EFFECT OF FENOFIBRATE ON THE LEVEL OF ASYMMETRIC DIMETHYLARGININE IN TYPE 2 DIABETES MELLITUS

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ABSTRACT

However, whether the beneficial effect of fenofibrate on the endothelium is related to reduction of ADMA level in individuals with diabetes mellitus (DM) is not known, and the current study aimed to address this issue. **Aim:** A total of 60 patients with type 2 DM and dyslipidemia (aged 45 ± 8 years; mean ± SD) were compared to 59 healthy controls without type 2 DM and normolipidemia (aged 44 ± 9 years). **Methods:** Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol, Apolipoprotein-A1 (Apo-A_1) and Apolipoprotein-B (Apo-B) were investigated using biochemical analyzer Konelab 60i, Thermo Electron Co, USA. The levels of asymmetric dimethylarginine (ADMA), sICAM-1, sVCAM were determined by ELISA. Determination of flow mediated vasodilatation of the brachial artery was performed based on Celermajer's guidelines and on %FMD manual (Celermajer et al., 1992; Corretti et al., 2002). The diameter of the brachial artery was measured using a 7.5 MHz transducer of Hewlett Packard 2 500 ultrasound equipment. Automatic computer software MedicaSoft. IMT.lab was applied. The DM group had significantly lower level of HDL cholesterol and significantly higher levels of total cholesterol, triglycerides, LDL-cholesterol, Apo-B, Apo-A_1, ADMA, sVCAM-1, sICAM-1. The DM patients showed significantly redused % FMD of the brachial artery in comparison to the controls. ($p<0.01$) Therapy with 200 mg Fenofibrate for 1 month statistic significant reduced total cholesterol, triglycerides, LDL-cholesterol, Apo-B, Apo-B/Apo-A_1, and ADMA. Same therapy statistical significant increased HDL-cholesterol, Apo-A_1 and %FMD. These therapy don't change sVCAM-1 and sICAM-1. **Conclusion:** Treatment with fenofibrate 200 mg/d for one month statistically significant reduction in plasma levels of ADMA. ($p<0.001$) The present results suggest that fenofibrate improves endothelium-dependent dilation of hypertriglyceridemic individuals, and the beneficial effect of fenofibrate may be related to a reduction of the level of ADMA.

Keys words: diabetes mellitus, dyslipidemia, flow-mediated vasodilation, fenofibrate,

BACKGROUND

There are abundant experimental data that endothelial dysfunction caused by reduced availability of NO is an early step in the course of atherosclerotic vascular disease. Evidence has accumulated that inhibition of NO synthesis by NOS inhibitors may be causally involved in this process.4 Recently, more and more attention has been paid to ADMA, an endogenous NOS inhibitor, contributing to endothelial dysfunction. It has been documented that plasma level of ADMA is significantly elevated in individuals with atherosclerosis, hypercholesterolemia, hyperhomocysteinemia and diabetes mellitus.6 Elevated ADMA level was correlated with major cardiovascular events after percutaneous coronary intervention and cardiovascular morbidity and mortality in middle aged men.14 With increasing knowledge of the role of ADMA in the pathogenesis of atherosclerosis, ADMA has become potential target for drug therapy.2 Fenofibrate, used widely as a lipid-lowering agent, has potent antioxidant and anti-inflammatory actions via activation of peroxisome proliferator-activated receptor-α (PPARα).12 Clinical studies have demonstrated that longterm treatment with fenofibrate can hinder the progress of atherosclerosis.13 It has been reported that fenofibrate attenuates oxidative-LDL-induced impairment of endothelium- dependent vasodilation in hyperlipidemic patients.9 One study has shown that fenofibrate attenuated endothelial dysfunction induced by injection of LDL via reduction of ADMA production in rats .16 However, whether the beneficial effect of fenofibrate on the endothelium is related to reduction of ADMA level in individuals with diabetes mellitus (DM) is not known, and the current study aimed to address this issue.

AIM

We investigated effect of 200 mg fenofibrate on asymmetric dimmethylarginine and %FMD in patients with diabetes mellitus and dyslipidemia..

PATIENTS

A total of 60 patients with type 2 DM and dyslipidemia (aged 45 ± 8 years) were compared to 59 healthy controls without type 2 DM and normolipidemia (aged 44 ± 9 years).

METHODS

Laboratory testing was performed at the Central Clinical Laboratory of University Hospital St George in

Plovdiv. Biochemical parameters: total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) cholesterol, Apolipoprotein-A1 (Apo-A_1) and Apolipoprotein-B (Apo-B) were investigated using biochemical analyzer Konelab 60i, Thermo Electron Co, USA. Determination of LDL cholesterol in serum was performed using direct automated analysis and reagents from Thermo Electron Co KonelabTM, Finland. The levels of ADMA, sICAM-1, sVCAM were determined by ELISA) using kits from DLD Diagnostika GMBH, Germany and BenderMed Systems, Germany and total homocysteine by the fluid chromatographic method.

