What Are the Effects of Peroxisome Proliferator-Activated Receptor Agonists on Adiponectin, Tumor Necrosis Factor-Alpha, and Other Cytokines in Insulin Resistance?

PATRICK J. BOYLE, M.D.

University of New Mexico School of Medicine, Albuquerque, New Mexico, USA

Summary: Patients with type 2 diabetes are at high risk of cardiovascular disease. In addition to treating hyperglycemia, the thiazolidinedione (TZD) class of antidiabetic agents may also benefit the cardiovascular complications associated with the disease. The two available TZDs, pioglitazone and rosiglitazone, are peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonists that influence gene expression of key proteins involved in regulating glucose and lipid metabolism. Tumor necrosis factor-alpha (TNF- α) and adiponectin are believed to be important in the development of insulin resistance and atherosclerosis. Understanding the role of these cytokines in the inflammatory processes that trigger plaque development might lead to identification of other potential mechanisms that could be exploited to enhance future treatments for patients with diabetes and atherosclerosis.

Key words: adiponectin, tumor necrosis factor-alpha, peroxisome proliferator-activated receptor-gamma, thiazolidinediones, atherosclerosis

Introduction

Because persons with type 2 diabetes are at high risk of developing macrovascular complications, it will be necessary for endocrinologists and cardiologists to work together closely to maximize patient care and outcomes. Recent

advances in our understanding of molecular biology have led to the development of pharmacotherapeutic options for diabetes, for example, the thiazolidinediones. These agents are peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonists, which, by modulating the expression of key proteins involved in carbohydrate and lipid metabolism, reduce peripheral insulin resistance and consequently reduce blood glucose concentrations. Their mechanism of action may also have an anti-inflammatory component, which may attenuate the atherogenic process. Accordingly, pioglitazone and rosiglitazone may be particularly beneficial for patients with type 2 diabetes who are at increased risk from cardiovascular complications/disease.

The PPAR- γ agonists are believed to inhibit the inflammatory processes involved in atherosclerosis by suppressing the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) by monocytes and by influencing the gene expression of certain key proteins such as adipocyte complement-related protein (adiponectin). Adipocytes have conventionally been considered simply as repositories for calories, but these cells have now been shown to have a major role in the etiology of insulin resistance. An increasing awareness of adipocyte function, not only in the development of cardiovascular complications associated with diabetes but also in the pathogenesis of diabetes itself, may lead to the need for closer scientific collaboration between diabetologists and cardiologists. Greater understanding of the roles the various cytokines and proteins play in the atherogenic process may also further define the close interrelationship that exists between development of insulin resistance and atherosclerosis.

With this in mind, this paper considers the role of adiponectin, TNF- α , and other cytokines in regulating glucose and lipid metabolism, and how PPAR- γ agonists can influence these processes.

Does TNF- α Play a Role in Insulin Resistance?

The association between TNF- α and insulin resistance has been researched intensively over recent years. An initial

Dr. Boyle is a clinical investigator for Takeda Pharmaceuticals North America, Inc.

Address for reprints:

Patrick J. Boyle, M.D.
Director, Diabetes Case Management
University of New Mexico School of Medicine
Department of Internal Medicine
2211 Lomas North East 5ACC
Albuquerque, NM 87131, USA
e-mail: pboyle@salud.unm.edu

hypothesis suggested that TNF- α was associated with impaired insulin signaling and insulin stimulation of glucose uptake by skeletal muscle cells,¹ but research findings have since been conflicting as to the exact mechanism.

If TNF- α is directly responsible for controlling insulin resistance associated with obesity, then as TNF- α levels increase, it would be expected that insulin sensitivity would decrease (with insulin levels rising in the face of either normal or only modestly elevated glucose concentrations). Accordingly, persons with relatively large amounts of body fat would be expected to produce more TNF- α and thus be more insulin resistant than people with less body fat.

Numerous studies in animal models have shown that TNF- α plays an important role in mediating the insulin resistance of obesity through its overexpression in fat tissue. However, the mechanisms linking obesity to insulin resistance and diabetes in humans have remained elusive.

