Physiol. Res. 55: 475-481, 2006

Folate Co-Administration Improves the Effectiveness of Fenofibrate to Decrease the Lipoprotein Oxidation and Endothelial Dysfunction Surrogates

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Received date September 6, 2005
Accepted date November 22, 2005
On-line available December 12, 2005

Summary

Fibrate therapy results in elevation of plasma total homocysteine (tHcy), which is known to induce oxidative stress and endothelial dysfunction. We aimed to establish whether fibrate-induced elevation of tHcy has also similar consequences and whether they may be prevented by folate co-administration. Eighteen subjects with hypercholesterolemia were included in an open, prospective, cross-over study. We compared intra-individually the effect of fenofibrate on tHcy, oxidative stress and endothelial dysfunction surrogates, in monotherapy and when combined with 10 mg of folate. These effects were also compared with fluvastatin monotherapy. Fenofibrate in monotherapy significantly decreased LDL cholesterol, increased the tHcy by 39.5 %, while oxidized LDL (oxLDL), malondialdehyde (MDA), von Willebrand factors (vWf) and thrombomodulin (TMD) remained unchanged. When fibrate was co-administered with folate, the tHcy remained on the initial post-diet level, while both the total and oxLDL as well as MDA, vWf and TMD decreased. In contrast to fenofibrate monotherapy, fluvastatin (80 mg) had a similar effect as combined therapy with fenofibrate and folate, while tHcy remained uninfluenced. In conclusion, fenofibrate decreases the LDL cholesterol, but in contrast to fluvastatin, has no significant antioxidative and endothelium-protective potential, probably due to a concomitant increase of tHcy. These effects may be improved by co-administration of folate.

Key words

Fenofibrate • Homocysteine • Folate • Lipoprotein oxidation • Endothelial dysfunction

Introduction

Several studies demonstrated that moderately elevated total plasma homocysteine (i.e. mild hyperhomocysteinemia) is an independent risk factor for coronary, cerebrovascular and peripheral vascular diseases (Graham *et al.* 1997, Nygard *et al.* 1997, Mayer *et al.* 1999, Stubbs *et al.* 2002). The vascular pathophysiological mechanisms of hyperhomocysteinemia are manifold. The vascular damage may be caused by hypercoagulation status, mitotic effect on smooth muscle cells, but especially important is the

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oxidative stress induced by homocysteine (Prasad 1999, Loscalzo 1999, Kanani *et al.* 1999). During the auto-oxidation of homocysteine in plasma, reactive oxygen species (superoxide anion radicals and hydrogen peroxide) are generated. These oxygen-derived molecules probably enhance the oxidative modification of low-density lipoproteins and initiate the lipid peroxidation in cell membranes that are responsible for endothelial injury, reduction of vascular nitric oxide production and its bioavailability (i.e. endothelial dysfunction) (Kanani *et al.* 1999).

Mild hyperhomocysteinemia can most frequently result from nutritional deficiency of B group vitamins (folate, vitamin B_6 and B_{12}) in combination with some genetic factors (mainly enzymatic deficiency of methylene-tetrahydrofolate reductase or cystathione β-synthase (Shimakawa *et al.* 1997, Homocysteine Lowering Trialists Collaboration 1998, Klerk *et al.* 2002), but it can also be drug-induced. It has been recently reported, that fibrates (with the exception of gemfibrozil) increase total homocysteine concentrations by about 17-57 % (de Lorgeril *et al.* 1999, Dierkes *et al.* 1999 Jonkers *et al.* 1999, Goffin *et al.* 1999, Westphal *et al.* 2001, Giral *et al.* 2001). In our previous study we observed that this adverse effect can be prevented by folate co-administration (Mayer *et al.* 2003). The aim of our study was to establish, whether co-administration of folate, which prevents the fibrate-induced total homocysteine increase, would also improve the effect of fenofibrate on lipoprotein oxidation and endothelium.

Methods

Selected population and design

The design and selection criteria were identical as in our previous study (Mayer *et al.* 2003), but different population sample was used. Eighteen volunteers (7 males, 11 females; mean age 54.7±3.03years; mean body mass index 27.9±1.93 kg/m^2) participated in an open, prospective, randomized, two-period, cross-over study. The selection was carried-out of 294 males and 287 females with age range 25-65 years, participants of a local sample of MONICA study (Cífková *et al.* 2000), examined in 2000´s, by the following criteria: age 40-65 years, total cholesterol levels more than 5.9 mmol/l, triglycerides less than 5.0 mmol/l, blood pressure less than 140/90 mmHg, fasting plasma glucose less than 5.6 mmol/l, no systematic pharmacotherapy and no clinical form of any vascular or metabolic disease. Of those, who met these inclusion criteria, 18 were willing to participate and signed an informed consent. All women included were post-menopausal. For calculation of necessary sample size we used data from our previous study (Mayer *et al.* 2002) and 18 subjects were sufficient to assess the variability with 90 % power and 5 % significance.