Determination of flow mediated vasodilatation of the brachial artery was performed based on Celermajer's guidelines and on %FMD manual (Celermajer et al., 1992; Corretti et al., 2002). The diameter of the brachial artery was measured using a 7.5 MHz transducer of Hewlett Packard 2 500 ultrasound equipment. Automatic computer software MedicaSoft. IMT.lab was applied. (Fig. 1).

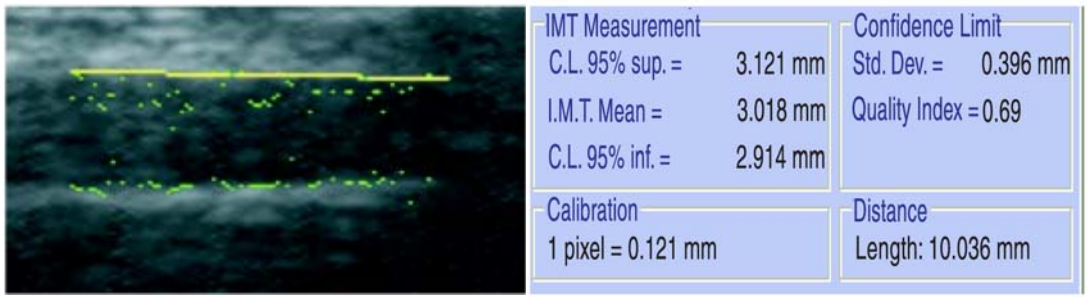

Fig. 1. Automatic measurement of the brachial artery diameter with MedicaSoft. IMT.lab. Statistic analysis

Data are expressed as means±SD. The data were analyzed by unpaired Student's t-test and paired Student's t-test. Mann-Whitney U-test was used for comparison of FMD between baseline and post-treatment values in individuals receiving fenofibrate. The statistical significance was considered if $P<0.05$.

RESULTS

Both groups did not differ from each other significantly with respect to age, sex and BMI.($p>0.05$) The DM group had significantly lower level of HDL cholesterol and significantly higher levels of total cholesterol, triglycerides, LDL-cholesterol, Apo-B, Apo-A1, ADMA, total homocysteine, sVCAM-1, sICAM-1 (Table 1). The DM patients showed significantly redused % FMD of the brachial artery in comparison to the controls. (Tabl. 1).

Therapy with 200 mg Fenofibrate for 1 month statistic significant reduced total cholesterol, triglycerides, LDL-cholesterol, Apo-B, Apo-B/Apo-A1, and ADMA. (Table 2) Same therapy statistical significant increased HDL-cholesterol, Apo-A 1 and %FMD. These therapy don't change sVCAM-1 and sICAM-1. (Table 2)

DISCUSSION

Asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS), is attributed to endothelial dysfunction and has been considered as a risk marker of atherosclerosis.8 Previous investigations have shown that the ADMA level was elevated in some cardiovascular disorders, such as atherosclerosis, hyperlipidemia, congestive heart failure and hypertension. It has been reported that the ADMA level was positively correlated with carotid artery intima-media thickness(IMT) and major adverse cardiovascular events in healthy subjects, or patients with stable angina treated with percutaneous coronary intervention, respectively.10 All these findings suggest that ADMA may be a risk factor of cardiovascular diseases and has been expected to become an important target for pharmacotherapeutic intervention[7]. In the present study, fenofibrate markedly reduced the elevated levels of triglyceride and increased the reduced HDL-C level. Recently, it was shown that treatment with fenofibrate in diabetes mellitus improved endothelial function in hyperlipidaemic or stroke patients or in rats treated with LDL.9,13 The beneficial effects of fenofibrate may be secondary to its lipid-lowering and non-lipid lowering effects such as potent antioxidant and anti-inflammatory actions via activation of PPARα receptor.12

