Both fenofibrate and atorvastatin improve vascular reactivity in combined hyperlipidaemia (fenofibrate versus atorvastatin trial — FAT)

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Received 26 October 2000; accepted 7 June 2001

Abstract

Objective: It has been repeatedly proven that statins improve endothelial function in isolated hypercholesterolaemia but there is far less evidence in the case of combined hyperlipidaemia. Studies assessing the effects of fibrates on endothelium have been neglected. Therefore, we conducted a trial in which the effects of fenofibrate and atorvastatin monotherapy on both endothelium-dependent vascular reactivity and biochemical parameters were compared in patients with combined hyperlipidaemia. Methods: 29 otherwise healthy males (aged 47.4±7.8 years) with combined hyperlipidaemia (total cholesterol 7.55±1.20 mmol/l, triglycerides 5.41±4.54 mmol/l) were included into the randomised, single-blind, cross-over study to receive either 200 mg of micronised fenofibrate or 10 mg of atorvastatin daily — each of the drugs for a period of 10 weeks. Analysed biochemical parameters were as follows: serum total-, LDL- and HDL-cholesterol, apolipoproteins A-I and B, triglycerides, fibrinogen, uric acid, C-reactive protein (CRP), insulin, and homocysteine. Endothelial function was investigated by duplex Doppler ultrasonography at the brachial artery. Two indices of endothelial-dependent postischaemic changes were used — the recently introduced index of peak blood flow (PBF) representing the level of reactive hyperaemia and traditional flow-mediated dilatation (FMD).

Results: We observed a small improvement in FMD after both fenofibrate and atorvastatin (from 2.26% to 2.98% and 2.87%, respectively; NS). PBF increased from 448 ml/min to 536 ml/min after fenofibrate (P=0.04) and to 570 ml/min after atorvastatin (P=0.03). The effects of both fenofibrate and atorvastatin on endothelial function did not differ significantly (P-values of 0.82 and 0.47 for FMD and PBF, respectively). Significant correlations (P<0.01) between the changes of vascular reactivity and biochemical indices were found between FMD and CRP (r=0.60) and between both FMD and PBF, and insulinaemia (r=−0.48 and −0.56, respectively) only during treatment with fenofibrate. Conclusions: Both fenofibrate and atorvastatin significantly improved endothelium-dependent vascular reactivity without mutual difference. The PBF was superior to FMD for the detection of this improvement. The beneficial effect of both drugs did not correlate with the change of lipid profile during therapy. The improvement of vascular reactivity during treatment with fenofibrate (opposed to atorvastatin) was related to the reduction of indirect marker of chronic vessel wall inflammation and of insulin resistance. The PBF was more reproducible than FMD because of considerably lower intra-subject variability. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Atherosclerosis; Cholesterol; Endothelial function; Regional blood flow; Statins

1. Introduction

There is growing evidence that endothelial dysfunction precedes the development of atherosclerosis [1] in subjects with cardiovascular risk factors. Endothelium-dependent vascular reactivity has been studied both in coronary and peripheral arteries. Flow-mediated dilatation (FMD) of brachial artery assessed by ultrasound represents a frequently used non-invasive method [2]. Recent evidence has suggested that the level of postischaemic hyperaemia assessed by Doppler ultrasound or by plethysmography, is also related to endothelial function [3,4]. The duplex Doppler ultrasonography can be used for the simultaneous assessment of both FMD and reactive hyperaemia.

Major functional consequences of endothelial dysfunc-
tion include reduced bioavailability of nitric oxide (NO) [5], increased expression of cytoadhesive molecules [6,7], and of components involved in both thrombosis and fibrinolysis [8]. Several studies have documented endothelial dysfunction in subjects with isolated hypercholesterolaemia [9,10]. The presence of highly atherogenic small, dense LDL-particles, accompanying elevated triglycerides, was related to endothelial dysfunction in diabetic patients [11]. The reports on the influence of elevated triglycerides on endothelium are controversial [12,13]. Endothelial dysfunction was also observed in insulin resistant subjects [14]. Chronic vessel wall inflammation, widely studied recently [15–17], was also closely related to endothelial dysfunction. The levels of C-reactive protein (CRP), interleukin-6, and fibrinogen — the markers of chronic vessel wall inflammation [18] — were decreased by lipid-lowering therapy [19].

