Bitter melon triterpenes work as insulin sensitizers and insulin substitutes in insulin-resistant cells

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ABSTRACT
The triterpenes 3β,25-dihydroxy-7β-methoxycucurbita-5,23(E)-diene (DHM) and 3β,7β,25-trihydroxycucurbita-5,23(E)-dien-19-al (THC) were previously isolated from Momordica charantia (bitter melon) and identified as hypoglycaemic principles. This study further investigated their hypoglycaemic mechanisms. FL83B cells were treated with tumour necrosis factor-α to result in insulin resistance, a feature of type 2 diabetes. DHM and THC increased the tyrosine phosphorylation of insulin receptor substrate isoform 1 and the phosphorylation of Akt only in the presence of insulin in insulin-resistant cells, suggesting that they are insulin sensitizers. However, they enhanced the phosphorylation of AS160 (Akt substrate of 160 kDa), the migration of glucose transporter-4 and the glucose uptake of insulin-resistant cells in the absence of insulin, suggesting that they can substitute for insulin to promote glucose clearance. The insulin substitution function was blocked by an AMP-activated protein kinase (AMPK) inhibitor, whereas the insulin-sensitizing function may involve the inhibition of protein-tyrosine phosphatase-1B (PTP-1B). The IC50 of DHM and THC to PTP-1B is 92.84 μM and 25.42 μM, respectively. In summary, DHM and THC have insulin-sensitizing and insulin-substitution functions, which are likely correlated with their effects on inhibiting PTP-1B and activating AMPK, respectively.

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Diabetes mellitus is one of the most prevalent chronic disorders worldwide, and type 2 diabetes accounts for over 90% of diabetic cases. Type 2 diabetes is featured by insulin resistance, which occurs when the insulin-responsive tissues, mainly skeletal muscle, adipose tissue, and the liver, cannot respond to insulin properly (Hirabara et al., 2012; Tang, Li, Liu, Huang, & Ho, 2013). Insulin promotes the glucose uptake of insulin-sensitive tissues and inhibits glucose export from the liver; thus, insulin resistance causes hyperglycaemia and stimulates pancreatic β cells to secrete more insulin for maintaining a normal level of blood glucose, which will result in β-cell damage and the development of type 2 diabetes without prompt treatment (Sidor & Granza-Michałowska, in press). Insulin resistance is also correlated with several other chronic diseases, such as dyslipidaemias, cardiovascular diseases, neurodegenerative disorders, and cancers (Hirabara et al., 2012). Agents or food supplements that can treat insulin resistance or provoke insulin-independent glucose disposal should be helpful in preventing β-cell damage, and managing type 2 diabetes and related disorders.

The precise mechanism of insulin resistance is not yet fully understood, whereas how obesity leads to the development of insulin resistance has been extensively investigated. When insulin binds to the cell-surface insulin receptor, the tyrosine-kinase activity of the receptor is activated, and several intracellular docking proteins are recruited to the receptor, among which insulin receptor substrates (IRSs), mainly IRS-1 and IRS-2, are closely related to glucose metabolism. The receptor catalyzes the tyrosine phosphorylation of the IRSs, resulting in the activation of P13K (phosphatidylinositol-3-kinase), PDK1 (P13K-dependent kinase), and subsequently Akt (protein kinase B). Akt catalyzes the phosphorylation of AS160 (Akt substrate of 160 kDa), which causes the migration of glucose transporter-4 (GLUT-4) from its cytoplasmic pool to the cell membrane. Cell-surface GLUT-4 facilitates the uptake of extracellular glucose by the cell, resulting in the reduction of blood glucose (Saltiel & Kahn, 2001; Whitehead, Clark, Urso, & James, 2000). In obese individuals, an overaccumulation of lipids in adipose tissue has been suggested to result in the production of proinflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β from the adipocytes and the infiltrating leukocytes. The circulating cytokines cause low-grade chronic inflammation throughout the body and interfere with the insulin-signalling pathway in cells, resulting in insulin resistance (Baboota et al., 2013; Kolb & Mandrup-Poulsen, 2010). TNF-α inhibits insulin signalling through several mechanisms, including the promotion in the expression of protein-tyrosine phosphatase-1B (PTP-1B). PTP-1B removes the phosphorylation on tyrosine residues of the insulin receptor and the IRSs, resulting in the down-regulation of insulin signalling (Nieto-Vazquez et al., 2007). Therefore, in previous studies, we used TNF-α to induce insulin resistance in FL83B liver cells (Chang et al., 2011, 2014). The insulin-stimulated glucose uptake and IRS-1 tyrosyl phosphorylation of the cells were obviously suppressed by TNF-α treatment. To explore the hypoglycaemic constituents in antidiabetic medicinal plants such as Momordica charantia, we used such cells as a model to screen for natural products that promoted the glucose uptake of insulin-resistant cells (Chang et al., 2011; Cheng, Huang, Chang, Tsai, & Chou, 2008).

