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REVIEW ARTICLE

Present status of clinical deployment of glucokinase activators

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ABSTRACT

Glucokinase is one of four members of the hexokinase family of enzymes. Its expression is limited to the major organs (such as the pancreas, liver, brain and the gastrointestinal tract) that are thought to have an integrated role in glucose sensing. In the liver, phosphorylation of glucose by glucokinase promotes glycogen synthesis, whereas in the β-cells, it results in insulin release. Studies of glucokinase-linked genetically-modified mice and mutations in humans have illustrated the important roles played by glucokinase in whole-body glucose homeostasis, and suggest that the use of pharmacological agents that augment glucokinase activity could represent a viable treatment strategy in patients with type 2 diabetes. Since 2003, many glucokinase activators (GKAs) have been developed, and their ability to lower the blood glucose has been shown in several animal models of type 2 diabetes. Also, we and others have shown in mouse models that GKAs also have the effect of stimulating the proliferation of β-cells. However, the results of recent phase II trials have shown that GKAs lose their efficacy within several months of use, and that their use is associated with a high incidence of hypoglycemia; furthermore, patients treated with GKAs frequently developed dyslipidemia. A better understanding of the role of glucokinase in metabolic effects is required to resolve several issues identified in clinical trials.

INTRODUCTION

The incidence of diabetes has been rising globally over the past approximately 30 years[1], and there are an estimated 371 million adults with diabetes around the world[2]. In regard to the medical treatment of hyperglycemia in type 2 diabetes patients, a position statement by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommended initial treatment with metformin monotherapy in patients where lifestyle interventions are inadequate, followed by additional agents[3]. Although several classes of oral therapeutic agents are currently available for the treatment of type 2 diabetes, in most cases, the initial improvements of the hyperglycemia are not sustained because of continued β-cell failure[4]. In addition, many of these therapeutic agents have adverse effects, such as hypoglycemia, weight gain, gastrointestinal disturbances, peripheral edema and potential cardiovascular effects[5]. Therefore, the search for new therapeutic agents with improved benefit–risk profiles continues.

Since the first report by Grimsby *et al.*[6] in 2003, many glucokinase activators (GKAs) have been developed, and their ability to lower the blood glucose has been shown in several animal models of type 2 diabetes[7]. Also, single-and multiple-dose placebo-controlled studies in humans have shown the effect of GKAs in reducing the fasting and postprandial glucose levels in patients with type 2 diabetes as well as in healthy adults[8,9]. Notably, however, in one trial of MK-0941, one of the GKAs, loss of efficacy of the drug over time and an increase of the serum triglyceride levels were observed, which led to termination of this particular trial[10]. Therefore, a better understanding of the mechanism is required to determine whether glucokinase activation with GKAs is a feasible treatment approach for patients with type 2 diabetes. Here, we briefly review the role of glucokinase in glucose metabolism, the effects of GKAs on the pancreatic β-cells and liver reported from animal studies, the current status of clinical trials on GKAs, and

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the problems and future prospects of clinical use of GKAs determined from these trials.

GLUCOKINASE

Glucokinase is one of the four members of the hexokinase family of enzymes. It operates as a monomer and phosphorylates glucose on carbon 6 with magnesium adenosine triphosphate as the second substrate, to form glucose-6-phosphate (G6P). Under physiological conditions, glucose is its preferred substrate; hence, its widely accepted name is glucokinase rather than hexokinase IV or D. Expression of glucokinase is limited to the major organs (such as the pancreas, liver, brain and the gastrointestinal tract) that are thought to have an integrated role in glucose sensing[11]. In the liver, phosphorylation of glucose by glucokinase promotes glycogen synthesis, whereas in the pancreatic β-cells, it results in insulin release. Glucokinase has unique biochemical kinetics (it shows non-Michaelis–Menten kinetics) that accounts for its role as a glucose sensor. It has a low affinity for glucose as a substrate, with a $K_{0.5}$ of ~7 mmol/L (which is within the physiological range for glucose). The enzyme is not inhibited by its end-product, G6P, and although it is a monomeric enzyme, it shows a sigmoidal saturation curve for glucose with a characteristic Hill slope of ~1.7. The inflection point of the sigmoidal curve (4–5 mmol/L) is close to the physiological threshold for glucose-stimulated insulin secretion in the pancreatic β-cells. Functionally, this positive co-operativity with glucose allows the enzyme to have an increased sensitivity to fluctuations in the blood glucose levels. Together, these kinetic properties enable glucokinase to be highly responsive to glucose levels, and to ensure that the glucose metabolic flux is closely tied to the blood glucose concentration.

