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Effect of simvastatin and fenofibrate on endothelium in Type 2 diabetes

Jan Škrha[a,b,*], Tomáš Štulc[b], Jiřina Hilgertová[a], Hana Weiserová[a], Jan Kvasnička[c], Richard Češka[b]

[a] *Laboratory for Endocrinology and Metabolism, Faculty of Medicine 1, Czech Republic*
[b] *Department of Internal Medicine 3, Faculty of Medicine 1, Czech Republic*
[c] *Department of Clinical Hematology, Faculty of Medicine 1, Charles University, Prague, Czech Republic*

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Abstract

Statins and fibrates influence endothelial activity and consequently atherogenesis but the mechanisms are not well understood. Twenty Type 2 diabetic patients with dyslipidemia were treated 3 months with simvastatin (20 mg daily) and then 3 months with fenofibrate (200 mg daily) with 2 months of wash-out between the two treatments. Laboratory parameters of oxidative stress, fibrinolysis and endothelial function were evaluated before and at the end of each treatment period. The significant decrease in serum total and LDL-cholesterol concentrations ($P<0.0001$) caused by simvastatin was associated with an increase in serum *N*-acetyl-β-glucosaminidase activity ($P<0.001$), ascorbic acid ($P<0.001$), plasminogen activator inhibitor (PAI-1) ($P<0.01$), vonWillebrand factor ($P<0.05$), E-selectin ($P<0.01$) and vascular endothelial growth factor ($P<0.05$) concentrations and with a decrease in plasma glutathione ($P<0.01$) levels. Fenofibrate caused a significant decrease in serum triglyceride concentration ($P<0.0001$) associated with a decrease in plasma malondialdehyde ($P<0.001$) and an increase in plasma PAI-1 ($P<0.05$) and P-selectin ($P<0.05$) concentrations. We conclude that simvastatin and fenofibrate interact, by different mechanisms, with oxidative stress, a key factor in the modification of fibrinolysis and endothelial function in Type 2 diabetes.

Keywords: Simvastatin; Fenofibrate; Type 2 diabetes; Oxidative stress; Endothelial function

1. Introduction

Dyslipidemia plays an important role in the development of atherosclerosis in diabetes mellitus. High blood concentrations of small dense low-density lipoprotein (LDL)-cholesterol, a low level of high-density lipoprotein (HDL)-cholesterol and hypertriglyceridemia represent typical features of Type 2 diabetes, especially as a part of the metabolic syndrome. The impaired lipid profile, together with other factors such as high blood pressure, obesity, hyperglycemia or impaired fibrinolysis, contributes to the development of the vascular pathology in affected subjects. Only intensive treatment of all pathogenic factors may delay the progression of atherosclerosis in Type 2 diabetic patients.

Early vascular changes are manifested by endothelial dysfunction prior to morphological changes of the vessel wall. Oxidative stress is suggested as the crucial pathogenic mechanism inducing impairment of vascular function (Nedeljkovic et al., 2003; Tomiyama et al., 2003). However, dyslipidemia together with hyperglycemia accelerates oxidative stress and both contribute to vascular dysfunction. An improvement in the lipid profile may therefore have a beneficial effect on oxidative stress as well as on endothelial or vascular function (Evans et al., 2000; Wassmann and Nickenig, 2003).

Statins and fibrates have different mechanisms of action and have different effects on the lipid profile (Schweitzer et al., 2002). The beneficial effects of statins are caused not only by the improved plasma lipid profile but also by a direct effect on vascular activity (Laufs, 2003; Mueck and Seeger, 2003). Pleiotropic effects of statins have been recognized in the last few years (Ambrosi et al., 2003). They may improve oxidative stress and endothelial function in subjects with hyperlipoproteinemia (Melenovský et al.,

* Corresponding author. Department of Internal Medicine 3, Faculty of Medicine 1, Charles University, Czech Republic, U Nemocnice 1, 128 08 Prague 2, Czech Republic. Tel./fax: +42-2-2491-6745.
E-mail address: jan.skrha@lf1.cuni.cz (J. Škrha).

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2002; Aviram et al., 2000). In contrasts, data on fibrates in this respect are scarce but they also support an antioxidant effect and improved vascular reactivity (Bae et al., 2001; Malík et al., 2001). It may be therefore worthwhile to compare both groups of drugs in a clinical trial evaluating different laboratory parameters of oxidative stress and endothelial function.