However, the precise mechanisms responsible for endothelial protection of fenofibrate are still unclear. There is evidence that an increase in ADMA level may be related to elevation of oxidant stress. ADMA concentrations were reported to be elevated not only in individuals with diabetes mellitus, but also in other conditions with increased oxidant stress, such as hypertension,hyperglycemia and hyperhomocysteinemia. Recently, it was reported that oxidant stress decreased the activity of dimethylarginine dimethylaminohydrolase (DDAH), an oxidant-sensitive enzyme responsible for degradation of ADMA, resulting in an accumulation of ADMA 11. In cultured endothelial cells, oxidant stress induced by ox-LDL or TNF-α caused an increase of ADMA production via decreasing the activity of DDAH, and antioxidants such as vitamin E, probucol, daviditin A and pyrollidine dithiocarbamate (PDTC) decreased levels of both

lipid peroxides and ADMA..2,7 In the present study, treatment with fenofibrate decreased the level of ADMA in diabetes mellitus individuals. Therefore, it is probable that the decreased level of ADMA by fenofibrate is related to reduction of oxidant stress via improving DDAH activity. ADMA, besides regulating NO synthesis, might be a novel inflammatory factor. Interactions of ADMA with cytokines were shown in the inflammatory processes of atherosclerosis, and ADMA up-regulates the expression of redox-sensitive genes encoding endothelial adhesion molecules.3

It was recently reported that fenofibrate increases Larginine/ ADMA ratio but has no effect on ADMA concentration, which is contradictory with the present results.5 Previous observations have shown that, in the rats treated with LDL, treatment with fenofibrate improved endothelial function, concomitantly with reduction of ADMA level. Recent work has also found that, in the cultured endothelial cells, incubation with fenofibrate induced a significant endothelial protection via reduction of ADMA and TNF-α level by inhibiting the activity of NF-kB, in support of the hypothesis that the beneficial effect of fenofibrate on the endothelium may be related to reduction of endogenous ADMA level.15

CONCLUSION:

Treatment with fenofibrate 200 mg/d for one month statistically significant reduction in plasma levels of ADMA. ($p<0.001$) The present results suggest that fenofibrate improves endothelium-dependent dilation of hypertriglyceridemic individuals, and the beneficial effect of fenofibrate may be related to a reduction of the level of ADMA.

Table 1. Baseline characteristics of the two investigated groups

Parameter	DM subjects	controls	p
Glucose (mmol/l)	5.55 ± 0.52	5.30 ± 1.04	<0.01
Glycated hemoglobin (%)	5.40 ± 0.03	5.06 ± 0.52	<0.01
Total cholesterol (mmol/l)	6.75 ± 0.13	4.52 ± 0.61	<0.001
LDL cholesterol (mmol/l)	5.38 ± 0.64	2.47 ± 0.05	<0.001
HDL cholesterol (mmol/l)	0.94 ± 0.02	1.58 ± 0.44	<0.001
Triglycerides (mmol/l)	3.19 ± 0.44	0.78 ± 0.24	<0.001
ADMA (µmol/l)	1.38 ± 0.34	0.47 ± 0.05	<0.01
Total homocysteine (µmol/l)	14.02 ± 0.22	10.55 ± 0.12	<0.01
Apo-A_1 (g/l)	1.20±0.16	1.51 ± 0.09	<0.001
Apo-B (g/l)	2.07±0.35	1.4 ± 0.22	<0.001
sVCAM-1 (ng/ml)	778.21±24.73	568.09 ± 20.66	<0.001
sICAM-1 (ng/ml)	329.52±16.40	239.33 ± 12.40	<0.001
Flow-mediated dilation (%)	4.88 ± 0.34	8. 47 ± 0.05	<0.01

Table 2. Effect of one month fenofibrate 200 mg on lipid and nonlipids biomarkers and %FMD in DM patients

Parameters		mean ± SEM	P
Total cholesterol (mmol/l)	at baseline	6 .75±0.13	< 0.01
	After 1 month	5.41±0.07	
Triglycerides (mmol/l)	at baseline	3.19 ± 0.44	< 0.001
	After 1 month	1.76±0.02	
HDL-cholesterol (mmol/l)	at baseline	0.94±0.02	< 0.001
	After 1 month	1.10±0.24	
LDL- -cholesterol (mmol/l)	at baseline	5.38±0.64	< 0.001
	After 1 month	3.61±0.09	

Apo-B (g/l)	at baseline	2.07±0.35	< 0.001
	After 1 month	1.06±0.30	
Apo-A_1 (g/l)	at baseline	1.20±0.16	< 0.001
	After 1 month	1.52±0.21	
Apo-B / Apo-A_1	at baseline	1.89±0.39	< 0.001
	After 1 month	0.66±0.19	
ADMA	at baseline	1.38 ± 34	< 0.001
	After 1 month	0.78 ± 0.21	
sICAM-1 (ng/ml)	at baseline	329.52±16.40	> 0.05
	After 1 month	309.50±16.30	
sVCAM-1 (ng/ml)	at baseline	778.21±24.73	> 0.05
	After 1 month	778.21±24.73	
% FMD	at baseline	4.88±0.34	< 0.001
	After 1 month	7.98±0.42	

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