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RESEARCH PAPER

Andrographolide derivative AL-1 improves insulin resistance through down-regulation of NF-κB signalling pathway

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BACKGROUND AND PURPOSE

Andrographolide is the most active constituent of the medicinal plant *Andrographis paniculata*. Previously, we synthesized a novel andrographolide derivative AL-1, conjugating andrographolide with lipoic acid. Although the antioxidative and/or anti-inflammatory activity of AL-1 contributes to its cytoprotective effects, whether AL-1 can improve insulin resistance and the mechanisms responsible for its action have not been elucidated.

EXPERIMENTAL APPROACH

We investigated the anti-hyperlipidaemic and anti-hyperglycaemic effects of AL-1 in a high-fat diet/streptozocin-induced animal diabetic model. In addition, we investigated the effect of AL-1 on the NF-κB signalling pathway in rat islet derived insulinoma cells (RIN-m cells) with a focus on the link between reactive oxygen species-associated inflammation and insulin resistance.

KEY RESULTS

AL-1, at doses of 40 and 80 mg·kg^{-1}, had a significant hypoglycaemic effect; it significantly reduced the level of cholesterol and increased HDL. AL-1 also reduced the homeostasis model assessment of insulin resistance and enhanced insulin sensitivity. In addition, AL-1 improved the morphology of pancreatic islets and their function. Furthermore, AL-1 suppressed high glucose-induced phosphorylation of p65 and IκBα in RIN-m cells.

CONCLUSION AND IMPLICATIONS

AL-1 has a hypoglycaemic effect and improves insulin resistance in type 2 diabetic rats. It protected islet from high glucose-induced oxidative damage by down-regulating the NF-κB signalling pathway. Further investigations of AL-1 as a promising new agent for treatment and/or prevention of diabetes are warranted.

Abbreviations

HOMA-IR, homeostasis model assessment of insulin resistance; ISI, insulin sensitivity index; STZ, streptozocin

Tables of Links

TARGETS
GLUT4

LIGANDS	
Glibenclamide	Insulin

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Diabetes is one of the major and common public health problems today. According to the statistics data of the International Diabetes Federation, the number of people with diabetes was 366 000 000 in 2011, which will grow to 552 000 000 in 2030 all over the world (Zimmet *et al.*, 2001). Diabetes mellitus is classified into two major categories: type 1 diabetes mellitus (T1DM), which is caused by complete insulin desensitization and deletion of pancreatic β-cell function, and type 2 diabetes mellitus (T2DM) whose causes are both genetic and environmental (Shaw *et al.*, 2010). The main causes of type 2 diabetes are insulin resistance and pancreatic β-cell dysfunction characterized by carbohydrate metabolic and lipid metabolism disorders (West, 2000; Liu *et al.*, 2013).

Reactive oxygen species (ROS) are one of the main concerns in diabetes over the years (West, 2000; Aruoma *et al.*, 2006). ROS can directly damage islet β-cells leading to β-cell apoptosis and reduction of β-cell mass (Hou *et al.*, 2008). NF-κB, an important nucleus transcriptional factor, is involved in the production of pro-inflammatory proteins and plays a pivotal role in inflammation and oxidative stress (Wei *et al.*, 2008; Remels *et al.*, 2009). In the inactivation state, NF-κB combines with the inhibitory cytoplasmic protein IκB. Once activated, the p50/p65 subunits of NF-κB are transferred into the nucleus to boost the expression of inflammatory proteins, following the occurrence of inflammation and oxidative stress.

Andrographolide (Andro) is a traditional drug in Chinese medicine. A large body of literature has reported that Andro has beneficial effects in diseases related to inflammation and oxidative stress, such as diarrhoea, rheumatoid arthritis and laryngitis (Chiou *et al.*, 2000; Lu *et al.*, 2012). Andro has both hyperlipidaemia and hyperglycaemia lowering effects. Andro reduces serum cholesterol, triglycerides and LDL-cholesterol in hypercholesterolaemic patients and high-fat diet-fed animals (Reyes *et al.*, 2006; Nugroho *et al.*, 2013). Andro attenuates oxidative stress through inhibition of the activation of the NF-κB signalling pathway and a post-transcriptional mechanism (Chen *et al.*, 2013; Zhu *et al.*, 2013a). We had previously synthesized a novel Andro derivative AL-1, conjugating Andro with lipoic acid (Jiang *et al.*, 2009). Although the antioxidative and/or anti-inflammatory activity of AL-1 contributes to its cytoprotective effects (Yan *et al.*, 2013; Zhu *et al.*, 2013b), the effect of AL-1 on insulin resistance and the mechanisms responsible for its action have not been elucidated to date. In the present study, we investigated the effect of AL-1 on hyperlipidaemia and hyperglycaemia in a high-fat diet/streptozocin (STZ)-induced animal diabetic model. Since oxidative stress and inflammation play a pivotal role in the initiation of insulin resistance, we investigated the effect of AL-1 on the NF-κB signalling pathway in rat islet derived insulinoma cells (RIN-m cells) with a focus on the link between ROS-associated inflammation and insulin resistance.

