Clinical Trials, Triumphs, and Tribulations of Glucagon Receptor Antagonists

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Diabetes Care 2016;39:1075–1077 | DOI: 10.2337/dci15-0033

Since the discovery of glucagon's opposing actions to insulin, drugs targeting the inhibition of glucagon action have been pondered. In recent years, several attempts to generate small molecules or antibodies that impair glucagon action have been pursued as potential therapeutics for type 2 diabetes. In the current issue of *Diabetes Care*, Kazda et al. (1) summarize the outcomes of the phase 2a and phase 2b clinical trials of LY2409021, a small-molecule glucagon receptor antagonist (GRA). This is the largest and longest trial for safety and efficacy of a GRA ever performed. Importantly, LY2409021 does not produce side effects on cholesterol homeostasis that have impeded the progress of other small-molecule GRAs. Here, we place their success in perspective and discuss the advantages and concerns relating to glucagon-based therapeutics as this line of drugs comes closer than ever to achieving their clinical potential.

In 1922, the first children with type 1 diabetes were treated with an insulin-containing pancreatic extract, preventing ketoacidosis and an insidious death. In addition to the discovery of insulin, the crew of Banting, Best, and Collip observed glucagon action, as they had noticed in their preclinical studies in canines that some of their crude insulin preparations would raise glucose levels in the dog briefly before glucose was lowered (2,3). This glucose-raising peptide was termed "glucagon" (4) and subsequently purified and identified as a 29–amino acid peptide (5). In 1959, the development of the radioimmunoassay made it possible to quantify the two major glucoregulatory hormones, insulin (6) and glucagon (7). It was quickly established that glucagon was in fact a true hormone responsible for maintaining the glucose supply to the brain via increased glycogenolysis and gluconeogenesis.

It has since been demonstrated that every form of diabetes is associated with hyperglucagonemia, the suppression of which eliminates hyperglycemia (8). After pancreatectomy, glucagon-producing α-cells proliferate in the fundus of the stomach, allowing for hyperglucagonemia (9). In other words, diabetes is a bihormonal disease rather than simply the result of insulin deficiency (10) (Fig. 1). It was further shown that glucagon-producing α-cells are topographically arranged for functional reasons with 91% of α-cells in the islets of Langerhans juxtaposed to β-cells. This close proximity permits insulin to tightly regulate glucagon secretion and precisely control the insulin-to-glucagon ratio in the healthy pancreatic islet. When insulin is present, the α-cells, juxtaposed to β-cells, receive the highest insulin concentration of any target cell in the body. The paracrine levels of insulin reaching α-cells have been estimated at between 2,000 and 4,000 μU/mL (11). This is almost impossible to achieve by administration of insulin into the periphery, which provides a substantial gap in our ability to treat diabetes effectively within the clinic. An alternative to higher doses of insulin for the treatment of hyperglycemia is to minimize the contributions of glucagon. Thus, the insulin-to-glucagon ratio is enhanced by minimizing the denominator and glucagon's effects on the liver.

Regulation of glucagon secretion occurs locally, within the islet, and via effects in the central nervous system (12). Insulin, γ-aminobyteric acid, and leptin are potent physiologic regulators that dampen glucagon secretion (13). In 1984, we conducted experiments that demonstrated the paracrine role of insulin on α-cell function (11). Using a potent anti-insulin neutralizing serum, we perfused normal pancreata and showed that when insulin inside the islet was neutralized, glucagon levels rose by 150% and remained elevated until the antiserum was stopped. Ablation of the

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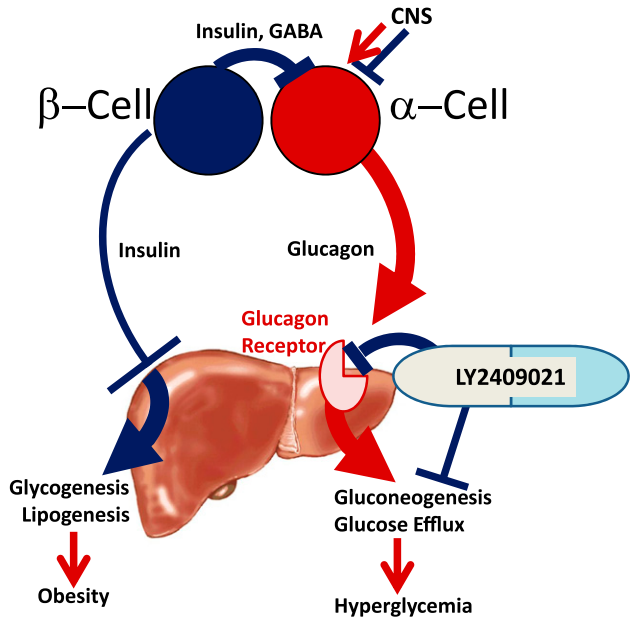

