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Beneficial effects of IH-901 on glucose and lipid metabolisms via activating adenosine monophosphate–activated protein kinase and phosphatidylinositol-3 kinase pathways

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Abstract

IH-901 is an intestinal metabolite of ginsenosides found in *Panax* ginseng. In the present study, effects of IH-901 on glucose and lipid metabolisms were examined using C2C12 myotubes and C57BL/ksJ *db/db* mice. A significant increase in phosphorylated adenosine monophosphate–activated protein kinase was observed when differentiated C2C12 myotubes were treated with IH-901. Glucose transporter 4 protein expressions were also up-regulated when muscle cells were treated with of IH-901 up to 60 minutes, resulting in stimulation of glucose uptake by 25% as compared with untreated cells. In addition, phosphatidylinositol-3 kinase and Akt protein expressions were increased when C2C12 myotubes were exposed to IH-901 for up to 3 hours; and these effects including glucose uptake were attenuated by pretreatment with LY294002, a selective phosphatidylinositol-3 kinase inhibitor. In animal study, IH-901 at 25 mg/kg lowered the plasma glucose, triglyceride, cholesterol, and nonesterified fatty acid levels by 20.7%, 41.6%, 20.2%, and 24.6%, respectively, compared with control mice. In the meantime, plasma insulin levels were significantly increased by 2.2 and 3.4 times in 10 and 25 mg/kg–treated mice, respectively, compared with control mice, in parallel with the histologic observation showing a preserved architecture of the pancreatic islet. Protein and gene expression patterns for adenosine monophosphate–activated protein kinase, sterol regulatory element binding protein–1a, and glucose transporter 4 in the liver and skeletal muscles were similar to those in cell studies. In summary, IH-901 might be a promising therapeutic agent improving altered glucose and lipid metabolisms revealed in type 2 diabetes mellitus patients.

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1. Introduction

Diabetes mellitus (DM) is increasing at alarming rates in the Westernized and developing countries. The increasing costs in terms of suffering as well as economics are well recognized [1]. Diabetes mellitus is a metabolic disease characterized by deranged glucose homeostasis due to an absolute or relative lack of insulin [2]. Commonly practiced pharmacologic treatment of DM includes insulin and oral hypoglycemics. There is an increasing demand for the use of natural products with antidiabetic activity because insulin and oral hypoglycemic agents had many undesirable adverse effects and certain oral agents are not effective in lowering the blood glucose level in chronic situation. Traditional herbal medicine has been widely used for disease treatment and is recognized as an interesting alternative to conventional medicine. The uncertainty about the efficacy and safety of the currently available oral hypoglycemic drugs has prompted a search for safer and effective agents in the treatment of diabetes [3]. Ginseng is one of the most widely used herbal medicines and is reported to have a wide range of pharmacologic applications. Ginsenosides, the major pharmacologically active ingredients of ginseng, appear to be responsible for most of the activities of ginseng including vasorelaxation, antioxidation, antidiabetes, anti-inflammation, and anticancer. Individual ginsenosides may have different effects in pharmacology and mechanisms because of their different chemical structures [4].

IH-901 is an intestinal metabolite of protopanaxadiol-type ginseng saponins after oral administration and is major form of protopanaxadiol saponins absorbed in the body (Fig. 1A). Recently, IH-901 has received increasing attention because

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Fig. 1. Effects of IH-901 on glucose uptake and expressions of AMPK and GLUT4 in C2C12 myotubes. A, Chemical structure of IH-901. B, IH-901 stimulates glucose uptake. C2C12 myotubes were incubated in 24-well for 3 hours with either IH-901 (5, 20 μ mol/L) or insulin (100 nmol/L) and assayed for glucose uptake. C and D, Concentration- and time-dependent phosphorylations of AMPK, ACC, and GLUT4. E and F, Glucose uptake and phosphorylations of AMPK and ACC were determined in the presence of a selective AMPK inhibitor, compound C (CC). ***P* < .01 compared with the control value.

various pharmacologic actions including anticancer, antiinflammation, and antidiabetes were shown to be mediated by this compound [5-9]. However, there are no reports documenting that IH-901 enhances the glucose uptake through overexpression of glucose transporter in C2C12 myotubes and C57BL/ksJ db/db mice via the adenosine monophosphate-activated protein kinase (AMPK) and phosphatidylinositol-3 kinase (PI3K) pathways. In the present study, we found that antidiabetic and antihyperlipidemic activities of IH-901 were due to enhancing glucose uptake through up-regulated glucose transporter and inhibiting lipogenesis in C2C12 myotubes and liver tissue, respectively, and preserved the architecture of the pancreas islets in C57BL/ksJ db/db mice via activating AMPK/ acetyl-coenzyme A (CoA) carboxylase (ACC) and PI3K/ Akt pathways.