M. charantia L., belonging to the Cucurbitaceae family, is also known as bitter gourd or bitter melon, and has been used as an herbal medicine to treat hyperglycaemia in India, South America, and Asia (Grover & Yadav, 2004; Xie & Du, 2011; Zhang et al., 2014). Various extracts and some ingredients of the bitter melon have been reported in scientific articles to have hypoglycaemic functions in animal models of type 1 or type 2 diabetes (Chang et al., 2011; Harinantenaina et al., 2006; Nerurkar, Lee, Motosue, Adeli, & Nerurkar, 2008). Several types of medicinal compounds have been isolated from the different parts of bitter melon, including triterpenoids, proteins, lipids, steroids, monoterpenes, alkaloids, etc. Some of them were shown to have hypoglycaemic effects, especially triterpenoids and proteins (Chang et al., 2008, 2011; Cheng et al., 2008; Lo et al., 2014; Zhang et al., 2014), whereas the action modes and mechanisms of most of these hypoglycaemic molecules, and whether they work under insulin-resistant conditions, remain undefined.

In our previous study, constituents in the crude extract of the M. charantia stem were screened, and three structurally similar triterpenes, namely (23E)-cucurbita-5,23,25-triene-3β,7β-diol (CTD), 3β,25-dihydroxy-7β-methoxycucurbita-5,23(E)-diene (DHM; Fig. 1A), and 3β,7β,25-trihydroxycucurbita-5,23(E)-

Fig. 1 – Activation of the insulin-signalling pathway by DHM and THC in the presence or absence of insulin. (A) Structures of DHM and THC. (B and D) Western blot analysis of tyrosine-phosphorylated and total IRS-1 (P-Y-IRS and IRS, respectively), phosphorylated and total Akt (P-Akt and Akt, respectively), and actin in FL83B cells. The cells were pretreated using the vehicle or TNF-α, followed by stimulation using insulin, troglitazone (TZD), DHM, or THC for 30 min, as indicated underneath the blots. The assays for DHM and THc were performed in duplicate. (C) Western blot analysis of phosphorylated AS160 (P-AS160) and actin in FL83B cells treated as indicated underneath the blots. The assays for DHM and THC were performed in triplicate. The data shown are from one of the experiments. (D) Western blot analysis of phosphorylated and total AS160, and actin in FL83B cells treated as indicated underneath the blots. Compound C was added with the chemical in Lanes 5–8. The band intensity of P-AS160 relative to Lane 1 was determined after normalization by that of actin. The data of the histogram represent the mean of two independent assays. The blots shown are from one of the experiments. (E) Western blot analysis of phosphorylated and total AS160, and actin in FL83B cells treated as indicated underneath the blots. Compound C was added with the chemical in Groups 5–8. The relative glucose uptake of each group versus Group 1 was determined. The experiments were performed twice independently, each in triplicate. The data represent the mean ± standard deviations of the experiments. *p < 0.05 versus Group 1; #p < 0.05 between Groups 6 and 2, Groups 7 and 3, and Groups 8 and 4.
dien-19-al (THC; Fig. 1A), were identified as potential hypoglycaemic principles. These molecules were demonstrated to raise the tyrosine phosphorylation of IRS-1 and the glucose uptake of insulin-resistant FL83B cells (Cheng et al., 2008). Moreover, THC was also isolated by another group from the M. charantia fruit as one of the major constituents, and its hypoglycaemic activity in vivo was confirmed in diabetic mice (Harinantenaina et al., 2006). However, the mechanisms underlying the hypoglycaemic activities of these triterpenoids were not clear. In this study, the hypoglycaemic actions and molecular mechanisms of DHM and THC were further characterized.

