ELSEVIER

Clinica Chimica Acta 332 (2003) 61–67

www.elsevier.com/locate/clinchim

Gemfibrozil reduces release of tumor necrosis factor-α in peripheral blood mononuclear cells from healthy subjects and patients with coronary heart disease

Shui-Ping Zhao*, Hui-Jun Ye, Hong-Nian Zhou, Sai Nie, Quan-Zhong Li

Department of Cardiology, The Second XiangYa Hospital, Central South University, Middle Renmin Road No. 86, ChangSha, Hunan 410011, People's Republic of China

Received 17 December 2002; received in revised form 27 February 2003; accepted 4 March 2003

Abstract

Background: Inflammatory process plays an important role in the pathogenesis of coronary heart disease (CHD). With the growing use of gemfibrozil and other fibrates, their anti-inflammatory effects have been noted. But little is known about the effect of gemfibrozil on tumor necrosis factor (TNF)-α secretion in peripheral blood mononuclear cells (PBMC) from patients with coronary heart disease. *Methods*: PBMC were obtained from CHD patients ($n=16$) and healthy controls ($n=13$). PBMC (2×10^6 cells/ml) were cultured in 24-well plates with or without Ang II (10^{-8}, 10^{-7}, 10^{-6} mol/l), or Ang II (10^{-6} mol/l) plus gemfibrozil (10^{-6}, 10^{-5}, 10^{-4} mol/l). After 24-h incubation, the supernatants were separated, and TNF-α was measured by an enzyme-linked immunosorbent assay (ELISA). *Results*: Spontaneous release of TNF-α was 299.2 ± 110.7 pg/ml in PBMC from CHD patients and 179.3 ± 78.2 pg/ml in PBMC from control subjects ($P<0.05$). Incubated with Ang II (10^{-8}, 10^{-7}, 10^{-6} mol/l), TNF-α secretion was 307.7 ± 141.8, 318.9 ± 135.6, 328.6 ± 123.9 pg/ml in PBMC from CHD patients, and 225.3 ± 135.4, 224.1 ± 141.0,218.7 ± 134.8 pg/ml in PBMC from control subjects, respectively. Ang II did not significantly trigger TNF-α secretion in both groups. Compared with that incubated with Ang II (10^{-6} mol/l) alone, release of TNF-α intervened by gemfibrozil (10^{-6},10^{-5},10^{-4} mol/l) decreased to 279.4 ± 132.2, 268.0 ± 132.7, 226.6 ± 102.7 pg/ml in PBMC from CHD patients, and 177.6 ± 94.4, 156.1 ± 69.4, 105.3 ± 52.7 pg/ml in the control group, respectively. Gemfibrozil (10^{-5},10^{-4} mol/l) significantly inhibited TNF-α secretion in both groups ($P<0.05$). *Conclusions*: Our data demonstrated that gemfibrozil reduced release of TNF-α in PBMC both from CHD patients and controls. This effect may partially be relevant to the clinical benefits of gemfibrozil in the treatment of dyslipidemia and atherosclerosis.

Keywords: Atherosclerosis; Inflammation; Fibrates; TNF-α; Cytokines

1. Introduction

The pathophysiology of coronary artery atherosclerosis is complex and multifactorial. Evidence is increasing that inflammatory process plays an important role in the pathogenesis of coronary heart disease (CHD) and in the prognosis of CHD patients [1–3].

* Corresponding author. Tel.: +86-731-4895989; fax: +86-731-4895989.

E-mail addresses: ZhaoSP@public.cs.hn.cn, dryhj@sohu.com (S.-P. Zhao).

0009-8981/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0009-8981(03)00123-2

Risk for the development and progression of CHD is strongly associated with increased concentrations of biochemical markers of inflammation, including tumor necrosis factor (TNF)-α, interleukin-6, C-reactive protein, modified low-density lipoprotein [4–6]. Recent studies have shown that increased concentrations of TNF-α are expressed in atherosclerotic plaques. TNF-α accompanied by other inflammatory cytokines may cause migration of leucocytes into the intima, which is a central step in the development of atherosclerosis. And they may also have biological effects on other cell types, modulating functions of endothelial cell, fibroblasts and proliferation of smooth muscle cell (SMC) [7,8]. Furthermore, persistent overexpression of TNF-α after ischemia might be relevant to adverse coronary outcomes, cardiomyopathy, left ventricular dysfunction, and advanced heart failure [9].

