

Congress Abstracts

PPAR alpha/beta/gamma and inflammation

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Scientific Committee: L. Baud, F. Berenbaum, C. Brink

Editors: Michel Chignard and Mustapha Si-Tahar
Unite de Défense Innée et Inflammation,
Inserm E336, Institut Pasteur,
25 rue du Dr Roux,
75724 Paris cedex 15,
France.
Tel: +33 1 45 68 86 88
Fax: +33 1 45 68 87 03
E-mail: chignard@pasteur.fr

Peroxisome proliferator-activated receptor-gamma (PPAR- γ) is a pivotal key for pro-inflammatory effect of leptin on human epithelial colonic cells

Abolhassani¹ M, Marie¹ JC, Ashktorab² H, Bado¹ A and Sobhani¹ I

¹Department of liver and Gastroenterology, Henri Mondor Hospital Créteil and INSERM U410, Bichat Hospital, Paris, France; ²Medicine and Cancer Center, Howard University Hospital, Georgia, Washington, DC, USA

Leptin, the product of the *ob* gene, regulates food intake, energy balance and lymphocyte functions. Receptor mutated animals have reduced production of proinflammatory cytokines. To analyze the direct effects of leptin (0.1 nM – 1 μ M) on cytokine production from colonic cells, we examined pro-inflammatory (IL-8, MCP-1, IL-6 and TNF-alpha) and anti-inflammatory (IL-10) cytokine production in normal human colonic epithelial fresh cells and in colon cancer cell lines (HT-29 and Caco-2) using an ELISA and the Rnase Protection Assay (RPA). Leptin increased the release of IL-8 and MCP-1 and slightly reduced IL-10 release, but did not affect both basal-stimulated and LPS-stimulated IL-6 and TNF-alpha in the supernatant of confluent cells. Up to 24 h after cell exposure to leptin, IL-8 significantly increased and IL-10 decreased, and these effects were dose and time dependently documented in cancer cell lines (HT29 and Caco2). To investigate the mechanisms of this

pro-inflammatory effect, by luciferase reporter gene assay, we showed an increase in the activity of NF-kappaB in response to leptin in a dose-dependent manner. This activity was inhibited in IkappaB super-repressor transfected cells as assessed by luciferase expression and IL-8 and MCP-1 production (ELISA and RPA). Furthermore, PPAR-gamma was reduced after leptin treatment as assessed by western blot while the addition of PPAR agonists (Rosiglitazone and MCC-555) induced a decrease of IL-8 and MCP-1 expression, suggesting PPAR-gamma suppression is required for cytokine productions. The failure of the PPAR-gamma antagonist (GW-9662) to inhibit cytokine production and to alter NF-kappaB activity suggest that PPAR-gamma suppression is required for cytokine productions. We demonstrate that leptin regulates pro-inflammatory cytokine production by colonic epithelial cells via PPAR-gamma modulation.

The PPAR- γ agonist, Rosiglitazone, upregulates the receptor for advanced glycated end-products (RAGE) in human macrophages

Ahluwalia MK, Roberts AW, Evans M, Webb R and Thomas AW

University of Wales Institute, School of Applied Sciences, Western Avenue, Llandaff Campus, Cardiff CF5 2YB, UK

Thiazolidinediones (PPAR- γ agonists, which ameliorate insulin resistance) have also been reported to possess anti-inflammatory properties. One of the most important inflammatory pathways in type 2 diabetes is the interaction of advanced glycated end-products (AGEs) with receptor for AGE (RAGE) in monocytes and macrophages, resulting in induction of inflammatory mediators. In this study we aimed to determine the effect of PPAR- γ agonists on expression of RAGE and subsequent AGE-induced TNF α release in the human macrophage-like cell lines, MM6 and differentiated THP1.

Simultaneous incubation of MM6 cells with Rosiglitazone (2–20 μ M) and AGE (500 μ g/ml) for 5 h significantly reduced AGE-induced TNF α secretion (maximum $33 \pm 3\%$, $p < 0.001$). Paradoxically, if cells were pre-incubated with Rosiglitazone (2–20 μ M) for 24 h prior to 5 h incubation with AGE, TNF α release was elevated by $63 \pm 4\%$

($p < 0.001$). PPAR- γ agonist significantly upregulated RAGE expression in these cells (maximum 2.8-fold increase, $p < 0.05$). Thus, the AGE-induced rise in TNF α correlated with the PPAR- γ agonist-dependent increase in RAGE expression. The mechanism by which the PPAR- γ agonist upregulates RAGE has not yet been fully elucidated but may possibly be due to PPAR- γ -independent phosphorylation events that result in the induction of RAGE-specific transcription factors. We have confirmed these data using the specific PPAR- γ agonist, GW7845, and in THP1 cells.

Our data demonstrating a PPAR- γ -dependent increase in RAGE expression may initially appear to be a pro-inflammatory event. However, RAGE expression in other tissues may provide a favourable mechanism for the removal of AGEs from the circulation and therefore a net anti-atherogenic effect.

Anti-inflammatory potency of PPAR γ ligands in articular cells

Bianchi A, Moulin D, Boyault S, Poleni PE, Bordji K, Netter P, Jouzeau JY and Terlain B

UMR 7561 CNRS-UHP Nancy 1, Laboratoire de Physiopathologie et Pharmacologie articulaire, Faculté de Médecine — BP 184, F-54505 Vandoeuvre les Nancy, France

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. They regulate lipid metabolism, glucose homeostasis, cell proliferation and differentiation and are thought to modulate inflammatory responses.

We studied the expression of PPAR isotypes (α , β , γ) in resident articular cells as well as the anti-inflammatory potency associated with PPAR γ agonists. Most experiments were performed on primary cultures of B synoviocytes (rat) or chondrocytes (rat and human) stimulated with bacterial endotoxin (LPS) or interleukin-1 β (IL-1 β), respectively. Our results showed that all isotypes of these nuclear receptors are constitutively expressed in chondrocytes and synoviocytes as well as in 'articular' adipocytes of the rat fat pad. Inflammatory stimulus (IL-1 β , LPS) decreased PPAR γ expression in all cell types whereas other isotypes remained unaffected. Their stimulating effect on inflammatory gene expression (mPGES, iNOS, COX-2, TNF α and IL-1) and corresponding proteins or mediators was inhibited

unequally by natural (15d-PGJ₂) or synthetic (Troglitazone, Rosiglitazone) PPAR γ agonists.

