MOLECULAR MEDICINE REPORTS 16: 1333-1339, 2017

Protective effects of asiatic acid in a spontaneous type 2 diabetic mouse model

WEN SUN[1*], GUANGYUAN XU[2*], XUAN GUO[2], GUANGBIN LUO[3], LILI WU[1], YI HOU[2], XIANGYU GUO[2], JINGXIN ZHOU[4], TUNHAI XU[5], LINGLING QIN[6], YIXIN FAN[6], LI HAN[1], MOTLALEPULA MATSABISA[7], XUESHENG MA[7] and TONGHUA LIU[1]

[1]Key Laboratory of Health Cultivation of the Ministry of Education, Beijing University of Chinese Medicine, Beijing 100029; [2]Department of Endocrinology, Dongfang Hospital of Beijing University of Chinese Medicine, Beijing 100078; [3]School of Basic Medical Sciences, Beijing University of Chinese Medicine, Beijing 100029; [4]Endocrinology Department, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 101100; [5]School of Chinese Pharmacy, Beijing University of Chinese Medicine; [6]Department of Science and Technology, Beijing University of Chinese Medicine, Beijing 100029, P.R. China; [7]School of Natural Medicine, University of The Western Cape, Cape Town 7535, South Africa

Received November 8, 2016; Accepted May 26, 2017

DOI: 10.3892/mmr.2017.6684

Abstract. Asiatic acid (AA) has been demonstrated to exhibit anti-diabetic activity. However, the mechanisms and underlying signaling pathways remain to be elucidated. The present study was performed to confirm the protective effect of AA and demonstrate its ability to regulate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/glycogen synthase kinase-3β (GSK-3β) signaling pathway in db/db mice. Db/db mice fed on a high-fat diet were used to model diabetes mellitus. Modeled mice were divided randomly into the model control, pioglitazone hydrochloride tablet (PH) and AA groups. Age-matched C57 BL/6J mice served as normal controls. Lipid and glucose levels, and glycogen synthesis rates were assessed following treatment. Pathological changes were detected using hematoxylin and eosin staining. Expression of the PI3K/AKT/GSK-3β signaling pathway at the mRNA level was measured using quantitative polymerase chain reaction analysis. The model control group revealed typical characteristics of obesity and diabetes, including high glucose and lipid levels, and decreased glycogen synthesis. Four weeks of treatment with AA or PH ameliorated these abnormalities. AA and PH treatments mitigated the upregulation of PI3K, AKT, insulin receptor, and insulin receptor substrate-1 mRNA expression in modeled mice. Furthermore, AA and PH treatments decreased GSK-3β and glucose-6-phosphatase mRNA expression compared with the normal control group. The results of the present study confirmed that AA possesses anti-diabetic activity in db/db mice. The PI3K/AKT/GSK-3β signaling pathway may mediate this protective effect.

Correspondence to: Dr Tonghua Liu, Key Laboratory of Health Cultivation of the Ministry of Education, Beijing University of Chinese Medicine, 11 North Third Ring Road East Road, Chaoyang, Beijing 100029, P.R. China
E-mail: thliu@vip.163.com

[*]Contributed equally

Key words: asiatic acid, phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase-3β, spontaneous type 2 diabetic model

Introduction

Diabetes mellitus is among the most common chronic metabolism diseases, and its incidence continues to increase. Epidemiologically, 250 million patients are diagnosed with diabetes annually, and this number is projected to increase to about 380 million by 2025 (1). Diabetes mellitus is divided into two subgroups: type 1 diabetes mellitus (T1DM) and types 2 diabetes mellitus (T2DM). T1DM is an autoimmune disease characterized by destruction of islet beta cells in the pancreas, which results in the complete cessation of insulin production (2). By contrast, T2DM is a heterogeneous disorder caused by a progressive decline in insulin resistance in the liver and peripheral tissues, accompanied by the inability of beta cells to compensate for insulin resistance (3). Abnormality of glucose metabolism is the central characteristic of diabetes mellitus. Accordingly, the pathogenesis of glucose metabolism is a major topic of research in this field.

The key enzymes, glucose-6-phosphatase (G-6-P) and glycogen synthase kinase-3 (GSK-3) regulate glycogen and gluconeogenesis. G-6-P is the rate-limiting enzyme for the transformation of glucose-6-phosphate to glucose, affecting glycogen output and blood glucose level (4). GSK-3 is the rate-limiting enzyme responsible for gluconeogenesis (5). Two types of GSK-3-GSK-3α and GSK-3β-have been reported to regulate G-6-P expression (6). GSK-3β can phosphorylate glycogen synthase and inhibit the synthesis of glycogen. In turn,

this process leads to the phosphorylation of insulin receptor substrate-1 (IRS-1), interrupting insulin signaling and producing insulin resistance (7). Thus, the regulation of key enzymes involved in the inhibition of glycogen synthesis is a critical step in the search for an effective treatment for diabetes mellitus.

