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PPARγ in coronary atherosclerosis: *In vivo* expression pattern and correlations with hyperlipidemic status and statin treatment

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Abstract

Objective

Peroxisome proliferator-activated receptor-γ (PPARγ) is involved in regulation of macrophage inflammation and in atherosclerosis. Herein we investigate the influence of statin treatment on PPARγ expression in coronary artery disease.

Method

PPARγ expression was investigated in coronary atherosclerotic atherectomies (N = 48) and arteries (N = 12) from patients with stable or unstable coronary syndromes or undergoing cardiac transplantation for end-stage ischemic cardiomyopathy, respectively, by immunohistochemistry. Plaque components and tissue factor immunoreactivity were also investigated. Atherectomies were obtained from *de novo* culprit lesions of hypercholesterolemic (16 statin-treated and 16 untreated) and normolipidemic (N = 16) patients. Furthermore, PPARγ expression was evaluated in patients peripheral blood monocytes and in monocytic U937 cells after atorvastatin incubation, by Western blot analysis.

Result

PPARγ expression was higher in coronary plaques and peripheral blood monocytes of statin-treated patients, and it significantly increased in monocytes after 24 h atorvastatin incubation ($p < 0.05$). Intra-plaque macrophage content, atheroma, neoangiogenesis and hemorrhage, and circulating CRP levels were lower in statin-treated than untreated hypercholesterolemic patients and comparable with normolipidemic subjects. PPARγ immunoreactivity was localized to neointima and media, its distribution pattern being different from that of tissue factor.

Conclusion

PPARγ expression was enhanced in statin-treated patients with different distribution and behavior as compared to atheroma, macrophage content, tissue factor immunoreactivity and serum CRP. *In vitro* studies showed increased PPARγ expression in monocytes after atorvastatin incubation. These findings provide further evidence as to the protective role of statins in coronary artery disease and their influence on PPARγ expression in coronary plaques and on the inflammatory status of patients.

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1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors. They regulate the expression of genes controlling lipid and glucose homeostasis, and exert additional anti-inflammatory and lipid-modulating effects in the arterial wall[1] and [2]. Their most investigated role is in the fatty acid oxidation, but they play anti-inflammatory effects, influence cytokine expression and vascular pathophysiology [3],[4] and [5]. In normal vasculature and in atherosclerotic vessels, PPARγ are expressed in macrophages, endothelial and vascular smooth muscle cells [6], [7] and [8]. PPARγ agonists (WY14,643 and rosiglitazone) have been shown to inhibit neointimal hyperplasia in coronary arteries injured by balloon inflation [7] and macrophage foam-cell formation in LDL receptor deficient mice [4]. In patients with coronary artery diseases (CAD), PPARγ expression has been recently shown to be enhanced in circulating monocyte/ macrophages [9].

Statin treatment contributes to stabilization of coronary plaques by lowering lipids, inflammatory burden, intraplaque neoangiogenesis and hemorrhage, and by improving the endothelial function [10], [11] and [12]. In carotid plaques, pravastatin increases collagen content and decreases lipids, inflammation, metalloproteinases, and cell death[13]. Statins regulate PPARγ transcription activity in macrophages by inducing the expression of cyclooxygenase-2 (COX-2), which converts arachidonic acid into various bio-active lipids (including prostaglandins), such activation resulting in the production of endogenous PPAR ligands [14].

Aim of the present study was to test the hypothesis that the expression of PPARγ might be related to statin treatment in patients affected by coronary atherosclerosis with stable or unstable coronary syndromes, and to investigate the possible correlations with plaque composition.

2. Method

2.1. Study cohort

Coronary plaque fragments were obtained from 48 patients with stable (SA, $N = 24$) or unstable (UA/NSTEMI, $N = 24$) coronary syndrome by directional coronary atherectomy (DCA) procedure. Clinical

data of patients and relationships between statin treatment, CRP serum levels and histopathological features of coronary plaque composition have been previously published [13]. DCA was part of the percutaneous revascularization procedure to treat a culprit lesion, before percutaneous stent implantation. The three study subgroups (16 individuals each) consisted of: a) patients with untreated primary hypercholesterolemia (fasting total cholesterol >200 mg/dL), b) patients treated with statins because of primary hypercholesterolemia (pre-treatment values: total cholesterol 256 ±7 mg/dL, LDL-cholesterol levels 160.5 ± 4.3 mg/dL), and c) normolipidemic patients. Patients groups were matched for age and clinical diagnosis. Statins (simvastatin – 20 mg, pravastatin – 40 mg or atorvastatin – 10/20 mg) were given continuously for ≥1 year before DCA in group (b) patients.

