recruits lymphocytes in atherosclerotic lesions [6] and promotes proliferation of macrophages within plaques [7].

1.1.2. LDL oxidation and association with atherosclerosis and diabetes

Small and dense LDL particles are considered more prone to oxidation than large ones and at least three studies have shown an inverse relationship between lag phase of LDL oxidation and LDL density [10]. Using LDL split, these studies show that small and dense LDL particles are less resistant to in vitro oxidation than large LDL particles, energetic harvested from the same individual.

Plasma levels of oxidized LDL, measured by ELISA-sandwich were increased in individuals with impaired glucose tolerance (TAG) compared with control subjects after adjusting for age and body mass.
index (BMI), and increased in patients with type 2 DM with macroalbuminuria than in those with normo-or microalbuminuria and healthy subjects [13]. Patients with well controlled DM have increased plasma levels of oxidized LDL compared with control subjects, when adjusted for LDL-cholesterol concentration [15, 16]. Moreover, it was observed an inverse relationship between circulating levels of LDL and oxidised LDL particle size in patients with DM 2 [16]. The middle-aged men observed an independent association between serum oxidised LDL and subclinical atherosclerosis assessed by intimate-media thickness at common carotid artery [14].

**1.1.3. Conclusions and perspectives**

There is evidence of increasingly numerous, showing that changes in LDL increase aterogenitatea. Since the modified LDL is not a homogeneous entity, no single diagnostic marker that adequately reflect the risk of cardiovascular disease associated with modified LDL.

The presence in plasma of small dense LDL particles is associated with hypertriglyceridaemia and an increased risk of coronary artery disease (CAD). Small LDL particles predominate in patients with insulin resistance and an inverse relationship has been observed between LDL particle size and circulating oxidised LDL particle number in patients with type 2 diabetes [16]. LDL particle size may be influenced favorably by establishing a strong control of blood glucose [17]. This supports the importance of glycemic control benefits above and beyond established microvascular disease. LDL particle size is increased fenofibrat [2], while therapy with statins, although significantly reduce the total level of LDL cholesterol, not affecting
their size [18].

In conclusion, LDL cholesterol is a poor predictive factor for CAD risk assessment associated with DM 2. Some oxidative and non-oxidative modification of LDL, as oxidation and glycation contributes to accelerating atherosclerosis in diabetes. Measuring these changes in LDL is very technically demanding and therefore unsuitable for routine practice. Serum triglycerides are closely related to the predominance of small LDL particles and increased levels of oxidised LDL. In addition, the number of LDL particles is positively correlated with risk of CAD.

1.2. Inflammatory biomarkers predictive of acute cardiovascular events

1.2.1. C-reactive protein (CRP) and cardiovascular risk in type 2 diabetes

Development high-sensitive CRP (hs-CRP) by a stable and cheaper method, increased ability to obtain more useful determinations of circulating levels of this cytokine and therefore redefine the assessment of cardiovascular risk, particularly those where the risk is intermediate, as individuals with medium LDL-cholesterol levels.

CRP is considered to be an important inflammatory cytokine, which acts as a nonspecific defense mechanism in response to permanent damage or infection tissue. Synthesized primarily in the liver, CRP activity is stimulated by other cytokines, particularly interleukin IL-6, IL-1β and tumor necrosis factor-α (TNF-α). CRP binds to a variety of other molecules, particularly liposomes and lipoproteins, the LDL and VLDL cholesterol and is a potent activator of classical complement pathway. The data accumulated suggest that CRP, which
is also found in macrophages from the ateroma plaque is associated atherothrombosis as concerned or mechanism.

1.2.2. CRP - predictor of cardiovascular events

Moderate increases in CRP can be found even in apparently healthy people [6]. A progressive increase of CRP may reflect vascular inflammation amplification stages, but specific clinical conditions in which the event is not fully understood. Although LDL cholesterol remains a major risk factor for cardiovascular disease, at least one third of coronary events occur in individuals with an LDL level below 130 mg/dl [25], which is generally considered a medium level in individuals without known coronary artery disease. Monitoring levels of CRP in these clinical conditions can be very useful for risk assessment. Evidence that CRP levels are elevated during cardiovascular and cerebrovascular events suggests that CRP is valuable in predicting the subsequent evolution of these events.

1.2.3. C-reactive protein and type 2 diabetes

Some data [26], reinforcing the concept that inflammation plays an important role in the pathogenesis of type 2 diabetes and linking diabetes with concomitant states there is an inflammatory component [27]. There is evidence that insulin resistance with normal glycemia is an proinflammatory state and there with long before the actual onset of type 2 diabetes [28]. The mechanism has a major role in the inflammatory cascade of events leading to rupture aterom plate. Up-regulation of receptor for advanced glycation and products (AGES) was associated with increased inflammatory reactions. Increased expression of these receptors was correlated with poor control of blood glucose and could contribute to the area of complex
mechanisms that lead to acceleration of atherosclerosis in patients with diabetes [29].

