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Insulin as an Anti-Inflammatory and Antiatherosclerotic Hormone

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Fasting hyperinsulinemia is associated with an increased risk of atherosclerotic complications, namely heart attack and stroke, which has led to the concept that insulin may promote atherosclerosis despite the absence of any evidence that insulin is atherogenic either in humans or in experimental models. Recent evidence shows that insulin exerts vasodilatory, antiplatelet, and anti-inflammatory effects at the cellular level in vitro and in humans in vivo. Because atherosclerosis is an inflammatory process, insulin is probably antiatherosclerotic in the long term. Recent data on experimental atherosclerosis in mice shows that (a) insulin administration reduces the number and the size of atherosclerotic lesions in apolipoprotein E null mice; and (b) in insulin receptor substrate-2 null mice, the interruption in insulin signal transduction results in enhanced atherogenicity. The use of a low dose of insulin infusion in patients with acute myocardial infarction (AMI) has been shown to markedly improve clinical outcomes both in diabetic and nondiabetic patients. The authors' most recent data show that a low-dose infusion of insulin in patients with AMI induces a reduction in inflammation (C-reactive protein and serum amyloid A) and oxidative stress and may have a role in myocardial protection. The authors conclude that insulin is both anti-inflammatory and antiatherogenic and may be of use in the treatment of cardiovascular inflammatory conditions, including AMI. Clinical Cornerstone® Supplement 4. Copyright © 2003 Excerpta Medica, Inc.

INSULIN ACTION

Based on the findings of 2 epidemiologic studies conducted 2 decades ago that fasting hyperinsulinemia is associated with accelerated atherosclerosis and an increase in cardiovascular events, it was suggested that insulin may be atherogenic (1–3). More recently, in the Quebec cardiovascular study fasting hyperinsulinemia has again been shown to be associated with an increase in cardiovascular

events (4). Thus, it is clear that fasting hyperinsulinemia, which is a reflection of insulin resistance, predicts atherosclerosis-related cardiovascular events. Persistent attempts have been made to provide mechanistic links between insulin and atherogenesis. Most of these attempts have involved studies on various cell lines in vitro in an endeavor to show that insulin causes specific changes in vascular cells (5,6). Thus, insulin-induced prolifera-

tion, especially in the vascular smooth muscle cells, was considered to be evidence of its atherogenicity. Most of these experiments were carried out at concentrations far greater than those used in a real pathophysiologic setting; these concentrations ranged between 10 to 1000 nM (1600 to 160,000 $\mu\text{U/mL}$) (5,6). Such concentrations of insulin are not observed even in the most insulin-resistant patients with the exception of patients with antibodies to the insulin receptor (4). At those concentrations, insulin binds to the insulin-like growth factor-1 receptor and may activate it. These studies focused on the stimulation of the mitogen-activated protein (MAP) kinase system, which was considered to be a surrogate for atherosclerosis. No study with insulin has been shown to induce mitosis or MAP kinase activity at concentrations usually found in obese patients with insulin resistance, and no study has demonstrated that insulin either causes an induction or acceleration of atherosclerosis in either humans or experimental animals.

Proinflammatory Process

Since atherosclerosis is an inflammatory process and the primary lesion of this process, the fatty streak, is formed by a subendothelial collection of lipid-laden macrophages, the foam cells, it is important to consider the proinflammatory process that leads to fatty streak formation (7). All the major classic risk factors for atherosclerosis—hypercholesterolemia, diabetes, hypertension, smoking, and menopause—are associated with (and probably cause) inflammation. Obesity, which only recently has been added to this list, is associated with insulin resistance, oxidative stress, and proinflammatory changes (8,9). Because oxidative stress and inflammation are integral to atherogenesis, it is possible they are the mechanisms underlying increased atherogenesis in obesity. Although increased macronutrient intake may account for increased oxidative stress (10–12) and inflammation (13,14), it is theoretically possible that insulin may also contribute to these 2 processes and thus to atherogenesis. However, insulin has recently been shown to exert an anti-inflammatory effect *in vitro* in human aortic endothelial cells and *in vivo* in human mononuclear cells. Insulin suppresses

