Journal of Developments in Sustainable Agriculture 11: 31−35 (2016)

The Type 2 Diabetes-preventive Effect of Cyanidin-3-glucoside on Adipocytes

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Type 2 diabetes mellitus (T2DM) is a serious health problem, and the number of affected persons is increasing annually worldwide. Obesity is the main cause of diabetes. As an individual gain weight, adipocytes are observed to become bigger. Hypotrophic adipocytes shows decreased adiponectin secretion and increased free fatty acids and inflammatory adipokines, which leads to lowered insulin sensitivity and metabolic activity. In contrast, having smaller and fewer adipocytes lead to increases in insulin sensitivity and adiponectin secretion and decreases in the release of inflammatory cytokines. Therefore, increasing the number of smaller adipocytes has been suggested as an effective strategy to prevent and treatment of T2DM. Although thiazolidinediones, such as troglitazone and pioglitazone, which are used currently to treat T2DM, can improve insulin sensitivity by increasing the number of small adipocytes, the side effects of these drugs include weight gain and liver failure. As an alternative treatment for T2DM, polyphenolic compounds, such as nobiletin and sakuranetin, reportedly induce preadipocytes to become small adipocytes. In addition, our recent study showed that the polyphenol cyanidin-3-glucoside (Cy3G), an anthocyanin, similarly induces 3T3-L1 preadipocytes to become small adipocytes, and several other researchers have obtained diverse evidence that supports the efficacy of Cy3G in the prevention or treatment of T2DM. Here, we summarize the activities of Cy3G that may support its use in the prevention of T2DM, focusing on the drug's effect on adipocytes.

Key words: Type 2 diabetes, adipocyte differentiation, adipokines, cyanidin-3-glucoside

Introduction

Type 2 diabetes mellitus (T2DM) is a serious health problem, comprising 75%～80% of all cases of diabetes. The prevalence of T2DM is estimated to reach 350 million cases by the year 2030, and the expenditure for diabetes treatment is projected to be $132 billion in the United States alone (Guilherme *et al*., 2008). Obesity is the main cause of T2DM (Kopelman, 2000; Nawrocki and Scherer, 2005), and adipocytes play an important role in maintaining lipid and glucose homeostasis and energy balance by storing triglycerides and releasing free fatty acids and adipokines. In obese persons, adipocytes become bigger and more numerous, accumulating triglycerides and increasing the secretion of inflammatory adipokines, including tumor necrosis factor (TNF-α), interleukin (IL-6), and monocyte chemotactic protein 1 (MCP-1). These increases lead to the development of insulin resistance, in which cells fail to respond to otherwise normal amounts of insulin; insulin resistance arises due to the inhibition of insulin-stimulated tyrosine phosphorylation, which is triggered through the activation of c-Jun NH_2 terminal kinase (JNK) (Nawrocki and Scherer, 2005; Zou and Shao, 2008). In addition, hypertrophic adipocytes demonstrate decreased secretion of adiponectin, a hormone that regulates energy expenditure, glucose homeostasis, and insulin sensitivity

Received: September 16, 2015, Accepted: February 29, 2016
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(Zou and Shao, 2008). As a countermeasure, increasing the number of small adipocytes has been suggested as a strategy to decrease insulin resistance, given that smaller adipocytes secrete more adiponectin and less inflammatory cytokines than do larger ones (Smith, 2003).

Thiazolidinediones (TZDs) such as troglitazone and pioglitazone, a class of drugs currently used in the treatment of T2DM, ameliorate insulin resistance by increasing the number of small adipocytes (Saltiel and Olefsky, 1996). However, because the adverse effects of TZDs include weight gain and edema, alternative methods for inducing the differentiation of preadipocytes into small adipocytes are desired. In this context, polyphenolic compounds have gained attention recently (Leiherer *et al*., 2013). Like TZDs, the polyphenols nobiletin (Saito *et al*., 2007) and sakuranetin (Saito *et al*., 2008) induce preadipocytes to become small adipocytes.

