**Mini-Reviews**

**Therapeutic Approaches to Target Inflammation in Type 2 Diabetes**

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**BACKGROUND:** Chronic inflammation may participate in the pathogenesis of insulin resistance, type 2 diabetes, and cardiovascular disease and may be a common denominator that links obesity to these disease states.

**CONTENT:** Epidemiologic studies have linked inflammatory biomarkers to incident diabetes and cardiovascular disease risk. Cellular and animal studies have provided support to the idea that inflammation mediates these disease processes, providing impetus to pharmacologically target these pathways for disease treatment and prevention. We review clinical strategies to target inflammation, with a focus on the antiinflammatory and antihyperglycemic effects of salicylates.

**SUMMARY:** The evolving concept of diet-induced obesity driving insulin resistance, type 2 diabetes, and cardiovascular disease through immunologic processes provides new opportunities for the use of antiinflammatory strategies to correct the metabolic consequences of excess adiposity.

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Obesity, which is occurring at epidemic rates in the US and worldwide, is associated with multiple disease conditions, including, importantly, type 2 diabetes mellitus and cardiovascular disease. The molecular link between obesity, diabetes, and atherosclerosis remains incompletely understood; however, multiple lines of evidence suggest inflammatory pathways as pathogenic mediators for both type 2 diabetes and cardiovascular disease, mechanistically providing a common soil for these disorders. Obesity-induced inflammation is chronic and indolent, features that differentiate it from the more acute types of inflammation commonly associated with infections, injury, and autoimmunity. In addition to providing new insights into disease mechanisms, this difference creates an opportunity to examine inflammation as a new therapeutic option for these conditions.

The most fundamental clinical measure of inflammation is the white blood cell count. Even within the reference interval, higher total leukocyte counts precede and predict the incident risk of type 2 diabetes (1) and coronary heart disease (2), primarily because of the granulocyte subpopulation, rather than the lymphocyte and monocyte subpopulations. More specifically, high concentrations of circulating cytokines, particularly C-reactive protein (CRP)(4) and interleukin-6 (IL-6), have been associated with the development of type 2 diabetes (3, 4) and cardiovascular disease (5).

Indeed, a variety of circulating proinflammatory cytokines and acute-phase reactants are increased in obesity, the metabolic syndrome, hypertension, nonalcoholic steatosis, polycystic ovarian syndrome, type 2 diabetes, and cardiovascular disease. Mechanistically, inflammation marks an acquired cause of both insulin resistance and impaired insulin secretion [reviewed in (6, 7)]. Together, these data raise the question of whether inflammation can be targeted to treat or reduce disease risk, given that the target is mild and chronic low-grade inflammation and is not overt local or systemic acute infections.

At the cellular and molecular level, nuclear factor κB (NF-κB) is the inflammation master switch that controls the synthesis of many proteins critical for the activation and maintenance of the inflamed state. The hypothesis is that obesity stimulates NF-κB activity and additional stress pathways in adipose tissue, liver, and leukocytes, thereby promoting insulin resistance. The first firm evidence to support inflammation, not only as a marker but also as a mediator of disease, was provided more than 17 years ago. Tumor necrosis factor α (TNF-α), a proinflammatory cytokine, was shown to be produced by adipose tissue and to promote insulin resistance. In preclinical rodent studies, blockade of TNF-α was shown to improve insulin resistance, a re-

