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Tanshinone IIA improves endoplasmic reticulum stress-induced insulin resistance through AMP-activated protein kinase

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ABSTRACT

The aim of the present study was to determine the effect of Tanshinone IIA (Tan IIA) on endoplasmic reticulum (ER) stress-induced insulin resistance in L6 myotubes and *db/db* mice. ER stress markers, RNA-activated protein kinase-like ER resident kinase (PERK), JNK, and AMPK activity were determined in tunicamycin–treated L6 myotubes. Insulin resistance was monitored using glucose uptake assays *in vitro* and blood glucose levels *in vivo*. Tan IIA clearly suppressed the phosphorylations of PERK and JNK and potentiated insulin-mediated Akt phosphorylation as well as glucose uptake via AMPK activation under ER stress. Furthermore, these effects are completely abrogated by siRNA-mediated knockdown of AMPK or LKB1. In addition, Tan IIA reduced blood glucose levels and body weights in *db/db* mice without altering food intake. These findings suggest that Tan IIA enhances insulin sensitivity and improves glucose metabolic disorders by increasing AMPK activity and attenuating ER stress-induced insulin resistance.

1. Introduction

Insulin resistance is major metabolic abnormality and leads to type 2 diabetes. Recently, various studies have shown that endoplasmic reticulum (ER) stress plays a crucial role in the development of insulin resistance and pathogenesis of type 2 diabetes [1–4]. Skeletal muscle glucose uptake is regulated by both intrinsic and circulating factors that are involved in the phosphatidylinositol (PI3)-kinase/Akt and the AMP-activated protein kinase (AMPK) signaling pathways [5]. In insulin-resistant skeletal muscle, reduced insulin receptor substrate (IRS) and Akt activation reduce skeletal muscle glucose uptake [6,7]. In contrast, the activation of AMPK by pharmacological agents, such as 5-amino-imidazole-4-carboxamide riboside (AICAR), increases muscle glucose uptake [8,9].

AMPK is a ubiquitously expressed serine/threonine protein kinase that has been implicated in the regulations of the metabolisms of glucose and lipid [10,11]. Upon activation by various stimuli, AMPK regulates a number of metabolic processes, such as, glucose uptake [12], fatty acid oxidation [13] and lipolysis [14]. In recent years, several major insulin-sensitizing agents, such as, metformin [15] and thiazolidinediones [16], have been developed that indirectly activate AMPK. These effects suggest that these agents improve insulin sensitivity and reduce plasma glucose and lipid contents caused, at least in part, by the activation of AMPK.

Tanshinone IIA (Tan IIA), a derivative of phenanthrenquinone, is one of the most abundant components of *Salvia miltiorrhiza bunge* (Danshen), which has been widely used in traditional Chinese medications to treat cardiovascular diseases [17]. In recent years, it is available for use in stroke, heart attack, and angina patients [18,19]. Furthermore, Tan IIA has been shown to protect against inflammation and oxidative stress [17,20]. However, the *in vitro* and *in vivo* effects of Tan IIA on insulin resistance have not been subjected to scientific study. Accordingly, in the present study, we investigated the effects of Tan IIA on ER stress-induced insulin resistance in L6 myotubes and on hyperglycemia and weight gain in *db/db* mice.

2. Materials and methods

2.1. Materials

Tan IIA (Fig. 1A) was isolated from the roots of *S. miltiorrhiza* and structurally identified according to the previous report [21].

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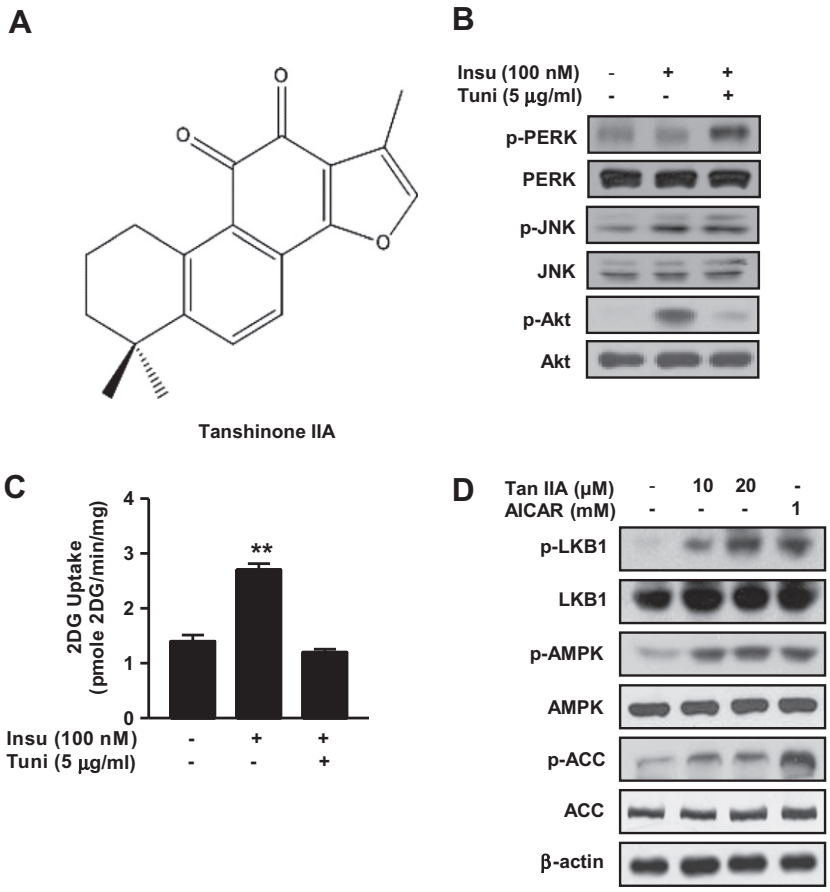