In present study we compared the effect of separately administered simvastatin and fenofibrate on biochemical parameters of oxidative stress, fibrinolysis and endothelial function in the same patients with Type 2 diabetes and dyslipidemia.

2. Methods

2.1. Patients

Twenty patients with Type 2 diabetes (12 men and 8 women) with proven dyslipidemia were selected for this pilot study. Their characteristics are shown in Table 1. Diabetes was treated in fourteen patients with oral agents (metformin was used in each of them and in 10 together with a sulfonylurea derivative) and in the remaining six subjects metformin was combined with insulin (mean dose 38 ± 8 units daily). This chronic antidiabetic therapy was not changed during the study. Twelve patients had arterial hypertension and their chronic antihypertensive medication was unchanged during the trial. They used angiotensin-converting enzyme inhibitors in combination with calcium channel blockers or beta-blockers. Only patients with normal renal function and liver tests were selected for this study.

None of the patients were treated with hypolipidemic drugs for at least 2 months prior to enrolment in the study. Serum total cholesterol and triglyceride concentrations were increased in all of them at baseline as the main inclusion criterion. After collection of blood for laboratory examination at baseline, the patients were randomized to simvastatin or fenofibrate treatment. Ten patients were treated with simvastatin (20 mg daily, Zocor, MSD, randomization Group A) for 3 months and the other 10 with fenofibrate (200 mg daily, Lipanthyl 200 M, Fournier, randomization Group B) during the same period. After 2 months of washout, when the hypolipidemic therapy was stopped, the drug administration was reversed. The patients in Group A were treated with fenofibrate for the following 3 months and those in Group B with simvastatin. Their life style did not change during the whole study to eliminate other factors (i.e. vitamin intake) that could influence the parameters measured. The control group, for comparison of the baseline data, consisted of 24 healthy people of comparable age and body mass index. Informed consent was given by all subjects examined in this study, which was approved by the Ethics Committee of our Medical Faculty, and the investigation conformed with the principles outlined in the Declaration of Helsinki.

Table 1
Characteristics of Type 2 diabetic patients and of the control subjects

	Type 2 diabetic patients ($n=20$)	Control subjects ($n=24$)
Age (years)	57 (44–70)	52 (39–69)
BMI (kg m^{-2})	30.4 ± 3.0	28.3 ± 2.7
Duration of diabetes (years)	9 ± 5	–
Blood pressure		
Systolic (mm Hg)	141 ± 15	136 ± 11
Diastolic (mm Hg)	84 ± 7	82 ± 6

2.2. Laboratory methods

Blood samples were drawn between 7:00 and 8:00 h after an overnight fast. Laboratory variables were examined at baseline before hypolipidemic drug administration and after 3 months of the respective treatment. After a wash-out period, all examinations were repeated in the same way. Fasting plasma glucose was determined by the glucose oxidase method with a glucose analyzer Super GLAmbulance (Dr. Müller Gerätebau, Freital, Germany) and glycated hemoglobin HbA_1c with the Imx GHb Assay System on an Abbott Analyzer (Abbott, Chicago, USA). Serum total cholesterol, HDL- and LDL-cholesterol, triglyceride, urea, creatinine, uric acid and total protein concentrations were estimated in the central laboratory by routine methods, using a Hitachi analyzer. The level of oxidative stress was determined by measuring the plasma malondialdehyde concentration, using fluorimetric method (Yagi, 1976). Superoxide dismutase activity in erythrocytes (EC 1.15.1.1) was evaluated by xanthine–xanthine oxidase system in a spectrophotometer Genesys 5 (USA) (McCord and Fridovich, 1969). Superoxide dismutase activity is expressed in units (1 unit is equivalent to 250 units superoxide dismutase/mg standardized hemoglobin (McCord and Fridovich, 1969). Plasma glutathione and homocysteine concentrations were determined by high-pressure liquid chromatography (HPLC) (Shimadzu LD-10A system) with fluorescent detection (Krijt et al., 2001). Serum for analysis of ascorbic acid levels was treated with trichloracetic acid immediately after centrifugation, then frozen until analyzed by spectrophotometric method (Nakagawa et al., 1997). Alpha-tocopherol was estimated by HPLC method (Catignani, 1986). Superoxide dismutase activity and glutathione, ascorbic acid and alpha-tocopherol concentrations were used as markers of the antioxidant system. Serum *N*-acetyl-beta-glucosaminidase (NAG, EC 3.2.1.30) activity was determined by spectrophotometric methods with *p*-nitrophenyl-*N*-acetyl-beta-D-glucosaminide (Sigma, St.Louis, MO, USA) as substrate (Škrha et al., 1987). Fibrinolysis was characterized by plasma concentrations of tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1). Both antigens were determined by enzyme-linked immunosorbent assay (ELISA) method using Coalisa tPA and PAI-1 kits (KABI

