Glucokinase as a therapeutic target based on findings from the analysis of mouse models

Akinobu Nakamura

Department of Rheumatology, Endocrinology and Nephrology, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

Abstract. I investigated mouse models to elucidate the pathophysiology and to establish a new treatment strategy for type 2 diabetes, with a particular focus on glucokinase. The decrease in pancreatic beta-cell function and mass are important factors in the pathophysiology of type 2 diabetes. My group have shown that glucokinase plays an important role in high-fat diet-induced and high-starch diet-induced beta-cell expansion. The findings indicated that the mechanism of short-term high-fat diet-induced beta-cell proliferation involved a glucokinase-independent pathway, suggesting that there are different pathways and mechanisms in the proliferation of pancreatic beta-cells during short-term versus long-term high-fat diets. Because enhancement of glucose signals via glucokinase is important for beta-cell proliferation, it was thought that beta-cell mass would be increased and insulin secretion would be maintained by glucokinase activators. However, sub-chronic administration of a glucokinase activator in db/db mice produced an unsustained hypoglycemic effect and promoted hepatic fat accumulation without changes in beta-cell function and mass. In contrast, my group have shown that inactivating glucokinase in beta-cells prevented beta-cell failure and led to an improvement in glucose tolerance in db/db mice. Regulation of glucokinase activity has an influence on the pathophysiology of type 2 diabetes and can be one of the therapeutic targets.

Key words: Beta-cell, Glucokinase, Knockout mouse

Introduction

The pathophysiology of type 2 diabetes is characterized by increased insulin resistance and decreased insulin secretion [1]. Regarding decreased insulin secretion, the function and mass of insulin-secreting pancreatic beta-cells progressively decrease before the onset of diabetes [2-7]. However, it is not easy to accurately evaluate beta-cell function over time to assess the pathophysiology and therapeutic intervention in humans. In particular, accurate evaluation of changes in human beta-cell mass over time is difficult. Therefore, evaluation using a mouse model of diabetes is useful, although close attention must be paid to the differences between mice and humans. This review article will describe recent findings from mouse models on the pathophysiology and treatment of type 2 diabetes, centered on pancreatic beta-cells, and with a particular focus on glucokinase.

Glucokinase and Corresponding Knockout Mice

Glucokinase is an enzyme that catalyzes the conversion of glucose to glucose 6-phosphate, the first step in glycolysis, and plays an important role in maintaining glucose homeostasis throughout the body [8, 9]. In pancreatic beta-cells, glucokinase plays the role of a glucose sensor that controls insulin secretion according to the glucose concentration. In the liver, the glucokinase reaction is rate-determining, and glycogen synthesis and glycolysis are both regulated. Glucokinase activity increases because of an increase in blood glucose after ingestion of food, insulin secretion from pancreatic beta-cells increases, and at the same time, glucose uptake and glycogen synthesis increase in the liver, and these cooperative actions lower blood glucose levels. In 1995, Terauchi et al. established glucokinase knockout mice by disrupting expression of exon 1a of the glucokinase (Gck) gene [10]. The mutant glucokinase allele in this model affected the expression of the neuroendocrine isoform, but not that of the hepatic isoform. Thus, these mice were not pancreatic beta-cell-specific glucokinase deficient. However, the phenotype of these mice was extremely similar to that of beta-cell-specific glucokinase knockout mice.
Indeed, mice with glucokinase haploinsufficiency (Gck+/-) exhibited hyperglycemia with decreased glucose-stimulated insulin secretion [10], indicating that glucokinase has an influence on beta-cell function. In humans, the effect of glucokinase on beta-cell function was shown by individuals with glucokinase-maturity-onset diabetes of the young, which is caused by heterozygous inactivating mutations in the GCK gene [12].

**Effect of Glucokinase on Diet-induced Beta-cell Mass Regulation**

Insulin secretion is regulated by pancreatic beta-cell function and mass [13]. When wild-type mice and the above-mentioned Gck+/- mice were loaded with a high-fat (HF) diet for 20 weeks, an increase in beta-cell mass was observed in wild-type mice because of compensatory beta-cell proliferation for the insulin resistance. In contrast, Gck+/- mice did not show such an increase in beta-cell mass [14]. Consistent with the presence or absence of this increase in beta-cell mass, insulin receptor substrate (Irs)-2 expression was increased in the pancreatic islets of HF diet fed wild-type but not Gck+/- mice [14]. Based on the above findings and further studies using Irs-2 knockout and overexpressing mice, it was revealed that glucokinase and Irs-2 play important roles in the mechanism of increased pancreatic beta-cell mass induced by a long-term HF diet [14-16] (Table 1). Combined, these findings suggested the importance of glucose signaling in beta-cell proliferation [17].