Fig. 1. ER stress inhibited insulin signaling in L6 myotubes. (A) Chemical structure of Tan IIA, (B) ER stress was induced in L6 myotubes by treating them with 5 μg/ml of tunicamycin for 3 h; cells were then stimulated with 100 nM of insulin. Levels of Akt phosphorylation (p-Akt) and the phosphorylations of PERK and JNK were examined by immunoblotting, (C) Cells were cultured as in (B), glucose uptake measurements was performed as described in the Section 2. All results are representative of at least three independent experiments. $^{*}P < 0.05$ and $^{\#}P < 0.05$ vs. untreated control and insulin-treated cells, respectively and (D) L6 myotubes were treated with Tan IIA (10, 20 μM) for 2 h. Total cell lysates were prepared, resolved by 8% SDS–PAGE, and immunoblotted with specific antibodies as indicated. Anti-β-actin antibody used as loading control.

Tunicamycin (an inhibitor of N-linked glycoprotein synthesis) and insulin were obtained from Sigma–Aldrich (St. Louis, MO). Fetal bovine serum (FBS), αMEM, trypsin/EDTA, and penicillin/streptomycin were from GIBCO (Auckland, NZ), and 2-Deoxy-[^{3}H] D-glucose was from Perkin-Elmer Life Sciences (Boston, MA, USA). Antibodies against total and phospho-AMPKα (Thr172), phospho-acetyl CoA carboxylase (pACC) (Ser79), phospho-Akt (Ser473), phospho-PERK (Tyr980), and phospho-JNK (Thr183/Tyr185) were purchased from Cell Signaling Technology (Beverly, MA, USA). β-actin was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA), and all other reagents were of the highest analytical grade available.

2.2. Animal care and experimental procedures

Male C57BL/KsJ *db/db* and lean C57BL/KsJ mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Mice were housed in a temperature-controlled room (22 ± 2 °C) under a 12 h light/dark cycle and allowed free access to water and standard rodent chow. The blood glucose levels were measured in blood obtained by tail clipping using a ONE TOUCH BASIC plus Glucose Monitor (Lifescan, Milpitas, CA, USA), unless otherwise specified. All animal experiments were approved beforehand by the Institutional Animal Care and Use Committee of Yeungnam University.

2.3. Glucose uptake assay

Radiolabeled 2-deoxyglucose uptake assay was carried out as previously described [22].

2.4. Cell culture

L6 myotubes were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell culture was performed as described previously [23].

2.5. Immunoblotting

Immunoblotting analysis was performed as described previously [23].

2.6. Transfection with small-interfering RNA (siRNA)

For siRNA experiments, SMARTpool for rat LKB1 (ONTARGET*plus* SMARTpool targeting rat LKB1, L-100539-01-0020), and AMPKα2 (L-100623-00-0020) were obtained from Dharmacon (Lafayette, CO). Nonspecific siRNA (ONTARGET*plus* siCONTROL Non-Targeting Pool, D-001810-10-20) were used as control.

2.7. Statistical analysis

All experiments were performed at least three times. Average values were expressed as mean ± SEMs and/or SDs. The analysis was performed using SPSS 9.0 (SPSS, Chicago, IL). The Student's *t*-test was used to compare two independent groups, statistically significant was accepted for P values of <0.05.

3. Results

3.1. ER stress inhibited insulin signaling in L6 myotubes

To understand the mechanisms underlying ER stress-induced insulin resistance, we treated L6 myotubes with tunicamycin, an ER stress inducer. Tunicamycin treatment markedly enhanced the phosphorylation levels of ER stress markers, including PERK and JNK. On the other hand, tunicamycin treatment markedly reduced insulin-stimulated Akt phosphorylation (Fig. 1B). Consistent with this, insulin-stimulated glucose uptake was reduced in tunicamycin-treated L6 myotubes (Fig. 1C), suggesting that ER stress negatively regulates insulin-stimulated Akt phosphorylation and glucose uptake. In addition, we examined the effects of Tan IIA on AMPK activation, which is related to insulin resistance. Treating the cells with Tan IIA led to substantial increases in the phosphorylations of AMPK and ACC (Fig. 1D). Furthermore, Tan IIA increased the level of phosphorylated from of LKB1, which is responsible for the phosphorylation of AMPK (Fig. 1D). Moreover, AICAR (5-aminoimidazole-4-carboxamide ribose), a well known AMPK agonist, increased the phosphorylations of LKB1 and AMPK (Fig. 1D). These results suggest that Tan IIA stimulates the AMPK signaling pathway.