Methods

Animals and study design

All animal welfare and experimental procedures were in strict accordance with the Research Ethics Committee of Jinan University. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of 110 animals were used in the experiments described here.

Specific pathogen-free, male Sprague-Dawley rats (aged 6–8 weeks) were purchased from the Animal Research Centre of Jinan University (Guangzhou, China). The rats were kept in a temperature-controlled room with 12 h dark/light cycles, and were allowed free food and water *ad libitum*. All rats were randomly divided into two groups: control group (group I) served as non-diabetic controls receiving a normal chow diet; and the challenge group were fed a high-fat diet *ad libitum* for a period of 8 weeks. In order to develop type 2 diabetes, the challenge group was subjected to an overnight fast and received a dose of STZ (35 $mg \cdot kg^{-1}$ in 0.1 M citrate buffer, pH 4.4) i.p., while group I was given vehicle citrate buffer (pH 4.4) i.p. The formation of type 2 diabetes was confirmed by measuring fasting serum glucose levels with a glucometer 7 days after the STZ injection. Rats with fasting blood glucose ≥ 11.1 $mmol \cdot L^{-1}$ were considered to be diabetic. The challenge group was randomly divided into six groups of 10 animals each. Group II diabetic rats given high-fat diet served as diabetic model rats. Group III-V diabetic rats were treated with AL-1 at a dose of 20, 40 and 80 $mg \cdot kg^{-1}$ respectively. Group VI diabetic rats were treated with Andro at a dose of 50 $mg \cdot kg^{-1}$ (equal molar dose to 80 $mg \cdot kg^{-1}$ of AL-1). Group VII diabetic rats were treated with glibenclamide at a dose of 1.2 $mg \cdot kg^{-1}$ for 4 weeks. The blood glucose levels of the rats were monitored at 2 and 4 weeks after STZ injection. The blood glucose levels were measured using an auto analyser (Roche Diagnostics GmbH, Penzberg, Germany).

Cell culture

RIN-m cells were maintained in RPMI-1640 medium with L-glutamine supplemented with 10% FBS (Gibco), 100 U mL^{-1}

penicillin and 100 μg·mL^{-1} streptomycin in a humidified atmosphere of 5% CO_2 at 37°C.

Biochemical analysis of blood

The rats were killed under deep anaesthesia by isoflurane inhalation. The blood was taken from the abdominal aorta via syringes and prepared for measurement of insulin, lipid profile and parameters related to insulin. Plasma insulin was quantified according to the manufacturer's protocol using a rat insulin ELISA kit. Total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C were detected by colorimetric assays. The marker of insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR).

Histopathological examinations

Pancreatic tissues were collected and fixed in 4% buffered formaldehyde solution overnight at 4°C. The fixed tissues were cut into small pieces and put in a labelled tissue cassette for dehydration processing. The tissues were cleaned twice with xylene before being embedded in paraffin. Paraffin sections were cut into slices of 4 μm and stained with hematoxylin-eosin (HE) staining solution. Finally, the stained sections were observed and photographed under a light microscope (with 200× magnification).

Western blot assay

To investigate the mechanism of how AL-1 improves insulin resistance, we examined the effect of AL-1 on the high glucose-induced NF-κB activation in RIN-m cells by Western blotting. AL-1-treated and untreated RIN-m cells were scraped and washed twice with an ice-cold PBS. The washed cell pellets were lysed in a lysis buffer before centrifugation at 14 000× *g* at 4°C. The protein concentration was then measured using a BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL, USA). Equal amounts of protein (20 μg) were boiled with loading buffer for 10 min. The protein extracts were subjected to electrophoresis on SDS-PAGE gel and then transferred to a nitrocellulose membrane. The membranes were incubated with skimmed milk (5%) for 4 h at room temperature and then with primary antibodies overnight at 4°C, followed by incubation with corresponding secondary antibodies. The signals were developed by using an ECL Western blot detection kit (Pierce, Rockford, IL, USA) and visualized on X-ray film.

Statistical analysis

The experimental data are expressed as the mean ± SD. ANOVA and least significant difference tests were used to make comparisons among the groups using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Differences with $P < 0.05$ were considered statistically significant.

Antibodies and other reagents

The rat insulin ELISA kit was purchased from R&D Systems Inc. (Minneapolis, MN, USA). AL-1 was synthesized in our laboratory. Andro and glibenclamide (Glib) were purchased from Alfa Aesar (Ward Hill, MA, USA). Total NF-κB/p65, NF-κB/IκBα, NF-κB/p50 and Phospho-NF-κB/IκBα, Phospho-NF-κB/p65 antibodies were purchased from Cell Signaling Technology Inc. (Boston, MA, USA). RPMI-1640 and DMEM were purchased from Gibco (Grand Island, NY, USA). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Results

AL-1 improved glucose metabolism in type 2 diabetic rats

After 4 weeks of intervention, the glucose level in control rats was maintained in a normal range throughout the experiment with a slight increase of 5.95%. Meanwhile, the blood glucose levels of diabetic rats were significantly higher than that in the control group, indicating that STZ had a harmful effect on pancreatic islets. However, the blood glucose was gradually reduced in the groups, which were treated with AL-1, Andro and glibenclamide. The blood glucose was decreased by 13.25, 15.13 and 21.81% after treatment with AL-1 at doses of 20, 40 and 80 mg·kg^{-1} (Table 1). In sharp contrast, Andro (50 mg·kg^{-1}), an equal molar dose of AL-1 (80 mg·kg^{-1}), only lowered blood glucose by 18%. The results showed that AL-1 had a significant hypoglycaemic effect.

