Atherosclerotic cardiovascular disease (CAD) represents the major cause of premature death in Europe [1]. Dyslipidaemia (DYS) represents a major risk factor for the development of atherosclerosis and therefore subsequent development of cardiovascular events. Dyslipidaemia is strongly correlated with endothelial dysfunction [2], a systemic pathological state characterized by the alteration of normal functionality of vascular endothelium caused by the decrease of the bioavailability of vasodilators (in special nitric oxide, NO), the increase of smooth muscle cell proliferation, cell adhesion and higher sensitivity to vasoconstrictors. Alteration of endothelial function is associated with the development of the coronary atherosclerosis and represents a predictive factor for future cardiovascular events [3]. The evaluation of the artery wall properties of treatment options [4]. The measuring of IMT can be used to evaluate the potential antiatherogenic properties of treatment options [4]. The measuring of IMT is used to evaluate the early arterial wall alteration of the carotid artery, the established atherosclerosis and to estimate the predilection for future coronary artery events [6, 7]. Preventing the development of atherosclerosis and also the subsequently reduction of cardiovascular events can be achieved by lowering the cholesterol levels [8]. It is demonstrated that statins (3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors), such as atorvastatin (3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid, play a major role in the treatment of hypercholesterolemia by lowering the levels of low-density lipoprotein (LDL) cholesterol [9]. This study aimed to evaluate the endothelial function and cIMT in dyslipidemic patients that were treated with statins for six months vs. non-treated dyslipidemic patients.

Experimental part
The study was performed over a 6 month period and included a number of 23 healthy patients that were included in the control group (CON), 32 subjects in the dyslipidemia group that were treated with statins (DYS+STAT) and 30 subjects in the dyslipidemia group non-treated with statins (DYS-). We administered every day for 6 months in the evening to the patients in the DYS+STAT group 20 mg/day of atorvastatin. Patients with dyslipidemia were diagnosed according to the criteria established by Japan Atherosclerosis Society that implies the presence of minimum one of the following: LDL-C >140 mg/dL, TG >150 mg/dL, and HDL-C <40 mg/dL [10]. The inclusion criteria was: subjects with minimum 55 years old and the presence of dyslipidemia accompanied with the absence of clinical atherosclerosis. We recorded patients’ demographic and clinical data and determined the blood pressure (BP) by measuring it 3 times at 30 s intervals, on the left arm, with the patient in a sitting position. For the determination of TC we used in vitro enzymatic colour test (Roche/Hitachi 717). LDL-cholesterol was determined using Freidwald formula [11].

Measurement of carotid IMT
We assessed the carotid IMT using an ultrasound system with a frequency linear probe (7 MHz), ALOKA ProSound SSD 4000 (Tokyo, Japan) equipped with software. The final IMT valued that was used in the study was a mean of 3 values obtained by investigating the wall of the common carotid artery (left and right). The measurements were conducted 10 mm proximal to the bifurcation of common carotid artery into the external and internal artery. On the
ultrasound system, the IMT is formed of 2 parallel lines that represent the edges of 2 anatomical lines: the interface lumen-intima and the interface media-adventitia. The early phase of the atherosclerotic plaque demonstrates a thickness greater than 1.3 mm.

**Measurement of FMD**

After a recovery time of 20 min, endothelium-dependent vasodilation of the left brachial artery was measured using high-resolution ultrasound, a technique introduced by Celermajer et al. [12]. Patients did not consume cigarettes, high-fat meals and caffeine beverages in the night before the study. We examined the brachial artery diameter on a relaxed patient that was in the supine position, with the aid of an experienced sonographer. We determined the brachial artery diameter 5 cm above the antecubital fossa (BAD1), using a high-resolution ultrasound equipment and a linear transducer (ALOKA ultrasound system, Tokyo, Japan). A cuff was inflated on the forearm for 5 min to obtain a 50 mmHg above systolic pressure to measure the brachial artery FMD response following the occlusion. The cuff was released and we measured the maximal arterial lumen diameter baseline flow hemodynamic parameters and the Doppler signal. There was determined BAD2 in the 45-60 s interval after cuff deflation. Arterial FMD was calculated using the formula: FMD = \( \frac{(BAD2 - BAD1)}{BAD1} \times 100\% \). Endothelium-dependent % FMD was appreciated as the maximal percent of basal vessel diameter after reactive hyperemia.

**Statistical analysis**

The variables were expressed as means ±SD. We compared the means using the analysis of variance (ANOVA) or the Student t-test. To test the bivariate correlations we used Pearson's correlation. Statistical analyses were performed using Excel Microsoft Office 2013 and Graph Pad Prism 5.

**Results and discussions**

The results show the response of dyslipidemic patients without clinical atherosclerosis to a 6 months treatment with 20 mg/day of atorvastatin. We considered a value of TC <200 mg/dL normal, a value of TC: 200-239 mg/dL as a border value and a value of TC >240 mg/dL as hypercholesterolemia. LDL-c <130 mg/dL represented a normal value, LDL-c: 130-159 mg/dL was a border value and LDL-c values >160 mg/dL was considered hypercholesterolemia.