2. Materials and methods

2.1. Chemical

IH-901 was obtained from the Central Research Center of ILHWA Pharmaceutical (Guri, Korea). The chemical identity and purity of IH-901 (>99%) were confirmed by proton nuclear magnetic resonance and high-performance liquid chromatography analysis, respectively. IH-901 was dissolved in 0.1% dimethyl sulfoxide (Sigma-Aldrich, St Louis, MO) for in vitro study and 1% Tween 80 (Yakuri Pure Chemicals, Kyoto, Japan) for in vivo study. Compound C (AMPK inhibitor) and LY294002 (PI3K inhibitor) were purchased from Calbiochem (Darmstadt, Germany) and Promega (Madison, WI), respectively. Other reagents were of the highest purity commercially available.

2.2. Cell culture

Skeletal muscle C2C12 myoblasts were bought from Korean Cell Line Bank (Seoul, Korea) and maintained in Dulbecco modified Eagle media (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C with 95% air and 5% CO₂. To induce differentiation of C2C12 myoblasts, media were replaced with DMEM containing 1% FBS when they reached to confluence. Experiments were performed in differentiated C2C12 myotubes after 7 days in 1% FBS/DMEM.

2.3. Cell viability assay

The cytotoxicity of IH-901 was determined by CellTiter 96 AQ_{ueous} One Solution Cell Proliferation Assay kit bought from Promega. C2C12 myotubes were incubated with IH-901 for 24 hours, and the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt reagent was added and incubated at 37°C for 1 hour. At the end of incubation period, absorbance was recorded at 490 nm.

2.4. Glucose uptake assay

C2C12 myotubes were cultured on 12-well plate, washed with Krebs-Ringer phosphate buffer (25 mmol/L HEPES, pH 7.4, 118 mmol/L NaCl, 4.8 mmol/L KCl, 1.3 mmol/L CaCl₂, 1.2 mmol/L KH₂PO₄, 1.3 mmol/L MgSO₄, 5 mmol/L NaHCO₃, 0.07% bovine serum albumin, and 5.5 mmol/L glucose), and incubated in serumfree media for 3 hours. After treatment with IH-901 for 3 hours, cells were incubated in Krebs-Ringer phosphate buffer containing 0.5 μ Ci of 2-deoxy-D-[³H] glucose for 20 minutes. The reaction was terminated by placing the plates on ice and adding ice-cold phosphate-buffered saline. After washing 3 times with phosphate-buffered saline, cells were dissolved in 0.1% sodium dodecyl sulfate; and trace activities were determined by liquid scintillation counter.

2.5. Animal treatment

Twelve-week-old C57BL/KsJ *db/db* mice (ORIENT BIO, Seoul, Korea) were used in this experiment. Experi-

mental protocol was approved by the Institutional Animal Ethics Committee of the Kyung Hee University. They were acclimatized for 2 weeks before being randomly assigned into the experimental groups. The animals were housed in a room with a 12-hour/12-hour light/dark cycle, a temperature of $24^{\circ}C \pm 1^{\circ}C$, and a humidity of $50\% \pm 5\%$. During the acclimatization period, animals were fed standard rodent chow (LabDiet, Richmond, VA) and water ad libitum. Mice were randomly divided into 3 groups—diabetic control group (DC), group treated with 10 mg/kg of IH-901 (IH-901L), and group treated with 25 mg/kg of IH-901 (IH-901H)—and IH-901 was orally administered once a day for 6 weeks.