Table 1

AL-1 improved glucose metabolism in type 2 diabetic rats

	Blood glucose level (mmol·L^{-1})				
Group (mg·kg^{-1})	Day 0	Day 14	Change (%)	Day 28	Change (%)
Control	4.87 ± 0.43	4.92 ± 0.71	+5.75	5.16 ± 0.51	+5.95
DM	24.10 ± 4.18	23.33 ± 4.45	−3.20	22.11 ± 5.34	−8.26
AL-1 (20 mg·kg^{-1})	24.83 ± 3.77	22.67 ± 3.95	−8.70	21.54 ± 3.89	−13.25
AL-1 (40 mg·kg^{-1})	23.60 ± 3.41	21.23 ± 2,78	−10.04	20.03 ± 3.30$^{\#}$	−15.13
AL-1 (80 mg·kg^{-1})	23.48 ± 3.95	16.99 ± 3.82$^{\#\#}$	−27.64	18.36 ± 2.83$^{\#}$	−21.81
Andrographolide (50 mg·kg^{-1})	23.83 ± 4.36	19.76 ± 4.35	−17.08	19.54 ± 4.97	−18.00
Glibenclamide (1.2 mg·kg^{-1})	24.06 ± 3.75	14.95 ± 3.50$^{\#\#}$	−37.86	16.40 ± 2.98$^{\#}$	−31.84

Values are expressed as means ± SD of 10 rats. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, significantly different compared with the DM group.

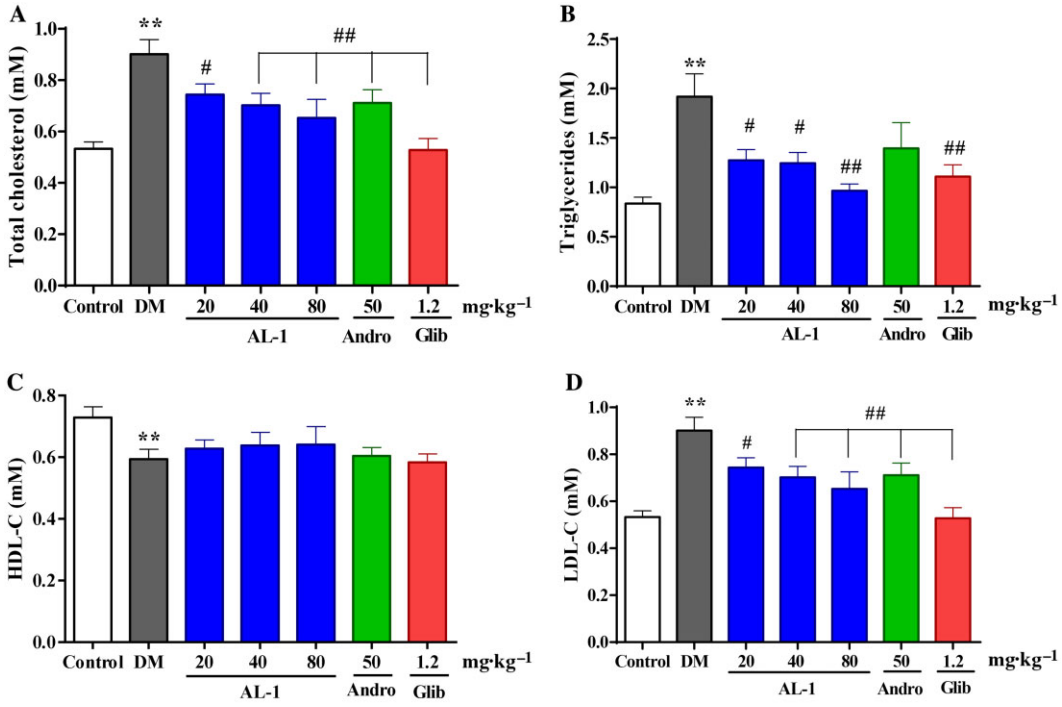

Figure 1

AL-1 ameliorated lipid metabolism in type 2 diabetic rats. AL-1 decreased the levels of (A) total cholesterol, (B) triglycerides and (D) LDL-C in a dose-dependent manner compared with those in the diabetic rats. (C) HDL-C level was slightly increased by AL-1 treatment. Values are expressed as means ± SD of 10 rats. $^{**}P < 0.01$ significantly different compared with control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ significantly different compared with the DM group.

AL-1 ameliorated the lipid levels in type 2 diabetic rats

Plasma TC, LDL-C and TG levels in diabetic rats were significantly increased by 102.97, 69.81 and 101.19 compared with that in the control rats, and plasma HDL level in diabetic rats was decreased by 19.18%. AL-1 (80 mg·kg^{-1}) significantly reduced the TC, LDL-C and TG levels (27.32, 27.78 and 42.6%), and elevated the HDL-C levels (8.47%) compared with those in the diabetic rats (Figure 1).