A high-starch diet (HSTD), which contains a large amount of glucose, also has been shown to increase body weights of mice [18, 19]. It has been reported that an increase in beta-cell mass was observed in mice fed a HSTD as well as a HF diet [20, 21]. Additionally, studies of Kir6.2 knockout mice showed that the adenosine triphosphate-sensitive potassium channel is required for the mechanism of increasing beta-cell mass induced by a HSTD [20]. These findings prompted me to investigate the role of glucokinase in HSTD-induced beta-cell mass expansion. When wild-type and Gck+/- mice were both fed a normal diet or a HSTD for 15 weeks, both types of mice showed body weight gain with the HSTD compared with the normal diet. In wild-type mice, a significant increase in pancreatic beta-cell mass was observed with the HSTD compared with the normal diet. In contrast, Gck+/- mice had no difference in pancreatic beta-cell mass on the normal diet or HSTD [22]. These results indicated that glucokinase is required for HSTD-induced beta-cell mass expansion, similar to HF diet-induced beta-cell mass expansion (Table 1).

**Short-term Diet-induced Beta-cell Proliferation**

As described above, when wild-type mice were fed a long-term HF diet for 20 weeks, pancreatic beta-cell mass expansion was accompanied by beta-cell proliferation [14]. For this HF diet model, it was reported that beta-cell proliferation was enhanced by long-term and also short-term HF diets [23-25]. My group investigated whether glucokinase was required for short-term HF diet-induced beta-cell proliferation. Wild-type mice had significantly increased pancreatic beta-cell proliferation after one week of a HF diet, although no difference was observed in insulin resistance, evaluated in the insulin tolerance test, compared with normal diet-fed mice. Interestingly, when Gck+/- mice were fed a HF diet for one week, beta-cell proliferation increased to the same extent as the observed in wild-type mice fed a HF diet compared with a normal diet. Because Irs-2 also plays an important role in the mechanism of beta-cell mass expansion induced by a long-term HF diet, the same study was performed using Irs-2-knockout mice. A significant increase in beta-cell proliferation was observed in Irs-2-knockout mice after a HF diet for one week, resembling the findings observed in wild-type and Gck+/- mice [26]. The above results indicated that the mechanism of the short-term HF diet-induced beta-cell proliferation involved a glucokinase- and Irs-2-independent pathway (Table 1).

Next, to clarify what pathway was involved in short-term HF diet-induced beta-cell proliferation, comprehensive gene expression in isolated islets was compared

**Table 1** Diet-induced increase in beta-cell mass in wild-type mice and mice with glucokinase haploinsufficiency (Gck+/-)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Duration (week)</th>
<th>Beta-cell mass</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat diet</td>
<td>20</td>
<td>Increase</td>
<td>14</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>15</td>
<td>Increase</td>
<td>22</td>
</tr>
<tr>
<td>High-starch diet</td>
<td>1</td>
<td>Increase*</td>
<td>26</td>
</tr>
</tbody>
</table>

* Increase in beta-cell proliferation
between normal diet-fed and 1-week HF diet-fed wild-type mice. Profiling of gene expression showed that the expression levels of forkhead transcription factor M1 (Foxm1) and its downstream genes were coordinately upregulated in islets of HF diet-fed compared with those of normal diet-fed mice. Because Foxm1 is a transcription factor involved in cell proliferation, and plays an important role in pancreatic beta cell proliferation [27-30], Foxm1 and its downstream cell cycle-related factors may be involved in the mechanism of beta-cell proliferation induced by the short-term HF diet. These findings suggested that there are different pathways involved in the proliferation of pancreatic beta-cells during short-term versus long-term HF diets [26].