In this study, we used the cross-over design with three months of initial diet period, two treatment branches and two months of wash-out between two six-month treatment periods. All study participants were instructed verbally and in written form on lipid-lowering diet and life-style, and a baseline examination was done. After 3 months, the post-diet follow-up examination was carried out and subjects were consecutively randomized into two treatment groups: ***A*** - subjects received 200 mg of micronized fenofibrate for 3 months and then for 3 months combination of 200 mg micronized fenofibrate and 10 mg of folate daily; ***B*** - subjects received for 3 months 40 mg of fluvastatin and then for 3 months 80 mg of fluvastatin daily. Follow-up examinations were done after 3 and 6 months. The second period started after 2 months of wash-out, after which the identical therapeutic scheme was used in reversed manner (***A*** group was rearranged for fluvastatin, while ***B*** group for fenofibrate in monotherapy and for fenofibrate with co-administered folate afterwards). The study protocol was approved by the local Ethical Committee and carried out respecting the Good Clinical Practice Regulation.

Laboratory examinations

At baseline, before and after each treatment period blood samples were obtained after a minimally 12 hours of overnight fast. The blood samples were immediately cooled, plasma or serum samples were separated within one hour and then, either analyzed or stored at –80 °C. Total (TCHOL), HDL cholesterol (HDL) and triglycerides (TG) were estimated by enzymatic methods (commercial kits of Roche Diagnostics, Mannheim, Germany) from fresh serum samples by Hitachi 800 analyzer, LDL cholesterol (LDL) was calculated by Friedewald equation. Total homocysteine (tHcy) and folate were estimated from frozen specimens in series by FPIA methods (commercial kits of Abbott Diagnostics, Wiesbaden, Germany) using AxSYM analyzer, the interassay coefficients of variations were 1.16 % and 2.2 %, respectively. Serum oxidized LDL, plasma von Willebrand factor (vWf) and plasma thrombomodulin (TMD) were estimated from frozen

Table 1. Comparison of parameters: the post-diet period vs. lipid lowering therapies.

	post-diet	FENOFIBRATE					FLUVASTATIN 80 mg	
		monotherapy	P1	with folate	P1	P2		P1
Homocysteine (μmol/l)	11.1±0.81	15.3±1.57	<0.003	10.2±0.68	0.54	<0.001	9.36±0.65	<0.004
Folate (ng/ml)	7.41±0.99	8.92±1.20	0.34	17.8±1.07	<0.001	<0.001	9.24±0.97	0.16
LDL-cholesterol (mmol/l)	4.08±0.14	3.58±0.15	<0.04	3.43±0.16	<0.001	0.62	2.94±0.17	<0.001
HDL-cholesterol (mmol/l)	1.81±0.10	2.00±0.12	<0.003	2.02±0.11	<0.001	0.50	1.83±0.12	0.67
Triglycerides (mmol/l)	2.06±0.35	1.46±0.22	<0.02	1.26±0.13	<0.004	0.64	1.62±0.26	<0.03
Oxidized LDL (mU/l)	96.6±6.72	90.8±9.25	0.61	68.5±5.52	<0.008	<0.007	75.4±7.28	<0.03
Malondialdehyde (μmol/l]	2.79±0.13	2.57±0.17	0.26	2.24±0.10	<0.002	<0.02	2.26±0.15	<0.002
von Willebrand factor (%)	220.4±35.8	186.8±31.6	0.10	160.9±22.9	<0.02	<0.05	121.3±22.5	<0.03
Thrombomodulin (ng/ml)	79.2±5.44	76.1±5.92	0.76	66.7±4.12	<0.05	<0.05	65.3±3.73	<0.05

Data are means ± S.E.M.; p_1 - post-diet vs. treatment, p_2 - fenofibrate in monotherapy vs. combination with folate (Wilcoxons paired test).

specimens using commercial ELISA kits (Mercodia AB, Uppsala, Sweden; IMTEC GmbH, Berlin, Germany; Diagnostica Stago, Asnieres-sur-Seine, France; respectively), the interassay coefficients of variations were 3.32 %, 3.03 % and 2.97 %, respectively. Plasma malondialdehyde (MDA) was estimated using incubation with 0.6 % thiobarbituric and 1 % phosphoric acid, precipitation with n-butanol (Jentzsch *et al* 1996) and spectrophotometrical detection by Specol 11 device, interassay coefficients of variations was 3.76 %. All laboratory variables were estimated by local laboratories of University Hospital in Plzeň. Quality of estimates is being regularly validated by external reference laboratories (namely total homocysteine estimation is validated by ERNDIM Laboratory, Nijmegen, The Netherlands).

