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Betulinic Acid Stimulates Glucose Uptake through the Activation of PI3K and AMPK in 3T3-L1 Adipocytes

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Hyperglycemia in type 2 diabetes can be alleviated by promoting cellular glucose uptake. Betulinic acid (3β,-3-hydroxy-lup-20(29)-en-28-oic acid) is a pentacyclic lupane-type triterpenoid compound. Although there have been studies on the antidiabetic activity of betulinic acid, studies on cellular glucose uptake are lacking. We investigated the effects of betulinic acid on glucose uptake and its mechanism of action in 3T3-L1 adipocytes. Betulinic acid significantly stimulated glucose uptake in 3T3-L1 adipocytes by increasing the phosphorylation of the insulin receptor substrate 1-tyrosine (IRS-1tyr) in the insulin signaling pathway, which in turn stimulated the activation of phosphoinositide 3-kinase (PI3K) and the phosphorylation of protein kinase B (Akt). The activation of PI3K and Akt by betulinic acid translocated glucose transporter 4 to the plasma membrane (PM-GLUT4), thereby increasing the expression of PM-GLUT4 and thus stimulating cellular glucose uptake. Betulinic acid also significantly increased the phosphorylation/activation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase. The activation of PI3K and AMPK by betulinic acid was confirmed using the PI3K inhibitor wortmannin and the AMPK inhibitor compound C. The increase in glucose uptake induced by betulinic acid was significantly decreased by wortmannin and compound C in the 3T3-L1 adipocytes. These results suggest that betulinic acid stimulates glucose uptake by activating PI3K and AMPK in 3T3-L1 adipocytes.

Key words : AMPK, betulinic acid, glucose uptake, PI3K, 3T3-L1 adipocytes

Introduction

Diabetes mellitus is a chronic metabolic disease that affects not only health but also quality of life of patients [20, 35]. Insulin promotes glucose uptake into muscle and adipose tissues and plays an important role in maintaining glucose homeostasis by reducing hyperglycemia. Insulin resistance is a characteristic of type 2 diabetes, which reduces the ability to promote cellular glucose uptake in insulin-sensitive target tissues, such as skeletal muscle and adipocytes. In the insulin-resistant state, glucose that enters the blood from carbohydrate digestion cannot enter cells, causing hyperglycemia. To reduce hyperglycemia in type 2 diabetes, it is important to promote cellular glucose uptake.

Insulin-stimulated glucose uptake involves a series of insulin-signaling cascades initiated by the binding of insulin to its receptor [6, 23, 27]. The binding of insulin to its receptor increases the phosphorylation of insulin receptor substrate 1-tyrosine (IRS-1tyr), which activates phosphoinositide 3-kinase (PI3K) and phosphorylates Akt, which in turn promotes the translocation of GLUT4 from intracellular sites to the plasma membrane. As a result, plasma membrane GLUT4 (PM-GLUT4) stimulates cellular glucose uptake and reduces hyperglycemia [14, 25].

Another major signaling pathway that promotes glucose uptake is activated by AMP kinase (AMPK), which plays an important role in maintaining energy homeostasis and increasing glucose and fatty acid uptake when intracellular energy levels are low [11]. AMPK activation promotes the translocation of GLUT4 to the plasma membrane and increases cellular glucose uptake [14]. The activation of AMPK leads to phosphorylation of its downstream substrate acetyl-CoA carboxylase (ACC). Through ACC phosphorylation, AMPK activation can be confirmed [33]. Because AMPK is an important regulator of glucose homeostasis, it has been considered a potential therapeutic target for improving insulin resistance in type 2 diabetes [2, 4].

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Betulinic acid (3β,-3-hydroxy-lup-20(29)-en-28-oic acid) is a pentacyclic lupane-type triterpenoid compound that is present in the bark of several plants, such as white birch (*Betula pubescens*), common self-heal (*Prunella vulgaris*), and ber tree (*Ziziphus mauritiana*) [24, 31]. Betulinic acid (BA) has anti-inflammatory, anti-retroviral, anti-malarial, and anti-diabetic properties. Recently, betulinic acid was found to have potential as an anti-cancer agent [1, 9, 12, 21, 28]. Although there are studies on the anti-diabetic activity of betulinic acid, studies investigating cellular glucose uptake are rare. In this study, we hypothesized that betulinic acid might exhibit anti-diabetic effects by stimulating glucose uptake. To test this hypothesis, we investigated the effect of betulinic acid on glucose uptake and by what mechanism betulinic acid increased the glucose uptake in vitro by using 3T3-L1 adipocytes.