3.2. Tan IIA improved ER stress-induced insulin resistance in L6 myotubes

ER stress has been shown to play a key role in the development of insulin resistance and in the pathogenesis of type 2 diabetes by impairing insulin signaling via JNK activation [24,25]. To characterize the molecular mechanisms involved in the anti-diabetic effects of Tan IIA further, we examined whether Tan IIA has an insulin-sensitizing effect under ER stress. Pretreatment with Tan IIA suppressed the tunicamycin-induced phosphorylations of PERK and JNK (Fig. 2A), and restored ER stress-reduced Akt phosphorylation in L6 myotubes (Fig. 3A). These results suggest that Tan IIA improves ER stress-induced insulin resistance in L6 myotubes. Several reports have shown that ER stress plays a causal role in the

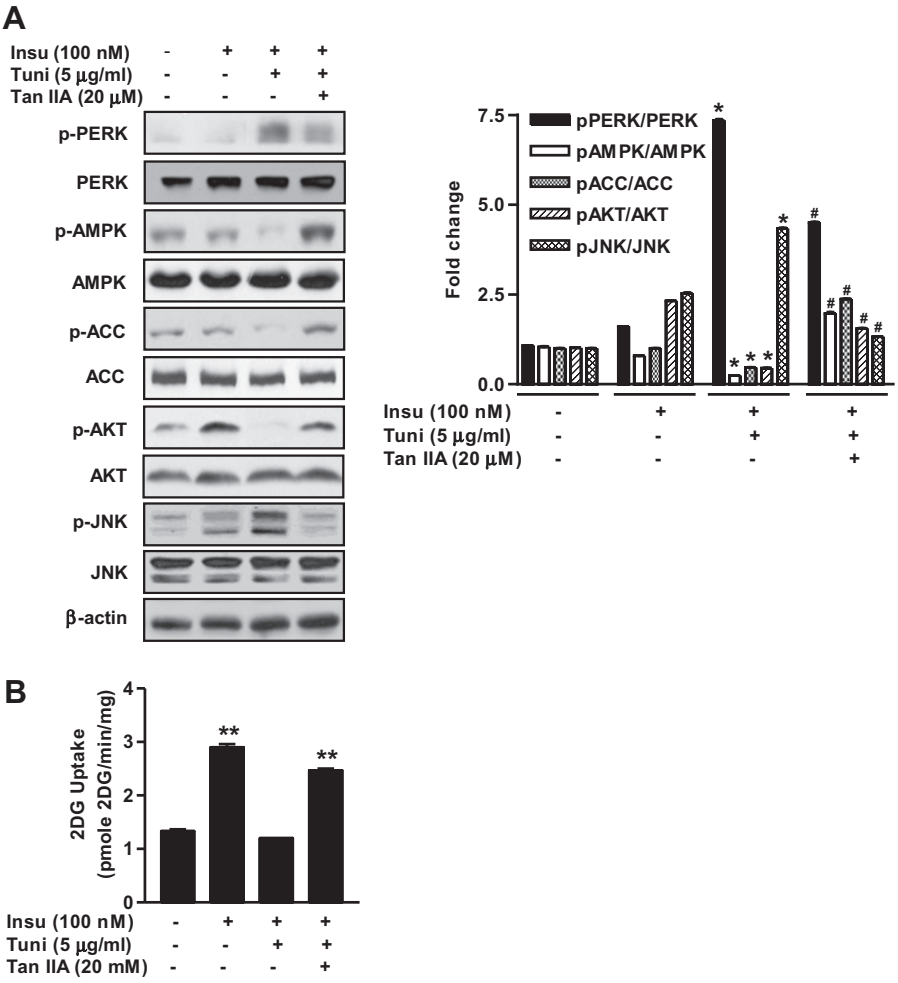

Fig. 2. Tan IIA protected L6 myotubes against insulin resistance by reducing ER stress. (A) L6 myotubes were incubated in the presence of 5 μg/ml of tunicamycin for 3 h with or without Tan IIA, and then stimulated with 100 nM of insulin. Phosphorylated PERK, JNK, Akt, AMPK, ACC, and their total protein levels were examined by direct immunoblotting (*left*). The blot shown is representative of three independent experiments performed in triplicate; $^{*}P < 0.05$, insulin vs. tunicamycin plus insulin; $n = 3$. $^{\#}P < 0.05$, tunicamycin plus insulin vs. tunicamycin plus insulin treated with Tan IIA; $n = 3$ (*right*). (B) Tan IIA improved insulin-mediated glucose uptake in tunicamycin-treated L6 myotubes. Cells incubated with Tan IIA (20 μM) for 2 h were treated with tunicamycin (5 μg/ml) for 3 h and then with insulin (100 nM) for 10 min. Representative glucose uptake and quantifications of five independent experiments are shown. Results are expressed as means ± SEMs ($n = 4$). $^{**}P < 0.01$ vs. untreated controls.