AL-1 promoted glycogen synthesis in type 2 diabetic rats

Under STZ stimulation, glycogen synthesis was significantly lower than that of the control group, indicating that STZ treatment induced insulin resistance in rats. AL-1 increased glycogen synthesis in a dose-dependent manner. Glycogen synthesis was enhanced by 40.91, 47.86 and 59.89% at the doses of 20, 40 and 80 mg·kg^{-1} of AL-1 respectively (Figure 2A).

Effects of AL-1 on insulin, insulin sensitivity index (ISI) and HOMA-IR in type 2 diabetic rats

As shown in Figure 2B, the plasma insulin level in diabetic rats was significantly increased. After 4 weeks of treatment, AL-1 reduced insulin levels in diabetic rats. Insulin levels were reduced by 23.31, 34.07 and 37.37% with 20, 40 and 80 mg·kg^{-1} of AL-1.The HOMA-IR level was significantly reduced after treatment with AL-1 (Figure 2C). The ISI level of diabetic rats was lower than that of control rats. However, the ISI level was significantly increased in diabetic rats treated with AL-1. AL-1 (20 mg·kg^{-1}) increased the ISI level by 4.07%, followed by AL-1 (40 mg·kg^{-1}; 7.56%), AL-1 (80 mg·kg^{-1}; 9.59%), Andro (50 mg·kg^{-1}; 4.36%) and glibenclamide (1.2 mg·kg^{-1}; 11.77%; Figure 2D). These results showed that AL-1 ameliorated insulin resistance in type 2 DM.

AL-1 improved pancreatic morphology in type 2 diabetic rats

Based on HE-stained tissue sections, vehicle administration alone caused no notable changes in pancreatic histology throughout the 4 weeks of study (Figure 3). In contrast, STZ administration elicited severe injury of the pancreas, decreased the number of islets cells and diminished the diameter of the pancreatic islets. The islets were shrunken in diabetic rats compared with those in control rat. Administration of AL-1, Andro and glibenclamide moderately enlarged the islets and significantly reduced the scores of the injuries of the pancreas. In light of these figures, AL-1 obviously ameliorated islet damage and effectively improved islet mass, which suggests that AL-1 had a potential therapeutic effect in type 2 diabetes.

AL-1 inhibited high glucose-induced NF-κB activation

Numerous studies have shown that NF-κB is involved in many inflammatory cascade events (Tantiwong *et al.*, 2010;

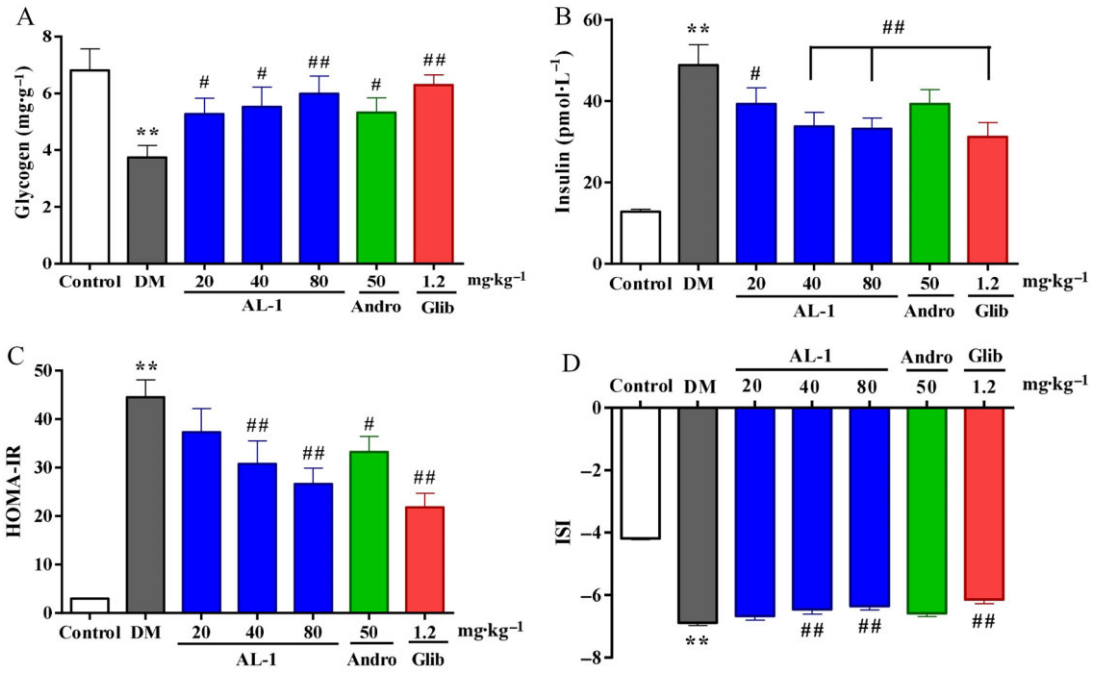

Figure 2

Effects of AL-1 on (A) glycogen synthesis, (B) insulin, (C) HOMA-IR and (D) ISI in type 2 diabetic rats. Values are expressed as means ± SD of 10 rats. $^{**}P < 0.01$ significantly different compared with control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ significantly different compared with the DM group.