Materials and Methods

Materials

Betulinic acid (99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Mouse 3T3-L1 preadipocytes were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). Fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), bovine calf serum, compound C, and wortmannin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies against IRS-1tyr, PI3K, Akt, and GLUT 4 were purchased from Abcam (Cambridge, UK). 2-Deoxy-2-[(7-nitro-2,1,3-benzoxadiazol-4-yl) amino]-D-glucose was purchased from Invitrogen (Carlsbad, CA, USA). All chemicals were of analytical grade and were used without further purification.

Cell culture and adipocyte differentiation

Mouse 3T3-L1 preadipocytes were maintained in DMEM supplemented with 10% FBS at 37℃ in a humidified environment with 5% CO_2 [26]. To differentiate preadipocytes into adipocytes, the preadipocytes were placed in DMEM with 10% FBS, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine, and 10 μg/mL insulin. After 2 days, the medium was replaced with 10 μg/mL insulin in DMEM supplemented with 10% FBS. Thereafter, the medium was replaced every two days, for nine days. And then, differentiated 3T3-L1 adipocytes were cultured with DMEM in plates and incubated with the following concentrations of betulinic acid, Insulin (100 nM, positive control), AICAR (0.5 mM, AMPK activator), wortmannin (PI3K inhibitor) and compound C (AMPK inhibitor).

Glucose uptake assay

Differentiated 3T3-L1 adipocytes were cultured in DMEM at a density of 1×10^4 cells/well in 96-well plates. Adipocytes were incubated with various concentrations of betulinic acid, betulinic acid + wortmannin (PI3K inhibitor), or betulinic acid + compound C (AMPK inhibitor) for 24 hr. Glucose uptake was initiated by adding 10 μM 2-(N-(7-nitrobenz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxy glucose (Invitrogen) to each well. After 90 min, glucose uptake was measured using a fluorescence spectrophotometer (Perkin Elmer, MA, USA) set at emission and excitation wavelengths of 535 and 485 nm, respectively.

Western blot analysis

Cells were seeded in a tissue culture plate at a density of 3×10^5 and incubated overnight at 5% CO2 and 37℃. Then, the cells were treated with growth medium that contained various concentrations of betulinic acid (10 and 20 μM), Insulin (100 nM, positive control), AICAR (0.5 mM, AMPK activator) for 24 hr. For whole protein extraction from adipocytes, the cells were washed twice with ice-cold phosphate-buffered saline (PBS) and harvested in a lysis buffer (RIPA, 1 mM EDTA, 150 mM NaCl, 50 mM Tris-HCl, 1 mM phenylmethylsulfonyl fluoride, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.1 mM sodium orthovanadate, 10 μg/ml leupeptin, and 10 μg/ml aprotinin at pH 7.4) on ice with gentle shaking. The protein content of the supernatant was determined using a BCA protein assay kit after centrifugation at 13,000× *g* for 10 min at 4℃. Lysates containing 20 μg of protein were subjected to electrophoresis on a 10% sodium dodecyl sulfate-polyacrylamide gel. The separated proteins were electrophoretically transferred to a pure nitrocellulose membrane, blocked with 5% skimmed milk solution for 1 hr, and incubated with the appropriate primary antibody overnight at 4℃. After washing, the blots were incubated with goat anti-mouse or goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody for 1 hr at room temperature. Each antigen–antibody complex was visualized using ECL western blot reagents and detected using a LAS-1000 Plus Lumino-Image analyzer (Fuji Film, Tokyo, Japan). Band density was determined using an image analyzer (Multi Gauge V3.1, Fujifilm, Valhalla, NY, USA) and normalized to β-actin for total and nuclear proteins.