1.2.4. Conclusions and perspectives

Current Opinions differ regarding the value of CRP test additive, in addition to classical risk factors for cardiovascular risk assessment. Continue discussion on the use of CRP as part of screening for global risk assessment.

1.3. Lipid and non-lipid effects of statins in the prevention of cardiovascular pathology

1.3.1. Statin-brief history, definition, mechanisms of action

First clinical experience of administration of statins was held in 1976, and since the 80 trials were initiated extensive research of the effects of statins in U.S. clinics. The emergence of 3-hydroxy-3 inhibitors -metilglutaril-coenzyme A reductase inhibitors (statins) has revolutionized treatment of hypercholesterolemia. In the liver, statins specifically inhibit competitive, reversible HMG-CoA reductase, the enzyme that catalyzes the conversion of HMG-CoA (hydroxymethyl-glutaryl coenzyme A) to mevalonic acid, limiting stage the formation of cholesterol [37]. By decrease in cholesterol synthesis, statins also reduce, the formation of lipoproteins, especially LDL and VLDL. Because diabetes is considered equivalent for heart disease, aggressive treatment of dyslipidemia (especially when using statins) to reduce coronary risk, is a priority in the care of diabetic patients. Observations of large-scale randomized clinical trials underlying of recommendations of international guidelines that support the need and importance of reaching lipid targets all people with diabetes. Lipid
control is part of multifactorial management of diabetes, which should be early and intensive.

1.3.2. Statins and diabetes

Endothelial dysfunction is representative of the DM and insulin resistance and is characterized by reduction effective action of nitric oxide. Hyperglycemia status implies a cascade of mechanisms leading to increased vascular tone. In patients with DM statins improve endothelial dysfunction by stimulating NO production by endothelial cells, modulating the release and action vasoconstrictors.

2. Own contributions

2.1. Motivating the study and current working hypothesis

Is well studied many effects of statins: lipid-lowering, anti-inflammatory, antiproliferative, antioxidant, immunosuppressive, positive influence on rheological parameters and thrombogenesys, correction of endothelial function. These effects have been well studied in patients with ischemic heart disease and less in patients with type 2 diabetes. How atherosclerosis is a diffuse process, serum biomarkers have the potential to provide a general measure of risk that can allow a more targeted, with a variety of techniques focused on those with abnormal levels. In addition, they can provide an overall assessment of treatment response to various interventions. Serum biomarkers are likely to play an important role in the future, risk stratification and prognosis of cardiovascular disease, especially when established risk factors are complementary. Compared to invasive or noninvasive procedures, their evaluation has the advantage of being relatively free
of risks, less expensive, and applicable to a wide range of populations at risk.

Although it was shown that treatment with statins reduce CRP levels in a manner that is largely independent of LDL cholesterol level, lack of evidence linking a greater reduction in CRP levels by reducing the rate of cardiovascular events.

Provocative information now suggests that hsCRP is useful in monitoring the effectiveness of statins in primary prevention, after the stent implantation, and in increased risk of acute coronary syndromes. Applying biology of inflammation in atherosclerosis has already provided a new perspective on how current interventions, both pharmacological and in lifestyle can reduce cardiovascular risk. There are no studies that examine various lipid or non-lipid effects of statins, according to the dose administered to prevent acute cardiovascular events (especially the influence of hs-CRP and oxidized LDL) in patients with type 2 diabetes with or without dyslipidemia or who suffered a heart attack or stroke.

There is data showing the association between blood levels of hs-CRP and LDL-ox, and that would be predictive value for the cardiovascular risk of the two biomarkers together, in diabetic patients.

2.2. Study Objectives

1. Evaluation of inflammatory biomarkers (hs-CRP), of oxidative stress (oxidized LDL), lipids (cholesterol, triglycerides), blood glucose (blood sugar, HbA1c) and hepato-renal (ALT, AST, microalbuminuria) in patients with diabetes tip2.

2. Setting changes inflammatory biomarkers (hs-CRP), oxidised LDL, the metabolism of blood glucose and lipid parameters between
patients with type 2 diabetes without manifest cardiovascular disease and those with heart attack or stroke.

3. The changes that inflammatory biomarkers (hs-CRP), oxidised LDL, the metabolism of blood glucose and lipid parameters in patients untreated with lipid-lowering drugs and patients treated with atorvastatin.

4. Analysis of lipid parameters, the hs-CRP and oxidised LDL in the treatment of long (12-24 weeks) with atorvastatin versus short-term treatment (4-8 weeks).

5. Assessing the influence of long-term therapy (12-24 weeks) with atorvastatin on glycemic metabolism parameters versus short-term treatment (4-8 weeks).