2. Materials and methods

2.1. Reagents

The following antibodies were used: antibodies for total or phosphorylated IRS-1 (YTr 895), total or phosphorylated Akt (Ser 473), total or phosphorylated AMPK α subunit (Thr 172), and total AS160 from Cell Signaling Technology (Beverly, MA, USA); horseradish peroxidase-conjugated secondary antibodies and GLUT-4 antibody for Western blotting from Santa Cruz Biotechnologies (Santa Cruz, CA, USA); GLUT-4 antibody for confocal microscopy from Abcam (Cambridge, UK); actin antibody from Chemicon (Temecula, CA, USA); phosphorylated AS160 (Thr 642) and FITC-conjugated secondary antibody from Invitrogen (Carlsbad, CA, USA). Foetal bovine serum, DAPI, and rhodamine phalloidin were purchased from Invitrogen. Recombinant human full-length PTP-1B expressed in Escherichia coli was ordered from Millipore (Bedford, MA, USA). Bovine insulin solution, troglitazone, cell culture media, mouse TNF-α, dimethylsulfoxide (DMSO), bovine serum albumin (BSA), and Compound C were from Sigma Chemical Company (St. Louis, MO, USA); B23 (3-(3,5-Dibromo-4-hydroxy-benzoyl)-2-ethylbenzofuran-6-sulphonicacid-(4-(thiazol-2-ylsulfamyl)-phenyl)-amide) was acquired from Calbiochem (Merck Millipore, Darmstadt, Germany). Glucose concentration was assayed using a glucose assay kit (Glucose GOD FS, Diagnostic Systems, Holzheim, Germany). CTD (10 mg), DHM (6 mg), and THC (52 mg) were previously isolated from 18 kg of M. charantia stems (Chang et al., 2008), each was dissolved in DMSO in a concentration of 10 mM as a stock solution. The final concentrations of troglitazone, CTD, DHM, THC, Compound C, and B23 were 50, 20, 20, 20, 8 μM, respectively, in all the assays except in Fig. 5A, B, and C.

2.2. Cell culture, glucose uptake assay, and statistical analysis

FL83B, C2C12, AML12, BNL CL.2, HepG2, and SK-Hep-1 cells were purchased from Bioresource Collection and Research Center (Hsinchu, Taiwan), and cultured at 37 °C in a humidified incubator supplied with 5% CO2 using the medium specified in the supplier’s instructions. The differentiation of C2C12 cells was performed as previously described (Cheng et al., 2008). Glucose uptake assays in insulin-resistant FL83B cells were performed as previously described with modifications (Chang et al., 2011; Cheng et al., 2008). Briefly, FL83B cells were seeded in 12-well plates (2 × 105 cells/well), cultured overnight, washed with phosphate-buffered saline (PBS, pH 7.4), then incubated in serum-free F12K medium containing 20 ng/mL of TNF-α for 5 h. After being washed twice with PBS, cells were incubated for 5 h in 450 μL of serum-free Eagle’s minimum essential medium (MEM) containing the investigated chemicals or without Compound C. In the control, an equivalent amount of the vehicle was added instead of the chemical. Before and at 5 h after the addition of the chemical, 30 μL of the medium was withdrawn and centrifuged at 500 × g for 5 min. Five microlitres of the resulting supernatant was mixed with 250 μL of Glucose GOD FS in a 96-well plate and incubated at 37 °C for 10 min. Absorbance at 500 nm was then determined using a microplate reader (Molecular Devices, Sunnyvale, CA, USA) to calculate glucose concentration. A standard curve was established simultaneously using solutions of various glucose concentrations. The amount of medium glucose consumed in each cell plate was calculated to determine the relative glucose uptake versus the control. Experiments were performed twice independently, each in triplicate. Data were analyzed using one-way analysis of variance (ANOVA) followed by Scheffe’s post hoc test. Significance was considered when p < 0.05 and F > 3.5546.