Anthocyanins, one group of polyphenolic compounds, are present as the water-soluble pigments in numerous colorful fruits and vegetables, including black soybeans, blueberries, and grapes. For the US population, the estimated daily intake of anthocyanins is 180−215 mg (Kühnau, 1976). Cyanidin is the most widespread anthocyanin in nature (Parkinson and Brown, 1981) and exists in plants in its glycated form, cyanidin-3-glucoside (Cy3G) (Fig. 1). Several studies have disclosed the numerous health benefits of Cy3G, including anti-oxidative (Tsuda *et al*., 1998; Slavin *et al*., 2013) and anti-inflammatory (Kim *et al*., 2008) effects. Pharmacokinetic studies in rats and humans (Miyazawa *et al*., 1999; Cao *et al*., 2001) have revealed that absorbed Cy3G is present in its unchanged, glycated form in plasma and urine. In addition, an investigation into the distribution of anthocyanins in various organs indicated that Cy3G is present in adipose tissue (Felgines *et al*., 2009). Together these studies clearly demonstrate that Cy3G can—and does—reach adipocytes through the blood serum.

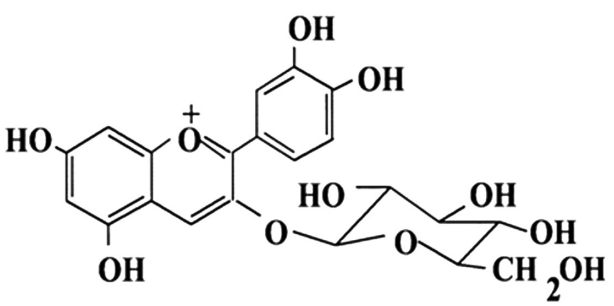

Fig. 1. Chemical structure of cyanidin-3-glucoside (Cy3G).

In our own recent study, we found that Cy3G can ameliorate T2DM by inducing 3T3-L1 preadipocytes to differentiate into small (rather than large) adipocytes, thereby increasing the secretion of adiponectin and decreasing the release of TNF-α (Matsukawa *et al*., 2015). Many other researchers similarly assert that Cy3G is likely to be effective in the treatment and prevention of T2DM (Sasaki *et al*., 2007; Guo *et al*., 2008; Wei *et al*., 2010; Guo *et al*., 2012; Matsukawa *et al*., 2015). In this review, we summarize the T2DM-preventive activities of Cy3G, focusing on its effects on adipocytes.

1. The effect of cyanidin-3-glucoside on the secretion of adipokines

Adipocytes function as an endocrine organ, and adipokines, the cytokines derived from adipocytes, regulate energy balance, insulin sensitivity, lipid metabolism, and inflammation (Zou and Shao, 2008). Inflammatory adipokines, such as TNF-α, IL-6, and MCP-1, decrease insulin sensitivity by inhibiting insulin-stimulated insulin receptor (IR) phosphorylation through the JNK pathway. In contrast, adiponectin, another adipokine, activates energy expenditure and promotes insulin sensitivity (Nawrocki and Scherer, 2005; Zou and Shao, 2008). Small adipocytes secrete more adiponectin and less inflammatory adipokines than do their larger counterparts (Smith, 2003). In fact, TZDs, a class of current therapeutic drugs for T2DM, increase adiponectin levels and decrease those of inflammatory cytokines by promoting the differentiation into small adipocytes (Saltiel and Olefsky, 1996).

Like TZDs, Cy3G increases the secretion of adiponectin *in vivo* (Guo *et al*., 2012) and *in vitro* (Matsukawa *et al*., 2015). Adiponectin activates mitochondrial biogenesis and oxidative capacity in skeletal muscle by increasing the protein and mRNA expression of peroxisome proliferator-activated receptor γ coactivator α (PGC-1α) (Iwabu *et al*., 2010). Consistent with these results, the addition of culture medium from Cy3G-treated adipocytes increased the expression of *PGC-1α* in C2C12 myotubes, a cellular model of skeletal muscle (Matsukawa *et al*., 2015). In addition to its effects on adiponectin, Cy3G modulates the secretion of inflammatory adipokines. In both *in vivo*

and *in vitro* systems, Cy3G treatment reduces the secretion of TNF-α (Guo *et al*., 2012; Matsukawa *et al*., 2015) as well as of MCP-1, which leads to inflammation through the infiltration of macrophages into adipose tissue (Guo *et al*., 2012). These combined results imply that Cy3G activates metabolic activity and alleviates insulin resistance and inflammation by regulating the secretion of adipokines.

