Asians, Insulin Resistance, and PPAR Gamma

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Cellular proliferation and migration are fundamental processes that contribute to the injury response in major blood vessels. The resultant pathologies are atherosclerosis and restenosis, which are accelerated, in insulin-resistant type 2 diabetic patients compared with nondiabetic subjects. Controlling glucose to nearly normal levels is crucial to attenuate atherosclerosis. This also prevents and slows the progression of microvascular complications. However, other factors in addition to glucose contribute to atherosclerotic complications. As we begin to understand the cellular changes associated with vascular injury, it is critical to determine whether the inhibition of growth and movement of cells in the vasculature could serve as a novel therapeutic strategy to prevent the vascular complications of diabetes.

The Insulin Resistance Syndrome –

Insulin resistance and diabetes increase the atherosclerotic process. Epidemiological studies demonstrate three- to fourfold increased rates of coronary artery disease mortality in type 2 diabetic patients compared with nondiabetic subjects. Prediabetic patients presenting with impaired glucose tolerance have a two fold increased rate of coronary heart disease mortality compared with subjects with normal glucose control, which suggests that atherosclerosis is enhanced in insulin resistance, even in the absence of frank hyperglycemia. Insulin resistance is associated with a constellation of factors that enhance atherosclerosis. These factors include a common dyslipidemia consisting of an elevated level of triglycerides, a low HDL, and increased oxidized LDL; an increased prevalence of hypertension; an increased thrombotic tendency due to an increased production of plasminogen activator inhibitor-1 (PAI-1) and fibrinogen; and endothelial dysfunction. Hyperinsulinemia paired with insulin resistance may further accelerate vascular injury. Insulin also is a modest vasodilator and stimulates nitric oxide production in endothelial cells. This process is mediated by the phosphatidylinositol 3-kinase (PI3-K) pathway, which also mediates insulin's ability to simulate glucose transport in a variety of tissues, including skeletal muscle and adipose. Insulin-resistant subjects, by definition, are defective in insulin-mediated glucose transport. Mounting evidence also suggests that insulin-resistant subjects have a defect in insulin-stimulated nitric oxide production, which parallels their defect in glucose transport. Altogether, these observations suggest that insulin resistance may be associated with a global defect of PI3-K signaling in response to insulin. In contrast, insulin stimulates vascular cell growth and migration. The mitogen-activated protein kinase (MAPK) pathway appears to function normally in these proatherosclerotic effects of insulin. Similar data in human studies indicate that insulin-resistant subjects have defective PI3-K activity in the skeletal muscle but normal insulin-stimulated MAPK activity. We have hypothesized that an imbalance between these two pathways in the insulin-resistant state shifts the balance toward enhanced proatherosclerotic effects of insulin with decreased nitric oxide activity to accelerate atherosclerotic processes.

Role of Cell Proliferation and Migration in Vascular Disease

<u>Atherosclerosis</u>

Atherogenesis consists of a cascade of events involving interactions of circulating cells and substances within the vascular wall. The first step of this cascade involves damage to the endothelium

caused by traditional cardiovascular risk factors including diabetes. These factors are mediated by the MAPK pathway. In insulin-resistant models, such as the Zukedr obese rat, the vascular PI3-K pathway is attenuated, whereas MAPK activity is enhanced. Insulin resistance, even in the absence of these risk factors, is associated with endothelial dysfunction in the peripheral arterial circulation, as well as in the coronary arterial bed. Endothelial damage results in a lower production of nitric oxide, which inhibits thrombosis, inflammation, and vascular smooth muscle cell (VSMC) growth and migration to prevent the vascular injury response. Another manifestation of injury to the endothelium is the expression of adhesion molecules: intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). VCAM-1 promotes the attachment of circulating monocytes, macrophages, and platelets. An inflammatory reaction ensues, along with the production of the chemokines, specifically monocyte chemoattractant protein-1 (MCP-1) which directs migration of the attached monocyte/macrophages into the vascular wall. Clot formation, in addition to inflammation, occurs in the vessel wall. LDL is incorporated into the vessel wall and is phagocytosed by macrophages to form foam cells. This phagocytosis is markedly enhanced when LDL is oxidized. The lipid-laden foam cells break down inside the vessel wall to form fatty streaks. VSMCs migrate from the media to the intima, where they proliferate and form a neointima with increased extracellular matrix production, leading to the development of an organized atherosclerotic plaque. These smooth muscle cell changes are thought to constitute a late event in the atherosclerotic process; however, recent evidence suggests that early formation of neointima may contribute to the enhancement of inflammatory and thrombotic processes, leading to atherosclerosis in the vessel wall.