2.3. Western blot analysis

Cells were seeded in 35- or 60-mm plates until they reached 80–90% confluence, washed with PBS, and incubated in a serum-free medium containing 20 ng/mL of TNF-α or an equal volume of the vehicle for 5 h. After being washed twice with PBS, the cells were incubated in a medium containing the investigated chemical or an equal volume of the vehicle for 30 min. Subsequently, the cells were washed twice with PBS, submersed in a lysis buffer [Cell Culture Lysis Reagent (Promega, Madison, WI, USA)] containing 1 mM phenylmethylsulphonyl fluoride, 1 μg/mL pepstatin, 1 μg/mL leupeptin, 1 μg/mL aprotinin, 10 mM NaF, 1 mM sodium orthovanadate, and 10 mM sodium pyrophosphate], and scraped off the plate on ice. The resulting suspension was centrifuged at 500 × g for 5 min at 4 °C. The supernatant was collected and the protein concentration was analyzed using Bradford assay reagent (Bio-Rad, Hercules, CA, USA). Equal amounts of proteins were sampled and subjected to electrophoresis and Western blotting as described previously (Cheng, Chang, Cheng, Liu, & Chen, 2006; Cheng, Kuo, Liao, & Lin, 2012). The resulting X-ray films were scanned and band intensities were analyzed using the software of an imaging system (UVBiospectrum, UVP, LLC, Upland, CA, USA). Alternatively, immunoreactive bands on the PVDF membrane were detected directly by the aforementioned imaging system, and band intensities were analyzed using the supplied software.

2.4. Confocal microscopy of GLUT-4

FL83B cells were grown on 18 mm × 18 mm glass coverslips pretreated with 1% gelatin A and placed in 35-mm dishes. After being treated using the investigated agents, the cells were washed twice with PBS, fixed with 4% (v/v) paraformaldehyde in PBS for 5 min, and washed three times with PBS again. The cells were then permeabilized using 0.3% (v/v) Triton X-100.
in PBS for 5 min, washed extensively with 1% BSA (in PBS), blocked with 1% BSA for 1 h, and incubated with anti-GLUT-4 antibody (dilution 1:100 in 1% BSA) at 4 °C overnight. After being extensively washed with 1% BSA, the following procedures were all performed in the dark. The cells were incubated with FITC-conjugated secondary antibody (dilution 1:50 in 1% BSA) at room temperature for 1 h, and extensively washed with 1% BSA afterward. Subsequently, the cells were stained with rhodamine phalloidin (dilution 1:80 in PBS) for 30 min, washed with 1% BSA twice, and stained with 1 μg/mL of DAPI in PBS for 1–3 min. After being extensively washed with 1% BSA, the cells were mounted in Mounting Medium (Vector Laboratories, Burlingame, CA, USA) onto glass slides. The localization of GLUT-4 was then examined using a confocal microscope (Leica TCS SPS, Leica Microsystems, Heidelberg, Germany) and the images were processed using the Leica Application Suite Advanced Fluorescence program.

2.5. PTP-1B inhibition assay

PTP-1B activity was assayed using 5 mM p-nitrophenyl phosphate (pNPP) as the substrate in an assay buffer (50 mM HEPES, pH 7.4; 100 mM NaCl; 2 mM EDTA; 1 mM DTT). Recombinant human PTP-1B (25 nM) was preincubated with various concentrations of DHM, THC, or BZ3 at 30 °C for 20 min in the assay buffer, whereas in the control, an equal volume of the vehicle was added instead of the compound. Afterward, pNPP was added to the solution to start the reaction, which was allowed to proceed at 30 °C for 10 min in a final volume of 100 μL, and stopped by adding 50 μL of 3M NaOH. The solution was transferred to a 96-well plate to determine the absorbance at 405 nm using a microplate reader. The activity relative to the control (as 100% activity) was calculated using the absorbance obtained. The experiments were performed in triplicate. Data were fitted and IC50 values were calculated using SigmaPlot (Systat Software Inc., San Jose, CA, USA) according to the equation Four Parameter Logistic.

