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Review Article

Salicylates targeting insulin resistance

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CONTENTS

Abstract

Insulin resistance is a condition in which there is a diminished ability to transport glucose from the bloodstream to muscle and tissues, while the pancreas tries to keep up with the demand for insulin by producing more. Several syndromes are related to insulin resistance, such as obesity, dyslipidemia, hypertension, glucose intolerance and diabetes. Nuclear factor κ B (NF-κB), a key proinflammatory transcription factor, has been implicated in the pathophysiology of insulin resistance. As salicylates have been shown to block NF-κB activation, preventing insulin resistance induced by proinflammatory mediators, they are currently being investigated in this field, in particular salsalate due to its safety profile and NCX-4016 on the basis of its dual antiinflammatory and nitric oxide (NO)-releasing properties.

Introduction

The prevalence of type 2 diabetes has reached epidemic proportions worldwide and is projected to increase dramatically (1). Furthermore, cases of insulin resistance, a major causative factor in the early development of this metabolic disorder and an independent risk factor for cardiovascular disease and the metabolic syndrome X, are even more widespread. As dietary modification and increased physical activity provide insufficient glucose control over the long term, the vast majority of patients require some form of pharmacological intervention. In response to this growing problem, efforts to identify and develop new pharmacological agents and to understand the mechanisms underlying insulin resistance have increased significantly in recent years.

Interestingly, a rationale seems to be re-emerging for the treatment of type 2 diabetic patients with antiinflammatory agents. An important role for subacute, chronic inflammation in the development of insulin resistance, type 2 diabetes mellitus and cardiovascular disease has been identified (2). Obesity and high-fat or "Western" diets activate subacute inflammatory processes in fat and liver tissue, as well as in mononuclear cells. The inflammatory mediators produced by these tissues and cells promote the development of insulin resistance both locally and at distant sites such as skeletal muscle (3). This article will piece together new ideas relating to the antidiabetic mechanisms of derivatives of salicylic acid, salicylates, and outline the clinical development program under advancement as a result.

Targeting IKK for treating insulin resistance

IκB kinase (IKK)-β is part of a multisubunit IKK complex that is responsible for regulating the nuclear translocation and activation of the key proinflammatory transcription factor nuclear factor κ B (NF-κB). Phosphorylation of IKK-β leads to the proteosome-catalyzed degradation of IκB, releasing NF-κB for translocation to the nucleus, where it can initiate the transcription of various genes to produce proinflammatory mediators, such as cytokines, to initiate the inflammatory cascade. The IKKs are themselves activated by several factors known to be involved in insulin resistance and diabetic complications, such as hyperglycemia, oxidative stress and specific isoforms of protein kinase C (PKC). IKK-β can also inhibit insulin signaling immediately downstream of the insulin receptor by regulating a pathway leading to serine phosphorylation of insulin receptor substrate-1 (IRS-1) (4-6).

IKKs are proposed to be targets of salicylates. Salicylates have been shown to inhibit IKK-β activity and block NF-κB activation (7, 8), preventing insulin resistance induced by proinflammatory mediators, such as tumor necrosis factor-α (TNF-α), and improving insulin signaling both *in vitro* and *in vivo* (9, 10). Furthermore, it has been shown that the effects of salicylates are attributable to their ability to inhibit IKK and not the cyclooxygenase enzymes COX-1 and -2 (10). Figure 1 depicts how salicylates interfere with the molecular signaling cascade involved in the development of insulin resistance. We will go on to look at early data originating from the use of the widely prescribed antiinflammatory agents aspirin

