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Peroxisome Proliferator-Activated Receptor Activators Target Human Endothelial Cells to Inhibit Leukocyte-Endothelial Cell Interaction

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Abstract

An early event in acute and chronic inflammation and associated diseases such as atherosclerosis and rheumatoid arthritis is the induced expression of specific adhesion molecules on the surface of endothelial cells (ECs), which subsequently bind leukocytes. Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily of transcription factors, are activated by fatty acid metabolites, peroxisome proliferators, and thiazolidinediones and are now recognized as important mediators in the inflammatory response. Whether PPAR activators influence the inflammatory responses of ECs is unknown. We show that the PPAR activators 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), Wyeth 14643, ciglitazone, and troglitazone, but not BRL 49653, partially inhibit the induced expression of vascular cell adhesion molecule-1 (VCAM-1), as measured by ELISA, and monocyte binding to human aortic endothelial cells (HAECs) activated by phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide. The "natural" PPAR activator 15d-PGJ2 had the greatest potency and was the only tested molecule capable of partially inhibiting the induced expression of E-selectin and neutrophil-like HL60 cell binding to PMA-activated HAECs. Intracellular adhesion molecule-1 induction by PMA was unaffected by any of the molecules tested. Both PPAR-α and PPAR-γ mRNAs were detected in HAECs by using reverse transcription-polymerase chain reaction and a ribonuclease protection assay; however, we have yet to determine which, if any, of the PPARs are mediating this process. These results suggest that certain PPAR activators may help limit chronic inflammation mediated by VCAM-1 and monocytes without affecting acute inflammation mediated by E-selectin and neutrophil binding.

Keywords: Atherosclerosis; Peroxisome proliferator-activated receptors; Endothelial cells; Adhesion molecules; Inflammation

Introduction

Acute inflammation is the short-term response to bacterial infections and injuries, whereas chronic inflammation is the longer-term response to sustained infections, chronic injury, or foreign bodies. When uncontrolled, chronic inflammation may lead to a variety of common, serious ailments such as atherosclerosis, arthritis, inflammatory bowel disease, and fibrotic lung disease. However, acute inflammation is essential for survival against bacterial infections. Development of new therapeutic agents that preserve the beneficial effects of acute inflammation while controlling the devastating effects of chronic inflammation would have major clinical value. Nonsteroidal anti-inflammatory agents and glucocorticoids achieve this goal to some degree, but with significant side effects. Although both acute and chronic forms of inflammation involve the endothelial-leukocyte interactions described above, E-selectin and neutrophils are associated with acute inflammation and VCAM-1 and monocytes are associated more closely with chronic inflammation [1-3].

Peroxisome proliferator-activated receptors (PPARs), members of the nuclear receptor superfamily, have a newly recognized role in inflammation. All 3 subtypes, α, δ, and γ, are ligand-activated transcription factors. PPAR-α and PPAR-γ are known to regulate the expression of genes involved in lipid metabolism. PPARs combine with the retinoid X receptor-α (RXR-α) to form a functional heterodimer bound to specific DNA sequences within the promoters of target genes. Activating ligands for PPARs are semiselective for the subtypes, and selectivity is ligand-concentration and cell-type dependent. The so-called "natural or endogenous ligand" prostaglandin 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2), is γ-selective at low concentrations but activates α at higher levels. Many of the eicosanoids, certain nonsteroidal anti-inflammatory drugs, and the long-chain fatty acids examined are activators of all PPAR subtypes, whereas peroxisome proliferators (such as Wyeth 14643) are considered α-selective at low concentrations. The

most selective γ-ligands examined are the insulin-sensitizing drugs, the thiazolidinediones (such as ciglitazone, troglitazone, and BRL 49653). Ligands for RXR-α, such as 9-cis- retinoic acid, can induce similar responses to PPAR ligands by activating the PPAR/RXR-α heterodimer [4-8].