3. Results and discussion

3.1. Activation of the insulin-signalling pathway by DHM and THC in the absence or presence of insulin

DHM and THC promoted the glucose uptake and the tyrosine phosphorylation of IRS-1 of insulin-resistant cells in the presence of insulin (Cheng et al., 2008). As shown in Fig. 1B, insulin elevated the tyrosine phosphorylation of IRS-1 (P-Y-IRS) and the phosphorylation of Akt (P-Akt) in normal FL83B cells (Lanes 2 and 9) compared with the control (Lanes 1 and 8), whereas when the cells were pretreated with TNF-α, insulin could no longer promote the phosphorylation of these proteins (Lanes 4 and 11), confirming that TNF-α induced insulin resistance in cells. However, when DHM (Lanes 6 and 7), THC (Lanes 13 and 14), or troglitazone (Lanes 5 and 12; a thiazolidinedione [TZD]-type medicine known to be an insulin sensitizer [Rangwala & Lazar, 2004]) was added simultaneously with insulin, the tyrosine phosphorylation of IRS-1 and the phosphorylation of Akt in TNF-α-treated cells were obviously increased, similar to that in insulin-stimulated normal cells (Lanes 2 and 9). Consistently, Fig. 1C shows that the insulin-stimulated phosphorylation of AS160 (Lane 2) was attenuated in TNF-α-pretreated cells (Lane 3), whereas the simultaneous addition of CTD (lane 4), DHM (Lane 5), THC (Lane 6), or troglitazone (Lane 7) with insulin recovered the phosphorylation of AS160 in TNF-α-treated cells compared with the control (Lane 3). On the other hand, in Fig. 1D, DHM (Lanes 6 and 7), THC (Lanes 13 and 14), or troglitazone (Lanes 5 and 12) was added to TNF-α-pretreated cells without insulin, whereas none of them elevated the tyrosine phosphorylation of IRS-1 and the phosphorylation of Akt compared with the control (Lanes 2 and 9). Together, these data revealed that DHM and THC increased the tyrosine phosphorylation of IRS-1 and the phosphorylation of Akt in insulin-resistant cells only in the presence of insulin, suggesting that DHM and THC are insulin sensitizers.

Nonetheless, in the absence of insulin, DHM and THC still enhanced the phosphorylation of AS160. Fig. 1E showed that DHM (Lane 2) or THC (Lane 3) increased the phosphorylation of AS160 in TNF-α-pretreated cells in the absence of insulin compared with the control (Lane 1), similar to the effect of troglitazone (Lane 4). Correspondingly, Fig. 1F exhibited that DHM (Group 2), THC (Group 3), or troglitazone (Group 4) increased the glucose uptake of TNF-α-pretreated cells in the absence of insulin compared with the control (Group 1).

Collectively, these data suggest that DHM and THC act as insulin sensitizers in the presence of insulin in insulin-resistant cells; whereas in the absence of insulin, DHM and THC still promoted the phosphorylation of AS160 and raised the glucose uptake of insulin-resistant cells although they could not activate IRS-1 and Akt. The latter function is defined as insulin substitution herein because they can replace insulin to promote the glucose intake of insulin-resistant cells. Thus, DHM and THC function as insulin sensitizers and insulin substitutes in insulin-resistant cells.

In a previous study, a steroid, (22E,24R)-24-methyl-6β-methoxy-5α-cholesta-7,22-diene-3β,5β-diol, isolated from Cucurbita moschata (pumpkin), was also shown to possess both insulin sensitizing and insulin substitution functions in insulin-resistant FL83B cells in a concentration identical with those of DHM and THC used in this study (Chang et al., 2014), but the mechanisms underlying its functions are not clear. This suggests that other sources may also contain natural compounds with both insulin sensitizing and insulin substitution functions and deserve to be explored.

