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의학석사 학위논문

Anti-diabetic effects of DMC-derivatives by activation of AMP-activated protein kinase and peroxisome proliferator activated receptor

DMC 유사물질을 통한 PPARγ/AMPK 이중 작용제의 항당뇨적 효능 분석 연구

2018년 8월

서울대학교 대학원

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최 동 락

Abstract

Type 2 diabetes is caused by insulin resistance and beta-cell dysfunction, leading to hyperglycemia. It is a complex metabolic disorder with various etiological pathways, impacting ectopic lipid depositions. Insulin resistance is very closely linked with T2D because it is predictor and therapeutic target of T2D. To treat and prevent T2D, insulin resistance and insulin signaling are needed to be improved. There are two representative insulin sensitizers in the market, metformin and TZDs. Metformin works as activator of AMPK which is a master regulator of energy metabolism. It is activated by increasing phosphorylation of AMPKα at Thr-172. Activation of AMPK increases fatty acid oxidation in skeletal muscle and regulates the hepatic glucogenesis in liver. TZDs work as activator of peroxisome proliferator activated receptor, PPARγ which is a nuclear receptor and is predominantly expressed in adipose tissues. Expressions of lipid transport related proteins are increased by its activation. It reduces lipid contents in circulation and improves insulin sensitivity by reducing lipotoxicity. However, TZDs have several side effects such as weight gain and fluid retention. In previous study, my lab studied about antidiabetic effects of DMC which is isolated from *cleistocalyx operculatus* plant by screening 14 putative PPARγ agonists. DMC worked as dual agonist of PPARγ and AMPK, increasing PPARγ transcriptional activity and AMPK activation. It also suppressed differentiation of adipocytes, promoted glucose uptake and increased the fatty acid oxidation in myotubes by AMPK activation in cell cultured system. It also improved insulin

sensitivity in high fed diet (HFD) induced obese mice. I received the B series of DMC derivatives form department of chemical engineering in Choong-Ang University. They were designed and synthesized with different functional groups according to the degree of electronic effect, hydroxylation, hydrophobicity and hydrophilicity. Purpose of my study is to investigate the antidiabetic effects of the B series of DMC derivatives in vitro and in vivo, having more efficacy than DMC. In in vitro experiment, most of DMC derivatives increased FAO ratio and phosphorylation of AMPK as much as DMC and AICAR. They also inhibited adipogenesis as much as DMC but they showed lower PPARγ transcriptional activities than DMC. For in vivo study, B1 and B7 selected as increasing FAO ratio as much as DMC and AICAR. In in vivo experiment, DMC derivatives showed similar effects on blood glucose tolerance with DMC. They tended to increase FAO in GM and decrease triglyceride (TG) levels in liver. In conclusion, the antidiabetic effects of B series of DMC derivatives were mainly mediated by AMPK activation.

Keywords: DMC, insulin resistance, type2 diabetes, metformin, TZDs, PPARγ, AMPK, fatty acid oxidation

Student number: 2016-29367

Contents

List of Abbreviations

T2D : type 2 diabetes

DMC : 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone

PPAR : peroxisome proliferator-activated receptor

FAO : fatty acid oxidation

FFA : free fatty acid

TG : triglyceride

GM : gastrocnemius muscles

AMPK: AMP-activated protein kinase

TZD : thiazolidinediones

ACC :Acetyl-CoA carboxylase

GAPDH : glyceraldehyde 3-phosphate dehydrogenase

HFD : high fat diet

H&E : Hematoxylin and Eosin

IP-GTT : intraperitoneal glucose tolerance test

WAT : white adipose tissue

BAT : brown adipose tissue

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Introduction

Metabolic syndrome including obesity and type 2 diabetes (T2D) has been rising rapidly and prevalence of T2D reached epidemic proportions worldwide. According to the International Diabetes Federation, the number of people who have T2D will rise to approximately 600 million by 2035 [1-2].

Development of T2D is caused by insulin resistance which is a complex metabolic disorder with various etiological pathways which can impact ectopic lipid deposition including lipogenesis, energy expenditure, and fatty acid uptake. Insulin resistance and insulin signaling can be aggravated by these cellular changes which can promote the accumulation of lipid metabolites in skeletal muscle and liver. Therefore, decreasing insulin resistance is key issue to treat and prevent T2D. There are two main insulin sensitizers improving insulin resistance in the market, metformin and TZDs (thiazolidinediones). Metformin works as an activator of AMP-dependent protein kinase (AMPK) and TZDs activate peroxisome proliferator activated receptor, PPARγ. [3–6].

Metformin has been widely used as an oral anti-diabetic treatment of T2D for a century [7]. It alleviates hyperglycemia without promoting weight gain and stimulates insulin secretion by decreasing hepatic glucose production and increasing glucose uptake in skeletal muscle [8-9]. It works as the activator of AMPK which is a multi-subunit enzyme composed of α, β, and γ subunits. AMPK is activated by phosphorylating AMPKα at Thr-172 and its activation

regulates lipid biosynthetic pathways and increases fatty acid oxidation and glucose uptake by phosphorylation and inactivation of Acetyl-CoA carboxylase (ACC) in skeletal muscle [10-13]. Skeletal muscle has critical role in regulating whole body homeostasis because it uptakes 80 % of the postprandial glucose [14]. Therefore, increasing FAO rates in skeletal muscle can be effective way to prevent insulin resistance and obesity [15].

TZDs have been used as antidiabetic drugs acting as full agonist of PPARγ, improving insulin action and hyperglycemia with T2D patients [16]. PPARγ, a member of nuclear receptor, is a dominant regulator of adipocytes, lipogenesis and insulin resistance [17-19]. It is widely known that insulin resistance is caused by elevated free fatty acids (FFAs), especially lipotoxic effect of FFAs, decreasing skeletal muscle glucose transport stimulated by insulin [20-21]. TZDs are well known drug to decrease FFAs and stimulate skeletal muscle insulin sensitization, by regulating lipid metabolism [22-23]. However, many patients who took TZDs kind of medications experience several side effects such as fluid retention, weight gain, congestive heart failure, and loss of bone mineral density [24]. It is unclear about action of PPARγ because some synthetic molecules with partial agonist retain their antidiabetic effects, blocking the Cdk5-mediated phosphorylation. Some of them have potent antidiabetic effect without side effects such as weight gain and fluid retention [25].