The treatment of isolated hypercholesterolaemia by statins improved endothelial function [20,21]. Only one recent study has documented the improvement of endothelial function by a fibrate [22]. However, this study was performed in a population of diabetic subjects. Generally, there is little data about the influence of lipid-lowering therapy on endothelial function in combined hyperlipidaemia [23].

The most frequently used lipid-lowering drugs in combined hyperlipidaemia are fibrates, statins (mainly atorvastatin), or combination of both. The dose of atorvastatin prescribed in clinical practice is usually much lower than that in clinical trials, where up to 80 mg daily were administered [24]. It raises a question whether endothelial dysfunction could be significantly improved by a standard-dose lipid-lowering therapy.

We conducted a study in a population of patients with combined hyperlipidaemia, in which the effects of micronised fenofibrate (200 mg o.d.) and atorvastatin (10 mg o.d.) were compared. The primary objective of this study was to compare the effects of atorvastatin and fenofibrate on endothelium-dependent vascular reactivity. The secondary objective was to investigate whether expected improvement of vascular reactivity is related to the drug-induced changes of lipid profile, insulinaemia or markers of chronic inflammation.

2. Patient population and study design

Participants of the study were recruited from patients referred to the university hospital-based lipid clinic. Only non-smoking otherwise healthy males with non-treated combined hyperlipidaemia (fasting plasma cholesterol >6.2 mmol/l and triglycerides >1.5 mmol/l) were included in the study. Subjects with secondary hyperlipidaemia, body mass index >32 kg/m², impaired glucose tolerance (according to the oral glucose tolerance test prior to the inclusion), alcohol abuse or apo E2/E2 genotype were excluded. Since we found considerably high intra-subject variability of FMD contrasting to that of Doppler indices (see Results and Discussion), the population size was calculated in order to detect significant changes of reactive hyperaemia during medical intervention.

After the initial examination the participants were randomised into two groups. Group I (n=15) received 200 mg of micronised fenofibrate once daily for 10 weeks followed by 10 mg of atorvastatin once daily for the next 10 weeks. Group II (n=14) received the same drugs but in the opposite way. The drugs were taken in the mornings except on the days of examination to eliminate their acute effect. All participants were instructed not to change their dietary and drinking habits during the whole study and not to take any additional medication including vitamin supplements. A physical examination was performed, blood samples taken and ultrasound examination were completed during each visit at baseline, at cross-over, and at the end of the study.

In the absence of a parallel placebo group, a control group was constituted. Fifteen healthy male volunteers aged 34.8±7.2 years (range 29–50) without cardiovascular risk factors and any medication were recruited from the hospital staff. Two ultrasound examinations were performed within 1 week using rigorously the same examination protocol as in patients.

The local ethical committee approved the design of the study and all participants signed informed consent. The study conformed to the principles outlined in the Declaration of Helsinki.

3. Methods

3.1. Ultrasound examination protocol

Ultrasound examination of vascular reactivity was performed in fasting state (at least 12 h) in the morning (07:00–08:00 h). The subjects remained at rest in the supine position for at least 15 min before the examination started. Subject’s right arm was comfortably immobilised in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. The artery segment was imaged using a 7.5-MHz linear array ultrasound transducer (Hewlett-Packard, SONOS 2000, USA). A special transducer fixing device developed by R. Meijer from Utrecht University, The Netherlands, was used to avoid arm or transducer movement and to minimise examiner fatigue. The transducer was immersed into a water-reservoir with a membrane-bottom that was also fixed to the device in order to ensure a constant pressure to the artery. Ultrasound gain and the section plane were optimised to provide clear arterial wall interfaces. A modified protocol described by Celermajer was used [9]. After resting records of both B-mode and pulsed Doppler
spectral curve, a sphygmomanometer cuff, placed around the forearm distal to the imaged artery segment, was inflated to the pressure of 200 mmHg for 4.5 min. The spectral curve was taken immediately after the cuff release for 10 s. Then the ultrasound device was switched back to B-mode and the recording continued for the next 5 min. All ultrasound images were stored on S-VHS videotape for subsequent off-line analysis.