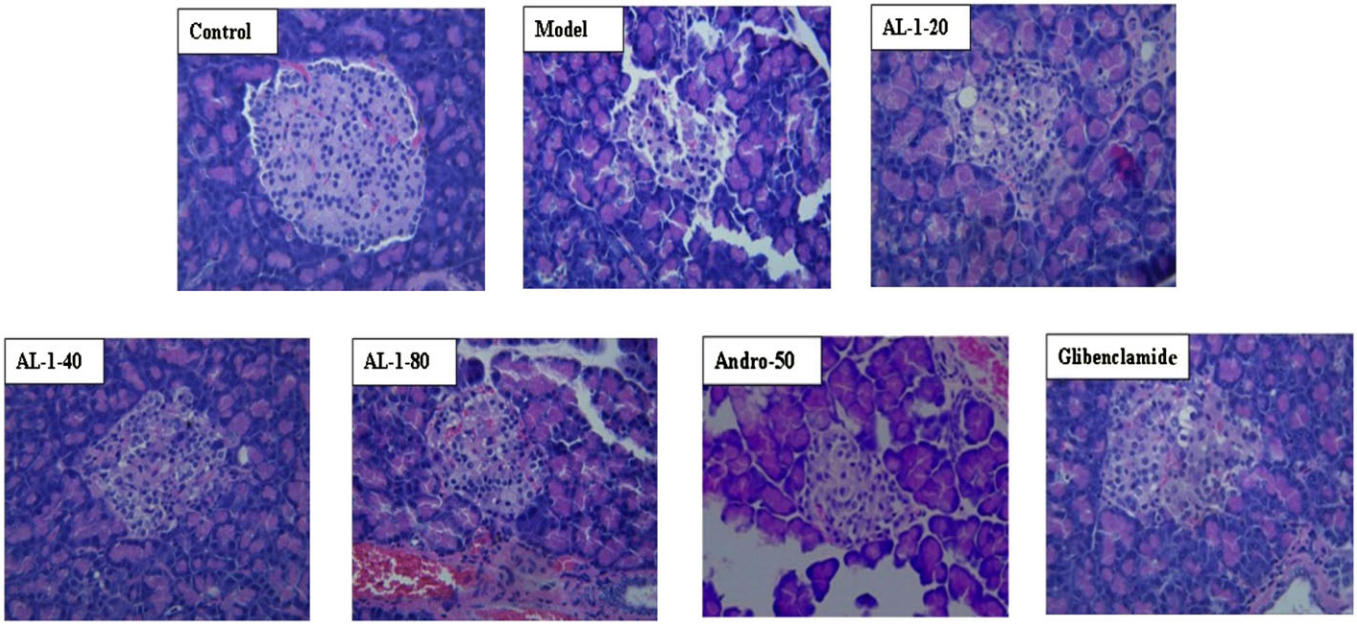

Figure 3

AL-1 improved pancreatic morphology in type 2 diabetic rats. Pancreatic tissues were collected and fixed into 4% buffered formaldehyde solution overnight at 4°C. The tissues were cleaned twice with xylene before being embedded in paraffin. Paraffin sections were cut into slices of 4 μm and stained with HE staining solution. Finally, the stained sections were photographed under a light microscope (with 200× magnification).

Shao *et al.*, 2012), so we investigated the effect of AL-1 on NF-κB activation in RIN-m cells (Figure 4). High glucose markedly increased the phosphorylation of IκBα and NF-κB p65 subunits compared with those in non-stimulated RIN-m cells. However, AL-1 effectively suppressed the phosphorylation of IκBα and p65, but had no effect on p50. These results indicate that AL-1 inhibited NF-κB activation induced by high glucose in RIN-m cells.