T2DM is a multifactorial disease, with genetic and environmental factors contributing to its development (8). Although western medicine has achieved much improvement in T2DM medications, side effects and high costs continue to pose challenges (9). As a chronic disease, T2DM requires long-term treatment, and side effects such as weight gain, bone loss, and increased cardiovascular risk are unavoidable. Moreover, continuous health care utilization poses huge economic burdens on societies and families. Hence, alternative agents with fewer side effects and lower costs are urgently needed. Herbal medications can be good alternatives, replacing or at least supplementing western medications (10,11). Because most of these medications are extracted from plants, they are characterized by low cost and few side effects. In Chinese medicine, several thousand years of history and experience in the use of herbal medications to prevent and treat T2DM have been documented.

Centella asiatica (*C. asiatica*) has been used widely in Chinese medicine to treat varicose veins and chronic venous insufficiency, and in ointments to treat psoriasis and help heal minor wounds (12). Asiatic acid (AA) is an active component of *C. asiatica* with many biological activities, including anti-oxidant (13), liver-protecting (14), lipid-lowering (15), anticancer (16), and anti-diabetic (17) effects. Mechanical studies have suggested that AA lowers glucose levels through anti-inflammatory action, regulation of glucose metabolism enzymes, and anti-fibrotic action in the pancreatic islets (15,18). However, the signaling pathway involved in the effects of AA on glucose level in diabetes mellitus remains unknown.

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway is important in the regulation of the insulin signaling cascade (19). The activation of PI3K/AKT negatively mediates GSK-3β and affects glycogen synthesis. In this study, a T2DM (db/db) mouse model was used to evaluate the PI3K/AKT/GSK-3β signaling pathway involved in the protective effects of AA on diabetes symptoms.

Materials and methods

Chemicals and reagents. AA (lyophilized powder) was purchased from Chengdu PuRuiFa Technology Development Co. Ltd (Chengdu, China). Pioglitazone hydrochloride (PH) (batch no. Zhunzi H20040631) was purchased from Jiangsu Hengrui Medicine Co., Ltd. (Jiangsu, China). Assay kits for glucose (glucose oxidase), total cholesterol (COD-PAP), triglycerides (TG; GOP-PAP), high-density lipoprotein (HDL) cholesterol (direct hydrogen peroxide scavenging method), and low-density lipoprotein (LDL) cholesterol (direct surfactant removal method), and hematoxylin and eosin (H&E) and glycogen staining kits were obtained from Beijing LEYBOLD Cable Technology Co., Ltd. (Beijing, China). An immunohistochemical kit and insulin receptor (InsR) antibody were obtained from Boosen Biological Technology Co., Ltd. (Beijing, China).

Animal modeling and treatments. Twelve-week-old male db/db mice and age-matched male C57BL/6J mice were provided by the Model Animal Research Center of Nanjing University. This study was approved by the ethics committee of Beijing University of Chinese Medicine. All animals were housed at the Beijing Animal Experimental Center at a temperature of 23±2˚C and humidity of 55±10% with a 12/12-h light/dark cycle. The animals were provided with food and water *ad libitum*. Db/db mice were fed a full-formula high-fat diet (composition: basal diet, cholesterol, egg-yolk powder, lard, bile salt). C57BL/6J mice were fed a common basal diet. After 1 week of feeding, tail blood was collected. A non-random blood glucose concentration >11.1 mmol/l was considered to indicate successful diabetic modeling.

Eighteen modeled mice were divided randomly into control, PH, and AA groups. C57BL/6J mice were used as normal controls and were given a normal diet. The other mice were fed a high-fat diet. PH was used as the positive control. PH (12 mg/kg) and AA (50 mg/kg) were administered orally once per day for 4 consecutive weeks. Saline served as the negative control. The animals' general condition (mental state, activity, hair color) was observed and body weight was recorded each week. After 4 weeks of treatment, the mice were fasted for 12 h. Tail blood was collected for the measurement of blood glucose and performance of oral glucose tolerance test (OGTTs) and insulin tolerance test (ITTs) at 0, 30, 60, and 120 min. Arterial blood was obtained for the measurement of fasting plasma glucose (FPG), carbohydrate (CHO), TG, HDL, LDL, insulin, and free fatty acids (FFAs). Insulin resistance was calculated using the following formula: homeostasis model assessment-estimated insulin resistance (HOMA-IR) index=glucose level x serum insulin level/22.5 (20).

H&E and periodic acid-Schiff staining. Liver tissue fixed in paraformaldehyde was used for H&E and periodic acid-Schiff (PAS) staining. After fixation in 10% neutral formalin, the tissues were embedded in paraffin, sectioned (5-μm slice thickness) and stained with H&E or PAS.

