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Peroxisome proliferator-activated receptor (PPAR) α and δ activators induce ICAM-1 expression in quiescent non stimulated endothelial cells

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Abstract

Background: Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are implicated in the regulation of lipid and glucose homeostasis. PPAR agonists have been shown to control inflammatory processes, in part by inhibiting the expression of distinct proinflammatory genes such as vascular cell adhesion molecule-1 (VCAM-1), IL-8, and intercellular adhesion molecule-1 (ICAM-1). ICAM-1 is an important endothelial membrane receptor that facilitates the transmigration of leukocytes across the endothelium. To date, the influence of PPAR α and δ activators on the expression of ICAM-1 in non-induced, quiescent endothelial cells has been unclear. Therefore, we examined the effects of various PPAR α and δ agonists on the expression of ICAM-1 in non-stimulated primary human endothelial cells.

Results: We found that PPAR α and PPAR δ agonists significantly induced ICAM-1 surface, intracellular protein, and mRNA expression in a time and concentration-dependent manner. The PPAR δ induced ICAM-1 expression could be paralleled with a significantly increased T-cell adherence to the endothelial cells whereas PPAR α failed to do so. Transcriptional activity studies using an *ICAM-1* reporter gene constructs revealed that PPAR δ , but not PPAR α agonists induced gene expression by stimulating *ICAM-1* promoter activity via an Sp1 transcription factor binding site and inhibit the binding of the transcription factors Sp1 and Sp3. Furthermore, we performed mRNA stability assays and found that PPAR α and PPAR δ agonists increased *ICAM-1* mRNA stability.

Conclusion: Therefore, our data provide the first evidence that PPAR α and PPAR δ agonists induce ICAM-1 expression in non-stimulated endothelial cells via transcriptional and posttranscriptional mechanisms.

Keywords: PPAR α , PPAR δ , mRNA stability, ICAM-1, Endothelial cell, Sp1, Promoter

Abbreviations: HUVEC, Human Umbilical Vein Endothelial Cell; ICAM-1, Intercellular Adhesion Molecule-1; PPAR, Peroxisome Proliferator Activated Receptors; VCAM-1, Vascular Cell Adhesion Molecule-1

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Background

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor-activated transcription factor superfamily comprised of three subtypes: PPAR α , PPAR δ , and PPAR γ . The role of PPARs was originally thought to be restricted to lipid metabolism, glucose homeostasis, and cellular differentiation [1–4]. PPARs can be activated by natural ligands such as eicosanoids or fatty acids. In addition, synthetic antidiabetic thiazolidinediones and lipid-lowering fibrates have been shown to act as activators of PPAR γ and PPAR α , respectively [5–7]. Recent evidence suggests that PPAR δ plays a crucial role in the regulation of differentiation, cell growth, and the metabolism of lipids and glucose [8–11]. Previous studies demonstrated that PPAR δ agonists improve insulin sensitivity and therefore might be interesting targets for the treatment of obesity-associated disorders [12–14].

In the last few years, several studies have revealed the impact of PPAR α and PPAR δ on endothelial cell function. During inflammation, proinflammatory stimuli, including LPS, TNF α or IL-1 β , cause phenotypic changes to the quiescent endothelium by inducing the expression of proinflammatory factors such as IL-6 and IL-8 or adhesion molecules such as ICAM-1 and VCAM-1. Fan et al. demonstrated that the PPAR δ agonist GW501516 suppressed IL-1 β -induced VCAM-1 and E-selectin expression in human umbilical vein endothelial cells (HUVECs) [15]. Furthermore, Rival et al. showed that the PPAR δ activator L-165041 suppressed TNF α -induced VCAM-1 and MCP-1 expression [16]. Huang et al. revealed that ICAM-1 expression could be attenuated by PPAR α activation via fenofibrate in TNF α -activated human aortic endothelial cells [17]. Piqueras demonstrated, that TNF α induced ICAM-1 expression in endothelial cells can be partly suppressed by PPAR δ agonists [18]. Taken together, these studies demonstrate that PPAR α/δ agonists play a role in suppressing the proinflammatory response in stimulated endothelial cells.

On the other hand, there is also evidence that PPAR agonists have proinflammatory properties in non-inflammatory, quiescent endothelial cells. Chen et al. demonstrated that PPAR γ agonists significantly induced ICAM-1 expression in human endothelial cells [19]. In addition, PPAR δ agonists were shown to induce IL-6 and IL-8 expression in non-stimulated human endothelial cells via increased induction and translocation of Nf κ B [20]. Recently, Gu et al. demonstrated, that the PPAR α agonists fenofibrate induces inflammation in experimental acute colitis mice [21]. Furthermore, Wang et al. demonstrated that PPAR δ promotes colonic inflammation and colitis-associated tumor growth via the COX-2-derived PGE2 signaling [22]. Therefore, it is important, that not only the anti-inflammatory action of PPARs but also the possible pro-inflammatory properties are investigated.