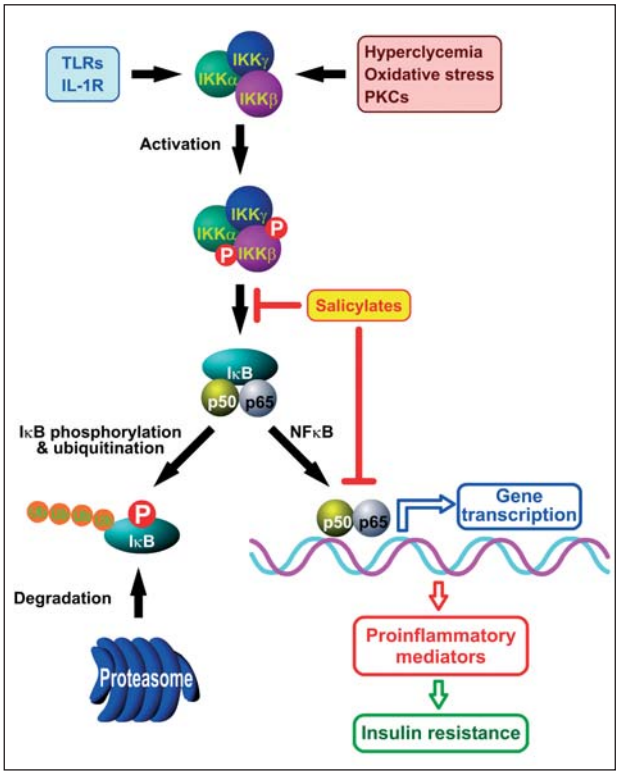

Fig. 1. Both ligand-related stimuli and intracellular factors have been shown to activate the IκB kinase (IKK)/nuclear factor κ B (NF-κB) pathway. The first include toll-like receptors (TLRs) and IL-1 receptor (IL-1R), while the second include hyperglycemia, oxidative stress and various protein kinase C (PKC) isoforms. Activation of IKK-β from the IKK complex (IKK-α, IKK-β and IKK-γ) leads to phosphorylation, ubiquitination and proteosomal degradation of IκB. As a consequence, NF-κB dimers (p50:p65) are released and translocate to the nucleus, where they bind to specific sites in promoter regions and initiate transcription of genes associated with the production of inflammatory mediators that can ultimately lead to insulin resistance. IKK and/or NF-κB are inhibited or repressed by the actions of salicylates.

and sodium salicylate, with further examination of the work stemming from these earlier observations, which now indicates potential clinical benefit for 'safer' salicylates. A summary of relevant completed and ongoing clinical studies of salicylates in insulin resistance is provided in Table I.

Salicylates as promising agents for the treatment or prevention of diabetes

Aspirin and sodium salicylate

Early evidence, dating back to 1876, described how high doses of salicylates (aspirin and sodium salicylate) could markedly attenuate hyperglycemia (for review see Ref. 11). Subsequent *in vivo* studies undertaken in several rodent models of diabetes provided further evidence to suggest that treatment with high doses of these antiinflammatory agents produces significant improvement in glucose intolerance, via an improvement in insulin action as opposed to enhanced secretion (10).

An open-label clinical study in 9 patients with type 2 diabetes treated with high doses of aspirin (mean of 6.77 g/day) for 2 weeks showed a reduction in fasting plasma glucose, insulin clearance and hepatic glucose production, and an improvement in insulin-stimulated peripheral glucose uptake (8). A recent open-label, controlled study in healthy volunteers receiving lipid loads and undergoing euglycemic hyperinsulinemic clamps reported that pretreatment with aspirin at a dose of 1 g t.i.d. improved fat-induced insulin resistance in these subjects, and that the insulin-sensitizing effect was not related to changes in circulating inflammatory markers (12). However, the potential for toxicity associated with high doses of salicylates administered chronically precludes consideration of these agents for therapy, although the results do support further investigation of antiinflammatory agents as useful pharmacological approaches to increase insulin sensitivity.

Interestingly, recent data have shown that clinically relevant doses of aspirin have the unique property of reversing the conformational change of the amyloidogenic peptide amylin, which can aggregate to form amyloid deposits commonly found in the pancreas of type 2 diabetic patients (13). Sodium salicylate has also been shown to protect human islets against the detrimental proapoptotic effects of the proinflammatory cytokine IL-1β and high glucose levels by blocking NF-κB activation (14).