Immunohistochemistry. Fixed liver tissue was cryoprotected in 30% sucrose for 1 h at 4˚C and sectioned at 20-μm intervals with a freezing microtome (Leica, Mannheim, Germany). The sections were then incubated with the primary antibody (1:1,000) at 4˚C overnight. After washout with phosphate-buffered saline, the second antibody was added and incubated for 2 h at room temperature. After 3,3-diaminobenzidine (DAB) colorization, the slices were covered and observed under a microscope (Olympus Corp., Tokyo, Japan). The optical density was analyzed using Image-Pro Plus software.

Quantitative PCR. Total RNA was extracted from liver tissues using TRIzol reagent. RNA concentrations were determined spectrophotometrically, and 1 μg total RNA was reverse transcribed using an avian myeloblastosis virus reverse-transcriptase kit (Promega Corp., Madison, WI, USA). PCR primers were as follows: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5'-TGAAGCAGGCATCTGAGGG-3' (sense), 5'-CGAAGGTGGAAGAGTGGGAG-3' (antisense); InsR: 5'-TTTGTCATGGATGGAGGCTA-3' (sense), 5'-CCT CATCTTGGGGTTGAACT-3' (antisense); IRS-1: 5'-TCCTAT CCCGAAGAGGGTCT-3' (sense), 5'-TGGGCATATAGCCAT CATCA-3' (antisense); PI3K: 5'-GCTCCTGGAAGCCAT

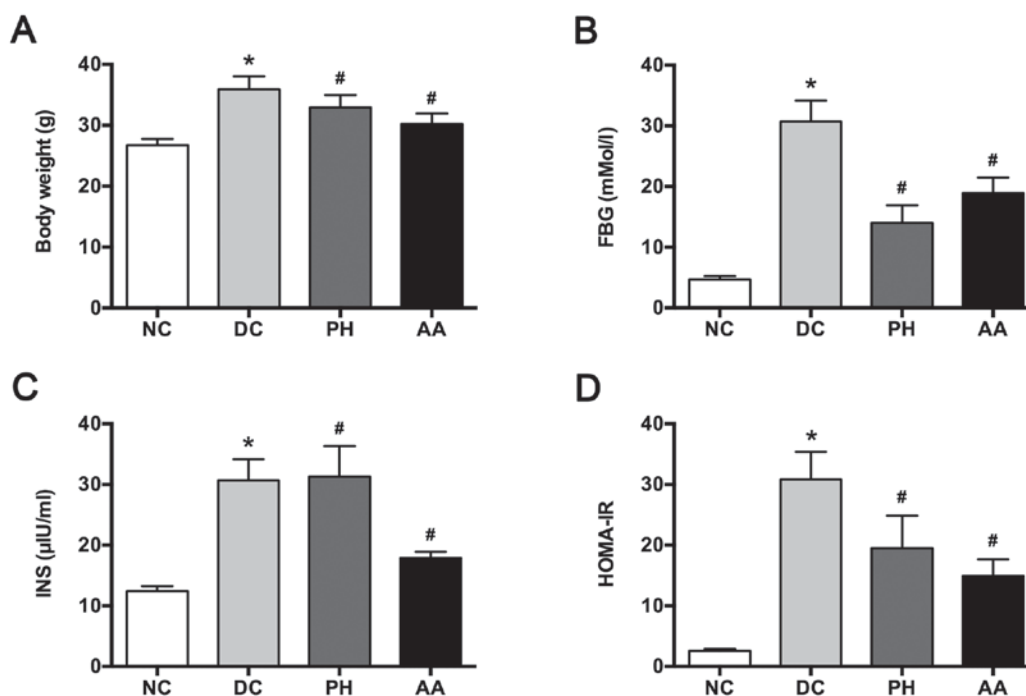

Figure 1. Effects of AA on (A) body weight, (B) FPG, (C) INS, and (D) HOMA-IR. Values are mean ± SD for six mice. *P<0.05 vs. NC, #P<0.05 vs. DC. NC, normal control; DC, diabetic control; PH, pioglitazone hydrochloride tablets; AA, asiatic acid; FPG, fasting plasma glucose; INS, insulin; HOMA-IR, homeostasis model assessment-estimated insulin resistance.

TGAGAA-3' (sense), 5'-CGTCGATCATCTCCAAGTCCAC-3' (antisense); Akt-1: 5'-CCCTTCTACAACCAGGACCA-3' (sense), 5'-ATACACATCCTGCCACACGA-3' (antisense); GSK-3β: 5'-TATTTCCTGGGGACAGTGGT-3' (sense), 5'-ATTTGCTCCCTTGTTGGTGT-3' (antisense); G-6-P: 5'-AGCTCCGTGCCTATAATAAAGCAG-3' (sense), 5'-CATACGTTGGCTTTTTCTTTCCTC-3' (antisense). The amplification reactions were carried out with a 7500 Real-Time PCR system (Applied Biosystems Life Technologies, Foster City, CA, USA), with initial hold steps (50˚C for 2 min, followed by 95˚C for 10 min) and 40 cycles of a two-step PCR (95˚C for 15 sec and 60˚C for 1 min). The comparative computed tomography method was used to determine the amount of target, normalized to an endogenous reference (GAPDH) and relative to a calibrator ($2^{-\Delta\Delta Cq}$).