The impact of PPAR α and PPAR δ agonists on ICAM-1 expression in quiescent non stimulated endothelial cells has yet to be assessed. Understanding the consequences of PPAR signaling is of importance due to the possible wide range use of PPAR agonists in various diseases such as chronic inflammation, glucose metabolism, dyslipidemia, obesity, cancer therapy, and potentially many more. In the present study, we analyzed the effects of PPAR α and PPAR δ activators on the expression of ICAM-1 in non-stimulated HUVECs. Furthermore, we investigated the mechanisms by which PPAR agonists exert their influence within these cells.

Methods

Reagents

Recombinant human TNF α was purchased from R&D Systems (Minneapolis, MN, USA). L-165041, GW501546, WY14643, Fenofibrate and Actinomycin-D were obtained from Sigma-Aldrich (Hamburg, Germany).

Cell culture

HUVECs were purchased from PromoCell (Heidelberg, Germany) and were cultured until the fifth passage at 37 °C and 5 % CO₂ in Endothelial Cell Growth Medium (Cambrex, East Rutherford, NJ, USA). Jurkat cells were obtained from ATCC (LGC Standards; Wesel, Germany) and cultured at 37 °C and 5 % CO₂ atmosphere in RPMI-1640 medium supplemented with 10 % fetal bovine serum (FBS) and 2 mM glutamine, 100 U/ml penicillin, and 100 μ g/ml streptomycin.

Fluorescence-activated cell sorting analysis

HUVECs were treated with PPAR α and PPAR δ agonists for 24 h. The treated cells were incubated with mouse anti-human ICAM-1 fluorescein-conjugated mAb (Clone #BBIG-1; 1:200 dilution; R&D Systems, Wiesbaden, Germany) or isotype control mouse anti-human IgG1 (R&D Systems, Wiesbaden, Germany) for 30 min on ice. Isotype control cells were then incubated with fluorescein-conjugated affinity-purified goat F(ab')₂ anti-mouse IgG (F0479; DAKO, Hamburg, Germany) at a 1:100 dilution for 30 min, and cells were subsequently analyzed by a BD FACScan Cytometer (Becton–Dickinson, Franklin Lakes, NJ, USA). Nonviable cells were identified and excluded by propidium iodide staining.

Western blot analysis

Whole cell protein was prepared as previously described [23]. Membranes were incubated with the indicated primary antibodies. Antibodies were as follows: anti-ICAM-1 (SC-107; 15.2) from Santa Cruz (Heidelberg, Germany) and anti-Tubulin α Ab-2 (DM1A) from LabVision (Fremont, CA, USA). Primary antibody application was followed by incubation with horseradish peroxidase-

conjugated secondary antibodies (anti-mouse and anti-rabbit IgG, Amersham, Uppsala, Sweden; anti-goat, Dako, Glostrup, Denmark). Blots were developed using an enhanced chemiluminescence detection system (ECL) (Amersham, Uppsala, Sweden), according to the manufacturer's instructions. Densitometry was used to quantify band intensities using ImageJ (v1.29 s). Optical densities of the bands were corrected for loading differences based on corresponding control bands.

Flow chamber assays

Adhesion was determined using Ibidi μ -slide VI chambers (Munich, Germany) [24]. Two days before treatment 1.8×10^5 HUVECs were seeded in each chamber. The day before treatment HUVECs were serum-starved (0.5 % FBS) for 24 h. After 24 h the HUVECs were treated with PPAR δ or PPAR α agonists (L-165,041 (50 μ M); GW501516 (20 μ M); Fenofibrate (100 μ M); WY14643 (200 μ M)) or TNF α (20 ng/ml) for 6 h. 5×10^4 Jurkat cells were allowed to attach on the endothelium for 3 min. Non-adherent cells were flushed away and shear stress was increased stepwise from 0.35 to 2, 5, 8 and 15 dyn/cm² for 30 s each. Every 30 s the number of adherent cells was quantified, with a charge-coupled device (CCD) camera (Sony, New Jersey, USA).

RNA extraction and RT-PCR

RT-PCR analyses were performed using total RNA (150 ng) extracted from sub-confluent cell cultures. Total cellular mRNA was isolated by the RNeasy Mini Procedure (Qiagen, Hilden, Germany) after DNase digestion. RT-PCR analyses for ICAM-1 and GAPDH were performed using the One Step RT-PCR Kit (Qiagen). PCR products were resolved by 1–2 % agarose gel electrophoresis, and ethidium bromide-stained bands were visualized with an ultraviolet transilluminator. The primer sets for ICAM-1 and GAPDH have been previously published [23]. Densitometry was used to quantify band intensities using ImageJ (v1.29 s). Optical densities of the bands were corrected for loading differences based on corresponding control bands.