2.6. Biochemical analysis

At the end of treatment, mice were anaesthetized by diethyl ether; and blood samples were collected by cardiac puncture before killing. Blood samples were centrifuged at 3000g for 15 minutes at 4°C; and plasma glucose, insulin, triglyceride (TG), total cholesterol (TC), and nonesterified fatty acid (NEFA) levels were measured. Plasma glucose and insulin concentrations were determined using an enzymatic GOD-PAP (glucose oxidase peroxidase) diagnostic kit (Asan Pharmaceutical, Seoul, Korea) and a mouse insulin enzyme immunoassay kit (Shibayagi, Gunma, Japan), respectively. Plasma TG and TC concentrations were determined using commercially available kits (Asan Pharmaceutical). Plasma NEFA levels were determined using an enzymatic colorimetric method (Eiken, Tokyo, Japan).

2.7. Western blot analysis

Total protein extracts were prepared using a protein extraction kit (Intron Biotechnology, Seoul, Korea), and insoluble protein was removed by centrifugation at 15 000g for 20 minutes. Protein concentrations were measured using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). For Western blotting, 40 μ g of protein was electroblotted onto a nitrocellulose membrane after separation on an 8% sodium dodecyl sulfate polyacryl-amide gel electrophoresis. Blotted membranes were incubated for 1 hour with blocking solution (Tris-buffered

Table 1

The	sequences	of primers	used for PCR	
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Gene	Forward primer (from 5' to 3')	Reverse primer (from 5' to 3')				
mSREBP1a	GCGCTACCGGTCTTCTATCA	TGCTGCCAAAAGACAAGGG				
mFAS	GATCCTGGAACGAGAACAC	AGACTGTGGAACACGGTGGT				
mSCD1	CGAGGGTTGGTTGTTGATCTGT	ATAGCACTGTTGGCCCTGGA				
mCD36	TCCTCTGACATTTGCAGGTCTATC	GTGAATCCAGTTATGGGTTCCAC				
mPPARα	CCCTGAACATCGAGTGTCGA	CTTGCCCAGAGATTTGAGGTCCT				
mGLUT4	CAACGTGGCTGGGTAGGCA	ACACATCAGCCCAGCCGGT				
mCPN	ATG GTC AAC CCC ACC GTG	TTA GAG TTG TCC ACA GTC GGA GA				

saline/Tween 20 [TBST]) containing 5% skin milk (wt/ vol) at room temperature, followed by incubation overnight at 4°C with 1:2000 diluted AMPK, pAMPK, ACC, pACC, glucose transporter 4 (GLUT4), PI3K, and pAkt primary antibodies (Cell Signaling, Beverly, MA). Membranes were washed 4 times with 0.1% TBST and incubated with 1:3000 diluted horseradish peroxidase– conjugated goat anti-rabbit or donkey anti-rabbit immunoglobulin G secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hour at room temperature. Membranes were washed 4 times in 0.1% TBST and developed by enhanced chemiluminescent solution (Amersham, Uppsala, Sweden).

2.8. Reserve transcription-polymerase chain reaction

Total messenger RNA was isolated using an Easy-Blue kit (Intron Biotechnology) according to the manufacturer's instructions. From each sample, total RNA (10 μ g) was reverse transcribed into complementary DNA using the Moloney murine leukemia virus transcriptase and oligo (15) dT primers. The complementary DNA fragment was amplified by polymerase chain reaction (PCR) using specific primers. The sequences of primers used for PCR are shown in Table 1. The PCR was performed at 95°C for 30 seconds, followed by 51°C (mCD36), 55°C (mFAS), or 57°C (mSREBP1a, mSCD1, mPPAR-a, mGLUT4, mCPN) for 30 seconds and 72°C for 1 minute. The last cycle was followed by a final extension step at 72°C for 10 minutes. The reverse transcriptase-PCR products were electrophoresed in 1% agarose gels under 100 V and stained with 0.5 μ g/ mL ethidium bromide. Scanning densitometry was performed with I-MAX Gel Image analysis system (Core-Bio, Seoul, Korea).