Statistical analyses. Data are presented as means ± standard deviations. One-way analysis of variance with post hoc Bonferroni tests for multiple comparisons was performed. P<0.05 was considered to indicate a statistically significant difference.

Results

After 4 weeks of treatment, mice in the normal control group displayed normal activity and appearance. In contrast, the activity and appearance of mice in the model control group were abnormal. AA and PH treatments ameliorated those abnormalities. Body weight increased significantly after modeling compared with the normal control (P<0.05), AA and PH treatments decreased body weight significantly (P<0.05; Fig. 1A). The FPG level was elevated in the model group, but this condition was reversed significantly by the AA and PH treatments (Fig. 1B). The increased INS level after modeling was attenuated by AA, but aggravated by PH (Fig. 1C). HOMA-IR values were elevated significantly in the model control group compared with the normal control group (P<0.05; Fig. 1D). PH and AA treatments significantly attenuated this elevation (P<0.05).

OGTT and ITT values were also higher in the model control group than in the normal control group at each time point (P<0.05). AA and PH treatments reversed those abnormalities (P<0.05 *vs*. model group; Fig. 2).

After 4 weeks of treatment, serum CHO, TG, HDL, LDL, and FFA levels were markedly elevated in the model group. AA treatment attenuated CHO, HDL, and FFA levels, and PH treatment attenuated CHO, TG, and FFA levels (P<0.05; Table I).

H&E staining showed that hepatic cells were arranged radially around the central vein in the normal control group (Fig. 3). Cellular structure was normal, with centralized nuclei. In the model control group, H&E staining displayed steatosis in hepatic cells. Moreover, the cells were arranged irregularly. Portal-area inflammation infiltration was observed in the model control group. AA and PH treatments ameliorated these pathological changes, and steatosis disappeared. Hepatic cells were arranged regularly in these groups, and minimal inflammation infiltration was observed in the portal area.

PAS staining revealed a large number of purple glycogen granules in hepatic cells in the normal control group (Fig. 4). In contrast, few such granules were found in the model control group. AA and PH treatments achieved remarkable recovery of glycogen, although the numbers of granules remained smaller than in the normal control (P<0.05).

Liver InsR, detected by immunohistochemical staining, was expressed mainly on cell membranes (Fig. 5). After 4 weeks of treatment, InsR expression was reduced significantly in the model group compared with the normal control.

Table I. Effects of asiatic acid on lipid levels in db/db mice.

Group	n	CHO (mM)	TG (mM)	HDL (mM)	LDL (mM)	FFA (mM)
NC	6	1.80±0.24	0.62±0.13	0.76±0.11	1.03±0.14	0.40±0.01
DC	6	2.40±0.61[a]	1.24±0.21[a]	0.56±0.15[a]	1.55±0.55[a]	0.49±0.04[a]
PH	6	1.86±0.20[b]	0.88±0.26[b]	0.63±0.20	1.32±0.12	0.56±0.06[b]
AA	6	1.67±0.46[b]	1.21±0.27	0.75±0.14[b]	1.53±0.26	0.45±0.01[b]

Data are presented as mean ± standard deviation. [a]P<0.05 vs. NC, [b]P<0.05 vs. DC. NC, normal control; DC, diabetic control; PH, pioglitazone hydrochloride tablet; AA, asiatic acid; CHO, cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; FFA, free fatty acids.

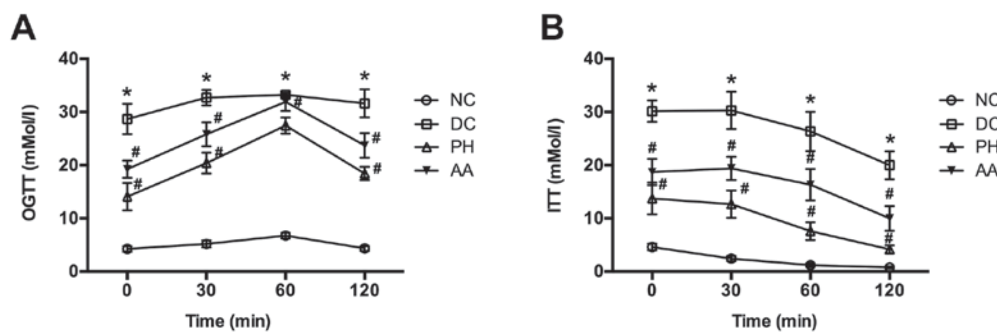

Figure 2. Effects of AA on (A) OGTT and (B) ITT values. Values are mean ± SD for six mice. *P<0.05 vs. NC, #P<0.05 vs. DC. NC, normal control; DC, diabetic control; PH, pioglitazone hydrochloride tablets; AA, asiatic acid; OGTT, oral glucose tolerance test; ITT, insulin tolerance test.