At similar concentrations, the inhibitory potency of glitazones was less than that observed with 15d-PGJ₂ except for TNF α production in response to LPS in synoviocytes. This result suggested strongly that the anti-inflammatory potency of 15d-PGJ₂ occurred independently from PPAR γ activation. We demonstrated further that 15d-PGJ₂ interacted with the NF- κ B pathway *via* several sites of inhibition, mainly by decreasing I κ B α phosphorylation and I κ B α degradation through the induction of stress proteins (HSP). In contrast, the inhibitory effect of glitazones on TNF α production by synoviocytes was PPAR γ dependent and could be due to the induction of the SOCS-1 (Suppressor of Cell Signalling-1) protein.

These results show that 15d-PGJ₂ inhibit effects of pro-inflammatory cytokines in a PPAR γ -independent manner whereas synthetic PPAR γ agonists inhibit cytokine production in articular cells.

15-Deoxy-delta 12,14-prostaglandin J2 inhibits glucocorticoid binding and signalling in macrophages through a peroxisome proliferator-activated receptor gamma-independent process

Adeline Cheron, Julie Peltier, Joëlle Perez, Agnès Bellocq, Bruno Fouqueray and Laurent Baud

INSERM U489, Hôpital Tenon, 4 rue de la Chine, 75020 Paris, France

15-Deoxy-delta 12,14-prostaglandin J2 (15d-PGJ2) is involved in the control of inflammatory reaction. We tested the hypothesis that 15d-PGJ2 would exert this control in part by modulating the sensitivity of inflammatory cells to glucocorticoids. Human U 937 cells and mouse RAW 264. Seven cells were exposed to 15d-PGJ2 and binding experiments were performed with [³H]-dexamethasone as a glucocorticoid receptor (GR) ligand. 15d-PGJ2 caused a transient and dose-dependent decrease in [³H]-dexamethasone-specific binding to either cell, through a decrease in the average number of GR per cell without significant modification of the K_d value. These changes were related to functional alteration of the GR rather than to decrease in GR protein. They did not require the engagement of peroxisome proliferator-activated receptor gamma (PPAR gamma), since the response to 15d-PGJ2 was neither mimicked by the PPAR gamma agonist ciglitazone

nor prevented by the PPAR gamma antagonist BADGE. 15d-PGJ2 altered GR possibly through the interaction of its cyclopentenone ring with GR cysteine residues since cyclopentenone ring *per se* could mimic the effect of 15d-PGJ2 and modification of GR cysteine residues with MMTS suppressed the response to 15d-PGJ2. Finally, 15d-PGJ2-induced decreases in glucocorticoid binding to GR resulted in parallel decreases in the ability of GR to transactivate [GRE]2 TK-Luc and to reduce the expression of monocyte chemoattractant protein-1 (MCP-1). Together, these data suggest that 15d-PGJ2 limits glucocorticoid binding and signalling in human and murine monocytes/macrophages through a PPAR gamma-independent and cyclopentenone-dependent mechanism. It provides a way by which 15d-PGJ2 would exert pro-inflammatory activities in addition to its known anti-inflammatory activities.

PPAR γ promotes macrophage mannose receptor gene expression by IL-13: consequences in the control of gastrointestinal candidosis in normal and immunodeficient RAG-2^{-/-} mice

Coste A, Authier H, Balard P, Cassaing S, Linas MD, Bernad J, Lepert JC, Dubourdeau M and Pipy B

EA 2405, INSERM IFR 31, Université Paul Sabatier, CHU Rangueil, 31403 Toulouse cedex 4, France

Effective host defense against microbial infection is characterized by rapid recognition and clearance of the pathogen involving increased expression of the pattern-recognition receptors. Thus, macrophage mannose receptor (MMR) is implicated in the recognition of unopsonized microorganisms, which as *Candida albicans* have mannose residues on the cell surface. MMR expression may be critical in phagocytosis process, in host defense functions and in antigen processing. MMR over-expression can be modulated by Th2 cytokines such as interleukin-13 (IL-13). The mechanisms involved in regulation of its expression are little known. We demonstrate in murine macrophages that IL-13 can positively regulate MMR surface expression by controlling the production of PPAR γ endogenous ligands, particularly 15d-PGJ2 via cPLA2 activation. This regulation of MMR, which is also obtained with synthetic

PPAR γ ligands, is PPAR γ dependent. In parallel, this signaling pathway promotes uptake and killing of *Candida* via reactive oxygen intermediates production. *In vivo* study on normal or immunodeficient RAG-2^{-/-} mice validates that the PPAR γ ligands as IL-13 treatments significantly decrease *C. albicans* infection in gastrointestinal tract. This reduction of *Candida* colonization is correlated with the increase of *Candida* phagocytosis and reactive oxygen intermediate production of macrophages *via* MMR induction. These *in vitro* and *in vivo* observations show that PPAR γ and the ligands of this nuclear receptor are involved in the regulation of innate immune response in increasing the expression of MMR. These results let us perceive the possibility that PPAR γ ligands may be of therapeutic value in human diseases due to the pathogens that are eliminated by the MMR.

Role of peroxisome proliferator-activated receptor- γ (PPAR- γ) ligands: the development of acute and chronic inflammation

Cuzzocrea¹ S, Dugo¹ L, Paola¹ R Di, Genovese¹ T, Caputi¹ AP and Christoph Thiemermann²

¹Dipartimento Clinico e Sperimentale di Medicina e Farmacologia, Torre Biologica, Policlinico Universitario, 98123 Messina, Italy; ²The William Harvey Research Institute, St Bartholomew's and The Royal London School of Medicine, London, UK

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors that are related to retinoid, steroid and thyroid hormone receptors. The PPAR- γ receptor subtype appears to play a pivotal role in the regulation of cellular proliferation and inflammation. We postulated that rosiglitazone, a synthetic PPAR- γ -specific agonist, and 15-deoxy- $\Delta^{12,14}$ PGJ₂ (15d-PGJ₂), which is a metabolite of the prostaglandin D₂, and functions as an endogenous ligand for PPAR- γ , would attenuate acute and chronic inflammation. We have investigated the effects of rosiglitazone and 15d-PGJ₂ in animal models of acute and chronic inflammation (carrageenan-induced pleurisy and collagen-induced arthritis, respectively). We report here that rosiglitazone given at (10, 30 or 100 μ g/kg i.p., or at 30 μ g/kg i.p. every 48 h in the arthritis model) and 15d-PGJ₂ (given at 10, 30 or 100 μ g/kg i.p. in the pleurisy model or at 30 μ g/kg i.p. every 48 h in the arthritis model) exerts potent anti-inflammatory effects (e.g. inhibition of pleural exudate formation, mononuclear cell infiltration, delayed development of clinical indicators and histological injury) *in vivo*.