2.9. Histologic analysis

For hematoxylin-eosin (HE) staining, the pancreas and liver were removed and fixed in 10% neutral buffered formalin. The tissues were subsequently embedded in paraffin, sectioned with 5- μ m thickness (Leica, Wetzlar, Germany), and stained with HE for microscopic assessment (Olympus, Tokyo, Japan). For Oil Red O staining, frozen liver tissue was cut into small cubes and embedded using the Optimal Cutting Temperature Compound (Sakura Finetechnical, Tokyo, Japan). Cryosection was performed at -25° C where the embedded tissues were sectioned into $15-\mu$ m slices and mounted on glass slides. To detect neutral lipid accumulation, sections were stained with Oil Red O and hematoxylin for microscopic assessment.

2.10. Statistical analysis

Data are expressed as mean values \pm SE, and differences between groups were analyzed using Student *t* test. Mean values were considered significantly different when P < .05.

3. Results

3.1. IH-901 increases glucose uptake in C2C12 myotubes by activating AMPK

Effects of IH-901 on glucose uptake and glucose transporter (GLUT4) expression were investigated in differentiated C2C12 myotubes. IH-901 stimulated 2-deoxy-D- $[^{3}H]$ -glucose uptake at a concentration of 20 μ mol/L as



Fig. 2. Effects of IH-901 on expressions of p85-PI3K and pAkt in C2C12 myotubes. A, C2C12 myotubes were treated with 20 μ mol/L of IH-901 for indicated period, and phosphorylated forms of PI3K and Akt were measured. B, C2C12 myotubes were preincubated with 30 μ mol/L of LY294002 (LY), a selective PI3K inhibitor, for 30 minutes and treated with 20 μ mol/L of IH-901 for 3 hours; and phosphorylated forms of PI3K and Akt were determined by the Western blot. C, In the same condition, glucose uptake was measured as described in "Materials and methods." The results represent the mean ± SE. **P < .01 and ***P < .001 compared with the control value.

shown in Fig. 1B. Next, we examined the protein expression levels of phosphorylated AMPK and ACC at indicated concentrations of IH-901 for 3 hours (Fig. 1C) or for indicated times at 20 μ mol/L of IH-901 (Fig. 1D). IH-901 markedly stimulated the phosphorylations of AMPK and ACC in concentration- and time-dependent manners. The GLUT4 protein expression was also increased and reached the maximum levels at 60 minutes when treated with IH-901. To confirm whether activated AMPK is responsible for the effect of IH-901 on glucose uptake, we attempted to inhibit AMPK by a pharmacologic approach. Pretreatment of C2C12 myotubes with compound C, an AMPK inhibitor, significantly attenuated the IH-901–induced glucose uptake, and AMPK and ACC phosphorylations (Fig. 1E and F).

3.2. IH-901 increases glucose uptake in C2C12 myotubes by activating PI3K-Akt pathway

Because the PI3K-Akt pathway is considered as a main signal for glucose uptake, we examined the effects of IH-901 on PI3K-Akt pathway. The phosphorylation levels of PI3K and Akt were determined by immunoblotting p85-PI3K and pAkt antibody. Compared with the basal level, IH-901 markedly stimulated the phosphorylations of PI3K and Akt in a time-dependent manner (Fig. 2A). Next, to confirm whether IH-901 activates PI3K-Akt pathway, we attempted to inhibit PI3K and Akt by a pharmacologic approach. Pretreatment of C2C12 myotubes with LY294002, a selective PI3K inhibitor, significantly attenuated IH-901–induced PI3K and Akt phosphorylations (Fig. 2B) and glucose uptake (Fig. 2C).

3.3. Effect of IH-901 on plasma parameters in db/db mice

Table 2 shows the effects of IH-901 on metabolic parameters in diabetic *db/db* mice treated for 6 weeks. To evaluate the potential role of IH-901 in glucose homeostasis, fasting blood glucose and insulin levels were measured at the end of experiment. Plasma glucose levels were significantly decreased by 15.4% (P < .001) in IH-901L and 20.7% (P < .001) in IH-901H when compared with diabetic control, and this blood glucose–lowering effect was accompanied by increase in plasma insulin levels. Plasma insulin levels were markedly increased by 2.2-fold (P < .01) in IH-901L and 3.4-fold (P < .001) in IH-901H compared with diabetic control. Plasma TG levels in IH-901L and IH-901H groups were decreased by 16.8% (P < .05) and 41.6% (P < .001),

respectively, when compared with control group. The TC levels were also significantly decreased by 13.1% (P < .05) in IH-901L and 20.2% (P < .05) in IH-901H compared with control group. Elevated NEFA inhibits insulin's ability to promote peripheral glucose uptake into muscle and fat to reduce hepatic glucose production. Plasma NEFA levels in IH-901L and IH-901H groups were decreased by 19.2% (P < .01) and 24.6% (P < .001) when compared with control group. These results suggested that IH-901 caused a positive influence on glucose and lipid homeostases.