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4 Nonstandard abbreviations: CRP, C-reactive protein; IL-6, interleukin-6; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; JUPITER, Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin; COX, cyclo-oxygenase; IKK, IκB kinase; TINSAL-T2D, Targeting Inflammation Using Salsalate in Type 2 Diabetes.
sult that raised hopes that TNF-α blockade would also work in humans (8). In the meantime, antibodies against TNF-α and related TNF-α–binding proteins, such as etanercept and infliximab, were developed for the treatment of rheumatoid arthritis, inflammatory bowel disease, and other inflammatory conditions. Despite their great efficacy in treating these inflammatory conditions, these antibodies have proved unsuccessful in treating obesity-related inflammation and type 2 diabetes (9, 10), perhaps because obesity-induced inflammation in humans is a more complex process that involves cytokines and chemokines other than TNF-α, such as adiponectin, leptin, IL-6, resistin, monocyte chemoattractant protein 1, plasminogen activator inhibitor 1, angiotensinogen, visfatin, retinol-binding protein 4, serum amyloid A, and many others. Although leptin and adiponectin are true adipokines that appear to be produced exclusively by adipocytes, other adipokines are also produced in activated macrophages and/or other cells; however, the relative amounts of each adipokine produced by the adipocyte and associated adipose tissue macrophages remain unknown (Fig. 1). In addition, obesity-mediated subacute chronic inflammation involves organs other than adipose tissue, such as the liver or endothelium, that may have important roles in the pathogenesis of diabetes and atherosclerotic processes. At the tissue level, multiple cellular stresses can modulate both phosphorylation and transcriptional events via activation of c-Jun N-terminal kinases and NF-κB, respectively, to regulate enzymatic functions and the concentrations of multiple cytokines, chemokines, and cellular receptors [reviewed in (6)]. In addition to c-Jun N-terminal kinase signaling, these cytokines and their signaling pathways may be novel targets of pharmacologic therapies, or at least they may be clinically useful as biomarkers of disease risk (11, 12).

Epidemiology-based studies suggest that factors besides obesity, including periodontal disease [reviewed in (13)], gut flora (14), and even exposure to air pollutants (15), may contribute to chronic inflammation and therefore may warrant alternative approaches to treatment.

**Antiinflammatory Effects of Diabetes Therapies**

Many therapeutic interventions are effective in reducing inflammation and improving diabetes via indirect or pleiotropic mechanisms. Not surprisingly, given that obesity promotes subacute chronic inflammation, weight loss can diminish the process. In patients with impaired glucose tolerance who participated in the Diabetes Prevention Program, lifestyle modifications that promoted weight loss, including caloric restriction and exercise, reduced the number of patients with high CRP values by approximately 31%, whereas metformin treatment reduced the number of such patients by approximately 13% (16). Both interventions reduced incident diabetes. Surgical weight-loss procedures can also lessen inflammatory processes (17). Besides metformin, insulin sensitizers of the thiazolidinedione class also reduce markers of inflammation. This effect is not merely due to glucose lowering, because the reductions observed for multiple inflammatory markers are greater than the reductions seen after comparable lowering of glucose concentrations achieved with other antihyperglycemic approaches (18). Furthermore, the change in CRP concentration as a marker of the anti-

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**Fig. 1. Adipocyte cell size and adipose tissue cellular content vary with diet-induced obesity.**

(Left), Adipose tissue from C57BL/6J mice fed regular chow; infiltrating inflammatory cells are not evident. (Right), Adipose tissue from age- and sex-matched, genetically identical mice fed a diet high in fat. On average, adipocytes are larger, and infiltrating inflammatory cells are readily apparent.
inflammatory effect may be related to weight changes produced by lifestyle alterations and metformin therapy, but it appears to be independent of weight for thiazolidinediones (19). The latter may be mediated by peroxisome proliferator–activated receptor γ transrepression of inflammatory-response genes (20). In addition to the antiinflammatory effects of insulin sensitizers, insulin itself can reduce the activity of NF-κB (the central transcriptional regulator of the inflammatory response) over the short term (21). This effect does not appear to be sustained (22), however, and/or it may require high intravenous doses of insulin. Interestingly, simple improvements in glycemic control with drugs other than thiazolidinediones or metformin (including subcutaneous basal insulin) do not improve abnormal values for biomarkers of inflammation (23).