As to evaluate and confirm the immunoreactivity pattern of PPARγ in full coronary segments, other 6 male patients (age 51.5 ± 14.3 years) undergoing cardiac transplantation because of end-stage (NYHA functional class IV) ischemic cardiomyopathy, entered the study. Their pre-transplant hearts were harvested, and atherosclerotic coronary arteries (5 left anterior descending coronary arteries, 5 right coronary arteries, one circumflex and one left main coronary artery) were transversally cut, serially sectioned and totally sampled for histology.

The study was approved by the institutional review committee and patients gave their written informed consent.

2.2. Histology and immunohistochemistry

DCA specimens and coronary artery segments were fixed in 10% buffered formalin and paraffin-embedded. Serial 3 µm sections were mounted on poly-l-lysine coated slides and stained with hematoxylin-eosin and the trichrome method. Immunohistochemistry was performed on adjacent sections. After microwave antigen retrieval, immunoreactivity for the primary antibodies was revealed by using a biotinylated link antibody directed against mouse/rabbit antigen with a peroxidase-based kit (LSAB, Dako, Glostrup, Denmark) and 3'-diaminobenzidine as chromogen substrate. PPARγ immunostaining was evaluated by using a specific antibody raised in rabbit at appropriate dilution (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Positive controls consisted of sections obtained from human adipose tissue and breast carcinoma. Negative controls were performed by replacing the respective primary antibodies by isotype and concentrations matched irrelevant antibody.

Intraplaque hemorrhage was assessed by using Glycophorin A immunostaining (Dako), and further antibodies against CD68 (KP 1), CD31 antigens and α-smooth muscle actin (Dako) were used to identify macrophages, endothelial and smooth muscle cells, respectively. Tissue factor immunoreactivity was investigated by using a monoclonal antibody (American Diagnostica Inc, Stamford, CT, USA). Positive controls consisted of sections obtained from human lymph node, bowel appendix and placenta. Negative controls were performed by replacing the respective primary antibodies by isotype and concentrations matched irrelevant antibody.

2.3. Morphometric analysis

Histological and immunohistochemical results were planimetrically quantified on DCA specimens by using a computer-based morphometry software (Image Pro Plus 4.5, Media Cybernetics) as previously described [12]. For PPARγ, the number of positive cells was counted in the entire tissue sections and expressed as number of cells per $mm^2$and relative data (immunoreactive area percentages) were calculated

as percentages of the total tissue area. Analysis of the tissue sections was performed blinded to clinical data.

2.4. Cells, culture conditions and cell treatment

Monocytes were isolated on a ficoll-hypaque (Lymphoprep) gradient at 1077 g/L. from the peripheral blood of patients. After isolation, total proteins were extracted for western blot analysis.

Monocytic U937 cells, obtained from European Collection of Cell Cultures (ECACC), were cultured at 37 °C in a humidified atmosphere of 5% CO_2 –air, using RPMI 1640 medium supplemented with 2 mM glutamine, 1% antibiotic mixture (penicillin–streptomycin) and 10% fetal calf serum (Biochrom AG Seromed, Berlin, Germany). U937 cells were incubated with 50 µM atorvastatin (Sigma, Milano, Italy), dissolved in DMSO. The concentration of vehicle in culture did not exceed 0.1%. Moreover U937 cells were treated with 0.1% DMSO alone to exclude vehicle's effects. Three independent experiments were carried out and in all of them, cells were collected after 24 h and total proteins were extracted for western blot analysis.

2.5. Western blot analysis

Fresh human blood monocytes and monocytic U937 cell line were resuspended in a lysis buffer containing 20 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% v/v Triton X-100, phosphatase and protease inhibitor cocktails (Sigma–Aldrich) and incubated for 15 min at 4 °C. Insoluble proteins were discarded by high-speed centrifugation at 4 °C. Protein concentration in the supernatant was measured in triplicate using a commercially available assay (Bio-Rad Laboratories). All proteins were separated by SDS–PAGE and electroblotted on nitrocellulose membrane (Bio-Rad Laboratories) with a semidry transfer apparatus (Biometra). Membranes were blocked overnight at 4 °C in Tris-buffered saline containing 5% milk plus 0.5% Tween 20 and then incubated at room temperature with primary anti-PPARγ (Santa Cruz Biotechnology, Inc.) and horseradish peroxidase-conjugated secondary (Bio-Rad Laboratories) antibodies. Detection was carried out by enhanced chemiluminescence according to the manufacturer's protocol (PerkinElmer), through film (Santa Cruz Biotechnology). Densitometric analysis was performed using a software program (Multi-Analyst, version 1.1; Bio-Rad Laboratories), results were normalized by using the β-actin signal (Sigma–Aldrich) and expressed as arbitrary densitometric units (UA).

2.6. Statistical analysis

Data are expressed as mean (±standard error) or as number (proportion). Univariate statistical analysis was performed in order to bring to light statistically significant differences between two groups. The methods used were standard: for continuous variables ANOVA; for dichotomous variables the chi-square test (with Yates' correction) or, when requested, the Fisher and Mid-P exact tests. Statistical significance was determined at a p value ≤0.05.