3.4. Effect of IH-901 on AMPK and SREBP1a target gene expressions in the liver and skeletal muscle

Adenosine monophosphate-activated protein kinase is a cellular fuel gauge and key regulator of both glucose and lipid metabolism. To confirm in vitro study results, we attempted to determine the effect of IH-901 on AMPK and GLUT4 expression in liver and skeletal muscles. As shown in Fig. 3, IH-901 markedly stimulated the phosphorylations of AMPK and ACC in the liver (Fig. 3A) and skeletal muscle (Fig. 3B). The GLUT4 protein and gene expressions in skeletal muscle were also increased in IH-901-treated groups (Fig. 3B, D). Of note, AMPK activation reduces expression of sterol regulatory element-binding protein (SREBP), transcription factor playing a key role in the transcriptional regulation of lipogenic and lipolytic genes. IH-901 significantly reduced the expressions of SREBP1a and its target genes such as fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1), and glycerol-3-phosphate acyltransferase (GPAT) in the liver of C57BL/ksJ db/db mice. In contrast, IH-901 markedly increased the expressions of lipolytic genes such as peroxisome proliferator-activated receptor- α (PPAR- α) and CD36 (Fig. 3C). These results suggested that IH-901 activates AMPK pathway to increase hepatic glucose utilization and fatty acid oxidation, and increases glucose uptake in the skeletal muscle.

3.5. Histologic analysis

Hematoxylin-eosin or Oil Red O staining was performed to compare morphology of liver and pancreas tissues between IH-901-treated and untreated groups. The untreated group (diabetic control) exhibited lots of lipid droplets and destructed pancreatic islets in the liver and pancreas, respectively (Fig. 4). However, the IH-901-treated groups

Table 2 Effects of IH-901 on diabetes-related plasma biomarkers in C57BL/KsJ *db/db* mice

	Glucose (mg/dL)	Insulin (ng/mL)	TG (mg/dL)	TC (mg/dL)	NEFA (µEq/L)
DC	629 ± 9	3.8 ± 0.7	197 ± 10	84 ± 10	$1812 \pm 62 \\ 1464 \pm 58^{\dagger} \\ 1366 \pm 31^{\ddagger}$
IH-901L	$532 \pm 18^{\ddagger}$	$8.4 \pm 1.3^{\dagger}$	$164 \pm 10^{*}$	73 ± 3*	
IH-901H	$499 \pm 21^{\ddagger}$	$13.1 \pm 0.6^{\ddagger}$	$115 \pm 7^{*}$	67 ± 1*	

Values represent mean \pm SE (n = 4).

* P < .05 vs DC.

[†] P < .01 vs DC.

[‡] P < .001 vs DC.



Fig. 3. Effects of IH-901 on AMPK and GLUT4 protein expressions or SREBP1a and GLUT4 gene expressions in the liver and skeletal muscle of db/db mice. At the end of treatment, AMPK, ACC, and GLUT4 protein expressions in the liver (A) and skeletal muscle (B) were measured by Western blot as described in "Materials and methods." Lipogenic and lipolytic gene expressions in the liver (C) and GLUT4 gene expression in the skeletal muscle (D) were measured by reverse transcriptase–PCR as described in "Materials and methods."

showed few lipid droplets and preserved architecture of islets when compared with the untreated group.

4. Discussion

Skeletal muscle is a major organ contributing toward whole-body energy homeostasis and is responsible for up to

80% of glucose uptake after a meal [10]. In a healthy, lean, and metabolically fit individual, skeletal muscle is able to switch between insulin-dependent glucose use after a meal and fatty acid use after a sustained fast. In a state of insulin resistance, glucose uptake and utilization are dramatically decreased and skeletal muscle becomes metabolically inflexible, unable to switch between glucose and fatty acid use [11,12]. In skeletal muscle of obese and type 2 DM subjects, defects in glycogen synthase activation, Akt activation, and insulin receptor substrate phosphorylation have been documented [13-16].