Statistical analysis

The Wilcoxon´s paired test was used to compare pair-wise mean values and relative changes after each treatment. Mann-Whitney U test was used for *post-hoc* comparisons to exclude potential differences between baseline characteristics of both treatment groups (***A*** vs. ***B***) and to exclude potential bias, resulting from treatment sequence.

Results

Both the treatment groups (A or B) did not statistically differ at baseline in age, blood pressure, body mass index, smoking habits, and in lipid or total homocysteine concentrations (data not given). Comparing with the post-diet values, fenofibrate monotherapy significantly decreased LDL cholesterol and triglycerides, but increased the tHcy concentrations and HDL. Comparing the post-diet values with those after combined therapy of fenofibrate with 10 mg of folate, the tHcy remained at the initial post-diet level, while plasma folate and HDL increased and both total and oxidized LDL (oxLDL) decreased, as well as triglycerides, malondialdehyde, von Willebrand factor and thrombo-modulin. In contrast to fenofibrate monotherapy, fluvastatine had virtually similar effects, as combined fenofibrate+folate therapy, while tHcy, folate and HDL

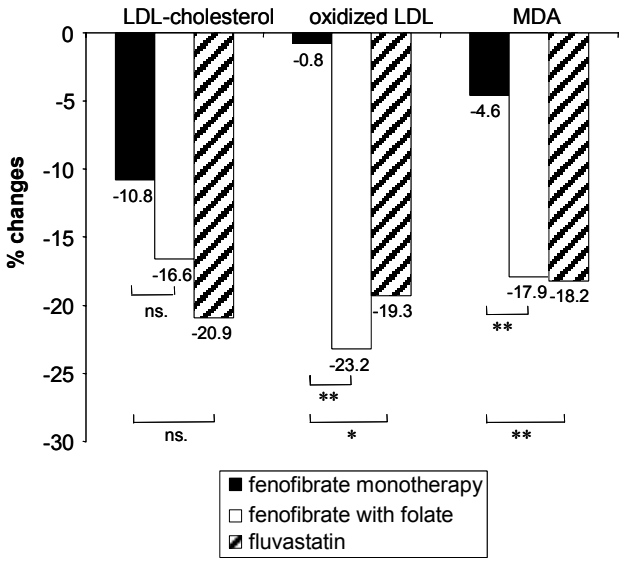

Fig. 1. Mean percentage changes (treatment versus post–diet values) in LDL cholesterol and its oxidized products. (** p<0.01, * p<0.05, Wilcoxon´s paired test)

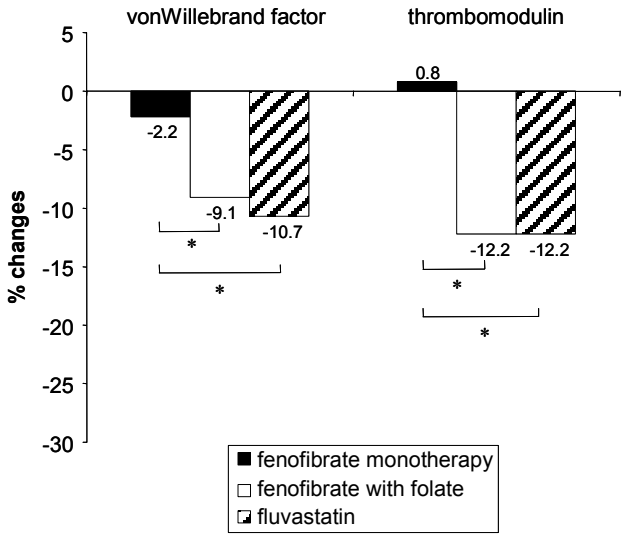

Fig. 2. Mean percentage changes in endothelial dysfunction surrogates. (* p<0.05, Wilcoxon´s paired test)