intranuclear nuclear factor kappa B (NF κ B) binding and intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) in human aortic endothelial cells *in vitro* (15,16). A low dose of insulin infused intravenously suppresses NF κ B, increases inhibitor κ B (I κ B), and suppresses reactive oxygen species (ROS) generation and p47^{phox} subunit of the essential protein components of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, the enzyme that converts molecular O₂ to the superoxide (O₂⁻) radical. Insulin also suppresses plasma concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) and MCP-1 (17).

Furthermore, insulin also causes the suppression of 2 other proinflammatory transcription factors, activator protein-1 (18) and early growth response-1 (19). These transcription factors regulate the genes for matrix metalloproteinases, the prothrombotic tissue factor, and the antifibrotic plasminogen activator inhibitor-1 (PAI-1). Thus, insulin is potentially antithrombotic as well as having an antiplatelet property (20). In addition, it is profibrinolytic since it suppresses PAI-1 (17,21). These properties of insulin are potentially anti-atherogenic. They are also of potential use in the treatment of acute myocardial infarction (AMI) in both diabetic and nondiabetic patients.

KEY POINT

Data obtained from human aortic endothelial cells *in vitro* and humans *in vivo* indicate that insulin may have an anti-inflammatory effect.

ANTI-INFLAMMATORY EFFECT OF INSULIN

On exploration of previous literature, few previous experimental studies have indicated an anti-inflammatory effect of insulin in experimental animals. For example, endotoxin-induced lung injury is significantly diminished following insulin treatment (22). Insulin inhibits carrageenan-induced inflam-

mation in rats (23). Insulin suppresses tumor necrosis factor α (TNF- α) generation by peritoneal exudate cells (24), prevents TNF- α -induced interstitial pneumonitis (25), and prevents periportal inflammation in the liver (26). In addition, insulin suppresses macrophage migration inhibiting factor (MIF) expression by adipocytes in vitro (27). Clearly, therefore, there are data that indicate insulin may have an anti-inflammatory effect in various experimental models of inflammation. Along with data obtained from human aortic endothelial cells (HAEC) in vitro and those from humans in vivo, these data point to a definitive anti-inflammatory effect of insulin.

In addition to anti-inflammatory effects, insulin also exerts a vasodilatory action. This action, which is exerted through a direct action on the blood vessel, has been observed in arterial and venous beds and in the microcirculation (28,29). Insulin has been shown to induce nitric oxide (NO) release and to induce nitric oxide synthase (NOS) expression in endothelial cells (30–34). Long-term use of insulin improves vascular reactivity in diabetic patients (35). Insulin also exerts an anti-platelet aggregatory effect that is reduced in diabetic patients due to hyperaggregability of the platelets. This antiplatelet effect is mediated by the nitric oxide–cyclic guanosine-3'-5'-monophosphate signaling (NO-cGMP) pathway. Platelets express NOS and have guanylate cyclase that responds to NO by generating cGMP (20,36).

In view of the comprehensive anti-inflammatory effect of insulin, an insulin-resistant state may be expected to be proinflammatory and atherogenic as well as exhibiting a precontractor vascular behavior. Thus, obesity (37,38) and other insulin-resistant states such as polycystic ovary syndrome (PCOS) (22) are proinflammatory and proatherogenic and are associated with abnormal vascular reactivity and platelet hyperaggregability. This concept is of great interest in terms of the pathogenesis of atherosclerosis in patients with the metabolic syndrome (insulin resistance syndrome) since 24% of the US population have this condition (39), 8% have diabetes mellitus type 2, (40) and 60% are either obese or overweight (41). Furthermore, this insulin-resistant population

would account for the significant number of people who develop congenital heart disease and in whom the traditional cardiovascular risk factors do not explain atherosclerosis.