Results from three major studies, published in the mid 1990s, that investigated the possible relationships between adipose tissue, TNF- α , obesity, and insulin resistance in humans consistently showed a relationship between overexpression of TNF- α and the pathogenesis of obesity-related insulin resistance.²⁻⁴ Adipocytes from obese subjects overexpressed TNF- α compared with adipocytes from lean individuals, with the extent of expression correlating with the level of hyperinsulinemia observed in these subjects.² Adipose TNF- α expression also correlated with percent body fat.³ Muscle cells from insulin-resistant and diabetic subjects also overexpressed TNF- α compared with muscle cells from nondiabetic subjects, suggesting another mechanism by which TNF- α may be associated with the insulin resistance of obesity.⁴

Another study published in 1998 compared intraperitoneal glucose tolerance in normal and TNF- α receptor-

deficient male mice fed a high-fat diet for 6 weeks to induce obesity.⁵ Specifically, glucose concentrations were compared in wild-type mice, mice with mutations or deletions in each of the two distinct TNF- α receptors, and mice that had both TNF- α receptors deleted. The hypothesis was that, if TNF- α were responsible for mediating insulin resistance, the mice unable to respond to circulating TNF- α due to deletions or mutations in their TNF- α receptors would have the lowest glucose concentrations. Surprisingly, 4 h after an intraperitoneal glucose injection, the mice with both TNF- α receptors deleted had significantly poorer glucose clearance than the wild-type mice ($p < 0.05$) (Fig. 1), indicating that TNF- α did not mediate insulin resistance.⁵

These workers also studied insulin concentrations in wild-type mice that had been crossed with obese (*db/db*) mice.⁵ These animals were genetically obese and had neither TNF- α receptors nor functional leptin receptors. As these mice are unable to respond to TNF- α , they were expected to have normal insulin levels even if TNF- α is responsible for insulin resistance. However, the mice showed marked fasting hyperinsulinemia, indicating that they were insulin resistant rather than insulin sensitive.⁵ This again contradicted the hypothesis that TNF- α is a mediator of obesity-associated insulin resistance.

More evidence accumulated against this hypothesis following the development of a recombinant-engineered human TNF- α -neutralizing antibody. When such an antibody (CDP571) was administered to 21 patients with type 2 diabetes, no effect was seen on fasting glucose concentrations, fasting serum insulin, and C-peptide levels during the following 4-week period.⁶ The prolonged half-life of this antibody ensured that adequate plasma levels were maintained throughout the study. Other more complex studies of

* $p < 0.05$ at 240 min by ANOVA

FIG. 1 Intraperitoneal glucose tolerance test in male C57BL/6 (*wild-type*) and tumor necrosis factor- α receptor-deficient mice. ANOVA = analysis of variance. Reprinted from Ref. No. 5 with permission.

anti-TNF- α antibodies have also shown that there was no change in insulin sensitivity.

Collectively, these studies suggest that TNF- α does not have a role in insulin resistance, because they show no correlation among TNF- α concentrations, glycemic control, and insulin levels. However, a recent study⁷ reported interesting findings from an experiment that measured forearm glucose uptake during an intra-arterial insulin infusion in healthy, lean humans without diabetes.

As expected, insulin infusion resulted in an approximate 200% increase in glucose uptake. The plasma insulin concentration in the infused forearm was approximately 100 μ U/ml (717.5 pmol/l), tenfold higher than fasting concentrations in normals, and glucose uptake in the forearm was increased by 220%. The systemic insulin concentration from this forearm infusion (measured in the contralateral arm) increased by 2 μ U/ml (14.35 pmol/l) only. When TNF- α was infused in addition to insulin, insulin-mediated glucose uptake was completely inhibited. This effect was also even more pronounced when TNF- α was infused alone (Fig. 2).⁷

This study suggests that TNF- α may indeed have a role to play in muscle glucose metabolism. As previous studies have shown contradictory findings, the exact relationship between insulin resistance and TNF- α is likely to be complex.

Mechanism of Action: PPAR- γ Activation

The first of the thiazolidinedione class of drugs was initially discovered during the development of a new fibrate lipid-lowering agent (a PPAR- α agonist). Pioglitazone and rosiglitazone are the two thiazolidinediones currently avail-

able for treating patients with type 2 diabetes. These drugs are PPAR- γ -activating compounds, which act in the same way as the naturally occurring ligands for PPAR- γ , and they include fatty acids and related icosanoids such as the prostaglandins.