Transient transfection and analysis of reporter gene expression

HUVECs (1.0×10^5 cells/well in 12-well plates) were transfected with 0.5 μ g of the appropriate *firefly* luciferase construct and 0.1 μ g pRG-TK vector (Promega, Madison, WI, USA) using the SuperFect transfection reagent (Qiagen). Human ICAM-1 full length reporter gene construct -1014 pIC was generously provided by Paul van der Saag, Hubrecht Laboratory, Utrecht, The Netherlands and subcloned in a pGL3 luciferase vector (Promega, Madison, WI, USA) [25]. In addition, four new deletional ICAM-1 promoter constructs containing 5' regulatory

elements were established in the pGL3 luciferase vector using PCR amplification (HotStar HiFidelity Polymerase Kit; Qiagen) and KpnI and BglII restriction sites. The -1014 gene construct was used as a template. All constructs were sequenced from the 5'- and 3'-ends to confirm orientation and sequence correctness. Twenty-four hours after transfection, cells were treated with vehicle (DMSO, 0.3 %) or the appropriate PPAR agonist for 24 h. Luciferase activities were measured with the Dual-Luciferase Reporter Assay System (Promega).

Preparation of nuclear extracts and electrophoretic mobility shift assay (EMSA)

HUVECs were treated with vehicle (0.1 % DMSO) or L-165041 for 30 min. Nuclear proteins were extracted as described previously [26]. An ICAM-1 promoter specific oligonucleotide between -69 and -45 bp containing the Sp1 site between -53 and -59 bp was constructed (5'-primer: GAAAGCAGCAC**CCGCCCTTGGCCC** (Sp1-site in bold letters); 3'-primer: GGGCCAAGG). In addition, a Sp1 mutated oligonucleotide was constructed carrying two nucleotide mutations within the Sp1 consensus sequence (5'-primer: GAAAGCAGCAC**AGACCCCTTGGCCC** (mutated Sp1-site in bold letters, exchanged nucleotides in italic); 3'-primer: GGGCCAAGG). DNA-binding reactions were performed with or without excess unlabeled competitor, Sp1 consensus-oligonucleotide (Promega), wildtype Sp1 oligo and mutated Sp1 oligo as well as Sp1 and Sp3 antibodies (SC-59 (PEP2); SC644 (D-20) Santa Cruz Biotechnology, Santa Cruz, CA).