Furthermore, Rosiglitazone and 15d-PGJ₂ reduced; (1) the increase in the staining (immunohistochemistry) for nitrotyrosine and poly (ADP-ribose) polymerase (PARP), and (2) the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in the lungs of carrageenan-treated mice and in the joints from collagen-treated mice. To confirm that the observed anti-inflammatory property of the two PPAR- γ agonists was related to the activation of the PPAR- γ receptor we have carried out a new set of experiments using a selective antagonist for the PPAR- γ (Bisphenol A Diglycidyl Ether). This compound, given (1 mg/kg i.v.) 30 min before rosiglitazone or 15d-PGJ₂ was able to revert the beneficial effect of the PPAR- γ ligand in the acute model of inflammation.

Taken together, our results clearly demonstrate that rosiglitazone and 15d-PGJ₂ treatment exerts a protective effect and part of this effect may be due to inhibition of the expression of adhesion molecules and peroxynitrite-related pathways with subsequent reduction of neutrophil-mediated cellular injury.

Impaired expression of the peroxisome proliferator activated receptor alpha during hepatitis C virus infection

Sébastien Dharancy¹, Mathilde Malapel¹, Gabriel Perlemuter², Tania Roskams³, Yang Cheng¹, Philippe Podevin⁴, Filoména Conti⁴, Valérie Canva¹, Luc Gambiez¹, Philippe Mathurin¹, Jean-Claude Paris¹, Kristina Schoonjans⁵, Yvon Calmus⁴, Stanislas Pol⁶, Johan Auwerx⁵ and Pierre Desreumaux¹

¹Equipe Mixte INSERM 0114, Centre Hospitalier Universitaire (CHU), 59037 Lille, France; ²INSERM U 370, Faculté de Médecine Necker-Enfants Malades, 75015 Paris, France; ³Department of Morphology and Molecular Pathology, KU Leuven, B-3000 Leuven, Belgium; ⁴Laboratoire de Biologie Cellulaire, Faculté de Médecine Cochin Port-Royal, 75014 Paris, France;

⁵Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), INSERM, CNRS, Université Louis Pasteur, 67404 Illkirch, France; ⁶Service de Hépatologie, Hôpital Necker-Enfants Malades, 75015 Paris, France

Background and aims: Liver inflammation and fibrosis are common features in patients with chronic hepatitis C virus (HCV) infection. Since the heterodimer peroxisome proliferator activated receptor (PPAR)alpha/retinoid X receptor (RXR) is expressed in the liver and is involved in the regulation of metabolism and inflammation, we studied its hepatic expression and function during liver injury in patients with HCV infection.

Methods: PPAR alpha/RXR mRNAs were quantified in liver biopsies of 46 untreated patients with HCV infection and compared with 40 controls. Heterodimer levels were analysed according to the intensity of liver inflammation/fibrosis. To determine whether HCV may influence PPAR alpha expression in the liver, we also quantified PPAR alpha and its liver target gene CPT1A in human hepatocellular carcinoma cell line stably expressing HCV core protein.

Results: Hepatic levels of RXR mRNA were similar in patients and controls. Using real-time PCR and western blot, levels of PPAR alpha and CPT1A mRNA and protein were significantly lowered in the liver of untreated patients with HCV infection compared with controls, and inversely associated with inflammatory and fibrosis scores. Similarly, an impaired expression of PPAR alpha and CPT1A mRNAs were found in hepatocytes stably expressing HCV core protein and activated with the PPAR alpha ligand fenofibric acid.

Conclusions: Impaired expression of the anti-inflammatory nuclear receptor PPAR alpha in the liver of patients with hepatitis C virus infection may be involved in the pathogenesis of HCV infection. This decreased transcriptional activity of PPAR alpha may be influenced at least in part by HCV core protein expression. Taken together, these results suggest that PPAR alpha activators may be an alternative to the traditional therapeutic approaches of HCV-induced liver injury.

Impaired expression of the peroxisome proliferator-activated receptor gamma in patients with ulcerative colitis: roles of the Toll-like receptor 4

Laurent Dubuquoy¹, Emmelie Jansson², Samir S. Deeb³, Jean-Frédéric Colombel¹, Johan Auwerx⁴, Sven Pettersson² and Pierre Desreumaux¹

¹Equipe INSERM 0114, CHU Lille, France; ²Karolinska Institutet, Stockholm, Sweden; ³University of Washington, Seattle, WA, USA; ⁴Institut de Génétique et Biologie Moléculaire et Cellulaire, Illkirch, France

Background: The peroxisome proliferator-activated receptor gamma is a nuclear receptor highly expressed in the colon and playing a central role in the regulation of colitis through attenuation of the NF- κ B pathway.^{1,2} NF- κ B is a transcription factor regulated by Toll-like receptors (TLRs), which are receptors for bacteria. TLR-4 is overexpressed by epithelial cells in patients with inflammatory bowel diseases (IBD).³

Aim: (1) To study the possible crosstalk between TLR-4 and PPAR gamma and (2) to evaluate the expression and genetic polymorphism of PPAR gamma in patients with Crohn's disease (CD) and ulcerative colitis (UC).

Methods: (1) Transfections of Caco-2 cells with TLR-4, PPAR gamma or the response element of PPAR (PPRE) were used to assess the regulation of PPAR gamma by TLR-4; (2) PPAR gamma mRNA and protein were quantified by competitive PCR, western blot and immunohistochemistry in peripheral blood mononuclear cells (PBMC), healthy and inflamed colon biopsies of 122 patients with IBD (53 UC and 69 CD) compared with 60 controls. The polymorphism of the PPAR gamma gene and promoter was studied in 53 patients with UC compared with 1158 controls.

Results: (1) Activation of TLR-4 transfected Caco-2 cells with LPS upregulated the expression of PPAR gamma and

activated the PPRE; (2) PPAR gamma concentrations were similar in PBMC of patients with UC, CD and controls. Expression of both PPAR gamma mRNA and protein was significantly decreased in the healthy and inflamed colon mucosa in UC as compared with CD and controls. No mutation of the PPAR gamma gene or its promoter was found in UC or controls.

Conclusion: PPAR gamma expression and activation is regulated by the TLR-4 and may be a key regulator of mucosal inflammation induced by bacteria. PPAR gamma expression is dramatically decreased in the colonic mucosa of patients with UC. A mucosal imbalance between TLR-4 and PPAR gamma may thus lead to colon inflammation induced by flora in patients with UC.