Diagnostics, Sweden). Serum concentrations of vascular endothelial growth factor (VEGF) and of cell adhesion molecules E-selectin, P-selectin, intercellular cell adhesion molecule (ICAM-1) and vascular cell adhesion molecule (VCAM) were measured with ELISA kits manufactured by RD System Europe (Abingdon, UK). Von Willebrand factor (vWF) was determined with a kit from Diagnostica Stago (Asnieres-sur-Seine, France). Serum *N*-acetyl-β-glucosaminidase activity and concentrations of VEGF, vWF and cell adhesion molecules were used as indicators of endothelial activity. The immunoassays had an intrassay variability below 5% and an interassay variability below 8%. All samples from the patients were measured in one assay to minimize the effect of variation.

2.3. Statistical analysis

The results are expressed as the means ± S.D. Student's *t*-test was used for comparison of biochemical variables in diabetic patients and in the control group. An analysis of covariance (ANCOVA) was applied to compare the baseline values and treatment groups as covariates in the model. Pearson correlation was applied to linear regression analysis between variables at baseline and to the differences (after treatment vs. baseline values) between variables.

3. Results

Biochemical variables at baseline and after hypolipidemic drug administration are given in Table 2. Diabetes control remained unchanged during treatment with the hypolipidemic drugs. Serum cholesterol and triglyceride concentrations were significantly higher at baseline in diabetic patients than in healthy subjects. Laboratory variables for oxidative stress, fibrinolysis and endothelial function are shown in Table 3. The plasma malondialdehyde concentration, as a measure of oxidative stress, was significantly higher ($P<0.001$) whereas superoxide dismutase activity in erythrocytes and serum ascorbic acid concentrations were lower in diabetic patients at baseline than in control subjects ($P<0.01$). These results confirmed that oxidative stress was greater in Type 2 diabetic patients. Serum alpha-tocopherol levels were significantly higher in diabetic patients than in control subjects ($P<0.01$). However, its concentration depends on plasma lipid levels, and therefore we calculated the ratio of alpha-tocopherol (αT) to the sum of serum cholesterol (CH) and triglyceride (T) levels (αT/(CH + T)). This ratio was lower in diabetic patients than in healthy control subjects ($P<0.01$). Higher serum *N*-acetyl-β-glucosaminidase activity, tPA and cell adhesion molecule concentrations at baseline indicated the presence of impaired endothelial function in diabetic patients.

Simvastatin administration caused a significant decrease in total and LDL-cholesterol ($P<0.0001$) without any changes in HDL-cholesterol and triglyceride concentrations (Table 2). This was accompanied by a non significant reduction in plasma malondialdehyde levels and by a highly significant increase in serum *N*-acetyl-β-glucosaminidase activity and ascorbic acid concentrations ($P<0.001$) (Table 3). Simvastatin treatment also reduced the alpha-tocopherol concentration. However, no changes in the alpha-tocopherol/(cholesterol + triglyceride) ratio were observed during treatment. Simvastatin caused an increase in PAI-1 antigen concentration ($P<0.01$) without any influence on tPA levels. A mild increase in vonWillebrand factor ($P<0.05$) and E-selectin ($P<0.01$) concentrations was also observed during treatment.

Fenofibrate administration was accompanied by a highly significant decrease in serum triglycerides ($P<0.0001$) whereas its effect on cholesterol and its fractions was much less pronounced than in the simvastatin-treated group (Table 2). The plasma malondialdehyde concentration was significantly decreased ($P<0.001$) but the slight increase in serum *N*-acetyl-β-glucosaminidase activity did not reach statistical significance and no changes in ascorbic acid concentration were observed after fenofibrate treatment (Table 3). The serum alpha-tocopherol concentration and

Table 2
Biochemical variables at baseline and after treatment with simvastatin or fenofibrate in Type 2 diabetic patients