Isolation of plasma membranes from 3T3-L1 adipocytes

Cells were seeded in a tissue culture plate at a density

of 3×10^5 and incubated overnight at 5% CO_2 and 37℃. The cells were treated with growth medium that contained various concentrations of betulinic acid (20 μM), insulin (100 nM), betulinic acid 20 μM + 10 μM compound C, or betulinic acid 20 μM + 20 μM wortmannin for 24 hr. The adipocytes were homogenized by sonication at 3 kHz/130 W (UCD-130TM, Cosmo Bio Co., Tokyo, Japan) in ice-cold HES buffer (0.02 M HEPES, 2 mM EGTA and 0.25 M sucrose, pH 7.4) for 5 min and centrifuged to remove nuclei and non-homogenized cellular debris from the homogenate at 700× *g* for 7 min. The collected supernatant was centrifuged again at 760× *g* for 10 min to remove the mitochondria. The pellet obtained from this process was used as the plasma membrane fraction of the cells after re-centrifugation at 35,000× *g* for 1 hr, and the remaining supernatant was used as the cytosolic fraction. The membrane and cytosolic fractions were subjected to western blotting to determine GLUT4 protein expression. Protein concentrations in the membrane pellet and cytosolic fraction were quantified using a BCA protein assay kit.

Statistical analysis

Data are presented as the mean ± standard deviation. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Differences between groups were evaluated for significance using one-way analysis of variance followed by Duncan's multiple range post-hoc tests.

Results

Cytotoxic effect of Betulinic acid

To evaluate if betulinic acid has toxic effects on the cells, betulinic acid was applied to 3T3-L1 cells for 24 hr, and the cytotoxic effects were measured using the MTT assay. Betulinic acid was not found to have any cytotoxic effect at all on the different concentrations (1, 5, 10, 20, 50 and 100 M) studied in the 3T3-L1 cells (Fig. 1).

Betulinic acid stimulates glucose uptake

Cellular glucose uptake in 3T3-L1 adipocytes was examined to determine whether betulinic acid takes part in its stimulation. Glucose uptake was stimulated by 143, 155, 175, and 185% in cells treated with 5, 10, 15, and 20 μM betulinic acid, respectively, compared with that in control cells. The results indicated that betulinic acid stimulated glucose uptake in 3T3-L1 adipocytes in a dose-dependent manner (Fig. 2).

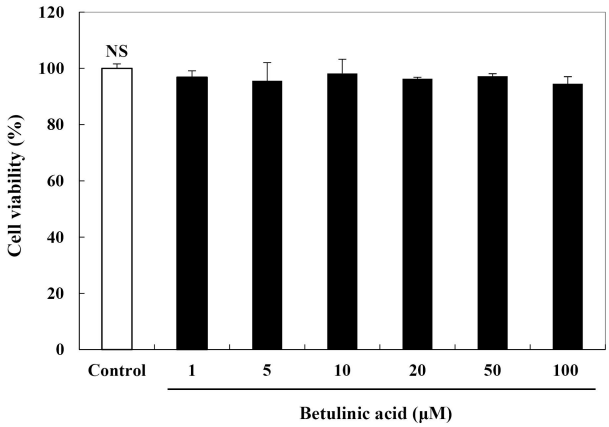

Fig. 1. Effects of betulinic acid on cytotoxic effects in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with betulinic acid at concentrations of 1 to 100 μM for 24 hr prior to the MTT assay. Each value is shown as the mean ± standard deviation (n=3). [a-e]Values tagged with different letters indicate significant differences ($p<0.05$) by Duncan's multiple range test. NS: not significant.

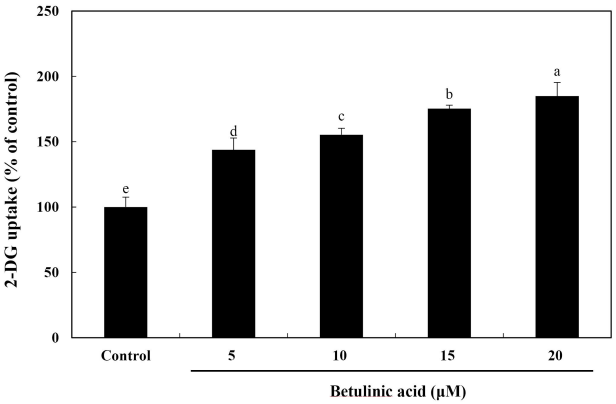

Fig. 2. Effect of betulinic acid on glucose uptake in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with betulinic acid at concentrations of 5 to 20 μM for 24 hr prior to the 2-DG uptake assay. Each value is shown as the mean ± standard deviation (n=3). [a-e]Values tagged with different letters indicate significant differences ($p<0.05$) by Duncan's multiple range test. 2-DG, 2-deoxyglucose.