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A PPAR alpha-dependent pathway increases IL-1ra production and protect from IL-1beta detrimental effects in articular chondrocytes

Mathias François, Pascal Richette, François Rannou, Lydia Tsagris and Marie-Therese Corvol

UMR-S 530 Inserm-Paris5, 45 rue des St Peres 75006 Paris, France

Cytokines, such as interleukin-1beta (IL-1beta), induce cartilage degradation and inflammatory process via metalloproteinases (MMPs) and COX2 gene expression in chondrocytes. We used Clofibrate (CloF), an activator of peroxysome proliferator-activated receptor alpha (PPAR-alpha), to assess the influence of PPAR-alpha on IL-1beta-mediated gene expression. By using northern blot analysis, electrophoretic mobility shift assay (EMSA) and transient transfection assay PPAR-alpha was shown to be expressed and functional in chondrocytes *in vitro*. CloF (50–500 μ M) displayed concentration-dependent inhibitory effects on IL-1beta-induced degradative and inflammatory markers. CloF inhibited IL-1beta-induced decrease in ³⁵S-sulfated proteoglycan production. CloF also counteracted gelatinolytic activities and down-regulated MMP and COX2 mRNA expression induced by IL-1beta. CloF maximized the endogenous production of IL-1 receptor antagonist (IL-1ra) induced by IL-1beta in chondrocytes, while CloF

alone had no effect. The mechanism of action of CloF effects was then explored in detail on IL-1ra gene expression. In transiently transfected chondrocytes, IL1beta-induced p1680 IL-1ra-Luciferase activity was enhanced by addition of CloF in a dose-dependent manner, indicating a transcriptional effect. Moreover, chondrocytes co-transfected with p1680 IL-1ra-Luc and a wild-type PPAR-alpha showed an enhanced response to CloF treatment in the presence of IL-1beta, while co-transfection with a dominant negative PPAR-alpha led to the loss of CloF effect. Two PPAR responsive elements (PPRE) were identified in the IL-1ra promoter, with binding activity to PPAR-alpha *in vitro* (EMSA). Mutations of this PPRE sites suppressed CloF stimulatory effects. Our data support a new PPAR-alpha-dependent inhibitory mechanism on IL-1beta-induced gene expression, which might act through maximization of IL-1ra production in chondrocytes.

A peroxysome proliferator-activated receptor gamma-dependent pathway mediates rosiglitazone inhibition of cytokine-induced metalloproteinase 1 expression in articular cartilage cells

Mathias François¹, Pascal Richette¹, Lydia Tsagris¹, Michel Raymondjean², Marie-Claude Fulchignoni-Lataud¹, Jean-François Savouret¹, Claude Forest¹ and Marie-Thérèse Corvol¹

¹Institut National de la Santé et de la Recherche Médicale (INSERM) UMR-S-530, Université Paris 5, UFR Biomédicale, 45 rue des Saints Pères, 75006 Paris, France; ²CNRS UMR 7079, 7 Quai Saint Bernard, 75005 Paris, France

Cytokines, such as interleukin-1beta (IL-1beta), induce cartilage degradation via metalloproteinase (MMP) gene expression by chondrocytes. We used Rosiglitazone (Rtz) to assess the influence of peroxysome proliferator-activated receptor gamma (PPAR-gamma) on IL-1beta-mediated regulations. We showed, using immunocytochemistry, electrophoretic mobility shift assay (EMSA) and transient transfection assays, that PPAR-gamma was expressed and functional in chondrocytes *in vitro*. Rtz displayed differential concentration-dependent effects. At 10 µM, Rtz inhibited IL-1beta-induced NO production and COX₂ mRNA expression. Rtz also inhibited IL-1beta-induced decrease in ³⁵S-sulfated proteoglycan production and gelatinolytic activities, and down-regulated MMP mRNA expression. When used at concentrations closer to Kd values, Rtz did not modify NO production nor COX₂ mRNA expression, whereas it maintained its anticytokine effects on proteoglycan production and MMP gene expression. The mechanism of action of Rtz anti-IL1beta effect was then

explored on MMP1 gene expression. In transiently transfected chondrocytes, IL1beta-induced pMMP1-Luciferase activity was inhibited by low concentrations of Rtz, indicating a transcriptional effect. EMSA analysis showed that Rtz did not modify IL-1beta-induced NF-κB complex binding at cognate DNA sites while AP-1 binding was reduced. The inhibitory effect of Rtz was PPAR-gamma dependent since it was increased by overexpression of wild-type PPAR-gamma and suppressed by a dominant negative PPAR-gamma. A PPAR responsive element (PPRE) was identified in the MMP1 promoter. Mutations of this PPRE site suppressed Rtz inhibitory effects. Our data support a new PPAR-gamma-dependent inhibitory mechanism on IL-1β-induced MMP1 expression, which acts through a composite PPRE/AP-1 site at a position (– 83 to – 71) upstream from the transcription start site.

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The PPAR_γ agonist pioglitazone reduces inflammation and Aβ_{1–42} levels in APP V717I transgenic mice

Michael T. Heneka, Magdalena Sastre, Lucia Dumitrescu-Ozimek, Anne Kreutz, Ilse Dewachter, Kerry O'Banion, Thomas Klockgether, Fred Van Leuven and Gary E. Landreth

Department of Neurology, University of Bonn, Bonn, Germany

Alzheimer's disease (AD) is characterized by deposition of β-amyloid in the brain and an associated glia-mediated inflammatory response. Non-steroidal anti-inflammatory drugs (NSAIDs) dramatically reduce AD risk and microglial reactivity. NSAIDs bind to and activate the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR_γ), which acts to inhibit the expression of pro-inflammatory genes. Recent *in vitro* data suggested that NSAIDs negatively regulate immunostimulated APP processing via PPAR_γ activation. In line with this result, we show that an oral seven day treatment of 10-month-old APP-V717I mice with the PPAR_γ-specific agonist pioglitazone or the NSAID ibuprofen resulted in a reduction of

activated microglia and reactive astrocytes in the hippocampus and cortex. Drug treatment reduced the expression of the inflammatory markers COX2 and iNOS. In parallel with the suppression of inflammatory markers, pioglitazone and ibuprofen administration decreased BACE-1 mRNA, protein and activity, and led to a significant reduction of focal Aβ_{1–42} deposits in the hippocampus and cortex. Furthermore, treatment with pioglitazone decreased the levels of soluble Aβ_{1–42} peptide by 27%. These findings demonstrate that anti-inflammatory drugs and PPAR_γ agonists inhibit inflammatory responses in the brain and modulate amyloidogenesis.