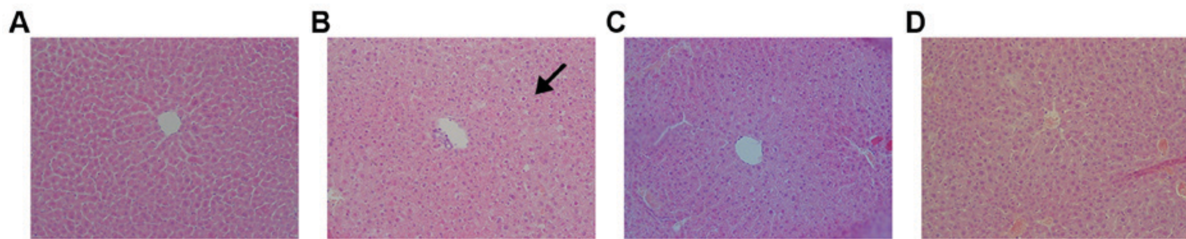

Figure 3. Liver hematoxylin and eosin staining in db/db mice in different groups. (A) Normal control, (B) diabetic control, (C) pioglitazone hydrochloride tablets, and (D) asiatic acid. Magnification, x200.

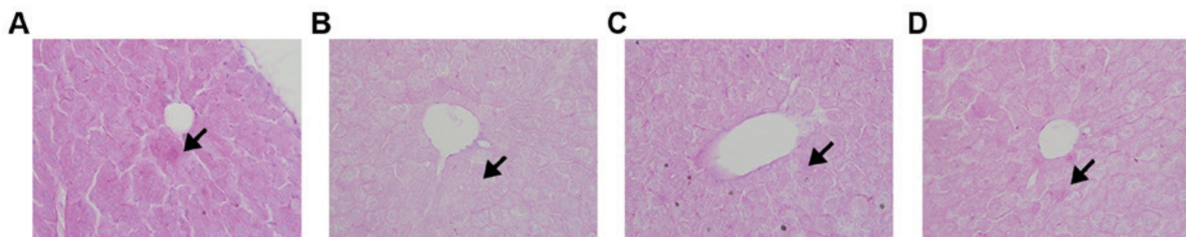

Figure 4. Liver periodic acid-Schiff staining in db/db mice in different groups. (A) Normal control, (B) diabetic control, (C) pioglitazone hydrochloride tablets, and (D) asiatic acid. Magnification, x400.

By contrast, AA and PH treatments significantly elevated InsR expression (P<0.05).

InsR, IRS-1, PI3K, and Akt-1 expressions were increased significantly in the model group compared with the normal control group (P<0.05; Fig. 6). After 4 weeks, AA and PH treatments significantly mitigated the expressions of InsR, IRS-1, PI3K, and Akt-1 (P<0.05). In addition, diabetic modeling elevated G-6-P and GSK3β expression, which was reversed by AA and PH treatments (P<0.05).

Discussion

The db/db mouse model is used widely to study spontaneous T2DM (21). Genetically, leptin gene mutation leads to

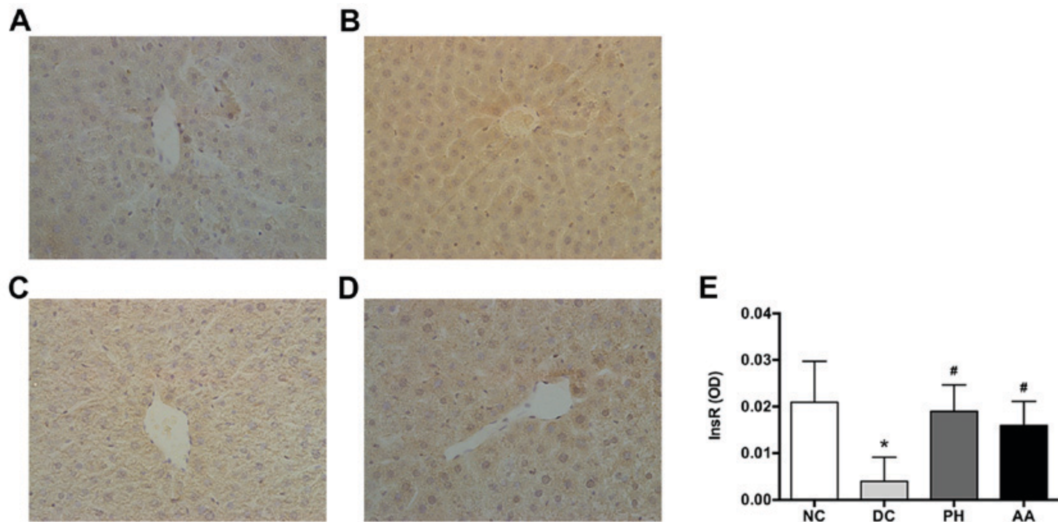

Figure 5. Effect of asiatic acid (AA) on InsR expression. (A) Normal control (NC), (B) diabetic control (DC), (C) pioglitazone hydrochloride tablets (PH), (D) asiatic acid (AA), and (E) quantification of OD. Values are means ± SDs for six mice. *P<0.05 vs. NC, #P<0.05 vs. DC.