Peroxisome proliferator-activated receptor-gamma is a protein found in the nucleus of many cell types, including adipocytes, smooth muscle cells, and macrophages. It usually exists as a heterodimer with retinoid X receptor-alpha (RXR- α). When bound to a ligand (such as pioglitazone), a three-dimensional conformational change occurs, which initiates the transcription activation process within the nucleus.

Certain genes are known to be PPAR- γ dependent, because they require PPAR- γ for their activation and initiation of the transcription process. During this process, PPAR- γ binds to the promoter sequence situated upstream of the transcription site. Once bound to the promoter sequence, PPAR- γ attracts a coactivator protein, which, in turn, leads to histone acylation and subsequent alterations in gene transcription. Ultimately, the production of corresponding protein product is either increased or decreased.

An increased understanding of this process has allowed for the development of new drugs for treating type 2 diabetes, a field few thought would develop in this way. By being able to influence such pathways, treatment options for conditions such as type 2 diabetes can be improved, and more specific drugs developed.

PPAR Action and the Adiponectin Gene

Research has shown that one of the genes regulated by this PPAR- γ -dependent mechanism is that encoding adiponectin.⁸ Little was known about adiponectin until recently,

FIG. 2 Forearm glucose uptake and tumor necrosis factor-alpha (TNF- α) infusion in humans without diabetes. Reprinted from Ref. No. 7 with permission.

although, on a quantitative basis, this is the adipocyte's main protein product. Given that adipocytes were considered to be mere inert repositories of calories for future utilization, this is perhaps not surprising. It is now recognized that adipose tissue is in fact highly specialized, having important roles in energy storage, fatty acid metabolism, and glucose homeostasis.

Adiponectin is an adipocyte-specific secretory protein of 247 amino acids, and has two distinct domains—a globular domain and a collagen-repeat domain. It normally circulates in the plasma as a homotrimer and shares structural homology with the complement factor C1q. Plasma concentrations of adiponectin decrease as fat levels accumulate within the body, and are lower in patients with diabetes and in those with coronary artery disease.⁹

Adiponectin levels are increased in the presence of a PPAR- γ agonist, as shown in a recent study by Iwaki *et al.*⁸ This study investigated the role of PPAR- γ in the induction of adiponectin in human kidney HEK293 cells, which lack most of the adipogenic transcription factors. The PPAR- γ /RXR- α heterodimer was shown to bind to a PPAR response element (PPRE) and increase adiponectin promoter activity, but PPAR- γ or RXR- α alone failed to do so.⁸ Addition of pioglitazone augmented the transcriptional activity induced by PPAR- γ /RXR- α , resulting in an increased level of adiponectin production.⁸

Further assays were performed in cells with mutations within the PPRE, that is, the section of the gene to which PPAR- γ binds. Addition of ligand plus PPAR- γ to these cells did not result in an increase in gene transcription,⁸ and no increase in adiponectin levels was seen. These findings therefore support the fact that PPAR- γ , with its coactivator RXR- α , regulates the adiponectin gene.

Typically, circulating adiponectin levels are approximately 7 $\mu\text{g/ml}$, but some individuals may have levels of only 2 $\mu\text{g/ml}$.¹⁰ These lower concentrations have been associated with a single-point mutation within the adiponectin gene. Specifically, a T-to-C substitution at nucleotide 517 in the adiponectin gene transcript, which results in an amino acid substitution from isoleucine to threonine at position 164 in the globular domain, is significantly more prevalent in patients with than in those without type 2 diabetes (3.2 vs. 0.4%, $p = 0.007$).¹⁰ Compared with age-matched controls, subjects with the I164T mutation have higher systolic and diastolic blood pressure, a greater body mass index, lower high-density lipoprotein (HDL) levels, and higher triglyceride levels.¹⁰ This single amino acid mutation is therefore clearly associated with the development of insulin resistance and the metabolic syndrome, and may potentially be one of several factors that contribute to the development of type 2 diabetes in susceptible individuals.