IMPORTANCE OF GLUCOKINASE IN REGULATING THE BLOOD GLUCOSE

In humans, the glucokinase gene (*Gck*) is located on chromosome 7p15.3–p15.1, and consists of 12 exons that span 45,168 bp and encode a 465-amino acid protein with a molecular weight of 52,191 Da, which is especially expressed in the pancreas, liver, brain and endocrine cells of the gut. The presence of tissue-specific promoters allows differential regulation and transcription of different transcripts giving rise to three different-sized versions of exon 1 (a, b and c). In the pancreas, the upstream promoter is functional, whereas in the liver, the downstream promoter is used. Exon 1a is expressed in the pancreatic β-cells, whereas exons 1b and 1c are expressed in the liver[12]. The critical role of glucokinase in regulating the blood glucose levels is shown by studies carried out in glucokinase-knockout or transgenic mice and in humans with glucokinase mutations.

Insights Obtained from Genetically Modified Mice

We generated mice carrying a null mutation of *Gck* in the pancreatic β-cells, but not in the liver, by disrupting exon 1a expression and thereby selectively eliminating expression of the pancreatic β-cell glucokinase isoform without affecting the expression of the liver isoform[13]. The heterozygous mutant mice showed normal glucokinase activity in the liver, but an approximately 50% reduction of the glucokinase activity in the pancreatic islets. At the age of 10 weeks, these mice showed mild diabetes as a result of impaired secretion of insulin in response to glucose. Homozygous null mutants developed postnatal metabolic disorders and died within a week after birth. In *ex vivo* experiments, glucose-induced insulin secretion from the islets of these animals was defective. Grupe *et al.*[14] reported the phenotypes of animals in which *Gck* had been inactivated in both the pancreatic β-cells and the liver by insertion of a neo cassette between exons 3 and 5. In these heterozygous mutant mice, the blood glucose levels were elevated and insulin secretion was reduced. Furthermore, these homozygous mutant mice developed severe diabetes with ketoacidosis and died within a week after birth, just like our β-cell-specific *Gck*-knockout mice. Thus, the findings suggest that expression of glucokinase in the pancreatic β-cells is crucial for survival. Also, β-cell-specific or liver-specific *Gck* knockout was generated using the Cre/loxP system[15]. Animals either globally deficient in *Gck* or lacking *Gck* in just the β-cells died of severe diabetes within a few days after birth. Mice that were heterozygous for the null mutation of *Gck*, either globally or in just the β-cells, survived but were moderately hyperglycemic, similar to our β-cell-specific *Gck*-deficient mice and mice lacking *Gck* in both the β-cells and the liver. In contrast, mice lacking *Gck* only in the liver were only mildly hyperglycemic, but showed pronounced defects in both glycogen synthesis and glucose turnover rates during a hyperglycemic clamp. These studies pointed to β-cell glucokinase having a greater impact on glucose homeostasis than liver glucokinase, and provided strong support for the concept that glucokinase is important for glucose sensing. In contrast, overexpression of glucokinase led to lower basal blood glucose levels and improved the glucose tolerance, even though the plasma insulin levels in the animals were similar to those in the non-transgenic mice. Furthermore, these animals also showed increased hepatic glycogen synthesis despite a smaller increment of the plasma insulin during a hyperglycemic clamp. These results indicated that overexpression of glucokinase activated hepatic glucose metabolism, and consequently led to a lower plasma glucose concentration. Increased insulin secretion was not observed, even though the transgene was expressed in the islets, because hypoglycemia caused a downregulation of the islet glucokinase content[16,17]. Also, these mice were protected against the development of both hyperglycemia and hyperinsulinemia associated with the feeding of a high-fat (HF) diet[18].

