Enhancement of Glucose Utilization by Loesenerine through AMPK Activation in Myotubes

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Type 2 diabetes is characterized by hyperglycemia derived from insulin resistance in periphery tissue. Effects of skeletal muscle on glucose disposal are closely related to insulin resistance. The potential effects on mitochondrial function of loesenerine, a macrocyclic spermidine alkaloid from the aerial part of Euonymus fortunei (Turcz.) Hand-Mazz were observed after a high-throughout screening based on mitochondrial membrane potential (MMP) assay. Further pharmacological studies revealed that loesenerine activates AMP-activated protein kinase (AMPK) pathway through increasing ADP/ATP ratio by inhibiting mitochondrial respiration. In addition, loesenerine induced 1.07-, 1.14-, and 1.22-fold increment of glucose uptake in C2C12 cells at the concentrations of 20, 40 and 80 µmol/L, respectively. Meanwhile, incubated with loesenerine for 12 h increased glucose consumption in a dose-dependent manner in C2C12 cells. This is the first report that macrocyclic spermidine alkaloid possesses potential hypoglycemic activity in vitro.

Key words loesenerine; spermidine alkaloid; AMP-activated protein kinase activation; glucose uptake

Type 2 diabetes is characterized by insulin resistance in peripheral tissue, which leads to high blood glucose level.1) Skeletal muscle is one of primary insulin-target tissues in maintaining glucose homeostasis, which contributes to up to 80% of blood glucose disposal.2) It has been reported that glucose disposal mediated by skeletal muscle is impaired in type 2 diabetic patients.3) AMP-activated protein kinase (AMPK), a critical cellular energy sensor and regulator of energy homeostasis, has been an attractive therapeutic target for treatment of diabetes.4) AMPK is activated by a rising AMP/ATP (or ADP/ATP) ratio.5) Mitochondrial membrane potential (MMP) resulted from the electric potential difference between the inside and outside of mitochondrial inner membrane,6) which provides the electric potential energy for ATP production. The inhibition of mitochondrial respiration would lead to the reduction of ATP production, increasing of AMP/ATP ratio, and thus activation of AMPK.7) Notably, glucose uptake is stimulated in skeletal muscle upon AMPK activation, an independent pathway from insulin.8)

Phytochemicals have attracted attention as source materials for the development of new antidiabetic drugs or alternative therapy for the management of diabetes.9) In present study, a high-throughput screening based on MMP was used for the screening of new potential mitochondrial modulators that might activate AMPK in our natural product extract library. As a result, the effect of loesenerine (Fig. 1A), a macrocyclic spermidine alkaloid isolated from the aerial part of Euonymus fortunei (Turcz.) Hand-Mazz on mitochondrial function and energy status was observed. Macro cyclic spermidine alkaloids mainly exist in a few kinds of plant species, such as the family Celastraceae.10) In the past decades, the structural complexity of macrocyclic spermidine alkaloids has attracted the interest of synthetic chemists.11) However, there are less reports concerning the bioactivities of these spermidine alkaloids. The identification of loesenerine with AMPK activation may also provide an opportunity to develop a new class of hypoglycemic agents.

Results and Discussion

Skeletal muscle is one of the major tissue that accounted for glucose disposal. AMPK is activated by the increment of ADP/ATP ratio, which might be partially resulted from inhibition of mitochondrial electron respiratory chain. To determination of the effects of loesenerine on mitochondrial function and energy status, MMP was firstly tested. MMP was decreased by 7.7 and 11.5% in the presence of loesenerine at concentrations of 20 and 40 µmol/L, respectively (Fig. S1A, Supplementary material). Then we examined the effects of loesenerine on mitochondrial respiration in L6 myotubes. Cellular oxygen consumption rate (primary in mitochondria) was significantly suppressed by loesenerine at the concentrations of 20 and 40 µmol/L in the presence of oligomycin and carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (Fig. S1B, Supplementary material). These results indicated that depolarizing of MMP by loesenerine may be owing to the inhibition of mitochondrial respiration. A statistical analysis of bioluminescent assay revealed that incubated with loesenerine for 3 h significantly increased ADP/ATP ratio in L6 myotubes at the concentrations of 20 and 40 µmol/L (Fig. SIC, Supplementary material).

In addition, loesenerine activated AMPK signaling in a dose-dependent manner, as evidenced by increased phosphorylation of Thr172 of the α-catalytic subunit of AMPK and Ser79 of its classical downstream substrate acetyl-CoA carboxylase (ACC) (Figs. 1B–D), which led to switch anabolism to catabolism.

Activation of AMPK partially contributed to stimulation of glucose uptake in skeletal muscle. As shown in Fig. 1E, loesenerine caused a dose-dependent increase in glucose uptake. It induced 1.07-, 1.14-, and 1.22-fold increment of glucose uptake in a dose-dependent manner, as evidenced by increased phosphorylation of Thr172 of the α-catalytic subunit of AMPK and Ser79 of its classical downstream substrate acetyl-CoA carboxylase (ACC) (Figs. 1B–D), which led to switch anabolism to catabolism.

Note

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glucose uptake in C2C12 cells at the concentrations of 20, 40, and 80 µmol/L, respectively. Meanwhile, co-incubation of loesenerine (20, 40, and 80 µmol/L) with C2C12 cells for 12 h increased glucose consumption in a dose-dependent manner (Fig. 1F). Because of perturbation of mitochondrial respiration, mitochondrial oxidative phosphorylation was suppressed and anaerobic glycolysis was stimulated as a compensatory way for ATP generation. As lactate derived from anaerobic glycolysis, loesenerine promoted lactate production in C2C12 cells, which suggest that glycolysis is stimulated by loesenerine (Fig. 1G). These results indicate that loesenerine promotes glucose utilization in C2C12 cells.

Conclusion
Loesenerine, a macrocyclic spermidine alkaloid isolated from *E. fortunei*, activates AMPK pathway through increasing ADP/ATP ratio by inhibiting mitochondrial respiration, which leads to increment of glucose utilization in C2C12 cells.

**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

**References**