Adiponectin levels are influenced by thiazolidinedione treatment, as illustrated by a recent study by Bajaj *et al.*¹¹ After a period of 16 weeks, pioglitazone treatment (45 mg/day) in 11 subjects with type 2 diabetes was associated with a significant increase in peripheral glucose utilization (5.2 ± 0.5 to 6.6 ± 0.6 mg/kg/min, $p < 0.01$) and a signifi-

* $p = 0.0001$

FIG. 3 Effect of pioglitazone treatment on plasma adiponectin in patients with type 2 diabetes. Adapted from Ref. No. 11 with permission.

cant decrease in A1C levels ($7.8 \pm 0.4\%$ to $6.6 \pm 0.3\%$, $p < 0.01$). However, in addition to these improvements in glycemic control, these subjects also showed significant increases in levels of circulating adiponectin (16.7 ± 2.3 to 51.0 ± 4.7 $\mu\text{g/ml}$, $p < 0.0001$) (Fig. 3).¹¹ Thus, the thiazolidinediones clearly have a significant effect on glucose metabolism, while their ability to influence adiponectin concentrations may also be clinically important from a cardiovascular perspective.¹¹

Adiponectin and Atherosclerosis

Adiponectin is one of many factors involved in the atherogenic process. A clear understanding of the role of adiponectin may aid in the development of alternative treatments for preventing and/or treating atherosclerosis.

Circulating low-density lipoprotein (LDL) particles gain access to the subendothelial space by crossing the coronary endothelium. An initial oxidative step occurs (largely driven by angiotensin II) that creates a metabolic environment in which large amounts of superoxide radicals are generated. Monocytes infiltrate this subendothelial space and, under the influence of interleukin 1 (IL-1) produced by the endothelial cells, mature into macrophages. The oxidized LDL particles are taken up by the macrophages, which in turn become foam cells. These foam cells secrete macrophage colony-stimulating factor (MCSF), with a subsequent increase in IL-1 and TNF- α production. The endothelial cells reciprocally increase their production of vascular cell adhesion molecules (VCAM), allowing T lymphocytes to adhere to the coronary endothelium. This cycle is repeated until the T lymphocytes in the subendothelial space produce interferon-gamma (INF- γ). In turn, the surrounding smooth muscle cells are stimulated to proliferate, which contributes to the development of a thick fibrous cap over the top of the developing atheroma.

Much of the action taken to prevent atherosclerosis focuses on reducing levels of circulating LDL, such as dietary advice and prescription of drugs to lower lipid levels. However, several other steps in this complex process could provide potential targets for inhibiting the development of atheromatous plaques. Treatments could perhaps focus more on the subendothelial compartment and the processes contained within.

Expression of the VCAM-1 molecule may be considered a potential target. By influencing VCAM-1, it may be possible to prevent or reduce the whole inflammatory cascade at an early stage of development, because VCAM-1 encourages T-lymphocyte adhesion to the coronary endothelium, thereby triggering inflammation and promoting atherosclerosis.

Both adiponectin and TNF- α can modulate VCAM-1 expression and thus reduce the extent of lymphocyte adhesion. Addition of TNF- α to isolated human aortic endothelium increases VCAM-1 production fourfold, causing a doubling in the number of white cells that adhere (Fig. 4).⁹ Adiponectin has no independent effect on VCAM-1 production on endothelium and, therefore, does not affect the level of white cell adhesion observed.⁹ However, when endothelium is exposed jointly to TNF- α and adiponectin, the increase in VCAM-1 production previously seen with TNF- α alone is nearly abolished as the concentration of adiponectin in the culture medium rises. Consequently, TNF- α -mediated increments in white cell adhesion are dramatically reduced (approximately 50%).⁹ Targeting adiponectin's involvement in the inflammatory process thus provides a potentially valuable means for influencing the inflammatory cascade associated with the development of atherosclerosis.

Peroxisome proliferator-activated receptor-gamma may also play an important role in this inflammatory process. Macrophages present in human atheromas have shown positive staining for PPAR- γ molecules.¹² Previously, the diabetes research community believed that PPAR- γ simply

had a role in glucose disposal and enhancement of muscle glucose uptake. However, it is now known that PPAR- γ activation is involved in the uptake of oxidized LDL by macrophages through alteration in the expression of scavenger receptor A1 (SRA1).