Anti-inflammatory and antiproliferative actions of peroxisome proliferator-activated receptor- γ (PPAR γ) agonists on T-lymphocytes in multiple sclerosis patients and healthy controls

Luisa Klotz, Stephan Schmidt, Martina Schmidt, Magdalena Sastre, Thomas Klockgether and Michael T. Heneka

Department of Neurology, University of Bonn, Bonn, Germany

Peroxisomal proliferator-activated receptors (PPARs) belong to the superfamily of nuclear receptors and act as ligand-activated transcription factors. The PPAR γ isoform is expressed in human T lymphocytes, and oral administration of PPAR γ agonists ameliorates the clinical course and histopathological features of experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis (MS), suggesting a potential role for PPAR γ agonists in the treatment of MS. In order to assess a potential therapeutic role of PPAR γ agonists in MS, we compared the immunomodulatory effects of the thiazolidinediones (TZD), pioglitazone and ciglitazone, and the non-TZD agonist GW347845 on human T-leukemia cells (Jurkat cells) and phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs) derived from 21 MS patients and 12 healthy controls. Pioglitazone, ciglitazone and GW347845 suppressed PHA-induced T-cell proliferation and secretion of interferon- γ and tumor necrosis factor- α .

Inhibition of proliferation and proinflammatory cytokine secretion was more pronounced when PBMCs were first pre-incubated with PPAR γ -agonists and re-exposed at the time of PHA stimulation, indicating a sensitizing effect of PPAR γ agonists. In order to assess the molecular mechanisms of PPAR γ -mediated anti-inflammatory effects, we analyzed the PPAR γ expression levels in PBMCs of MS patients and healthy controls. Surprisingly, all patients exhibited decreased PPAR γ expression levels compared with controls, and PHA stimulation of PBMCs in healthy controls resulted in a significant decrease of PPAR γ expression. Pre-incubation of PBMCs with PPAR γ agonists almost completely prevented this inflammation-induced decrease in PPAR γ expression, suggesting that the anti-inflammatory and antiproliferative effects of PPAR γ agonists may result at least in part from an inhibition of inflammation-induced decrease of PPAR γ expression levels.

PPAR beta has an important role in promoting preservation of kidney function during acute ischemic renal failure

Emmanuel Letavernier¹, Joëlle Perez¹, Elisabeth Joye², Julie Peltier¹, Agnès Bellocq¹, Bruno Fouqueray¹, Jean-Philippe Haymann¹, Béatrice Desvergne², Walter Wahli² and Laurent Baud¹

INSERM U 489, Hôpital Tenon, 4 rue de la Chine, F 75020 Paris¹; Centre Intégréatif de Génomique, Université de Lausanne, CH 1015 Lausanne, Switzerland²

Epithelial cells from the proximal tubule express both PPAR alpha and PPAR beta. In ischemic acute renal failure, PPAR alpha expression and activation afford a protection against necrosis process. Whether PPAR beta exerts a similar role is still unknown. To answer this question, we analyzed the renal dysfunction and injury caused in mice by 30 min ischemia and 24/48 h reperfusion, using both a pharmacological and a genetic approach. Administration of the specific PPAR beta agonist L-165 041 limited tubular necrosis and protected kidney function as evidenced by a significant decrease in plasma creatinine levels (26.0 ± 2.1 versus 44.4 ± 6.4 μ M). Conversely, PPAR beta^{-/-} and PPAR beta^{+/-} mice exhibited significantly greater kidney dysfunction, as assessed by enhanced tubular apoptosis/necrosis and higher plasma creatinine levels (88.0 ± 12.5 and 70.3 ± 10.5 , respectively versus 39.5 ± 5.8 μ M). To

identify the mechanisms whereby PPAR beta exerts a cytoprotection, we analyzed *in vitro* epithelial cells from proximal tubule (HK-2 cells) exposed to different PPAR beta ligands. Neither L-165 041 nor cPGI2 (0.03–1 μ M) affected cell proliferation and viability under control conditions, but both PPAR beta ligands protected HK-2 cells from oxidative stress (0.5 mM H₂O₂ for 24 h) in a dose-dependent manner. This response involved the control of Akt signaling since: (1) L-165 041 increased phosphorylated Akt cell content and (2) L-165 041 effect on HK-2 cell apoptosis/necrosis was completely prevented by the addition of Akt inhibitor. In conclusion, the present study demonstrates that PPAR beta is potentially involved in the repair process of tubular epithelium. This role involves antiapoptotic signals, including Akt activation.

The PPAR ligand, conjugated linoleic acid, reduces expression of the receptor for advanced glycosylated end-products (RAGE) in monocytic cells

Thet-Thet Lin, N. Singh, R. Al-Nasri, A.W. Thomas and K. Morris

School of Applied Sciences, UWIC, Western Avenue, Cardiff CF5 2YB, UK

Conjugated linoleic acid (CLA), a dietary fatty acid, is a natural ligand for peroxisome proliferator-activated receptors (PPARs). Increasingly, CLA has received considerable attention due to its important anti-tumour and anti-inflammatory effects. CLA is a mixture of mainly cis-9, trans-11 and trans-10, cis-12 isomers and these species are known to bind to both PPAR-alpha and gamma. It has also been observed that PPARs have an important role in regulating the inflammatory response in monocytic cells treated with advanced glycosylated end-products (AGEs). We have previously shown in the human monocytic cell line Monomac 6 (MM6), that incubation of CLA at a concentration of 60 μ M, for 3 and 24 h, was able to significantly suppress AGE-induced secretion of the inflammatory cytokines, TNF- α and IL-1 β . We therefore suspected that

CLA may be able to affect (AGE) induced inflammatory activity by regulating RAGE expression. MM6 cells were treated with CLA at 60 μ M for 3 and 24 h and their RAGE expression determined by western blotting. After 3 h incubation with CLA, RAGE expression in MM6 cells was reduced by 22% (range 18–26%), and by 26% after 24 h (range 22–30%), as compared with cells not treated with CLA. This reduction in RAGE expression was found to be significant ($p < 0.01$) when the data was analysed by ANOVA. The mechanism by which CLA down-regulates RAGE has not been fully elucidated, but may be in part due to its PPAR ligand properties of this fatty acid. Furthermore, these processes may be important in regulating inflammation in monocytic cells.

Anti-inflammatory effects of peroxysome proliferator-activated receptor gamma in CCl₄-induced hepatitis

Mathilde Malapel¹, Sébastien Dharancy¹, Tania Roskams², Johan Auwerx³, Philippe Mathurin¹ and Pierre Desreumaux¹

¹Laboratoire INSERM 0114, CHU Lille, France; ²Laboratoire d'Anatomie Pathologique, Université de Louvain, Belgique;

³Institut de Génétique et Biologie Moléculaire et Cellulaire, Université Louis Pasteur, Illkirch, France

Introduction: PPAR gamma is a nuclear receptor mainly expressed in colon and fat adipose tissue. It controls many physiologic processes such as lipid storage, glucid metabolism and inflammation through regulation of inflammatory cytokine production and inhibition of the NF- κ B pathway. The levels of PPAR gamma in the liver remain still unknown and the potential anti-inflammatory effects of PPAR gamma activators *in vivo* in the liver have not yet been investigated.