Fibrates, also known as fibric acids, are capable of modulating the serum concentrations of triglyceride and cholesterol and are successfully used to treat dyslipidemia and atherosclerosis. Several clinical trials demonstrated that gemfibrozil and other fibrates reduced the mortality and morbidity of cardiovascular diseases [10–12]. A number of additional effects have been noted including their anti-inflammation effects [13–15], but little is known about the effect of fibrates on TNF-α secretion in peripheral blood mononuclear cells (PBMC) from patients with coronary heart disease patients.

2. Methods

2.1. Subjects

Sixteen patients with coronary heart disease were studied, including male ($n=10$) and female ($n=6$) patients, aged 46 to 74 years (mean 60.4 years). The patients had new-onset ischemic chest pain within 15 days, and had been stable >3 days before the participation. Transient ST-T segment depression and/or T-wave inversion was present in all cases, but no evidence of myocardial necrosis was detected by cardiac enzyme measurements. The diagnosis of coronary artery disease was confirmed in all patients by coronary angiography, showing at least one main coronary artery lesion (>75% narrowing of luminal diameter). Exclusion criteria were myocardial infarction (old or acute), ECG abnormalities invalidating ST-segment analyses, thrombolytic therapy, lipid-lowering therapy using statins, anti-inflammation and anti-infection therapy during previous 15 days. All patients with heart failure, inflammatory manifestations (e.g., acute or chronic infectious and autoimmune disorders), neoplasm, hematological, liver or kidney disease, diabetes, hypertension, or with severe hyperlipidemia, needed to be treated with drugs immediately, were also excluded.

Control subjects in the study were healthy volunteers. They were matched to subjects according to age (mean 55.2 years) and gender (seven men and six women). The protocol for the present study was approved by the regional committee of medical ethics.

2.2. Reagents

Flat-bottomed 24-well trays were from Corning. Isopaque-Ficoll and endotoxin-free fetal calf sera were from Sangong. Gemfibrozil was from Xiangjiang Pharm. Dimethyl sulfoxide (DMSO), Roswell Park Memorial Institute (RPMI) 1640 medium and angiotensin II was from Sigma. TNF-α enzyme-linked immunosorbent assay (ELISA) kits were from Jingmei.

2.3. Laboratory assays

Blood samples for measurement of lipids, cardiac enzymes, renal function and liver function were drawn after a 12-h fast. Serum was separated by centrifugation of the blood samples from CHD patients and controls. All serum samples for each individual were stored at -70 °C and were measured at the same time to avoid variation of assay conditions. Biological markers were measured with established methods [16].

2.4. Isolation and culture of human peripheral blood mononuclear cells

Peripheral blood mononuclear cells were isolated as described previously [17]. Briefly, peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by Isopaque-Ficoll gradient centrifugation. The cells were washed three times with phosphate buffered saline (PBS), and were incubated in 24-well trays (2×10^6 cells/ml, Costar, Corning), resuspended in RPMI 1640 medium (Sigma) supple-

mented with 10% heat-inactivated fetal calf serum (FCS), penicillin 100 U/ml, and streptomycin 100 mg/ml, and cultured at 37 °C in a humidified 5% CO_2 atmosphere. Cell viability was always >95%, as measured by trypan blue exclusion.

2.5. Peripheral blood mononuclear cells culture and intervention

Angiotensin II (Ang II) was dissolved in sterile water of triple distillation. Gemfibrozil were dissolved in 0.1% DMSO. This concentration of DMSO did not influence the production of cytokines by PBMC. PBMC (2×10^6 cells/ml) were cultured in 24-well plates with or without Ang II (10^{-8}, 10^{-7}, 10^{-6} mol/l), or Ang II (10^{-6} mol/l) plus Gemfibrozil (10^{-6}, 10^{-5}, 10^{-4} mol/l). After 24-h incubation, the supernatants were separated from cells by centrifugation, harvested and stored at -70 °C until performance of the cytokine assay. None of the agents affected monocyte viability, verified by staining with trypan blue.