KEY POINT

The anti-inflammatory effect of insulin also opens up the possibility that insulin may be used as an anti-inflammatory agent in clinical practice.

Insulin Use in Clinical Practice

In explaining the proatherogenic behavior of insulin-resistant states, the anti-inflammatory effect of insulin also opens up the possibility that insulin may be used as an anti-inflammatory agent in clinical practice. This principle has been effectively used in the Diabetes and Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study (42) and the Estudios Cardiológicos Latinoamericana (ECLA) study (43) in which small doses of intravenously delivered insulin markedly improved clinical outcomes in patients with AMI. The DIGAMI study involved patients with type 2 diabetes who had AMI whereas the ECLA study involved patients with AMI irrespective of the presence of diabetes. Since AMI is an acute inflammatory state, it is likely that the anti-inflammatory and antithrombotic effects of insulin are important mechanisms underlying its beneficial action. It is worth noting that the inclusion of insulin in the perfusion fluid at the time of reperfusion in a rat model of coronary ischemia and MI substantially reduced myocardial muscle loss by ~50% (44). It has also been shown in this model that insulin has a potent antiapoptotic effect (45). This may explain a part of its protective effect in AMI. Similarly, insulin improved outcomes in patients in a surgical intensive care unit with a reduction in plasma C-reactive protein (CRP) concentrations (46). Thus, in a series of 1500 patients, half of whom were infused with low doses of insulin aiming to maintain blood glucose concentrations at <110 mg/dL,

the mortality rate was reduced by half when compared with the conventionally treated group with blood glucose concentrations of 153 mg/dL.

In a study just completed in our unit in patients with AMI, half of whom were treated with conventional fibrinolytic therapy and the other half with fibrinolytic therapy plus a low-dose infusion of insulin, the insulin-treated group had significantly smaller elevations of CRP, serum amyloid A, PAI-1, creatine kinase (CK), and creatine kinase with muscle and brain subunits (CKMB). These observations are consistent with an anti-inflammatory, profibrinolytic, and potential cardioprotective effect of insulin in AMI (unpublished data).

ANTIATHEROGENIC EFFECT OF INSULIN

The demonstration that the administration of insulin to apolipoprotein (apo E) null mice reduces the number and the size of atherosclerotic lesions induced by a 2% cholesterol diet illustrates an antiatherogenic effect of insulin in direct fashion (47). In addition, insulin administration suppresses macrophage cholesterol synthesis and cholesterol content, macrophage lipid peroxide content, and macrophage ROS generation. The next challenge is to selectively eliminate insulin action in the mononuclear cell (MNC) and the endothelial cell in apo E null mice to determine whether, (a) a proinflammatory state ensues; and (b) atherosclerosis occurs in an accelerated fashion. Another interesting experimental approach has been utilized by Kubota et al (48), who demonstrated that in insulin receptor substrate-2 (IRS-2) null mice, the application of a perivascular cuff around the aorta, a known method for inducing atherosclerosis, leads to more accelerated atherosclerosis than that observed in normal mice. Therefore, an interruption of insulin signal transduction leads both to severe insulin resistance and to an enhanced tendency to atherosclerosis.

The timely arrival of data showing an antiatherosclerotic effect of insulin in the apo E null mouse, soon after the demonstration of the anti-inflammatory effect of insulin in HAEC in vitro and in humans in vivo, allows the development of novel concepts and potential therapies in

the areas of inflammation and atherogenesis. It also rationalizes the association of insulin resistance with inflammation and allows us to use insulin in patients with type 2 diabetes without the often held fear that “adding” exogenous insulin in a hyperinsulinemic state may worsen atherosclerosis.

INSULIN RESISTANCE

In this perspective of insulin action, insulin resistance, inflammation, and atherosclerosis, it is of interest that insulin sensitizers have also been shown to exert anti-inflammatory (49–53) and potential antiatherosclerotic effects (54,55).