Figure 1—The bihormonal regulation of glucose by insulin and glucagon. Insulin suppresses glucagon secretion while promoting lipid and carbohydrate storage. Glucagon prompts gluconeogenesis and glucose efflux from the liver. LY2409021 decreases serum glucose by preventing glucagon receptor activation and alleviating excess gluconeogenesis. CNS, central nervous system; GABA, γ-aminobyteric acid.

insulin receptor from the α-cell has supported the importance of insulin as a negative regulator of α-cell secretion (14,15).

Glucagon raises blood glucose through its cognate receptor in the liver (rev. in 16). This G-protein–coupled receptor is responsible for driving gluconeogenesis by upregulating glucose-6-phosphatase and PEPCK, key enzymes that are activated through adenylyl cyclase signaling to protein kinase A and subsequently CREBP and peroxisome proliferator–activated receptor γ coactivator (PGC)1a. Salt-inducible kinase (SIK)2 is also activated through protein kinase A. SIK2 phosphorylates and activates CREBP-regulated transcription coactivator 2 (CRTC2), another cotranscriptional regulator of gluconeogenic genes. By blunting glucagon action on the liver, these signals are diminished and hepatic glucose efflux is reduced.

Since the discovery of glucagon's counterregulatory effects that oppose the glucose-lowering actions of insulin, strategies for blunting glucagon secretion or glucagon action have been imagined as potential therapies for diabetes. Over the past decade, several GRAs have emerged from the pharmaceutical industry and have been evaluated both preclinically and in clinical trials. These approaches have included small-molecule antagonists, which can work through allosteric or competitive inhibition; fully human antibodies, which assuage side effects from chimeric or humanized antibodies against the glucagon receptor; and antisense oligonucleotides to downregulate the expression of the glucagon receptor. All of these appear to have glucose-lowering properties and have recently been reviewed (17).

In this issue of *Diabetes Care*, Kazda et al. (1) describe the most recent clinical trials by Eli Lilly and Co. using the small-molecule GRA LY2409021. The initial randomized, double-blind phase 2a clinical study compared the effects of three doses of LY2409021 with placebo over a 12-week study period. Patients were either naïve to antidiabetes medications or on metformin (59%). Dose-dependent lowering of HbA_{1c} was achieved with 10, 30, or 60 mg LY2409021, while HbA_{1c} rose 11% in patients receiving placebo. Fasting blood glucose and self-monitored blood glucose were lowered by all three doses. During the phase 2b study, 2.5-, 10-, or 20-mg doses of LY2409021 produced a dose-dependent improvement in glycemia at the two higher doses that was sustained for the 24-week treatment period. One-third of patients achieved HbA_{1c} levels $<6.5\%$, and one-half of the patients achieved HbA_{1c} levels $<7\%$. Dose-dependent increases in total glucagon-like peptide 1 (GLP-1) and alanine aminotransferase (ALT) were observed and returned to basal levels during a posttreatment washout period. No significant changes in body weight, blood pressure, heart rate, electrocardiogram, or plasma lipid were detected for any dose of LY2409021 compared with the placebo control group.

The involvement of GLP-1 has been investigated as a contributor to GRA-mediated improvements in glucose metabolism. As glucagon and GLP-1 are derived from the same preproglucagon precursor, endocrine feedback stemming from blunted glucagon action prompts increases in both GLP-1 and glucagon production from the α-cell. In preclinical models, both fibroblast growth factor 21 (FGF21) and GLP-1 are known to increase after GRA treatment. Although data have suggested that these hormones may contribute to the glycemic improvements evoked by GRAs (18,19), conflicting results exist (20) (M.Y. Wang and R.H.U., unpublished observations). LY2409021 did not alter levels of active GLP-1, suggesting that it is dispensable for the glycemic improvements in humans.