Insights Obtained from Mutations in Humans

The crucial role of glucokinase in regulating the blood glucose levels is illustrated by the fact that over 600 mutations in *Gck* cause different monogenic glycemic disorders[12]. Thus, heterozygous inactivating mutations cause familial, mild fasting

hyperglycemia, also known as maturity-onset diabetes of the young type 2 (MODY2). MODY2 patients show mild fasting hyperglycemia from birth, although they are usually asymptomatic and most remain undiagnosed until later in life[19]. These patients show little deterioration with age, and usually do not require any specific treatment. Recently, a cross-sectional study carried out in the UK revealed that patients with a glucokinase mutation showed a low prevalence of microvascular and macrovascular complications, despite a median duration of hyperglycemia of 48.6 years[20]. The principal pathophysiological mechanism of altered glycemia in patients with glucokinase mutations is β-cell dysfunction, characterized by a modification of the blood glucose threshold that triggers insulin secretion, which is consistent with a defect in glucose sensing[21]. In addition, abnormalities in liver glucose metabolism contribute to the hyperglycemia in patients with MODY2. Furthermore, homozygous inactivating glucokinase mutations result in a more severe phenotype presenting at birth as permanent neonatal diabetes mellitus. So far, a total of 10 glucokinase mutations have been reported that lead to neonatal diabetes mellitus. Homozygosity or compound heterozygosity for nonsense, missense or frameshift mutations is found in these cases, which result in a deficiency of glucokinase activity[22]. In contrast, heterozygous activating mutations cause persistent hyperinsulinemic hypoglycemia of infancy. Activating glucokinase mutations increase the affinity of glucokinase for glucose and alter the threshold for glucose-stimulated insulin secretion[23]. Thus, insulin continues to be produced at lower blood glucose levels. All reported activating mutations are clustered in a region of the enzyme, which has been termed as the allosteric activator site and is remote from the substrate-binding site.

SMALL-MOLECULE GLUCOKINASE ACTIVATORS

Studies carried out in the glucokinase-linked genetically-modified mice and mutations in humans show the important role that glucokinase plays in whole-body glucose homeostasis, and suggest that the use of pharmacological agents that augment glucokinase activity might represent a viable treatment strategy in patients with type 2 diabetes. Grimsby *et al.*[6] reported that Ro-28-1675, which has been identified as a GKA for the first time, activated glucokinase by increasing the affinity for glucose and maximal velocity. It has been shown to enhance glucose-stimulated insulin secretion from isolated rat pancreatic islets, increase hepatocyte glucose uptake and exert antihyperglycemic effects in various rodent models. Furthermore, chronic administration of Ro-28-1675 prevented the development of hyperglycemia in diet-induced obese mice. Like Ro-28-1675, other GKAs also bind to an allosteric site in the region of clustering of the activating mutations described above, and have been shown to lower the blood glucose in several animal models[6,24–33]. In excess of 150 patents for novel GKAs have since been recorded[7], and GKAs are classified into several classes according to the chemical structure (Table 1) [34].