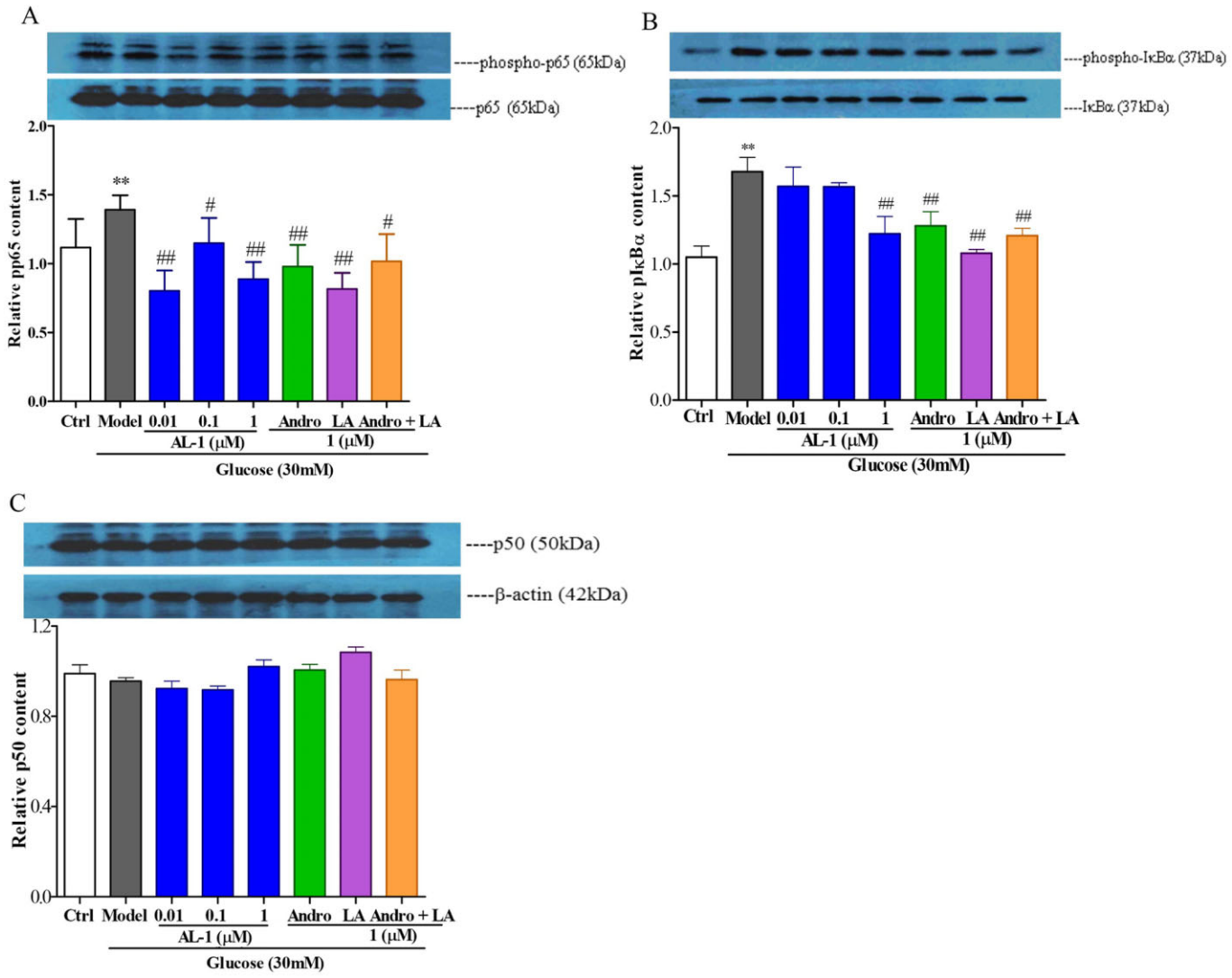

Figure 4

Effects of AL-1 on protein expression of pp65 (A), pIκBα (B) and p50 (C) in high glucose treated RIN-m cells. Data are expressed as means ± SD $^{**}P < 0.01$ significantly different compared with control group. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ significantly different compared with the DM group.

Discussion

This study first demonstrated that AL-1 had a hypoglycaemic effect and improved insulin resistance in the type 2 diabetic rats. It also significantly reduced HOMA-IR and enhanced insulin sensitivity, leading to an improvement in the morphology and function of pancreatic islets. Moreover, AL-1 suppressed phosphorylation of the p65 and IκBα proteins, suggesting that AL-1 protected the islets from high glucose-induced oxidative damage via down-regulation of the NF-κB signalling pathway.

High-fat diet-fed/STZ-induced type 2 diabetes rats are a well-recognized model for the screening of antidiabetic agents. STZ specially penetrates the β-cells via glucose transporter and induces the DNA strand breakage in β-cells causing a decrease in endogenous insulin release (Ferber *et al.*, 2000). Many studies have reported that a long-term high-fat diet leads to insulin resistance and hyperinsulinaemia (Srinivasan *et al.*, 2005; Feng *et al.*, 2014). Under the strain of compensatory hyperinsulinaemia, β-cells were easily damaged by low doses of STZ. In other words, the high-fat diet combined with low doses of STZ-induced diabetic rats have the characteristics of later-stage T2DM including hyperglycaemia, moderate impairment of insulin secretion, abnormalities in lipid metabolism, destruction of islet cells and reduced glycogen synthesis (Whitton and Hems, 1975).

In a previous study, we investigated the antidiabetic effects of AL-1 in type 1 diabetic mice induced with alloxan (Zhang *et al.*, 2009). We found that AL-1 lowered blood glucose, prevented loss of β-cells and their dysfunction, and stimulated the glucose transport protein subtype 4 (GLUT4) membrane translocation in soleus muscles. In the present study, the results also showed that the blood glucose level of diabetic rats was significantly decreased after treatment with

AL-1, providing direct evidence for the hypoglycaemic effect of AL-1. We propose that there are two potential mechanisms accounting for the hypoglycaemic action of AL-1. Firstly, it may stimulate insulin release or regenerate β-cells. Secondly, it may enhance the sensitivity of target tissues to insulin. Therefore, we determined the serum insulin level and the histology of the pancreas. Our results showed that the serum insulin level was increased and the number and size of islets were decreased in DM group. However, AL-1 significantly increased the number of islets and inhibited the secretion of insulin in the diabetic rats.