2. The effect of cyanidin-3-glucoside on glucose uptake

Glucose uptake has an important role in maintenance of blood glucose levels. In general, after insulin is secreted from the β cells of the pancreas, it binds to IR and thus activates the phosphorylation of the insulin signaling cascade, which includes IR, insulin receptor substrate 1 (IRS-1), and Akt (Huang and Czech, 2007). The activation of insulin signaling promotes the translocation of glucose transporter 4 (GLUT4) into cell membranes. During insulin resistance, a hallmark of T2DM, cells fail to respond to the otherwise normal actions of insulin, leading to the inhibition of GLUT4 translocation and therefore decreased glucose uptake in adipocytes and myocytes (Huang and Czech, 2007). As evidence of its potential use in treating T2DM, feeding Cy3G to db/db mice, an animal model of diabetes, lowered their fasting glucose levels (Guo *et al*., 2012). In addition, glucose and insulin tolerance tests revealed that Cy3G improved the insulin sensitivity and glucose clearance of db/db mice (Guo *et al*. 2012).

Like the db/db mouse experiments, our previous study (Matsukawa *et al*., 2015) supports the claim that Cy3G decreases the serum glucose level during T2DM. Using 3T3-L1 cells, an in vitro model of adipocytes differentiation, we found that Cy3G increases their insulin sensitivity and glucose uptake. In addition, we revealed that the expression of *GLUT4* in 3T3-L1 adipocytes were up-regulated by Cy3G treatment during adipocytes differentiation; GLUT4 expression is downregulated in the adipose tissue of patients with T2DM (Yang *et al*., 2005). Furthermore we found that Cy3G increases the gene expression of CCAAT/enhancer binding protein α (*C/EBP*α) (Matsukawa *et al*., 2015). C/EBPα influences not only adipocyte differentiation but also insulin sensitivity: C/EBPα binds to the promotors of the insulin receptor and GLUT4 genes and thus upregulates their expression (Wu *et al*., 1999; Karnieli and Armoni, 2008). Therefore, Cy3G appears to promote the insulin sensitivity of adipocytes by increasing the gene expression of *C/EBP*α and thus of *GLUT4*.

Several adipokines, including TNF-α and retinol binding protein 4 (RBP4), decrease the glucose uptake ability and insulin sensitivity of adipocytes and myocytes (Zou and Shao, 2008). In particular, TNF-α suppresses the expression of many proteins associated with glucose uptake, including IR, IRS-1, and GLUT4, by activating JNK and thus suppressing peroxisome proliferator-activated receptor γ (PPARγ) and C/EBPα, which are transcriptional factor for regulating the gene expression of associated with various adipocyte function, including GLUT4 and adiponectin (Cawthorn and Sethi, 2008; Cristancho and Lazar, 2011). In addition, TNF-α promotes the generation of reactive oxygen species, which are involved in JNK activation and thus play an important role in the progression of insulin resistance (Houstis *et al*., 2006). However, because Cy3G has anti-oxidant activity (Tsuda *et al*., 1998; Slavin *et al*., 2013), it protects against a TNF-α-induced decrease in insulin sensitivity by inhibiting the activation of JNK (Guo *et al*., 2008). Furthermore, although the adipokine RBP4 decreases insulin sensitivity and glucose uptake by inhibiting GLUT4 expression (Yang *et al*., 2005), Cy3G curtails the secretion of RBP4 (Sasaki *et al*., 2007). Overall, these results indicate that Cy3G promotes glucose uptake and insulin sensitivity by increasing GLUT4 expression and inhibiting the activation of the inflammatory reaction.

3. The effect of cyanidin-3-glucoside on adipocyte differentiation

Adipocyte differentiation plays an important role in the regulation of adipokine secretion and in the insulin sensitivity of adipocytes (Farmer, 2006; Siersbaek *et al*., 2012). In turn, adipokine secretion is regulated by PPARγ and C/EBPα, which also modulate adipocyte differentiation (Wu *et al*., 1999; Farmer, 2006). During terminal adipocyte differentiation, PPARγ and C/EBPα regulate the gene expression of various metabolic proteins and adipokines associated with adipocyte function, including GLUT4, fatty acid-binding protein 4, and adiponectin (Cristancho and Lazar, 2011). In particular, a complex comprising PPARγ, PPARγ ligand, and retinoic acid receptor binds to peroxisome proliferator response elements (PPRE) and leads to their transcription (Houseknecht *et al*., 2002). TZDs act as PPARγ agonists by activating the tran-

scriptional activity of PPARγ and thus increasing insulin sensitivity and promoting adipocyte differentiation (Saltiel and Olefsky, 1996). Similar to TZDs, polyphenols such as phloretin (Hassan *et al*., 2008) and flavanone (Saito *et al*., 2009) exhibit PPARγ agonist activity, induce PPARγ transcriptional activity, and promote adipocyte differentiation.