3. Results

Coronary atherosclerotic segments from pre-transplant hearts showed complex and critically or sub-critically stenosing fibro-adipose or fibrous plaques, with or without a variable amount of calcium deposits. Immunohistochemical expression of PPARγ was found in neointima and media of all examined coronary segments (data not morphometrically analyzed), including endothelial cells. PPARγ was mainly localized to cells corresponding to foam cells, monocytes and myofibroblasts/smooth muscle cells on adjacent serial sections. Immunostaining was mainly nuclear and peri-nuclear, but cytoplasmic positivity was detected in foam cells (Fig. 1A,B). As to tissue factor immunoreactivity in this pre-transplant group, it was detected in

macrophages, in atheromatous gruel or necrotic debris of plaques and in areas of thrombotic depositions or intraplaque hemorrhage (data not morphometrically analyzed, Fig. 2A,B).

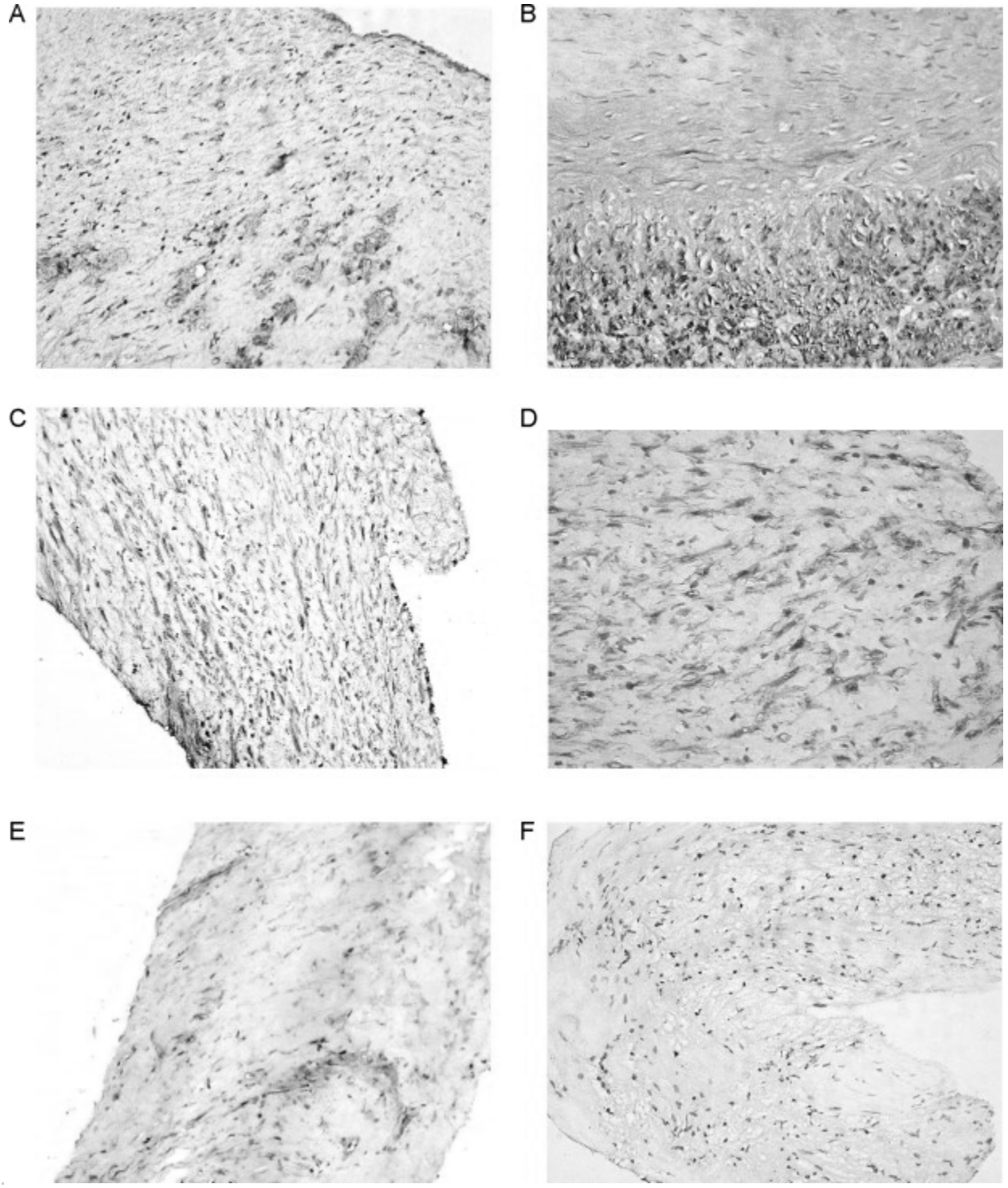

Fig. 1.