The amelioration of insulin sensitivity is an important therapeutic approach for type 2 diabetes. HOMA-IR is a common index of insulin resistance, derived from fasting insulin and glucose concentrations (Matsuda and DeFronzo, 1999). In our current studies, high glucose had an adverse effect on insulin resistance and ISI in the rat model. AL-1 significantly reduced the HOMA-IR and increased the level of ISI. These results suggested that AL-1 could ameliorate the insulin sensitivity of diabetic rats. In addition, T2DM patients are often prone to suffer from dyslipidaemia (Goldberg *et al.*, 2005). Insulin resistance contributes to the development of lipid accumulation in hepatocyte through impairing the capacity of insulin to repress lipolysis. This event leads to elevated lipid accumulation in livers. In the present study, AL-1 significantly reduced the concentrations of TC, TG and LDL-C, and increased HDL-C concentration. This may be due to the indirect anti-hyperglycaemic effect of AL-1 in diabetic rats.

NF-κB, a vital signalling pathway, regulates the expression of a large number of genes and its over-expression is implicated in many diseases (Hu *et al.*, 2012; Samy *et al.*, 2012). In unstimulated cells, The NF-κB p50/NF-κB p65 dimer is bound to IκBα. After stimulation, IKKα and IKKβ are phosphorylated, promoting the phosphorylation of IκB. The phosphorylated IκBα is then rapidly degraded via the ubiquitin-proteasome pathway. The degradation of IκBα leads to NF-κB p50/NF-κB p65 dimer phosphorylation and translocation, resulting in the transcription of related genes (Grilli *et al.*, 1993; Baeuerle and Baichwal, 1997). In this regard, we wondered whether AL-1 inhibited NF-κB activation in RIN-m cells. Using RIN-m cells, we discovered that high glucose markedly promoted NF-κB p65 phosphorylation and AL-1 suppressed NF-κB p65 phosphorylation induced by high glucose. To gain insight into the biochemical mechanism, we studied the effects of AL-1 on the phosphorylation of IκBα. Our data showed that high glucose led to rapidly activation of the NF-κB pathway, involving IκBα and NF-κB p65 phosphorylation. Nevertheless, the high glucose-induced phosphorylated proteins were attenuated by AL-1 administration. These results demonstrated that the antidiabetic effect of AL-1 was probably mediated by inactivation of NF-κB.

In conclusion, AL-1 facilitated the responsiveness to insulin, decreased insulin resistance and blood glucose level in rats with high-fat diet/STZ-induced diabetes. AL-1 also ameliorated lipid metabolism, promoted glycogen synthesis and maintained the normal structure of islet and β-cells. Regulating the expressions of p65 and IκBα phosphorylation to improve the pancreatic system might be the underlying mechanisms of these effects. The results suggest that maintaining a stable environment for β-cells might be a new insight for the prevention and treatment of diabetes.

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Author Contributions

Y. L. performed the animal experiments and wrote the first draft of the paper. H. Y. performed cell experiments. Z. Z. and G. Z. assessed the results and performed the statistical analyses. Y. S. and P. Y. contributed to discussion and technical guidance or assistance. Y. W. and L. X. conceived, guided and supported experiments, assessed the results and gave the final version of the paper. All authors read and approved the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013). The Concise Guide to PHARMACOLOGY 2013/14: Transporters. Br J Pharmacol 170: 1706–1796.

Aruoma OI, Grootveld M, Bahorun T (2006). Free radicals in biology and medicine: from inflammation to biotechnology. Biofactors 27: 1–3.

Baeuerle PA, Baichwal VR (1997). NF-κB as o frequent target for immunosuppressive and anti-inflammatory molecules. Adv Immunol 65: 111–137.

Chen Y-Y, Hsu M-J, Sheu J-R, Lee L-W, Hsieh C-Y (2013). Andrographolide, a novel NF-κB inhibitor, induces vascular smooth muscle cell apoptosis via a ceramide-p47phox-ROS signaling cascade. Evid Based Complement Alternat Med 2013: 1–10.

Chiou WF, Chen CF, Lin JJ (2000). Mechanisms of suppression of inducible nitric oxide synthase (iNOS) expression in RAW 264.7 cells by andrographolide. Br J Pharmacol 129: 1553–1560.

Feng X, Scott A, Wang Y, Wang L, Zhao Y, Doerner S *et al.* (2014). PTPRT regulates high-fat diet-induced obesity and insulin resistance. PLoS ONE 9: e100783.

Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I *et al.* (2000). Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med 6: 568–572.

Goldberg RB, Kendall DM, Deeg MA, Buse JB, Zagar AJ, Pinaire JA *et al*. (2005). A comparison of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care 28: 1547–1554.

Grilli M, Chiu JJ-S, Lenardo MJ (1993). NF-κB and Rel: participants in a multiform transcriptional regulatory system. Int Rev Cytol 143: 62.