Aim and methods: To quantify PPAR gamma mRNA levels in different human tissues and to evaluate the involvement of PPAR gamma in a murine model of CCl₄-induced acute hepatitis (1 ml/kg). PPAR gamma was quantified by quantitative PCR in human blood mononuclear cells, pancreas, ileum, skin, liver kidney, colon and adipose tissues. We tested whether PPAR gamma heterozygous mice (PPAR gamma^{+/-}) were more sensitive to CCl₄-induced liver damage than wild type littermates (WT). We also evaluated the anti-inflammatory effects of a PPAR gamma agonist (pioglitazone, 50 mg/kg/day) administered preven-

tively 2 days before hepatitis induction in Balb/c mice. The severity of hepatitis was assessed by mortality rates and evaluation of histologic lesions using the necro-inflammatory score (Ishak score).

Results: PPAR gamma mRNA were highly expressed in human liver at similar levels to kidney and skin. Compared with wild type animals, PPAR gamma^{+/-} mice displayed a significantly enhanced susceptibility to CCl₄-induced acute hepatitis, reflected by intense hepatic lesions (10.6 versus 4) leading to a 50% mortality rate 2 days after CCl₄ administration. Similar results were provided using the PPAR gamma activator pioglitazone, which reduced significantly the mortality rate and the liver injury in treated mice compared with untreated mice with hepatitis.

Conclusion: This study demonstrates that PPAR gamma is highly expressed in human liver and is a new factor involved in the regulation of hepatic inflammation. These data suggest that PPAR gamma activators may be use as new therapeutic agents in liver injury.

Effect of conjugated linoleic acid, a natural PPAR ligand, on cytokine production in normal human cells

G. Martinasso, M. Maggiora, A. Trombetta, G. Gassino, R.A. Canuto and G. Muzio

Dipartimento di Medicina ed Oncologia Sperimentale, Università degli Studi di Torino, Corso Raffaello 30, 10125 Torino, Italy

The therapeutic protocol for head and neck cancer requires, in most cases, surgical exeresis of the neoplasm. This procedure can determine serious functional and aesthetic alterations, and it must be followed by reconstructive surgery, using a skin graft. Since reconstructive surgery can restore only a part of the anatomical defect, implant methods are of fundamental importance in restoring entirely it. However, intraoral implants surrounded by skin graft tissue have a low percentage of success, for inflammation processes and rejection of the implant. Our research examined *in vivo*, on biopsies of skin present around the implants, the modifications of cytokine and PPAR content in order to find substance(s) able to reduce the inflammation process improving the implant success. In the patients showing implant failure, an increase of pro-

inflammatory cytokines (TNF α , IL-1 β , IL-6, IL-8) was evident, coupled with an increase of PPAR β expression. This observation was confirmed *in vitro* by treating cultured normal epithelial cells (NCTC 2544) with lipopolysaccharide. Studies investigating the effect of conjugated linoleic acid, a PPAR ligand, on the content of cytokines and PPAR are currently being carried out on NCTC 2544 cells and fibroblasts isolated from the same biopsies used to determine the cytokine content. Preliminary data on fibroblasts showed that conjugated linoleic acid is able to reduce the content of pro-inflammatory cytokine, IL- β .

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15d-PGJ2 inhibits IL-1beta-induced PGE2 production in chondrocytes via a PPARgamma-independent pathway

Véronique Meynier de Salinelles¹, C. Salvat¹, M. Goldring², M. Raymondjean¹ and F. Berenbaum¹

¹UMR 7079 CNRS/Paris 6, 7 quai Saint-Bernard, 75005 Paris, France; ²Rheumatology Division, Beth Israel Deaconess Medical Center, and New England Baptist Bone & Joint Institute, Harvard Institutes of Medicine, Boston, MA, USA

The cyclopentenone 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) has been shown to have anti-inflammatory effects in different models. Prostaglandin E2 (PGE2) is involved in many biological processes and is overproduced in inflammatory conditions. The pro-inflammatory interleukin-1beta (IL-1beta) is a potent mediator of cartilage degradation and increases PGE2 production, partly through inducing secreted phospholipase A2 type IIA (sPLA2 IIA) and cyclooxygenase-2 (COX-2) expressions in chondrocyte. In this study, we investigated whether 15d-PGJ2 was able to inhibit IL-1beta-stimulated PGE2 production and type 1 microsomal PGE2 synthase (mPGES-1) in human chondrocytes. 15d-PGJ2, at the micromolar level, suppressed PGE2 production in a time-dependent and dose-dependent manner, both in rabbit primary cultured chondrocytes and in T/C28a2 human chondrocyte cell line.

RT-PCR showed that, in the T/C28a2, mPGES-1 expression was stimulated by IL-1beta and that 15d-PGJ2 inhibited this IL-1beta-induced expression. We also provide evidence that 15d-PGJ2 effect is independent from peroxylsome proliferator-activated receptor γ (PPAR γ) activation since rosiglitazone, a ligand for PPAR γ , was unable to inhibit IL-1beta-induced mPGES-1 expression. Moreover, we show that 15d-PGJ2 reduced IL-1beta-stimulated sPLA2-IIA activity, suggesting that 15d-PGJ2 would also have the ability to decrease the quantity of AA provided to the COX-2/mPGES-1 enzymatic pathway. Taken together, these results suggest that 15d-PGJ2 at pharmacological concentration should have anti-inflammatory and/or anti-degradative properties in arthritic conditions such as osteoarthritis or rheumatoid arthritis.

Variations in the genes encoding the peroxisome proliferator-activated receptors α and γ in psoriasis

Rotraut Mössner¹, Rolf Kaiser², Philipp Matern², Ullrich Krüger¹, Götz A. Westphal³, Jürgen Brockmüller², Andreas Ziegler⁴, Christine Neumann¹, Inke R. König⁴ and Kristian Reich¹

¹Department of Dermatology, Georg-August-University, Von-Siebold-Strasse 3, D-37075 Göttingen, Germany; ²Department of Clinical Pharmacology, Georg-August-University, Robert-Koch-Strasse 40 D-37075 Göttingen, Germany; ³Department of Occupational Health, Waldweg 37, D-37073 Göttingen, Germany; ⁴Institute of Medical Biometry and Statistics, University at Lübeck, Ratzeburger Allee 160, Haus 4, D-23538 Lübeck, Germany

The three peroxisome proliferator-activated receptor (PPAR) subtypes α , β (or δ), and γ belong to the group of nuclear receptors that act as ligand-activated transcription factors. Recently, expression of PPAR α and PPAR γ in keratinocytes has been demonstrated, and ligands of PPAR α and PPAR γ were found to enhance epidermal maturation and protect against cutaneous inflammation. There is first evidence for a possible role of PPARs in psoriasis, as expression of PPAR α and PPAR γ is decreased in lesional skin and treatment with PPAR γ agonists improves psoriatic

keratinocyte pathology *in vitro* and *in vivo*. We performed a case-control study to search for possible associations between variations in the genes encoding PPAR α and PPAR γ and psoriasis. Seven variations in these genes were analyzed in 192 patients with chronic plaque-type psoriasis and 330 healthy controls by PCR-based methods. No association between any of the investigated PPAR variants and psoriasis was found. Our findings argue against a significant contribution of the investigated PPAR variations to the genetic basis of psoriasis.