PPARγ expression in the atherosclerotic left anterior descending coronary artery from a cardiac transplant recipient (A,B) and in the directional coronary atherectomies (C–F). In the cardiac transplant recipient, scattered nuclear and peri-nuclear immunostaining in cells of neointima, and perinuclear/cytoplasmic positivity in foamy cells (A) is shown, such as nuclear and perinuclear immunoreactivity in smooth muscle cells from medial layer adjacent to intimal plaque (B). In directional coronary atherectomies, predominantly nuclear staining in a fibrocellular plaque from a statin-treated dyslipidemic patient, at low (C) and intermediate (D) magnification; (E) few, scattered positive nuclei in a fibrous plaque from a normolipdemic patient; (F) in an untreated dyslipidemic patient, the cells of the plaque fragment, including foam cells, do not show immunostaining. Immunoperoxidase, Hematoxylin counterstaining, original magnification: A, C, E, F 20×; B, D 40×.

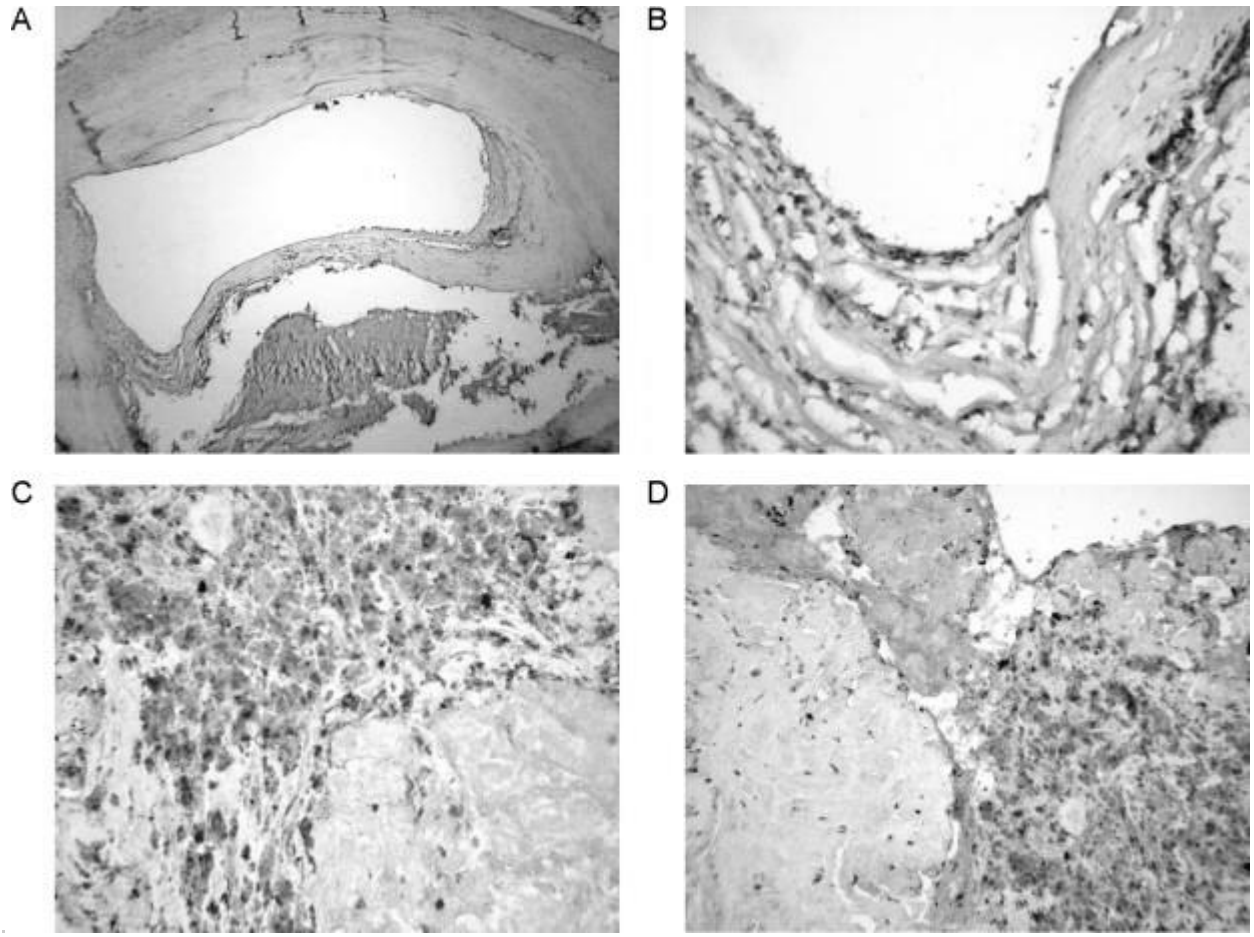

Fig. 2.