3.2. Biochemistry

All blood samples were collected in the morning hours (07:00–08:00 h) after overnight fasting and processed immediately. Total serum cholesterol (T-C), HDL cholesterol (HDL-C), triglycerides (TG), and uric acid were determined enzymatically using a multianalyser HITACHI 717 and commercially available kits. LDL cholesterol (LDL-C) was calculated according to the Friedewald’s formula and non-HDL-C as the difference of T-C and HDL-C. Serum levels of apolipoprotein AI (Apo AI) and apolipoprotein B (Apo B) were determined by Laurell’s rocket immunoelectrophoresis, using commercially available antisera (Immuno, Austria).

Plasma fibrinogen was determined according to Clauss (Immuno, Austria). Serum CRP was determined by immunoturbidimetric assay using a commercially available kit (Lachema, Czech Republic). Total serum homocysteine (HCY) was determined by a modification of the method developed by Araki and Sako [25]. The thiol compounds in serum were derivatised with SBD-F (a thiol-specific fluorogenic reagent). The derivatives were separated and quantified by reversed-phase high-performance liquid chromatography using a HPLC Chrompack GRAS (Chrompack, Holland) apparatus and fluorescence detector FL 2000 (SP-Thermo, Separation Products, California). Fasting serum insulin was determined by RIA method using a commercially available kit (CIS Bio International, France).

3.3. Image processing

All ultrasound images stored on videotapes were examined off-line by a single observer blinded to the randomisation code and biochemical data. Digitised B-mode scans were analysed using the Image-Pro Plus software, v. 4.0 (Media-Cybernetics, L.P.) with automatic border detection function. The perpendicular distance between the M-lines was measured at end-diastole detected by the R wave of a single precordial ECG lead. The averaged value of vessel diameter was calculated from six resting cycles taken before the cuff inflation and from six post-ischaemic cycles within the interval of 60–80 s after cuff release. Flow-mediated dilatation (FMD) was expressed as the percentage of postischaemic increase of the resting diameter.

The pulsed-Doppler spectral scans were analysed using a built-in electronic calliper of the ultrasound device. Blood flow was measured at rest and immediately after the cuff release, as a mean of three cardiac cycles (excluding the first, usually deformed cycle). Peak blood flow (PBF, ml/min) in early post-ischaemic period was calculated as the velocity time integral multiplied by the vessel cross-sectional area. Blood flow increase (BFI) was calculated as the percentage increase of resting blood flow.

3.4. Statistical methods

For the randomisation, the dynamic allocation procedure was used, in which the allocation was influenced by the current balance of pre-specified factors — age, T-C, TG, and body-mass index. All results are presented as means±standard deviations (S.D.). Comparison of the effects of both drugs, period effect and carry-over effect were tested using cross-over T statistics [26]. Unpaired- and paired t-test were used as appropriate. Pearson’s correlation was used to test the relationship between absolute changes of biochemical parameters and of ultrasound surrogates of vascular reactivity. The normal values of ultrasound indices and their intra-subject repeatability were calculated from control group. The intra-subject repeatability was determined as a mean of the coefficients of variation, which were separately calculated for each pair of values of FMD, PBF, and BFI obtained during both examinations. The inter-subject variability was calculated as the standard deviation of differences between two measurements over the pooled mean of all measurements. The intra-observer reproducibility of the measurement of vessel diameter and mean blood velocity for both resting and hyperaemia period were expressed as coefficient of variations which were calculated separately for every subgroup of readings consisting of six measurements in six consecutive heart cycles in all available records.