**Effect of Glucokinase Activation on Beta-cell Proliferation**

Pancreatic beta-cell mass expansion due to beta-cell proliferation induced by a long-term HF diet suggested that the beta-cell mass may be increased and insulin secretion may be maintained by enhancing the glucokinase- and Irs-2-mediated pathway. It was predicted that enhancing this signaling pathway could prevent beta-cell failure, which is one of the important features of the pathophysiology of type 2 diabetes. Glucokinase activators (GKAs), which have been developed as a therapeutic agent for diabetes, provide a hypoglycemic effect by augmenting insulin secretion and enhancing hepatic glucose utilization, consistent with the action of glucokinase [16, 31, 32]. It has been shown that GKAs lowered blood glucose by both these actions in animal models [16, 31, 33-36].

I investigated whether GKA had a proliferative effect on pancreatic beta-cells, and showed that the proliferation evaluated by 5-bromo-2-deoxyuridine incorporation was enhanced by the administration of GKA in vitro and in vivo [36]. Furthermore, GKA increased Irs-2 expression in vitro and ex vivo, and Irs-2 knockout mice did not show the elevated proliferation of pancreatic beta-cells after GKA administration, as was observed in wild-type mice [36, 37]. These results revealed that GKA has a beta-cell proliferative effect and the Irs-2-mediated pathway is important for this proliferative effect of GKA [16] (Fig. 1).

**Chronic Glucokinase Activation in Pancreatic Beta-cells**

As described above, I found that GKA induced pancreatic beta-cell proliferation in mice, but found no difference in beta-cell mass between the GKA-administered and non-administered groups [36, 37]. Initially, I thought that no increase in beta-cell mass would reflect GKA lowering blood glucose, which would not lead to sustained glucokinase activity in pancreatic beta-cells. However, a study using mice with beta-cell-specific activation of glucokinase showed that continuous activation of glucokinase promoted beta-cell proliferation, as well as pancreatic beta-cell apoptosis, resulting in beta-cell toxicity [38]. A recent report from the same group has shown that short-term activation of glucokinase resulted in increased beta-cell proliferation and mass and decreased blood glucose levels, whereas long-term activation resulted in a reduced beta-cell mass and increased blood glucose levels in mice with a conditional heterozygous beta cell-specific mutation of glucokinase activation [39]. Therefore, it was suggested that long-term activation of glucokinase may lead to beta-cell failure [16, 40]. This proposal may be related to the lack of GKAs’ long-term efficacy in several clinical studies [16, 40-44].

In prediabetes or the early stages of type 2 diabetes, the sensitivity of beta-cells to glucose can be increased, with the insulin response curve to glucose shifted to the left, resembling the response to glucokinase activation [45]. If sustained activation of glucokinase leads to beta-cell failure, I considered that inactivation of glucokinase may prevent beta-cell failure in the long term [40]. To test this hypothesis, the above-mentioned Gck+/- mice were bred with db/db mice (a model of obese and type 2 diabetes), and Gck+/-db/db mice were established [46]. Blood glucose increased with age in db/db mice, whereas Gck+/-db/db mice exhibited a lower level of hyperglycemia. Although there was no difference in insulin resistance between db/db mice and Gck+/-db/db mice, glucose-stimulated insulin secretion and beta-cell mass were increased in Gck+/-db/db mice compared with those in
from inactivating glucokinase in beta-cells prevented beta-cell failure and led to improvement in glucose tolerance in *db/db* mice [40, 46]. Inactivating glucokinase in beta-cells may be a new therapeutic approach in the pathophysiological conditions of increased glucose sensitivity of beta-cells observed in prediabetes and the early stages of type 2 diabetes [40, 46].

**Glucokinase Activation in Liver**

The loss of efficacy of GKA observed in some previous clinical trials may be related to effects on pancreatic beta-cells and also on the liver. In fact, it has been proposed that activation of glucokinase may cause a fatty liver [47, 48]. My group studied 6-week-old *db/db* mice sub-chronically treated with GKA and found that compared with non-treated *db/db* mice, GKA-treated *db/db* mice showed a decrease in blood glucose immediately after the start of administration, and the decrease was sustained until 6 days after the administration [49]. However, after 6 days, the hypoglycemic effect of GKA disappeared, and there was no difference in glucose tolerance between the treated and non-treated mice. This study design reproduced the loss of long-term efficacy of GKA observed in some clinical trials. To determine the mechanism of disappearance of the sub-chronic efficacy of GKA, the pancreatic beta-cells were studied initially. However, there was no difference in beta-cell function or mass between the treated and non-treated mice. These findings indicated that the unsustained glucose-lowering effect of GKA in this study design was not due to a negative effect of GKA on beta-cells.