PPAR-alpha decreases airway inflammation in a model of asthma

Carine Orthez¹, Julien Becker¹, Johan Auwerx², Nelly Frossard¹ and Françoise Pons¹

¹EA3771 'Inflammation et environnement dans l'asthme', Faculté de Pharmacie, Illkirch, France; ²Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France

We have addressed the role of PPAR-alpha in inflammation associated with asthma using both PPAR-alpha^{-/-} versus PPAR-alpha^{+/+} mice, and mice treated with PPAR-alpha agonists. Naive PPAR alpha^{-/-} mice exhibited no difference in total and differential cell counts in bronchoalveolar lavage (BAL) fluids, as compared with PPAR-alpha^{+/+} animals. Histological examination of their airways confirmed the absence of inflammation, and showed no tissue remodeling. Upon allergen sensitization and challenge with ovalbumin (OA), PPAR-alpha^{-/-} mice displayed, however, increased total cell number and eosinophilia (1.6-fold and 2.1-fold, respectively, $p < 0.05$) in BAL fluids, and greater peribronchial and perivascular eosinophil infiltrates in tissue, as compared with sensitized and challenged PPAR-alpha^{+/+} animals. PPAR-alpha^{-/-} mice also exhib-

ited increased levels of the pro-inflammatory cytokines TNF- α and IL-5 (1.9-fold and 1.8-fold, respectively) in BAL fluids. Conversely, allergen sensitized and challenged C57BL/6 mice treated with the PPAR-alpha agonist, fenofibrate (5–500 mg/kg, p.o.) displayed a dose-dependent decrease in total cells and eosinophilia in BAL fluids. BAL eosinophilia was also significantly reduced in mice that received another fibrate, ciprofibrate. In animals treated with 500 mg/kg fenofibrate, reduction in eosinophilia reached 70% ($p < 0.001$), as compared with 90% in mice treated with dexamethasone (5 mg/kg p.o.). Levels of TNF- α and IL-5 in BAL fluids were also significantly reduced (by 63% and 46%, respectively). Our data provide evidence of a beneficial and potent effect of PPAR-alpha activation on airway inflammation associated with asthma.

Oxidized low-density lipoprotein inhibit trophoblastic cell invasion: implication of PPAR γ

Pavan¹ L, Tsatsaris¹ V, Hermouet¹ A, Therond² P, Evain-Brion¹ D and Fournier¹ T

¹INSERM U427 and ²Laboratoire de Biochimie, Faculté des Sciences Pharmaceutiques et Biologiques, Université René Descartes, Paris 5, 75006 Paris, France

Human implantation involves a major invasion of the uterine wall and remodelling of the uterine arteries by trophoblastic cells. Abnormalities in these early steps of placental development lead to placental ischemia, foetal growth defects, and are frequently associated with pre-eclampsia, a serious disease specific to human pregnancy. Lipid metabolism is altered during normal human pregnancy, with low-density lipoproteins (LDL) becoming more susceptible to oxidation. The aim of this study was to localize oxidized LDL (oxLDL) at the implantation site and to investigate the role of oxLDL in human trophoblast invasion *in vitro*. We showed by immunohistochemistry that oxLDL was present in trophoblastic cells involved in

invasion. Incubation of these primary invasive cells with oxLDL showed a concentration-dependent inhibition in cell invasion, whereas non-oxidized LDL had no effect. oxLDL-mediated effect was abrogated by pre-incubation of cells with a pan-RXR antagonist. These results and our recent report that PPAR γ is involved in the control of trophoblast invasion suggest that human trophoblastic cell invasion may be modulated by oxLDL *in vivo* through activation of PPAR γ /RXR heterodimers, and provide new insights into the pathophysiology of pre-eclampsia associated with oxidative stress, excessive lipid peroxidation and defective trophoblast invasion.

Multiple mechanisms involved in the effects of the PPAR agonist 15-deoxy-Delta12,14-prostaglandin J2 in mesangial cells

Francisco J. Sánchez-Gómez, Eva Cernuda-Morollón, Konstantinos Stamatakis and Dolores Pérez-Sala

Departamento de Estructura y Función de Proteínas, Centro de Investigaciones Biológicas, C.S.I.C., Ramiro de Maeztu, 9, 28040 Madrid, Spain

Mesangial cells (MC) are key players in the inflammatory and proliferative diseases of the renal glomerulus. These cells express PPAR α , β and γ forms, and agonists of these receptors have been shown to modulate the response of mesangial cells to pro-inflammatory stimuli. We have observed that cytokine stimulation of MC increases the generation of prostaglandins of the J series, like 15-deoxy-Delta12,14-prostaglandin J2 (15d-PGJ2), as detected by enzyme immunoassay of the cell culture supernatants. The increase of 15d-PGJ2 is delayed with respect to the onset of COX-2 expression and is inhibited by indomethacin. 15d-PGJ2 is an agonist of the transcription factor PPAR γ as well as a reactive electrophile due to its cyclopentenone structure. Incubation of MC in the presence of 15d-PGJ2 at concentrations above 1 micro-

molar leads to the activation of PPAR, as measured with a PPRE reporter construct, and increases the expression of PPAR target genes. In addition, 15d-PGJ2 forms covalent adducts with a number of cellular proteins distributed both in nuclear and cytosolic compartments. Protein modification by exogenous 15d-PGJ2 can be detected using nanomolar concentrations of a biotinylated 15d-PGJ2 analog. Covalent binding of 15d-PGJ2 to cellular proteins may modulate transcription factor activity by direct or indirect mechanisms. We have observed that 15d-PGJ2 directly binds to components of NF- κ B and AP-1 transcription factors, an effect that is associated with inhibition of DNA binding. The contribution of these various mechanisms to the modulation of gene expression in MC will be discussed.