However, Nagy, Tontonoz showed that PPAR-γ activators promote monocyte differentiation into macrophages and promote uptake of lipids by the scavenger receptor, thus leading to increased foam cell formation. These results suggest the converse, that PPAR-γ activators would promote development and growth of atherosclerotic plaque. There is preliminary evidence suggesting that ECs express PPARs. However, it is not known what affect PPAR activators have on ECs and whether the former can affect even earlier stages of atherogenesis and other chronic inflammatory diseases, specifically leukocyte-EC interaction. This information would be critical in predicting the effects of PPAR activators on chronic inflammatory diseases. In this study, we show that PPAR activators directly affect leukocyte interaction with ECs, partly through preventing up regulation of adhesion molecules by ECs in response to inflammatory stimuli. Most synthetic PPAR activators tested inhibited only the chronic inflammatory mediators-monocyte binding and VCAM-1 expression-and not the acute inflammatory

mediators-neutrophil binding and E-selectin expression. These results suggest that PPAR activators may be beneficial in ameliorating chronic inflammatory disease such as atherosclerosis by reducing extravasation of monocytes to the involved tissue without limiting the response to acute infection [9].

Overview

These results indicate that some synthetic PPAR activators, such as a peroxisome proliferator and certain thiazolidinediones, inhibit monocyte binding by activated HAECs and their expression of VCAM-1, an adhesion molecule relatively specific for monocyte and lymphocyte adhesion and chronic inflammation. These activators showed no effect on neutrophil binding by activated HAECs or their expression of E-selectin, an adhesion molecule associated with acute inflammation. A natural PPAR activator, 15d-PGJ2, a prostaglandin metabolite of PGD2, inhibited both monocyte and neutrophil binding concomitant with the inhibition of both induced VCAM-1 and E-selectin expression. These results further fuel the discussion on the positive versus negative aspects of PPAR activation on atherosclerosis (Figure 1).

Induction of adhesion molecules by stimuli such as LPS and various cytokines is mediated by signal transduction pathways that subsequently regulate the activity of transcription factors. There are various binding sites for oxidation, proliferation, and inflammation-related transcription factors in the promoter elements of the adhesion molecule genes. The transcription factor nuclear factor-nB (NF-nB) is critical for the induction of adhesion molecules; however, it works in concert with other transcription factors that are specific for each adhesion molecule. Author postulated that PPAR-γ activation inhibited the activity of transcription factors such as NF-nB, activating protein-1, and STAT, subsequently inhibiting the inducible form of NO synthase, gelatinase B, and scavenger receptor A expression in activated monocytes. Such a molecular mechanism may apply here; however, NF-nB is required for induction of all of the adhesion molecules examined in this study. So why do we observe the specific inhibition of VCAM-l?. We can only speculate that because NF-nB is a dimeric complex that can comprise a range of distinct subunits, such as p50 (NF-nB1), p52 (NF-nB2), p65 (Rel A), c-Rel, and Rel B, the mechanism for the specificity of the synthetic PPAR activators may be due to an inhibitory interaction with a specifically composed NF-nB complex or specific auxiliary transcription factors on the VCAM-1 promoter. A similar specificity of inhibition of adhesion molecule expression was previously observed after EC treatment with all-trans-retinoic acid or certain antioxidant molecules. Troglitazone contains a vitamin E moiety that endows it with some antioxidant properties, perhaps partially accounting for its activity in this study. The other PPAR activators used in our study have no reported antioxidant activity. In addition, using an assay that detects lipid hydroperoxide formation (in LDL, in this case), we found that although troglitazone prevented lipid hydroperoxide formation in LDL, the activators Wyeth 14643, ciglitazone, and 15d-PGJ$_2$ at the concentrations used in the adhesion experiments did not (data not shown). This result suggests that an antioxidant effect is not a major factor accounting for the observed effects of PPAR activators. The mechanism by which certain PPAR activators induce E-selectin expression and HL60 binding to ECs in our study may also pertain to specific interactions with the transcription factor complexes on the promoter or with the promoter itself. Further studies are required to define the mechanism(s) involved (Figure 2).