Recently, Kim et al [17] reported that ginsenoside Rg3 increased glucose uptake both in the basal and insulininduced states of L6 myotubes. Consistent with the increase in glucose uptake, Rg3 stimulated the phosphorylation of insulin receptor substrate-1 and Akt. However, they found that this effect of Rg3 on insulin signaling was not mediated by the AMPK pathway [17]. In the mean time, our data show a significant increase in glucose uptake by IH-901 via the AMPK pathway without insulin (Fig. 1). Adenosine monophosphate-activated protein kinase is an important energy sensor in mammalian cells. In skeletal muscle cells, AMPK may be activated by contraction or 5-aminoimidazole-4-carboxamide-1-b-D-ribofuranoside (AICAR), leading to increase in glucose uptake [18,19]. Metformin and thiazolidinediones (rosiglitazone and pioglitazone) have been shown to increase glucose uptake via AMPK [20,21]. The present study showed a rapid and significant increase in AMPK phosphorylation by IH-901 consistent with our recent study in hepatoma cells [22]. Using compound C, a selective AMPK inhibitor, we demonstrated a significant reduction in IH-901-stimulated glucose uptake (Fig. 1E), providing support of the notion that AMPK is a mediator of the IH-901 effects on glucose uptake. In addition, IH-901 also activated PI3K-Akt pathway; and this effect was abolished in the presence of LY294002, a selective PI3K inhibitor (Fig. 2). Insulin enhances the glucose uptake by activating the insulin receptor that stimulates PI3K/Akt pathway leading to inhibition of the vesicle translocation suppressor TBC1D4/AS160 and membrane translocation of glucose transporter GLUT4 [23-25]. Impaired activation of this pathway is a hallmark of obesityassociated insulin resistance and contributes to glucose intolerance and hyperglycemia [26-28]. Accordingly, induction of glucose uptake is probably ascribed to translocation of GLUT4 to the plasma membrane by insulin-like action of IH-901 (Fig. 1D). However, the cellular mechanism through which AMPK activation leads to increased glucose uptake in response to IH-901 is unclear at this time. AICAR increased glucose uptake but not GLUT4 translocation to surface area of skeletal muscle [29], raising the possibility that AMPK may regulate glucose transporter activity. Interestingly, we observed that IH-901 stimulated glucose uptake with parallel increase in GLUT4 translocation to the plasma membrane possibly via activating PI3K/Akt and AMPK pathways.



Fig. 4. Microscopic views of the liver and pancreas obtained from untreated (DC) and treated (IH-901L and IH-901H) mice. First and third lanes denote HE staining, and second lane denotes oil red O and hematoxylin double staining. Magnification × 200.

Hepatic metabolism plays a key role in the control of whole-body energy status because liver is the major site for storage and release of carbohydrates and for the synthesis of fatty acid. In the liver, AMPK coordinates the changes in the activity of enzymes of the lipid metabolism and regulates the partitioning of fatty acids between oxidative and biosynthetic pathways [30,31]. Acetyl-CoA carboxylase is an important rate-controlling enzyme for the synthesis of malonyl-CoA, which is both a critical precursor for biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation. Phosphorylation and inhibition of ACC by AMPK lead to a fall in malonyl-CoA content and a subsequent decrease in TG synthesis concomitant with an increase in β -oxidation [32]. This was evidenced by the decrease in plasma TG levels during IH-901 administration in db/db mice (Table 2). Although the action of AMPK in systemic energy balance is achieved by rapid and direct phosphorylation of metabolic enzymes, long-term effects have also been clearly demonstrated on gene expression. It is now clearly established that AMPK plays an important role in the repression of glycolytic and lipogenic gene expression in the liver. The intracellular and membrane levels of fatty acids and cholesterol are under constant surveillance coordinated with de novo lipid biosynthesis controlled by ER-bound SREBPs [33]. Recently Kim et al [22] evaluated gene