Betulinic acid increases PI3K activation and Akt phosphorylation

To elucidate the mechanism by which betulinic acid stimulates glucose uptake, the expression levels of phosphorylated IRS-1tyr, PI3K, and Akt were examined. Insulin (100 nM) was used as a positive control to compare with betulinic acid in 3T3-L1 adipocytes. Betulinic acid significantly increased the phosphorylation of IRS-1tyr and activation of PI3K in 3T3-L1 adipocytes (Fig. 3). IRS-1tyr phosphorylation was

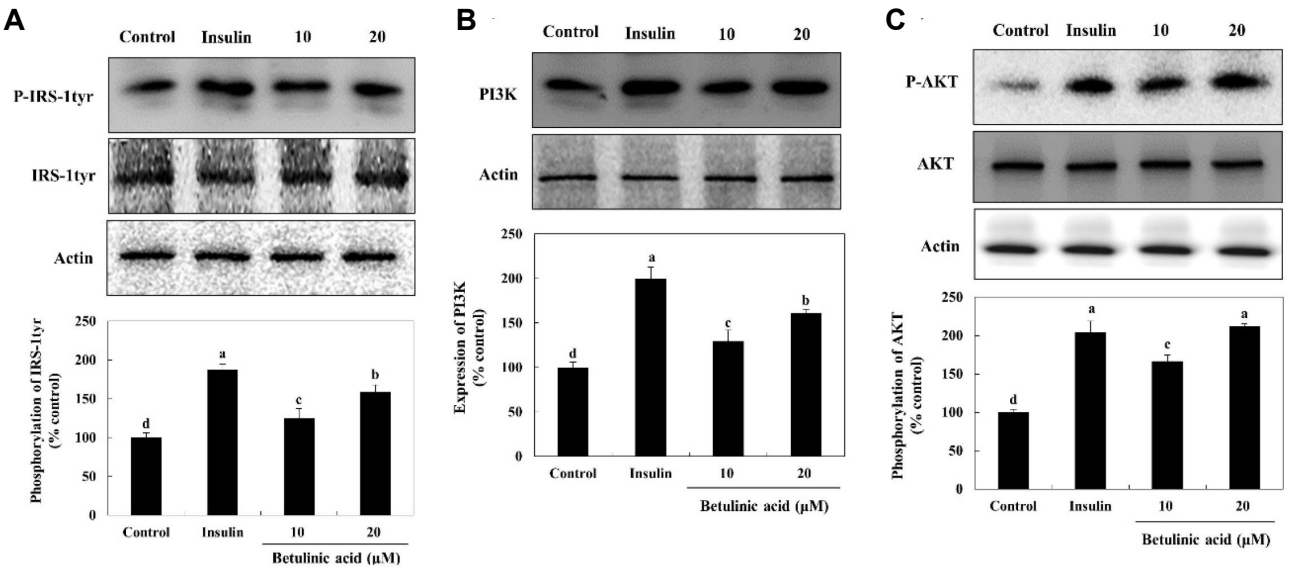

Fig. 3. Effect of betulinic acid on the expression of proteins involved in insulin signaling in 3T3-L1 adipocytes. Betulinic acid at 10 and 20 μM or insulin at 100 nM were applied to 3T3-L1 adipocytes for 24 hr, and the lysed cells were used in Western blot analysis. (A) Phosphorylation value of IRS-1tyr, (B) Expression value of PI3K, (C) Phosphorylation value of Akt. Each value is shown as the mean±standard deviation (n=3). [a-d]Values flagged with different letters indicate significant differences (p<0.05) by Duncan's multiple range test. IRS-1tyr, insulin receptor substrate 1-tyrosine; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B.

significantly upregulated by 125 and 159% after treatment with 10 and 20 μM betulinic acid, respectively, compared with the control. PI3K activation was also significantly upregulated by 161% after treatment with 20 μM betulinic acid. Interestingly, Akt phosphorylation markedly increased. As shown in Fig. 3C, treatment with 10 and 20 μM betulinic acid significantly increased Akt phosphorylation by 162 and 213%, respectively, compared with the control. These results indicate that betulinic acid could effectively upregulate IRS-1tyr and Akt phosphorylation and PI3K activation in the insulin signaling pathway.