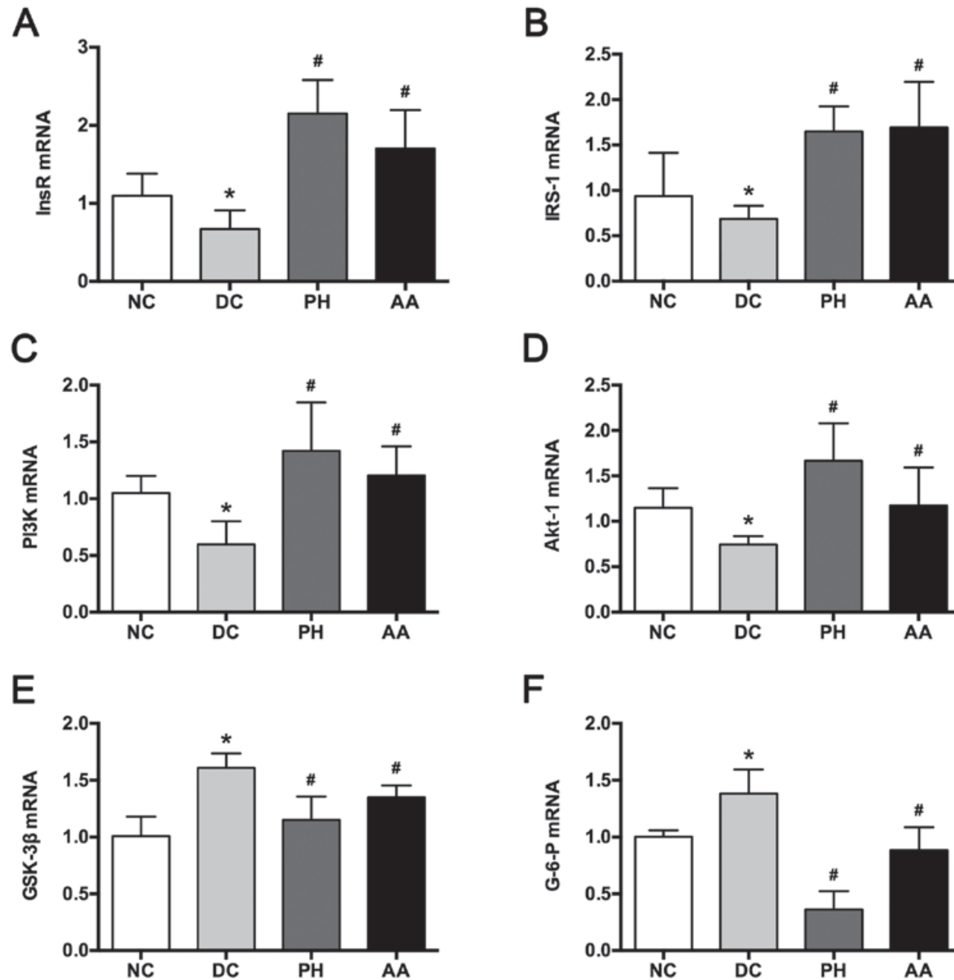

Figure 6. Effects of AA on (A) InsR, (B) IRS-1, (C) PI3K, (D) Akt-1, (E) GSK-3β and (F) G-6-P mRNA expression. Values are mean ± SD for six mice. *P<0.05 vs. NC, #P<0.05 vs. DC. NC, normal control; DC, diabetic control; PH, pioglitazone hydrochloride tablets; AA, asiatic acid; InsR, insulin receptor; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; Akt-1, protein kinase B; GSK-3β, glycogen synthase kinase-3β; G-6-P, glucose-6-phosphatase.

abnormality of the leptin signaling pathway. Major symptoms that appear in this model include obesity, insulin resistance, high glucose levels, and fatty liver, which are always found in 6-week-old and typically displayed in 8-12-week-old mice (22). T2DM is characterized by absolute or relative deficiency of insulin secretion. Previously, AA was reported

to mitigate the symptoms of streptozotocin (STZ)-induced diabetes, possibly through anti-oxidant and anti-inflammatory actions, amelioration of glycogen metabolism, protection of islets, and insulin resistance (23). In the present study, we further demonstrated the protective effects of AA against diabetic symptoms in a genetic model. Moreover, we showed that the PI3K/AKT/GSK-3β signaling pathway plays a role in these protective effects.