Hydroxy-chalcones have diverse biological activities including antioxidant, anti-inflammatory, anticancer, antihepatotoxic and antimalarial activities. Furthermore, they are well known to have inhibitory activity against diabetic

complications and also known as potential dietary PPARγ ligands [26].

In previous study, my lab showed that 2′,4′-dihydroxy-6′-methoxy-3′,5′-dimethylchalcone (DMC) isolated from *Cleistocalyx operculatus* by screening 14 putative PPARγ agonists and showed 10 times lower binding activity compared to rosiglitazone. It also has an anti-diabetic effect by increasing FAO rates in skeletal muscle, improving glucose tolerance via activation of AMPK in vivo and vitro. It also inhibits adipogenesis by activation of AMPK in a cell culture system [27]. DMC and its derivatives were received from chemical engineering department of the Choong-Ang University to find out molecules which have more antidiabetic effect by activating not only AMPK but also PPARγ with less cell cytotoxicity than DMC. DMC derivatives were designed and synthesized with different functional group according to degree of electronic effect, hydroxylation, hydrophobicity, and hydrophilicity. First, the effect of DMC derivatives on FAO rates and activation of AMPK by phosphorylating alpha subunit of Thr 172 were examined in fully differentiated C2C12 cell. Second, PPRE TK luciferase assay was performed to find out PPARγ transcriptional activities. Third, Effect of adipocyte differentiation by DMC derivatives in 3T3-L1 was also examined. To find out antidiabetic effects in high fat diet fed (HFD)-induced obese mice, DMC and selected derivatives, B1 and B7, were injected with oral-gavage for 4 weeks to mice fed with HFD for 14 weeks.

Materials and Methods

Cell culture and treatment

Cos-7 and C2C12 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). C2C12 myoblasts were differentiated into myotubes by incubation in DMEM supplemented with 2% horse serum for 5 days. 3T3-L1 preadipocytes were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum. DMI solution, 0.25 μM of dexamethasone (Dex), 0.5 mM of 3-isobutyl-α-methylxanthine (IBMX), and 5 μg/mL insulin in DMEM supplemented with 10 % fetal bovine serum, induced to confluent preadipocytes after 2 days. Differentiation of adipocytes was monitored with Oil red O staining. After cells were washed with PBS 2 times, cells were fixed with 4 % of formaldehyde for 1 h and stained with 0.5 % oil red O solution in propylene glycol for 30min. For the quantitative analysis of neutral lipid contents, 0.2 ml of isopropanol was treated to the stained cells and optical density was monitored spectrophotometrically at 510 nm.

Cell viability assay

C2C12 myotubes were treated with 0.1% DMSO (vehicle), DMC and its derivatives (1 and 10 μ mol/L) for 24 h, and cell viability was determined using a CellTiter96 non-radioactive cell proliferation assay kit (Promega, Madison, WI, USA).

Western blot analysis

Differentiated C2C12 myotubes were lysed with 1XRIPA buffer containing 0.5M Tris-HCl pH 7.4, 1.5M NaCl, 2.5% deoxycholic acid, 10% NP-40, 10mM EDTA (Merck Millipore, Temecula, CA) with protease inhibitors (10 ug/ul aprotinin, 10 ug/ul leupeptin and 1mM PMSF) and phosphatase inhibitor cocktail (Thermofisher). Bicinchoninic-acid (BSA) assay was used to measure concentration of proteins. 20ug of proteins were loaded to SDS-PAGE per lanes and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). The membranes were incubated with antibodies specific to pAMPK and total AMPK. The membranes were then incubated with anti-GAPDH antibody to confirm the same amount of protein loading in each lane. Protein bands were visualized using the enhanced chemiluminescence kit (Thermo, Rockford, IL, USA).

Transient transfection and Luciferase reporter assay

COS7 cells with 80 % confluency were transfected with pPPRE-tk-Luc (0.3 μg), pcDNA-HA-PPARγ (0.1 μg) and pCMV- β-gal (0.1 μg) using Lipofectamin and PLUS reagent for 3 h. 0.1% DMSO, 1μM of DMC derivatives and 10μM of rosiglitazone were treated for 24 h. Luciferase activities were measured with luciferase assay system kit and normalized by β-galactosidase activity

Animal experiments

8week C57BL/6 male mice were purchased from Center for Animal Resource Development, SNU, Seoul, Korea and were fed high fat diet (HFD), consisting of 58% fat and 25% sucrose (Research diet, New Brunswick, NJ) for 14 weeks. The mice were treated with 30 mg/kg/day DMC and its derivatives dissolved in Cremophor EL (CrEL) (Sigma) by oral gavage for 4weeks. CrEL has been used as a vehicle for oral gavage. After 3week treatment, intraperitoneal glucose tolerance test (IP-GTT) was performed. Mice were fasted for 17 h and then injected with 1 g/kg glucose (Sigma) solution. The blood glucose levels were measured at the indicated time points with blood glucose meter (Lifescan, Milpitas, CA). The next week, mice were starved for 6 h and sacrificed for tissue sampling. FAO ratio in GM was measured and triglyceride levels in liver were measured using triglyceride colorimetric assay kit (Cayman). Each WAT, BAT and liver tissues of mice were stained with hematoxylin and eosin (H&E) and observed using bright field microscopy (100X). All animal studies were approved by the Institutional Animal Care and Use Committee of Seoul National University Hospital.

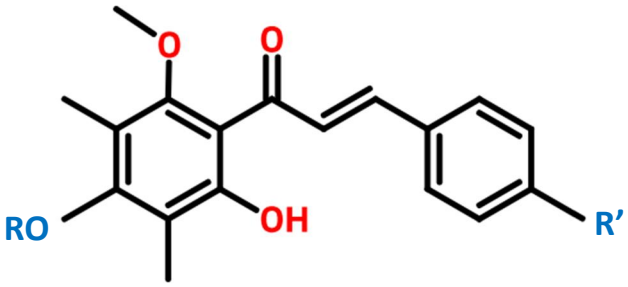
Measurement of FAO

For assessing FAO rates in skeletal muscle cells and gastrocnemius muscles, lysates were homogenized in ice-cold mitochondria isolation buffer (250 mmol/L sucrose, 10mmol/L Tris-HCl, and 1mmol/L EDTA). And the homogenized lysates were with 0.2 mmol/L [1-^{14}C] palmitate for 2 hours in incubation of 37 °C. By a liquid scintillation counter, ^{14}CO2 and ^{14}C-labeled acid-soluble metabolites were measured. The radioactivity of each lysate was standardized by protein amount.