Salsalate

Salicylate in its prodrug form salsalate (salicylsalicylic acid), which is much safer than aspirin (as it does not irritate the gastric mucosa nor alter bleeding times), has also been identified as an inhibitor of NF-κB and has shown promise in the treatment of diabetes. In 6 patients with type 2 diabetes, salsalate (3 g/day) combined with misoprostol over a period of 2 weeks significantly reduced serum levels of triglycerides and fasting glucose, diastolic blood pressure and glucose disposal rate during euglycemic hyperinsulinemic clamp procedures. Salsalate therapy was safe and induced no significant changes in weight, systolic blood pressure or hepatic and renal function of the patients (15, 16). In another double-blind, placebo-controlled study, the effects of salsalate (4 g/day) were assessed in 20 overweight nondiabetic adults over 1 month. Results showed a marked improvement in insulin sensitivity, a reduction in inflammatory markers and a significant increase in adiponectin levels in the salsalate group compared to placebo. The authors concluded that these findings supported the role of inflammation in the pathogenesis of obesity-related insulin resistance and early dysglycemia (17).

The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) is currently recruiting for a clinical study aimed at determining whether administration of the antiinflammatory drug salsalate improves insulin sensitivity in obese nondiabetic individuals and whether this

Table I: Clinical studies assessing the use of salicylates for insulin resistance (from Prous Science Integrity®).

Drug	Design	Treatments	n	Condition	Conclusions	Ref.
Aspirin	Open	Aspirin, 1.24 g p.o. 5x/d x 2 wks	9	Type 2 diabetes	High-dose aspirin improved glucose metabolism in patients with type 2 diabetes by reducing basal rates of hepatic glucose production and insulin clearance and enhancing peripheral insulin sensitivity	8
	Open Crossover	Aspirin, 1 g p.o. t.i.d. x 4 d Aspirin, 1 g p.o. t.i.d. x 4 d + Lipid infusion	12	Healthy	Aspirin pretreatment attenuated lipid-induced insulin resistance in healthy volunteers independently of changes in circulating inflammatory markers	12
Salsalate	Open	Salsalate, 3 g o.d. + Misoprostol, 200 µg q.i.d. x 2 wks	6	Type 2 diabetes	The combination of salsalate and misoprostol was well tolerated and significantly reduced serum levels of triglycerides and fasting glucose, diastolic blood pressure and glucose disposal rate during euglycemic hyperinsulinemic clamp procedures in patients with type 2 diabetes	15
	Randomized Double-blind	Salsalate, 4 g p.o. o.d. x 1 mo Placebo	20	Obesity	Salsalate significantly reduced systemic inflammation and improved glucose metabolism in overweight nondiabetic patients	17
	Randomized Double-blind	Salsalate, 3 g/d p.o. x 7 d Placebo	92	Obesity	This study will evaluate if salsalate can improve insulin sensitivity in obese nondiabetic subjects	18
	Randomized Double-blind	Salsalate x 12 wks Placebo	80	Impared glucose tolerance	A phase II/III study will evaluate if salsalate may be a safe and effective means of modulating inflammation in metabolically critical tissues and reducing insulin resistance and related complications in patients with impaired glucose tolerance	19
	Multicenter Randomized Double-blind Dose-finding	Salsalate, 3 g/d p.o. x 2 wks Salsalate, 3.5 g/d p.o. x 2 wks Salsalate, 4 g/d p.o. x 2 wks Placebo	402	Type 2 diabetes	This study will evaluate the optimal dose of salsalate that is both well tolerated and improves glycemic control in 120 patients with type 2 diabetes. In a further stage of the study the effects of salsalate on measures of inflammation, metabolic syndrome and cardiac risk will be assessed in a cohort of 282 patients	20, 21
NCX-4016	Comparative Randomized Double-blind Crossover	NCX-4016, 800 mg b.i.d. x 4 wks Aspirin, 325 mg o.d. x 4 wks Placebo	13	Type 2 diabetes	NCX-4016 was associated with improvement in insulin resistance and systemic hemodynamics, and it reduced oxidative stress and prevented aspirin-associated changes in albuminuria and blood pressure in patients with type 2 diabetes and early nephropathy	24