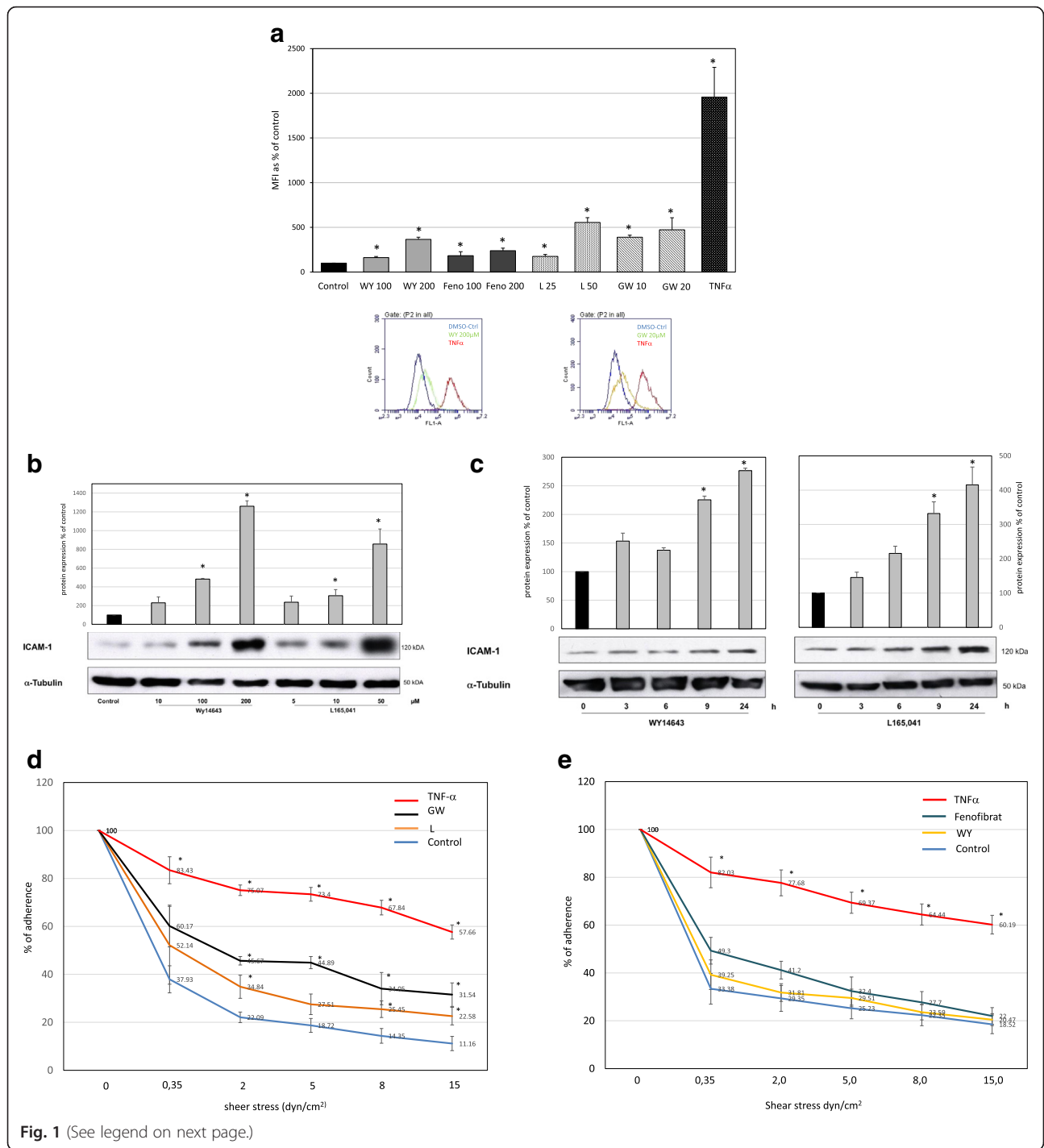
Statistical analyses

The data are expressed as means \pm SEM from at least three independent experiments. Statistical analyses were performed using the ANOVA test.

Results

PPAR α and δ agonists induce ICAM-1 surface and whole cell protein expression in non-stimulated human endothelial cells

We performed FACS analysis to evaluate the effects of the PPAR α agonists fenofibrate and WY14643 as well as the PPAR δ agonists L165041 and GW501516 on the surface expression of ICAM-1 in non-stimulated, quiescent HUVECs. The concentrations of the PPAR agonists were used as published and do not induce any cytotoxicity as previously shown [12, 20, 26–29]. Treatment of non-stimulated HUVECs with each agonist resulted in an induction of ICAM-1 surface expression (Fig. 1a). The level of induction was comparable between the two agonists of each PPAR, indicating that the observed effects are specific to PPAR α and PPAR δ . We also examined the effects of PPAR α and PPAR δ agonists on whole cell protein expression of ICAM-1. Western blot analysis



(See figure on previous page.)

Fig. 1 Effects of PPAR α and δ agonists on the ICAM-1 surface and protein expression as well as function in non-stimulated HUVEC. **a** Flow-cytometric analyses of ICAM-1 expression; HUVECs were left untreated (solvent only) or were treated with different concentrations of PPAR α agonist (WY14643 (100 and 200 μ M) and Fenofibrate (100 and 200 μ M) or PPAR δ agonists (L-165041 (25 and 50 μ M) and GW501514 (10 and 20 μ M)). As positive control TNF α (20 ng/ml) was used. Mean values from triplicate experiments performed four times are depicted \pm SEM. * $p < 0.05$ was considered significant. Exemplary plots for the PPAR α agonist WY14643 and the PPAR δ agonist GW501514 are depicted. **b** Representative western blot analyses of endothelial cells that were left untreated (solvent only) or were treated with WY14643 and L-165041 for 24 h at different concentrations. Comparable results were obtained from three independent experiments. The results were normalized to the expression of tubulin. The relative expression of ICAM-1 is presented in % of control. The mean values from three independent experiments are presented as the mean \pm SEM. * $p < 0.05$. **c** Representative western blot analyses of endothelial cells that were left untreated (solvent only) or were treated with WY14643 (200 μ M) and L-165041 (50 μ M) at different time points. Comparable results were obtained from three independent experiments. The results were normalized to the expression of tubulin. The relative expression of ICAM-1 is presented in % of control. The mean values from three independent experiments are presented as the mean \pm SEM. * $p < 0.05$. **d** Adhesion assay for the functional interaction between T cells (Jurkat) and endothelial cells; HUVECs were left untreated (solvent only) or were treated with PPAR δ agonists for 24 h (L-165041 (50 μ M) and GW501514 (20 μ M)) or **e**) PPAR α agonists for 24 h (Fenofibrate (100 μ M) and WY14643 (200 μ M)). As positive control TNF α (20 ng/ml) was used. Jurkat cells were allowed to adhere to the endothelial cells for 3 min following stepwise increase of shear stress. The number of adherent cells was quantified. The mean values from five independent experiments are presented as the mean \pm SEM. * $p < 0.05$ was considered significant