The study was designed to detect a 20% change of PBF at the 5% significance level (α=5%, two-sided) with 80% power. Minimum sample size was calculated by nQuery Advisor, v. 4.0 (Statistical Solutions, USA) on the basis of the estimate of inter-subject variability in the control group.

4. Results

It was calculated that 16 patients would be needed to prove the change of PBF. As 29 males were enrolled into the study, the power of the study increased to 98%. Baseline characteristics of total population were as follows: age 47.4±7.8 years, BMI 27.8±2.7 kg/m², systolic blood pressure 124.8±12.0 mmHg, and diastolic blood pressure 82.3±6.9 mmHg.

There were neither drop-outs from the study nor serious adverse events. Two participants complained of mild abdominal pain when receiving atorvastatin and discontinued the treatment for 2 and 3 days, respectively. Safety
screening biochemistry (including creatin-phosphokinase and transaminases) did not change significantly during the trial.

4.1. Ultrasound characteristics of endothelial function

In the group of healthy volunteers, the indices of vascular reactivity were as follows: PBF 896±251.8 ml/min, BFI 550.9±190.0%, and FMD 4.75±2.52%. The intra-subject repeatability was 12.8, 24.5, and 41.0%, for PBF, BFI, and FMD, respectively. The inter-subject variability of differences was 25.3, 40.5, and 59.1% for PBF, BFI, and FMD, respectively. The intra-observer reproducibility was 0.87, 3.7, and 1.8% for the measurement of vessel diameter, mean blood velocity at rest and during hyperaemia, respectively.

In patients, only three of 87 ultrasound B-mode records and nine of 87 spectral records had insufficient quality for the assessment of FMD and indices of blood flow, respectively. The results are shown in Table 1. There were no differences between drugs in all ultrasound parameters. We observed only a trend in FMD to increase after both drugs. On the contrary, significant post-treatment changes of indices of reactive hyperaemia were observed. PBF increased significantly after both drugs. BFI increased significantly after atorvastatin, while its increase after fenofibrate reached a borderline statistical significance.

4.2. Biochemical parameters

The results of biochemical analysis are shown in Table 2. Atorvastatin was superior to fenofibrate in reducing serum T-C, LDL-C and non-HDL-C. Fenofibrate was more efficient in reducing serum TG and in elevating HDL-C, although the beneficial effect of both drugs on Apo-AI was comparable. Both drugs decreased CRP and insulinaemia. However, this decrease was statistically significant only for fenofibrate. Adverse effects of both fenofibrate and atorvastatin were observed — increase in homocysteine after fenofibrate and increase in fibrinogen after atorvastatin.

4.3. Correlation of biochemical parameters and indices of vascular reactivity

At baseline, the only significant correlation between biochemical and ultrasound parameters was found for CRP and FMD (negative relationship) as we reported elsewhere [27].

Analysis of the effects of treatment revealed significant correlation between the decrease of both CRP and insulinaemia and the improvement of vascular reactivity.

Table 1
Changes of ultrasound parameters of vascular reactivity after 10 weeks of treatment with fenofibrate or atorvastatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (B)</th>
<th>After fenofibrate (F)</th>
<th>P-value (F vs. B)</th>
<th>After atorvastatin (A)</th>
<th>P-value (A vs. B)</th>
<th>P-value (F/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMD (%)</td>
<td>2.26±2.1</td>
<td>2.98±1.91</td>
<td>0.21</td>
<td>2.87±1.9</td>
<td>0.19</td>
<td>0.82</td>
</tr>
<tr>
<td>PBF (ml/min)</td>
<td>448±216</td>
<td>536±172</td>
<td>0.04</td>
<td>570±180</td>
<td>0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>BFI (%)</td>
<td>497±247</td>
<td>664±270</td>
<td>0.06</td>
<td>671±293</td>
<td>0.01</td>
<td>0.93</td>
</tr>
</tbody>
</table>

* Paired t-test was used for evaluation of effects of each of the two drugs.
† Cross-over T-statistics were used for between-drugs comparison.
‡ FMD, flow-mediated dilatation; PBF, peak blood flow; BFI, blood flow increase.

