Tea Prevents Hyperglycemia through Promoting Glucose Uptake in Skeletal Muscle Cells

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Summary

Tea prevents hyperglycemia and glucose intolerance. However, underlying molecular mechanism is not fully understood yet. Here, we show the prevention mechanism of tea on hyperglycemia through promoting glucose uptake in skeletal muscle cells. In cultured L6 myotubes, catechins promoted glucose uptake accompanied by translocation of glucose transporter (GLUT) 4 to the plasma membrane at physiological concentration: e.g., EGCg promoted significant glucose uptake and GLUT4 translocation at 1 nM. As the underlying mechanism for GLUT4 translocation, EGCg activated PI3K and aPKC without affecting phosphorylation of IRβ. LY294002, a PI3K inhibitor, suppressed EGCg-caused translocation of GLUT4 and phosphorylation of aPKC. Moreover, EGCg also activated AMPK. These results indicated that EGCg promoted GLUT4 translocation through dual-pathways: i.e., both PI3K- and AMPK-dependent pathways in L6 myotubes. This mechanism will contribute to prevention of hyperglycemia and glucose intolerance by tea.

Introduction

Epigallocatechin gallate (EGCg) is a major polyphenol in green tea and may account for 50–80% of the total catechin content in tea. EGCg was reported to possess various beneficial effects such as anti-oxidative, anti-tumor, and anti-inflammatory activities. Recent studies have also shown that EGCg has antidiabetic effects. For example, Wolfram *et al*. reported that EGCg supplementation prevented obesity in rodents by reducing adipose tissue mass. Kao *et al*. reported that the injection of EGCg significantly lowered blood glucose and insulin levels in obese Zucker rats, an animal model of type 2 diabetes,. Our previous study (Ueda, M. *et al*.) showed that the treatment with physiologic concentrations of EGCg promoted GLUT4 translocation in normal and insulin-resistant L6 cells, and the same phenomenon was observed both *in vivo* and *ex vivo*. These studies suggest that EGCg has the potential to reduce hyperglycemia in type 2 diabetes mellitus by promoting GLUT4 translocation in skeletal muscle. However, the mechanism by which EGCg promotes GLUT4 translocation is not yet fully understood. Therefore, in this study, we investigated the effects of EGCg on the insulin- and AMPK-signaling pathways in cultured L6 myotubes.

Materials and methods

GLUT4 translocation was detected by immunofluorescence microscopy using an L6-derived cell clone stably expressing GLUT4myc7−GFP (Ueda, S. *et al.*) and quantified by western blot after the cells were treated with anti-myc antibody. Translocation of endogenous GLUT4, and phosphorylation of insulin signaling pathway and AMPK were also determined by western blot. Uptake of glucose was estimated by measuring the incorporation of [1,2-^{3}H]-2-deoxy-D-glucose (2-DG) into the L6 cells.

Results and discussion

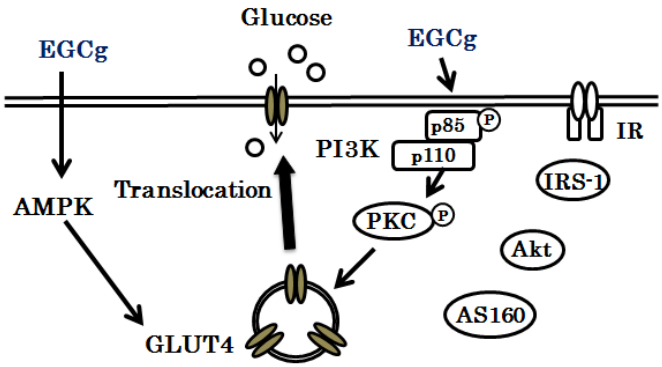
EGCg promoted translocation of endogenous GLUT4 to the plasma membrane of L6 myoblasts by western blot. We confirmed EGCg-caused GLUT4 translocation to the cell surface in GLUT4myc7−GFP cells by immunofluorescence microscopy and quantify the translocation level on the cell-surface of GLUT4myc7–GFP cells by immunestaining with anti-myc antibody. In GLUT4myc7–GFP cells treated with EGCg, activation of Rac1 and membrane ruffling were observed. It was reported that activation of Rac1 is

an inducer of membrane ruffle formation in skeletal muscle and is regulated by PKCλ/ξ [25]. Therefore, EGCg seems to activate Rac1, which subsequently induces actin remodeling and GLUT4 translocation.

To clarify the mechanism by which EGCg promotes GLUT4 translocation in L6 cells, we examined the phosphorylation status of several proteins in the insulin-signaling pathway. As the results, insulin induced the phosphorylation of insulin receptor (IR) and its substrate (IRS-1), as expected, but EGCg did not affect the phosphorylation of either protein at any of the concentrations tested in this study. However, EGCg dose-dependently induced the phosphorylation of PI3K and PKCλ/ξ in a similar manner to insulin. However, there is a difference between the action of EGCg and that of insulin: EGCg did not affect the phosphorylation of Akt at Thr308 or Ser473, whereas insulin phosphorylated Akt at both residues. In addition, EGCg did not affect the phosphorylation of AS160, which is located downstream of Akt. Neither EGCg nor insulin affected the overall expression level of these proteins. These results indicate that EGCg acts through an insulin-independent pathway in L6 myotubes.

To confirm that PI3K and PKCλ/ξ phosphorylation is involved in EGCg-stimulated glucose uptake and GLUT4 translocation, the inhibitor of PI3K or PKC was treated to the L6 cells and measured glucose uptake. A PI3K inhibitor, LY294002 decreased glucose uptake activities in cells treated with EGCg or insulin to a level similar to that in DMSO-treated control cells. GLUT4 translocation and PKCλ/ξ phosphorylation, as induced by EGCg and insulin, were also inhibited by LY294002, indicating that activation of PI3K is involved in EGCg-induced GLUT4 translocation and glucose uptake. EGCg- and insulin-stimulated glucose uptake activities and GLUT4 translocation were also decreased by a PKC inhibitor, Go6983 to a level similar to that in DMSO-treated control cells, indicating that activation of PKCλ/ξ is involved in EGCg-induced GLUT4 translocation and glucose uptake. We also confirmed that IR was not involved in EGCg-induced GLUT4 translocation in L6 cells by using IR siRNA.

GLUT4 translocation is also triggered through an AMPK-dependent pathway, which is activated by exercise and muscle contraction. Therefore, we investigated the effects of EGCg on phosphorylation of AMPK. EGCg induced AMPK phosphorylation in a dose-dependent manner. Taken together, these results indicate that EGCg stimulates glucose uptake in skeletal muscle cells by activating PI3K through part of the insulin-signaling pathway and by the AMPK-driven insulin-independent pathway. Thus, EGCg is a unique dual-activator for both PI3K- and AMPK-pathways to promote GLUT4-dependent glucose uptake in skeletal muscle as shown in the illustration.



A proposed model for EGCg-induced GLUT4 translocation

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