of whole-cell extracts demonstrated that both PPAR agonists induced ICAM-1 expression in a concentration and time-dependent manner (Fig. 1b,c). To analyze whether the general ICAM-1 induction on endothelial cells has a functional consequence, we performed adhesion assays with the T-cell line Jurkat and PPAR δ and PPAR α agonists treated HUVEC as a proof of concept (Fig. 1d,e). Here we could demonstrate, that the PPAR δ agonist induced ICAM-1 expression elevates the adherence of Jurkat cells significantly whereas PPAR α agonists failed to increase T-cell adherence.

PPAR α and δ agonists induce ICAM-1 steady-state mRNA expression

We examined whether PPAR α and PPAR δ agonists affect the steady-state mRNA levels of ICAM-1 in HUVECs.

Consistent with our protein expression data, treatment with both PPAR α and PPAR δ agonists induced ICAM-1 mRNA expression in a concentration-dependent manner (Fig. 2).

PPAR δ , but not PPAR α agonists upregulate ICAM-1 promoter activity via a Sp1 binding site between -59 and -53 bp

We hypothesized that PPAR α and PPAR δ agonists induce ICAM-1 expression through transcriptional regulation of the ICAM-1 promoter. To test this possibility, a luciferase reporter construct containing all transcription factor binding sites of the ICAM-1 promoter was transiently transfected into vehicle- and agonist-treated HUVECs. Analysis of luciferase expression revealed a significant 1.4-fold induction of ICAM-1 promoter activity in response to PPAR δ agonist. However, PPAR α agonist treatment failed

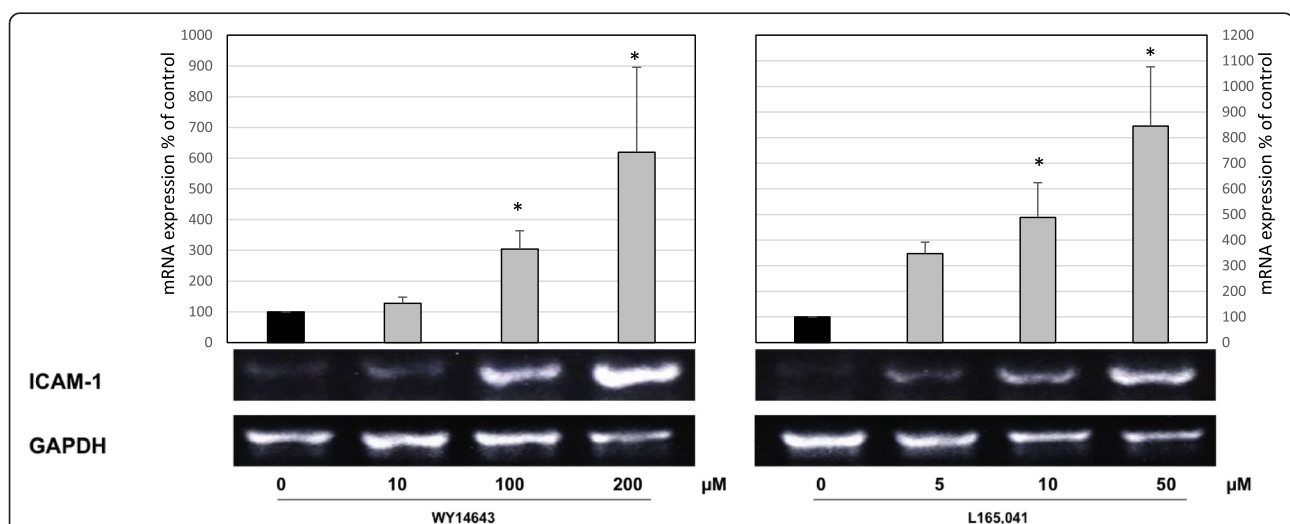


Fig. 2 Effects of PPAR α and δ agonists on the ICAM-1 mRNA expression. RT-PCR analyses of total mRNA extracted from HUVECs that were treated with vehicle (solvent only) or L165041 and WY14643 for varying concentration as indicated for 24 h. Results were confirmed in three independent sets of experiments. The results were normalized to the expression of GAPDH. The relative expression of ICAM-1 is presented in % of control. The mean values from four independent experiments are presented as the mean \pm SEM. * $p < 0.05$

to activate transcriptional activity of the *ICAM-1* promoter (Fig. 3a). To further analyze the underlying mechanisms of PPAR δ agonist induced *ICAM-1* promoter activity a series of *ICAM-1* promoter deletions was introduced into a luciferase reporter construct and transiently transfected into HUVECs (Fig. 3b). Interestingly, the deletion of a Sp1-binding site located between -59/ -53 bp led to a complete loss of PPAR δ agonist induced *ICAM-1* promoter activity. In contrast, constructs containing this site demonstrated a significant induction of *ICAM-1* promoter activity during PPAR δ agonist treatment. Therefore, this single Sp1 site seems to be responsible for PPAR δ agonist conveyed *ICAM-1* promoter activity.

