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COMMENTARY

Metformin induces insulin secretion by preserving pancreatic aquaporin 7 expression in type 2 diabetes mellitus

Evidence of the efficacy of metformin in diabetes patients has been accumulating since its launch in the 1950s. Recently, it was shown that metformin, at least partially, exerts hypoglycemic effects through the changing of gut microbiota. Ogawa *et al*[1]. reported that metformin altered glucose absorption from the intestine in people with type 2 diabetes. It has also been reported that the appetite-suppressing effect of metformin, whose mechanism of action was previously ambiguous, is mediated by the increase in serum growth and differentiation factor-15 concentration[2]. Aquaporin 7 (AQP7), a member of the aquaglyceroporin family, is localized to the plasma membrane and allows the transfer of water, glycerol, and urea across the cell membrane. AQP7 is predominantly expressed in adipocytes, and it is also expressed in testis and pancreatic β-cells. Studies in mice have confirmed their involvement in insulin secretion, triacylglycerol synthesis and proliferation in β-cells. Therefore, AQP7 is currently considered an important regulator of β-cell protein and islet glycerol content[3]. Recently, He *et al.*[4] reported the relationship between metformin and pancreatic AQP7 expression for the first time in the world. They reported that metformin rescued pancreatic AQP7 expression and maintained insulin secretion in the diabetic state. The key pathway between metformin and AQP7 expression is through p38 and c-Jun-NH2-terminal kinase (JNK) mitogen-activated protein kinases (MAPKs), which are activated *in vivo* and *in vitro* in response to hyperglycemia and hyperlipidemia. Metformin inhibited MAPK signaling and rescued pancreatic AQP7 expression in the hyperglycemic state, leading to inducing insulin secretion.

First, He *et al.*[4] checked the presence of pancreatic AQP7 by co-staining with insulin in diabetic rats and diabetic rats treated with metformin. Diabetic rats did not express AQP7 in the β-cell area, although it was expressed in the control normal glycemic group. In contrast, the expression of AQP7 in the β-cell area was maintained in a metformin concentration-dependent manner. They also evaluated AQP7 protein expressions both in rat pancreatic islets and rat insulinoma cell line (INS-1) cells. As a result, metformin rescued AQP7 levels in a concentration-dependent manner in both cells. Namely, AQP7 was co-localized with insulin-producing cells, and metformin normalized hyperglycemia-induced downregulation of AQP7.

Next, He *et al.*[4] evaluated the effect of metformin on MAPK signaling with western blotting in pancreatic islets. The p38 and JNK pathways, but not the extracellular signal-regulated kinase (ERK) pathway, were activated in the type 2 diabetes group. Interestingly, metformin suppressed the p38 and JNK phosphorylation in a concentration-dependent manner. In addition, metformin did not affect the ERK phosphorylation. Taken together, metformin inhibited hyperglycemia-induced activation of p38 and JNK MAPKs, but not ERK MAPK. The same phenomenon has been confirmed in INS-1 cells.

Next, He *et al.*[4] used INS-1 cells to confirm the time course of intracellular glycerol levels in the presence of 50 mmol/L glycerol under glucolipotoxicity conditions with and without metformin. Glycerol content was low under glucolipotoxicity conditions, but the content was increased in a metformin concentration-dependent manner at all time points. Then, as expected, glucose-stimulated insulin secretion under these circumstances showed a strong insulin secretory response in the presence of metformin.

Next, they evaluated the relationship between AQP7 expression and JNK, and p38 MAPKs in INS-1 cells using some compounds. Anisomycin (a potent agonist of p38 and JNK) significantly reduced AQP7 expression to levels comparable to glucolipotoxicity state. In contrast, the expression level of AQP7 was restored to the same level as that of the control in the presence of SP600125 (JNK inhibitor) and SB203580 (p38 inhibitor), even under glycotoxic conditions, respectively. The activity of JNK and p38 was suppressed in the presence of each inhibitor, respectively. JNK and p38 MAPKs were considered to mediate the expression of AQP7 under the glucolipotoxicity conditions in INS-1 cells.

To confirm whether AQP7 is involved in insulin secretion, the authors carried out a glucose-stimulated insulin secretion test using both AQP7 knockdown (KD) and overexpression (OE) INS-1 cells. First, they checked the glycerol content in both KD and OE. The glycerol content was significantly reduced in AQP7 KD cells compared with the control and was significantly increased in OE cells. Glucose-stimulated insulin secretion certainly deteriorated in AQP7 KD cells compared with the control. In AQP7 OE cells, the intracellular glycerol content was slightly increased, but no increase in insulin secretion was observed.