Betulinic acid activates AMPK and ACC

To elucidate the mechanism by which betulinic acid increased glucose uptake, we examined AMPK and ACC activation/phosphorylation. AICAR-treated cell culture (0.5 mM) was used as a positive control. 5-aminoimidazole-4-carboxamide ribonucleotide, AICAR, is a metabolic intermediate in the purine nucleotide synthesis process. AICAR is an analog of adenosine monophosphate (AMP) that can stimulate the activity of AMPK. Betulinic acid significantly increased AMPK and ACC phosphorylation in 3T3-L1 adipocytes (Fig. 4). AMPK activation was significantly upregulated by 136 and 178% after treatment with 10 and 20 μM betulinic acid, respectively, compared with control. ACC phosphorylation was also significantly upregulated by 153 and 206% after treatment with 10 and 20 μM betulinic acid, respectively. These results demonstrate that betulinic acid could also stimulate glucose uptake by upregulating AMPK and ACC phosphorylation.

Betulinic acid increases PM-GLUT4 expression

The expression of PM-GLUT4 was examined to determine whether betulinic acid translocated GLUT4 to the plasma membrane, stimulating glucose uptake. As shown in Fig. 5, 20 μM betulinic acid significantly increased PM-GLUT4 expression in 3T3-L1 adipocytes by 215%, as compared with control. However, PM-GLUT4 expression significantly decreased by 141%, by co-treatment with betulinic acid and the PI3K inhibitor wortmannin. Furthermore, PM-GLUT4 expression significantly decreased by 173%, after co-treatment with betulinic acid and an AMPK inhibitor, compound C. These results indicate that betulinic acid increases the expression of PM-GLUT4 through the activation of PI3K and AMPK.

Betulinic acid combined with PI3K or AMPK inhibitor inhibits glucose uptake

To confirm that betulinic acid promoted glucose uptake by activating PI3K and AMPK, the cells were treated with