Statistical analyses

Statistical analysis of the data was examined with SPSS version 12.0 (SPSS, Chicago, IL). Mean-Whitney U test was used to measure the differences between means. Data were expressed as means standard error, and data with p-Value less than 0.05 are denoted as statistically significant. For in vivo experiments, statistical analysis was performed using ANOVA by the Student-Neumann-Keuls test using GraphPad InStat software (San Diego, CA).

Results



Parent nucleus of derivatives

A1: R=H, R’=F
A2: R=H, R’=H (DMC)
A3: R=H, R’=CH_3
A4: R=H, R’=$CH(CH_2)_2$
A6: R=H, R’=OCH_3
A7: R=H, R’=OH

B1: R=OCH_3 R’=F
B2: R=OCH_3 R’=H
B3: R=OCH_3 R’=CH_3
B4: R=OCH_3 R’=$CH(CH_2)_2$
B5: R=OCH_3 R’=OMOM
B6: R=OCH_3 R’=OCH_3
B7: R=OCH_3 R’=OH
C2: R=OCH_3 R’=H
D2: R=OMOM R’=H

Figure 1. Chemical structures of DMC and its derivatives.

Designed and synthesized by Prof. Kwang Yong Park, School of chemical engineering and materials science, Chung Ang University.

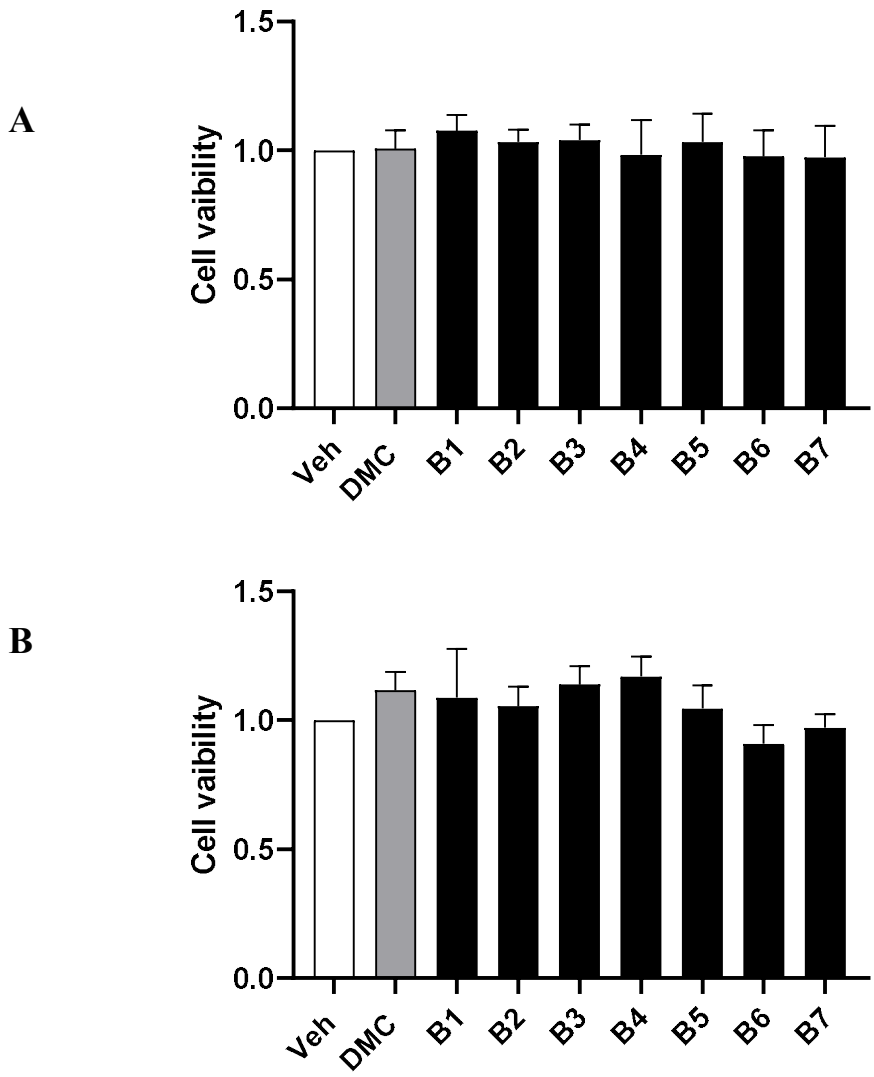


Figure 2. Cytotoxicity of the DMC and its derivatives in C2C12 myotubes. C2C12 myotubes were treated with 0.1% DMSO (Vehicle), DMC and its derivatives (A) (B1– B7, 1 μmol/L), (B) (B1– B7, 10 μmol/L) for 24 h, and then cell viability assays were performed. The value of vehicle considered 1 and the other values were expressed as its relative value. Data represent the means ± standard error (SEM) of four independent experiments (n=4).