improvement is related to a decrease in serum markers of inflammation (18). Researchers from the Department of Veterans Affairs are planning a study to fully characterize the benefits of high-dose salsalate therapy for modulating inflammation in metabolically critical tissues and thus reduce insulin resistance and related complications (19). The Joslin Diabetes Center and the NIDDK are also conducting a multicenter clinical trial, known as TINSAL-T2D (Targeting InflammatioN with SALsalate in Type 2 Diabetes), to investigate whether salsalate can reduce blood glucose levels in subjects with type 2 diabetes. This 2.5-year study is expected to enroll 402 adults with poorly controlled blood glucose levels who are not taking insulin. The study will be conducted in two

stages: stage 1 will last 14 weeks and 120 participants will be randomized to placebo or salsalate 3.0, 3.5 or 4.0 g/day; the optimal dose selected from stage 1 will be tested in the second stage in a second cohort of 282 participants, and salsalate or placebo will be administered for 26 weeks (20, 21).

NCX-4016

NCX-4016 (NicOx), or nitric oxide (NO)-releasing aspirin, has demonstrated beneficial effects on diabetes-induced vascular complications. NCX-4016 has been shown to prevent defects in NO-mediated, endothelium-dependent relaxation due to acute elevations in glucose *ex vivo*, and *in vivo* in a model of chronic streptozotocin-induced diabetes mellitus (22). Further *in vivo* studies in the same model demonstrated that, in contrast to NCX-4016, aspirin has no protective action, suggesting that the beneficial effects of NCX-4016 may be related to its NO-releasing capacity (23).

In patients with type 2 diabetes and early nephropathy (n=13), NCX-4016 (800 mg b.i.d.) administered over a period of 4 weeks was associated with an improvement in insulin resistance and systemic hemodynamics, and it reduced oxidative stress and prevented aspirin-associated changes in albuminuria and blood pressure (24).

New clinical data from a substudy of study NCX 4016-X-207 in 40 type 2 diabetic patients supported further development of NCX-4016 as an insulin-sensitizing agent for the treatment of type 2 diabetes. These patients underwent a 4-h hyperglycemic clamp procedure following 2 weeks of treatment with NCX-4016, NCX-4016 plus aspirin, aspirin alone or placebo. These investigations revealed that plasma insulin levels were approximately 40% higher in the two groups that received NCX-4016, probably due to decreased insulin clearance. Glucose infusion rates were also approximately 50-60% higher in those receiving NCX-4016, indicating increased glucose utilization (25, 26). In a second separate crossover study conducted in 12 healthy obese subjects, subjects received NCX-4016 or placebo over 2 weeks before undergoing a hyperglycemic clamp procedure. Subjects receiving NCX-4016 displayed a significant improvement in glucose uptake and a trend for lower fasting blood glucose levels (26).

NicOx plans to initiate two phase II studies in 2007. The first study will be a single-center, crossover trial, designed to confirm the mechanism of action of NCX-4016 as an insulin-sensitizing agent using a hyperinsulinemic euglycemic clamp. The second trial will be a placebo- and active-controlled, double-blind, parallel study to demonstrate clinical benefit of NCX-4016 via the measurement of glycosylated hemoglobin (HbA1c) and blood glucose in the fasting state and after meals. NicOx also reports a good safety and tolerability profile for long-term NCX-4016, which has been shown to be metabolized to release higher and more sustained levels of salicylate than previously thought, in addition to an NO-donating moiety (27).

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