Table 1 | Some classes of glucokinase activators classified by the chemical structures

Carbon centered
Amino acid based
Urea centered
1,3,5-Substituted aryl-centered
1,2,4-Substituted aryl-centered
Other templates

Effects of GKAs on Insulin Secretion from the Pancreatic β-Cells

The rate of phosphorylation of glucose in the pancreatic β-cells is directly related to the concentration of glucose over a range of physiological glucose concentrations (4–15 mmol/L). Phosphorylation of glucose by glucokinase is the rate-limiting step in insulin secretion. Glucokinase controls glycolytic and oxidative adenosine triphosphate production and thereby determines the ratio of adenosine triphosphate to adenosine diphosphate, which in turn regulates the K channel, closing it and depolarizing the cell gradually as the ratio increases. The L-type Ca channel opens when its membrane potential threshold is reached, which then triggers insulin release. Regarding β-cell function, GKA-50 has been shown to stimulate insulin secretion in a Ca^{2+}-dependent manner in rodent islets and MIN6 cells[35]. Also, we investigated the changes in the expression levels of the genes involved in the functions of the β-cells in the islets of mice in the absence and presence of GKA. Pancreatic and duodenal homeobox-1 is the major regulator of glucose-stimulated insulin gene transcription, and its messenger ribonucleic acid (mRNA) level was significantly increased by GKA, indicating that GKA improved the functions of the β-cells at the transcriptional level[36]. Furthermore, Doliba *et al.*[37,38] investigated the effects of piragliatin, one of the GKAs, on glucose-stimulated insulin secretion, respiration and calcium metabolism of islets isolated from the pancreas of individuals with type 2 diabetes. These results showed that piragliatin repairs defective insulin secretion, oxygen consumption and calcium response to glucose in the islets isolated from type 2 diabetic organ donors.

Effects of GKAs on Glucose Uptake in the Liver

To investigate the mechanism of action of GKA in liver, the interaction between glucokinase and glucokinase regulatory protein (GKRP) is key. It is well known that hepatic glucokinase activity is modulated by the endogenous inhibitor, GKRP. Glucokinase is localized in the nucleus as an inactive complex with GKRP at low glucose concentrations, and is dissociated from the glucokinase/GKRP complex and translocated to the cytoplasm at high glucose concentrations, which triggers glucose disposal[39]. It has been proposed that the glucokinase/GKRP complex dissociates as GKAs bind to the allosteric site. GKAs could activate glucokinase both directly and by destabilizing the

glucokinase/GKRP complex. Studies using isolated rat hepatocytes have shown that GKAs stimulate glycolysis and glycogen synthesis, and effectively dissociate the nuclear glucokinase/GKRP complex[40]. The data on stimulated glycogen synthesis are consistent with the findings in the study in which GKA (PSN-GK1) increased the hepatic glycogen levels in mice[27]. Also, glucose production was shown to be reduced by another GKA (compound A) in rat-cultured primary hepatocytes[26], and GKA23 improved glucose tolerance not only by stimulating insulin secretion, but also by reducing the hepatic glucose output *in vivo*[33].

IMPACT OF GKAS ON β-CELL PROLIFERATION

Previously, we demonstrated that wild-type mice fed a HF diet showed marked β-cell hyperplasia, whereas the islets of the mice with haploinsufficiency of β-cell-specific glucokinase ($Gck^{+/-}$) showed insufficient β-cell hyperplasia, decreased β-cell replication and impaired upregulation of insulin receptor substrate-2 (Irs2), despite the presence of a similar degree of insulin resistance (Figure 1)[41]. Taken together with the role of Irs2 in β-cell growth and survival[42–44], it might be suggested that the combination of glucokinase and Irs2 is critical for β-cell hyperplasia to occur in response to HF diet-induced insulin resistance. Based on the results in $Gck^{+/-}$ mice fed a HF diet, stimulating glucokinase-dependent pathways could be a powerful therapeutic strategy for type 2 diabetes to the extent of proliferating β-cells. Interestingly, Kassem *et al.*[45] showed that gain-of-function mutations of *Gck* in humans was associated with increased β-cell proliferation. These backgrounds prompted us to investigate the effects of GKAs on the β-cell proliferation in addition to β-cell function in mouse models.