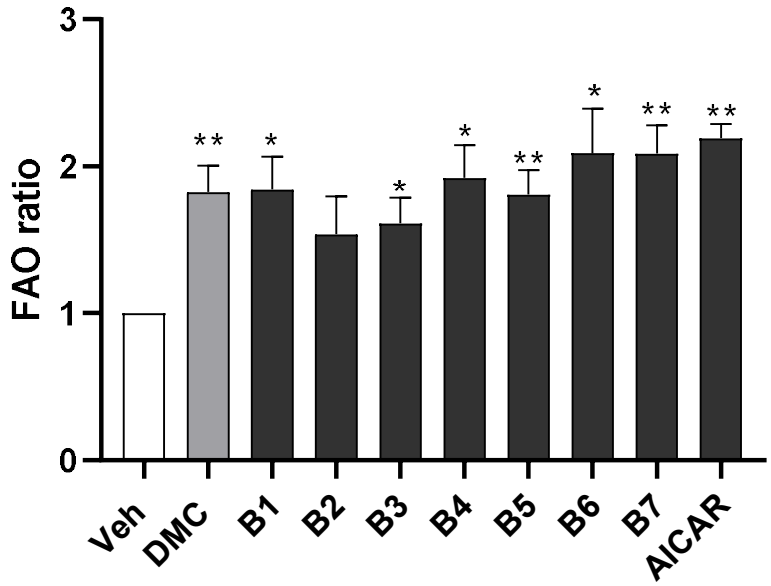


Figure 3. Effect of DMC derivatives on fatty acid oxidation in C2C12

DMC, its derivatives (10μ mol/L) and AICAR (1m mol/L) were treated to differentiated C2C12 myotubes for 24 h. FAO rates were measured. The value of vehicle (0.1% DMSO) considered 1 and the other values were expressed as its relative value. Data represent the means ± standard error (SEM) of five independent experiments. * $p<0.05$ vs. Vehicle, ** $p<0.01$ vs. Vehicle.

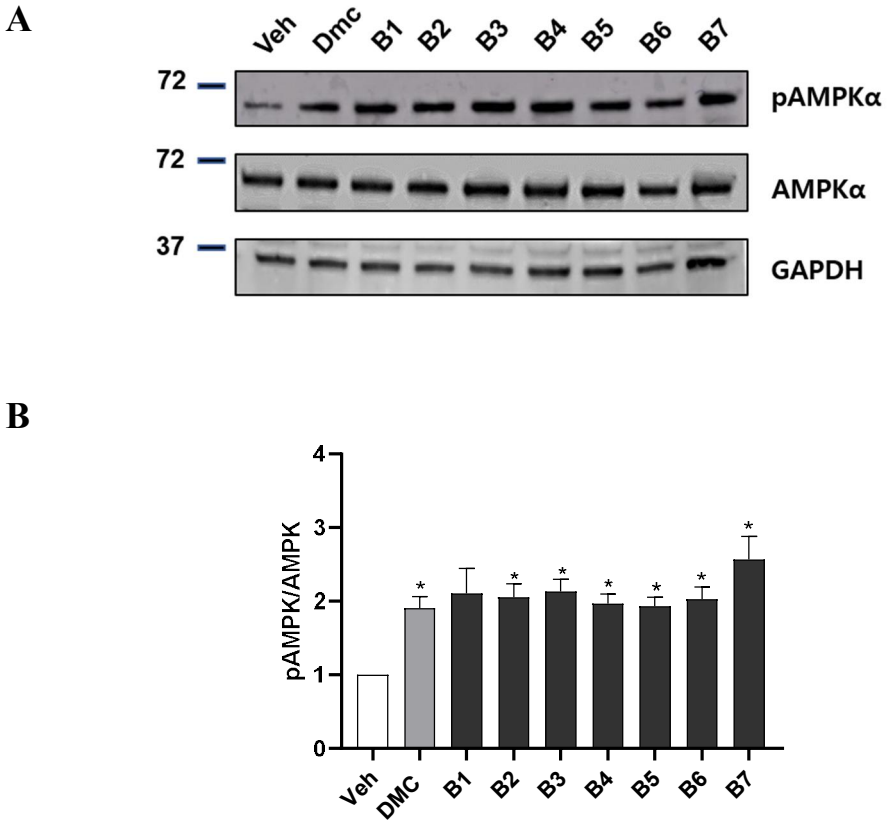


Figure 4. Effects of DMC derivatives on AMPK phosphorylation in C2C12 myotubes

DMC and its derivatives (10μ mol/L) were treated to differentiated C2C12 myotubes for 24 h. AMPK activations were measured. (A) Cell lysates were subjected to Western blot analyses with specific antibodies. (B) The value of vehicle (0.1% DMSO) considered 1 and the other values were expressed as its relative value. Data represent the means ± standard error (SEM) of three independent experiments. * $p<0.05$vs. Vehicle