Tissue factor immunoreactivity in a pre-transplant coronary artery with thin-cap fibroatheroma (A,B) and in a coronary atherectomy case (C,D). Intraplaque hemorrhage mixed to atheromatous gruel is intensely positive (A); scattered cells in the thin cap are also positive (B). Thrombus positivity is also shown in a coronary atherectomy from an untreated dyslipidemic patient (C) with very few positive cells in the adjacent fibrous plaque (D). Immunoperoxidase, hematoxylin counterstaining, original magnification: A 5×; B,C,D 40×.

As to DCA patients groups, clinical characteristics and coronary risk factors are listed inTable 1. Plaque components were characterized as previously reported and results are summarized in Table 2[12]. Immunostaining for PPARγ was detected in all cases but two untreated dyslipidemic patients (Fig. 1C–F). Cellular localization was similar to pre-transplant coronary arteries and a higher expression was found in statin-treated group as compared to untreated dyslipidemic or normolipidemic patients ($p < 0.05$), with no statistically significant difference between the two latter groups. Tissue factor immunostaining was also present, localized to those areas of thrombus, intra-plaque hemorrhage, and/or atheroma, which were more frequently observed in untreated dyslipidemic patients (Fig. 2C,D). The different behaviours of the investigated variables in the three DCA groups are summarized in Fig. 3A. PPARγ reached its maximum expression in statin-treated patients, whereas the other parameters exhibited a steady decrease from untreated to statin-treated dyslipidemic patients and finally to normolipidemic patients. No relationship was found between clinical diagnosis of SA or UA/NSTEMI and PPARγ expression.

Table 1.

Baseline characteristics of patients undergoing coronary atherectomy.

Variables	Non-treated hyperlipidemic pts *N* = 16	Statin-treated hyperlipidemic pts *N* = 16	Normolipidemic pts*N* = 16
Age, yrs	64 (2.6)	66 (2.2)	63 (3.0)
Sex (F/M)	0/16	5/11	0/16
SA	8	8	8
UA/NSTEMI	8	8	8
Diabetes	5	6	3
Familial CAD	5	3	5
Hypertension	10	12	8
Smoking	6	7	6
CRP (mg/dL)	1.42 (0.47)	0.67 (0.17)	0.23 (0.05)
Total-C (mg/dL)	245 (5)	188.37 (1.18)	175.87 (2.83)
LDL-C (mg/dL)	156.2 (3.26)	89.7 (1.12)	80.9 (2.9)

Values are expressed as mean (standard error) or *n* (%). CRP—C-reactive protein. Total-C—total cholesterol. LDL-C—low density lipoprotein cholesterol. SA—stable angina. UA/NSTEMI—unstable angina/non-ST elevation myocardial infarction.

Table 2.

Histological and immunocytochemical characteristics of coronary atherectomy specimens.

Parameter	Non-treated dyslipidemic pts (*N* = 16)	Statin-treated dyslipidemic pts (*N* = 16)	Normolipidemic pts (*N* = 16)	*p*

Parameter	Non-treated dyslipidemic pts (*N* = 16)	Statin-treated dyslipidemic pts (*N* = 16)	Normolipidemic pts (*N* = 16)	*p*
PPARγ				
a) N of cases (%)	14(87.5)	16(100)	16(100)	0.12 (n.s.)
b) Cell counts: N/mm^2	6.86(1.47)	18.17(2.20)	9.74(2.30)	8×10^{-4}
c) Area percentage	5.00(1.61)	16.11(2.04)	8.22(1.79)	3×10^{-4}
Atheroma				
a) N of cases (%)	15(93.7)	7(43.7)	5(31.2)	0.0015
b) Area Percentage	19.37(2.49)	9.06(3.14)	2.5(1.02)	0.001
CD68 (macrophages)				
a) N of cases (%)	16 (100)	14 (87.5)	12 (75)	0.1 (n.s.)
b) Area percentage	28.75(2.12)	13.12(2.49)	7.18(1.44)	0.0001
CD31 (neoangiogenesis)				
a) N of cases (%)	16 (100)	11 (68.75)	9 (56.25)	0.013
b) Vascular counts: N/mm^2	14.54(1.16)	6.45(1.40)	4.6(1.26)	0.0001
Glycophorin A (hemorrhage)				
a) N of cases (%)	14 (87.5)	9 (56.25)	9 (56.25)	0.1 (n.s.)
b) Area percentage	28.18(2.81)	10.37(3.73)	9.75(2.92)	0.0028
Fibrous tissue				

Parameter	Non-treated dyslipidemic pts (*N* = 16)	Statin-treated dyslipidemic pts (*N* = 16)	Normolipidemic pts (*N* = 16)	*p*
a) N of cases (%)	8(50)	10(62.5)	15(93.7)	0.023
b) Area percentage	13.12(4.83)	19.06(5.24)	32.19(5.7)	0.041
Hypercellular tissue				
a) N of cases (%)	8(50)	6(37.5)	6(37.5)	0.71 (n.s.)