	Simvastatin ($n=20$)		Fenofibrate ($n=20$)		Controls ($n=24$)
	Baseline	After treatment	Baseline	After treatment	
FPG (mmol/l)	11.9 ± 4.0^{z}	10.9 ± 3.4	10.9 ± 3.4^{z}	10.7 ± 3.0	4.6 ± 0.4
HbA1c (%)	9.0 ± 1.7^{z}	9.0 ± 1.9	9.0 ± 1.8^{z}	8.9 ± 1.7	5.0 ± 0.4
fS-Urea (mmol/l)	6.0 ± 1.5	5.7 ± 1.7	5.9 ± 1.7	6.4 ± 1.6	4.9 ± 0.9
fS-Creatinine (μmol/l)	96 ± 17	93 ± 17	95 ± 13	105 ± 17^{c}	86 ± 8
fS-Uric acid (μmol/l)	364 ± 97^{x}	375 ± 106	370 ± 90^{x}	313 ± 107^{b}	309 ± 68
T-Cholesterol (mmol/l)	6.62 ± 0.83^{x}	5.19 ± 0.75^{d}	6.52 ± 0.65^{x}	5.97 ± 0.92^{b}	4.99 ± 0.60
HDL-chol (mmol/l)	1.34 ± 0.21^{y}	1.31 ± 0.29	1.31 ± 0.26^{y}	1.34 ± 0.31	1.62 ± 0.34
LDL-chol (mmol/l)	4.08 ± 0.77^{z}	2.88 ± 0.74^{d}	4.18 ± 0.88^{z}	3.72 ± 0.71^{a}	2.87 ± 0.56
Triglycerides (mmol/l)	3.61 ± 2.0^{z}	3.25 ± 1.96	4.14 ± 2.56^{z}	2.46 ± 1.42^{d}	1.11 ± 0.62
Homocystein (μmol/l)	11.4 ± 2.7^{y}	11.0 ± 2.6	11.9 ± 2.8^{y}	16.0 ± 4.5^{c}	9.0 ± 2.8

Values of the control group are shown for comparison.
The results are expressed as means ± S.D. Statistical significance between baseline values of diabetic patients compared to control subjects: $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$, and between post treatment vs. baseline values: $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$, $^{d}P<0.0001$.

Table 3
Laboratory variables of oxidative stress, fibrinolysis and endothelial function in Type 2 diabetic patients at baseline and after simvastatin or fenofibrate treatment

	Simvastatin ($n=20$)		Fenofibrate ($n=20$)		Controls ($n=24$)
	Baseline	After treatment	Baseline	After treatment	
Malondialdehyde (μmol/l)	2.56 ± 0.45^{z}	2.39 ± 0.50	2.78 ± 0.40^{z}	2.36 ± 0.36^{c}	2.0 ± 0.3
Superoxide dismutase (U)	0.85 ± 0.29^{y}	1.00 ± 0.36	0.92 ± 0.35^{x}	0.93 ± 0.37	1.2 ± 0.3
Glutathion (μmol/l)	14.4 ± 5.0^{y}	11.4 ± 5.4^{b}	14.3 ± 5.5^{y}	13.6 ± 6.2	10.4 ± 2.7
Ascorbic acid (μmol/l)	56.4 ± 23.2^{y}	75.0 ± 20.6^{c}	53.8 ± 21.0^{y}	51.4 ± 18.1	72 ± 17
α-Tocopherol (mg/l)	18.3 ± 5.5^{y}	14.9 ± 3.9^{c}	18.8 ± 5.2^{y}	14.7 ± 3.6^{c}	14.3 ± 2.3
αT/(CH + T) (mg/mmol)	1.77 ± 0.20^{y}	1.79 ± 0.24	1.81 ± 0.26^{x}	1.76 ± 0.25	2.0 ± 0.3
NAG (U/l)	23.4 ± 5.7^{x}	31.4 ± 7.3^{c}	26.2 ± 7.4^{y}	29.9 ± 8.3	18.5 ± 2.8
tPA (ng/ml)	6.46 ± 1.86^{z}	7.19 ± 2.38	7.06 ± 3.09^{z}	6.89 ± 2.68	4.9 ± 2.0
PAI-1 (ng/ml)	94.4 ± 22.3^{z}	116.8 ± 37.5^{b}	97.9 ± 26.6^{z}	113.9 ± 27.6^{a}	45 ± 18
vWF (%)	105 ± 13	112 ± 15^{a}	107 ± 14	109 ± 16	108 ± 20
E-selectin (ng/ml)	65 ± 19^{y}	70 ± 19^{b}	65 ± 20^{y}	60 ± 14	40 ± 12
P-selectin (ng/ml)	190 ± 54^{y}	199 ± 45	188 ± 31^{y}	214 ± 49^{a}	140 ± 48
ICAM-1 (ng/ml)	277 ± 57^{y}	299 ± 67	286 ± 84^{y}	310 ± 105	214 ± 43
VCAM-1 (ng/ml)	541 ± 131^{z}	582 ± 149	566 ± 112^{z}	568 ± 89	365 ± 56
VEGF (ng/ml)	260 ± 137^{x}	341 ± 202^{a}	276 ± 104^{y}	254 ± 110	195 ± 108