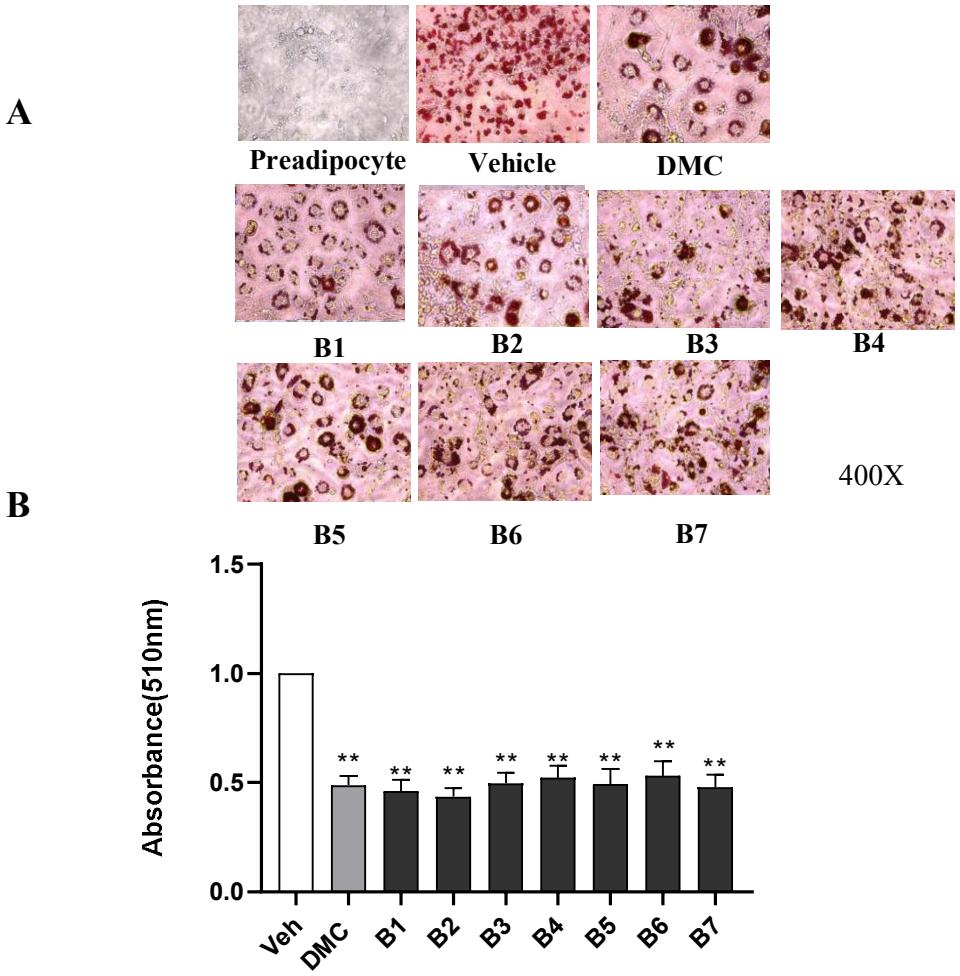


Figure 5. Effects of adipocyte differentiation by DMC derivatives in 3T3L-1. DMC and its derivatives (10μ mol/L) were treated to the confluent preadipocytes with differentiation induction media for 8 days. Intracellular lipid was stained with Oil Red O. (A) Lipid droplets, dyed with Oil Red O, observed by microscopy at 400X. To determine the extent of adipose conversion, 0.2 ml of isopropanol was added to the 12 well plates (B) Its optical density was monitored spectrophotometrically at 510 nm. The value of vehicle (0.1% DMSO) considered 1 and the other values were expressed as its relative value. Data represent the means ± standard error (SEM) of four independent experiments. * $p<0.05$ vs. Vehicle ** $p<0.01$ vs. Vehicle

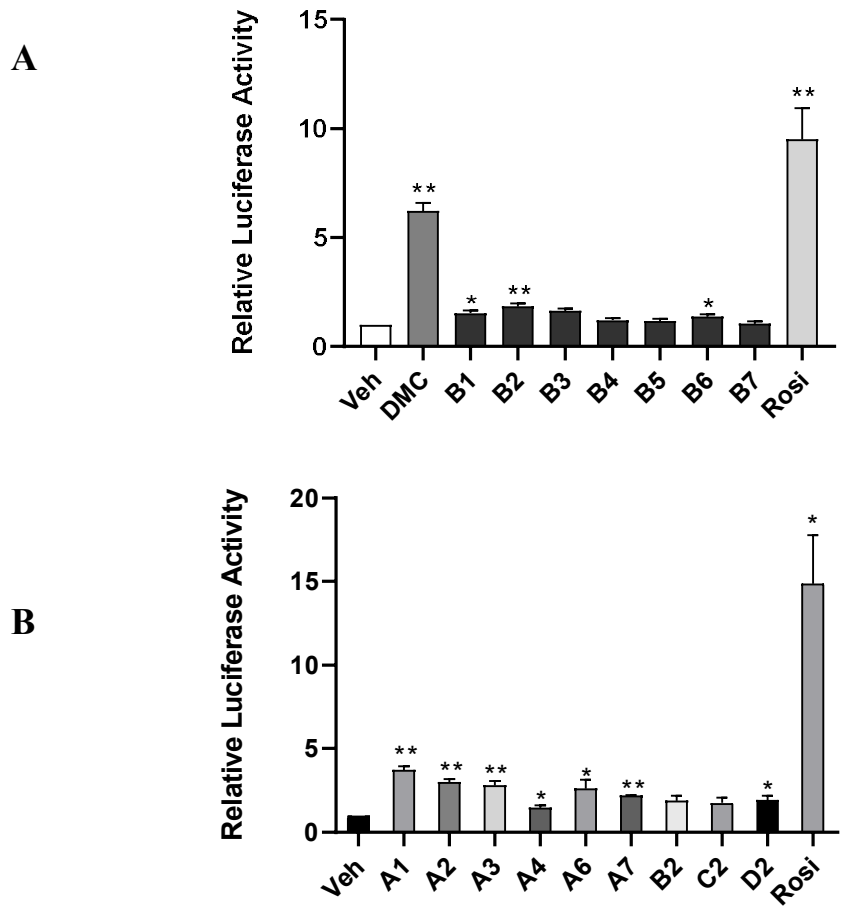


Figure 6. Effects of DMC derivatives on PPARγ transcriptional activities.

Luciferase assay was performed with DMC and its derivatives (A and B series) in Cos-7 cells which were co-transfected with a PPARγ expression vector, PPRE-luciferase vector and CMV- β-gal for transfection efficiency control. DMC, its derivatives (1μ mol/L) and Roisglitazone (10μ mol/L), were treated for 24 h after transfection. The value of vehicle (0.1% DMSO) considered 1 and the other values were expressed as its relative value. Data represent the means ± standard error (SEM) of four independent experiments. * $p<0.05$ vs. Vehicle, ** $p<0.01$ vs. Vehicle.

Cytotoxicity of DMC derivatives in C2C12 myotubes.

First, the cytotoxicity of DMC and its derivatives were examined by measuring cell viability. After C2C12 myoblasts were fully differentiated during 5days, DMC and its derivatives (B1-B7) were treated 24h with 1μ mol/L and 10μ mol/L (Fig. 2A, B). None of them affects cell viability with 1μ mol/L and 10μ mol/L.

Effects of Fatty acid oxidation in C2C12 myotubes by DMC derivatives

As DMC increased FAO ratio significantly by AMPK activation, FAO ratios were measured with DMC derivatives. DMC and its derivatives (10μ mol/L) were treated to fully differentiated C2C12 myotubes for 24h and AICAR used as a positive control (1mmol/L). Most of DMC derivatives increased FAO ratio as much as DMC and AICAR (Fig. 3).

Effects of AMPK activation in C2C12 myotubes by DMC derivatives

As DMC showed to increase FAO ratio via activation of AMPK, phosphorylating alpha subunit of Thr172, the effects of AMPK activation by DMC derivatives (10μ mol/L) were measured in fully differentiated C2C12 myotubes. Most of derivatives promoted phosphorylation of AMPK as much as DMC (Fig. 4).