2.6. Cytokine assays

Supernatant concentrations of TNF-α were determined with a specific ELISA manufactured by Jing-

Table 1
Characteristics of participants

	CHD group ($n=16$)	Control group ($n=13$)	P value
Demographic			
Male/female (number)	10/6	7/6	NS
Age (year)	60.4 ± 7.9	55.2 ± 8.1	NS
Height (cm)	165.6 ± 5.6	164.6 ± 7.2	NS
Weight (kg)	64.3 ± 6.9	62.9 ± 8.4	NS
Systolic blood pressure (mm Hg)	137.7 ± 20.6	121.9 ± 15.9	NS
Diastolic blood pressure (mm Hg)	78.0 ± 9.1	76.9 ± 6.2	NS
Liver function tests			
Alanine amiotransferase (U/l)	27.2 ± 16.0	22.2 ± 11.1	NS
Aspartate amiotransferase (U/l)	27.3 ± 9.4	24.9 ± 6.4	NS
Total protein (g/l)	69.2 ± 5.8	68.6 ± 3.8	NS
Prealbumin (g/l)	282.4 ± 51.9	278.8 ± 53.3	NS
Albumin (g/l)	44.5 ± 2.8	44.9 ± 2.1	NS
Globulin (g/l)	24.8 ± 4.1	23.9 ± 4.3	NS
Total bilirubin (μmol/l)	13.4 ± 7.8	16.9 ± 8.8	NS
Direct bilirubin (μmol/l)	5.3 ± 2.8	6.6 ± 2.9	NS
Total cholic acid (μmol/l)	6.7 ± 5.7	8.1 ± 11.9	NS
Renal function tests			
Blood urea nitrogen (mmol/l)	6.2 ± 1.2	5.9 ± 1.3	NS
Creatinine (μmol/l)	106.9 ± 11.5	105.3 ± 15.8	NS
Uric acid (μmol/l)	412.3 ± 81.7	381.5 ± 115.8	NS
Lipid parameters			
Triglyceride (mmol/l)	1.60 ± 0.80	1.59 ± 1.20	NS
Total cholesterol (mmol/l)	4.38 ± 0.66	4.54 ± 0.89	NS
HDL-C (mmol/l)	1.15 ± 0.15	1.16 ± 0.31	NS
LDL-C (mmol/l)	2.63 ± 0.58	2.64 ± 0.93	NS
Apoprotein A1 (g/l)	1.43 ± 0.11	1.48 ± 0.23	NS
Apoprotein B (g/l)	0.69 ± 0.24	0.77 ± 0.22	NS
Lipoprotein (a) (mg/l)	237.87 ± 128.48	201.48 ± 164.83	NS
Fasting blood glucose (mmol/l)	5.7 ± 0.7	5.3 ± 0.9	NS
Cardiac markers			
Lactate dehydrogenase (U/l)	167.3 ± 65.8	134.5 ± 29.9	NS
Creatine kinase (U/l)	63.1 ± 28.1	59.1 ± 14.0	NS
CK-MB (U/l)	13.4 ± 8.1	15.2 ± 7.3	NS
Myoglobin (U/l)	43.1 ± 12.7	35.9 ± 7.8	NS

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Mei. All samples were analyzed in duplicate. The amount of cross-reactivity was assessed by comparison with the concentration yielding a 50% inhibition of binding. Sensitivity was 10 pg/ml; intra-assay and inter-assay precision variability was $<9.6\%$.

2.7. Data analysis

Statistical analysis was performed by using SPSS 10.0 statistical software (SPSS). First of all, numeric values were analyzed for the presence of normal distribution. In cases of normal distribution, testing differences between patients and controls, one-way ANOVA was used and values were stated as mean ± S.D., or data were log-transformed before testing. The significance of differences concerning intervention in the same group was evaluated by paired *t*-test. A $P<0.05$ was considered significant for all tests.

3. Results

3.1. Characterization of subjects

The characteristics of the participants in this study were summarized in Table 1. Compared with healthy control subjects, the patients with coronary heart disease were slightly older and had higher systolic blood pressure on average, but this did not reach statistical significance. Though dyslipidemia was established risk factor for coronary heart disease, lipid parameters were not significantly different between patients and controls in this study, which was partly due to exclusion of severe hyperlipidemia. Subjects in those two groups were well matched.

Table 2
Effects of Ang II on the release of TNF-α in PBMC

	TNF-α in CHD group (pg/ml, $n=16$)	TNF-α in control group (pg/ml, $n=13$)	*P*
Baseline	299.2 ± 110.7	179.3 ± 78.2	<0.05
Ang II (10^{-8} mol/l)	307.7 ± 141.8	225.3 ± 135.4	NS
Ang II (10^{-7} mol/l)	318.9 ± 135.6	224.1 ± 141.0	NS
Ang II (10^{-6} mol/l)	328.6 ± 123.9	218.7 ± 134.8	<0.05

Ang II: angiotensin II; TNF-α: tumor necrosis factor-α; PBMC: peripheral blood mononuclear cells; CHD: coronary heart disease.