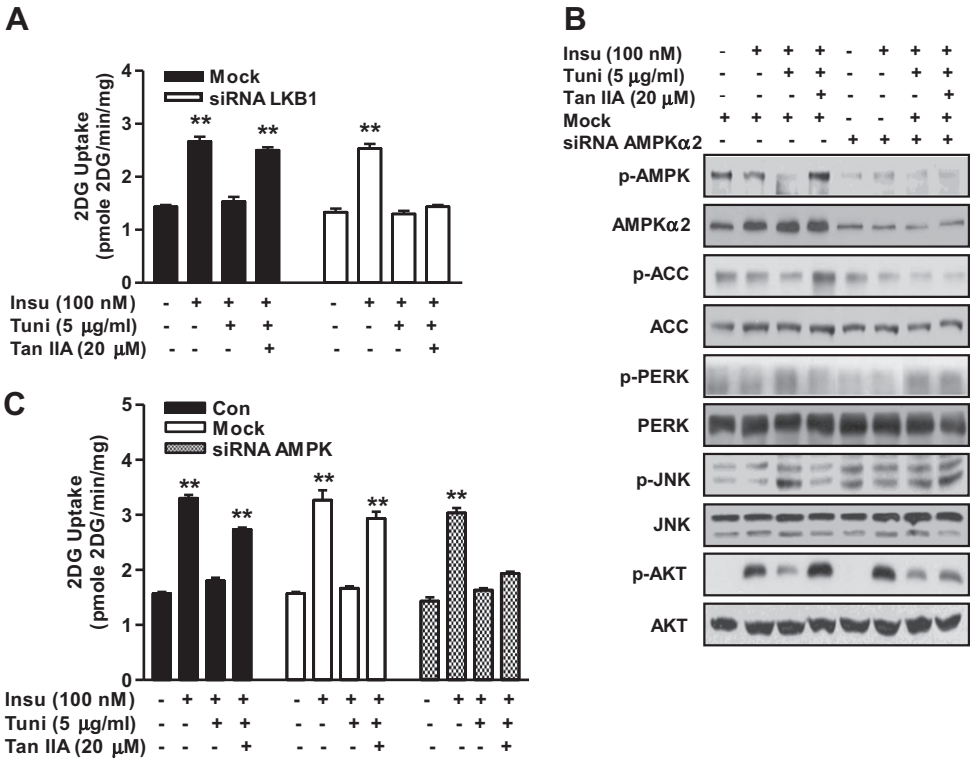

Fig. 3. Tan IIA reversed ER stress-reduced glucose uptake in L6 myotubes via the LKB1-AMPK pathway. (A) L6 myotubes were transfected with mock or LKB1 siRNA for 48 h, pretreated with or without Tan IIA for 2 h, treated with tunicamycin for 3 h and then stimulated with or without insulin (100 nM) for 10 min. Representative glucose uptake and quantifications of five independent experiments are shown. Results are expressed as means ± SEMs ($n = 4$). $^{**}P < 0.01$ vs. untreated controls, (B) L6 myotubes were transfected with mock or AMPKα2 siRNA for 48 h. Cells incubated with Tan IIA (20 μM) for 2 h were treated with tunicamycin (5 μg/ml) for 3 h and then treated with insulin (100 nM) for 10 min. The phosphorylated (p-AMPK, p-ACC, p-PERK, p-JNK, p-Akt) and total levels of each protein were determined by immunoblotting using specific antibodies and (C) L6 myotubes were transfected with mock or siRNA AMPKα2 for 48 h, pretreated with or without Tan IIA for 2 h, treated with tunicamycin for 3 h and then stimulated with or without insulin (100 nM) for 10 min. Representative glucose uptake and quantifications of five independent experiments are shown. Results are expressed as means ± SEMs ($n = 4$). $^{**}P < 0.01$ vs. untreated controls.

loss of AMPK activity in response to ER stress inducers, such as, thapsigargin and tunicamycin [26]. As was expected, the phosphorylations of AMPK and ACC were significantly reduced by tunicamycin, and this was prevented by pretreating the cells with Tan IIA (Fig. 3A). These results demonstrate that ER stress is associated with reduced insulin signaling and AMPK phosphorylation, which suggest that Tan IIA might be used to prevent metabolic abnormalities by reducing ER stress associated with insulin resistance (Fig. 3A). Next, we investigated the effect of ER stress on insulin-stimulated glucose uptake. Cells were incubated in the presence of tunicamycin for 3 h, and then treated with insulin (100 nM) for 10 min. As shown in Fig 3B, tunicamycin dramatically reduced insulin-stimulated glucose uptake in L6 myotubes, but pretreatment with Tan IIA significantly prevented this response (Fig. 3B). These results demonstrate that Tan IIA can relieve ER stress and restore both insulin signaling and AMPK activity.

3.3. LKB1 is required for Tan IIA-induced AMPK activation

LKB1 is the major upstream kinase of AMPK [27,28] and regulates AMPK activity by phosphorylating its Thr^{172} regulatory site [29,30]. We used siRNA-mediated knockdown of LKB1 to explore the participation of LKB1 in Tan IIA signaling. Tan IIA-treatment rescued insulin-stimulated glucose uptake, but had no significant rescuing effect on ER stress-reduced insulin signaling in L6 myotubes pretreated with siRNA LKB1 (Fig. 4A). These findings suggest that LKB1 is essential for Tan IIA-improved ER stress-reduced insulin sensitivity in L6 myotubes.

To assess whether AMPK was involved in the inhibitory effects of Tan IIA on the ER stress-induced activations of PERK and JNK, we silenced AMPKα2 expression using siRNA. AMPK was highly phosphorylated in L6 myotubes treated with Tan IIA, whereas knockdown with siRNA AMPKα2 nearly abolished this phosphorylation vs. control siRNA (Fig. 4B). Furthermore, the inhibitory effects of Tan IIA on the ER stress-induced activations of PERK and JNK were abolished in L6 myotubes transfected with AMPKα2 siRNA (Fig. 4B). Furthermore, siRNA AMPKα2 markedly reduced Tan IIA-mediated Akt phosphorylation and glucose uptake under ER stress (Fig. 4B and C). Taken together, these results suggest that Tan IIA-mediated AMPK activation is sufficient to prevent insulin-signaling impairment by ER stress.