**Antiinflammatory Effects of Statins**

In addition to its established cholesterol-lowering effects, statin therapy has more recently been recognized for its ability to reduce CRP. Cholesterol lowering is mediated by inhibiting hydroxymethylglutaryl-CoA reductase, the rate-controlling enzyme of the mevalonate pathway and the primary molecular target for the drug class. The mechanism underlying CRP lowering remains incompletely understood; however, statins effectively lower CRP concentrations by approximately 25% to 30% (24). All statins reduce CRP concentrations, and the effect does not appear to be dose dependent. CRP lowering is not correlated directly with lipid lowering. Statins do not affect fibrinogen concentrations or other inflammatory biomarkers, however. Lowering LDL and CRP concentrations has been shown to be of clinical benefit in settings of acute coronary syndrome (25).

Because statins lower the CRP concentration and CRP is an independent predictor of cardiovascular events, the JUPITER (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin) trial assessed the effect of rosuvastatin on rates of primary cardiovascular events in people with high CRP concentrations but without hyperlipidemia (CRP > 2 mg/L; LDL < 130 mg/dL) (26). Rosuvastatin prevented the primary composite cardiovascular end point of myocardial infarction, stroke, arterial revascularization, hospitalization for unstable angina, or death from cardiovascular cause (hazard ratio for rosuvastatin, 0.56; 95% CI, 0.46–0.69; P < 0.00001). Likewise, many of the components of the primary composite outcome were significantly reduced. Although the CRP concentration was reduced by 37%, however, the LDL concentration was reduced by 50%, so whether the benefit of statins is truly mediated via the antiinflammatory process, compared with the lipid-lowering process, remains uncertain. Notably, incident type 2 diabetes increased in the statin-treated patients, an effect seen with other agents in the statin class (27). This finding demonstrates a divide in the association of inflammation, diabetes, and cardiovascular disease that may be explained by the potent effects of statins on lipids.

**Antiinflammatory Strategies for Treating Type 2 Diabetes**

Although inhibiting TNF-α improves the inflammation of rheumatoid arthritis and other conditions, effects on glycemia are controversial and may depend on dose and duration (9, 10). A retrospective study suggested that the use of chloroquine to treat rheumatoid arthritis is associated with a lower incidence of type 2 diabetes (28). Prospective studies of chloroquine are ongoing. Importantly, inhibition of the IL-1 receptor shows therapeutic promise (7). The IL-1 receptor antagonist is endogenously produced, and its concentrations are reduced in the pancreatic islets of patients with type 2 diabetes mellitus. High glucose concentrations induce the production of the proinflammatory cytokine IL-1β in human pancreatic β cells, a process that may contribute to impaired insulin secretion, decreased cell proliferation, and apoptosis [reviewed in (29)]. In a double-masked, randomized parallel-group trial involving 70 patients with type 2 diabetes, anakinra (a recombinant human IL-1 receptor antagonist) reduced glycohemoglobin, IL-6 concentrations, and the ratio of proinsulin to insulin while increasing C-peptide secretion.

The known antiinflammatory drugs are generally separated into the steroid-based glucocorticoids, the nonsteroidal antiinflammatory drugs, and the more potent immunosuppressive drugs, such as cyclosporine and methotrexate. Glucocorticoids and most immunosuppressive drugs in current clinical practice lack glucose-lowering efficacy and have many long-term deleterious consequences. Similarly ineffective at insulin sensitization and glucose lowering are the commonly used nonsteroidal antiinflammatory drugs, including the nonselective cyclo-oxygenase (COX) inhibitors (e.g., ibuprofen, naproxen) and the selective COX2 inhibitors (e.g., celecoxib).

A recent series of studies suggests, however, that the nonacetylated salicylates, an older class of antiinflammatory drugs, infrequently used today, show promise as potential new treatments for diabetes. Two case reports published well over a century ago demonstrated that sodium salicylate lowered blood glucose (30, 31). No subsequent studies were conducted, however, and this finding was lost in the old medical literature. The effect was rediscovered when high doses of
aspirin used for rheumatoid arthritis were noted to lower the fasting glucose concentration in patients with type 2 diabetes (32), although the molecular mechanism underlying the glucose-lowering effects remained unknown. At about this time, sulfonylureas, with their better safety and tolerance profiles, became available as the first oral antidiabetes agents, and the effects of salicylates appeared to have been largely forgotten.