PPAR δ agonists induce SP1 and Sp3 transcription factor binding to the Sp1 binding site at -59/-53 bp

To determine the nuclear factor binding to the Sp1 binding site we performed EMSAs using nuclear extracts from control and PPAR δ agonist treated HUVEC, a wildtype Sp1-oligonucleotide, a mutated Sp1-oligonucleotide and a consensus Sp1 oligonucleotide (Fig. 3c). Here we could demonstrate that the treatment with PPAR δ agonists induces transcription factor (TF) binding to the Sp1 binding site. Via supershift analysis we could show, that not only Sp1 but also Sp3 TFs bind to the wildtype *ICAM-1* Sp-1 binding site between -59/-53 bp. A mutation of the Sp1 binding site abolished the binding of the TFs.

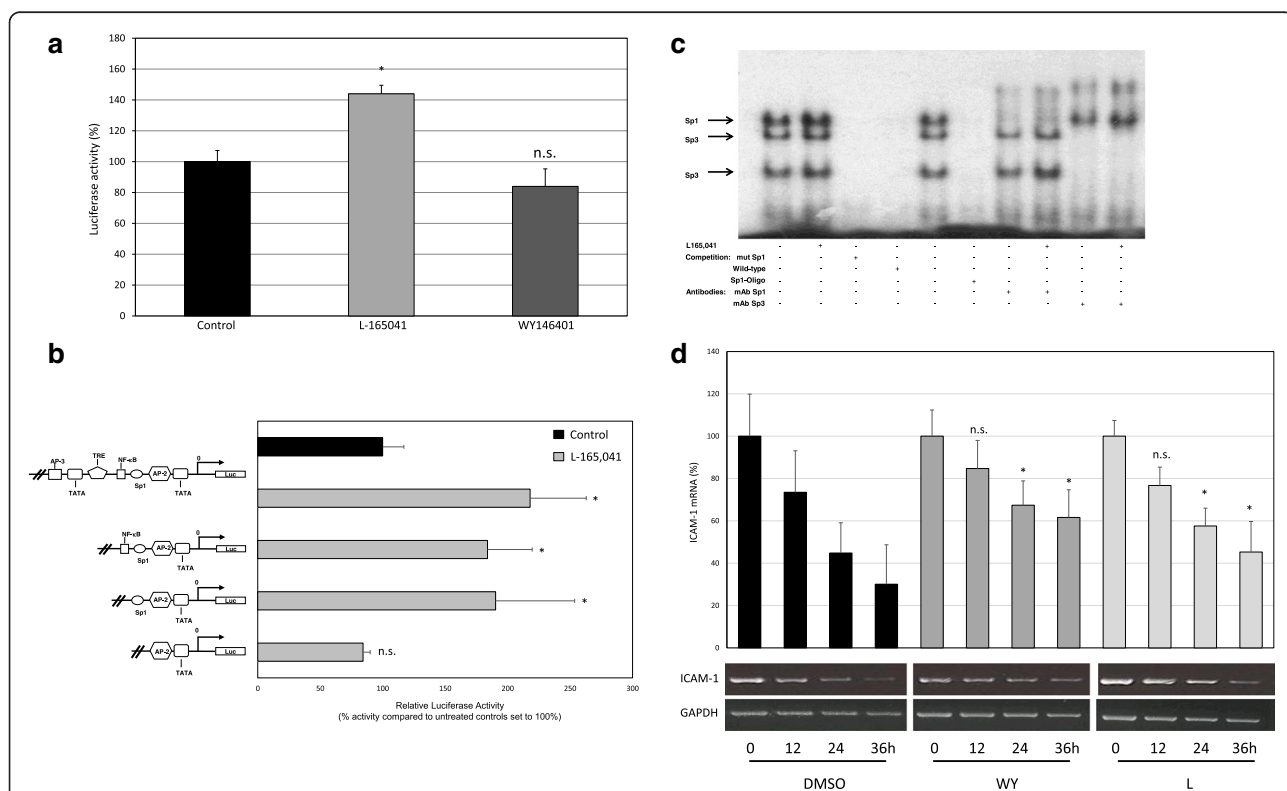


Fig. 3 Effects of PPAR α and δ agonists on the *ICAM-1* promoter activity and mRNA half-life. **a** Analyses of wildtype *ICAM-1* luciferase (Luc) reporter construct in HUVECs. The Luc activity is expressed in percent of control (mean \pm SEM of three independent experiments each performed in triplicate). HUVECs were left untreated (solvent only) or were treated with different concentrations of PPAR α agonist (WY14643 (200 μ M) and PPAR δ agonist (L-165041 (50 μ M)) for 24 h. As positive control TNF α (20 ng/ml) was used. Mean values from triplicate experiments with four independent experiments are depicted \pm SEM. * $p < 0.05$ was considered significant. **b** Analyses of 5'-deletional *ICAM-1* promoter-based luciferase constructs in HUVECs. Schematic representation of the respective reporter gene constructs on the left and the relative Luc activities (expressed as % activity of the control cells) in graphic format on the right. HUVECs were left untreated (solvent only) or were treated with the PPAR δ agonists (L-165041 (50 μ M)) for 24 h. Results were confirmed in four independent sets of experiments. * $p < 0.05$. **c** Representative EMSAs using nuclear extracts of HUVECs that were left untreated (solvent only) or were treated with PPAR δ agonists L-165041 (50 μ M) for 24 h (lane 1,2): mutated labelled Sp1 DNA (lane 3), competition with unlabelled wild-type DNA (lane 4, at 100 molar excess) or with unlabelled excess double-stranded Sp1 consensus oligonucleotides (lane 6, at a final concentration of 0.35 μ M / l). Supershift analyses were performed by addition of specific Sp1 (lane 7 and 8) or Sp3 antibody (lanes 9 and 10, all from Santa Cruz) at a final concentration of 100 ng / ml. Formation of Sp-dependent binding complexes is indicated by arrows to the left. A representative autoradiography from three independent experiments is shown. **d** HUVEC were incubated with vehicle, L165041 (50 μ M) or WY14643 (200 μ M) for 1 h, followed by incubation with fresh media containing Act D (10 μ g/ml) for 0, 12, 24 and 36 h. RT-PCR analyses for *ICAM-1*/GAPDH of total RNA extracted from subconfluent cell cultures were performed. The PCR products were separated by 2 % agarose gel electrophoresis, and ethidium bromide stained bands visualized using an ultraviolet transilluminator. *ICAM-1* bands were quantified by densitometric scanning, the results of which were normalized to amounts of GAPDH mRNA. The mean values from five independent experiments are presented as the mean \pm SEM. * $p < 0.05$