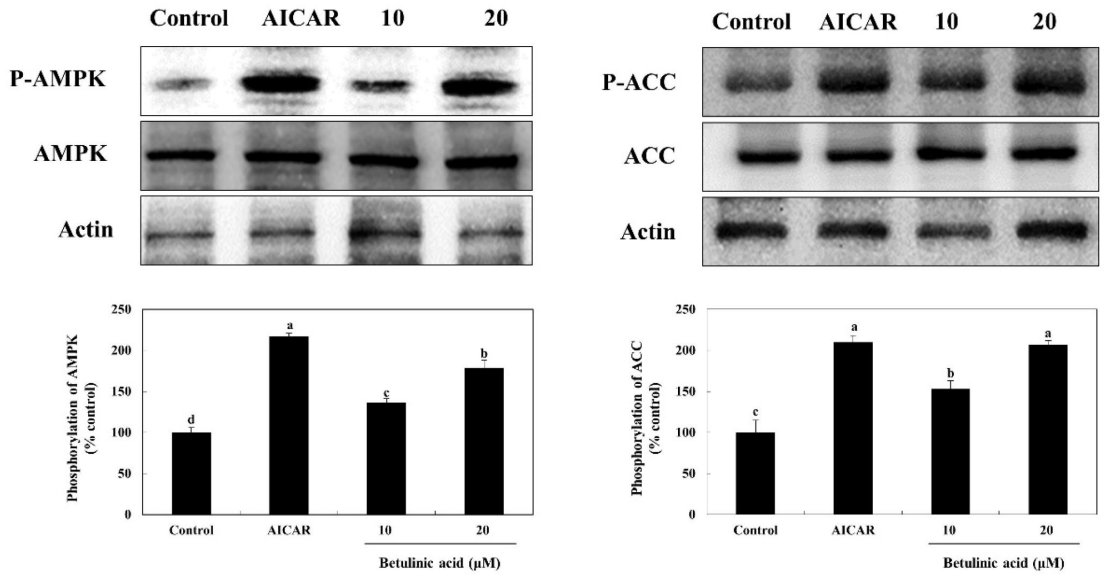

Fig. 4. Effect of betulinic acid on the expression of AMPK and ACC in 3T3-L1 adipocytes. Effects of betulinic acid on the expression of AMPK and ACC in 3T3-L1 adipocytes. Betulinic acid at 10 and 20 μM or AICAR at 0.5 mM were applied to 3T3-L1 adipocytes for 24 hr, and the lysed cells were used in Western blot analysis. (A) Phosphorylation value of 5 AMPK, (B) Phosphorylation value of ACC. Each value is shown as the mean ± standard deviation (n=3). AICAR-treated cell culture (0.5 mM) was used as a positive control. [a-d]Values flagged with different letters indicate significant differences (p<0.05) by Duncan's multiple range test. AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; AICAR, 5-Aminoimidazole-4-carboxamide ribonucleoside.

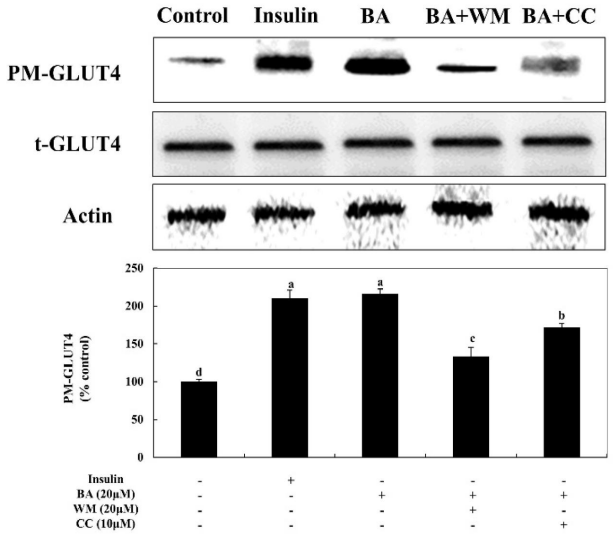

Fig. 5. Effect of betulinic acid on PM-GLUT4 expression in 3T3-L1 adipocytes. Effect of betulinic acid on PM- GLUT4 expression in 3T3-L1 adipocytes. 3T3-L1 adipocytes were treated with 20 μM betulinic acid and its combination with 20 μM WM or 10 μM CC and 100 nM insulin for 24 hr, after that western blot analysis was performed on lysed cells. Each value is shown as the mean±standard deviation (n=3). [a-d]Values composed of different letters indicate significant differences (p<0.05) by Duncan's multiple range test. PM-GLUT4, plasma membrane glucose transporter 4; WM, wortmannin; CC, compound C.

PI3K or AMPK inhibitors, respectively. As shown in Fig. 6, treatment with betulinic acid significantly increased glucose uptake in 3T3-L1 adipocytes. Betulinic acid (20 μM) significantly increased glucose uptake by 185%, compared with control. However, glucose uptake was significantly inhibited by 111 and 128%, respectively, when betulinic acid was treated with PI3K inhibitor wortmannin and AMPK inhibitor compound C, in the adipocytes. These results suggest that betulinic acid promotes glucose uptake by activating the PI3K and AMPK pathways in adipocytes.

Discussion

Type 2 diabetes is characterized by hyperglycemia due to insulin resistance. To reduce hyperglycemia, it is important to promote glucose uptake into insulin-sensitive tissues, such as skeletal muscle cells, adipocytes, and hepatocytes [3, 22]. Oral hypoglycemic agents, such as thiazolidinediones, have

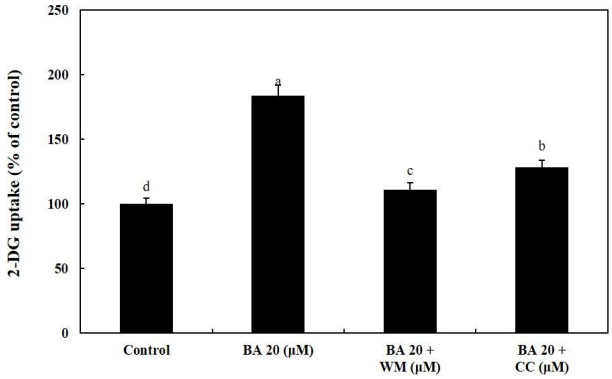

Fig. 6. Effect of betulinic acid combined with WM or CC on glucose uptake in 3T3-L1 adipocytes. 20 μM betulinic acid, its combination with 20 μM WM or 10 μM CC were applied to 3T3-L1 adipocytes, after that, 2-DG uptake assay was conducted. Each value is shown as the mean±standard deviation (n=3). $^{a-d}$Values composed of different letters indicate significant differences (p<0.05) by Duncan's multiple range test. WM, wortmannin; CC, compound C; 2-DG, 2-deoxyglucose.