Effect of GKA on β-Cell Proliferation

To examine the ability of GKA (3-[(1S)-2-hydroxy-1-methylethoxy]-5-[4-(methylsulfonyl)phenoxy]-N-1,3 -thiazol-2-ylbenzamide) to induce β-cell proliferation *in vitro*, we initiated studies on INS1 cells. INS1 cell proliferation, as assessed by 5-bromo-2′-deoxyuridine incorporation, was significantly increased at 11 mmol/L glucose as compared with that at 2.8 mmol/L glucose. GKA increased INS1 cell replication in a dose-dependent manner. Also, we evaluated β-cell proliferation after 3 days' administration of GKA in mice fed a HF diet for 20 weeks. Interestingly, the 5-bromo-2′-deoxyuridine incorporation ratio was markedly increased in certain $Gck^{+/-}$ mice, as well as wild-type mice administered GKA for 3 days as compared with that in the mice not administered GKA[29]. These results lend support to the notion that GKA stimulates β-cell proliferation *in vivo*. Some other GKAs (Ro-28-1675 and YH-GKA) have also been shown to have the ability to induce proliferation of β-cells *in vivo*[46,47]. Therefore, GKAs represent a class of compounds that not only augments insulin secretion in pancreatic β-cells and enhances glucose utilization in the liver, but also stimulates β-cell proliferation (Figure 2).

Mechanisms Underlying Stimulation of β-Cell Proliferation by GKA

Although we have hypothesized that glucose metabolism through glucokinase increases the expression of Irs2, at least in part through cyclic adenosine monophosphate response element binding protein phosphorylation, and that the increased Irs2 activity in turn activates a signaling cascade that induces β-cell proliferation[41,48], the detailed mechanisms are largely unknown. Here, we investigated the changes in the expression levels of genes related to β-cell proliferation in mouse islets in response to stimulation by GKA. The results showed that GKA stimulated phosphorylation of cyclic adenosine monophosphate response element binding protein in the isolated islets from wild-type mice. This phosphorylation of cyclic adenosine monophosphate response element binding protein also paralleled the upregulation of Irs2. GKA, but neither gliclazide nor glibenclamide, which are sulfonylureas, significantly increased *Irs2* mRNA expression. When glucose was replaced by 2-deoxyglucose, which is a non-metabolizable analog of glucose, GKA failed to upregulate the *Irs2* mRNA levels, showing that glucose metabolism was necessary for GKA-induced upregulation of Irs2 expression. Furthermore, we showed that the GKA-induced Irs2 upregulation in the islets is at least

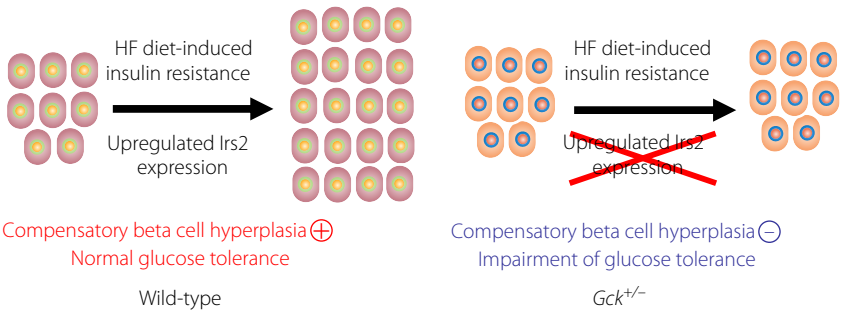

Figure 1 | Compensatory β-cell hyperplasia in response to high-fat (HF) diet-induced insulin resistance. When fed a HF diet, wild-type mice showed marked β-cell proliferation, whereas β-cell specific glucokinase heterozygous mice ($Gck^{+/-}$) showed insufficient β-cell proliferation, and impaired upregulation of insulin receptor substrate-2 (Irs2) in islets, despite the presence of a similar degree of insulin resistance.