Down regulation of adhesion molecule expression for therapeutic purposes has followed a number of strategies. Monoclonal antibodies to adhesion molecules were shown to significantly reduce leukocyte binding, and oligonucleotide tides were utilized as transcription factor decoys. Most pertinent to the present study, the author showed that certain lipids could block the induction of VCAM-1 expression on activated ECs These authors suggested that this may partially explain the purported protective effect of various unsaturated dietary lipids in atherogenesis. Such lipids, for instance, docosahexaenoic acid, are now understood to be activators of PPARs.

In contrast to the synthetic PPAR activators tested, the natural PPAR activator 15d-PGJ2 blocked both E-selectin as well as VCAM-1 expression and acted without the need for pretreatment of the cells. This difference may be related to the prostaglandin nature of 15d-PGJ2, which, by analogy with other prostaglandins such as PGE2, may interact with a cell surface receptor to generate an increase in intracellular cAMP levels. Such an increase in cAMP in this manner has been shown previously to block induction of VCAM-1 and E-selectin but not ICAM-1 expression on activated ECs. However, no known specific cell surface receptor for 15d- PGJ2 has been identified. It is also possible that 15d-PGJ2 has other pharmacological properties, such as conjugation with glutathione, as occurs with prostaglandin metabolites contain- ing an α,β-unsaturated carbonyl group resulting in varied biological activity. Alternatively, one might argue that these effects are solely mediated by PPAR and that 15d-PGJ2 may be a very effective PPAR activator in ECs. Even though 15d-PGJ2 is less effective than most thiazolidinediones at inducing adipocyte differentiation or transactivation of a PPAR-γ promoter-reporter construct in fibroblast-type cells, it has often been shown to have the greatest efficacy in other processes in various cell types, such as inhibiting cytokine production by activated monocytes, inducing the differentiation of monocytes into macro- phages, and inhibiting growth of breast cancer cells. The reason for this paradox is currently unknown [10-12].

We are unable to state, as yet, which PPAR subtype might be mediating this process in ECs. The ability of the peroxisome proliferator Wyeth 14643 to have an effect suggests that PPAR-α is involved, whereas the effects of the thiazolidinediones indicate that PPAR-γ is involved. The potency of 15d-PGJ$_2$ also predicts a role for PPAR-γ in this process; however, the ineffectiveness of BRL 49653, the most potent γ-activator in most systems, is puzzling. Analysis of the mRNA levels indicates that PPAR-α is more abundant and perhaps more active than PPAR-γ in ECs; however, this is not definitive, since we have as yet to determine the functional protein levels of the PPARs in ECs. Given that there is no truly specific activator of a particular PPAR, we can only conclude that the process described in this study may be mediated by any of the PPARs expressed in ECs.

Our results do not exclude a role for the effects of PPAR activators on the expression of other factors involved in leukocyte-EC interaction, such as inhibition of endothelium- derived chemokines and other adhesion molecules. Nor do they rule out the possibility that the molecules tested may act through unrelated mechanisms in addition to PPAR activation. Regardless of the exact mechanism(s), it is hoped that such molecules will have the same effects in vivo.

Because inflammatory lipid factors such as prostaglandins, eicosanoids, and leukotrienes are well-recognized activators of PPARs these transcription factors are poised to mediate inflammatory responses in general and in specific diseases. The present results suggest that, even though certain PPAR activators may promote foam cell formation in the atherosclerotic lesion, they may also block the initial

entry of monocytes into the artery wall, thus preventing the onset of atherosclerotic lesion formation but aggravating preexisting lesions. These findings carry enormous clinical significance, as increasing numbers of diabetic patients are receiving PPAR agonists as routine therapy for insulin resistance.

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