Hou ZQ, Li HL, Zhao JJ, Li GW (2008). Impairment of pancreatic islet beta cell function induced by intermittent high glucose through oxidative and endoplasmic reticulum stress: experiment with rat pancreatic islet beta cells]. Zhonghua Yi Xue Za Zhi 88: 2002–2004.

Hu J, Luo CX, Chu WH, Shan YA, Qian ZM, Zhu G *et al*. (2012). 20-Hydroxyecdysone protects against oxidative stress-induced neuronal injury by scavenging free radicals and modulating NF-kappaB and JNK pathways. PLoS ONE 7: e50764.

Jiang X, Yu P, Jiang J, Zhang Z, Wang Z, Yang Z *et al*. (2009). Synthesis and evaluation of antibacterial activities of andrographolide analogues. Eur J Med Chem 44: 2936–2943.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting in vivo experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Liu Z, Li W, Li X, Zhang M, Chen L, Zheng YN *et al*. (2013). Antidiabetic effects of malonyl ginsenosides from Panax ginseng on type 2 diabetic rats induced by high-fat diet and streptozotocin. J Ethnopharmacol 145: 233–240.

Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR (2012). Suppression of NF-kappaB signaling by andrographolide with a novel mechanism in human platelets: regulatory roles of the p38 MAPK-hydroxyl radical-ERK2 cascade. Biochem Pharmacol 84: 914–924.

Matsuda M, DeFronzo RA (1999). Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22: 1462–1470.

McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Nugroho AE, Lindawati NY, Herlyanti K, Widyastuti L, Pramono S (2013). Anti-diabetic effect of a combination of andrographolide-enriched extract of *Andrographis paniculata* (Burm f.) Nees and asiaticoside-enriched extract of *Centella asiatica* L. in high fructose-fat fed rats. Indian J Exp Biol 51: 1101–1108.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al*.; NC-IUPHAR (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl. Acids Res 42 (Database Issue): D1098–D1106.

Remels AH, Langen RC, Gosker HR, Russell AP, Spaapen F, Voncken JW *et al*. (2009). PPARgamma inhibits NF-kappaB-dependent transcriptional activation in skeletal muscle. Am J Physiol Endocrinol Metab 297: E174–E183.

Reyes B, Bautista N, Tanquilut N, Anunciado R, Leung A, Sanchez G *et al*. (2006). Anti-diabetic potentials of *Momordica charantia* and *Andrographis paniculata* and their effects on estrous cyclicity of alloxan-induced diabetic rats. J Ethnopharmacol 105: 196–200.

Samy RP, Rajendran P, Li F, Anandi NM, Stiles BG, Ignacimuthu S *et al*. (2012). Identification of a novel *Calotropis procera* protein that can suppress tumor growth in breast cancer through the suppression of NF-kappaB pathway. PLoS ONE 7: e48514.

Shao ZJ, Zheng XW, Feng T, Huang J, Chen J, Wu YY *et al*. (2012). Andrographolide exerted its antimicrobial effects by upregulation of human beta-defensin-2 induced through p38 MAPK and NF-kappaB pathway in human lung epithelial cells. Can J Physiol Pharmacol 90: 647–653.

Shaw JE, Sicree RA, Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 87: 4–14.

Srinivasan K, Viswanad B, Asrat L, Kaul C, Ramarao P (2005). 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.

Tantiwong P, Shanmugasundaram K, Monroy A, Ghosh S, Li M, DeFronzo RA *et al*. (2010). NF-kappaB activity in muscle from obese and type 2 diabetic subjects under basal and exercise-stimulated conditions. Am J Physiol Endocrinol Metab 299: E794–E801.

Wei Y, Sowers JR, Clark SE, Li W, Ferrario CM, Stump CS (2008). Angiotensin II-induced skeletal muscle insulin resistance mediated by NF-kappaB activation via NADPH oxidase. Am J Physiol Endocrinol Metab 294: E345–E351.

West IC (2000). Radicals and oxidative stress in diabetes. Diabet Med 17: 171–180.

Whitton PD, Hems DA (1975). Glycogen synthesis in the perfused liver of streptozotocin-diabetic rats. Biochem J 150: 153–165.

Yan GR, Zhou HH, Wang Y, Zhong Y, Tan ZL, He QY (2013). Protective effects of andrographolide analogue AL-1 on ROS-induced RIN-mbeta cell death by inducing ROS generation. PLoS ONE 8: e63656.

Zhang Z, Jiang J, Yu P, Zeng X, Larrick JW, Wang Y (2009). Hypoglycemic and beta cell protective effects of andrographolide analogue for diabetes treatment. J Transl Med 7: 62.

Zhu T, Wang D-X, Zhang W, Liao X-Q, Guan X, Bo H *et al*. (2013a). Andrographolide protects against LPS-induced acute lung injury by inactivation of NF-κB. PLoS ONE 8: e56407.

Zhu YY, Yu G, Zhang Y, Xu Z, Wang YQ, Yan GR *et al*. (2013b). A novel andrographolide derivative AL-1 exerts its cytotoxicity on K562 cells through a ROS-dependent mechanism. Proteomics 13: 169–178.

Zimmet P, Alberti KG, Shaw J (2001). Global and societal implications of the diabetes epidemic. Nature 414: 782–787.