In our previous study (Matsukawa *et al*., 2015), Cy3G induced the differentiation of preadipocytes to small adipocytes by increasing the expression of *PPARγ* and *C/EBPα*. Cy3G, however, does not act as a PPARγ agonist. Instead, Cy3G increases the expression of *C/EBPβ* (unpublished data); C/EBPβ is important for early-phase adipocyte differentiation and regulates *PPARγ* and *C/EBPα* (Siersbaek *et al*., 2012). Like Cy3G, the polyphenol nobiletin does not act as a PPARγ agonist but instead promotes adipocyte differentiation by increasing C/EBPβ expression through the activation of cAMP-responsive element binding protein (Saito *et al*. 2007). Cy3G may likewise influence the early phase of adipocyte differentiation, but additional research is needed to reveal the underlying mechanism.

Conclusion

Cy3G is an anthocyanin that might be effective for preventing or ameliorating T2DM owing to its abilities to activate insulin sensitivity, glucose uptake, and adiponectin secretion and to decrease the secretion of RBP4 and inflammatory cytokines, such as TNF-α and MCP-1. These effects of Cy3G are achieved through the promotion of adipocytes differentiation, given that Cy3G upregulates the expression of PPARγ and C/EBPα, which are key modulators of adipocyte differentiation (Fig. 2). However, Cy3G does not function as a PPARγ agonist, as do TZDs. Additional research is needed to clearly define the the mechanism underlying the function of Cy3G and how this compound can be used effectively in the treatment of T2DM. The use of vegetables and fruits, such as black soybeans, blueberries, and grapes, as sources of Cy3G contributes to the development of agriculture.

Acknowledgments

We thank the Nagao Laboratory (Kyoto University, Japan) for measuring the PPARγ agonist activity of cyanidin-3-glucodside.

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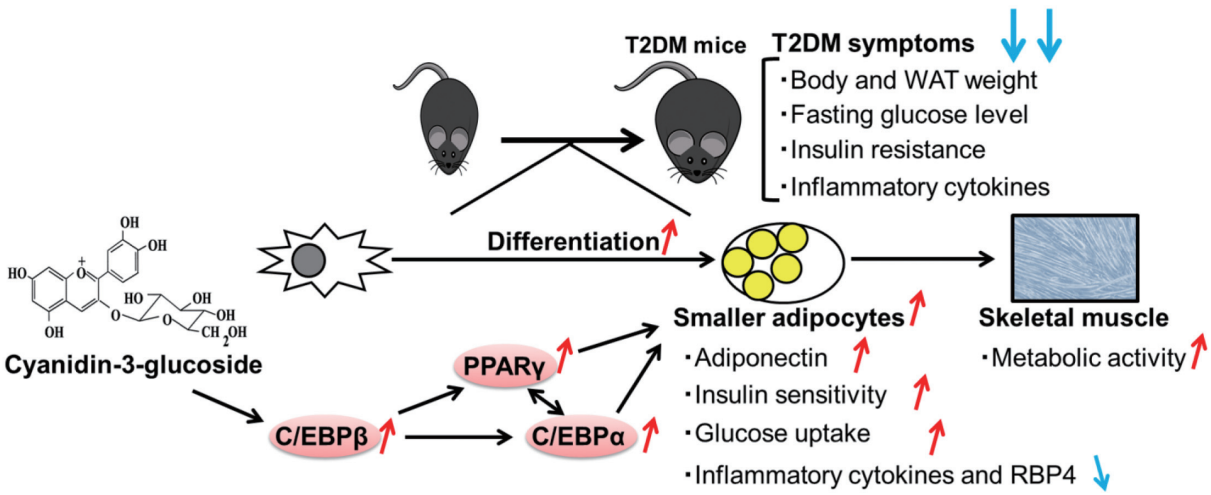

Fig. 2. Schematic diagram of the effect of cyanidin-3-glucoside (Cy3G) on adipocytes.
Cy3G promotes adipocyte differentiation, consequently ameliorating type 2 diabetes mellitus (T2DM) by increasing insulin sensitivity, glucose uptake, and adiponectin secretion and decreasing the secretion of RBP4 and inflammatory cytokines, including TNF-α and MCP-1.

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