The results are expressed as means ± S.D. Statistical significance between baseline values in diabetic patients and in control subjects: $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$, and in post treatment vs. baseline values: $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$.

its ratio (alpha-tocopherol/cholesterol + triglyceride) were similar to those obtained after simvastatin administration. A slight increase in PAI-1 was also observed ($P<0.05$). In contrast to simvastatin, an increase in P-selectin concentration ($P<0.01$) was found in fenofibrate-treated patients.

Multiple regression analysis showed several relationships between biochemical variables at both baseline and after treatment as well as between their differences. A significant positive correlation was observed between malondialdehyde and triglyceride concentrations ($r=0.71$, $P<0.001$) whereas the correlation between malondialdehyde and cholesterol levels was weaker ($r=0.35$, $P<0.05$) (Fig. 1). A positive correlation was found between serum triglyceride concentration and *N*-acetyl-β-glucosaminidase activity ($r=0.54$, $P<0,01$), and between cholesterol and tPA concentrations

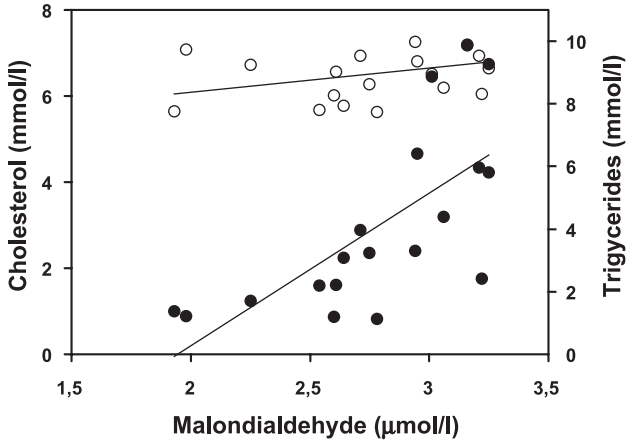

Fig. 1. Correlation between plasma malondialdehyde and serum triglyceride ($r=0.71$, $P<0.01$, black circles) or cholesterol ($r=0.35$, $P<0.05$, white circles) concentrations (logarithmically transformed) at baseline in Type 2 diabetic patients.

($r=0.45$, $P<0.01$). A strong relationship was observed between both cholesterol or triglyceride and alpha-tocopherol concentrations ($r=0.64$ and 0,61, $P<0.001$, respectively).

Hypolipidemic treatment was accompanied by several significant associations (Table 4). A decrease in triglyceride levels (difference between post treatment vs. baseline values, ΔTG) was related to a decrease in malondialdehyde (ΔMDA) concentrations only in fenofibrate-treated patients (r=0.58, P<0.001). No such relationship was found between a decrease in cholesterol (ΔCH) and ΔMDA values for either treatment group. A strong correlation was observed between a decrease in triglyceride (ΔTG) concentrations and an increase in *N*-acetyl-β-glucosaminidase (ΔNAG) activity in the fenofibrate group (r=−0.61, P<0.001) but the correlation was smaller in the simvastatin group (r=−0.38, P<0.05). The decrease in serum cholesterol concentration was related to changes in tPA in the simvastatin-treated group (r=0.36, P<0.05) whereas the decrease in triglycerides correlated with tPA levels in the fenofibrate group (r=0.45, P<0.01). Significant relationships were found between post- vs. pretreatment differences in cholesterol or triglycerides and alpha-tocopherol concentrations (Table 4). Ascorbic acid and cell adhesion molecules were not related to changes in serum cholesterol and triglyceride concentrations. E-selectin correlated with P-selectin (r=0.53, P<0.01) and ICAM-1 (r=0.55, P<0.01) before and after treatment with simvastatin or fenofibrate.