Table 3
Effects of Gemfibrozil on the release of TNF-α in PBMC

	TNF-α in CHD group (pg/ml, $n=16$)	TNF-α in control group (pg/ml, $n=13$)	*P*
Ang II	328.6 ± 123.9	218.7 ± 134.8	<0.05
Ang II + Gem (10^{-6} mol/l)	279.4 ± 132.2	177.6 ± 94.4	<0.05
Ang II + Gem (10^{-5} mol/l)	268.0 ± 132.7*	156.1 ± 69.4*	<0.05
Ang II + Gem (10^{-4} mol/l)	226.6 ± 102.7*	105.3 ± 52.7*	<0.05

Ang II: angiotensin II (10^{-6}mol/l); TNF-α: tumor necrosis factor-α; Gem: gemfibrozil; PBMC: peripheral blood mononuclear cells; CHD: coronary heart disease.

*$P<0.05$ vs. that incubated with Ang II alone.

3.2. Effect of Ang II on the release of TNF-α in PBMC

Spontaneous release of TNF-α in PBMC from CHD patients and control subjects was 299.2 ± 110.7, 179.3 ± 78.2 pg/ml, respectively. Stimulated by Ang II (10^{-8}, 10^{-7}, 10^{-6} mol/l), TNF-α secretion slightly increased, but it did not reach statistic significance (Table 2).

3.3. Effect of gemfibrozil on the release of TNF-α in PBMC

Compared with that stimulated by Ang II (10^{-6} mol/l) alone, release of TNF-α in PBMC both from CHD patients and healthy controls decreased with increased concentration of gemfibrozil. Gemfibrozil (10^{-5}, 10^{-4} mol/l) significantly inhibited TNF-α secretion ($P<0.05$). The effect of gemfibrozil was dose-related (Table 3).

4. Discussion

A number of factors, including lipid accumulation and local factors in the vascular wall, contribute to the pathophysiology and development of atherosclerosis [18]. Recently, it is postulated and emphasized that inflammation is part of the process that leads to the progression and rupture of atherosclerotic lesions. A lot of studies have shown that the atherosclerotic diseases have several features in common with inflammatory reactions [3]. A number of cytokines, including

TNF-α, have been detected in human atherosclerotic lesions. TNF-α is assumed to play an important role, especially during the early phases. TNF-α is a potent activator of endothelial adhesion molecule expression [19], and is also known to activate SMC migration and proliferation [20]. In addition, increased levels of TNF-α have been detected in serum of hypercholesterolemic and CHD patients, compared with healthy subjects. Persistent serum TNF-α elevation might be found in CHD patients with adverse coronary outcomes, cardiomyopathy, and advanced heart failure. And those CHD patients with the highest levels of TNF-α were found to have a three-fold increase in the risk of recurrent MI or coronary death [4,9]. Up to now, increased serum TNF-α concentration has been considered as a characteristic feature of CHD and a prediction factor for CHD outcomes. In the present study, we found that the release of TNF-α in PBMC from CHD patients was significantly higher than that from healthy controls. And this may at least partially explain that in vivo TNF-α level is higher in CHD patients vs. healthy subjects.

The expression of TNF-α may be modulated by several factors. TNF-α production was found to be regulated by lifestyle such as obesity, weight loss, and smoking [21]. Expression of TNF-α increased in cultured, aortic SMCs grown in the presence of LDL. Accumulation of LDL in the vascular wall in vivo also resulted in the activation of TNF-α expression [8]. Oxidized LDL (ox-LDL) stimulates human monocytes and macrophages to release TNF-α [22]. Some studies showed that lipopolysaccharide (LPS) and Ang II induces TNF-α biosynthesis and secretion [23,24]. It is generally believed that Ang II provokes TNF-α biosynthesis through a pathway that involves the sequential activation of protein kinase C (PKC), followed by activation of nuclear factor-kB (NF-κB). In addition, previous study showed that activation of NF-kB by Ang II in PBMC was due to activation of monocytes and that Ang II did not stimulate NF-kB activation in lymphocytes [25]; monocytes may play a major role in regulating TNF-α secretion in PBMC stimulated by Ang II. Since Ang II was involved in the development of CHD [26], we used Ang II as stimulation in the present study. However, we did not find that Ang II induced the release of TNF-α in PBMC. In accordance, Dorffel et al. [27] also found that Ang II did not trigger TNF-α secretion in monocytes.