3.2. DHM and THC promote GLUT-4 translocation in FL83B cells

The phosphorylation of AS160 is known to enhance the translocation of GLUT-4 to the cell membrane, which results in the increase of a cell’s glucose uptake. However, GLUT-4 is mainly found in skeletal muscles, cardiac muscles, and adipose tissues, and its expression in the liver was considered insignificant or undetectable in earlier studies (Gould & Holman, 1993; Nevada, Valverde, & Benito, 2006; Zhao & Keating, 2007). Nevertheless, several recent articles have reported GLUT-4 expression in hepatic cell lines as well as in liver tissues (Chen et al., 2013; Chung, Cho, Bhuiyan, Kim, & Lee, 2010; Hoffler et al., 2009;
Fig. 5C shows that in the presence of Compound C, DHM, THC, and troglitazone could no longer promote the phosphorylation of AS160 (Fig. 1E, Lanes 6, 7, and 8, respectively) compared with the respective controls (Fig. 1E, Lanes 2, 3, and 4, respectively). In agreement with this, Fig. 1F showed that in the presence of Compound C, DHM (Group 6), THC (Group 7), and troglitazone (Group 8) could not increase the glucose uptake of TNF-α-treated cells as they did without Compound C (Groups 2, 3, and 4, respectively). Together, these data suggest that AMPK plays a crucial role in mediating the insulin substitution function of DHM and THC.

Several other plant-based natural products are also reported to activate AMPK in concentrations of μM ranges in cells, including berberine from Berberis aquifolium, resveratrol from red grapes, and quercetin from many fruits and vegetables. They have been shown to have beneficial and/or hypoglycaemic effects in type 2 diabetes (Ding et al., 2014; Hardie, 2013). However, whether they can act as insulin substitutes in insulin-resistant tissues is not clearly defined, although berberine was reported to mimic insulin action by increasing glucose uptake of 3T3-L1 adipocytes and L6 myocytes in an insulin-independent manner (Chen, Zhang, & Huang, 2010). It will be interesting to compare the insulin substitution effects of these compounds with those of DHM and THC in the same model, such as insulin-resistant cells utilized here.

PTP-1B decreases the tyrosine phosphorylation of insulin receptor and IRSs, which was suggested to be involved in the development of TNF-α-induced insulin resistance (Zabolotny et al., 2008). TNF-α has been demonstrated to enhance the expression of PTP-1B in FL83B cells (Cheng et al., 2012). Some natural products were shown to inhibit the activity of PTP-1B (Popov, 2011), and PTP-1B inhibitors were reported to have hypoglycaemic effects in diabetic animal models (Jiang, Guo, Shi, Guo, & Wang, 2013). DHM and THC promoted the insulin-stimulated tyrosyl phosphorylation of IRS-1 in insulin-resistant cells (Fig. 1B). Thus, whether they inhibit the activity of PTP-1B was determined. Consequently, DHM (Fig. 5A) and THC (Fig. 5B) both inhibited the enzymatic activity of human PTP-1B in vitro. The half maximal inhibitory concentration (IC50; the concentration of the compound required for achieving a 50% inhibition of PTP-1B activity) of DHM is 92.84 μM, and that of THC is 25.42 μM. Thus, THC has a stronger inhibitory effect on PTP-1B than DHM does. In the literature, the IC50 values of various synthetic or natural PTP-1B inhibitors range from smaller than 1 μM to approximately 200 μM (Li, Li, Wang, Asada, & Koike, 2010; Popov, 2011; Reddy, Chakshusmathi, & Narasu, 2012). Therefore, THC and DHM displayed substantial PTP-1B inhibitory activities compared to other PTP-1B inhibitors. As a reference, the IC50 of BZ3, a synthetic PTP-1B-specific inhibitor, was also evaluated under the same assay condition and determined to be 3.36 μM (Fig. SC), which is approximate to its documented IC50 (4.0 μM according to the manufacturer’s manual). Consistently, in Fig. 5D, when insulin-resistant FL83B cells were treated with BZ3 and insulin (Lane 5), the tyrosine phosphorylation of IRS-1 and the phosphorylation of Akt were raised compared with those of the control (Lane 4), and were similar to those of DHM- (Lane 6), THC- (Lane 7), or troglitazone-treated (Lane 8) cells, or to those of the insulin-stimulated normal control (Lane 2), supporting that the inhibition of
Fig. 3 – The confocal microscopic analysis of GLUT-4 translocation in FL83B cells. (A and B) The cells were treated using the vehicle (A) or insulin (Ins; B) for 5 h. (C to H) The cells were pretreated using TNF-α for 5 h to induce insulin resistance (Res), followed by treatments using insulin (C), insulin and DHM (D), insulin and THC (E), insulin and troglitazone (F), DHM alone (G), or THC alone (H) for 5 h. The cells were then labelled using a GLUT-4-specific antibody and an FITC-conjugated secondary antibody, followed by staining with rhodamine phalloidin (actin labelling) and then with DAPI. Images of the FITC fluorescence (Column GLUT-4), the superimposed images of DAPI staining and rhodamine phalloidin staining (Column DAPI+actin), and the superimposed images of FITC, DAPI, and rhodamine phalloidin staining (Column Superimpose) are shown. The scale bars represent 25 μm (A to E, and H) or 50 μm (F and G). The arrows indicate example areas with visible GLUT-4 labelling on the cell membrane.
PTP-1B can relieve TNF-α-induced insulin resistance in FL83B cells. Thus, PTP-1B inhibitory activity may play a role in the insulin sensitizing function of DHM and THC. However, the PTP-1B inhibitory activity of DHM is obviously weaker than that of THC, yet their insulin sensitizing effects seemed to be similar (Fig. 1B, Fig. 3, and Fig. 5D). Whether these triterpenoids have insulin-sensitizing or insulin-substitution function resembling those of DHM and THC deserves to be addressed.