4. Discussion

Although this pilot study was done in a small number of Type 2 diabetic patients with dyslipidemia, a large number of different laboratory parameters revealed differences be-

Table 4
Relationships between the differences in post treatment vs. baseline values (Δ) of biochemical variables in the simvastatin (A) and fenofibrate (B) treatment groups

	ΔMDA	ΔNAG	ΔtPA	ΔPAI-1	ΔvWF	ΔAA	ΔAT
(A)							
ΔCH	0.15 (NS)	− 0.32 (NS)	**0.36** (0.05)	0.30 (NS)	0.25 (NS)	0.23 (NS)	**0.78** (0.001)
ΔTG	− 0.03 (NS)	− **0.38** (0.05)	0.21 (NS)	0.06 (NS)	0.16 (NS)	0.15 (NS)	**0.81** (0.001)
ΔMDA	X	− 0.19 (NS)	− 0.29 (NS)	− 0.32 (NS)	− 0.11 (NS)	− 0.14 (NS)	0.01 (NS)
ΔNAG	− 0.19 (NS)	X	0.22 (NS)	0.31 (NS)	0.25 (NS)	0.31 (NS)	0.24 (NS)
(B)							
ΔCH	0.22 (NS)	−0.09 (NS)	0.13 (NS)	0.23 (NS)	0.21 (NS)	− 0.08 (NS)	**0.44** (0.01)
ΔTG	**0.58** (0.001)	−**0.61** (0.001)	**0.45** (0.01)	0.32 (NS)	0.27 (NS)	− 0.29 (NS)	**0.74** (0.001)
ΔMDA	X	− 0.30 (NS)	0.10 (NS)	− 0.12 (NS)	0.04 (NS)	− 0.16 (NS)	**0.43** (0.01)
ΔNAG	− 0.30 (NS)	X	**0.56** (0.001)	0.02 (NS)	0.25 (NS)	− 0.01 (NS)	**0.46** (0.01)

Statistical significance of the relationship is shown in parentheses. NS—not significant. Abbreviations: CH—cholesterol, TG—triglycerides, MDA—malondialdehyde, NAG—*N*-acetyl-β-glucosaminidase, tPA—tissue plasminogen activator, PAI-1—plasminogen activator inhibitor, vWF—von Willebrand factor, AA—ascorbic acid, AT—alpha-tocopherol.

tween the effects of simvastatin and fenofibrate on oxidative stress, fibrinolysis and endothelial activity in these subjects. We confirmed that simvastatin treatment predominantly reduced serum total and LDL-cholesterol levels with a limited effect on triglycerides. This was associated with a trend to a decreased plasma malondialdehyde level with a significant decrease in the reduced form of glutathione and with a significant increase in serum ascorbic acid levels. In parallel, a highly significant increase in serum *N*-acetyl-β-glucosaminidase activity and plasma PAI-1 concentrations was observed in these subjects after treatment. In addition, a mild but significant increase in levels of von Willebrandt factor, E-selectin and VEGF was found following simvastatin administration.

This complex of laboratory changes has not been described previously in Type 2 diabetic patients although separate effects of statins on oxidative stress, fibrinolysis or endothelial dysfunction have been described recently (Malík et al., 2001; Streja et al., 2003). Decreased oxidative stress due to improved antioxidative properties and restoration of nitric oxide bioavailability after statin administration has been reviewed (Schmieder, 2003). The beneficial effect of atorvastatin on superoxide anion formed by endothelium may be independent of its lipid-lowering action (Christ et al., 2002). The role of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors on superoxide anion generation and endothelial dysfunction has been demonstrated in mice (Vecchione and Brandes, 2002). Statins may attenuate oxidative stress as a causative factor in endothelial dysfunction.

Improved fibrinolysis in patients on dialysis and favorable effects on hemostasis have been reported after statin treatment (Malyszko et al., 2001; Koh, 2000). However, controversial results of statins affecting endothelial dysfunction have been recently published (de Jongh et al., 2002; van Venrooij et al., 2002). While in hypercholesterolemic subjects without diabetes atorvastatin administration may improve endothelial function (de Jongh et al., 2002), in diabetic patients the endothelial dysfunction persists despite a significant improvement in serum lipid levels (van Venrooij et al., 2002). A similar insufficient effect on endothelial function was described after simvastatin treatment (Sheu et al., 1999; van de Ree et al., 2001).