3.4. Tan IIA had anti-diabetic activity in db/db mice

To evaluate whether and how Tan IIA is able to reduce blood glucose levels, we examined its effects in *db/db* mice, which exhibit extreme insulin resistance. Mice were treated orally daily for 2 weeks (from 10 to 12 weeks of age) with Tan IIA (100 mg/kg). To assess the effect of Tan IIA on glucose homeostasis and insulin sensitivity, we measured blood glucose levels. Plasma glucose levels were compared to control and rosiglitazone (10 mg/kg) treatment in *db/db* mice. Interestingly, Tan IIA significantly reduced blood glucose levels vs. untreated *db/db* mice (Fig. 4A), and importantly, food intake was no different between Tan IIA and rosiglitazone treated or untreated *db/db* mice (Fig. 4B). Interestingly, oral administration of Tan IIA reduced body weights slightly, whereas the body weights of rosiglitazone-treated and untreated *db/db* mice increased (Fig. 4C). We next examined whether Tan IIA altered the phosphorylation levels of AMPK, ACC, Akt, and ER stress markers in skeletal muscle tissues. As was expected, Tan IIA suppressed phosphorylated PERK and JNK levels vs. untreated *db/db*

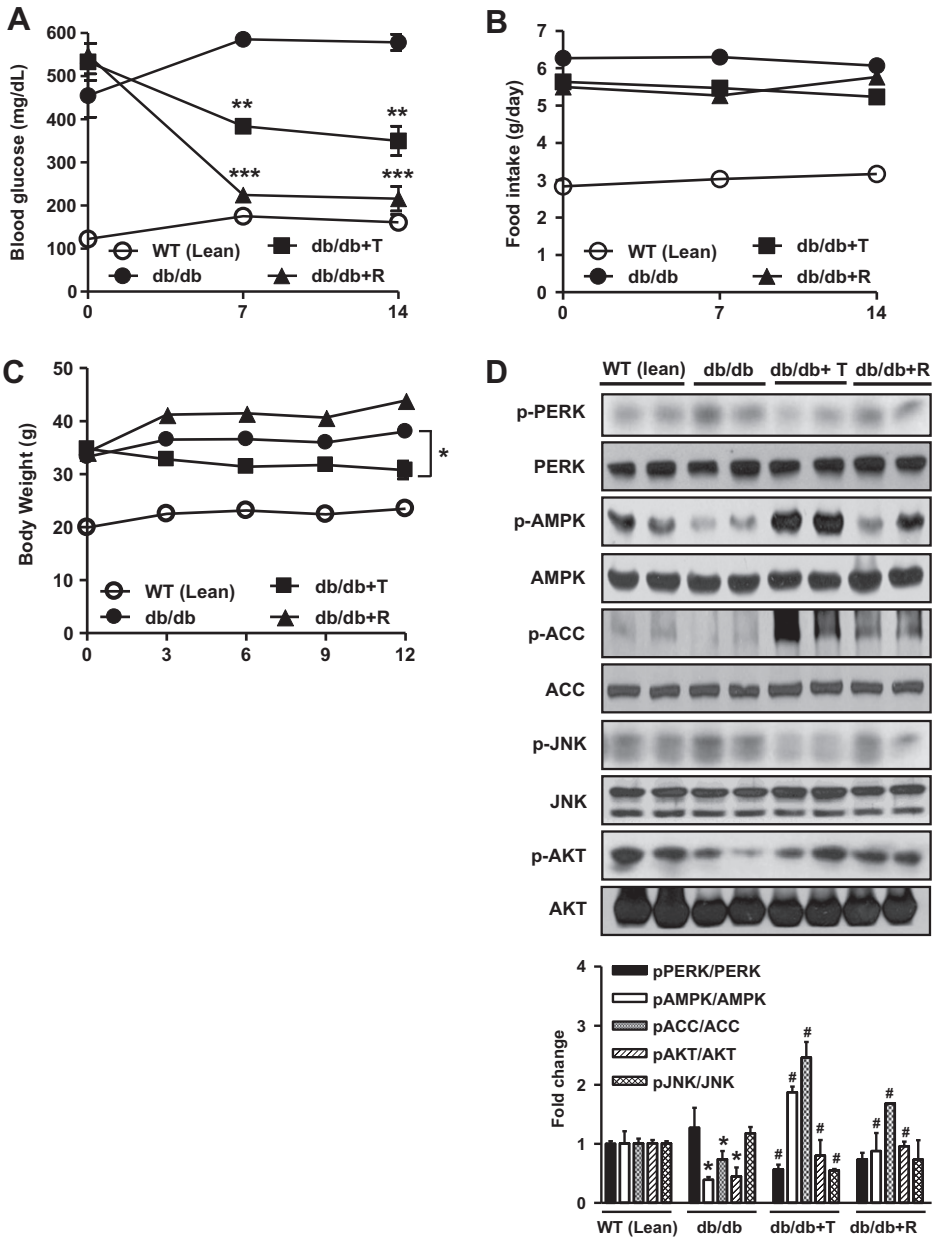

Fig. 4. Effect of Tan IIA treatment on systemic glucose metabolism and insulin sensitivity in *db/db* mice. Eight-week-old lean WT and *db/db* mice were orally adminstered Tan IIA (100 mg/kg/day) or rosiglitazone (5 mg/kg/day) for 14 days. (A) Effect of Tan IIA on nonfasting blood glucose levels. ○, Lean; ●, *db/db*; ■, *db/db* + Tan IIA; ▲, *db/db* + rosi. Results are presented as means ± SEMs ($n = 8$). $^{**}P < 0.01$; $^{***}P < 0.001$ vs. untreated *db/db* mice, (B) Effect of Tan IIA on food intake. ○, Lean; ●, *db/db*; ■, *db/db* + Tan IIA; ▲, *db/db* + rosi, (C) Effect of Tan IIA or rosiglitazone on body weight. ○, Lean; ●, *db/db*; ■, *db/db* + Tan IIA; ▲, *db/db* + rosi. Results are presented as means ± SEMs ($n = 8$). $^{*}P < 0.05$ vs. untreated *db/db* mice and (D) Effects of Tan IIA treatment on markers of ER stress in the skeletal muscle tissues of *db/db* mice. Phosphorylations of PERK (p-PERK) and JNK (p-JNK) in the muscle tissues of Tan IIA- or vehicle-treated *db/db* mice and lean controls. Phosphorylations of Akt and AMPK in the muscle tissues of Tan IIA- and vehicle-treated *db/db* mice and lean WT controls. Total proteins were obtained from *db/db* mice muscle tissues after orally administering Tan IIA for 14 days, and immunoblotted with corresponding antibodies. Phosphorylated AMPK, ACC, Akt, PERK, and JNK were examined in skeletal muscle extracts by direct immunoblotting (*top*). The blot shown is representative of three blots experiments performed in triplicate; $^{*}P < 0.05$, WT vs. *db/db* (control); $n = 3$. $^{\#}P < 0.05$, *db/db* (control) vs. *db/db* treated with Tan IIA or *db/db* treated with rosiglitazone; $n = 3$ (*bottom*).

mice, whereas phosphorylated AMPK and Akt levels increased in Tan IIA-treated *db/db* mice (Fig. 4D). Similar results were observed in skeletal muscle tissue obtained from rosiglitazone-treated *db/db* mice (Fig. 4D). These results support the notion that Tan IIA-mediated AMPK activation suppresses ER stress and increases insulin signaling in *db/db* mice.

4. Discussion