Other natural compounds reported to improve insulin sensitivity include 1,2,3,4,6-penta-O-galloyl-D-glucopyranose from Paeonia lactiflora roots (Baumgartner et al., 2010), dehydrotrametenolic acid from Poria cocos Wolf (Sato et al., 2002), berberine (Chen et al., 2010), and resveratrol (Bagul et al., 2012). The mechanisms underlying the insulin sensitizing function of these natural compounds include PTP-1B inhibition and the activation of peroxisome proliferator-activated receptors (PPARs). Thus, whether the insulin sensitizing effects of DHM and THC involve the activation of PPARs should be investigated.

AMPK activation and PTP-1B inhibition are both suggested to be potential strategies for treating and/or preventing type 2 diabetes (Hardie, 2013; Popov, 2011; Wu et al., 2013; Yang, Jang, & Hwang, 2012). Therefore, as AMPK activators and PTP-1B inhibitors, DHM and THC have the potential to be applied in treating insulin resistance and type 2 diabetes. Many other triterpenoids were isolated from M. charantia, and several of them were also shown to have potential hypoglycaemic effects (Chang et al., 2011; Tan et al., 2008; Zhang et al., 2014). Whether these triterpenoids have insulin-sensitizing or insulin-substitution function resembling those of DHM and THC deserves to be addressed.

Liver and skeletal-muscle insulin resistance are both important in the development of type 2 diabetes (Jornayvaz, Samuel, & Shulman, 2010; Leclercq, Da Silva Morais, Schroyen, Van Hul, & Geerts, 2007). In our previous study, DHM and THC have been shown to promote the glucose uptake of insulin-resistant C2C12 cells, a skeletal muscle cell line. Thus, it will be valuable to study whether DHM and THC have similar functions and mechanisms in insulin-resistant muscle cells as they do in liver cells. It will further our understanding on the effects of bitter melon hypoglycaemic constituents on different tissues under insulin-resistant condition. Meanwhile, it is also important to study the synergistic effects of DHM and THC along with other antidiabetic compounds or medicines. The results of these researches will help optimize the clinical application of these compounds in treating diabetes-related disorders.

4. Conclusion

DHM and THC, two structurally similar triterpenes isolated from the bitter melon, have dual functions in insulin-resistant cells: insulin sensitizer and insulin substitute. The insulin substitution function likely correlates with their ability to activate AMPK. Meanwhile, DHM and THC are PTP-1B inhibitors, and this property likely contributes, at least in part, to their insulin-sensitizing function. DHM and THC may be two of the principles that contribute to the hypoglycaemic function of the bitter melon.

Conflict of interest

The authors declare no conflicts of interest.

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REFERENCES