6. Comparison of atorvastatin action, depending on dose, the values of hs-CRP, oxidized LDL and lipoproteins in patients with acute cardiovascular events.

7. Comparison of atorvastatin action, depending on dose, the values of hs-CRP, oxidized LDL, lipoproteins in patients without manifest cardiovascular disease.

8. Highlighting the type and dynamics of adverse reactions, depending on the dose of atorvastatin administered and diabetic features.

4. Results

4.1. Demographic and clinical-biological characteristics of patients with type 2 diabetes study group

4.1.1. Demographic characteristics of patients

The study included 84 patients with type 2 diabetes, of whom 43 patients without acute cardiovascular events (group A) and 41
patients with acute cardiovascular events (group B). In each of the two study groups were distinguished two subgroups: patients who not received lipid-lowering medication (A1, B1) and patients treated with atorvastatin (A2, B2).

Diagnosis of type 2 diabetes was based on historical data, clinical and laboratory explorations. The absence of cardiovascular disease was defined as documented absence of myocardial infarction, stroke or peripheral arterial disease. Retinopathy and nephropathy have been refuted by practitioners.

The average age of patients included in the study was 61.12 ± 11.38 years (age limits - 39 and 84 years), with significant difference between the two groups: the group A (without acute cardiovascular events) mean age was 53.61 ± 6.68 years and in group B (acute cardiovascular events) mean age was 69.88 ± 9.27 years. The gender distribution showed a predominance of women in group A vs. 61.9%. 38.1% and a high proportion of men in group B 72.22% vs. 27.78%.

4.1.2. Clinical and biological characteristics of patients with type 2 DM without manifest cardiovascular disease (subgroups A1 and A2)

Table 1. Characteristics of patients with type 2 DM without manifest CVD.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>A1F</th>
<th>A1B</th>
<th>A1T</th>
<th>A2F</th>
<th>A2B</th>
<th>A2T</th>
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<tr>
<td>Glucose [mg/dl]</td>
<td>143,22</td>
<td>156,25</td>
<td>147,23</td>
<td>136,750</td>
<td>132</td>
<td>134,37</td>
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<td></td>
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<td>+24,22</td>
<td>+22,39</td>
<td>+12,72</td>
<td>+13,70</td>
<td>+8,89</td>
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<tr>
<td>HbA1c [%]</td>
<td>6,45</td>
<td>6,75</td>
<td>6,54</td>
<td>6,77</td>
<td>6,59</td>
<td>6,68</td>
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<tr>
<td></td>
<td>+0,39</td>
<td>+0,43</td>
<td>+0,41</td>
<td>+0,26</td>
<td>+0,42</td>
<td>+0,25</td>
</tr>
<tr>
<td>Col. total [mg/dl]</td>
<td>255,66</td>
<td>234,25</td>
<td>249,07</td>
<td>204,25</td>
<td>199,255</td>
<td>201,750</td>
</tr>
<tr>
<td></td>
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<td>+56,80</td>
<td>+38,26</td>
<td>+2,82</td>
<td>+35,33</td>
<td>+11,88</td>
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<tr>
<td>LDL col [mg/dl]</td>
<td>133,95</td>
<td>99,47</td>
<td>123,34</td>
<td>90,65</td>
<td>88,4</td>
<td>89,520</td>
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<td>+39,49</td>
<td>+33,32</td>
<td>+4,80</td>
<td>+31,80</td>
<td>+12,34</td>
</tr>
</tbody>
</table>
4.1.3. Clinical and biological characteristics of patients with type 2 DM with acute cardiovascular events (B1 and B2)

Subgroup B included 41 patients who suffered an acute myocardial infarction (AMI) or stroke (AVC) at inclusion in study group. The median age group was 69.88 ± 9.56 years and the distribution by sex showed a predominance of men 72.22% vs. 27.78%.

Table 2. Characteristics of patients with type 2 DM with acute cardiovascular events.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Glucose [mg/dl]</td>
<td>163,00</td>
<td>166,12</td>
<td>165,5</td>
<td>148</td>
<td>179,60</td>
<td>167,75</td>
</tr>
<tr>
<td></td>
<td>±11,38</td>
<td>±6,32</td>
<td>±12,87</td>
<td>±10,14</td>
<td>±21,73</td>
<td>±23,80</td>
</tr>
<tr>
<td>HbA1c</td>
<td>7,29</td>
<td>7,43</td>
<td>7,4</td>
<td>6,91</td>
<td>7,94</td>
<td>7,55</td>
</tr>
</tbody>
</table>
Note: CVE = cardiovascular events, B1 = subgroup with acute CVE untreated with ATV, B2 = subgroup with acute CVE treated with ATV, F = female, M = male, T = total, ATV = atorvastatin.