Typical symptoms of diabetes were observed in 6-week-old db/db mice fed a high-fat diet, including elevated FPG, INS, HOMA-IR, and glucose levels and ITT values. The protective effects of AA observed in this model provide support for the potential effects of this acid in glucose metabolism. In addition to its support of glucose control, AA also decreased FFA and CHO levels and increased HDL levels. In combination with morphological findings, these results suggest that AA is capable of lowering lipid levels. Ramachandran also reported that AA prevents lipid peroxidation in STZ-induced diabetes (24).

Glucose metabolism is regulated by the insulin signaling pathway. When combined with InsR, insulin initiates the phosphorylation of its intracellular substrates. PI3K serves as a docking protein for InsR. Activation of PI3K and its downstream protein kinase B (Akt) is essential for almost all insulin-induced glucose or lipid metabolism, such as glucose uptake, glycogen synthesis, and suppression of triglyceride synthesis (25,26). GSK-3 differs from other kinases in that it remains active in its dephosphorylated form. It is inactivated by phosphorylation by other protein kinases, such as AKT. Insulin activates Akt to inhibit the activity of GSK-3α or GSK-3β isoforms by phosphorylating their NH_2-terminal serine residues, terminating their inhibition of glycogen synthase (27). GSK-3β is the predominant regulator of glycogen synthase in skeletal muscle (28). In diabetes, GSK-3β level and activity are increased (29). In the present study, we demonstrated for the first time that AA decreased PI3K and AKT expression and regulated GSK-3β expression in diabetes.

G-6-P is a rate-limiting enzyme for gluconeogenesis and glycogen decomposition, which directly affects the hydrolysis of G-6-P to glucose. Consistent with previous reports (30), we showed that G-6-P expression was up-regulated in our diabetic model. Considering the inhibition of G-6-P by PI3K, GSK-3β might be important in this process. The effects of AA on G-6-P expression may be exerted *via* PI-3K, AKT, and GSK-3β.

Although our data support the effect of AA on PI3 K/AKT/GSK-3β in ameliorating diabetic symptoms, direct evidence of how AA functions through PI3K/AKT/GSK-3β remains lacking. AA and its derivatives as potential inhibitors of glycogen phosphorylases (31). Future studies should examine the mechanism by which AA regulates the expression of PI3K/AKT/GSK-3β.

In conclusion, we demonstrated that AA had clear lipid- and glucose-lowering effects in a db/db mouse model. AA functions through PI3K/AKT/GSK-3β to facilitate glycogen synthesis. This experimental evidence provides new clue for the development of anti-diabetic drugs.

Acknowledgements

This study was supported by grants from the Innovation Team Project of Beijing University of Chinese Medicine (no. 2011-CXTD-19) and the International Scientific Collaborative Project (no. 2012DFG31550).