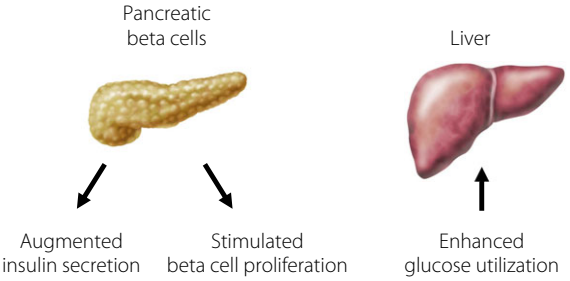

Figure 2 | Effects of glucokinase activators on the pancreatic β-cells and liver. Glucokinase activator represents a class of compounds that not only augments insulin secretion in pancreatic β-cells and enhances glucose utilization in liver, but also stimulates β-cell proliferation.

partly Ca^{2+}-dependent and mediated by calcineurin, based on the results obtained using an L-type calcium channel blocker and calcineurin inhibitor. In a previous study, we showed using deoxyribonucleic acid microarray analysis, that the expression levels of 3-phosphoinositide-dependent kinase 1 (*Pdpk1*) and cyclin D2 (*Ccnd2*) were decreased in $Gck^{+/-}$ mice as compared with those in wild-type mice on a HF diet[41]. GKA stimulation significantly increased the expression levels of *Pdpk1*, *Ccnd1*, *Ccnd2* and *Ccnd3* mRNA. Cyclin D2 protein levels were also increased by the GKA stimulation. These results suggest the involvement of cell cycle signaling, such as by cyclin D2, in GKA-stimulated β-cell proliferation[36].

Next, we used $Irs2^{-/-}$ mice to determine whether Irs2 was required for the GKA-stimulated β-cell proliferation. As was the case in $Gck^{+/-}$ mice, we evaluated the β-cell proliferative activity after GKA administration for three consecutive days to mice fed a HF diet for 10 weeks. There was a significant increase in the 5-bromo-2′-deoxyuridine incorporation ratio in the wild-type mice given GKA for 3 days, as compared with that in the wild-type mice not given GKA, but not in the $Irs2^{-/-}$ mice. These results support the concept that Irs2 could have an influence on the β-cell proliferation induced by GKA *in vivo*[36].

Anti-Apoptotic Effects of GKA on the β-Cells

Decline of β-cell mass as a result of increased apoptosis is an important pathological characteristic of type 2 diabetes[49,50]. Thus, protection against β-cell apoptosis could be a valid therapeutic strategy in patients with type 2 diabetes. Wei *et al.*[51] showed, for the first time, that GKAs play an important role in preventing apoptosis induced by chronic exposure to high glucose levels in a model INS1 β-cell line. The anti-apoptotic effect was probably as a result of an increase in the glucokinase protein levels, and normalization of the apoptotic protein BCL2-associated agonist of cell death and its phosphorylation level[51]. In cell culture, GKA prevented β-cell death induced by oxidative stress, probably through accelerated production of reduced-form nicotinamide adenine dinucleotide and reduced-form nicotinamide adenine dinucleotide phosphate[32]. Also, GKA ameliorated endoplasmic reticulum stress-mediated apoptosis by harmonizing Irs2 upregulation and the Irs2-independent control of β-cell apoptosis[52].

CLINICAL TRIALS OF GKAS

Since 2008, single- and multiple-dose placebo-controlled studies in humans have reported the effect of GKAs of reducing the fasting and postprandial blood glucose levels in both patients with type 2 diabetes and healthy adults[8]. Some of the important clinical trials are described here.