been developed to improve insulin resistance and promote cellular glucose uptake [8]. However, treatment with these agents can result into harmful side effects, such as edema, fracture risk, and hepatotoxicity; therefore, studies are being actively conducted to find substances derived from natural products that could promote glucose uptake into cells [31]. Betulinic acid (3β,-3-hydroxy-lup-20(29)-en-28-oic acid) is a naturally occurring pentacyclic lupane-type triterpenoid compound that is abundantly present in the bark of some woody plants [24, 31]. Betulinic acid is known to have various biological effects, including anti-inflammatory, anti-tumor, anti-viral, and anti-diabetic effects [1, 9, 12, 21, 28]; however, the effect of betulinic acid on the promotion of cellular glucose uptake has not yet been studied. This study investigated the effect of betulinic acid on cellular glucose uptake and on cellular mechanisms mediating this effect.

Glucose uptake into adipocytes and skeletal muscle cells is important for reducing hyperglycemia and maintaining glucose homeostasis. Adipocytes are insulin target cells widely used to study the promotion of cellular uptake of glucose [18]. In the present study, betulinic acid significantly promoted glucose uptake by 3T3-L1 adipocytes. Glucose uptake into cells generally occurs via two pathways: the insulin signaling pathway and AMPK pathway [5, 22]. The primary pathway that promotes glucose uptake is the insulin signaling pathway; insulin being an important hormone for regulating blood glucose levels and maintaining glucose homeostasis in the body [18]. Glucose uptake by insulin is promoted by translocating GLUT4 from the intracellular cytosol to the plasma membrane through the activation of proteins involved in insulin signaling pathways, such as IRS-1tyr, PI3K, and Akt in adipocytes. AMPK activation also translocates GLUT4 from the intracellular cytosol to the plasma membrane, which promotes glucose uptake into the cells [32].

To elucidate the mechanism of action of betulinic acid on glucose uptake, the activation of proteins involved in the insulin-signaling pathway was investigated. Betulinic acid significantly increased the phosphorylation of IRS-1tyr and Akt, and the activation of PI3K, in 3T3-L1 adipocytes. In the insulin-signaling pathway, insulin activates the insulin receptor by binding to its β subunit. Activated insulin receptors trigger the phosphorylation of IRS-1tyr [29, 30]. Phosphorylated IRS-1tyr offers a binding site for PI3K, and the binding causes the activation of PI3K, which phosphorylates Akt with high affinity for Akt, its downstream target [7]. Phosphorylation of Akt can translocate GLUT4 to the plasma membrane and promote glucose uptake into adipocytes [19]. In the present study, we found that betulinic acid increased PM-GLUT4 expression by activating IRS-1tyr/PI3K/Akt signaling. These results suggest that betulinic acid promotes glucose uptake through PM-GLUT4, which is increased by the activation of IRS-1tyr/PI3K/Akt signaling in adipocytes.

Betulinic acid is a naturally occurring pentacyclic triterpenoid widely distributed in plants. Pentacyclic triterpenoids from *Astilbe rivularis* increase glucose uptake by activating Akt in myotubes [15]. Triterpenoids isolated from *S. chinensis* also alleviate insulin resistance via the IRS-1/PI3K/Akt and AMPK pathways in HepG2 cells [16]. Ursolic acid, a pentacyclic terpenoid, increases autophosphorylation of the insulin receptor β subunit, which activates the insulin receptor, leading to phosphorylation of IRS-1tyr and Akt. Akt phosphorylation translocates GLUT4 from the intracellular cytosol to the plasma membrane, which promotes glucose uptake [15]. The hydroxyl group at the C3 position of a pentacyclic triterpenoid could have a significant effect on the autophosphorylation of the insulin receptor β subunit [17]. Betulinic acid is a pentacyclic triterpenoid with a β-phenolic hydroxyl group at the C3 position. Thus, the results suggest that the hydroxyl group at the C3 position of betulinic acid could promote glucose uptake via the activation of IRS-1tyr/PI3K/Akt signaling in adipocytes.