expression of SREBP1c, a transcriptional factor for TG biosynthesis in HepG2 cells. Gene expressions of SREBP1a and its target proteins, such as SCD1 and FAS, were decreased; but gene expressions of PPAR- α and CD36, a transcriptional regulator for lipid uptake and catabolism in the liver, were markedly enhanced. In addition, IH-901induced decrease in SREBP1c and increases in PPAR-a and CD36 gene expressions were reversed by treatment of compound C. These in vitro data were confirmed by in vivo experiment showing decreased TG deposition in the liver (Fig. 3C and 4). These observations suggest that IH-901 regulates lipogenic and lipolytic gene expressions via the AMPK cascade. These results are consistent with ex vivo findings demonstrating the AICAR-induced inhibition of mitochondrial GPAT activity and subsequent inhibition of triacylglycerol synthesis [34]. In addition, overexpression of AMPK α 2 in the liver decreases plasma TG levels and increases plasma ketone bodies levels, a surrogate marker for hepatic β -oxidation [35]. Conversely, liver-specific AMPKa2 deletion leads to increased plasma TG levels and reduction in plasma ketone bodies levels. This emphasizes the critical role for AMPK α 2 subunit in the control of the balance between hepatic lipogenesis and β -oxidation.

It is known that 25% of all drugs prescribed today come from plants. This estimate suggests that plant-derived drugs

make up a significant segment of natural product-based pharmaceuticals. This representation of natural productderived drugs begs the question of whether plant secondary metabolites and related synthetic compounds perform better as drugs than randomly synthesized compounds. An argument favoring plant-derived drugs is that they provide multiple actions that can simultaneously target various elements of human diseases, providing efficacy and safety unmatched by new chemical entities. This multitargeted approach of IH-901 may provide a valuable alternative to the therapeutic agent designed to work with one symptom at a time. Taken together, having beneficial effects on glucose and lipid metabolisms through activating AMPK and PI3K pathways, IH-901 has a potential as a therapeutic agent for hyperglycemic and hyperlipidemic patients.

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References

- Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782-7.
- [2] Donath MY, Halban PA. Decreased beta-cell mass in diabetes; significance, mechanisms and therapeutic implications. Diabetologia 2004;47:581-9.
- [3] Krentz AJ, Bailey CJ. Oral antidiabetic agents: current role in type 2 diabetes mellitus. Drugs 2005;65:385-411.
- [4] Lu JM, Yao Q, Chen C. Ginseng compounds: an update on their molecular mechanisms and medical applications. Curr Vasc Pharmacol 2009;7:293-302.
- [5] Wakabayashi C, Murakami K, Hasegawa H, Murata HJ, Saiki I. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumor cells. Biochem Biophys Res Commun 1998;246:725-30.
- [6] Lee SJ, Ko WG, Kim JH, Sung JH, Moon CK, Lee BH. Induction of apoptosis by a novel intestinal metabolite of ginseng saponin via cytochrome c-mediated activation of caspase-3 protease. Biochem Pharmacol 2000;60:677-85.
- [7] Choi K, Kim M, Rhy J, Choi C. Ginsenosides compound K and Rh (2) inhibit tumor necrosis factor-alpha-induced activation of the NFkappaB and JNK pathways in human astroglial cells. Neurosci Lett 2007;421:37-41.
- [8] Yoon SH, Han EJ, Sung JH, Chung SH. Anti-diabetic effects of compound K versus metformin versus compound K-metformin combination therapy in diabetic *db/db* mice. Biol. Pharm. Bull 2007;30:2196-200.
- [9] Han GC, Ko SK, Sung JH, Chung SH. Compound K enhances insulin secretion with beneficial metabolic effects in *db/db* mice. J Agric Food Chem 2007;55:10641-8.
- [10] Ryder E, Gómez ME, Fernández V, Campos G, Morales LM, Valbuena H, et al. Glucose/insulin response to a glucose overload in subjects at risk for type 2 diabetes. Invest Clin 2002;43:53.
- [11] Simoneau JA, Colberg SR, Thaete FL, Kelley DE. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. FASEB J 1995;9:273-8.
- [12] Kelley DE, Goodpaster B, Wing RR, Simoneau JA. Skeletal muscle fatty acid metabolism in association with insulin resistance, obesity, and weight loss. Am J Physiol 1999;277:1130-41.