Interestingly, the present study has also demonstrated that gemfibrozil reduced TNF-α secretion in PBMC, either from CHD patients or from healthy subjects. Gemfibrozil is one of fibrates, which are peroxisome proliferator-activated receptor-α (PPAR-α) activators. The PPAR family consists of three proteins, PPAR α, β/Δ and γ. Recent work from several groups implicates PPAR-α and PPAR-γ as anti-inflammatory mediators in atherosclerosis-associated cells [13,28–30], although PPAR-α and PPAR-γ activators exhibit some different function [31,32]. Fibrates, as PPAR-α activators, negatively interfere with NF-κB transcription activity [33,34]. While both gemfibrozil and Ang II may interfere with NF-kB transcription, the present study showed that the release of TNF-α in PBMC was reduced by gemfibrozil but was not induced by Ang II. It is not likely that gemfibrozil and Ang II share wholly the same pathway in regulating TNF-α secretion. Cunard et al. [35] supposed that PPAR-α activator modulated inflammatory responses partly in a PPAR-α independent manner, which provides a clue to do further investigation.

Gemfibrozil along with other fibrates are initially prescribed as lipid-lowing drugs. And some clinical studies have affirmed that fibrates improve the cardiovascular risk profile. Fibrate treatment decreased incidence of cardiovascular events, such as MI, stroke, and death [11–13,36]. The improvement of lipid parameters, however, only partially explained the observed benefits of gemfibrozil and other fibrates. Recent data suggest that apart from their lipid-lowering effects, fibrates can act as anti-inflammatory agents [12,13]. Potentially, reduced release of TNF-α from PBMC due to gemfibrozil, as found in the present study, may also account for part of such effects.

In the present study, we also demonstrated that the effect of gemfibrozil on TNF-α secretion not only in CHD patients, but also in healthy subjects. It is reasonable to suppose that gemfibrozil may prevent inflammation and this effect may contribute to the benefit with gemfibrozil in the primary prevention trials.

References

[1] Lindahl B, Toss H, Siegbahn A, Venge P, Wallentin L. Markers of myocardial damage and inflammation in relation