5. Discussion
In this study we evaluated the efficacy and safety of atorvastatin in lowering cardiovascular risk, given at a dose of 10 mg/day and 20 mg/day on lipid metabolism, glycemic and inflammatory biomarkers in patients with type 2 diabetes without manifest cardiovascular disease and in patients with acute cardiovascular events, which were given treatment for a period between 4 and 24 weeks.

In our study we found evidence of benefit for patients whose treatment with statins resulted in concentrations of hs-CRP of less than 2 mg/l, whether levels of LDL cholesterol were reduced to the target value of less than 70 mg/l or not. In this respect, our data are consistent with research indicating inflammation as a factor of plaque instability and experimental data showing that statins, in addition to lipid lowering effect, also have important anti-inflammatory effects [42].

In our study, oxidized LDL levels were significantly higher values in diabetic patients untreated with lipid-lowering compared with diabetic patients treated with atorvastatin, regardless of cardiovascular status. Significant changes in this biomarker were recorded depending on the dose of atorvastatin in type 2 DM patients without manifest cardiovascular disease (A2) - 10 mg/day: 70.75 ± 3.32 U/l vs. 20 mg/day: 60.24 ± 7.54 U/l.

6. Conclusion

1. Type 2 diabetes is a complex disease associated with an increased risk of ischemic heart disease and premature death. Among patients with acute coronary syndrome and stroke have predominantly
men aged 60-80 years.

2. In acute cardiovascular events diabetes is not only an important cardiovascular risk factor, but has a strong impact on other risk factors- hypertension, elevated total cholesterol, triglycerides, low HDL-cholesterol- showing a tendency to associate their the same patient.

3. Glicate hemoglobin and blood sugar levels, total cholesterol and LDL cholesterol were not significantly affected by the dose of atorvastatin administered, but blood glucose was as high as the effectiveness of statins in reducing lipids was lower.

4. Patients with received low-dose atorvastatin (10 mg/day or 20 mg/day) long term (12-24 weeks) had lower blood glucose levels (statistically insignificant) than those who received short-term treatment (4-8 weeks)

5. The group of patients without manifest cardiovascular disease, diabetes mean age was 42.61 ± 57.86 months and the group of patients with acute cardiovascular events 29.05 ± 49.49 months, which proves, surprisingly and contrary data literature, that age of type 2 diabetes does not influence the incidence of acute cardiovascular events. Myocardial infarction or stroke, in diabetic patients, may occur regardless of age diabetes, but dependent on the existing metabolic imbalance.

9. Oxidized LDL and hs-CRP were significantly elevated in patients with acute myocardial infarction or stroke than those without manifest cardiovascular disease, irrespective of lipid profile of patients, suggesting that may be considered independent predictive factors for cardiovascular risk.

10. Our research findings suggest a higher sensitivity of hsCRP at atorvastatin therapy compared with oxidized LDL, both in patients
without manifest cardiovascular disease, and especially in patients with acute MI or stroke.

11. Treatment with statins reduce hsCRP levels in a manner that is largely independent of LDL cholesterol level, but lack of evidence linking a greater reduction in hsCRP levels by reducing the rate of cardiovascular events.

15. Statins exercise beneficial pleiotrope effects on vascular wall cells. Treatment with statins was one of the most significant advances in prevention and treatment of atherosclerosis. The benefits of statin therapy in reducing cardiovascular risk extend beyond their effects on serum lipids.

16. In general, statins are well tolerated drugs, the primary side effect is hepatotoxicity, which increases in direct proportion to the dose of statin administered and seems to not be influenced by the duration of treatment.

17. Atorvastatin, at doses of 10 mg/day and 20 mg/day, did not cause a deterioration in the biological samples of liver (ALT, AST), requiring discontinuation of therapy.

18. Because clinical trials with statin therapy was initiated only on total cholesterol and LDL cholesterol, suggest that evaluation of biomarkers hsCRP and oxidized LDL is an objective way to assess the need for administration of statins, and establishing the effectiveness of statins in preventing acute cardiovascular events.
Selective Bibliography


33. Yue CC, Muller-Greven J, Dailey P, Lozanski G, Anderson V, Macintyre S: Identification of a C-reactive protein binding site in
two hepatic carboxylesterases capable of retaining C-reactive protein within the endoplasmic reticulum. J Biol Chem 271:22245-22250, 1996.


Curriculum vitae

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List of works published

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Full papers published in journals listed CNCSIS:
Type B:

Type C:


Abstracts of some papers (communications/posters) published in volumes of international scientific meetings (with ISBN):


Abstracts of some papers (communications/posters) published in volumes of scientific national (with ISBN):


4. Floriana Ionică, Florica Popescu, Eliza Gofiţă: ”The action of HMG-CoA reductase inhibitors on increased levels of C-reactive protein” - Scientific Symposium" 10 years of pharmaceutical education university Craiova ”UMF Craiova, p. 28-29, 18-20 May, 2006.