Subsequently, a critical question emerges: Which step in the atherosclerotic cascade should be targeted to inhibit atherosclerosis? Considerable data from clinical trials indicate that lowering LDL levels has a major impact: it decreases myocardial infarction, stroke, and cardiovascular mortality. Decreasing other risk factors, such as hypertension and hyperglycemia, also significantly attenuates both macrovascular and microvascular diseases in diabetes. Inhibiting the thrombotic reaction with aspirin or other agents, which in turn inhibits platelet function may decrease coronary artery disease events and mortality in subjects without diabetes. The American Diabetes Association has recommended aspirin therapy to prevent cardiovascular disease in patients with diabetes. Recent evidence in genetically altered animal models of atherosclerosis underscores the role of inflammation. A knockout of apolipoprotein E in mice results in the development of hypercholesterolemia and advanced atherosclerotic plaques similar to those seen in humans. These animals can be rescued from atherosclerosis by knockout of either MCP-1 or macrophage colony-stimulating factor, which would prevent the movement of monocytes attached to the endothelial surface into the vessel wall. Thus inhibition of inflammation in the vascular wall may be an important therapeutic target to stop the atherosclerotic process.

Restenosis

When the endothelium is injured by balloon catheterization or stent placement, VSMCs migrate from the medial (and possibly the adventitial) layer to the intimal layer of the vessel wall where they proliferate. The formation of this neointima is an important architectural change in the vessel wall that leads to restenosis after angioplasty or stenting. Neointima formation is accelerated in diabetic patients, who, thus, have increased rates of restenosis compared with nondiabetic patients. Mechanical injury to the vessel wall provokes the release of growth factors that stimulate VSMC movements to, and replication within, the thickening lumina through the activation of key cell signaling pathways, including the MAPK cascade. Reentry of quiescent VSMC into the cell cycle is a hallmark molecular event in the restenotic process. Thus, pharmaceutical interventions targeting VSMC growth or movement are a promising new approach to reduce the risk for restenosis in people with diabetes.

PPAR-g Expression and Function in the Vasculature

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of the nuclear receptor superfamily, which when activated by ligends, regulates gene expression. Thiazollidinediones (TDZs) bind with high affinity to PPAR-γ to enhance insulin-mediated glucose transport into adipose and skeletal muscle and are clinically used pharmacological ligands. In human and animal models with insulin resistance and type 2 diabetes, TZDs decrease hyperglycemia and hyperinsulinemia. PPAR-γ is expressed abundantly in adipose tissue, where it promotes the adipocyte differentiation and the expression of genes involved in fatty acid metabolism. PPAR-γ is important to normal human physiology. Dorminant-negative mutations of PPAR-γ result in an abnormal ligand-binding domain of the receptor, which inactivates the receptor in humans. This dominant-negative mutation results in the development of severe insulin resistant, type 2 diabetes, and hypertension in the absence of obesity. These patients also exhibited elevated triglycerides and low HDL levels and, thus, expressed a number of components of the metabolic syndrome. In contrast, the presence of a constitutively active receptor in human models results in obseity without insulin resistance or hypertension. These seminal observations suggest that this nuclear receptor is not only important to maintain insulin-mediated glucose transport, but also plays a role in the regulation of vascular tone and potentially other vascular activities.

PPAR-γ is expressed on all major cells of the vasculature, including endothelial cells, VSMCs and monocytes / macrophages. Human coronary artery smooth muscle cells, umbilical artery smooth muscle cells, umbilical endothelial cells, and aortic smooth muscle cells all express PPAR-γ, which is present in nuclear but not cystolic fractions from these cells. In early human atherosclerotic lesions, PPAR-γ expression is present in VSMCs in the neointima as well as in macrophages. In addition, PPAR-γ expression is upregulated in neointima that forms after balloon injury of rat endothelium; these data suggest that PPAR-γ expression is enhanced in vascular cells when the vasculature is damaged. Thus, it is important to define the role of PPAR-γ in vascular injury.