Table 2
Changes of biochemical parameters after 10 weeks of treatment with fenofibrate or atorvastatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline (B)</th>
<th>After fenofibrate (F)</th>
<th>After atorvastatin (A)</th>
<th>P-value F vs. A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>7.55±1.20</td>
<td>6.64±0.89***</td>
<td>5.44±0.95***</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>5.41±4.54</td>
<td>2.72±2.10**</td>
<td>3.67±3.24**</td>
<td>0.0047</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)†</td>
<td>4.39±0.89</td>
<td>4.05±0.76</td>
<td>2.90±0.65***</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.26±0.32</td>
<td>1.42±0.36***</td>
<td>1.25±0.32</td>
<td>0.003</td>
</tr>
<tr>
<td>Non-HDL-cholesterol (mmol/l)</td>
<td>6.3±1.3</td>
<td>5.2±1.00***</td>
<td>4.2±0.90***</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.30±0.25</td>
<td>1.52±0.26***</td>
<td>1.45±0.25**</td>
<td>n.s.</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>1.35±0.25</td>
<td>1.28±0.17</td>
<td>1.04±0.20***</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>2.70±0.49</td>
<td>2.67±0.46</td>
<td>3.15±0.75***</td>
<td>0.0017</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>11.9±7.11</td>
<td>5.8±2.99**</td>
<td>8.8±8.0</td>
<td>0.028</td>
</tr>
<tr>
<td>Homocysteine (µmol/l)</td>
<td>12.4±2.7</td>
<td>16.9±3.7***</td>
<td>12.3±2.4</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Insulinaemia (mUI/l)</td>
<td>26.2±12.0</td>
<td>22.9±7.2*</td>
<td>23.5±8.2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Uric acid (µmol/l)</td>
<td>418±205</td>
<td>308±60*</td>
<td>387±82</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

* Paired t-test was used for evaluation of effects of both drugs: P-values *<0.05; **<0.005; ***<0.001. Cross-over T-statistics were used for between-drugs comparison: P-values (F vs. A) are given in exact numbers; n.s., non-significant (P>0.05).
† LDL calculation according to Friedewald’s formula was possible in 18 blood samples at baseline, in 26 blood samples after fenofibrate and in 24 blood samples after atorvastatin.
Table 3
Correlation between biochemical and vascular effects of fenofibrate and atorvastatin (univariate regression analysis)

<table>
<thead>
<tr>
<th></th>
<th>FMD</th>
<th>PBF</th>
<th>BFI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fenofibrate</td>
<td>Atorvastatin</td>
<td>Fenofibrate</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.24</td>
<td>0.34</td>
<td>0.27</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.04</td>
<td>0.07</td>
<td>−0.24</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.43</td>
<td>0.35</td>
<td>0.32</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>−0.05</td>
<td>0.38</td>
<td>0.06</td>
</tr>
<tr>
<td>Non-HDL-cholesterol</td>
<td>0.07</td>
<td>0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>Apolipoprotein A-I</td>
<td>−0.37</td>
<td>−0.09</td>
<td>−0.12</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>−0.02</td>
<td>0.15</td>
<td>0.42</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>0.22</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>−0.60</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>0.34</td>
<td>−0.03</td>
<td>0.14</td>
</tr>
<tr>
<td>Insulinaemia</td>
<td>−0.48</td>
<td>−0.00</td>
<td>−0.56</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.06</td>
<td>−0.06</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Values represent Pearson’s correlation coefficients. *Significant correlation (P<0.01); P>0.01 in all others.

during treatment with fenofibrate (Table 3, Figs. 1–3). The improvement of vascular reactivity was not significantly correlated with the change of lipid parameters for both drugs.