We found a negative, moderate and significant correlation between FMD and TC in CON group (\( R^2=0.5975 \) and \( p<0.001 \)), DYS-STAT group (\( R^2=0.6851 \) and \( p<0.01 \)) and also for DYS+STAT group (\( R^2=0.683 \) and \( p<0.001 \)). The correlation between FMD and LDL-c in CON group was negative, weak but significant (\( R^2=0.1136 \) and \( p<0.05 \)), in DYS-STAT group the correlation was negative but not significant (\( R^2=0.183 \) and \( p<0.1 \)) and in DYS+STAT group there was found a negative, moderate and significant correlation (\( R^2=0.5437 \) and \( p<0.001 \)). The correlation between IMT and TC was positive, weak but significant for the CON group (\( R^2=0.0151 \) and \( p<0.01 \)), positive, moderate and significant for DYS-STAT group (\( R^2=0.5196 \) and \( p<0.001 \)) and also for DYS+STAT group (\( R^2=0.5647 \) and \( p<0.001 \)).

**Table 1**

BASELINE CHARACTERISTICS OF STUDY GROUP SUBJECTS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON group (n=23)</th>
<th>DYS-STAT group (n=32)</th>
<th>DYS+STAT group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.00±3.23</td>
<td>60.00±4.88</td>
<td>57.00±1.15</td>
</tr>
<tr>
<td>Male (%)</td>
<td>59</td>
<td>69</td>
<td>65</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>113.00±7.92</td>
<td>125.00±5.38</td>
<td>124.00±4.65</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70.00±4.14</td>
<td>75.00±2.64</td>
<td>73.00±3.93</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>93.00±23.92</td>
<td>259.00±6.37</td>
<td>201.00±25.71</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>40.00±4.75</td>
<td>31.00±8.95</td>
<td>38.00±5.82</td>
</tr>
</tbody>
</table>

Values are means ±SD or frequency (%). BP = blood pressure.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CON group (n=23)</th>
<th>DYS-STAT group (n=32)</th>
<th>DYS+STAT group (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mg/dL)</td>
<td>180.00±11.28</td>
<td>244.00±21.53</td>
<td>197.00±26.49</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>13.00±1.47</td>
<td>10.00±4.19</td>
<td>11.00±3.23</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.72±0.26</td>
<td>0.73±0.34</td>
<td>0.72±0.19</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>110.00±11.57</td>
<td>152.00±29.50</td>
<td>134.00±21.34</td>
</tr>
</tbody>
</table>

Fig. 1. Correlation between FMD and LDL-c in DYS-STAT group
The correlation between IMT and LDL cholesterol was positive, weak but significant for the CON group ($R^2=0.338$ and $p<0.05$) and positive, moderate and significant for DYS-STAT group ($R^2=0.556$ and $p<0.05$) and also for DYS+STAT group ($R^2=0.5664$ and $p<0.001$).

High TC values (>240 mg/dL) are present majorly in the DYS-STAT group vs. DYS+STAT group.

There was observed a higher prevalence of increased LDL values (>159 mg/dL) in the DYS-STAT group vs. DYS+STAT group. Also, normal and border values (<130 mg/dL and 130-159 mg/dL) are higher DYS+STAT group vs. DYS-STAT group.

FMD values are increased in the dyslipidemic patients treated with atorvastatin (DYS+STAT group) vs. patients with dyslipidemia non-treated (DYS-STAT group) ($p>0.01$).

IMT values are slightly, but not significant ($p>0.1$) increased in the dyslipidemic patients non-treated (DYS-STAT group) vs. treated dyslipidemic patients with atorvastatin (DYS+STAT group).

Statins are compounds capable to determine the complete inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme that represents a major limiting step in the cholesterol biosynthesis, catalysing the conversion of HMG-CoA to mevalonate (fig. 9). This inhibition will further determine the decrease of hepatic cholesterol (fig. 10b) synthesis and LDL plasmatic concentration and also, an increased LDL cholesterol uptake [13]. Atorvastatin (fig. 10a) is a lipophilic compound that binds to the active site on HMG-CoA [13]. It presents an extra hydrogen bond than fluvastatin and simvastatine, thus the different type of binding interaction can be a reason for its superior efficacy [14].

Carotid IMT measurements are used as an indicator of early phase atherosclerosis and to determine the risk of future coronary events [15]. The measurement of IMT indicated the degree of atherosclerosis disorder in patients. Improvement of IMT levels can be achieved by statin therapy but only in the sense that they lower the TC and LDL values and also the consecutive formation of atherosclerotic plaque. In DYS+STAT group we did not obtain a significant change of IMT compared with DYS-STAT group. IMT was slightly decreased, but not significant,
in the patients with treatment compared with non-treated patients.

The measurement of FMD in the brachial artery was used to assess the endothelial function. Various studies showed the improvement of endothelial function in patients that were receiving statin treatment [16, 17]. In the percentage distribution of FMD we observed a higher prevalence of FMD $\geq 11\%$ in the patients that were treated for 6 months with atorvastatin compared to the dyslipidemic patients that were not receiving any treatment. The negative correlation between FMD and TC and LDL indicated that the presence and degree of endothelial dysfunction is associated with high levels of TC and LDL, values that are associated with the formation of atherosclerotic plaque and also the risk of developing coronary events. After the treatment with atorvastatin we observed a lowering in TC, LDL and triglycerides levels, finding described by many other studies [18].

There were obtained decreased values of FMD in patients that were in DYS-STAT group vs. CON (we considered the normal value of FMD as 11%). In the DYS+STAT group, the FMD values were moderately increased, values that indicate that the response of the patients to the treatment is not equally and also the positive effect of 6 month treatment with atorvastatin on improving the endothelial function in patients with dyslipidemia.

Conclusions

These findings can suggest that impairment of endothelial function by the presence of dyslipidemia can be improved by the treatment of the patients with atorvastatin.

References


5. KUVIN J.T., KARAS R.H., Circulation, 107, 2003, p. 3243