PPAR-g Inhibits VSMC Proflifération

Pharmacological ligands to PPAR-γ, troglitazone, rosiglitazone, and pfoglitazone all inhibit VSMC proliferation in vitro at drug levels that are achieved when patients are given these agents in antidiabetic doses. Furthermore, examination of the mechanism by which PPAR-γ activation inhibits VSMC proliferation indicates that TZDs block the events that are critical for the reentry of quiescent VSMCs into the cell cycle. This step in the cell cycle requires the formation and activation of cyclin and cyclin-dependent kinase (CDK) complexes that result in phosphorylation of the retinoblastoma (Rb) protein, which functions as a gatekeeper for progression into the DNA synthesis (S phase) segment of the cell cycle. Phosphorylation of Rb releases the sequestered transcription factor E2F, which activates genes that participate in cell cycle progression. CDK inhibitors (CDKIs) $p21^{Cip1}$, $p27^{Kip1}$, and $p15/p15^{NK4}$, regulate this process by inhibiting cyclin/CDK activity and phosphorylation of Rb, resulting in G_1 arrest. We recently found that both troglitazone and rosiglitazone attenuated the mitogen-induced degradation of $p27^{Kip1}$. There was no effect of these agents on levels of cyclins or CDKs. The CDKI$p27^{Kip1}$ negatively regulates growth in a variety of cell types, including VSMCs. Overexpression of $p27^{Kip1}$ inhibits serum-stimulated DNA synthesis in VSMCs, and in a porcine balloon-injury model, $p27^{Kip1}$ expression was markedly reduced in the intima and media after injury. Thus, $p27^{Kip1}$ downregulation is necessary for cell cycle progression, and the activation of PPAR-γ controls cell cycle events through the regulation of this important CDKI.

PPAR-g Activation Inhibits VSMC Migration

The migration of cells involves 1) attaching to the extracellular matrix, 2) chemotaxis, or locomotion, and 3) burrowing through the extracellular matrix or other barriers. Cells attach to matrix proteins in their environment through cell surface receptors called integrins, which bind to amino acid sequences on the surrounding matrix proteins. Chemotaxis involves the cytoplasmic activation of myosin light-chain kinase and other kinases that are activated by chemoattractants. Invasion allows VSMCs to digest through extracellular matrix as cells move from the media, across the internal elastic lamella, and into the intima. Nuclear events and activation of the MAPK pathway are required for invasion, which involves the production and secretion of matrix metalloproteinases (MMPs). The transcription factor, Ets-q, is known to regulate MMP9 as well as expression of other MMPs. Ets-1 is upregulated by growth factors that activate the extracellular signal-regulated kinase MAPK pathway. Platelet-derived growth factor (PDGF) is a potent chemoattractant for VSMCs. Ets-1 is upregulated by PDGF in VSMCs, which is inhibited by both troglitazone and rosiglitazone. Consistent with their nuclear distribution in cells, PPAR-γ activation does not affect VSMC attachment or locomotion, but it inhibits invasion by transrepression of Ets-1 and subsequent inhibition of MMP production. Thus, activation of PPAR-γ may be an important strategy to control MAPK-mediated VSMC migration.

PPAR-g In the Macrophage: From Cell Formation Versus Inhibition of Inflammation

PPAR-γ is expressed in monocytes and is upregulated during monocyte differentiation into macrophages. In human atherosclerotic lesions, PPAR-γ expression occurs predominantly in macrophages with lower levels detected in VSMC. The pathophysiological role of PPAR-γ in atherogenesis is a subject of intensive investigation. Early *in vitro* studies of PPAR-γ function in macrophages identified a variety of antinflammatory and, therefore, potentially antiatherogenic activities including the following: an inhibition of cytokine production; an attenuation of cytokine-inducible expression of nitric oxide synthase, gelatinase B1, and scavenger A receptor; and an antagonism of AP-1, signal transducer and activator of transcription (STAT), and nuclear factor-kB transcription activity. The binding of monocytes to proinflammatory adhesion molecules expressed on the surface of endothelial cells and their subsequent infiltration into the subendothelial space may also be negatively regulated by PPAR-γ. The cytokine-induced expression of VCAM-1 and ICAM-1 by endothelial cells and the MCP-1-directed transendothelial migration of monocytes are both potentially inhibited by PPAR-γ ligands. Thus, pharmacological activation of PPAR-γ may suppress some of the earliest cellular events in the pathological sequelae leading to the formation of atherosclerotic lesions. In marked contrast, activation of PPAR-γ in cultured monocytes increased monocyte uptake of oxidized LDL, induced transcription of the scavenger receptor CD36, and promoted monocyte differentiation into foam cells. These actions tend to promote the atherosclerotic process. Clearly, the potentially important role of PPAR-γ in attenuating atherosclerosis can only be defined in vivo.