Numerous studies have shown that Tan IIA protects against vascular disease, such as, atherosclerosis or blood cotting abnormalities [31,32]. Liu et al. recently found that Tan IIA markedly improved impaired nerve functions [33], and Zhang et al. found that Tan IIA significantly prevented cardiac dysfunction in streptozocin-induced diabetic rats [34]. Pan et al. found that Tan IIA increased eNOS dependent nitric oxide (NO) production via phosphorylation of AMPK/PI3K/Akt pathway [35]. These investigations indicate that Tan IIA exhibits a wide range of activities on pathological state of the organism. The present study extends this by showing that Tan IIA can have a negative impact on metabolic disorders, including obesity and insulin resistance. Furthermore, our *in vitro* and *in vivo* studies show that suppression of ER

stress-induced insulin resistance by Tan IIA is associated with the activation of the LKB1-AMPK axis.

ER stress was also found to play a causal role in the loss of AMPK activity response to the ER stress inducers, thapsigargin and tunicamycin [26], which suggests agents that alleviate ER stress might be used to protect cells from ER stress-induced insulin resistance [36]. Previous studies have shown that the administration of active chemical chaperones to obese or diabetic mice can reduce ER stress by increasing folding capacity, which can restore systemic insulin sensitivity in liver, skeletal muscle, and white adipose tissues [4]. Base on these studies, we further investigated the effect of Tan IIA on JNK activity and on PERK under ER stress. Our results show that Tan IIA can suppress the ER stress-induced phosphorylations of PERK and JNK (Fig. 2A).

In insulin-resistant skeletal muscle, there appears to be a decrease in the insulin-stimulated associations between insulin receptor substrate (IRS) proteins and PI3-kinase, in the activation of Akt, and glucose uptake [37,38]. Surprisingly, we found that Tan IIA markedly rescued insulin-stimulated phosphorylation of Akt and glucose uptake under ER stress (Fig. 2A and B), which suggests that Tan IIA might improve impaired insulin signal transduction.

In a separate experiment, we found that Tan IIA failed to reduce ER stress-induced insulin resistance in L6 myotubes transfected with LKB1 siRNA (Fig. 3A), which shows that LKB1 is required for the activation of AMPK by Tan IIA. To determine whether the alleviation of ER stress by Tan IIA is mediated by AMPK activation, we also examined the effect of the siRNA-mediated knockdown of AMPKα2. It was found that transfection with siRNA AMPKα2, but not with control siRNA, blocked the Tan IIA-mediated preservation of the action of insulin under ER stress (Fig. 3B and C). These results suggest that Tan IIA suppresses ER stress-induced insulin resistance by activating of LKB1-AMPK axis.

Finally, we investigated that Tan IIA could suppress hyperglycemia using a type 2 diabetes model. The oral administration of Tan IIA to *db/db* mice reduced blood glucose in hyperglycemic animals (Fig. 4A), suggesting that the blood glucose-lowering effect of Tan IIA is due to an increase in insulin sensitivity. We considered that if the reversal of hyperglycemia and insulin sensitivity were related to a decrease ER stress, Tan IIA-treated *db/db* mice should display a reduction in the indicators of ER stress. We found the phosphorylations of PERK and JNK were markedly lower and that the phosphorylations of AMPK and Akt were higher in Tan IIA-treated *db/db* mice than in vehicle-treated controls (Fig. 4D). These results suggest that Tan IIA significantly improved insulin resistance in diabetic mice at least in part via insulin/Akt pathways and AMPK signaling.