Mechanistic Study of Piragliatin

To elucidate the mechanisms of the acute glucose-lowering action of piragliatin in patients with mild type 2 diabetes (glycated hemoglobin [HbA1c] was 6.1 ± 0.2%), Bonadonna *et al.*[9] carried out a phase Ib randomized, double-blind, modified three-way cross-over clinical trial to evaluate the effects of piragliatin on glucose regulation in the fasting state and on an oral glucose tolerance test, using a double-tracer glucose technique and mathematical modeling of glucose-stimulated insulin secretion to assess the glucose fluxes and β-cell function. Piragliatin caused a dose-dependent reduction of the blood glucose levels in both the fasting and fed states. In the fasting state, piragliatin caused a dose-dependent increase of the β-cell function, a fall in the endogenous glucose output, and a rise in glucose use. In the fed state, the primary effects of piragliatin were on the β-cell function. These results showed that piragliatin had an acute glucose-lowering action in patients with mild type 2 diabetes, mainly mediated through a generalized enhancement of the β-cell function. Administration of 100 mg piragliatin was also associated with a higher incidence of patients requiring rescue glucose infusion to prevent hypoglycemia.

Long-Term Efficacy and Safety of MK-0941

To assess the efficacy and safety of MK-0941 in patients with type 2 diabetes receiving insulin glargine who showed inadequate glycemic control, Meininger *et al.*[10] carried out a double-blind study, in which 587 patients taking stable-dose insulin glargine (±metformin ≥1,500 mg/day) were randomized (1:1:1:1:1) to MK-0941 10, 20, 30, 40 mg or matching placebo three times a day before meals. At week 14, the HbA1c and 2-h postmeal glucose was significantly improved in all the MK-0941 dose groups vs the values in the placebo group. No significant effects on the fasting plasma glucose were observed in any of the dose MK-0941 groups vs the placebo group. Surprisingly, by 30 weeks, the initial glycemic response noted at 14 weeks was no longer observed. Also, the incidence of hypoglycemia was significantly increased in some of the MK-0941 dose groups. Furthermore, a 6–19% increase of the plasma triglyceride levels was observed in the active treatment arms. After a review of the efficacy and safety results, the study was terminated owing to the lack of sustained glycemic efficacy.

Dose-Range Studies on AZD1656

To investigate the effect of AZD1656 on the HbA1c as an add-on to metformin in patients with type 2 diabetes, a randomized, double-blind, placebo-controlled multinational phase II study was carried out over 4 months with an optional 2-month extension[53]. The results showed a significant reduction of HbA1c, which was comparable with that in a reference group (glipizide) after 4 months of treatment. AZD1656 was well-tolerated, causing hypoglycemia at a lower incidence than glipizide. In the extension population, HbA1c was still reduced in the AZD1656 group vs the placebo group after 6 months, but the effect on the glucose control was not sustained over time.

Furthermore, to assess the glucose-lowing effects, safety and tolerability of AZD1656 monotherapy in Japanese patients with type 2 diabetes, a 4-month, randomized, double-blind, placebo-controlled phase II study was carried out in Japan[54]. Although favorable plasma glucose reductions were observed initially, the effect was not sustained over time. Thus, the change in the HbA1c from the baseline to 4 months in HbA1c was not significant in the AZD 40–200 mg group relative to that in the placebo group. Cases of hypoglycemia were rare after treatment with AZD1656, and there were no safety concerns.