4.4. Carry-over and period effect

The carry-over, i.e. the drug-specific effect, the presence of which means that treatment with one drug in the first period of cross-over trial influences the effect of subsequently used alternate drug, was not observed for any of the biochemical and ultrasound parameters. The period effect, i.e. the effect of intervention on a particular parameter (assessed in the whole population and irrespective of drug used) at cross-over is different from that at the end of the study, was found for insulinaemia. The level of serum insulin was 24.2±8.2 mU/l at cross-over and further decreased to 21.9±7.0 at the final visit (P=0.032).

5. Discussion

The study was designed to investigate the changes of vascular reactivity during lipid-lowering therapy with drugs from different classes in patients with combined hyperlipidaemia and to find out the relations between vascular reactivity and biochemical parameters. The detrimental vascular effect of hypertriglyceridaemia is mainly explained by the preponderance of atherogenic small, dense LDL particles, that was proved for triglyceride levels above 1.5 mmol/l [28]. Consequently, we chose this relatively low cut-off point of TG for inclusion into the study. Relatively high baseline levels of CRP (upper limit of normal in our laboratory is 10 mg/l) explain the fact that consistent changes of CRP were obtained despite the use of the less sensitive immunoturbidimetric method.
Based on our assessment of repeatability, some previous studies using FMD were underpowered due to small sample size. Therefore we decided to study the vascular reactivity primarily by PBF with the appropriate predetermined sample size. As FMD is widely used and gives easily assessed Doppler indices, we left it as a part of the study protocol to reveal whether at least the trend in its improvement will appear.

Although the drugs studied differed in metabolic action, their effects on vascular reactivity were similar. 

**Atorvastatin** decreased T-C and non-HDL-C significantly more than fenofibrate. In previous studies, the statins directly decreased the expression of endothelin-1 and increased the activity of endothelial NO-synthase [29], improved endothelial function [20] in isolated hypercholesterolaemia and in hypertriglyceridaemia, and generally reduced the mortality [30]. Neither the cholesterol reduction, nor other metabolic effects of atorvastatin, was significantly related to the changes of vascular reactivity in our study. Lack of correlation between the increase of FMD and the reduction of T-C and LDL-C was already described by others [31]. These findings may support the theory of non-lipid effects of statins including their influence on the monocyte–macrophage system and inhibition of the expression of cytoadhesive molecules [32].

**Fenofibrate** decreased triglycerides, CRP and increased HDL-C significantly more than atorvastatin. Fenofibrate did not increase plasma fibrinogen in comparison to atorvastatin. Fenofibrate also significantly reduced insulinemia, although this effect was not significantly different from that of atorvastatin. As we have demonstrated earlier, there is no difference between the effect of
fenofibrate and atorvastatin on mean LDL-particle size [33].

It has been documented by other authors that high triglycerides [12], increased CRP [17], elevated insulinaemia [14], low HDL-C and low mean LDL particle [11] size are all separately associated with endothelial dysfunction. The pleiotropic metabolic action of fenofibrate that is mediated through peroxisome-proliferator-activated receptor (PPAR) alpha could therefore partly explain the effect of fenofibrate on vascular reactivity [34]. However, in our study, the changes of vascular reactivity were significantly related only to the changes of CRP and insulinaemia.

The analysis of the period effect suggests that the insulin resistance in combined hyperlipidaemia continues to improve during lipid-lowering therapy beyond the treatment period of 10 weeks. However, our data do not authorise us to conclude whether this observation is inherent to fenofibrate, atorvastatin, or both drugs.

Vascular reactivity was studied by three variables — FMD, PBF and BFI. Others have already used PBF and BFI for the assessment of vascular reactivity [35], but until lately these indices have not been fully accepted as markers of endothelial function [36]. Recently, it was proven that PBF correlates tightly with the flow changes induced by intraarterial infusion of acetylcholine, a method widely accepted for the assessment of endothelial function [3,4]. Opposed to FMD, the parameters of blood flow reflect ‘mean’ vascular reactivity of a relatively large portion of the arterial tree. This is probably the basis of their robustness and better intra-subject repeatability in comparison to FMD. BFI is apparently less reproducible than PBF because it represents a relative number, in which two measurement errors (of resting and hyperaemic phase) are incorporated.