Summarizing, Tan IIA was found to improve ER stress-induced insulin resistance in L6 myotubes by activating AMPK, and to reduce body weight and serum glucose levels in an animal model of insulin resistance. Taken together, our findings indicate that Tan IIA might be useful as a therapeutic agent for the treatment of metabolic disorders, including type 2 diabetes and insulin resistance.

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References

[1] U. Ozcan, Q. Cao, E. Yilmaz, A.H. Lee, N.N. Iwakoshi, E. Ozdelen, G. Tuncman, C. Gorgun, L.H. Glimcher, G.S. Hotamisligil, Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes, Science 306 (2004) 457–461.

[2] Y. Nakatani, H. Kaneto, D. Kawamori, K. Yoshiuchi, M. Hatazaki, T.A. Matsuoka, K. Ozawa, S. Ogawa, M. Hori, Y. Yamasaki, M. Matsuhisa, Involvement of endoplasmic reticulum stress in insulin resistance and diabetes, J. Biol. Chem. 280 (2005) 847–851.

[3] K. Ozawa, M. Miyazaki, M. Matsuhisa, K. Takano, Y. Nakatani, M. Hatazaki, T. Tamatani, K. Yamagata, J. Miyagawa, Y. Kitao, O. Hori, Y. Yamasaki, S. Ogawa, The endoplasmic reticulum chaperone improves insulin resistance in type 2 diabetes, Diabetes 54 (2005) 657–663.

[4] U. Ozcan, E. Yilmaz, L. Ozcan, M. Furuhashi, E. Vaillancourt, R.O. Smith, C.Z. Gorgun, G.S. Hotamisligil, Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes, Science 313 (2006) 1137–1140.

[5] A.J. Rose, E.A. Richter, Skeletal muscle glucose uptake during exercise: how is it regulated?, Physiology (Bethesda) 20 (2005) 260–270

[6] C. Yu, Y. Chen, G.W. Cline, D. Zhang, H. Zong, Y. Wang, R. Bergeron, J.K. Kim, S.W. Cushman, G.J. Cooney, B. Atcheson, M.F. White, E.W. Kraegen, G.I. Shulman, Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle, J. Biol. Chem. 277 (2002) 50230–50236.

[7] H. Cho, J. Mu, J.K. Kim, J.L. Thorvaldsen, Q. Chu, E.B. Crenshaw 3rd, K.H. Kaestner, M.S. Bartolomei, G.I. Shulman, M.J. Birnbaum, Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta), Science 292 (2001) 1728–1731.

[8] T. Hayashi, M.F. Hirshman, E.J. Kurth, W.W. Winder, L.J. Goodyear, Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport, Diabetes 47 (1998) 1369–1373.

[9] S.B. Jorgensen, B. Viollet, F. Andreelli, C. Frosig, J.B. Birk, P. Schjerling, S. Vaulont, E.A. Richter, J.F. Wojtaszewski, Knockout of the alpha2 but not alpha1 5'-AMP-activated protein kinase isoform abolishes 5-aminoimidazole-4-carboxamide-1-beta-4-ribofuranosidebut not contraction-induced glucose uptake in skeletal muscle, J. Biol. Chem. 279 (2004) 1070–1079.

[10] D.G. Hardie, J.W. Scott, D.A. Pan, E.R. Hudson, Management of cellular energy by the AMP-activated protein kinase system, FEBS Lett. 546 (2003) 113–120.

[11] D. Carling, The AMP-activated protein kinase cascade–a unifying system for energy control, Trends Biochem. Sci. 29 (2004) 18–24.

[12] J. Mu, J.T. Brozinick Jr., O. Valladares, M. Bucan, M.J. Birnbaum, A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle, Mol. Cell 7 (2001) 1085–1094.

[13] G.F. Merrill, E.J. Kurth, D.G. Hardie, W.W. Winder, AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle, Am. J. Physiol. 273 (1997) E1107–1112.

[14] R.R. Russell 3rd, R. Bergeron, G.I. Shulman, L.H. Young, Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR, Am. J. Physiol. 277 (1999) H643–649.

[15] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, J. Clin. Invest. 108 (2001) 1167–1174.

[16] L.G. Fryer, A. Parbu-Patel, D. Carling, The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways, J. Biol. Chem. 277 (2002) 25226–25232.

[17] S.I. Jang, S.I. Jeong, K.J. Kim, H.J. Kim, H.H. Yu, R. Park, H.M. Kim, Y.O. You, Tanshinone IIA from salvia miltiorrhiza inhibits inducible nitric oxide synthase expression and production of TNF-alpha, IL-1beta and IL-6 in activated RAW 264.7 cells, Planta Med. 69 (2003) 1057–1059.

[18] G. Zhou, W. Jiang, Y. Zhao, G. Ma, W. Xin, J. Yin, B. Zhao, Sodium tanshinone IIA sulfonate mediates electron transfer reaction in rat heart mitochondria, Biochem. Pharmacol. 65 (2003) 51–57.

[19] X.Y. Ji, B.K. Tan, Y.Z. Zhu, Salvia miltiorrhiza and ischemic diseases, Acta Pharmacol. Sin. 21 (2000) 1089–1094.

[20] S.I. Jang, H.J. Kim, Y.J. Kim, S.I. Jeong, Y.O. You, Tanshinone IIA inhibits LPS-induced NF-kappaB activation in RAW 264.7 cells: possible involvement of the NIK-IKK, ERK1/2, p38 and JNK pathways, Eur. J. Pharmacol. 542 (2006) 1–7.