Values are expressed as mean ± standard error (in brackets) or *n* (%). Cell counts, number of PPARγ positive cells/mm^2. Vascular counts: number of microvessels (CD31 positive cells)/mm^2.

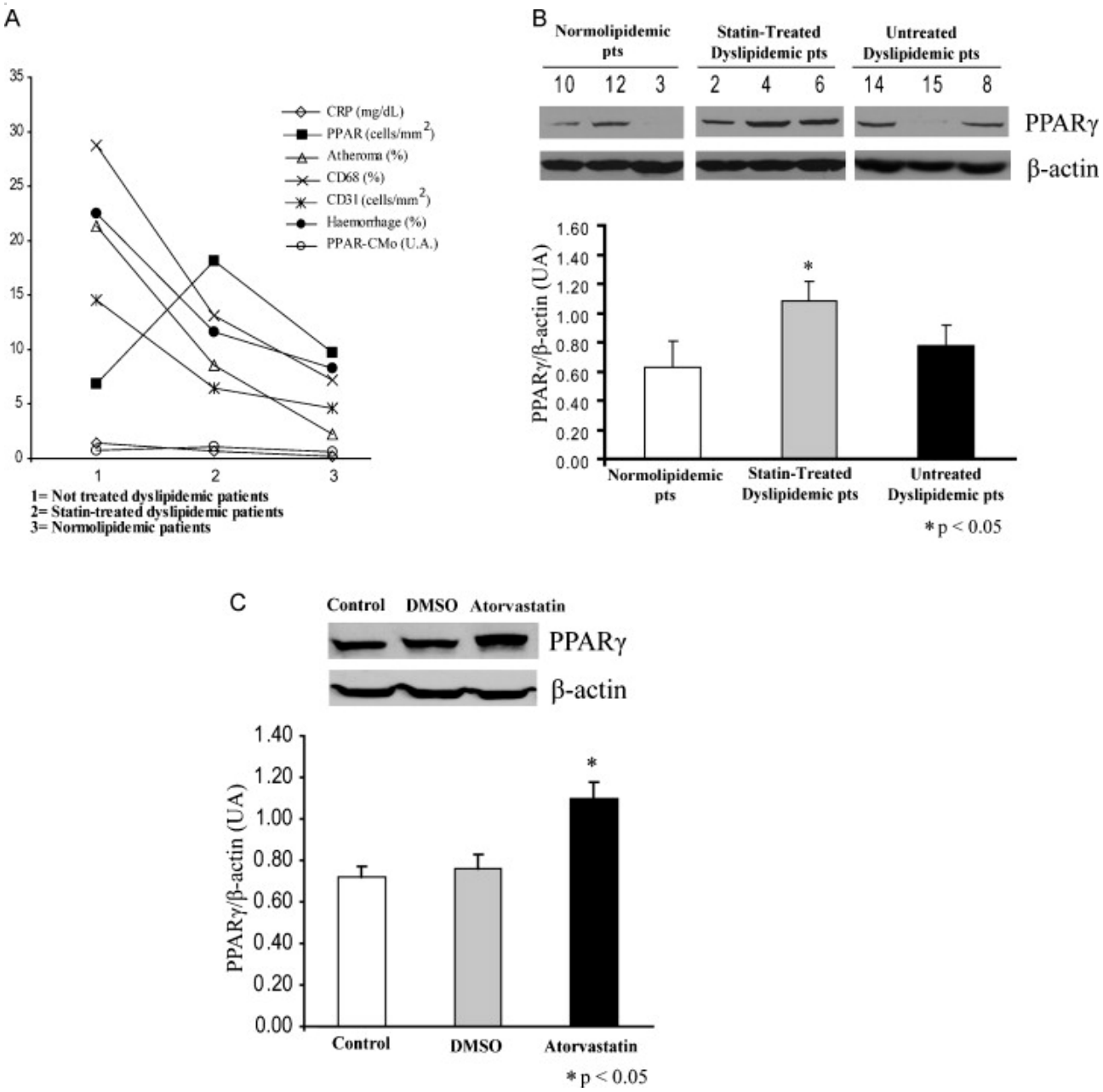

Fig. 3.