to long-term mortality in unstable coronary artery disease. FRISC study group. Fragmin during instability in coronary artery disease. N Engl J Med 2000;343:1139–47.
[2] Vorchheimer DA, Fuster V. Inflammatory markers in coronary artery disease: let prevention douse the flames. JAMA 2001; 286:2154–6.
[3] Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999;340:115–26.
[4] Koukkunen H, Penttila K, Kemppainen A, et al. C-reactive protein, fibrinogen, interleukin-6 and tumour necrosis factor-alpha in the prognostic classification of unstable angina pectoris. Ann Med 2001;33:37–47.
[5] Aukrust P, Berge RK, Ueland T, et al. Interaction between chemokines and oxidative stress: possible pathogenic role in acute coronary syndromes. J Am Coll Cardiol 2001;37: 485–91.
[6] Balbay Y, Tikiz H, Baptiste RJ, Ayaz S, Sasmaz H, Korkmaz S. Circulating interleukin-1 beta, interleukin-6, tumor necrosis factor-alpha, and soluble ICAM-1 in patients with chronic stable angina and myocardial infarction. Angiology 2001; 52:109–14.
[7] Rus HG, Niculescu F, Vlaicu R. Tumor necrosis factor-a in human arterial wall with atherosclerosis. Atherosclerosis 1991;89:247–54.
[8] Niemann-Jönsson A, Dimayuga P, Jovinge S, et al. Accumulation of LDL in rat arteries is associated with activation of tumor necrosis factor-a expression. Arterioscler Thromb Vasc Biol 2000;20:2205–11.
[9] Irwin MW, Mak S, Mann DL, et al. Tissue expression and immunolocalization of tumor necrosis factor-a in postinfarction dysfunctional myocardium. Circulation 1999;99:1492–8.
[10] The BIP Study Group. Secondary prevention by raising HDL cholesterol and reducing triglycerides in patients with coronary artery disease: the bezafibrate infarction prevention (BIP) study. Circulation 2000;102:21–7.
[11] Robins SJ, Collins D, Wittes JT, et al. Relation of gemfibrozil treatment and lipid levels with major coronary events: VA-HIT: a randomized controlled trial. JAMA 2001;285:1585–91.
[12] Malik J, Melenovsky V, Wichterle D, et al. Both fenofibrate and atorvastatin improve vascular reactivity in combined hyperlipidaemia (Fenofibrate Versus Atorvastatin Trial-FAT). Cardiovasc Res 2001;52:290–8.
[13] Marx N, Kehrle B, Kohlhammer K, et al. PPAR activators as antiinflammatory mediators in human T lymphocytes. Circ Res 2002;90:703–10.
[14] Takano H, Nagai T, Asakawa M, et al. Peroxisome proliferator-activated receptor activators inhibit lipopolysaccharide-induced tumor necrosis factor-alpha expression in neonatal rat cardiac myocytes. Circ Res 2000;87:596–602.
[15] Delerive P, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors in inflammation control. J Endocrinol 2001;169:453–9.
[16] Zhao SP, Liu L, Gao M, Zhou QC, Li YL, Xia B. Impairment of endothelial function after a high-fat meal in patients with coronary artery disease. Coron Artery Dis 2001;12:561–5.
[17] Zhao SP, Xie XM. Captopril inhibits the production of tumor necrosis factor-a by human mononuclear cells in patients with congestive heart failure. Clin Chim Acta 2001;304:85–90.
[18] Kaul D. Molecular link between cholesterol, cytokines and atherosclerosis. Mol Cell Biochem 2001;219:65–71.
[19] Wertheimer SJ, Myers CL, Wallace RW, Parks TP. Intercellular adhesion molecule-1 gene expression in human endothelial cells: differential regulation by TNF-α and phorbol myristate acetate. J Biol Chem 1992;267:12030–5.
[20] Jovinge S, Hultgardh-Nilsson A, Regnstrom J, Nilsson J. Tumor necrosis factor-alpha activates smooth muscle cell migration in culture and is expressed in the balloon-injured rat aorta. Arterioscler Thromb Vasc Biol 1997;17:490–7.
[21] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-a in human obesity and insulin resistance. J Clin Invest 1995;95:2409–15.
[22] Jovinge S, Ares MP, Kallin B, Nilsson J. Human monocytes/macrophages release TNF-α in response to ox-LDL. Arterioscler Thromb Vasc Biol 1997;16:1573–9.
[23] Kalra D, Sivasubramanian N, Mann DL. Angiotensin II induces tumor necrosis factor biosynthesis in the adult mammalian heart through a protein kinase C dependent pathway. Circulation 2002;105:2198–205.
[24] Wagner DR, Combes A, McTiernan C, Sanders VJ, Lemster B, Feldman AM. Adenosine inhibits lipopolysaccharide-induced cardiac expression of tumor necrosis factor-a. Circ Res 1998;82:47–56.
[25] Kranzhofer R, Browatzki M, Schmidt J, Kubler W. Angiotensin II activates the proinflammatory transcription factor nuclear factor-kappa B in human monocytes. Biochem Biophys Res Commun 1999;257:826–8.
[26] Gavras H, Brunner HR. Role of angiotensin and its inhibition in hypertension, ischemic heart disease, and heart failure. Hypertension 2001;37:342–5.
[27] Dorffel Y, Latsch C, Stuhlmuller B, et al. Preactivated peripheral blood monocytes in patients with essential hypertension. Hypertension 1999;34:113–7.
[28] Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998;391:79–82.
[29] Jiang C, Ting AT, Seed B. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391:82–6.
[30] Marx N, Sukhova G, Collins T, Libby P, Plutzky J. PPARalpha activators inhibit cytokine-induced vascular cell adhesion molecule-1 expression in human endothelial cells. Circulation 1999;99:3125–31.
[31] Marx N, Schonbeck U, Lazar MA, Libby P, Plutzky J. Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. Circ Res 1998;83:1097–103.
[32] Staels B, Koenig W, Habib A, et al. Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. Nature 1998;393:790–3.
[33] Delerive P, De Bosscher K, Besnard S, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk

with transcription factors NF-kB and AP-1. J Biol Chem 1999;274:32048–54.
[34] Delerive P, Gervois P, Fruchart JC, Staels B. Induction of IkappaBalpha expression as a mechanism contributing to the anti-inflammatory activities of peroxisome proliferator-activated receptor-alpha activators. J Biol Chem 2000;275: 36703–7.
[35] Cunard R, Ricote M, DiCampli D, Archer DC, Kahn DA, Glass CK, et al. Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors. J Immunol 2002;168:2795–802.
[36] Steiner G. Diabetes Atherosclerosis Intervention Study Investigators. Effect of fenofibrate on progression of coronary-artery disease in type 2 diabetes: the Diabetes Atherosclerosis Intervention Study, a randomised study. Lancet 2001;357: 905–10.