[21] H.T. Trinh, S.J. Chae, E.H. Joh, K.H. Son, S.J. Jeon, D.H. Kim, Tanshinones isolated from the rhizome of Salvia miltiorrhiza inhibit passive cutaneous anaphylaxis reaction in mice, J. Ethnopharmacol. 132 (2010) 344–348.

[22] R. Somwar, G. Sweeney, T. Ramlal, A. Klip, Stimulation of glucose and amino acid transport and activation of the insulin signaling pathways by insulin lispro in L6 skeletal muscle cells, Clin. Ther. 20 (1998) 125–140.

[23] S.L. Hwang, H.W. Chang, I.K. Lee, B.K. Yang, J. Magae, Y.C. Chang, Ascofuranone prevents ER stress-induced insulin resistance via activation of AMP-activated protein kinase in L6 myotube cells, Biochem. Biophys. Res. Commun. 396 (2010) 967–972.

[24] X. Shen, R.E. Ellis, K. Lee, C.Y. Liu, K. Yang, A. Solomon, H. Yoshida, R. Morimoto, D.M. Kurnit, K. Mori, R.J. Kaufman, Complementary signaling pathways regulate the unfolded protein response and are required for *C. elegans* development, Cell 107 (2001) 893–903.

[25] J. Hirosumi, G. Tuncman, L. Chang, C.Z. Gorgun, K.T. Uysal, K. Maeda, M. Karin, G.S. Hotamisligil, A central role for JNK in obesity and insulin resistance, Nature 420 (2002) 333–336.

[26] S.M. Rahman, I. Qadri, R.C. Janssen, J.E. Friedman, Fenofibrate and PBA prevent fatty acid-induced loss of adiponectin receptor and pAMPK in human hepatoma cells and in hepatitis C virus-induced steatosis, J. Lipid Res. 50 (2009) 2193–2202.

[27] H.J. Koh, D.E. Arnolds, N. Fujii, T.T. Tran, M.J. Rogers, N. Jessen, Y. Li, C.W. Liew, R.C. Ho, M.F. Hirshman, R.N. Kulkarni, C.R. Kahn, L.J. Goodyear, Skeletal

muscle-selective knockout of LKB1 increases insulin sensitivity, improves glucose homeostasis, and decreases TRB3, Mol. Cell. Biol. 26 (2006) 8217–8227.
[28] K. Sakamoto, A. McCarthy, D. Smith, K.A. Green, D. Grahame Hardie, A. Ashworth, D.R. Alessi, Deficiency of LKB1 in skeletal muscle prevents AMPK activation and glucose uptake during contraction, EMBO J. 24 (2005) 1810–1820.
[29] S.A. Hawley, J. Boudeau, J.L. Reid, K.J. Mustard, L. Udd, T.P. Makela, D.R. Alessi, D.G. Hardie, Complexes between the LKB1 tumor suppressor, STRAD alpha/beta and MO25 alpha/beta are upstream kinases in the AMP-activated protein kinase cascade, J. Biol. 2 (2003) 28.
[30] A. Woods, S.R. Johnstone, K. Dickerson, F.C. Leiper, L.G. Fryer, D. Neumann, U. Schlattner, T. Wallimann, M. Carlson, D. Carling, LKB1 is the upstream kinase in the AMP-activated protein kinase cascade, Curr. Biol. 13 (2003) 2004–2008.
[31] S. Ling, A. Dai, Z. Guo, P.A. Komesaroff, A preparation of herbal medicine Salvia miltiorrhiza reduces expression of intercellular adhesion molecule-1 and development of atherosclerosis in apolipoprotein E-deficient mice, J. Cardiovasc. Pharmacol. 51 (2008) 38–44.
[32] C.M. Yu, J.C. Chan, J.E. Sanderson, Chinese herbs and warfarin potentiation by 'danshen', J. Intern. Med. 241 (1997) 337–339.
[33] Y. Liu, L. Wang, X. Li, C. Lv, D. Feng, Z. Luo, Tanshinone IIA improves impaired nerve functions in experimental diabetic rats, Biochem. Biophys. Res. Commun. 399 (2010) 49–54.
[34] Y. Zhang, L. Wei, D. Sun, F. Cao, H. Gao, L. Zhao, J. Du, Y. Li, H. Wang, Tanshinone IIA pretreatment protects myocardium against ischaemia/reperfusion injury through the phosphatidylinositol 3-kinase/Akt-dependent pathway in diabetic rats, Diabetes Obes. Metab. 12 (2010) 316–322.
[35] C. Pan, L. Lou, Y. Huo, G. Singh, M. Chen, D. Zhang, A. Wu, M. Zhao, S. Wang, J. Li, Salvianolic acid B and tanshinone IIA attenuate myocardial ischemia injury in mice by NO production through multiple pathways, Ther. Adv. Cardiovasc. Dis. 5 (2011) 99–111.
[36] D.M. Muoio, C.B. Newgard, Biomedicine. Insulin resistance takes a trip through the ER, Science 306 (2004) 425–426.
[37] K. Cusi, K. Maezono, A. Osman, M. Pendergrass, M.E. Patti, T. Pratipanawatr, R.A. DeFronzo, C.R. Kahn, L.J. Mandarino, Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle, J. Clin. Invest. 105 (2000) 311–320.
[38] G.I. Shulman, Cellular mechanisms of insulin resistance, J. Clin. Invest. 106 (2000) 171–176.