In Vivo Translation

TZDs improve insulin sensitivity, decrease circulating insulin levels, and lower fasting blood glucose in type 2 diabetic individuals. These changes are associated with reversal of many of the components of the insulin resistance syndrome, including lowered triglycerides, increased HDL, decreased small dense LDL, decreased circulating PA1-1 levels, and decreased blood pressure. These observations suggest that reversal of the insulin resistance syndrome is associated with an improvement in the cardiovascular risk factors associated with insulin resistance. However, activation of PPAR-γ in the vasculature may have direct effects on blood pressure and PA1-1 expression.

Troglitazone was the first TZD demonstrated to prevent neointima formation after endothelial balloon injury of the aorta in rats. Subsequently, pioglitazone was shown to prevent neointima formation in carotid artery injury in rats and to prevent neointima formation in hypertensive rat models. Recently, Tagaki et. al explored these phenomena in humans. They demonstrated that type 2 diabetic patients requiring coronary artery stent placement had less neointima formation 6 months after stent placement if they were on troglitazone plus diet compared with type 2 diabetic patients on diet alone. The patients on troglitazone had decreases in blood glucose compared with those patients on diet alone and significant decreases in fasting insulin levels. Conceivably, metabolic changes could contribute to the differences in neointima formation, although direct effects of troglitazone on the vasculature are also likely to have played an important role.

In the LDL receptor (LDLR) knockout mice, a high-fat diet induces hyperglycemia and hyperinsulinemia; subsequently, the animal served as a model of both atherosclerosis and type 2 diabetes. Troglitazone decreases circulating insulin levels in this model but not in the models presenting with hyperglycemia. A high-fructose diet elevates cholesterol in LDLR knockout mice but does not increase insulin or glucose; therefore, the high-fructose diet is a model of atherosclerosis without diabetes. Administration of troglitazone of the LDLR knockout mice on the high-fructose diets resulted in a 45% decrease in atherosclerotic lesions. Lesions from the troglitazone-treated animals on either a high-fat or high-fructose diet accumulated significantly fewer macrophages than lesions from untreated animals. Rosigliazone and a nonthiazohdinedione PPAR-γ ligand, GW7845, have also recently been shown to suppress atherogenesis in high-fat LDLR knockout mice. Thus, inhibition of macrophage accumulation, and possibly the direct anti-inflammatory effects of TZDs, contributed to the significant attenuation of atherosclerotic lesion formation in this model. Two observations suggest that PPAR-γ ligands may have similar activities in humans. First, troglitazone has been demonstrated to decrease carotid intimal medial wall thickness (IMT) after 3 months of treatment in type 2 diabetic patients. However, this observation was based on only a small number of patients for a short period of time, making it difficult to demonstrate consistent changes in carotid IMT. Second, PPAR-γ ligands have been demonstrated to decrease albuminuria in type 2 diabetic patients when compared with the use of other oral antihyperglycemic agents. When troglitazone or metformin were given for 8 weeks to patients with type 2 diabetes and microalbuminuria, both agents similarly decreased blood glucose and HbA. However, troglitazone, but not metformin, reduced albumin excretion rates. Similarly, when glyburide or rosiglitazone was given to patients with type 2 diabetes and microalbuminuria, rosiglitazone, but not glyburide, was associated with substantial reductions in albumin excretion. Albuminuria is considered not only a marker of nephropathy in type 2 diabetes, but also a marker of widespread vascular disease; as such, it correlates with endothelial dysfunction. Thus the decrease in albumin excretion may not only have implications for renal protectin but for vascular protectin as well. Troglitazone has also been demonstrated to improve endothelial cell dysfunction in patients with insulin resistance and type 2 diabetes. In addition, we demonstrated that troglitazone improved coronary artery endothelial function in insulin-resistant subjects. (*reprinted from Dr. Hsueh's review article which appeared in Diabetes Care, 24:2, 2001)*

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