The observation of low repeatability of FMD is in contradiction with some previous reports. However, others have found even worse reproducibility of this parameter (CV above 50%) [37]. As the optimum experimental settings (experienced examiner, fixing device, and computer assisted tracking of vessel wall) was used and intraobserver reproducibility of measurement of vessel diameter measurement was low, the major source of high intra-subject variability of FMD is obviously due to its high spontaneous variability. It can be explained by the fact that FMD reflects both the local increase of wall shear stress due to the increase of blood flow velocity in hyperaemic phase and the ability of the arterial segment to respond to this change by its dilation. Therefore its variability depends on both variability of postischaemic hyperaemia and variability of local response. The considerable number of published ‘positive’ findings in small groups of patients could be partly explained by the ‘publication bias’. A larger study of FMD repeatability is needed to explain whether or not FMD represents a valuable and reproducible method.

6. Limitations

This trial compared the effects of two lipid-lowering drugs in a group of 29 males with moderate combined hyperlipidaemia. The endothelium-dependent vascular reactivity was investigated by a non-invasive method. Strong evidence is missing that similar changes may occur in other parts of the arterial tree (e.g. coronary arteries). However, a non-invasive method is relatively cheap and easily repeatable for studying the treatment effects in ‘primary prevention’ patients. The power of the study was calculated to detect change in PBF, but not for FMD.

We tested treatment effects on 15 variables and were aware of the problem of multiple testing. However, even after Bonferroni [38] adjustment was used, changes in biochemical variables remained significant. Similar problems were related to the correlations. Therefore, we accepted only those having a stricter $P$-value (below 0.01). The number of patients was not sufficient for reliable calculation of all links between changes of vascular reactivity and metabolic changes. Probably for this reason the significant correlation between cholesterol decrease and vascular reactivity improvement was not proven.

The study was primarily designed to compare effects of two lipid-lowering drugs. Therefore, no parallel placebo group was included. On the other hand, placebo effect is unlikely to occur for biochemical parameters and the spontaneous improvement of ultrasound indices was not observed in the control group. However, the subjects of control group were recruited from the population of healthy and younger people and the findings in this group may not be valid for the group of patients with combined hyperlipidaemia.

Target goal levels of cholesterol and triglycerides recommended by guidelines were not achieved due to relatively low doses of trial medications. Therefore, more intensive treatment could lead to more pronounced improvement of endothelial function. The period of 10 weeks appeared to be sufficient to reach the maximum effect for all parameters, except for insulinaemia, in which a longer period of treatment might probably cause a more prominent effect. Although the carry-over effect can occur in the cross-over trial design, it was not confirmed statistically. Thus, missing the washout period at cross-over should not have influenced the final results and 10 weeks of the second period of treatment was sufficiently long not to ‘carry’ the effect of the drug used in the first period.

7. Conclusions

This trial was designed to compare the effects of fenofibrate and atorvastatin, given at standard doses, on endothelium function. Both drugs increased the level of postischaemic hyperaemia significantly. Although, they expectedly differed in their biochemical effects, there was
no difference in the effects on vascular reactivity between them. Since there are still little data available regarding the effect of fibrates on endothelium, the finding of comparability of fenofibrate and atorvastatin effects on vascular wall reactivity is of considerable value. The secondary objective was to investigate the links between biochemical and vascular changes. We observed that the decrease in indirect markers of chronic inflammation and insulin resistance by fenofibrate is significantly related to the improvement of endothelial function. Surprisingly, there was no significant correlation between the beneficial effect of both drugs on lipid profile and the improvement of vascular reactivity. Finally, parameters of reactive hyperaemia were more reproducible than FMD because of lower spontaneous variability. This fact should be taken into account if similar studies are designed in future.

Acknowledgements

This study was supported by the research project J13/98 1111 0000 2-1 of the Ministry of Education, Czech Republic.

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