(A) Graphical representation of PPARγ immunoreactivity, atheroma, macrophage content, hemorrhage, vascular counts in coronary atherectomies and serum CRP in the 3 patients groups undergone directional coronary atherectomy. CRP, serum C-Reactive protein, mg/dL; PPAR, cells/mm^2; atheroma, area percentage; CD68, area percentages; CD31, cells/mm^2; hemorrhage, area percentage; PPAR-CMo, PPAR in monocytes, arbitrary units. (B) PPARγ expression in normolipidemic, statin-treated and untreated dyslipidemic patients. Western blot analysis of PPARγ protein levels in three patients from each group; equal protein loading was confirmed by exposure of the membranes to the anti β-actin antibody. Quantification of PPARγ protein levels was performed by densitometric scanning, data were normalized by using the β-actin signal and expressed as arbitrary densitometric units (UA); results of all analyzed samples are expressed as mean values ± standard error (SE). (C) Western blot analysis of PPARγ protein levels in monocytic U937 cells: control (untreated cells), DMSO (treated with 0.1% DMSO, i.e., vehicle), or atorvastatin (cells incubated with 50 μM atorvastatin) after 24 h. Equal protein loading was confirmed by exposure of the membranes to the anti β-actin antibody, data were normalized by using the β-actin signal and densitometric values of PPARγ protein levels were expressed as arbitrary densitometric units (UA). Results are expressed as mean values ± SE of the data obtained from three independent experiments.

Western blot analysis highlighted a higher expression of PPARγ in circulating monocytes isolated from statin-treated patients (mean 1.09, SE 0.12) as compared to untreated dyslipidemic patients (mean 0.78, SE 0.17) ($p = 0.014$) or normolipidemic patients ($p = 0.06$) who showed the lowest values (mean 0.63, SE 0.25) with no statistically significant difference between the two latter groups ($p = 0.39$) (Fig. 3B). In monocytic U937 cells, PPARγ protein expression increased after 24 h incubation with 50 μM atorvastatin (1.07 ± 0.1 UA), as compared to untreated U937 cells (0.72 ± 0.05 UA) and 0.1% DMSO (vehicle) treated (0.74 ± 0.08 UA) ($p = 0.003$, Fig. 3C). Equal protein loading was confirmed by exposure of the membranes to the anti β-actin antibody.

Plaque composition in DCA cases and relationships with hyperlipidemic status, statin treatment and serum CRP levels, have been previously reported (Table 2) [12]. Briefly, atherectomies from statin-treated hyperlipidemic patients contained lesser atheroma ($p = 0.008$), macrophage ($p < 0.0001$), intraplaque neovessels ($p = 0.0001$) and hemorrhage ($p = 0.02$) than those from untreated dyslipidemic patients. No statistically significant difference was found between statin-treated and normolipidemic patients apart from macrophage content ($p = 0.05$), whereas relevant differences did obviously exist between untreated dyslipidemic and normolipidemic patients for atheroma ($p = 0.001$), fibrous tissue ($p = 0.006$), macrophages ($p = 0.0004$)), intraplaque angiogenesis ($p = 0.0008$) and hemorrhage ($p = 0.009$). Finally, statin-treated patients showed serum CRP levels significantly lower than untreated dyslipidemic patients but higher than normolipidemic patients ($p < 0.001$).

4. Discussion

In the present study we showed the influence of statin treatment on the *in vivo* expression of PPARγ in patients with non-fatal acute coronary syndromes. PPARγ immunoreactivity (either number of positive cells/mm^2 and area percentages) was increased in coronary plaques of patients under statin treatment as compared with non-treated dyslipidemic patients or normolipidemic patients ($p < 0.05$) independently from stable or unstable coronary syndrome. Although the number of the enrolled patients was quite limited ($N = 48$), the present results are corroborated by data relative to increased PPARγ protein levels in both blood monocytes of statin-treated dyslipidemic patients and monocytic U937 cells after 24 h atorvastatin incubation. Furthermore, PPARγ expression showed a different behavior as compared with the circulating inflammatory marker CRP, and its increased expression was found to be associated with decreased intra-plaque content of inflammation (i.e., macrophages), hemorrhage and neoangiogenesis.

PPARs act as ligand-activated transcription factors, control the expression of specific target genes and regulate a variety of cell functions [23], [24] and [25]. PPARγ agonists might interfere with monocyte recruitment, smooth muscle cell proliferation, cholesterol efflux from macrophages, and blood cholesterol transport to the liver [27]. And PPARγ plays a central role in adipogenesis and lipid metabolism. Although PPARγ activators failed to decrease macrophage-laden atherosclerotic lesions in a dyslipidemic nondiabetic murine model [26], in LDL receptor deficient mice PPARγ agonists were shown to strongly inhibit foam cell formation through distinct ABCA1-independent pathway [4]. PPARγ may also reduce inflammation by regulating gene expression and decreasing inflammatory cytokines produced by macrophages (i.e., TNF-α, IL-6, IL-1β)[20] and [21].