New ideas about inflammation as a pathogenic mediator in type 2 diabetes provided an impetus for reinvestigating this approach, first in preclinical animal studies and subsequently in clinical trials. At the end of the 19th century, German chemists first acetylated salicylic acid to form acetylsalicylic acid, or aspirin, which soon became, through intensive marketing, the most widely used drug in the history of the world. Aspirin is a very effective antiinflammatory drug because it covalently and irreversibly modifies COX1 and COX2, thereby blocking the rate-limiting step of prostaglandin synthesis. Unlike aspirin, however, sodium salicylate neither effectively inhibits the COX enzymes nor blocks prostaglandin synthesis. Instead, sodium salicylate and other nonacetylated salicylates, including salaslate, inhibit the activity of the transcription factor NF-κB (33).

Preclinical Studies with Salicylates

Given that increased adiposity promotes subacute/chronic inflammation and NF-κB activation, one would expect the antiinflammatory actions of salicylate to inhibit NF-κB, thereby suppressing insulin resistance and lowering the blood glucose concentration (34). The initial preclinical rodent studies of the effect of salicylate on insulin resistance were performed with Zucker fatty rats and ob/ob mice, both of which have inactive leptin signaling that causes hyperphagia and severe obesity. The use of high-dose salicylates in these obese, severely insulin-resistant mice significantly lowered blood glucose concentrations, improved glucose tolerance, and increased insulin sensitivity (34). These early studies also noted significantly lowered circulating concentrations of triglycerides and nonesterified fatty acids, which may also help to improve glycemic control. Heterozygous deletion of the enzyme IκB kinase (IKK) that activates NF-κB (IKKB–/– mice) also improved blood glucose concentrations, glucose tolerance, and insulin sensitivity in obese mice. These results support hypotheses that NF-κB is involved in the pathogenesis of obesity-induced insulin resistance and that reductions in NF-κB activity would be beneficial for patients with type 2 diabetes (34).

Subsequent preclinical studies identified target tissues and cell types for both NF-κB–mediated insulin resistance and salicylate’s ability to reverse obesity-induced insulin resistance. The tissues commonly considered to have important roles in the development of type 2 diabetes include skeletal muscle, liver, fat, and pancreatic β cells. Muscle is a primary site of glucose disposal and utilization. The liver is a major site of gluconeogenesis, the dysregulation of which in type 2 diabetes contributes to fasting hyperglycemia. Adipose tissue is the primary energy depot and is thought to secrete substances that promote insulin resistance. The β cells of the pancreas produce insulin, the prime regulator of glucose homeostasis. Modern genetic methods of tissue-specific gene activation and inhibition have demonstrated that NF-κB activity is modulated in each of these tissues.

Selective NF-κB activation causes extreme muscle wasting in rodents, which can be reversed by selective NF-κB inhibition. Neither activation nor inhibition appears to be related to glycemic regulation, however, because no changes in insulin sensitivity, glucose tolerance, or lipid concentrations specifically accompany genetic modulation of NF-κB activity in muscle (35). On the other hand, activation of NF-κB in the liver, fat, or macrophages causes insulin resistance similar to that seen in obesity, and NF-κB inhibition decreases obesity-induced insulin resistance. These results suggest that these tissues play prominent roles in the pathogenesis of insulin resistance (36,37). Although the molecular effects of salicylates in modulating inflammatory metabolic processes are thought in principle to be mediated through IKK/NF-κB signaling, other signaling events may also participate in metabolic effects, including inhibition of cellular kinases (38) or up-regulation of the heat shock response (39).