References

1. Anna V, van der Ploeg HP, Cheung NW, Huxley RR and Bauman AE: Sociodemographic correlates of the increasing trend in prevalence of gestational diabetes mellitus in a large population of women between 1995 and 2005. Diabetes Care 31: 2288-2293, 2008.
2. Kaul K, Apostolopoulou M and Roden M: Insulin resistance in type 1 diabetes mellitus. Metabolism 64: 1629-1639, 2015.
3. Srinivasan K, Viswanad B, Asrat L, Kaul CL and Ramarao P: Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol Res 52: 313-320, 2005.
4. von Wilamowitz-Moellendorff A, Hunter RW, García-Rocha M, Kang L, López-Soldado I, Lantier L, Patel K, Peggie MW, Martinez-Pons C, Voss M, *et al*: Glucose-6-phosphate-mediated activation of liver glycogen synthase plays a key role in hepatic glycogen synthesis. Diabetes 62: 4070-4082, 2013.
5. MacAulay K and Woodgett JR: Targeting glycogen synthase kinase-3 (GSK-3) in the treatment of Type 2 diabetes. Expert Opin Ther Targets 12: 1265-1274, 2008.
6. Ciaraldi TP, Carter L, Mudaliar S and Henry RR: GSK-3beta and control of glucose metabolism and insulin action in human skeletal muscle. Mol Cell Endocrinol 315: 153-158, 2010.
7. Patel S, Doble BW, MacAulay K, Sinclair EM, Drucker DJ and Woodgett JR: Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. Mol Cell Biol 28: 6314-6328, 2008.
8. Park KS: The search for genetic risk factors of type 2 diabetes mellitus. Diabetes Metab J 35: 12-22, 2011.
9. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R and Matthews DR: Management of hyperglycaemia in type 2 diabetes: A patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). Diabetologia 55: 1577-1596, 2012.
10. Yang LX, Liu TH, Huang ZT, Li JE and Wu LL: Research progress on the mechanism of single-Chinese medicinal herbs in treating diabetes mellitus. Chin J Integr Med 17: 235-240, 2011.
11. Prabhakar PK and Doble M: Mechanism of action of natural products used in the treatment of diabetes mellitus. Chin J Integr Med 17: 563-574, 2011.
12. Orhan IE: *Centella asiatica* (L.) Urban: From traditional medicine to modern medicine with neuroprotective potential. Evid Based Complement Alternat Med 2012: 946259, 2012.
13. Pakdeechote P, Bunbupha S, Kukongviriyapan U, Prachaney P, Khrisanapant W and Kukongviriyapan V: Asiatic acid alleviates hemodynamic and metabolic alterations via restoring eNOS/iNOS expression, oxidative stress, and inflammation in diet-induced metabolic syndrome rats. Nutrients 6: 355-370, 2014.
14. Li Y, Yang F, Yuan M, Jiang L, Yuan L, Zhang X, Li Y, Dong L, Bao X and Yin S: Synthesis and evaluation of asiatic acid derivatives as anti-fibrotic agents: Structure/activity studies. Steroids 96: 44-49, 2015.
15. Ramachandran V, Saravanan R and Senthilraja P: Antidiabetic and antihyperlipidemic activity of asiatic acid in diabetic rats, role of HMG CoA: In vivo and in silico approaches. Phytomedicine 21: 225-232, 2014.
16. Zhang J, Ai L, Lv T, Jiang X and Liu F: Asiatic acid, a triterpene, inhibits cell proliferation through regulating the expression of focal adhesion kinase in multiple myeloma cells. Oncol Lett 6: 1762-1766, 2013.
17. Wang X, Lu Q, Yu DS, Chen YP, Shang J, Zhang LY, Sun HB and Liu J: Asiatic acid mitigates hyperglycemia and reduces islet fibrosis in Goto-Kakizaki rat, a spontaneous type 2 diabetic animal model. Chin J Nat Med 13: 529-534, 2015.
18. Ramachandran V and Saravanan R: Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Phytomedicine 20: 230-236, 2013.
19. Ramachandran V and Saravanan R: Glucose uptake through translocation and activation of GLUT4 in PI3K/Akt signaling pathway by asiatic acid in diabetic rats. Hum Exp Toxicol 34: 884-893, 2015.

20. McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PW and Jacques PF: Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. Diabetes Care 27: 538-546, 2004.
21. King AJ: The use of animal models in diabetes research. Br J Pharmacol 166: 877-894, 2012.
22. Wang YW, Sun GD, Sun J, Liu SJ, Wang J, Xu XH and Miao LN: Spontaneous type 2 diabetic rodent models. J Diabetes Res 2013: 401723, 2013.
23. Liu J, He T, Lu Q, Shang J, Sun H and Zhang L: Asiatic acid preserves beta cell mass and mitigates hyperglycemia in streptozocin-induced diabetic rats. Diabetes Metab Res Rev 26: 448-454, 2010.
24. Ramachandran V and Saravanan R: Asiatic acid prevents lipid peroxidation and improves antioxidant status in rats with streptozotocin-induced diabetes. J Funct Foods 5: 1077-1087, 2013.
25. Guilherme A, Virbasius JV, Puri V and Czech MP: Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nat Rev Mol Cell Biol 9: 367-377, 2008.
26. Guo S: Insulin signaling, resistance, and the metabolic syndrome: Insights from mouse models into disease mechanisms. J Endocrinol 220: T1-T23, 2014.
27. Fornoni A, Pileggi A, Molano RD, Sanabria NY, Tejada T, Gonzalez-Quintana J, Ichii H, Inverardi L, Ricordi C and Pastori RL: Inhibition of c-jun N terminal kinase (JNK) improves functional beta cell mass in human islets and leads to AKT and glycogen synthase kinase-3 (GSK-3) phosphorylation. Diabetologia 51: 298-308, 2008.
28. Bertsch S, Lang CH and Vary TC: Inhibition of glycogen synthase kinase 3[beta] activity with lithium in vitro attenuates sepsis-induced changes in muscle protein turnover. Shock 35: 266-274, 2011.
29. Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L and Henry RR: Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. Diabetes 49: 263-271, 2000.
30. Jiang SJ, Dong H, Li JB, Xu LJ, Zou X, Wang KF, Lu FE and Yi P: Berberine inhibits hepatic gluconeogenesis via the LKB1-AMPK-TORC2 signaling pathway in streptozotocin-induced diabetic rats. World J Gastroenterol 21: 7777-7785, 2015.
31. Zhang L, Chen J, Gong Y, Liu J, Zhang L, Hua W and Sun H: Synthesis and biological evaluation of asiatic acid derivatives as inhibitors of glycogen phosphorylases. Chem Biodivers 6: 864-874, 2009.