Finally, the authors showed a schema of this story (Figure 1). Under glycolipotoxic (type 2 diabetes) conditions, AQP7

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expression is reduced by the activated JNK and p38 pathways, resulting in reduced glycerol influx, and possibly reduced adenosine triphosphate production[5], which causes decreased insulin secretion. However, in the presence of metformin, the JNK and p38 pathways in β-cells are inhibited, resulting in preserved AQP7 expression and improved glycerol influx, thus maintaining insulin secretion (Figure 1). This study shows that the novel mechanism of metformin treatment of type 2 diabetes and selective regulation of AQP7 function might have important implications for type 2 diabetes treatment strategies.

Regarding diabetes and AQP7, several reports on their role in adipocytes began to be reported around 2006. AQP7 roles in adipocytes have been elucidated to some extent, and it functions as a glycerol channel and is involved in the release of glycerol. It has been reported that knocking out the AQP7 gene prevents the release of glycerol extracellularly, promotes intracellular fat accumulation and promotes adipocyte hypertrophy. That is, AQP7 deficiency independently induces obesity and β-cell dysfunction[3].

There were some limitation to this study. First, when 750 mg of metformin was given to humans, the maximum concentration in the blood was approximately 0.012 mmol/L, and in this experiment, they were carrying out *in vitro* studies at a concentration of 0.5–2.0 mmol/L, which is at least 40-fold higher. In addition, administration to rats was not effective at 100 mg/kg, but was effective at 300–500 mg/kg. This is at least 10-fold higher than that of humans. Further investigation is required to determine whether metformin is involved in the expression of AQP7 in humans at physiological concentration levels. Next, in this study, glucolipotoxicity did not change ERK phosphorylation in the main manuscript. The effect of β-cells on ERK in diabetic conditions is controversial; there are reports that it both promoted phosphorylation and that it did not. In this study, glycolipotoxicity did not affect the activity of ERK, and metformin was not involved in the phosphorylation of ERK in that state. This phenomenon might be due to different culture conditions or different time duration. Although authors have carried out knockdown experiments on INS-1 cells, it is interesting to see if the intracellular glycerol influx is reduced and the favorable effects of metformin are counteracted, even with AQP7 inhibitor.

The key molecule that connects JNK, p38 MAPK and AQP7 is extremely important, and is expected to be reported in the future. Adenosine triphosphate is considered to be important as a substance involved in insulin secretion due to glycerol influx[5], but there is no description of the adenosine triphosphate content in β-cells. Despite these limitations, He *et al.* discovered that metformin suppressed the JNK and p38 MAPKs pathways activated by glycotoxicity to maintain AQP7 expression and regulate insulin secretion by glycerol influx.

Taken together, we believe that He *et al.* provided important insights into the expression of AQP7 regulated by

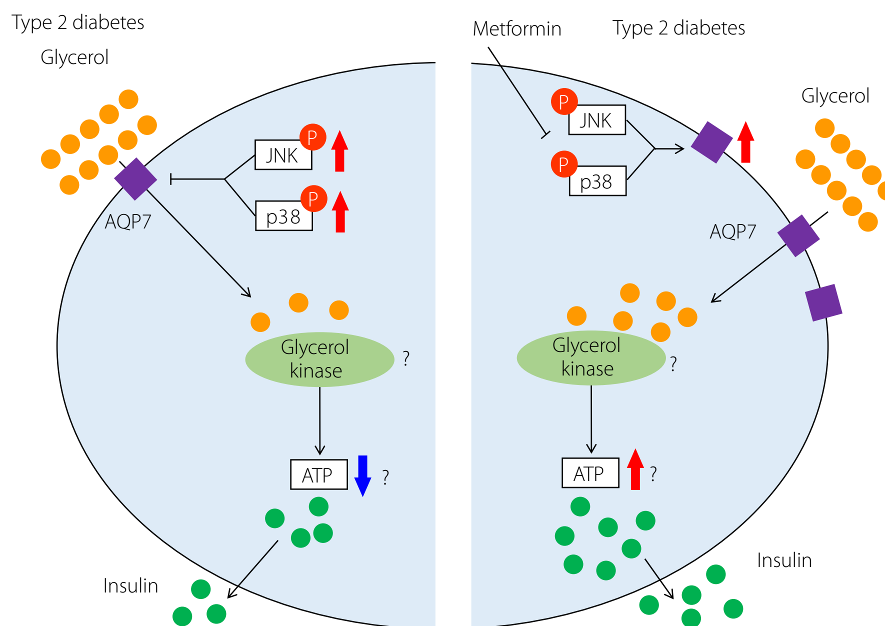

Figure 1 | Schema for regulations of metformin on pancreatic β-cells under glucolipotoxicity. Metformin suppresses the p38 and c-Jun-NH2-terminal kinase (JNK) mitogen-activated protein kinases (MAPK) pathways, thereby upregulating pancreatic aquaporin 7 (AQP7) expression, promoting the influx of glycerol into pancreatic β-cells, possibly maintaining adenosine triphosphate (ATP) production. As a result, it promotes insulin secretion in type 2 diabetes.

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metformin, and that further advances in this area would lead to the identification of new drug targets.

DISCLOSURE

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