Bench to Bedside—Clinical Trials of Salicylates

The epidemiologic findings in humans coupled with the results of the preclinical studies described above have set the stage for translation to clinical studies targeting inflammation. An initial clinical study documented that high-dose aspirin improves insulin resistance. A 2-week course significantly improved glucose concentrations and insulin sensitivity in obese patients with type 2 diabetes (40). Although the negative side effects of prolonged high-dose aspirin intake, which include potentially life threatening gastrointestinal bleeding, preclude its chronic use in patients with type 2 diabetes, the clinical confirmation that salicylates improve insulin resistance has prompted an examination of safer salicylate forms for efficacious treatment.

Clinical trials with the prodrug salaslate, which is marketed in the US for the treatment of joint pain, have recently been conducted. Salsalate has fewer side effects than aspirin or salicylate and is inexpensive to manufacture. Pilot clinical trials to test the efficacy of salaslate were conducted with small groups of patients for
periods of 2 to 4 weeks. Administration of salsalate to patients with type 2 diabetes or to obese prediabetic patients reduced the blood concentrations of glucose, triglycerides, and nonesterified fatty acids (41, 42). Adiponectin, a potentially cardioprotective protein produced by adipose tissue, was increased by up to 45%, a result consistent with insulin sensitization. NF-κB-binding activity was reduced (41, 43). Higher salsalate doses increased insulin secretion (41), a result that has been observed in other studies of salicylates (44, 45). In humans, reduced metabolic clearance of insulin leads to increased circulating insulin concentrations, which may also participate in glycemic improvement (40, 41). These pilot studies observed no seriously detrimental side effects, although some participants who took higher doses experienced tinnitus (ringing in the ears), a well-known reversible accompaniment to salicylate therapy. Likewise, short-term administration of high salsalate doses also improves endothelial function, a biomarker of early atherosclerosis, in obese patients (43).

The results of these small proof-of-concept studies were sufficiently encouraging that the NIH funded larger clinical trials for both type 2 diabetes and cardiovascular disease. The TINSAL-T2D (Targeting Inflammation Using Salsalate in Type 2 Diabetes) study was conducted as a multicenter, double-masked, multidose randomized trial of placebo or salsalate (3.0, 3.5, or 4.0 g/day in 3 doses) administered for 14 weeks as an add-on to current therapy in patients with type 2 diabetes. All 3 salsalate doses lowered glycohemoglobin concentrations (46). Each dose also significantly decreased fasting blood glucose and triglyceride concentrations and increased adiponectin concentrations. Considerations of renal safety will require further evaluation because the investigators found a small increase in urinary albumin. Although the number of patients studied and the trial duration were insufficient to warrant recommending the use of salsalate for type 2 diabetes at this time, these findings support the search for additional antiinflammatory strategies for treating patients with type 2 diabetes. Fully recruited trials involving more patients and longer exposure times are now under way (http://clinicaltrials.gov/ct2/show/NCT00799643). Given the common soil shared by obesity, chronic subacute inflammation, diabetes, and cardiovascular disease, trials are also under way to examine the effects of targeting inflammation via the effects of salsalate on endothelial function and on the coronary artery plaque burden, as assessed with a new imaging modality (multidetector computed tomography angiography), in patients with atherosclerosis (http://clinicaltrials.gov/ct2/show/NCT00624923). Depending on the results of these ongoing investigations, a large simple trial of this inexpensive approach may be warranted.

As discussed above, other strategies (e.g., monoclonal antibodies, chloroquine, periodontal treatment) are also being explored as modulators of insulin resistance and as potential treatments for type 2 diabetes.

Conclusions

Inflammation appears to participate in the pathogenesis of both type 2 diabetes and cardiovascular disease. Multiple basic-science laboratories are investigating the underlying mechanisms that initiate inflammation and link it to insulin resistance and vascular impairment. These new findings may provide new opportunities for treating patients with type 2 diabetes and/or cardiovascular disease and may provide prevention strategies as well. Ongoing trials with salsalate will determine whether this particular antiinflammatory agent is safe and effective. If it is, a new pharmacologic class will have been added to the clinical armamentarium.

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