Next, my group focused on the liver, which is another important organ for glucokinase-mediated regulation of blood glucose. A significant increase in liver weight was observed in GKA-treated *db/db* mice compared with that in non-treated *db/db* mice [49]. Histological analysis revealed a marked accumulation of lipid droplets and a significant increase in triglyceride content was observed in the livers of GKA-treated mice. Regarding hepatic gene expression, a significant increase in carbohydrate response element-binding protein beta (Chrebp-b) and a significant decrease in phosphoenolpyruvate carboxykinase (Pepck) expression levels were observed on the first day after GKA treatment. On day 6 after GKA treatment, the expression levels of these two genes had returned to normal, and expression levels of lipogenesis-related genes, including acetyl-CoA carboxylase, fatty acid synthase, elongation of very long chain fatty acid-like family member 6 and stearoyl-CoA desaturase 1, were increased. Based on these results, my group proposed that the mechanism for the loss of sub-chronic efficacy of GKA involves increased production of glycolytic intermediate metabolites by GKA-induced activation of hepatic glucokinase, followed by increased Chrebp-b expression and decreased Pepck expression. The downregulation of Pepck would lead to the suppression of gluconeogenesis, resulting in a decrease in blood glucose immediately after the start of administration. Subsequently, the repression of glucokinase activity induced by elevated levels of glycolysis metabolites would offset the increased Chrebp-b expression and decreased Pepck expression, resulting in the disappearance of the hypoglycemic effect of GKA. However, temporarily increased Chrebp-b expression would upregulate the expression of downstream lipogenesis-related genes, resulting in hepatic fat accumulation [40, 47, 49-52] (Fig. 2).

**Fig. 2** Glucose utilization and lipogenesis by glucokinase activation in the liver. ChREBP: Carbohydrate-responsive element-binding protein, ELOVL6: Elongation of chain fatty acids family member 6, FAS: Fatty acid synthase, Gck: glucokinase, GKA: glucokinase activator, G6P: glucose-6-phosphate, SCD1: Stearoyl-CoA desaturase 1.
It should be noted that the efficacy and safety of GKA may differ depending on the compound, patient background, and timing of GKA administration. Recently developed hepatoselective GKA (TTP399) has been shown to be effective for a relatively long period of time without causing fatty liver or dyslipidemia [53]. Additionally, a recent phase 1b/2 adaptive study has confirmed the efficacy and safety of TTP339 in patients with type 1 diabetes [54]. Therefore, this compound is one of promising GKAs for the future therapeutic application.

Conclusions and Perspectives

This review has focused on my recent findings obtained from mouse models used to study the pathophysiology and treatment of type 2 diabetes, with a particular focus on glucokinase. Although enhancement of glucose signals via glucokinase is important for beta-cell proliferation, chronic glucokinase activation was unable to improve pancreatic beta-cell function or increase beta-cell mass in my studies [36, 37, 49]. Additionally, it may cause a fatty liver [49]. In contrast, my group found that inactivation of pancreatic beta-cell glucokinase led to improvement in the pathophysiology of type 2 diabetes [46]. Regulation of glucokinase activity has an influence on the pathophysiology of type 2 diabetes and can be one of the therapeutic targets.

Acknowledgments

This work was supported in part by Grants-in-Aid for Young Scientists (B) 23791040, (B) 26860683 and Scientific Research (C) 19K08992 from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, a Grant-in-Aid for the Translational Research program; Strategic PRomotion for practical application of INnovative medical Technology (TR-SPRINT) from the Japan Agency for Medical Research and Development (AMED), a Grant-in-Aid for Young Researchers from the Japan Association for Diabetes Education and Care, Grants-in-Aid from the Japan Diabetes Foundation, the Front Runner of Future Diabetes Research from the Japan Foundation for Applied Enzymology, the MSD Life Science Foundation, the Suzuken Memorial Foundation, the Akiyama Life Science Foundation, the Takeda Science Foundation and the Suhara Memorial Foundation (to A.N). We thank Edanz (http://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure

The author declares no conflict of interest.

References

individuals with hyperglycemia because of a heterozygous glucokinase mutation. *Diabetes Care* 38: 1383–1392.


activation or inactivation: which will lead to the treatment of type 2 diabetes? Diabetes Obes Metab 23: 2199–2206.