Thiazolidinediones (TZDs) have been shown to exert anti-inflammatory effects at the molecular and cellular levels. They suppress NF κ B, ROS generation, p47^{phox}, induce I κ B in MNC, and also suppress TNF- α , ICAM-1, MCP-1, and CRP in plasma (52,53). Similarly, in the UK Prospective Diabetes Study (54) and the recent retrospective study from Saskatchewan (55), metformin has been shown to reduce cardiovascular morbidity and mortality and also suppress MIF and CRP in plasma. Studies are under way to test the hypothesis that TZDs will also impede the progress of atherosclerosis and reduce cardiovascular morbidity and mortality. In the short term, pioglitazone and troglitazone have been shown to reduce the progression of intima-media thickness in the internal carotid artery of diabetic patients over a period of 3 to 6 months.

If insulin is not the culprit behind atherogenesis in insulin-resistant states, what is? Most insulin-resistant states have been associated with an increase in proinflammatory mediators. Animal models of obesity—the ob/ob mouse, the db/db mouse, and the fa/fa Zucker rat—have been shown to express increased amounts of TNF- α constitutively in adipose tissue (56,57). Human adipose tissue also expresses TNF- α constitutively; this expression increases in the obese and decreases following weight loss (58). Plasma TNF- α , interleukin-6 (IL-6), and CRP concentrations are increased in the obese and decrease with weight loss (59–61). Plasma TNF- α and CRP concentrations are also increased in patients with PCOS, another insulin-resistant state (62). High concentrations of CRP and IL-6 predict the development

of type 2 diabetes and atherosclerotic events (63). Is it possible therefore that inflammation may be the mediator of insulin resistance? Data to demonstrate this are beginning to accumulate. The infusion of soluble receptor of TNF- α into obese animals to bind and neutralize TNF- α action restores insulin sensitivity in these animals (56). TNF- α induces serine phosphorylation of IRS-1, which in turn causes serine phosphorylation of the β subunit of the insulin receptor in the adipocyte (64). TNF- α also reduces insulin receptor tyrosine phosphorylation and insulin receptor protein in human aortic endothelial cells. In addition, TNF- α reduces insulin-induced NOS expression in HAEC (65). IL-6 induces suppressor of cytokine signaling-3 (SOCS-3) expression in cells. SOCS-3 in turn results in diminished IRS-1 tyrosine phosphorylation, phosphatidylinositol 3 kinase-activation, and Akt kinase activation (66,67). Thus, it is likely that the interference of insulin signal transduction by proinflammatory mediators, known to be increased in obesity, PCOS, and type 2 diabetes, is responsible for insulin resistance. Conversely, the administration of the classic anti-inflammatory drug, aspirin, results in the resolution of insulin resistance (68).

We have recently demonstrated that glucose, fat, protein, and mixed meal intake induce ROS generation and oxidative stress as well as inflammatory changes in circulating MNC with an elevation of NF κ B, a decrease in I κ B, an increase in p 47^{phox}, and an increase in I κ B kinase- α and β (10,11,69,70). This cluster of effects is similar to endotoxin-induced inflammatory responses. Since macronutrient intake exerts a proinflammatory effect, and insulin, the hormone secreted in response to macronutrient intake, is anti-inflammatory, it is possible that insulin exerts a continuous tonic anti-inflammatory effect, especially in postprandial states. This balance is likely impaired in insulin-resistant states.

CONCLUSIONS

On the basis of the above, we now have data that insulin is anti-inflammatory and antiatherogenic. These facts therefore should encourage us to further increase our understanding of the novel effects of

insulin so that we have an improved conceptualization of inflammation in states of insulin resistance and the relationship of these states to atherogenesis; and we explore the potential therapeutic role of insulin in inflammatory conditions such as AMI and septicemia.

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