Our results demonstrate slightly different effects of simvastatin. Malondialdehyde, as a measure of oxidative stress, was highly dependent on serum triglyceride and less on cholesterol levels. The decrease in malondialdehyde concentrations mirrored changes in triglyceride concentrations. However, a decrease in the reduced form of glutathione as a possible consequence of its oxidation as well as an increase in ascorbic acid levels may be suspected from the activation of antioxidant mechanisms. We have no other explanation for the increased ascorbic acid concentration after simvastatin except of its reduced elimination. These changes may secondarily improve oxidative stress after simvastatin. The increase in PAI-1 concentration without a significant increase in tPA values may be a consequence of activated anioxidative mechanisms although not dependent solely on endothelium because of the heterogeneity of PAI-1 sources.

The significant increase in serum *N*-acetyl-β-glucosaminidase activity after simvastatin was not expected and remains unclear. We described earlier that serum *N*-acetyl-β-glucosaminidase activity was associated with oxidative stress in diabetic patients (Škrha and Hilgertová, 1999). In the present study, a strong relationship was demonstrated between posttreatment vs pretreatment changes in *N*-acetyl-β-glucosaminidase activity and triglyceride concentrations. However, its increased activity after simvastatin could be explained better by a pleiotropic effect of the drug or as a consequence of activated antioxidative mechanisms rather than by the limited changes in serum triglyceride levels. The increase in von Willebrand factor, VEGF or E-selectin concentrations means that endothelial cells did not improve their function after three months of simvastatin treatment. We suppose that an activated endothelium plays a substantial role after statin administration.

In the fenofibrate-treated group a decrease in triglycerides predominated whereas the decrease in total and LDL-cholesterol concentrations was less pronounced than in the simvastatin group. These changes were accompanied by a significant decrease in malondialdehyde levels but by no differences in ascorbic acid or glutathione concentrations. In addition, we confirmed previous observations of increased serum creatinine and homocysteine as well as of lowered uric acid concentrations following fenofibrate administration (Melenovský et al., 2002; Elisaf, 2002).

In our study, fenofibrate caused a decrease in malondialdehyde, as an indicator of oxidative stress, without any activation of the measured parameters of antioxidant defence mechanisms. This was markedly different from the effect of simvastatin treatment. The mild but significant increase in PAI-1 concentration after fenofibrate treatment caused worsening of the fibrinolytic index (PAI-1/tPA) compared with the effect of simvastatin (15.2 ± 3.9 vs. 18.2 ± 4.9 after fenofibrate, 15.1 ± 3.4 vs. 16.6 ± 3.9 after simvastatin, respectively). We did not find any other changes in laboratory parameters indicative of an impairment of endothelial activity after fenofibrate.

Hypolipidemic therapy to lower serum cholesterol and triglyceride concentrations brings benefit to patients because of protective effect against atherosclerosis. However, the pathogenic mechanisms participating in this process have not been resolved yet. While the role of statins is more complex than only a decrease in lipid concentrations and statin pleiotropic effects have been described (Mueck and Seeger, 2003), the functions of fibrates in this process remain to be discovered. A decrease in oxidative stress and an improvement of endothelial function have been suggested (Tomiyama et al., 2003; Bae et al., 2001).

Our results could be influenced by bias. Firstly, our patients had poor diabetes control but it did not change during hypolipidemic drug administration and was comparable in both treatment groups. Secondly, we used medium doses of statin and the maximum effect may not have been obtained. Nevertheless, our results showed activation of antioxidative factors by statin administration. We demonstrated that the highly significant lowering of serum triglycerides by fenofibrate was associated with a decrease in malondialdehyde, as an indicator of oxidative stress. We speculate that simvastatin may activate directly (by its pleiotropic effect) or indirectly powerful antioxidant factors that inhibit oxidative stress, whereas fenofibrate may reduce pro-oxidative mechanisms. The difference between simvastatin and fenofibrate may be associated with a different modulation of other processes such as fibrinolysis or endothelial activity. We did not observe an improvement in biochemical variables indicative of persistently increased endothelial activity.

Our pilot study demonstrates that simvastatin and fenofibrate influence several laboratory parameters of oxidative stress, fibrinolysis and endothelial activity in different ways. Endothelial activation persisted despite hypolipidemic therapy. A change in the serum lipid profile could not explain these changes. Further investigation of laboratory parameters after prolonged administration of hypolipidemic drugs will be necessary.

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