The SRA1 is found on the surface of macrophages and binds to oxidized LDL from the interstitium of the developing atheroma. Following endocytosis of the oxidized LDL particle, this receptor is recycled, returning to the cell surface to repeat the cycle. Ultimately, cholesterol esters in LDL accumulate to such an extent that the macrophage becomes a foam cell.

By studying the promoter activity of the SRA1 protein, Ricote *et al.*¹³ showed that a modest increase in the activation of PPAR- γ led to decreased gene expression of the SRA1 surface receptor, which reduces the uptake of oxidized LDL by the macrophage. The macrophage is therefore prevented from becoming a foam cell, thus delaying or preventing the atherosclerotic process.

The PPAR- γ protein is an α/β heterodimer, with one subunit capable of binding to a thiazolidinedione. The other subunit is a retinoic acid-binding protein, and a series of retinoids have now been developed and studied. Retinoid exposure increases production of the ATP-binding cassette protein in isolated macrophages. This is the docking protein for HDL on the macrophage's surface. Were this pathway to be accelerated, reverse cholesterol transport out of the macrophage would be activated, thus preventing foam cell development. These processes that occur within macrophage represent two further metabolic targets for potential exploitation by thiazolidinedione therapy.

Adiponectin Treatment in Apolipoprotein E Deficiency

The impact of adiponectin has also been studied in animals that are deficient in apolipoprotein E (ApoE).

FIG. 4 Adiponectin regulates vascular inflammatory cell adhesion. TNF- α = tumor necrosis factor-alpha. Reprinted from Ref. No. 9 with permission.

Without ApoE, these animals are unable to regulate circulating LDL levels, giving rise to a tendency to develop severe atherosclerosis.

Recent studies have investigated the role of RXR¹⁴ and adiponectin¹⁵ in ApoE-deficient animals. For example, Claudel *et al.* demonstrated that ApoE-deficient animals treated with a retinoic acid derivative do not develop atherosclerosis. This study also showed that ApoE-deficient animals treated with a PPAR agonist had a greatly attenuated rate of atherosclerosis development as well.

In a similar study by Okamoto *et al.*¹⁵ ApoE-deficient animals were injected with either β -galactosidase (control group) or adiponectin (treatment group), and the extent of the inflammatory response at the coronary surface was compared. Stained sections of the coronary surface showed a much greater inflammatory response in the control animals, but the corresponding staining in the adiponectin-treated group was much more condensed. Immunofluorescent staining of the same cross section of the coronary surface showed that adiponectin was present in the area where cell accumulation occurred. Macrophages colocalized to this same region, suggesting that there is an interaction between adiponectin and macrophages. Production of VCAM-1 on the endothelium of ApoE-deficient animals was also reduced.¹⁴ Adiponectin was also found to impede the expression of SRA1 on the macrophage surface and to reduce macrophage TNF- α production (an effect that would also reduce the expression of VCAM-1 on the coronary endothelium).

Conclusions

The role of TNF- α in insulin resistance remains unclear, with findings from recent research apparently contradictory. The TNF- α may well have a role to play, but this is likely to be one of many factors in a complex cytokine cascade. Thiazolidinedione treatment in insulin resistance is now well established, and the effect of this class of drugs on glucose and insulin levels is benefiting many patients with type 2 diabetes. The exact mechanism of action in which blood glucose is reduced by these drugs may well involve TNF- α .

In addition to having an effect on insulin resistance, thiazolidinediones may also attenuate the cardiovascular complications associated with insulin resistance and type 2 diabetes. Acting as PPAR- γ agonists, drugs such as pioglitazone and rosiglitazone have been shown to upregulate the production of adiponectin and downregulate SRA1 production. Adiponectin diminishes the ability of TNF- α to upregulate VCAM-1 production at the coronary endothelium. This, along with their ability to reduce SRA1 expression in macrophages (a surface receptor essential for foam cell development in the inflammatory cascade leading to atherosclerosis), suggests that thiazolidine-

diones may have potential for preventing and/or treating atherosclerosis.

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