- [13] Wright J. Effect of high-carbohydrate versus high-monounsaturated fatty acid diet on metabolic control in diabetes and hyperglycemic patients. Clin Nutr 1998;17:35-45.
- [14] Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB. Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. J Clin Invest 1999;104:733-41.
- [15] Goodyear LJ, Giorgino F, Sherman LA, Carey J, Smith RJ, Dohm GL. Insulin receptor phosphorylation, insulin receptor substrate-1 phosphorylation, and phosphatidylinositol 3-kinase activity are decreased in intact skeletal muscle strips from obese subjects. J Clin Invest 1995;95:2195-204.
- [16] Björnholm M, Kawano Y, Lehtihet M, Zierath JR. Insulin receptor substrate-1 phosphorylation and phosphatidylinositol 3-kinase activity in skeletal muscle from NIDDM subjects after in vivo insulin stimulation. Diabetes 1997;46:524-7.
- [17] Kim M, Ahn BY, Lee JS, Chung SS, Park SG, Jung HS, et al. The ginsenoside Rg3 has a stimulatory effect on insulin signaling in L6 myotubes. Biochem Bioph Res Co 2009;389:70-3.
- [18] Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 2007;100:328-41.
- [19] Musi N, Goodyear LJ. AMP-activated protein kinase and muscle glucose uptake. Acta Physiol Sacnd 2003;178:337-45.
- [20] Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167-74.
- [21] Fryer LG, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002;277: 25226-32.
- [22] Kim DY, Yuan HD, Chung IK, Chung SH. Compound K, intestinal metabolite of ginsenoside, attenuates hepatic lipid accumulation via AMPK activation in human hepatoma cells. J Agric Food Chem 2009;57:1532-7.
- [23] Cartee GD, Wojtaszewski JF. Role of Akt substrate of 160 kDa in insulin stimulated and contraction-stimulated glucose transport. Appl Physiol Nutr Metab 2007;32:557-66.
- [24] Ishiki M, Klip A. Minireview: recent developments in the regulation of glucose transporter–4 traffic: new signals, locations, and partners. Endocrinology 2005;146:5071-8.
- [25] Zierath JR, Krook A, Wallberg-Henriksson H. Insulin action and insulin resistance in human skeletal muscle. Diabetologia 2000;43: 821-35.
- [26] Biddinger SB, Kahn CR. From mice to men: insights into the insulin resistance syndromes. Annu Rev Physiol 2006;68:123-58.
- [27] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-7.
- [28] Steinberg GR, Jorgensen SB. The AMP-activated protein kinase: role in regulation of skeletal muscle metabolism and insulin sensitivity. Mini Rev Med Chem 2007;7:519-26.
- [29] Lemieux K, Konrad D, Klip A, Marette A. The AMP-activated protein kinase activator AICAR does not induce GLUT4 translocation to transverse tubules but stimulates glucose uptake and p38 mitogenactivated protein kinase alpha and beta in skeletal muscle. FASEB J 2003;17:1658-65.
- [30] Viollet B, Foretz M, Guigas B, Horman S, Dentin R, Bertrand L, et al. Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. J Physiol 2006;574:41-53.
- [31] Brusq JM, Ancellin N, Grondin P, Guillard R, Martin S, Saintillan Y, et al. Inhibition of lipid synthesis through activation of AMP kinase: an additional mechanism for the hypolipidemic effects of berberine. J Lipid Res 2006;47:1281-8.
- [32] Velasco G, Geelen MJ, Guzmán M. Control of hepatic fatty acid oxidation by 5'-AMP-activated protein kinase involves a malonyl-CoA-dependent and a malonyl-CoA-independent mechanism. Arch Biochem Biophys 1997;337:169-75.

- [33] Eberle D, Heqarty B, Bossard P, Ferre P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie 2004;86:839-48.
- [34] Muoio DM, Seefeld K, Witters LA, Coleman RA. AMP-activated kinase reciprocally regulates triacylglycerol synthesis and fatty acid oxidation in liver and muscle: evidence that sn-glycerol-3-

phosphate acyltransferase is a novel target. Biochem J 1999;338: 783-91.

[35] Foretz M, Ancellin N, Andreelli F, Saintillan Y, Grondin P, Kahn A, et al. Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. Diabetes 2005;54:1331-9.