levels remained uninfluenced (Table 1). Treatment sequences (i.e. fenofibrate first or fluvastatin first) had no significant influence on the results in the three treatment groups (data not given). Relative changes in LDL, its oxidized products and endothelial dysfunction surrogates after three treatment regimes (i.e. fenofibrate monotherapy, fenofibrate with folate and fluvastatin) are compared in the Figures 1 and 2. Fenofibrate decreased oxidized LDL, MDA, vWf, and TMD significantly more if combined with folate than in monotherapy. Effect of fluvastatin on above mentioned parameters was almost the same as combined administration of fenofibrate with folate. However, the greater extent of LDL cholesterol lowering by fluvastatin versus fenofibrate have not reached statistical significance.

Discussion

The main finding of this study showed that fenofibrate, which is a potent lipid-lowering drug, increased total homocysteine concentrations and in contrast to fluvastatin lacked a significant antioxidative effect. On the other hand, when co-administered with folate, the potential to increase total homocysteine concentrations disappeared, and moreover, antioxidative effect together with improvement in endothelial dysfunction surrogates (von Willebrand factor and thrombomodulin) were comparable to fluvastatin therapy.

Lipoprotein oxidation and endothelial dysfunction have been considered as important vascular-damaging mechanisms. The uptake of oxidized LDL particles by scavenger receptors present in macrophages triggers a series of events, leading to production of foam cells and therefore the formation of atherosclerotic plaque (Kanani *et al.* 1999). The endothelium balances the counterregulatory pathways that control vasomotion, cell proliferation, thrombosis, inflammation, and oxidation. The arterial endothelium plays a crucial role in the regulation of vascular tone in part by the release of vasoactive substances, notably NO, endothelin, prostacyclin, and angiotensin II. Moreover, it is involved in the modulation of platelet activation, leukocyte adhesion and thrombosis. It is evident that elevated total homocysteine concentrations are positively associated with increased lipid oxidation and endothelial dysfunction (Chambers *et al.* 1998, Kanani *et al.* 1999). A question remains, whether also fibrate-induced elevation of total homocysteine has similar unfavorable effects, which could potentially diminish the cardiovascular benefit, resulting from lowering of blood lipids. Fibrate-induced elevation of total homocysteine could hypothetically explain the ambiguous results of randomized prevention trials using fibrates. Three trials with clofibrate or bezafibrate (which had an evidently adverse effect on tHcy concentrations), i.e. WHO Cooperative Trial of Clofibrate (Report from the Committee of Principal Investigators 1978), Bezafibrate Infarction Prevention Study (BIP Study Group 2000) and LEADER trial (Meade *et al.* 2002) had not shown any significant reduction of fatal coronary events. On the other hand, two trials, i.e. Helsinki Heart Study (Frick *et*

al. 1987) and Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (Rubins *et al.* 1999) that used gemfibrozil (which has neutral effect on total homocysteine) showed a significant reduction of all clinical end-points, including mortality.

It is evident that administration of folate decreased not only the total homocysteine concentration, but also concomitant vascular pathophysiological processes. In our previous study we found that folate administration was followed not only by decrease of tHcy, but also of biochemical markers of oxidative stress, endothelial dysfunction and hypercoagulation (Mayer *et al.* 2002). It was also reported, that supplementation with folate was followed by a significant decrease of *in vitro* LDL-oxidation (Bunout *et al.* 2000) and an improvement of endothelial dysfunction measured by flow-mediated vasodilatation (Chao *et al.* 1999). Wilmink *et al.* (2000) reported that folate prevented the MDA increase (reflecting oxidative stress) in urine after an acute fat load, while another study (Constans *et al.* 1999) observed that treatment with folate and pyridoxine resulted in a decrease of von Willebrand factor and prevented the rise of this factor after a methionine load.

In conclusion, folate co-administration represents a simple, safe and cheap measure which may improve the outcomes of fenofibrate treatment. Our study is limited by a rather small sample size, which was only sufficient to prove an immediate pharmacological effect and does not allow inferences for a large population. Thus, further evidence is needed to assess the potential benefit of combined therapy of fibrate and folate on long-term cardiovascular outcomes, such as mortality and morbidity.

Acknowledgement

The study was supported by Internal Grant Agency of the Czech Ministry of Health (grant No. 7534-3). Estimation of homocysteine was supported by Diagnostic Division of Abbott Laboratoires Ltd., Czech Republic, providing the kits free-of-charge. The excellent technical assistance of Mrs. Jitka Nováková is gratefully acknowledged.

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