DMC derivatives inhibit differentiation of adipocytes

As DMC inhibits adipogenesis via AMPK activation, I tested whether the DMC derivatives also inhibit the adipogenesis. They were treated to 3T3-L1 preadipocytes with (10 μmol/L) simultaneously with the induction of adipocyte differentiation (DMI treatment) and continuously thereafter. Eight days after adipogenesis induction, cells were stained with Oil red O. It showed that vehicle (0.1% DMSO) cells had more lipid droplets than other cells which had been treated with DMC and derivatives. Negative control which had grown with only FBS had no lipid droplets (Fig. 5A). DMC derivatives inhibited adipocyte differentiation as much as DMC. Quantitative analysis of neutral lipid contents supported this result by measuring the absorbance at 510 nM. (Fig 5B).

Effects of transcriptional activities of PPARγ by DMC derivatives

To find out whether DMC derivatives promote the transcriptional activities of PPARγ, luciferase assays were performed with DMC and its derivatives at 1 μM compound concentrations in Cos-7 cells which were co-transfected with a PPARγ expression vector, PPRE-luciferase vector and CMV- β-gal for transfection efficiency control. Rosi(10 μM), a PPARγ full agonist, was also used as positive control. The results showed that DMC increases transcriptional activity of PPARγ, 2 times lower transcriptional activity compared to rosiglitazone. However, effects of DMC derivatives (B1-B7) were very weak (Fig 6A). I also measured the transcriptional activities of A series

DMC derivatives and they showed higher transcriptional activities than B series, 3 times lower transcriptional activities compared to rosiglitazone (Fig 6B).

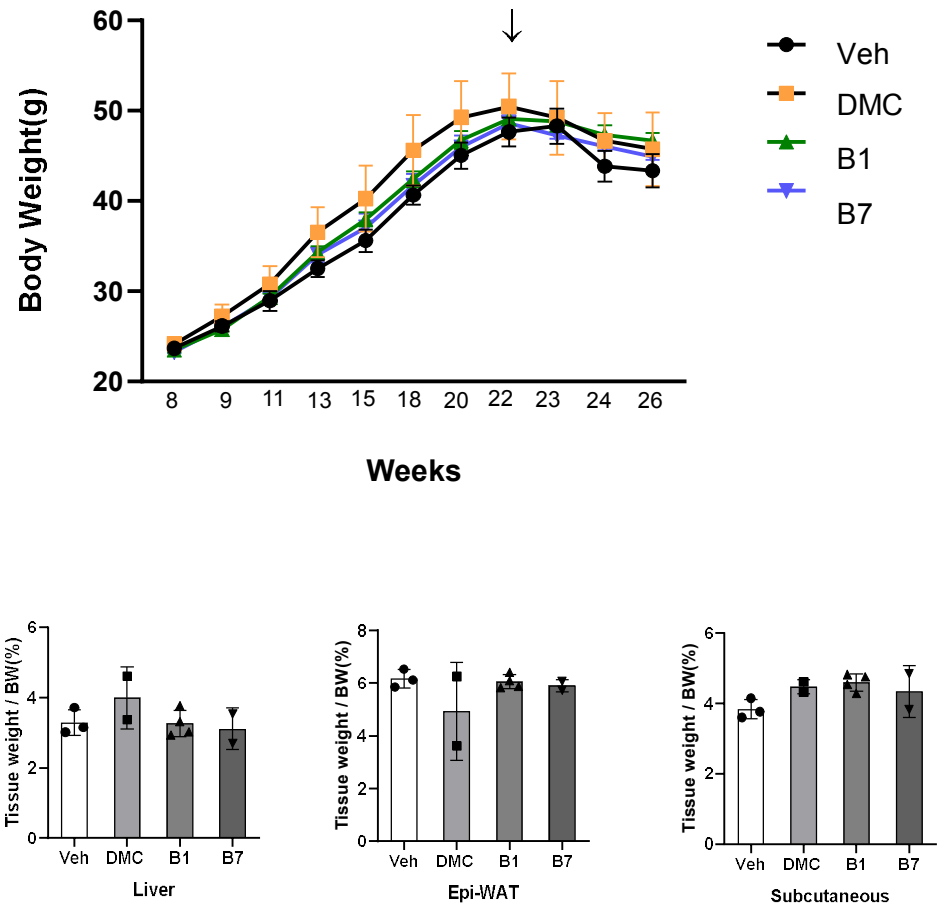


Figure. 7 Differences of body and tissue weights between control and treated groups

Mice were fed with HFD for 14 weeks and DMC and its derivatives (30mg/kg/day) were treated on every second day for 4 weeks by oral gavage. (A) Body weights of mice were measured during the treatment. (B) Each tissue weights were measured and represented as a percentage of the total body weight. Data represent the means ± standard error (SEM) of individual mice. (Veh, n=3, DMC n=2, B1 n=4, B7 n=2)

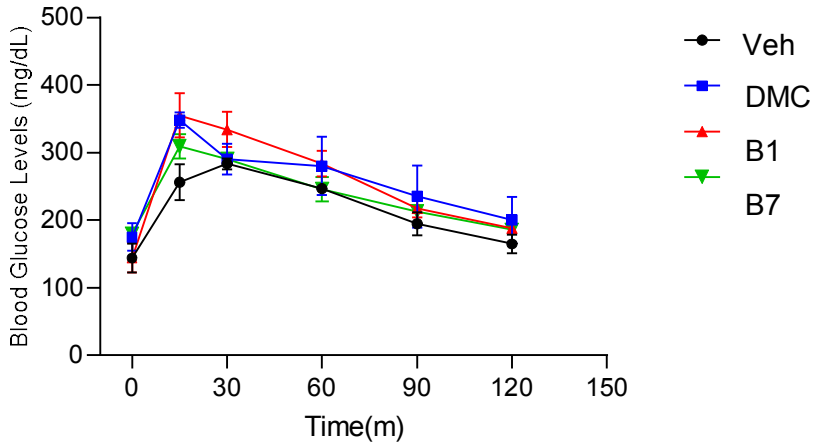


Figure.8 Effect of DMC derivatives on blood glucose tolerance test in HFD induced obese mice

After 3 weeks treatment of DMC and its derivatives, glucose solutions were injected after 17 h fasting. Blood glucose levels were measured at time course (0, 15, 30, 60, 90, and 120 min) from mice tail blood vain. Data represent the means ± standard error (SEM) of individual mice. (Veh, n=3, DMC n=2, B1 n=4, B7 n=2)