PROBLEMS AND FUTURE PROSPECTS OF GKAS IDENTIFIED FROM THE RESULTS IN CLINICAL TRIALS

These clinical trials identified some issues with the use of GKAs. First, MK-0941 and AZD1656 could not yield sustained glycemic control. In regard to MK-0941, the disease-related characteristics of the trial patients were a mean baseline HbA1c of 9.0%, mean duration of diabetes of 12 years and a mean insulin glargine dose of 45 units/day. These data suggest that insulin secretion or the β-cells themselves were severely impaired in these patients. Thus, it is possible that the pancreatic effect of GKAs was not evident, because the pancreatic β-cells were severely impaired. In contrast, the study on AZD1656 in Japan involved patients with early-stage disease. Thus, the reasons for the lack of sustained efficacy are currently unclear. In the pancreatic β-cells, augmentation of glucose signaling by glucokinase activation leads to an initial boost of insulin secretion and β-cell proliferation, and later fueling of a vicious cycle of glucotoxicity that precipitates β-cell failure and hyperglycemia. Recently, it was reported that genetic activation of β-cell glucokinase resulted in a rapid and significant decrease of the blood glucose levels in a mouse model[55]. However, this decrease was not sustained, and the blood glucose levels returned to normal. Histological analysis showed that genetic activation of glucokinase in the β-cells, which initially triggered proliferation, caused double-strand breaks in the deoxyribonucleic acid, likely accounting for activation of the p53 tumor suppressor and triggering β-cell death. These results seem to be consistent with the transient benefit of the GKAs observed in the clinical trials. Therefore, the reason for loss of efficacy might be the toxicity of GKAs on the β-cells. To eliminate this major drawback, GKA could be used in combination with incretin therapy in the event of GKA monotherapy being ineffective. In the aforedescribed mouse model, liraglutide treatment reduced β-cell death and rescued β-cell function[55]. Also, the results of our study showed that GKA was able to upregulate Irs2 expression in the islets of *db*/*db* mice pretreated with exendin-4[36], suggesting that combined GKA and incretin therapy might be useful in the treatment of type 2 diabetes.

Second, administrations of piragliatin and MK-0941 were associated with an increased incidence of hypoglycemia. GKAs modify the sigmoidal activity curve of glucokinase into a hyperbolic activity curve and consequently alter the sensing level of blood glucose by glucokinase in the pancreatic β-cells, causing hypoglycemia. To minimize the hypoglycemia risk, several groups have focused on developing partial activators that would increase the enzyme activity, but maintain a greater degree of glucose dependency to minimize the risk of activation at low glucose concentrations[34,56]. An alternative approach to minimize the risk of hypoglycemia is to use liver-specific GKAs. Indeed, in early clinical studies, liver-selective activators have also shown promising efficacy, producing dose-dependent reductions of both fasting and postprandial glucose levels in type 2 diabetic patients, with favorable safety profiles[34,57,58].

Third, in a phase II clinical study of GKAs (MK-0941), increase of the plasma triglyceride was observed in the active treatment arms[10]. A similar result was observed in a study of AZD1656 carried out in a diverse European and Latin American population[53]. Because overexpression of glucokinase increases hepatic lipogenesis and the circulating levels of lipids in animals[59], and increased serum levels of triglycerides have been observed in humans with an activating glucokinase gene mutation[60], it is suggested that administration of GKA might increase serum triglyceride levels through its effect on liver glucokinase. Furthermore, acute and chronic administration of several different GKAs has been shown to increase the hepatic triglyceride content in animal models[61]. By contrast, in a phase II clinical study carried out in a Japanese population, AZD1656 had no effect on the triglycerides[54], and several studies have showed that subchronic or chronic GKA treatment of mice fed a HF diet was not associated with any increase of the plasma or hepatic triglyceride levels[29,31,33]. The issue of triglyceride accumulation associated with the use of GKAs therefore appears inconclusive, and could benefit from further investigation. As it has been reported that treatment with exendin-4 reversed the increase in hepatic triglyceride content in GKA-treated *db*/*db* mice[62], combined GKA plus incretin therapy might be useful for preventing the harmful side-effects of GKAs, such as hepatic steatosis.

CONCLUSIONS

Our studies in mouse models showed that GKAs improve glucose metabolism and stimulate β-cell proliferation in the pancreatic islets (Figure 2). As reduction of the β-cell mass has been shown in patients with type 2 diabetes, GKAs could hold great promise for development as treatment agents for type 2

diabetes. However, the results of recent phase II trials have shown that GKAs lose their efficacy within several months of use, and that their use is associated with an increased incidence of hypoglycemia and dyslipidemia. A better understanding of the role of glucokinase in metabolic effects is required to resolve several issues identified in clinical trials.

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