Several concerns with previous GRAs have been noted. These include increased serum cholesterol, increases in body weight, glycogen storage alterations, enhanced α-cell hyperplasia, and increases in serum transaminase levels. The most obvious concern to most clinicians is an enhanced risk of hypoglycemia. No severe hypoglycemic episodes occurred in the study by Kazda et al. (1). During the phase 2a trial, four symptomatic hypoglycemic events were reported. Overall, the incidence of hypoglycemia was not statistically different from placebo. Importantly, exogenous glucagon can still rescue patients treated with LY2409021, as its affinity for the glucagon receptor is still low enough to allow for increased glycemia after intramuscular injection of glucagon.

An important critique overcome by the study by Kazda et al. (1) is the fear that all GRAs will cause increases in serum lipids. Most notably, Merck's small-molecule GRAs revealed increases in LDL cholesterol during phase 2 clinical trials. It is reasonable to speculate that insulin may drive lipogenesis (through sterol response–binding protein 1c) more strongly in the absence of glucagon action during the acute period before insulin secretion wanes to match the decreased demand for insulin. However, the Merck team showed that their small molecule, MK-0893, increases

cholesterol absorption while lowering blood glucose (21). Importantly, LY2409021 did not increase LDL, and data trended toward improvements in cholesterol.

Another concern of GRA therapy would be the development of glycogen storage disease. In mice, a fourfold increase of glycogen has been reported in glucagon receptor–null mice (22). With LY2409021, changes were not seen in rodents but were noted in cynomolgus monkeys (23). This will be important to investigate, as it may offer a potential explanation for the elevated ALT levels. Notably, most glycogen storage diseases present with elevated ALT and aspartate aminotransferase (AST). Glycogen content in human subjects has not been reported yet with this candidate drug.

Increased liver enzymes are perhaps the most commonly observed side effect of GRA treatment. Three of the 85 patients in the study by Kazda et al. (1) showed ALT levels more than three times the upper limit of normal. AST also increased, albeit to a lesser degree. In the phase 2b study, 8 of the 191 patients showed increases in ALT, which exceeded three times the upper limit of normal. The U.S. Food and Drug Administration guidelines for drug-induced liver injury are defined by a threefold ALT/AST increase, with concurrent increases in alkaline phosphatase by twofold and total bilirubin by twofold. None of the subjects with high ALT levels showed concomitant increases in bilirubin, alkaline phosphatase, or symptoms of liver disease. It is possible that increased glycogen content may account for some changes in liver enzymes. Alternatively, this may be driven by an alternative means of handling the amino acids, which would otherwise be used for gluconeogenesis. Regardless, the small degree of change in AST/ALT, in the absence of other markers of liver toxicity (alkaline phosphatase or bilirubin), suggests that these drugs would not exceed U.S. Food and Drug Administration guidelines for drug-induced liver injury.

The most severe concern with GRA therapy is malignant transformation of α-cells, as they undergo marked hyperplasia when the action of their secretory product is blocked. As glucagon promotes depletion of amino acids by stimulating their use as gluconeogenic substrates (24), GRAs promote an increase in hepatic and circulating amino acids. In turn, this can lead to enhanced α-cell proliferation by enhancing mechanistic target of rapamycin activation within the α-cell (25). Although we have yet to encounter a glucagonoma in any of the animals treated chronically with glucagon receptor antibodies, the disease (glucagonoma) is such a terrible one that one may consider monitoring patients treated with GRAs. A very early marker of glucagonoma is easily detectable by monitoring a COOH-terminal extension of the glucagon molecule.

An exciting direction that remains underexplored is the potential for GRAs to treat type 1 diabetes. We have seen in rodent models that have been treated with antibodies against the glucagon receptor that such treatment can improve glycemic control far better than insulin monotherapy alone (13). The primary advantage provided by the GRAs is the prevention of glycemic volatility, as oscillations in glucose are greatly minimized and glucose levels remain consistently between 80 and 120 mg/dL. LY2409021 has been revealed at conference proceedings to diminish the need for insulin in patients with type 1 diabetes (26). We remain optimistic that GRAs will find their way into the clinic to provide a potent glycemic therapy for the treatment of diabetes.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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