PPAR α and PPAR δ agonists induce *ICAM-1* mRNA stability

ICAM-1 expression is not only controlled on the transcriptional level, but can also be influenced by mRNA stability. We therefore used actinomycin-D, a transcription inhibitor, to determine whether PPAR α or δ agonists treatment increased *ICAM-1* mRNA stability in HUVECs. We found that *ICAM-1* mRNA stability was significantly increased in PPAR α as well as PPAR δ agonist treated cells (Fig. 3d).

Discussion

Insights into the function of PPAR activators have rapidly grown over the past decade. Recently, the impact of PPAR agonists especially in endothelial cell function and angiogenesis has been addressed [27–31]. These findings are critical for uncovering the breadth of PPAR functions in multiple cellular processes as PPAR agonists become increasingly more prevalent in clinical practice. Important pro-inflammatory cytokines, such as TNF α , IL-1 β , and LPS, are known to induce *ICAM-1* expression, and PPAR agonists are capable of potently suppressing these inflammatory effects [32–35]. Therefore, PPAR agonists are promising anti-inflammatory compounds for conditions such as chronic inflammatory diseases, cancer or obesity. However, the effects of PPAR activation in a non-inflammatory setting have not been previously addressed. This is an important consideration as PPAR agonists enter clinical testing for treatment and preventative strategies in various non-inflammatory diseases.

In the current study, we demonstrated that PPAR α and PPAR δ agonists effectively induced *ICAM-1* expression in non-stimulated, quiescent endothelial cells. We identified two mechanisms underlying this effect: a transcriptional promoter-based mechanism for PPAR δ agonists via increased binding of the TF Sp1/Sp3 at the *ICAM-1* promoter between –59/53 bp and a posttranscriptional mechanism mediated by increased mRNA stability for PPAR α and PPAR δ agonists. In addition, we could demonstrate, that the increase in *ICAM-1* surface expression leads to an increased T-cell adherence in PPAR δ treated cells. Therefore, the current work supports the possibility of a dual function of the PPAR α and δ agonists in endothelial cells that is dependent on the activation status of the cell. This novel information may have important implications for the responsiveness of different disease types to treatment with these compounds. The dual action of PPAR agonists may also account for the variable effects of PPAR activation in the treatment and development of distinct tumor entities [36–38]. It is possible that the activation status of the cancer cells or tumor stromal cells, including macrophages, fibroblasts, endothelial cells, and others, may influence PPAR function.