Peroxisome proliferator-activated receptor- γ (PPAR γ) agonists modulate immunostimulated processing of APP through regulation of β -secretase (BACE)

Magdalena Sastre, Ilse Dewachter, Evan Rosen, Gary Landreth, Timothy M. Willson, Thomas Klockgether, Fred van Leuven and Michael T. Heneka

Department of Neurology, University of Bonn, Bonn, Germany

Prolonged intake of non-steroidal anti-inflammatory drugs (NSAIDs) reduces the risk for Alzheimer's disease (AD). To determine the mechanisms by which inflammation affects AD and how NSAIDs protect against it, we stimulated N2a neuroblastoma cells stably transfected with amyloid precursor protein (APP) with pro-inflammatory cytokines, which increased the secretion of amyloid- β (A β) and APP ectodomain. Addition of ibuprofen, indomethacin, peroxisome proliferator-activated receptor- γ (PPAR γ) agonists or cotransfection with PPAR γ cDNA reversed this effect. The inhibitory action of ibuprofen and indomethacin was

suppressed by PPAR γ antagonists. We observed that the mRNA levels, expression and enzymatic activity of β -secretase (BACE) were increased by immunostimulation and normalized by NSAIDs. We performed the same experiments using mouse embryonic fibroblasts from PPAR γ knockout mice and N2a cells transfected with siRNA for PPAR γ . In these conditions, we did not find modulation of BACE by inflammation and ibuprofen. In conclusion, pro-inflammatory cytokines activate β -secretase and NSAIDs inhibit that effect through PPAR γ .

PPAR α agonists induce generation of hydrogen peroxide in human macrophages via NADPH oxidase stimulation

Elisabeth Teissier, Atsushi Nohara, Giulia Chinetti, Jean-Charles Fruchart, Ajay Shah and Bart Staels

UR 545 INSERM-Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, Lille 59019, France

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors regulating genes involved in lipid and glucose metabolism. PPAR α and PPAR γ are expressed in macrophages where they control inflammation and cholesterol homeostasis. Since macrophages have the ability to generate reactive oxygen species (ROS), the aim of this study was to investigate whether PPAR α or PPAR γ influence the production of hydrogen peroxide, a marker of ROS, in macrophages. An increase in the production of H₂O₂, measured using dichlorofluorescein fluorescence, was observed in THP-1 monocytes, primary human macrophages, as well as in murine bone marrow-derived, peritoneal and Raw 264.7 macrophages, incubated with different PPAR α but not with PPAR γ agonists. PPAR α activation did not induce cellular toxicity, oxidation of the cellular membrane nor ATP depletion. However, after 24 h a large decrease in

intracellular glutathione levels was observed after treatment with PPAR α ligands. The increase in H₂O₂ production was not due to inherent pro-oxidant effects of the PPAR α agonists tested, but was mediated by PPAR α since the effects were lost in bone marrow-derived macrophages from PPAR α -deficient mice. The induction of H₂O₂ by PPAR α is mediated by NADPH oxidase, since (1) preincubation with the NADPH oxidase inhibitor diphenyleneiodinium prevented the increase in H₂O₂ production; (2) PPAR α agonists induce mRNA levels of the NADPH oxidase subunits p47^{phox}, p67^{phox} and gp91^{phox}; and (3) induction of H₂O₂ production is abolished in p47^{phox} subunit-deficient mice. In conclusion, PPAR α but not PPAR γ gene activation stimulates H₂O₂ production via NADPH oxidase in macrophages, which results in the modification of the redox status.

Impact of phytanic acid and BRL49653 on the induction of inflammatory markers in epididymal adipose tissue of obese mice

Sandra R. Teixeira¹, Mareike Preller¹, Ying Wang¹, Joseph Schawger¹, Marie-France Champy², Johan Auwerx², Volker Elste¹, Peter Weber¹ and Beat Fluhmann¹

DSM Nutritional Products, R&D, Human Nutrition and Health, P.O. 3255, CH-4002 Basel, Switzerland¹;
Mouse Clinical Institute Strasbourg, F-67404 Illkirch, France²

In this study, we examined the effect of phytanic acid and BRL49653 on gene expression of inflammatory markers in adipose tissue in mice with diet-induced obesity. Forty-eight male C57BL/6J mice were assigned to four groups ($n = 12/\text{group}$). One group received chow (lean control [LC]), while three groups received a high-fat (HF) diet. One of the HF groups served as the fat control (FC), whereas the other two received additionally either phytanic acid at 150 mpk or BRL49653 at 10 mpk (TZD). Mice receiving HF became obese and diabetic during the study period. After 23 weeks, epididymal adipose tissue was collected from six mice per group and analyzed using Affymetrix Genechip. Genes known to be involved in inflammatory responses were selected and

further filtered to include only those with change factors < -0.5 or > 0.5 and $p < 0.05$. The HF diet resulted in upregulation of the acute-phase proteins haptoglobin, and orosomucoid 1 and 2, the lipopolysaccharide (LPS) binding protein, and heat-shock protein (HSP) 72. Treatment with either PPAR γ agonist resulted in a downregulation of the expression of most of these markers to levels close to LC. Other classical inflammatory markers were not regulated.

Our results suggest that the obesity-induced persistent acute-phase reaction in adipose tissue can drastically be reduced by treatment with either phytanic acid or BRL49653.

Apoptosis and G1-arrest of human renal interstitial fibroblast cells induced by PPAR-gamma activation

Weiming Wang, Jiayun Chen, Feng Liu and Nan Chen

Department of Nephrology, Ruijin Hospital, Shanghai Second Medical University, Shanghai 200025, P.R. China

The peroxisome proliferators activated receptors (PPARs) are a ligand activated transcription factor superfamily regulating cellular proliferation and apoptosis. PPAR-gamma-mediated signals play an important role in ameliorate inflammation and fibrosis. The aim of this study is to investigate the effect of PPAR-gamma agonists on human renal interstitial fibroblast (HFB).

Methods: HFB were treated with ligands for PPAR-gamma: 15d-PGJ2 (50 μM), and its agonists troglitazone (50 μM) and ciglitazone (10 μM) for different times (24-72 h). The cell viability was measured by trypan blue exclusion. Cell apoptosis were assessed by flow cytometry using annexin V/propidium iodide. The cell cycle was determined by flow cytometry.

Results: When HFB were exposed to PPAR-gamma agonists, the cellular viabilities are much lower than the control group ($p < 0.05$). All PPAR-gamma agonists induced more cell apoptosis compared with the control group (15d-PGJ2, 16.84%; ciglitazone, 14.78%; and troglitazone, 11.26% versus control, 3.49%; $p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively) and increased the percentage of G0/G1 cells.

Conclusion: We conclude that PPAR-gamma agonists can increase cell death by inducing the apoptosis of HFB and inhibit the cellular proliferation by G1-arrest. Thus, PPAR-gamma can be a potential target for new therapeutic approaches in the renal interstitial fibrosis.

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