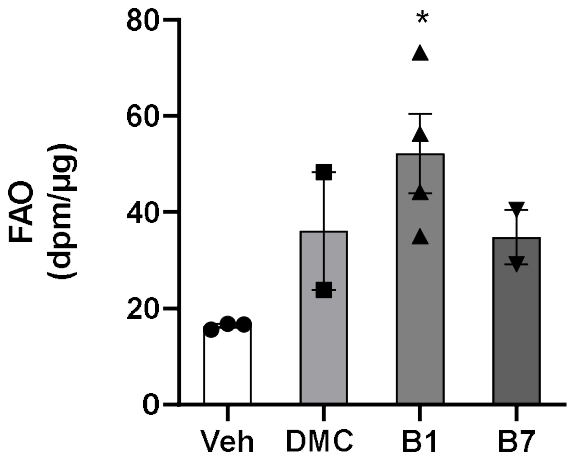


Figure 9. Effect of DMC derivatives on FAO ratio of gastrocnemius muscle (GM) .

After 4weeks treatment of DMC and its derivatives, mice were sacrificed. FAO were measured in GM. Data represent the means ± standard error (SEM) of individual mice. (Veh, n=3, DMC n=2, B1 n=4, B7 n=2) * $p<0.05$ vs. Vehicle.

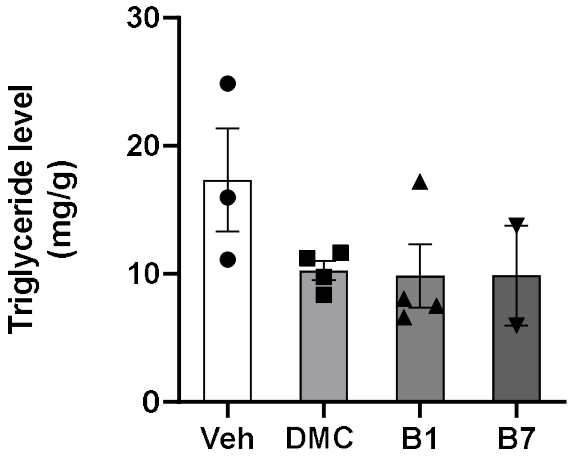


Figure 10. Effect of DMC derivatives on triglyceride levels of liver

After 4weeks treatment of DMC and its derivatives, mice were sacrificed. TG levels were measured in liver. Data represent the means ± standard error (SEM) of individual mice. (Veh, n=3, DMC n=4, B1 n=4, B7 n=2).

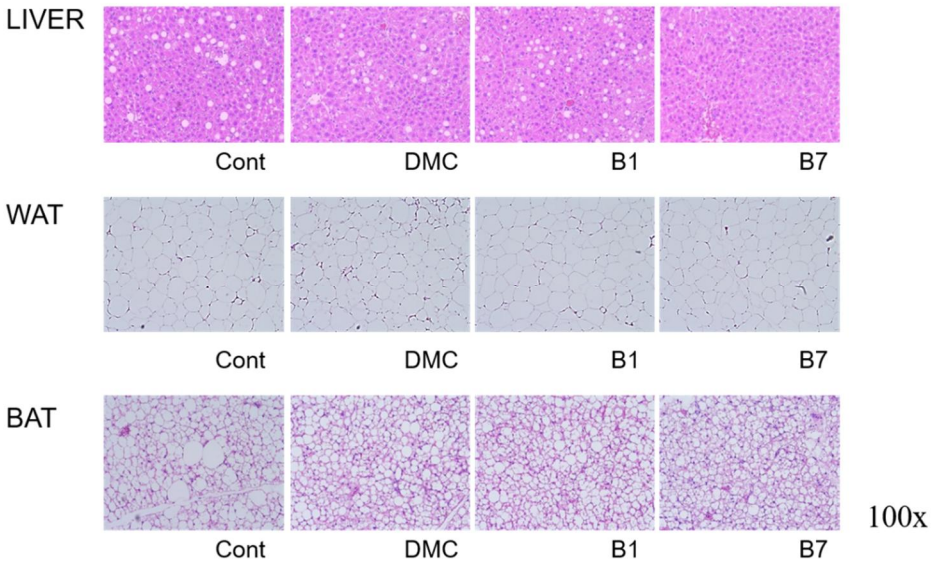


Figure.11 Histological analysis of each tissue, Liver, WAT and BAT.

H&E stained each tissue was observed using bright field microscope with 100x magnification.

Antidiabetic effects of DMC derivatives in HFD-induced obese mice.

For in vivo study, B1 and B7 selected as increasing FAO ratio as much as DMC and AICAR. To evaluate the anti-diabetic effects of DMC and its derivatives, mice were fed with HFD for 14weeks and DMC and its derivatives were injected with oral-gavage every other day for 4 weeks. Difference of body and tissue weight such as epididymal white and brown adipose tissue, subcutaneous fat and liver were not observed between control and treated groups (Fig. 7).

After 3 weeks treatment of DMC and its derivatives, glucose tolerance test was performed. DMC derivatives showed similar effects on blood glucose tolerance with DMC (Fig. 8). After 4 weeks treatment of DMC and its derivatives, FAO ratios of each mice in gastrocnemius muscle (GM) and triglyceride (TG) levels in liver were measured. DMC derivatives tended to increase FAO in GM and decrease TG levels in liver. (Fig. 9,10). To evaluate tissue histology, each tissue, brown and white adipose tissue and liver, were stained by hematoxylin and eosin (H&E). Significant histological differences of each tissue between control and treated groups were not observed. (Fig. 11).

Conclusion

B series of DMC derivatives activated AMPK and showed similar effects on FAO and adipogenesis inhibition but they showed lower transcriptional activities of PPARγ than DMC in in vitro experiments. They also showed similar effects on blood glucose tolerance compared to DMC and tended to increase FAO in GM and decrease TG levels in liver. Therefore, the anti-diabetic effects of B series of DMC derivatives were mainly mediated by AMPK activation.