In unstable carotid atheromas, PPARγ, together with PPARα ligands might have beneficial effects by acutely reducing tissue factor, the key initiator of the clotting cascade[19]. In the present study, tissue factor immunoreactivity was mainly localized to areas with thrombus, intra-plaque hemorrhage and/or atheroma, that were particularly represented in untreated dyslipidemic patients, with an immunostaining pattern distinct from that of PPARγ.

Statins have been shown to induce PPARγ activation through extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase–dependent cyclooxygenase-2 expression in macrophages [14]. Activation of PPARγ by atorvastatin is solely mediated by 15DPGJ2 in cultivated human umbilical venous endothelial cells[15]. PPARs are involved in the anti-inflammatory effect of statins; Grip et al. have previously shown that atorvastatin activates PPARγ, and inhibits pro-inflammatory cytokine production (i.e., tumor necrosis factor-alpha, monocyte chemoattractant protein-1 and gelatinase) in a concentration-dependent manner in human monocytes[16]. Statins and fibrates activate the PPAR system in platelets, and the ability of statins to lower blood levels of inflammatory mediators such as IL-6, IL-8, etc, is likely attributable to their influence on platelets [17]. A reduced platelet activation causes a reduced platelet release of CD40L, one of the major cellular stimuli capable of activating many cell types to produce proinflammatory and prothrombotic mediators.

In patients with coronary artery diseases, statin treatment maintains the stability of athermanous plaque, and this efficacy is related to its ability to reduce the plasma levels of inflammatory markers [18]. Accordingly, we have shown that in statin-treated patients, a decrease of circulating CRP is associated with an increase in intra-plaque PPARγ and with a decreased extent of histological features of plaque instability, such as macrophage content, hemorrhage and neoangiogenesis.

We confirmed the expression of PPARγ in neointima and media of coronary vessels, accordingly to previous molecular (real-time PCR) and immunohistochemical studies in dilated and ischemic cardiomyopathies, in vascular smooth muscle cells, but also in endothelial cells and macrophages, with cellular localization of immunostaining either nuclear and peri-nuclear or (in foam cells) cytoplasmic, such features being similar to other reported results in various tissues and organs [5], [8], [19] and [20].

PPARγ was expressed by vascular smooth muscle cells in both DCA and coronary arteries of the present study. PPARγ activation inhibits proliferation and migration of vascular smooth muscle cells and promote cell apoptosis [7] and [28], thus it is likely to contribute to coronary plaque stabilization, whereas smooth muscle cell abundance has been claimed to play a role in the transformation from stable to unstable clinical state in nonfatal UA [29] and to be associated with UA/NSTEMI diagnosis [12].

The present demonstration of PPARγ immunostaining in foam cells of both DCAs and whole atherosclerotic coronary arteries might be related to the possible effects of these receptors on lipid uptake and/or metabolism within lipid-rich plaques. In humans, statins have been advocated to play a major role in stabilization and regression of lipid-rich plaques [10], [12] and [13] and the combination of statins plus PPARγ agonists has been shown to have additive regression effects on plaque, i.e., decreased macrophage content and matrix metalloproteinases activity and increased collagen content of lesions[22].

As to the expression of PPARγ in blood monocytes, we have shown increased protein levels either in statin-treated patients and in monocytic U937 culture cells after 24 h stimulation with atorvastatin. Other Authors have shown that in human blood monocytes atorvastatin activates PPARγ and attenuates the inflammatory response, suggesting that statins may control inflammatory responses by the regulation of intracellular lipid homeostasis [16] Also Pravastatin increased PPARγ levels in non-stimulated monocytes, and, added to monocytes prior to or after treatment with non-oxidized or oxidized LDL, significantly inhibited generation of matrix metalloproteinases (MMPs), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor alpha (TNF-α) [30]. Altogether, the present and previous data corroborate the pleiotropic role of statins and suggest the involvement of PPARγ in the modulation of the inflammatory processes by statins. Previous studies have shown that statins (including atorvastatin) may activate PPARγ through COX-2 and prostaglandin systems [14] and [15], and also the present *in vitro* results are apparently supporting a

cholesterol-independent effect of statin on PPARγ release, although this study design was not addressed to investigate the cellular mechanisms of atorvastatin on PPARγ.

4.1. Limitations of the current study

The present *in vivo* study on coronary atherosclerotic plaques did not investigate the molecular mechanisms of PPARγ in complex human coronary atherosclerosis or the effects of PPARγ on plaque regression, no follow-up DCA being available Nevertheless, our results might contribute to the knowledge and definition of the role of PPARγ and statins in this multi-factorial disease.

5. Conclusion

Although the present results have been obtained on a limited series of patients, the immunoreactivity pattern of PPARγ we found in coronary atherosclerosis and its associations with statin treatment and coronary plaque histology as well as the *in vitro* results obtained on monocytes support the potentially beneficial role of PPARγ in adjunct to the protective role of statins in coronary artery disease and plaque stabilization.

Conflict of interests

None.

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