Concerning adipocytes and endothelial cells such a dual mechanism has been already described. Rodriguez-Calvo et al. demonstrated a reduced IL-6 expression after the previously LPS-stimulated adipocytes were treated with the PPAR δ activator GW501516 [39]. The same group also showed an increase of IL-6 mRNA and protein in the control group treated only with the PPAR δ agonist. Chen et al. demonstrated, that PPAR γ agonists significantly induce *ICAM-1* expression in non-stimulated human vascular endothelial cells [19]. In addition, it could be demonstrated that PPAR δ agonists induce IL-6 and IL-8 expression in non-stimulated human endothelial cells [20]. On the other hand Piqueras et al. demonstrated in TNF α stimulated endothelial cells a suppression of the induced *ICAM-1* expression via PPAR δ agonist treatment [18].

Our results further demonstrated that PPAR α and PPAR δ agonists influences *ICAM-1* mRNA stability. The regulation of *ICAM-1* mRNA stability is a well-accepted and important mechanism underlying posttranscriptional control of *ICAM-1* gene expression [40, 41]. PPAR α agonists have already been implicated in regulating mRNA stability of other genes. Ren et al. demonstrated that activation of PPAR α resulted in reduced *nephrin* mRNA stability, and therefore a decrease in *nephrin* expression in kidney epithelial cells [42]. Meissner et al. showed that *IL-8* mRNA stability was increased by PPAR δ agonist treatment [20]. These results demonstrate the various, and likely context-dependent, mechanisms by which PPAR agonists regulate gene expression.

Besides mRNA stability, we could demonstrate that PPAR δ agonists induce promoter activity via increased binding of the TFs Sp1/Sp3 at the Sp1 binding site in the proximal *ICAM-1* promoter. Sp1 is one of the important transcription factors of *ICAM-1* expression in endothelial cells. Brendji-Grün et al., demonstrated in murine aortic endothelial cells that IL-1 β induces *ICAM-1* expression via critical Sp1 binding sites [43]. Kornschnabl et al. showed that the Sp1 site between –59 and –53 bp is essential for the *ICAM-1* induction in cytomegalovirus infected HUVEC [44]. Furthermore, Zhang et al recently demonstrated that melanoma CD44 engagement with endothelial E-selectin leads to the induction of *ICAM-1* via increased binding of the TF Sp1 on the *ICAM-1* promoter [45]. Therefore, Sp1 is an important TF for *ICAM-1* regulation. We could demonstrate that the binding of Sp1/Sp3 on the Sp1 site is essential for PPAR δ induced *ICAM-1* transcriptional expression. Recently, Okazaki et al., demonstrated that PPAR δ agonists increase human SIRT-1 transcription via increased Sp1 promoter binding [46]. Comparable results were demonstrated from Bonofiglio et al., who showed that rosiglitazone, a PPAR γ agonist, induces fas ligand promoter activity via an increased Sp1 promoter binding [47]. Hong et al. demonstrated a comparable

mechanism of PPAR γ dependent p21 increase via Sp1 dependent p21 promoter activity [48].

Interestingly, only the induction of ICAM-1 expression by PPAR δ agonists increased T-cell adhesion. PPAR α agonists failed to increase T-cell adherence. This might be explained by the influence of PPAR α agonists on the expression of further adhesion molecules overriding the effect of ICAM-1. Nevertheless, the induction of ICAM-1 by PPAR α agonists might be relevant in a multi-drug setting. It could be demonstrated that besides the facilitating of leukocyte trans-endothelial migration ICAM-1 can for example bind fibrinogen preventing endothelial cell apoptosis and influencing vasomotorical reactions [49].

Conclusion

Taken together, we have shown that treatment with PPAR δ agonists results in transcriptional and posttranscriptional and the use of PPAR α agonists in posttranscriptional induction of ICAM-1 expression in non-stimulated, quiescent human endothelial cells. Furthermore, the effects of PPAR agonists may depend on the activation status of endothelial cells, and this status may dictate pro-inflammatory versus anti-inflammatory responses. Therefore, these findings may influence the future development and application of PPAR agonists in the clinic.

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Availability of data and material

Not applicable.

Authors' contribution

MM designed the study, performed the data analysis, interpreted and wrote the manuscript. JN acquired the data, performed the data analysis, interpreted and wrote the manuscript. NZ, RK, SK, IH, JK designed the study and interpreted the data. MD, VL, THJ acquired the data, performed the data analysis. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent of publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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