Discussion

DMC derivatives were synthesized and designed with different functional groups of DMC molecule according to degree of electronic effect, hydroxylation, hydrophobicity, and hydrophilicity. To find molecules with having more antidiabetic effect than DMC, FAO experiment was performed with differentiated C2C12 myotubes because skeletal muscle has a critical role of whole body homeostasis and uptakes 80 % of the postprandial glucose [14]. Increasing FAO rates in skeletal muscle can be effective way to prevent insulin resistance and obesity [15].

As DMC increased FAO ratio via activation of AMPK, phosphorylating alpha subunit of Thr172, FAO ratio and AMPK activation of DMC and its derivatives (10μ mol/L) were measured in C2C12 myoblasts. Most of DMC derivatives increased the fatty acid ratio and phosphorylation in C2C12 myotubes.

One of the most fatal side effects of TZDs is weight gain. As DMC inhibits adipogenesis via AMPK activation, DMC and its derivatives were treated with DMI, adipogenesis induction media. The quantitative result showed that DMC derivatives inhibited adipogenesis in 3T3L-1 as much as DMC.

As DMC increased PPARγ transcriptional activity, luciferase assay was performed with DMC and derivatives at 1 μM compound concentrations in Cos-7 cells which were co-transfected with a PPARγ expression vector, PPRE-luciferase vector and CMV- β-gal for transfection efficiency control. Rosi(10 μM), a PPARγ agonist, was also used as positive control. DMC

increased PPARγ transcriptional activity, 2 times lower transcriptional activity compared to rosiglitazone but its derivatives unexpectedly increased only 1.5~2.0 times compared to Vehicle. However, the action of PPARγ is still unclear because some molecules with partial or non-agonist of PPARγ retain their antidiabetic effects in lipid metabolism, blocking the Cdk5-mediated phosphorylation and some of them have potent antidiabetic effect without side effects such as weight gain and fluid retention [25]. I also received the A series, C2 and D2 of DMC derivatives and measured the transcriptional activities of PPARγ by luciferase reporter assay. The result showed that 2',4' –hydroxyl groups are important for PPARγ activation because the A series DMC derivatives with hydroxyl groups showed higher transcriptional activities than others. (Fig.6B)

As DMC showed antidiabetic effect with HFD mice, DMC derivatives were selected to examine the in vivo experiment. B1 and B7 selected as increasing FAO ratio as much as DMC and AICAR. They showed similar effects on blood glucose tolerance with DMC. They also tended to increase FAO in GM and decrease TG levels in liver.

However, there were several problems in in vivo experiments. The maximum point of the blood glucose level in control group did not reach as much as the maximum points of DMC and its derivates treated groups. Some of results such as FAO ratio and TG levels were not statistically significant and variations of individual mice were large in several experiments. I think these problems are caused by my unskillfulness of treating mice and using plastic oral

gavage needle in early treatment because the several mice were dead during oral administration and body weight of all groups also decreased after treatment during even HFD. Flexible plastic oral gavage needles could hurt mice stomach during oral administration and make stressful situation to the mice.

Future study can be the completion of in vivo experiment of B series by measuring of AMPK activities and TG levels in GM because FAO ratio of GM increased significantly by DMC derivatives. Surface plasmon resonance assay can be examined as future study to measure the binding affinities of AMPK and PPARγ with DMC derivatives because previous study showed that DMC activates AMPK with direct binding unlike metformin which activates AMPK indirectly by changing ADP/ATP ratio. Furthermore, B series derivatives showed less transcriptional activities of PPARγ than A series derivatives (Fig.6). Measuring binding affinities with DMC derivatives and rosiglitazone can show the important functional groups for binding affinity of PPARγ and whether DMC derivatives bind with PPARγ competitively with rosiglitazone or not.

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국문초록

대사성 질환인 제 2형 당뇨병은 인슐린 저항성과 밀접한 연관관계를 갖고 있다. 대표적인 인슐린 증감제로는 Metformin 과 TZDs가 존재한다. Metformin은 대사성 질환의 주요 표적인 AMP-dependent protein kinase (AMPK) 인산화를 촉진시키며 대사조절과 밀접하게 연관되어 있는 표적장기에 관여하며 인슐린 저항성을 개선시킨다. TZDs는 PPARγ의 활성을 증가시켜 지방세포를 분화시키고 피하지방에 유리지방산을 축적시킴으로써 인해 혈중 유리지방산을 낮추어 인슐린 저항성을 개선한다.

이전 실험에서 Cleistocalyx operculatus 라는 작물에서 추출된 단일화합물 DMC (2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone)의 효능을 기반으로 본 연구가 시작되었다. DMC 물질은 PPARγ와 AMPK의 이중작용제로서 인슐린 저항성을 개선시키는 것을 최진우 박사에 의하여 입증되었다.

DMC 보다 더 효능이 좋은 DMC 유사 물질을 찾기 위해 본 연구가 시작되었다. DMC유사물질은 중앙대학교 화학과에서 전자효과에 따른 작용기의 치환을 통해 만들어졌다. B 시리즈 DMC 유사물질은 근육세포내에서 지방산 산화와 AMPK의 인산화를 DMC 만큼 촉진 시켰으며 지방세포 분화를 억제하는 작용도 DMC와

유사한 결과를 보였다.

다음으로 14주간 고지방사료를 먹인 동물모델에서 B 시리즈의 DMC유사물질을 4주간 동물모델에 경구 투여한 후 인슐린 저항성의 미치는 영향을 관찰하였다. 비록 B 시리즈 DMC 유사물질이 DMC 보다 더 크게 인슐린 저항성을 개선시키지 못하였지만 간에서 중성지방 수치를 감소시키는 경향을 보였고 골격근에서 지방산 산화를 증가시키는 경향을 보였다. 결론적으로 B 시리즈의 항당뇨적인 효능은 주로 AMPK 활성에 의해 매개되었다.

DMC 유사물질을 연구함으로써 비만, 체액 저류 등의 부작용 없이 인슐린 저항성을 개선시키는 새로운 치료제 개발에 큰 도움이 될 것으로 기대된다.

주요어 : DMC, 인슐린 저항성, 제 2 형 당뇨병, metformin, TZDs, PPARγ, AMPK, 지방산 산화

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석사과정을 잘 마칠 수 있게 도와주셨던 많은 분들께 감사 드립니다.

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