AMPK is an important serine/threonine protein kinase that maintains energy homeostasis and promotes glucose and fatty acid uptake when intracellular energy levels are low. It is also a major enzyme that regulates chronic metabolic dis-

eases, such as insulin resistance in type 2 diabetes, and its activation regulates glucose transport [28]. Oral hypoglycemic agents, such as metformin, can activate AMPK, which translocates GLUT4 to the plasma membrane and promotes glucose uptake into cells, thereby alleviating hyperglycemia [10, 34]. To elucidate the effect of betulinic acid on AMPK activation, AMPK levels and ACC phosphorylation were examined in 3T3-L1 adipocytes. Betulinic acid significantly increased AMPK phosphorylation, which was confirmed by the increase in ACC phosphorylation, a downstream target of AMPK. The activation of AMPK by the increase in AMPK phosphorylation can translocate GLUT4 to the plasma membrane and promote glucose uptake in adipocytes. Our results suggest that betulinic acid promotes glucose uptake through activation of PI3K and AMPK in 3T3-L1 adipocytes. To confirm this, adipocytes were treated with the PI3K and AMPK inhibitors, wortmannin and compound C. Treatment with a PI3K or AMPK inhibitor combined with betulinic acid significantly decreased the expression of PM-GLUT4 and also reduced glucose uptake in adipocytes. These results indicate that betulinic acid could enhance the activation of IRS-1tyr/PI3K/Akt and AMPK and translocate GLUT4 to the plasma membrane, thereby promoting glucose uptake into adipocytes.

In conclusion, betulinic acid promotes glucose uptake by activating IRS-1tyr/PI3K/Akt and AMPK in 3T3-L1 adipocytes. The activation of the PI3K and AMPK pathways by betulinic acid was confirmed by the PI3K inhibitor wortmannin or AMPK inhibitor compound C. These results suggest that betulinic acid may promote glucose uptake and improve insulin sensitivity.

Acknowledgement

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : Betulinic acid의 PI3K와 AMPK경로 활성화를 통한 3T3-L1 지방세포에서 포도당 흡수 촉진 효과

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제 2형 당뇨병은 고혈당을 특징으로 하는 만성 대사성 질환으로 인슐린 민감성및 저항성을 개선하여 세포속으로 포도당 흡수를 촉진시킴으로서 완화될 수 있다. 본 연구는 triterpenoid 화합물인 betulinic acid가 3T3-L1 지방세포에서 인슐린 신호전달체계를 개선하여 포도당 흡수를 촉진시키는지를 조사하고 그 작용기전을 규명하였다. Betulinic acid는 3T3-L1 지방세포에서 농도의존적으로 포도당 흡수를 유의하게 증가시켰으며, 이는 PM-GLUT4의 발현 증가와 관련이 있음을 관찰하였다. Betulinic acid는 인슐린 신호전달 경로에서 PI3K의 활성화 및 IRS-1tyr, Akt의 인산화를 대조군에 비해 유의하게 증가시켰다. 또한 AMPK의 활성화를 나타내는 pAMPK와 AMPK 하위인자인 pACC의 수준을 유의하게 증가시켰다. Betulinic acid에 의한 PI3K 및 AMPK 경로의 활성화를 증명하기 위해, PI3K 억제제(Wortmannin)와 AMPK의 억제제(Compound C)를 사용하여 이들 처리에 의한 포도당 흡수능과 PM-GLUT4의 발현을 측정한 결과 이들의 발현이 유의하게 저해되었다. 본 연구에서 betulinic acid는 3T3-L1 지방세포에서 PI3K 및 AMPK 경로의 활성화를 통해 세포막으로 포도당 수송체인 GLUT4 전위를 촉진시키고 포도당 흡수를 증가시킬 수 있음을 나타내었다. 이러한 결과는 betulinic acid가 인슐린 민감성을 증진시키고 고혈당을 완화하는데 도움이 될 수 있음을 시사한다.